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RESPONSE OF THE INTERMEDIATE PLEXUS OF THE GUINEA PIG MOLAR TO EXPERIMENTAL TRAUMA

by

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INTRODUCTION

The existence of an intermediate plexus in the periodontal membrane of continuously erupting molars in the guinea pig was suggested by *Sicher* (1923). He described a zone in the middle of the periodontal membrane where the collagen fibers from the alveolar bone and those from the cementum buttons seemed to interlace. *Sicher* (1923) stated that this zone was functioning as a rearrangement area for the periodontal fibers from both the cementum buttons and the bone to maintain the direction of the periodontal fibers during the continuous eruption of the tooth. The concept of an intermediate plexus in the guinea pig molar has been supported by *Hunt* (1961), and similar interpretation of histologic slides have been made in rabbits by *Eccles* (1961), rats by *Sicher* (1942), monkeys by *Goldman* (1962) and in mice by *Stallard* (1963). Furthermore, *Orban* (1962) proposed that a somewhat modified intermediate plexus is present in the human periodontal membrane.

On the other hand, many workers (*Crumley*, 1964; *Zwarych & Quigley*, 1965; *Ramos & Hunt*, 1967) dispute the presence of an intermediate plexus. Recently *Hindle* (1967) would not exclude the possibility of an intermediate plexus in the periodontal membrane of the rat incisor after using polarized

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light microscopy, and *Melcher* (1968) supported the same view after experimental trauma to the periodontal membrane in the same species.

From a review of the literature, it becomes evident that most of the authors that maintain the concept of the intermediate plexus have used an experimental approach (*Goldman*, 1962; *Stallard*, 1963; *Melcher*, 1968) which has supplied new information about the function of the intermediate plexus under varying conditions.

The continuous eruption of the guinea pig teeth necessitates a continuous remodelling of either the cementum, the alveolar bone or the periodontal fibers, or all of these tissues in order to maintain a constant direction of the periodontal fibers. The present paper describes the results of mechanical injury to the periodontal membrane of the guinea pig molars following a technique introduced by the author (*Johansen*, 1960).

MATERIAL AND METHODS

Twenty-four healthy guinea pigs of both sexes, weighing between 300—400 grams, were anesthetized with intraperitoneal Nembutal or by inhalation of an ether-air mixture. A slowly rotating small fissure bur was introduced between the right maxillary first and second molars, starting in the middle of the palate. By this procedure the laceration of the interdental papilla was avoided. The bur was used to produce a mark in both the tooth surface and the interdental bony septum. Since the teeth are continuously erupting, the distance between the bur mark in the tooth and in the septum served as a measurement of the distance of the eruption during the post experimental periods. By this technique, an undamaged part of the tooth would erupt opposite the defect in the septum, and the marked tooth would be carried coronally opposite an undamaged part of the interdental septum. This procedure allowed a study of the response of the various parts of the periodontium during eruption. The animals were kept in separate cages after the experimental procedure. The left maxillary molars and the second and third molars of the experimental side were used for control. The animals were sacrificed after observation periods varying from 2—20 days. The jaws were dissected, fixed in 10 % neutral buffered formalin, decalcified in 5.2 % nitric acid (or in a formic acid-sodium citrate solution), and serially sectioned longitudinally at 5 microns.

The sections were stained with hematoxylin and eosin for orientation and general morphology, with elastic tissue stains (Weigert's and Verhoeff's and with connective tissue stains (Mallory's, Masson's, van Gieson's and Gomori's silver stain).

OBSERVATIONS

All animals survived the experiment. It turned out to be difficult to produce wounds which were exactly alike in size and location. In some animals the lesions were made either too far apically or too far coronally whereby the optimal conditions for studying the periodontal membrane changes were reduced. However, the findings throughout the series were so consistent that this lack of reproducibility was not an important factor.

