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THE REGENERATION OF THE MARGINAL PERIODONTIUM AFTER FLAP OPERATION

AN EXPERIMENTAL STUDY ON DOGS

by

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The relative merits of radical elimination of pathological periodontal pockets, i.e. gingivectomy, compared with more conservative surgical methods involving the reflection of flaps and the curettage of the soft and hard tissue walls of the pocket with subsequent re-positioning and suturing of the flaps, are of great importance in the field of periodontology.

Experimental studies of the tissue regeneration, after each of these types of operation, on the clinically healthy periodontium of man or animals would obviously be the best method to elucidate this problem. The use of clinically healthy tissues for the experiments would show the different phases of healing under similar conditions and serve as a starting point for studies of pathologically affected tissues.

Relatively few experimental studies of gingivectomy have been carried out. A review of published work on this subject was included in a paper by the present author (*Persson, 1959*) and the results compared with his own observations.

Many more papers dealing with flap operations have been pub-

lished. Most attention has been directed towards the possibility of obtaining re-attachment by this type of operation and a variety of opinions have been put forward.

Meyer (1932) seems to have been the first to investigate experimentally the results of flap operations. He carried out these operations on the clinically healthy periodontium of two dogs and found that, even if a clinically satisfactory result was obtained, there was always histologic evidence of chronic inflammation and proliferation of epithelium down over cementum, and that "... ein bis dahin gesunder Zahnfleischsaum in einen kranken umgewandelt werden muss". *Stones* (1934) produced experimentally deepened pockets on monkeys, either by simple separating the subgingival epithelium and connective tissue from the cementum or by also scraping away the cementum lining the artificial pocket. In the first series he found that "... occasionally, complete re-attachment of the epithelium and underlying connective tissue to cementum was effected.", but in the second series that "Partial re-attachment is frequently observed and the usual site is at the base of the pocket or injury..."

Skillen & Lundquist (1935, 1937) produced artificial periodontal pockets on dogs with healthy gingivae and studied the results histologically after varying periods of time. They concluded that connective tissue re-attachment can only take place in a very small area at the apical end of the artificial pocket because of the rapid proliferation of the pocket epithelium. *Morgenroth* (1940) carried out flap operations on dogs and studied the histology of the healing wound two months after operation. He found that, after this period, there was chronic inflammation in the area around the experimental lesion with proliferation of the pocket epithelium down along the cementum. Thus, he believed that the previously healthy tissues had been converted to a permanent state of chronic inflammation. *Borden* (1948) performed flap operations on both man and dogs and showed histologically that "The connective tissue fibres of the periodontal membrane will re-attach themselves to the tooth cementum." He also drew the rather surprising conclusion that "New cementum, bone and connective tissue are not essential for re-attachment." The only explanation for this conclusion would appear to be that *Borden* had not in fact produced "detachment" by breaking the union

between the cementum and the Sharpey's fibres so that all that he had studied was the healing of a simple wound. *Beube* (1947) studied the regeneration of the marginal periodontium in dogs. He made a curved incision in the mucosa, reflected a soft-tissue flap and removed buccal bone, periodontal membrane, cementum and some dentine and then sutured the flap back into place. After 27 days, he observed new cementum formation on the damaged dentine surface and deposition of new alveolar bone. "At 2 years, a structurally wellarranged, new periodontal membrane was present." In this series of experiments, the operation did not involve the gingival margin so that the type of healing is not representative of the healing after flap operation since it is the periodontal pocket which provides the major problem in the regeneration of the periodontal tissues. These experiments of *Beube* are, however, very interesting since the healing process in the periodontium was studied from as early as 24 hours after operation and thereafter at varying intervals for up to 2 years. These results thus provide a good basis for comparison of the healing after reflection of this type of flap with the type of healing which follows a "flap operation" involving the marginal periodontium.

Glickman & Lazansky (1950) compared the histologic picture of healing after gingivectomy with that after flap operation and found that there was good restitution of the periodontal tissues after both types of operation; these experiments were carried out on dogs with chronic marginal gingivitis. *Linghorne & O'Connell* (1950) reflected muco-periosteal flaps from the marginal periodontium of dogs, exposed and removed bone to a depth of about 6 mm and removed the periodontal membrane to the level of the resected bone. In some cases the tooth was scaled, in others, no scaling was done. After 18 days, the gingiva in the upper jaw appeared clinically normal but healing in the lower jaw was less satisfactory. There was histologic evidence of "successful" connective tissue attachment in the upper jaw with deposition of new cementum, preceded by resorption of both cementum and dentine. In the lower jaw where the result was poorer, there was an epithelialized pocket and only little re-attachment.

A more recent study by the same workers (*Linghorne & O'Connell*, 1951) using the same methods except that bone chips were

packed into the pockets in some cases, confirmed in general principle the results of the earlier investigation.

In his re-attachment experiments, *Waerhaug* (1952) produced artificial pockets by removing periodontal membrane and cementum and studied the results histologically. Out of 21 cases, some re-attachment was attained in 13, while in 8 it failed complete. He concluded that this method was very unreliable in producing re-attachment.

In a series of experiments on clinically healthy human gingivae, *Morris* (1953) studied the histology of the healing of surgically created pockets. In some cases, cementum was removed from the root surface, in other cases this was left undisturbed, except that a groove was cut in the tooth with a bur at the apical border of the wound. A clinically satisfactory result was obtained in 19 out of the 20 cases. Five of these were studied histologically ". . . after healing intervals ranging from 21 to 106 days . . . Connective tissue healing took place in all surgical pockets against both dentine and cementum . . . Cellular cementum and cementoid were deposited in all types of root surfaces." *Morris* (1957) also studied the healing of human periodontal tissues following surgical detachment from non-vital teeth. He observed that "Healing occurs directly with the dentine of vital teeth and cementum of vital and non-vital teeth, and does not occur with dentine of non-vital teeth." In subsequent studies (*Morris*, 1960) he found that there was a difference in the reaction of non-vital teeth from which the pulp had been extirpated but no root-filling inserted, and root-filled teeth. In the former, there was deposition of new cementum on parts of the exposed dentine and the old cementum while new cementum was deposited only on the old cementum of root-filled teeth.

Jansen, Copples, & Verdenius (1955) carried out flap operations on dogs. They burred away the upper part of the buccal alveolar bone and periodontal membrane and cut a groove in the tooth opposite the bony defect. Thereafter, the flap was carefully sutured back into place so that ". . . contamination and the ingrowth of oral epithelium could be excluded." They found that the repair of alveolar bone proceeded much more rapidly than the apposition of cementum in the groove. "After two or three months the gap in alveolar bone is completely filled . . . The root defect on

the contrary remains clearly discernible as only the shallowest defects are completely filled up with cementum."

An investigation which did not deal with the healing after flap operation, yet is of some interest in this connection, was carried out by *Linghorne & O'Connell* (1955). They produced pathological pockets in dogs by reflecting a gingivo-periosteal flap, removing some marginal alveolar bone, applying a "periodontal cement pack" at the base of the incision and suturing the flap back into position. This pack was removed three weeks later, and, after three months, a periodontal pocket was seen to have developed. A new pack was then inserted and was removed after 24 hours, when the root surface was scaled. The animals were sacrificed after a further 15 months and material was taken for histologic examination. It was found that regeneration of the periodontal tissues had taken place where there had previously been an epithelialized pocket even though the pocket epithelium had not been removed. Complete re-attachment had taken place with formation of new cementum. *Linghorne & O'Connell* explained this by suggesting that the connective tissue beneath the epithelium at the base of the artificial pocket begins to proliferate and gradually pushes the epithelium up in a coronal direction. Thereafter, dentine is resorbed and connective tissue cells with "osteogenic potentiality" differentiate into cementoblasts. These form new cementum which fixes the connective tissue fibres to the tooth.

Similar experiments were carried out by *Linghorne* (1957) although, in this series, he used "autogenous bone grafts". Prior to inserting these, a new gingival flap was made, the alveolar border "freshened" and the tooth scaled. Then the flap was sutured into place. Here again *Linghorne* obtained re-attachment with regeneration of bone and periodontal membrane although the epithelial lining of the pocket had not been removed.