Fig. 1 shows the normal interdental septum between the first and second maxillary molars in a control specimen. The principal fibers (f) are running from the cementum buttons (c b) through the region of the intermediate plexus (p), to the alveolar bone (a b). Within the alveolus the fibers are oriented obliquely in contrast to the fibers at the alveolar crest which are horizontal.

Fig. 2 shows the lacerated papilla between the first and second molar after two days observation time. The normal orientation of the principal periodontal fibers is interrupted. The horizontal arrangement as seen in Fig. 1 is not present, and a zone with vertically orientated fibers is predominant. This zone (p) is clearly seen at a higher magnification (Fig. 3). The normal orientation of the principal fibers has changed. Instead of running horizontally or slightly obliquely they now run in a changed direction, i.e. from the cementum buttons and apically.

Fig. 4 shows a specimen at the three day observation time. Close to the wound the inflammation has destroyed the normal arrangement of the periodontal membrane (Fig. 5). Fig. 6 is from the same specimen in a more coronal position. It represents a transition zone where the fibers again appear and are orientated. Further coronally in the same specimen the direction of the fibers is again normal, (Fig. 7).

After five days observation time (Fig. 8), the distance of eruption of the tooth should be particularly noted. In contradistinction from the animal with a two day observation time (Figs. 2 and 3), no fibers or cementum buttons are seen on the tooth in wound area.

Fig. 9 is a high power illustration from an area apically to the wound in Fig. 8, and shows the fibers originating from the tooth and the bone intervening in a plexus with no distinct orientation. Coronal to the wound the cemental fibers have followed the tooth in its eruption (Fig. 10), and their direction is changed. The zone of the plexus is not discernible. Further coronally (Fig. 11) a transitional zone is seen where the intermediate plexus zone reappears. A normal arrangement of the fibers is found still further coronally (Fig. 13).