Marfino, Orban, & Wentz (1959) questioned the validity of *Linghorne & O'Connell's* (1955) studies and carried out a similar series themselves. Their results did not vary those of *Linghorne & O'Connell*. They found that new attachment of both epithelium and connective tissue took place in the apical third of the area between the original gingival margin and the base of the experi-

mental wound. Gradually the epithelium spread apically. Its position, however, was dictated by the position of the apical end of the pack. For about two months after the surgical detachment the depth of the remaining pocket was reduced by recession of the soft-tissue wall. They concluded that "... reparative healing of the dento-gingival junction is complete and functionally acceptable in this experiment but with deformity, since a total regeneration of the original morphology did not occur."

Krømer (1958) transplanted bone into surgically produced pockets and found that "... in dogs it is possible to achieve reattachment both *with* and *without* bone-transplantation by using a certain technique." He added also that "In humans, however, blind curettage has been successful only in a few selected cases so why not make the curettage directly by sight, reflecting the gingiva."

Kohler & Ramfjord (1960) studied the clinical and histologic healing of human gingival muco-periosteal flaps "... avoiding any deliberate injury or curettage of the root surfaces." They found that these flaps healed "... without any significant loss of periodontal attachment ..."

It will be seen from these studies that there is great variation in opinion on the changes taking place during tissue regeneration after flap operation. The most pessimistic authors believe that the periodontium remains in a state of permanent disease while others believe that complete regeneration and healing may take place.

The operative technique is obviously of great importance to the final result. Some carry out simple detachment without damage to cementum or Sharpey's fibres while others curette cementum, periodontal membrane and alveolar bone. Conditions for regeneration of a functional periodontium are obviously much more favourable after simple detachment since simple healing is all that needs take place. After the more radical operative techniques, however, there must be formation of new cementum, periodontal membrane and alveolar bone before reattachment can take place and restore the damaged periodontium to function.

Before a valid comparison between the tissue regeneration after flap operation and that after gingivectomy can be made, both

types of operation must be carried out under the same conditions. A suitable way of standardizing these is to resect the margin of the alveolar bone at operation and also to cut a groove through the cementum into the dentine thus damaging both tissues as well as the periodontal membrane. In man, they are frequently damaged at operation in addition to being involved to some extent by the disease process.

OWN INVESTIGATIONS

The present author previously carried out an experimental investigation of tissue regeneration after experimental gingivectomy in dogs (*Persson, 1959*). A groove was cut in the cementum to mark the apical border of the wound, and the healing process was studied at varying time intervals from 2 to 76 days after operation. The most significant features of the healing process were the appearance of marked inflammatory infiltration around the bur-groove and the proliferation of pocket epithelium down along the wall of the groove. Towards the end of the observation period, the inflammation had become chronic in character and the epithelium exhibited pathologic changes. This state of affairs gradually worsened as healing progressed. Fig. 1 shows the appearance of the tissues at the last observation period, 76 days after the gingivectomy.

As the healing wound was left undisturbed in these studies, these undesirable changes are probably attributable to the groove forming an area in which blood clot, food remnants, calculus, etc. would be retained. What difference would a bur groove or similar accidental defect make to the healing process after a flap operation in which the flap is sutured back into place and can thus act to some extent as a shield for the underlying tissues?

In an attempt to answer this question, a series of experimental flap operations were carried out on dogs with clinically healthy gingivae. These were performed under virtually identical conditions to those under which the earlier gingivectomy operations were performed and the tissue regeneration after the two types of operation was compared.

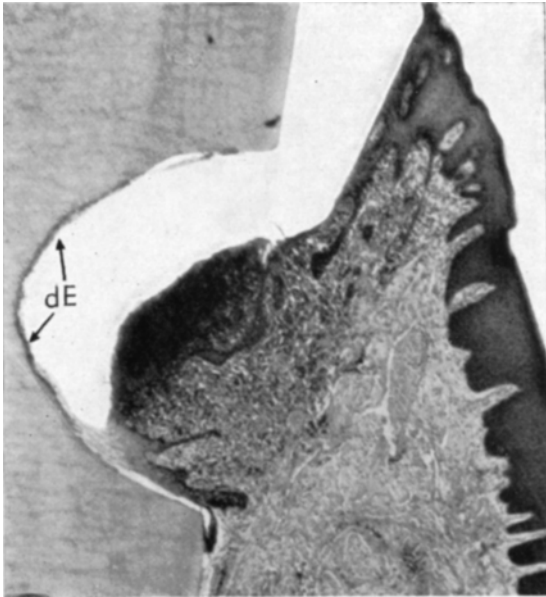


Fig. 1. 76 days after gingivectomy. Epithelium has become fastened to the dentine in the apical part of the groove and has proliferated a short distance along the cementum. Degenerated epithelial cell (dE) can be seen on the surface of the dentine in the more coronal part of the groove. There is marked inflammation and degeneration of the tissues in the groove. (from Persson, 1959). $\times 45$.

Experimental Material and Methods

The experiments were carried out on 7 dogs between the ages of 1 and 7 years, with clinically healthy gingivae. These same animals were used in the earlier gingivectomy studies. Out of a total of 20 flap operations, 13 were performed in the upper jaw and 7 in the lower jaw. Varying numbers of teeth from one to six were involved in each operation. Altogether 35 teeth were studied histologically; 20 were from the upper jaw and 15 from the lower jaw.

The operations were carried out under pentothal anaesthesia combined with local infiltration with 2 % xylocaine-exadrine. An incision was made on the facial aspect of one or more teeth and a muco-periosteal flap reflected.

The alveolar margin was usually exposed when the flap was retracted and an alveolotomy was generally carried out. In most cases a groove was also cut in the tooth with a small rose-head bur to mark the apical border of the wound. Thereafter the flap was replaced and fixed with interdental sutures.

The wounds were allowed to heal for varying periods before the animals were sacrificed. Then, the teeth and surrounding tissues which had been involved in the experiments were removed. In this way material removed at the following time intervals after operation was obtained: 1, 2, 6, 8, 9, 10, 14, 15, 22, 42, 50, 56, 57, 64, 71, 96, 103, 110, and 125 days.

The tissues were fixed in 10 % neutral formalin, decalcified and embedded in paraffin. Serial sections 5 microns thick were cut and most were stained with *Mayer's* haematoxylin-eosin.

Results

A study of the histologic material revealed that the regeneration followed a certain pattern. For the first 8 days after operation, the connective tissue was extensively infiltrated by inflammatory cells and the groove in the tooth was filled by a blood clot which gradually became converted to granulation tissue. After approximately 10 days, the intensity of the inflammation was reduced and some differentiation of collagen fibres was apparent. At this stage the first signs of resorption of the hard tissue were seen in the apical part of the groove. The pocket epithelium showed only a slight tendency to proliferate down along the cementum and remained, in most cases, coronal to the groove. After approximately 50 days, a thin layer of cementum had been formed over the walls of the groove, and in resorption lacunae. Cementum formation continued until it eventually filled the lacunae completely and thus formed a cementum layer of considerable thickness with a smooth surface along the wall of the groove. The connective tissue gradually became more organized, and collagen fibres became embedded in cementum. Towards the end of the observation period, new bone formation was seen. The proliferating epithelium always stopped coronal to the new cementum and there was little or no inflammation. True re-attachment had thus taken place with re-formation of a functional periodontium.

Variations, however, from this scheme of healing were occasionally seen. In one case, the epithelium had proliferated down the wall of the groove by 10 days after operation. In a further 5

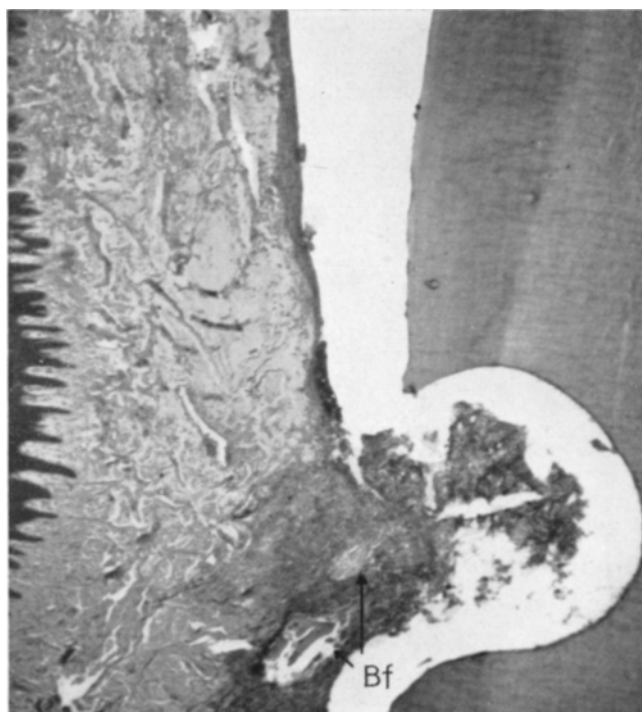


Fig. 2. One day after flap operation. The soft-tissue wall of the artificial pocket is well defined by a small area of haemorrhage and there is a blood clot containing some inflammatory cells in the groove. Fragments of alveolar bone (Bf) can be seen. $\times 48$.

cases after a longer period, proliferating epithelium had reached the apical border of the groove. This epithelium must prevent re-attachment so that a satisfactory result would not be obtained.