PLATE I

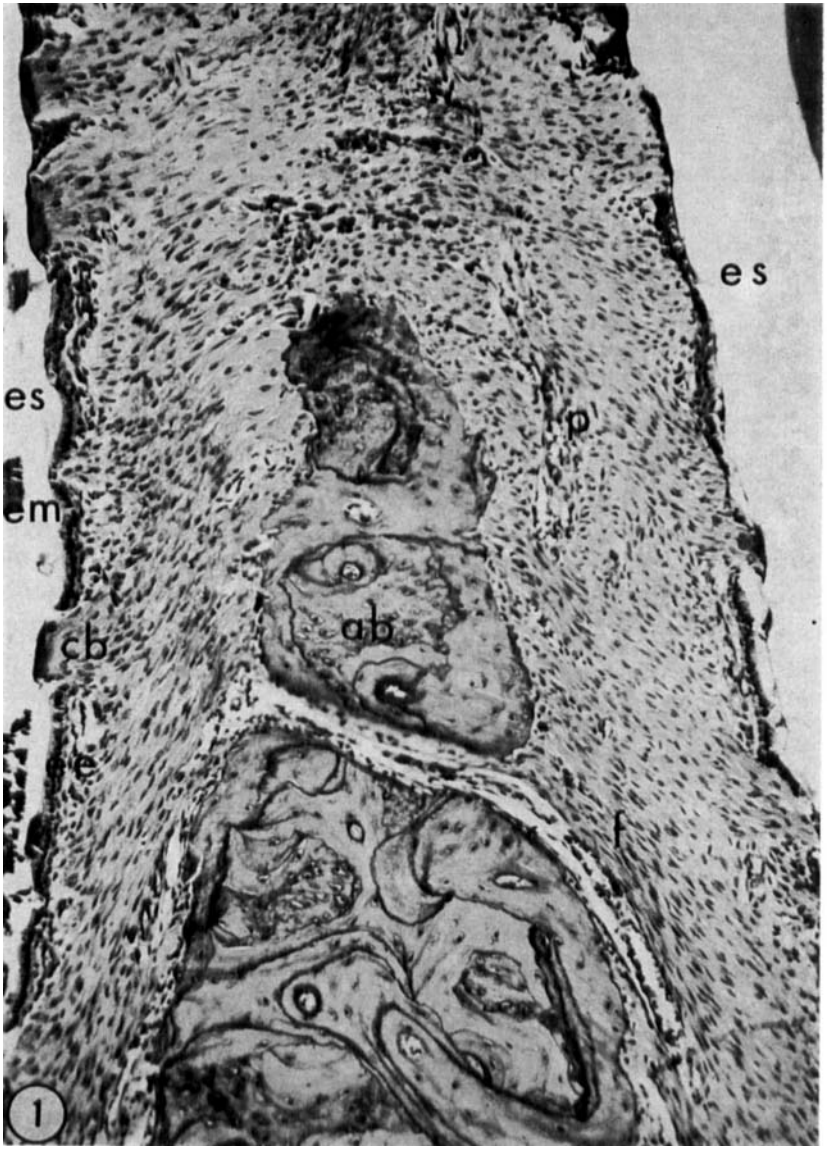


Fig. 12 shows the mesial part of the periodontal membrane from the first maxillary molar on the experimental side of the same animal as in Fig. 8. There is a total dearrangement of the periodontal membrane. Fibers profusely intermingled with inflammatory cells can be distinguished, and there is a decreased number of connective tissue cells as compared to Fig. 13.

Sequestration of the marginal crest was observed after eleven days observation time (Fig. 14). The principal fibers, attached to the sequestra, had changed direction. There was apposition of bone at the top of the new septal margin, and there was normal fiber direction in this area.

Sections stained with Gomori's silver stain showed more clearly the fiber direction which was changed in the specimen from an animal with six days observation time (Fig. 15). A neighbouring section from the same specimen (Fig. 16) stained with hematoxylin-eosin, does not show the fiber direction so clearly, but other tissue elements are more easily distinguishable.

PLATE I*)

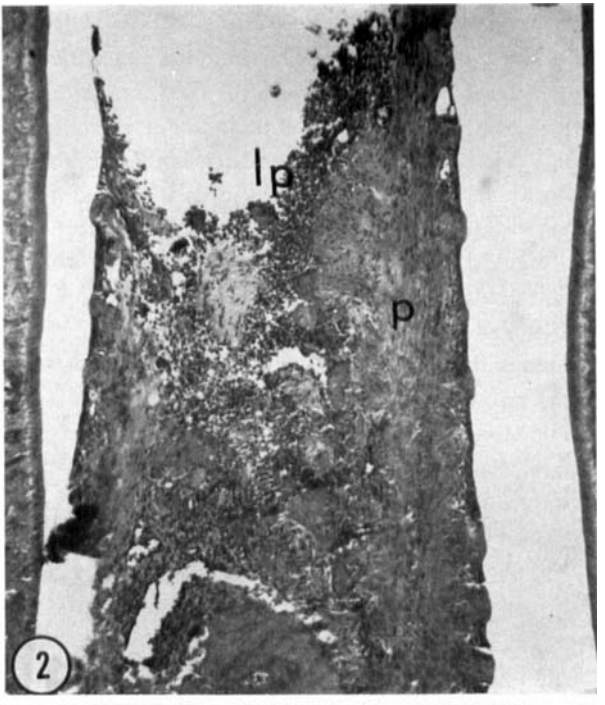
Fig. 1. Shows the marginal septum from a control animal between the maxillary right first and second molars. The space occupied by enamel before decalcification, (e s) is seen on both sides together with the enamel epithelium (e e) and the remnants of the incompletely calcified enamel matrix (e m). The principal periodontal fibers (f) are running in a coronal direction from the cementum buttons (c b) through the intermediate plexus zone (p) to the alveolar bone (a b). Above the septum the fiber direction is more horizontal. Hematoxylin and eosin (H+E).