These unsuccessful cases will be discussed in greater detail on page 72 in relation to the other cases in which re-attachment over a greater or smaller area had been obtained. These latter comprised such a large majority of the total number studied that they can be taken as representative of the healing process. This process will now be described and illustrated in detail.

Fig. 2 shows the artificial periodontal pocket with the groove at its base, one day after operation. Along the soft tissue wall of

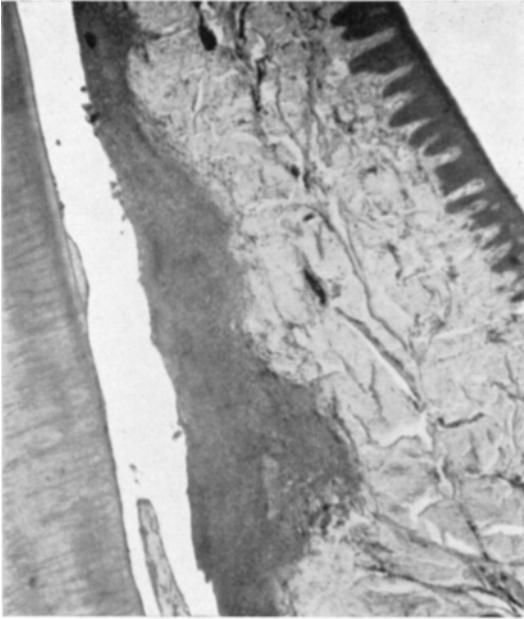


Fig. 3. Two days after flap operation. No groove was cut and there is well-marked area of haemorrhage, containing inflammatory cells, along the soft-tissue wall of the pocket. At the bottom of the wound where the haemorrhage is more diffuse the cementum has been accidentally damaged. $\times 48$.

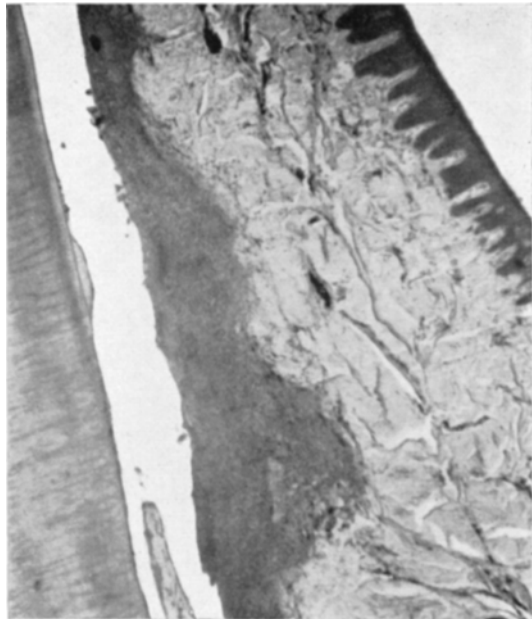


Fig. 4. 8 days after flap operation. The clot in the groove has been organized to granulation tissue which is extensively inflamed like the surrounding connective tissue. The pocket epithelium (pE) has begun to proliferate. $\times 70$.

the pocket, there is a small sharply defined area of haemorrhage extending down to the groove around which the haemorrhage is more diffuse. The groove contains a blood clot with evidence of early inflammation, and a few fragments of the alveolar bone which had been trimmed away. The stage of healing after two days is illustrated in Fig. 3. Along the soft tissue wall of the pocket there is a well-defined area of haemorrhage containing many inflammatory cells. In this case there is no groove at the base of the pocket but the cementum has been accidentally damaged and, around this area of damage, the haemorrhage is most severe and most diffuse. The underlying connective tissue shows early signs of inflammation.

The connective tissue around the site of the operation became progressively more inflamed and proliferation of the pocket epithelium was apparent after 8 days, as can be seen in Fig. 4. Here, the pocket epithelium is proliferating down into the inflamed connective tissue but is still some distance from the coronal edge of the groove. Organization of the blood clot in the groove has commenced, and many new capillary loops and numerous inflammatory cells, mainly polymorphonuclear leucocytes, are to be seen. The inflammatory infiltration extends a short distance apical to the groove.

In all the sections examined up to this stage, the union between the blood clot, and later the granulation tissue, and the underlying hard tissue seems to be very weak. Indeed in many sections a split had developed between them.

Fig. 5 shows the healing wound 10 days after operation. An area of dentine coronal to the groove has been exposed by accidental removal of cementum. The pocket epithelium has proliferated a short distance over the exposed dentine but has not reached the coronal edge of the groove. Granulation tissue fills the groove and some differentiating collagen fibres are visible in it. At the apical edge of the groove, resorption lacunae can be seen in both dentine and cementum (Fig. 6). In this area, the granulation tissue is more firmly joined to the tooth substance than it is in other parts of the groove, where splits between the dentine and the poorly differentiated granulation tissue have developed during preparation of the sections. The inflammation

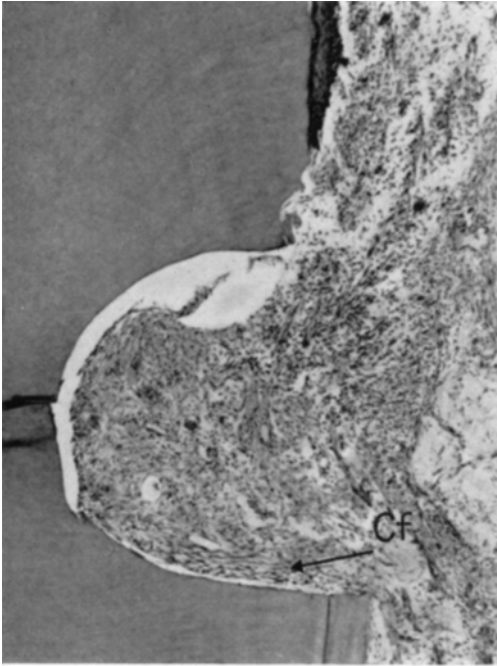


Fig. 5. 10 days after flap operation. The pocket epithelium has proliferated a short distance along the exposed dentine but has not reached the coronal edge of the groove. The granulation tissue in the groove is only slightly inflamed and there is differentiation of collagen fibres (Cf) in its apical part. $\times 70$.

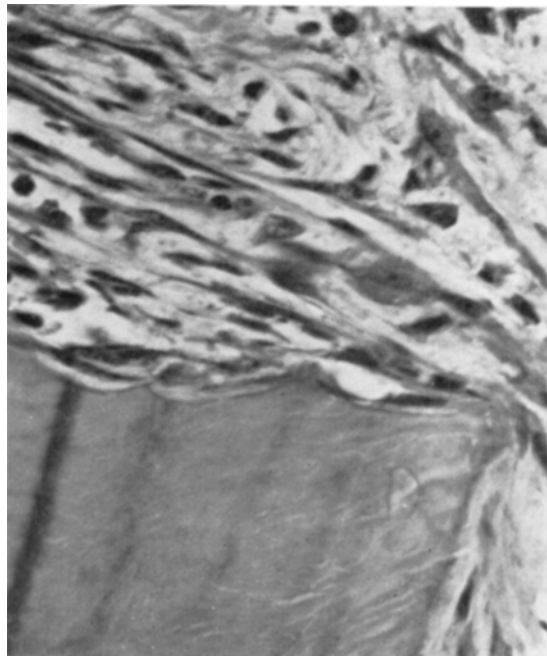


Fig. 6. 10 days after flap operation. A higher magnification of an area from Fig. 5 showing differentiation of collagen fibres and the resorption lacunae in the apical part of the groove. $\times 720$.