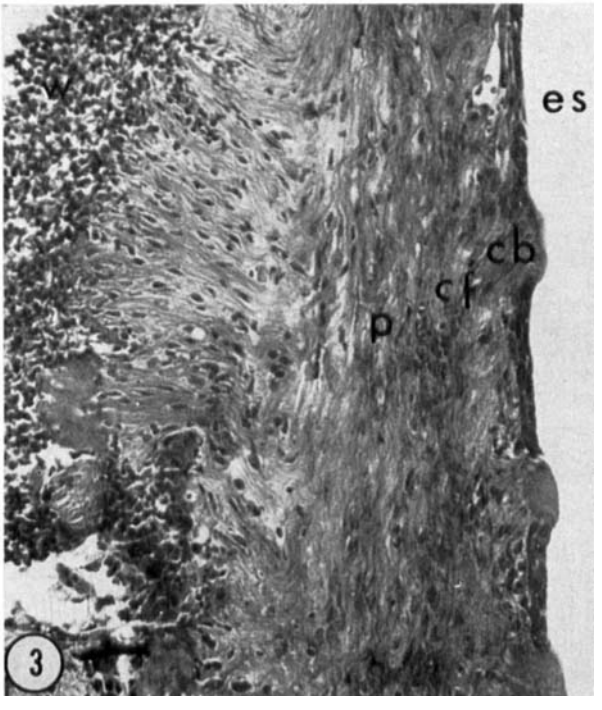
*) All illustrations are orientated with the apex of the tooth towards the bottom and the coronal portion towards the top of the page.

Abbreviations

a b	alveolar bone	f	principal fibers
c b	cementum button	l p	lacerated papilla
c f	changed fiber direction	n b	apposition of bone
d	distance of eruption	p	intermediate plexus
e e	enamel epithelium	s	sequestra
e m	enamel matrix	t	transitional zone
e s	enamel space	w	wound

PLATE II





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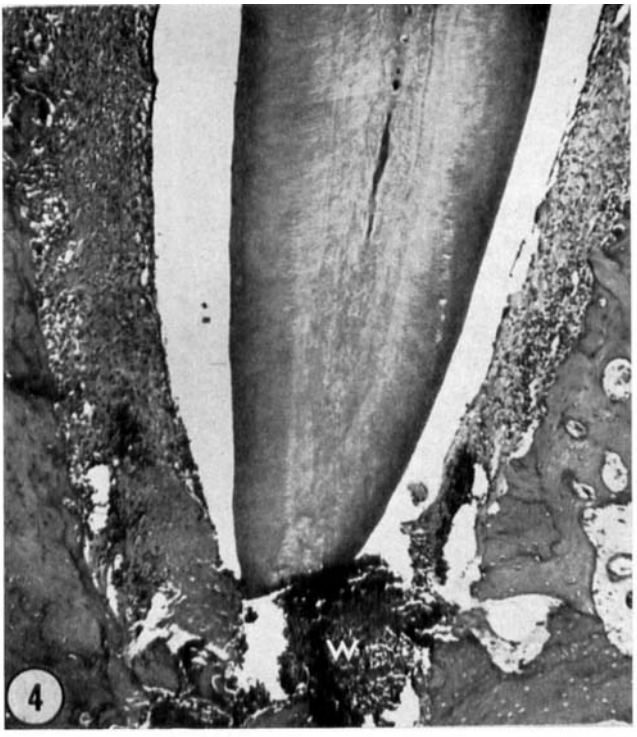