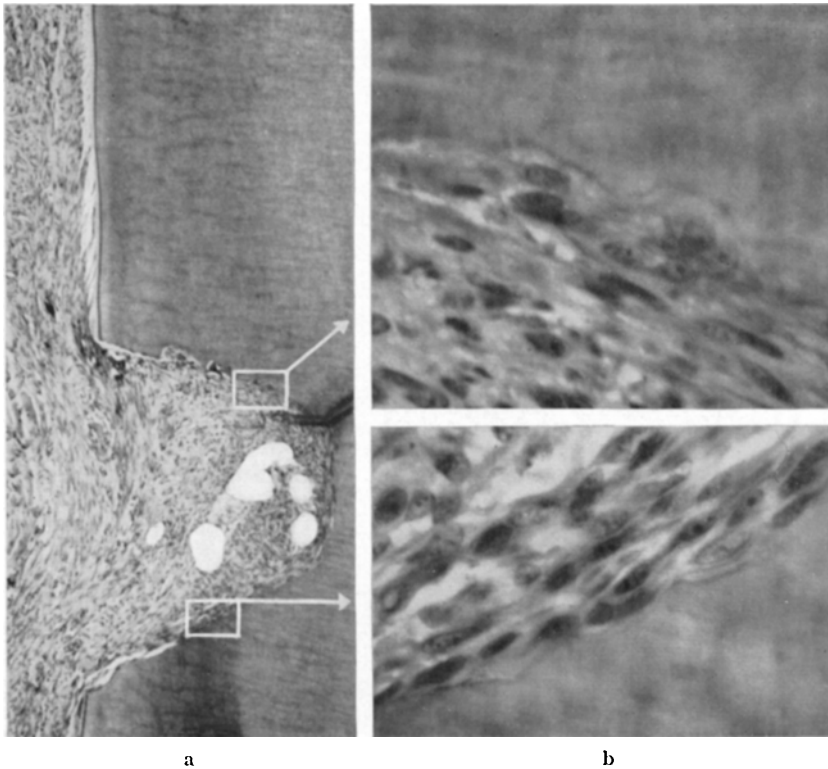


Fig. 7. a) 15 days after flap operation. There is no epithelial proliferation in the artificial pocket. Primary healing has taken place in the area coronal to the groove. There are resorption lacunae along the wall of the groove and the connective tissue lies in intimate contact with the dentine. $\times 70$.
 b) A higher magnification of an area in the apical part of the groove showing resorption lacunae and possibly cementoblasts starting cementum formation. $\times 690$.
 c) A higher magnification of an area in the coronal part of the groove showing resorption lacunae with osteoclasts. $\times 690$.

in the groove is considerably milder than during the earlier stages of healing.

Fig. 7 shows the state of affairs 15 days after operation. The epithelium has not proliferated down into the artificial periodontal pocket in this case, and it is obvious that, during reflection



Fig. 8. 50 days after flap operation. The pocket epithelium has proliferated down to the coronal border of the groove. The sub-epithelial and supra-alveolar connective tissue is inflamed but there is no evidence of inflammation along the wall of the groove. The connective tissue in the groove is closely applied to the hard tissue. $\times 65$.

Fig. 9. 50 days after flap operation. A higher magnification of an area from Fig. 8. Cementoblasts lie in a row along the wall of the groove and cementogenesis is active. Connective tissue fibres are embedded in the cementum and lie at a very acute angle to the tooth. $\times 690$.



of the flap, the cementum and Sharpey's fibres in the area coronal to the groove have not been damaged (Fig. 7 a). The only damage has been to the periodontal fibres some little distance from the cementum and these have healed simply.

The healing in the groove is most interesting, for here the cementum has obviously been removed. Re-union between tooth and connective tissue thus requires true "re-attachment", i.e. formation of new cementum incorporating Sharpey's fibres.

Resorption lacunae are visible in most of the wall of the groove. Unlike the earlier stages, the connective tissue appears to be closely and firmly adapted to the dentine, and a layer of cells can be seen in close apposition to this dentine. The general impression is that early cementogenesis is taking place in the resorption lacunae in the apical part of the groove (Fig. 7 b). In the coronal part of the groove, however, there are still osteoclasts in the lacunae (Fig. 7 c).

Fig. 8 shows a case 50 days after operation. The pocket epithelium has proliferated down to the coronal border of the groove where it has been halted by a connective tissue barrier. Many plasma cells and lymphocytes can be seen in the connective tissue outside the groove. Inside the groove, the inflammation is much milder while, along the wall of the groove, it is almost negligible.

The wall of the groove is covered by a thin layer of newly formed cementum which is thickest in the resorption lacunae suggesting that there is a tendency for the surface of the groove to be smoothed out (Fig. 9). Fibres pass from the newly formed connective tissue into the cementum. During that early stage of cementogenesis, they lie at a very acute angle to the tooth and, at times, appear almost parallel to it suggesting that the periodontal tissue in this area have not yet become functional. True connective tissue re-attachment has, however, taken place so that further proliferation of epithelium is prevented.

The next observation time is 56 days after operation (Fig. 10). Mild inflammatory infiltration persists in the sub-epithelial connective tissue and the epithelium has sent long finger-like processes down into this inflamed connective tissue. This proliferation has, however, been halted coronal to the groove in an area which has an uneven surface suggestive of sites of resorption. The surface down which epithelium has proliferated is smooth and even.



Fig. 10. 56 days after flap operation. The sub-epithelial connective tissue is slightly inflamed and the pocket epithelium has proliferated down to the coronal border of the groove. Newly formed connective tissue fibres are visible in the coronal part of the groove while unorganized granulation tissue remains in its apical part. Here too there is a layer of epithelium (pE) without any visible connection with the pocket epithelium. $\times 70$.

Fig. 11. 56 days after flap operation. A higher magnification of an area from Fig. 10. There are resorption lacunae with new cementum and cementoblasts (cB) in the coronal part of the groove. The dentine in the apical part is smooth and covered by epithelium (pE). $\times 190$.

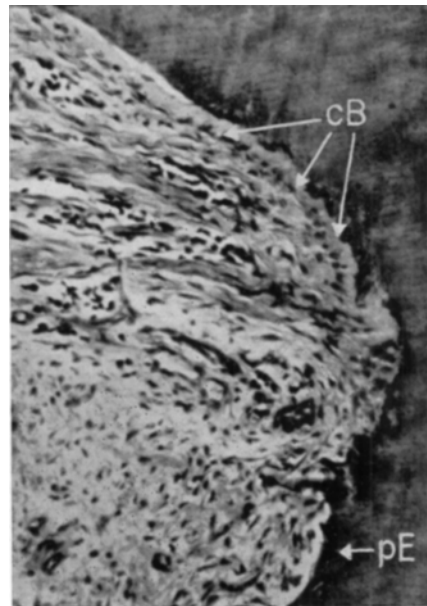




Fig. 12. 56 days after flap operation. Another section from the same block as Fig. 11. The pocket epithelium has proliferated over the whole of the wall of the groove. $\times 65$.

Fig. 13. 57 days after flap operation. No groove was cut but cementum was accidentally removed and re-attachment has taken place. The union between the new cementum and the connective tissue fibres must be weak since they have been torn apart during preparation. $\times 68$.





Fig. 14. 64 days after flap operation. Cementum has been removed from an area coronally to the groove. There is re-attachment along the whole dentine surface in that area and in the coronal part of the groove (at x). $\times 70$.