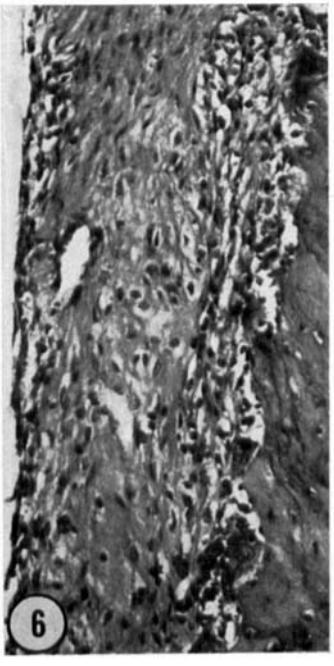

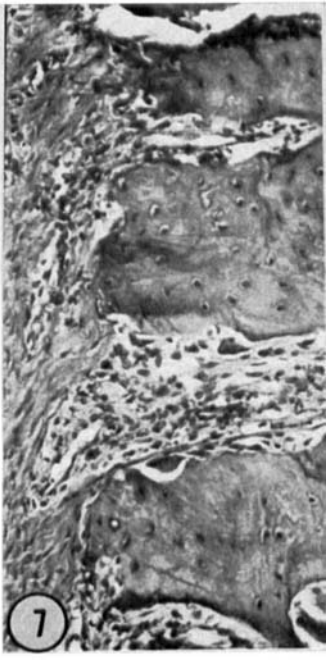

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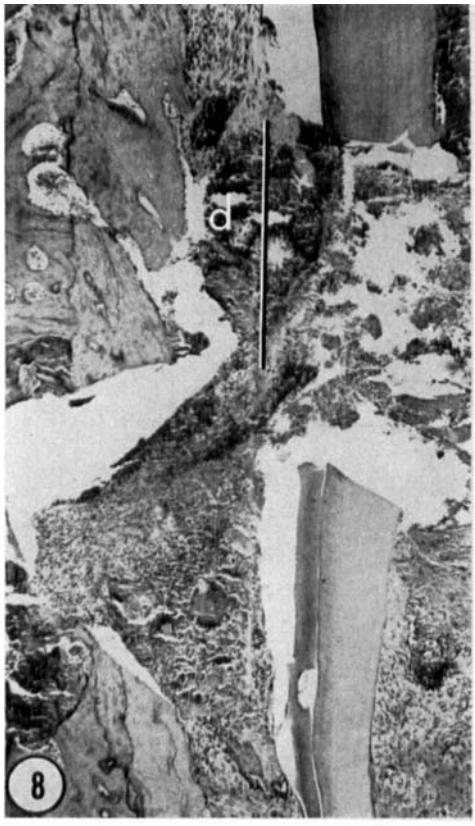
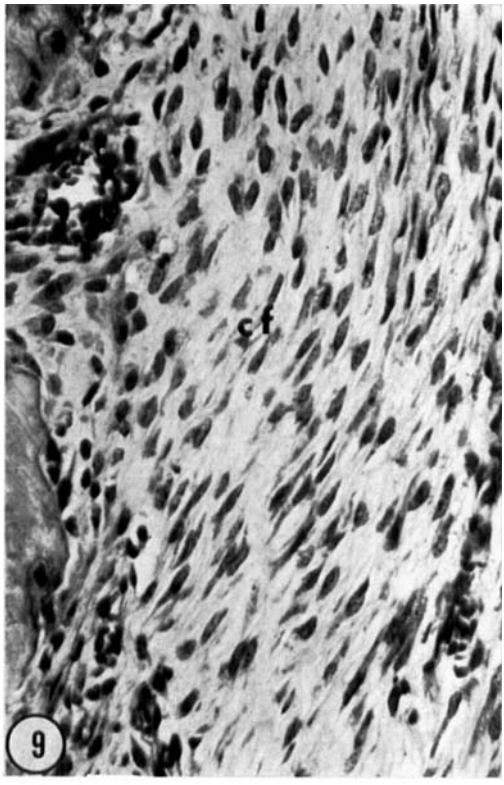
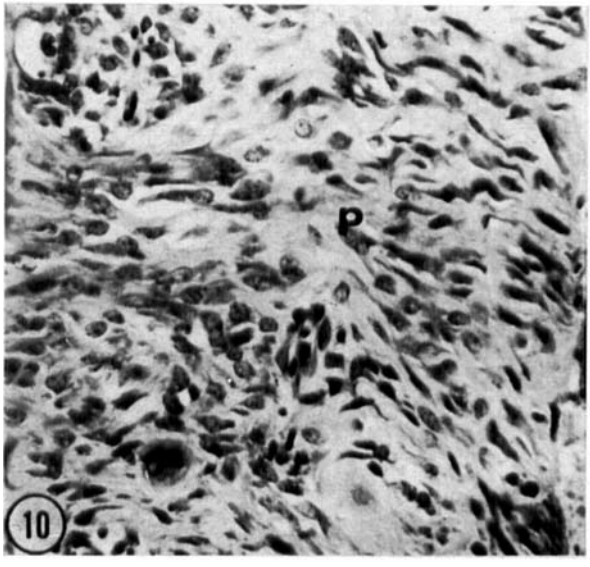






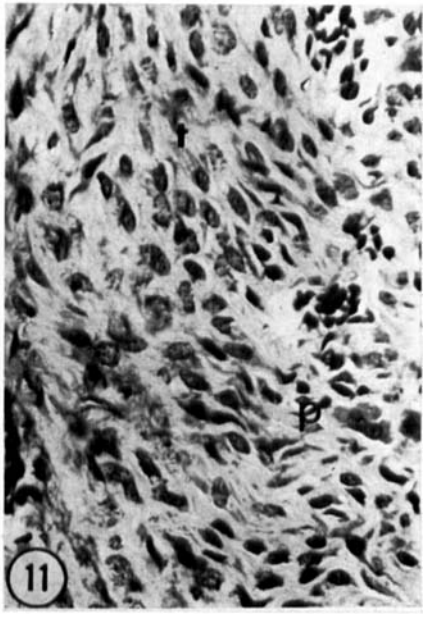


Plate II.

Fig. 2. Is a survey photomicrograph of the interdental bony septum with a lacerated papilla (l p) from an animal with two days observation time (H+E).

Fig. 3. Shows the upper right part of the section at a higher magnification. The fiber direction is now changed (c f), from the cementum buttons (c b), to the intermediate plexus (p) which is seen as a longitudinally orientated zone, more prominent than in Fig. 1. Compare this to the direction of the principal fibers (f) in Fig. 1. The wound (w) is seen in the upper left corner of the illustration. (H+E).

Plate III. Three days observation time.

Fig. 4. Is a survey of an apically induced wound (w) after three days observation time, showing destruction of the normal anatomy of the periodontal membrane.

Fig. 5. Higher magnification of an apical part of the same section showing disorganization of the principal fibers, inflammation and extravasation of red blood cells. (H+E).

Fig. 6. High power photomicrograph a little more coronally than the previous illustration. Inflammation is less pronounced, the intermediate plexus is not discernible. At the top some principal fibers are seen. (H+E).

Fig. 7. Is taken a little more coronally than Fig. 6. Here the principal fibers are again normally oriented, running from the tooth, (left side of the illustration) in an oblique direction to the alveolar bone (right side). Some inflammatory cells are seen. (H+E).

Plate IV. Five days observation time.

Fig. 8. Survey of a wound from an animal after five days observation time. The distance of eruption (d) and the tissue changes around the wound are seen. (H+E).

Fig. 9. Higher magnification of the periodontal membrane apical to the wound in Fig. 8. The intermediate plexus (p) is seen between the principal fibers from the cementum and the bone. (H+E).

Fig. 10. Shows change in fiber direction (c f) and no intermediate plexus coronal to the wound in Fig. 8. The fibers have followed the tooth in its eruption, no rearranging has taken place; therefore the fibers show changed orientation. (H+E).