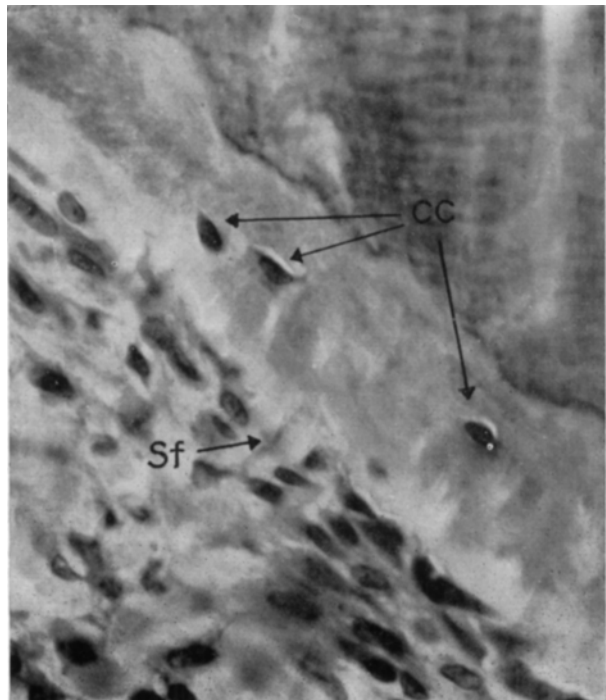


Fig. 15. 64 days after flap operation. A higher magnification of an area from the groove in Fig. 14. New cementum has been formed and Sharpey's fibres (Sf) are embedded in it. Cementocytes (CC) have been incorporated into this new cementum. $\times 690$.

In the groove which contains connective tissue with new-formed fibres, there is little evidence of inflammation. New cementum has been formed along the coronal two-thirds of the groove and lively cementogenesis is still in progress. A row of cementoblasts can be seen in Fig. 11 lying along the new cementum with connective tissue fibres passing between the cells.

A surprising state of affairs can be seen in the apical part of the groove. A layer of epithelium lies against the dentine and the connective tissue is not organized in bundles in the way that it is in the coronal part where re-attachment has taken place. This means that a process must have proliferated down from the pocket epithelium in an adjacent portion into this more apical area of connective tissue. Another section in the same series (Fig. 12) shows how the epithelium has proliferated down to the apical border of the groove and prevented re-attachment. Admittedly the connective tissue is relatively free from inflammatory cells but there is no evidence of any functional organization of the collagen fibres. Only partial re-attachment to this tooth has taken place, probably to its most proximal part due to better nutrition because of the proximity of the interdental papilla.

It is significant that, where re-attachment had taken place there were resorption lacunae in the dentine (Fig. 11), but hardly any in the areas over which epithelium had proliferated (Fig. 12).

The next photomicrograph (Fig. 13) shows the wound, 57 days after operation. As no groove was cut in this case, an exact assessment of the apical limit of the incision is not possible. The old cementum had been removed at operation and the dentine surface is irregular. No significant proliferation of the pocket epithelium down along the root has taken place. Immediately apical to the epithelium, new cementum has been formed on some parts of the damaged root surface. Partial re-attachment thus seems to have taken place although it must be fairly weak since large areas of soft tissue have become separated from the tooth during preparation of the sections. The union between cementum and connective tissue is considerably stronger in cases where defects such as grooves have been produced in the dentine (Fig. 8).

Fig. 14 shows the stage of healing 64 days after operation. Cementum and some dentine have been removed from an area extending from the cemento-enamel junction down to the groove.

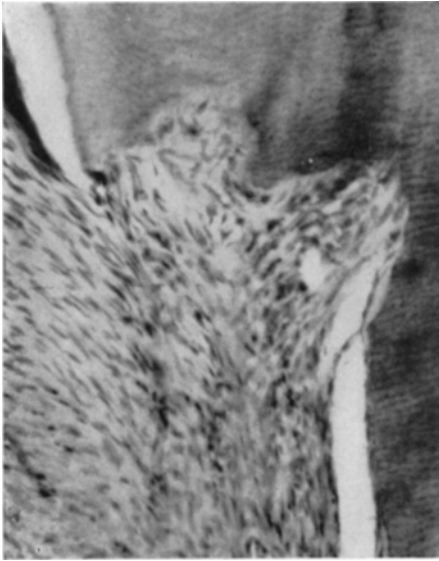
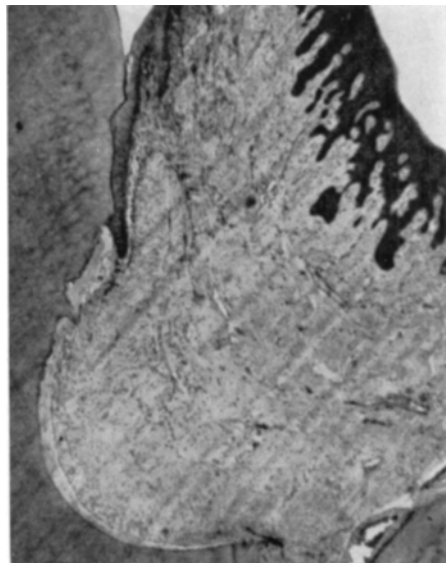


Fig. 16. 71 days after flap operation. No groove was cut but the dentine was severely damaged. The pocket epithelium has been halted immediately coronal to the defects. New cementum formation is most advanced in the greatest defects. $\times 190$.

Fig. 17. 96 days after flap operation. The new cementum has evened out the dentine defects in the wall of the groove. $\times 45$.



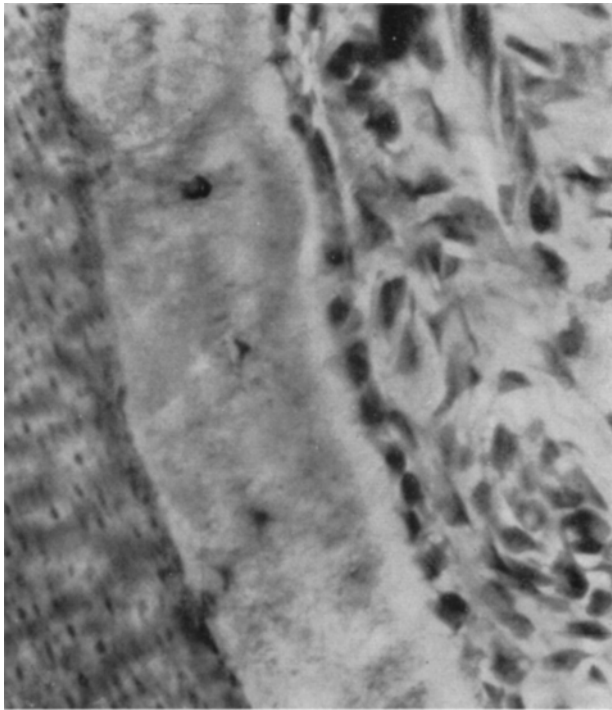


Fig. 18. 96 days after flap operation. A higher magnification of an area from Fig. 17. Inside the cementoblast layer there is a pale-staining zone and, inside this, are two layers of cementum. Cementocytes have been incorporated into the new cementum. $\times 690$.

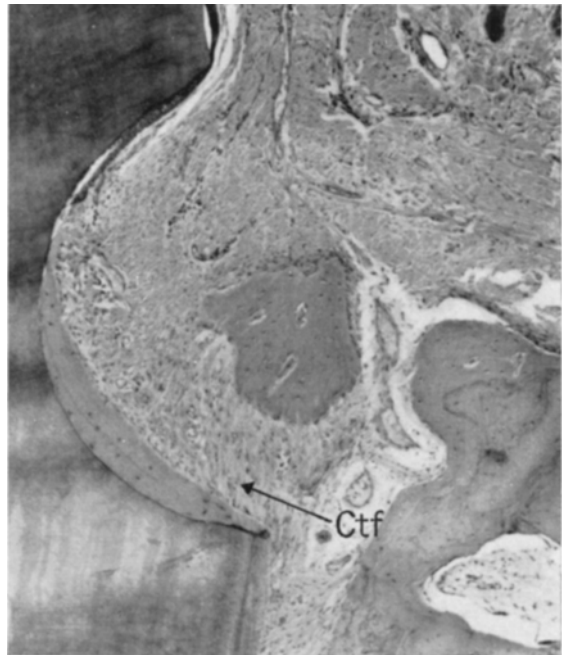
The pocket epithelium has not proliferated, to any significant extent, below the cemento-enamel junction. Along the whole of the damaged dentine surface there is a series of irregularities which resemble lacunae left by previous osteoclastic action. These irregularities have, however, been filled in by an unbroken layer of new cementum which is, of course, thickest in the groove. Some cementocytes have been incorporated in the new cementum in the groove and the regular layer of cementoblasts over the defect indicates active cementogenesis (Fig. 15). The anchorage of the Sharpey's fibres in this cementum can clearly be seen.