Fig. 11. Even more coronal than Fig. 10, from the same specimen as Fig. 8. Showing a transitional zone (t) where the fibers are not normally orientated, but not as changed as in Fig. 10. The intermediate plexus (p) is seen. (H+E).

Plate V.

Fig. 12. Extravasation and inflammation have disturbed the intermediate plexus and the normal arrangement of the principal fibers in the periodontal membrane of a neighbour tooth. Five days observation time. (H+E).

Fig. 13. Taken more coronally than Fig. 11 from the same specimen as Fig. 8. The intermediate plexus (p) is evident and the principal fibers (f) are normal in their direction. The transitional zone (t) also seen in Fig. 11 is seen further apically. (H+E).

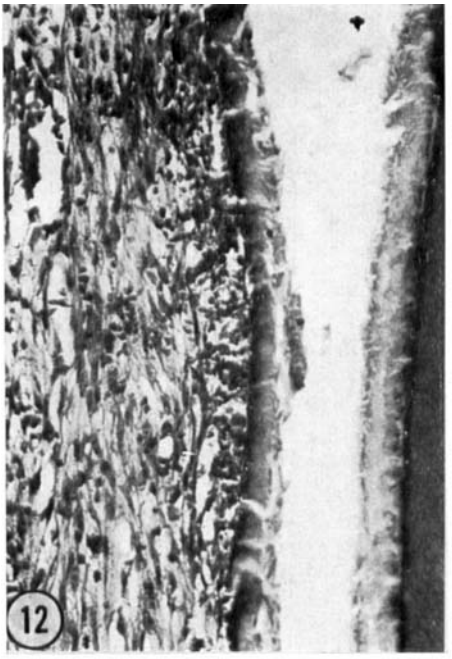
Fig. 14. Sequestration of the marginal crest after 11 days. The principal fibers, with changed direction (c f) remain attached to the sequestra (s). There is apposition of bone (n b) at the crest and here the fibers (f) are normally orientated.

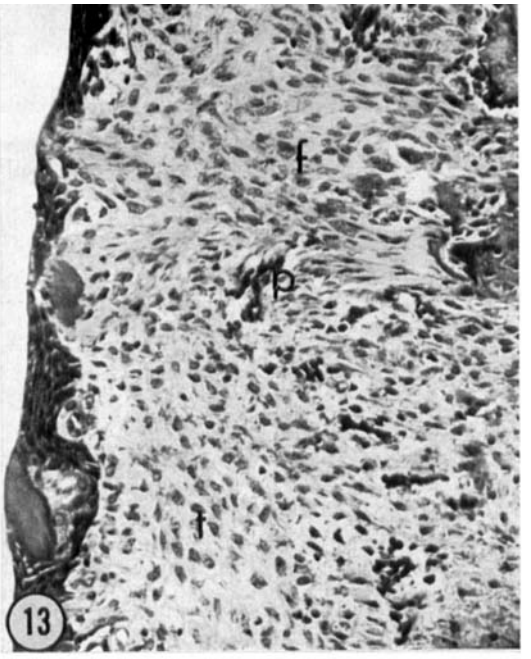
Plate VI.

Fig. 15. Wound (w) after 6 days observation stained with Gomoris' silver stain. There is changed direction (c f) of the principal fibers and no intermediate plexus in the vicinity of the wound.

Fig. 16. A neighbouring section from the same specimen as Fig. 15. The cellular details are more clear, but the fibers are not as well seen as in Fig. 15.