Fig. 16 shows a case without a groove but with hard tissue



Fig. 19. 96 days after flap operation. The new cementum consists of three layers which are readily distinguished from each other by demarcation lines. $\times 70$.

Fig. 20. 103 days after flap operation. The pocket epithelium has proliferated down about $\frac{1}{2}$ of the wall of the groove. Beneath this there is new cellular cementum. Regeneration of alveolar bone has taken place and, near the groove, there is a bone fragment showing lively osteoblastic activity. From this fragment, connective tissue fibres (Ctf) pass into the new cementum in the apical part of the groove. $\times 70$.



defects caused at operation. This is 71 days after the operation and there is no evidence of inflammation in the connective tissue round the defect. Here too, there has been some re-attachment. The formation of cementum incorporating the ends of the Sharpey's fibres can best be seen in the large defect just apical to the pocket epithelium. In this case as in most others, cementogenesis is most active in the larger defects; the smooth surfaces show less evidence of cementum formation. Re-attachment seems to be firmer in the greater irregularities as there is little tendency for the connective tissue fibres to tear away during histologic preparation although this is common where the root surface is smooth.

As the formation of new periodontal tissues progresses, the defects in the dentine wall of the groove are gradually filled out with new cementum. This can be seen in Fig. 17 which shows conditions 96 days after operation.

Along the cementum lies a row of cementoblasts. Immediately inside these is a lighter coloured zone which is probably partially mineralized cementum or pre-cementum, and below this there are two well-defined layers of cementum (Fig. 18). The demarcation lines between the different layers of cementum are more evident in Fig. 19. Here and there in the new cementum lie embedded cementocytes.

Fig. 20 shows a case 103 days after operation. The pocket epithelium has proliferated down about $\frac{1}{3}$ of the wall of the groove, and below the epithelium new cellular cementum is being formed. Three cementum layers can be distinguished and Sharpey's fibres are embedded in the outer two (Fig. 21).

In this case some regeneration of alveolar bone has also taken place (Fig. 20). Bone was resected at operation to the level of the apical border of the groove but has now re-formed up to a higher level. Several zones can be seen in this new-formed bone and, along its surface lies a row of osteoblasts indicating that bone formation is continuing. Nearer the groove and partly inside it lies a fragment of bone around which active bone formation is taking place. Bundles of connective tissue fibres pass between this and the newly formed cementum.

The longest observation time was 125 days after operation (Fig. 22). No groove was cut in this tooth but two accidental defects have been produced in the dentine. The relationship of

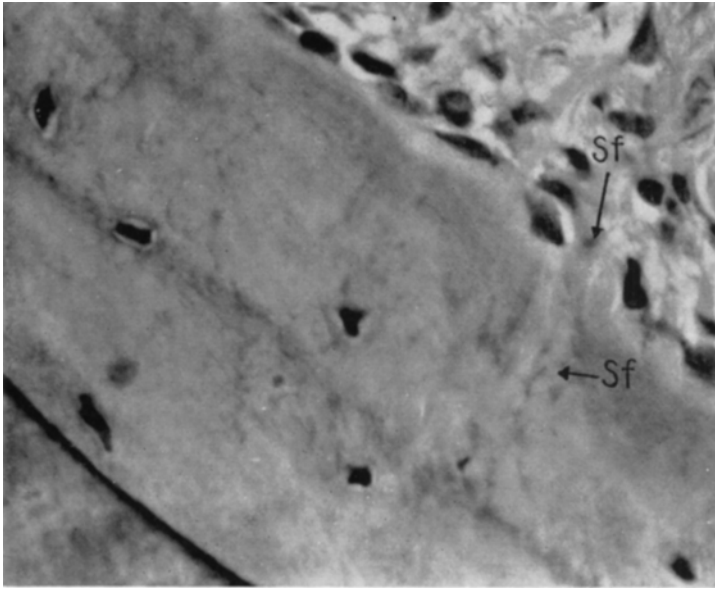


Fig. 21. 103 days after flap operation. A higher magnification of an area from Fig. 20 showing three layers of new cementum with Sharpey's fibres (Sf) in both outer layers. $\times 690$.

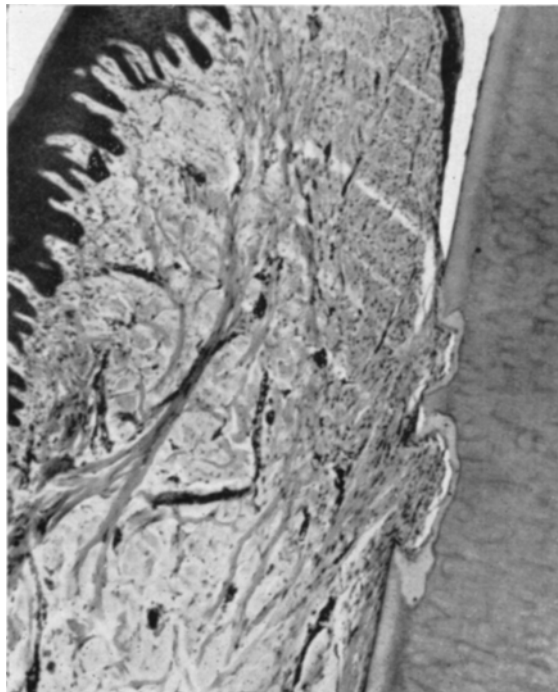


Fig. 22. 125 days after flap operation. There is no groove but there are two accidental defects in the dentine. Connective tissue fibres pass into the newly formed cementum and have assumed their typical direction running obliquely upwards to the tooth. $\times 70$.

the deeper defect to the apical limit of the lesion cannot be determined.

New cellular cementum has been formed in the defects and, as in the previous cases, this is tending to even out the irregularities produced by earlier resorption.

Unlike most of the cases described earlier, the connective tissue fibres run in regular bundles from bone to tooth. They run obliquely in a coronal direction towards the tooth in a manner typical of the undamaged periodontium.

Like most cases in which re-attachment has taken place, the supra-alveolar connective tissue is relatively free from inflammatory cells.

DISCUSSION

In the present investigation which was of a purely experimental nature, the regeneration of the periodontal tissues after flap operations was studied. Since the object of the investigation was to compare the type and extent of healing after flap operation with that after gingivectomy, the flap operations were carried out under conditions similar to those pertaining in an earlier series of experimental gingivectomies (*Persson, 1959*).

Striking histological differences in the healing process after the two types of operation were observed, particularly in the later stages after 14 days.

After gingivectomy, there was extensive inflammation around the groove in every case from 4 days after operation up to the longest time of observation, 76 days. This inflammation was acute in the earlier stages but gradually became chronic in type. After about 7 days, the pocket epithelium began to proliferate down into the groove. As healing progressed, this proliferating epithelium became more and more abnormal in appearance with long irregular finger-like processes extending down into the greatly inflamed connective tissue over the alveolar crest. This state of affairs gradually worsened and, at the last observation period, epithelium covered the whole of the wall of the groove extending even a short distance apical to it. Degenerative changes were evident in this epithelium. The surrounding connective

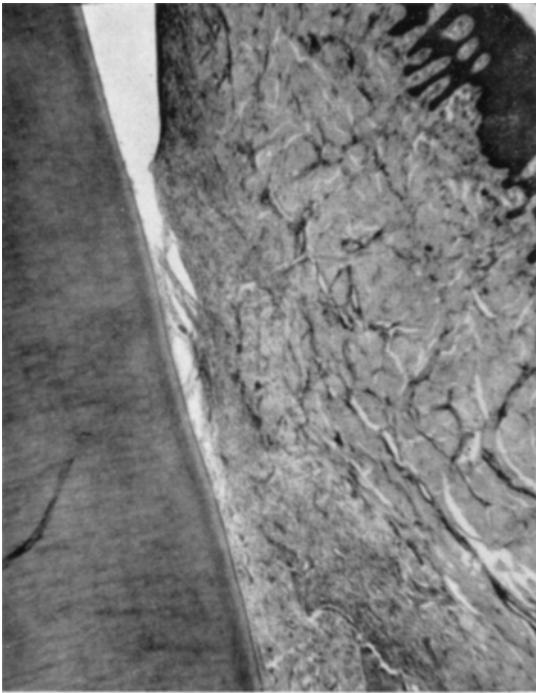


Fig. 23. 9 days after flap operation. There is no groove and no proliferation of pocket epithelium. The cementum and Sharpey's fibres are undamaged and no "detachment" has taken place — only tearing of the connective tissue fibres outside the cementum. A simple healing is developing. $\times 45$.



Fig. 24. 110 days after flap operation. There is no groove and no proliferation of pocket epithelium. The cementum is undamaged and simple healing has taken place close to the cementum. $\times 45$.

tissue was extensively inflamed and some inflammatory cells had even penetrated into the epithelium. In this way, a condition which would inevitably lead to the formation of a pathologically deepened gingival pocket arose. Fig. 1 shows the experimental area 76 days after operation, and is fairly representative of the histological picture of wound healing during the later stages of the study. In only one of the 57 experimental cases, was there any connective tissue re-attachment in the groove and, in this case, there was no evidence of inflammation.

The healing after flap operation was quite different. Up to 10 days after operation, there was some inflammation in the tissue around the groove, but after that it gradually disappeared. The epithelium showed no appreciable tendency to proliferate along the root and in most cases remained at the coronal border of the groove.

During the first 7 days after operation when the epithelium had not started to proliferate down into the groove, the healing after both types of operation was very similar. Thereafter, the type of healing after flap operation was much more favourable than that after gingivectomy.

A total of 30 teeth were studied at stages later than 7 days after operation. In 6 of these no groove was cut in the tooth to mark the apical limit of the wound and the cementum was not damaged. No "detachment" had been produced in these areas since the cementum and Sharpey's fibres were not damaged. All that had happened was that the connective tissue fibres had been torn where they entered the cementum so that only a simple type of wound healing took place. This can be seen in Figs. 23 and 24.

Fig. 23 shows a case 9 days after operation. The pocket epithelium has not started to proliferate and there is only mild inflammation around the line of the incision. The connective tissue fibres are joined to the undamaged cementum and simple healing is developing. Fig. 24 shows the state of affairs after 110 days. The pocket epithelium has not proliferated, the supra-alveolar connective tissue is free from inflammation and complete healing of the connective tissue along the cementum surface has taken place.

The remaining 24 cases are of considerably greater interest. In 19 of them a groove had been cut in the cementum and dentine

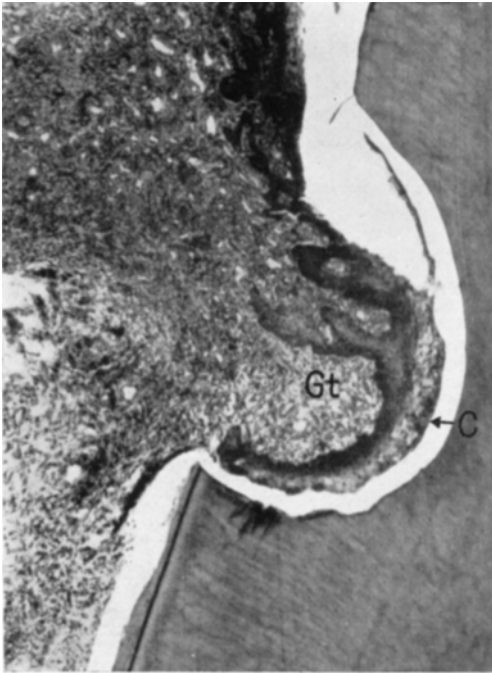


Fig. 25. 10 days after flap operation. The pocket epithelium has proliferated down into the groove between the granulation tissue (Gt) and the clot (C) in the base of the groove. The connective tissue round the groove is markedly inflamed. $\times 65$.

Fig. 26. 42 days after flap operation. The pocket epithelium has proliferated down to the apical border of the groove (at x). The dentine surface is even and devoid of resorption lacunae. $\times 65$.



with a bur and, in the other 5, these tissues had been accidentally damaged at operation. This produced a defect similar to a groove in that the attachment of the Sharpey's fibres to the cementum had been loosened.

Epithelium had proliferated down along the wall of the defect reaching its apical border in 6 out of the 24 cases so that re-attachment was prevented. Fig. 25 shows such a case 10 days after operation. The pocket epithelium has proliferated down the whole of the wall of the defect and both the sub-epithelial and supra-alveolar connective tissue show marked signs of inflammatory infiltration. The general picture is very reminiscent of that after gingivectomy with epithelium growing in between the granulation tissue and the clot in the depths of the groove.

Fig. 26 is from another case in which the epithelium proliferated down to the apical border of the groove. The observation time is 42 days. The wall of dentine in the groove is smooth and even without any signs of resorption.

In the other 18 cases, however, epithelial proliferation had stopped before it reached the apical border of the defect and had indeed often remained at its coronal border. There was little evidence of inflammation in the defect after 15 days and even this completely disappeared as healing continued.

The first signs of re-attachment can be seen 15 days after operation. Resorption lacunae containing osteoclasts can be seen in the coronal part of the groove (Fig. 7 c). In the apical part of the groove, a layer of cells lies over the irregular dentine surface and, inside this, connective tissue fibres lie in intimate contact with the dentine in the resorption lacunae (Fig. 7 b). As yet there is no evidence of cementum formation but the arrangement of the cells in a layer over the dentine surface suggests that it is about to begin. Cementum formation can be seen after 50 days, especially in those areas where resorption has taken place (Figs. 8 and 9). Cementoblasts have differentiated and lie in a layer over the new cementum and connective tissue fibres can also be seen embedded in this cementum indicating that re-attachment has begun. This is even more obvious in Figs. 10 and 11 which show the increase in cementum thickness after a further week. The progressive increase in cementum thickness continues and each new layer is separated from the previous one by prominent demarca-

tion lines (Figs. 19, 20, and 21). Cementocytes are often seen embedded in the cementum.

An interesting question in this connection is whether the Sharpey's fibres pass in a straight line through the different layers or whether they change direction at the junction between each two layers. Their paths could not be demonstrated with the techniques used in this study but would probably be revealed by polarized light (see *Gustafson & Persson, 1957*).

The appearance of the dentine surface in this study was particularly interesting. In most cases in which re-attachment took place, the dentine surface looked as though it had been nibbled away in a manner very suggestive of resorption. These lacunae in the dentine were later filled by new cementum until finally they were completely leveled out so that dentine was covered by a smooth layer of cementum as seen in Fig. 17.

It would thus appear that re-attachment is more likely to take place if the surface of the exposed tooth is irregularly pitted—either mechanically or by osteoclastic resorption.

Where the irregularities were not so marked and the dentine surface was more even, the cementum was not joined so firmly to the underlying dentine and often split away during preparation of the sections (Fig. 19). The same observation was made by *Björn (1957)*.

Where there had been no re-attachment and the pocket epithelium had proliferated down along the dentine, the wall of the groove was generally smooth and even without any signs of previous resorption lacunae. Fig. 26 shows such a case.

No resorption seemed to take place in the groove for about the first 10 days after operation whether epithelium had proliferated down or not. From 10 days onwards, however, resorption lacunae were visible along the surface of the dentine in the groove which had not been covered by epithelium, and it seems as if the resorption process began in the most apical part of the incision (Fig. 5).

It appears as if the proliferating epithelium is first halted by a commencing osteoclastic activity on the surface of the exposed dentine. If, however, epithelium has grown down over the dentine, no resorption can take place in that area.

This histological study of healing at varying time intervals has shown that the regeneration in the marginal periodontium is considerably more favourable after a flap operation than after a gingivectomy, if the two types of operations had been carried out under the following conditions: a clinically healthy periodontium, defects in cementum and dentine, and no application of surgical pack after operation.

It seems most likely that the absence of surgical pack has had the most damaging effect on the course of healing after the gingivectomies. Investigations by *Bernier & Kaplan* (1947), *Frleigh* (1956), and *Waerhaug & Loe* (1957) also suggest that a surgical pack after gingivectomy promotes the healing. However, *Waerhaug* (1956) was of the opinion that in experiments on dogs with healthy gingivae, "... a surgical pack does not seem to influence the final result of healing in any direction", if the teeth are given a daily brushing with a soft brush from the first day after operation.

In the present investigation the absence of surgical pack would probably not have the same unfavourable effect on the course of healing after flap operation, since the sutured flap offers good protection to the regenerating periodontal tissues. On that account the defects in the hard tooth substance may not have exerted the same damaging influence as after gingivectomy. Thus the results of this work suggest that a flap operation is to be preferred, if there are any defects in cementum or dentine under the gingival margin.

It is too early yet to apply the results of these experiments to clinical practice. Before this is done, further experiments must be carried out to compare healing after the two types of operations, both with and without defects in the root surface and after application of a surgical pack.

SUMMARY

Flap operations were carried out on dogs with clinically healthy periodontal tissues and the stages of healing were studied at relatively short intervals for the first 15 days, and thereafter at progressively longer intervals up to 125 days after operation.

1. The operations were performed around a total of 35 teeth. Of these, 30 were studied for intervals longer than 7 days after operation. The cementum was left intact in 6 of these 30 and simple healing was seen to take place.
2. Defects in the cementum and dentine were produced in the remaining 24 cases, either by cutting a groove with a bur at the apical border of the experimental wound or by accidental damage to the hard tissues in the wound. Epithelium proliferated down to the apical border of the defect in 6 of these 24 cases. Re-attachment was thus prevented and a pathologic periodontal pocket produced. In the remaining 18 cases, epithelial proliferation had been halted before it reached the apical border of the defect. Thus the conditions necessary for re-attachment had been obtained.
3. Where epithelium proliferated down over defects, the walls of these defects were generally found to be smooth and even, while in the cases in which re-attachment had taken place, the dentine surface was usually irregular, either because of mechanical damage or because of osteoclastic action.
4. After approximately 10 days, resorption lacunae were usually visible in the walls of the defects. No cementum formation was then evident but about a week later the presence of a layer of connective tissue cells arranged along the surface of the dentine suggested the onset of cementogenesis.
5. After approximately 50 days, differentiation of cementoblasts was evident and new cementum had been formed, especially where resorption had taken place. This cementum gradually became thicker and levelled out the irregularities in the dentine wall so that after about 95 days, a many-layered covering of cementum, with cementocytes embedded in it, had been formed.
6. These studies were carried out under the same conditions and on the same animals as earlier studies on gingivectomy (*Persson, 1959*). The results demonstrate considerably better healing after flap operation than after gingivectomy.

RÉSUMÉ

RÉGÉNÉRATION DU PARODONTE MARGINAL APRÈS OPÉRATION PAR LA MÉTHODE DU LAMBEAU

ETUDE EXPÉRIMENTALE SUR DES CHIENS

Des chiens présentant à l'examen clinique des tissus parodontaux sains ont subi l'opération par la méthode du lambeau et les étapes de la cicatrisation ont été étudiées à des intervalles relativement rapprochés pendant les 15 premiers jours, puis à des intervalles de plus en plus écartés jusqu'à 125 jours après l'opération.

1. Les opérations exécutées ont intéressé en tout 35 dents. 30 de ces dents ont été étudiées pendant des périodes de plus de 7 jours, après l'opération. Le ciment a été laissé intact sur 6 de ces 30 dents, et on a observé une cicatrisation sans complication.

2. Dans les 24 cas restant, des altérations ont été produites dans le ciment et dans la dentine, soit en découpant à la fraise un sillon au niveau du bord apical de la plaie expérimentale, soit à la suite d'une détérioration accidentelle du ciment dans la plaie. Une prolifération de l'épithélium se prolongeant jusqu'au bord apical de l'altération s'est produite dans 6 de ces 24 cas. Le réattachement s'est ainsi trouvé entravé et il s'est produit un cul-de-sac pathologique. Dans les 18 cas restant, la prolifération épithéliale s'était arrêtée avant d'atteindre le bord apical de l'altération. Les conditions nécessaires au réattachement étaient ainsi réalisées.

3. Dans les cas où la prolifération épithéliale recouvrait les altérations, les parois de celles-ci étaient en général lisses et unies, tandis que dans les cas où le réattachement s'était produit, la surface dentinaire était en général irrégulière, soit à cause d'une détérioration mécanique, soit en raison d'une action ostéoclastique.

4. Au bout d'environ dix jours, on pouvait généralement voir des lacunes de résorption dans les parois des altérations. On ne pouvait alors pas constater de formation de ciment, mais, au bout de quelques semaines, la présence d'une couche de cellules de tissu conjonctif disposées le long de la surface de la dentine semblait indiquer une cémentogénèse commençante.

5. Au bout d'à peu près 50 jours, la différenciation de cémentoblastes était évidente, et on constatait la formation de nouveau

cément, en particulier aux endroits où une résorption s'était produite. Ce cément s'est épaissi progressivement et a nivelé les irrégularités des parois dentinaires, de sorte qu'au bout de 95 jours un revêtement composé de nombreuses couches de cément avec inclusion de cémentocytes s'était formé.

6. Ces études ont été effectuées dans les mêmes conditions et sur les mêmes animaux que les études antérieures sur la gingivectomie (*Persson 1959*). Les résultats témoignent d'une cicatrisation nettement meilleure après opération par la méthode du lambeau qu'après gingivectomie.

ZUSAMMENFASSUNG

ÜBER DIE REGENERATION IM MARGINALEN PARODONTIUM NACH GINGIVALRANDSCHNITT MIT AUFKLAPPUNG DES ZAHNFLEISGHES EIN EXPERIMENTELLES STUDIUM AN HUNDEN

Gingivalrandschnitte mit Aufklappung des Zahnfleisches wurden an Hunden mit klinisch gesundem Parodontium durchgeführt, danach wurde der Heilungsverlauf mit dichtliegenden Intervallen während der ersten 15 Tage nach dem Eingriff studiert. Der Zeitabstand der Beobachtungen wurde nach diesen ersten 15 Tagen immer länger und war maximal 110 Tage nach dem Eingriff.

1. Es wurden 35 Zähne untersucht. Von diesen wurden 30 später als 7 Tage nach dem Eingriff untersucht. Bei 6 Zähnen dieser 30 Fälle wurde das Zement nicht beschädigt und eine einfache parodontale Wundheilung war im Gange oder hatte sich etabliert.

2. An den verbliebenen 24 Fällen wurden Defekte im Zement und Dentin entweder in Form einer Bohrrinne an der apikalen Grenze des Eingriffes oder akzidentell an den Hartsubstanzen des Eingriffes verursacht. Bei 6 dieser 24 Fälle war Epithel entlang des Defektes proliferiert und die apikale Grenze des Eingriffes erreicht. Ein Reattachment wurde dadurch verhindert und eine pathologische Zahnfleischtasche war entstanden. Bei den restlichen 18 Fällen war dagegen die Epithelproliferation zum Stillstand gekommen, bevor sie die apikale Kante des Defektes erreicht hatte. Hier waren offensichtliche Voraussetzungen für ein Reattachment geschaffen worden.

3. Bei den Fällen wo das Epithel in apikale Richtung über den Defekt proliferierte, war die Wand dieses Defektes im allgemeinen glatt und eben, während bei den Fällen, an denen ein Reattachment stattfand, die Dentinwand gewöhnlich mit Unebenheiten versehen war, die entweder mechanisch oder durch eine Osteoklasie verursacht waren.

4. Nach ungefähr 15 Tagen konnte man an der Wand des Defektes Resorptionslakunen wahrnehmen. Eine Zementneubildung konnte da noch nicht nachgewiesen werden, aber eine Anzahl Bindegewebszellen angeordnet an der Dentinwand gaben den Eindruck einer beginnenden Zementogenese.

5. Nach ungefähr 50 Tagen konnte man deutlich sehen, dass Zementblasten ausdifferenziert waren und dass auch neues Zement gebildet war, speziell in den früheren Resorptionslakunen. Dieses nahm an Dicke zu und glich die Unebenheiten in der Dentinwand aus, um schliesslich nach ungefähr 95 Tagen verschiedene Schichten zu zeigen, die durch Demarkationslinien getrennt waren. In dem neuen Zement waren auch Zementozyten eingelagert.

6. Die vorliegenden Untersuchungen wurden unter den gleichen Voraussetzungen und an den gleichen Versuchstieren durchgeführt wie bei den früher veröffentlichten Untersuchungen über Gingivektomien (Persson 1959). Die Untersuchungsergebnisse zeigen ein bedeutend günstigeres Heilungsbild nach Gingivalrand-schnitten als nach Gingivektomien.

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