PLATE V





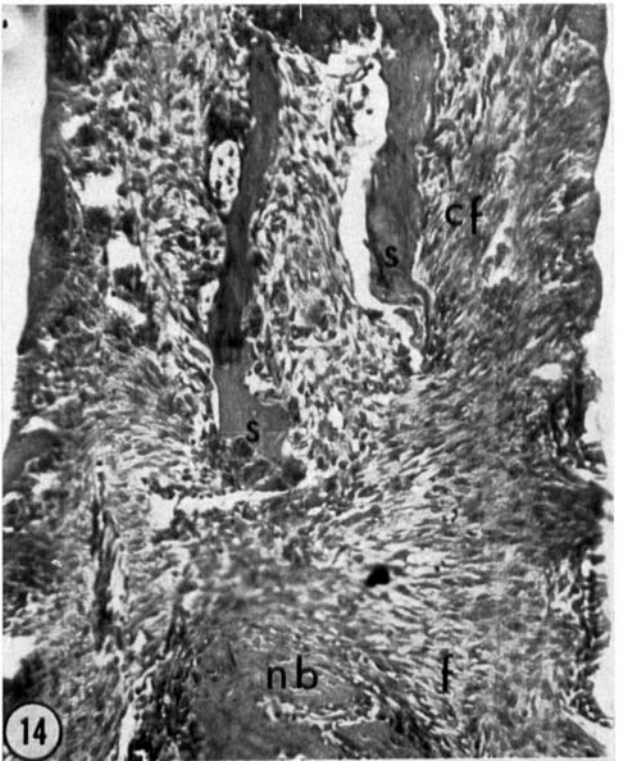
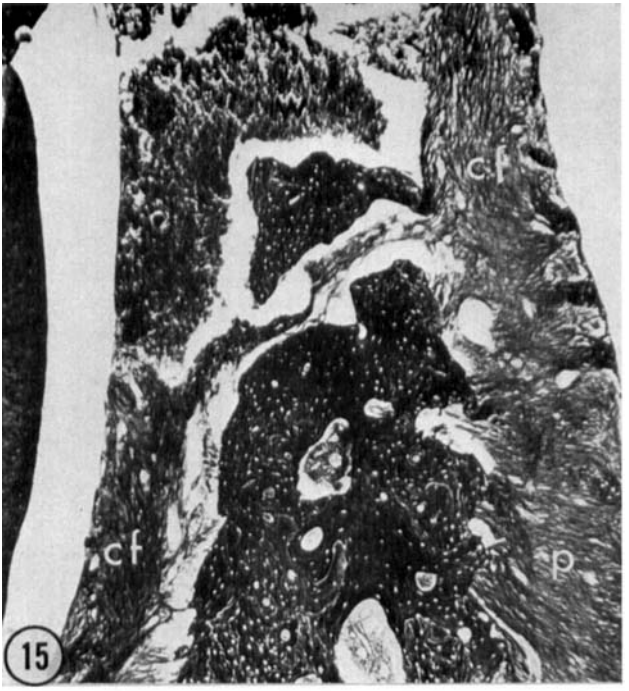


PLATE VI



DISCUSSION

A rearrangement of the periodontal membrane in the guinea pig takes place constantly under normal conditions. This is necessary to maintain a constant direction of the principal periodontal fibers during the continuous eruption of the teeth. Under the experimental conditions a complete change in the direction of these fibers has been demonstrated. The experimental interference with the normal function of the periodontal membrane resulted in a disturbance of the normal arrangement process of the fibers. The fibers remained attached to the bone and to the cementum above the wound, but with a distinct change in their orientation. An explanation for this change in direction cannot be forwarded without accepting a rearrangement capacity of a zone between the cementum buttons and the septal bone. This zone is highly susceptible to changes in the environment. It looses its rearranging function close to the wound, it is less influenced at some distance from the wound (the transition zone) and still further away from the wound it is not affected.

Autoradiographic studies in the guinea pig have shown an even distribution in the uptake of 3H proline by cementum, periodontal fibers and septal bone (*Ramos* & *Hunt*, 1967). A higher uptake of 3H proline along the alveolar wall was reported by *Crumley* (1964), who suggested that the normal periodontium is forming collagen at a rate very near its potential capacity. However, this does not exclude the possibility of an existing intermediate plexus with a rearranging function. The even uptake of 3H proline in the entire width of the periodontal membrane can be explained by an existing pool of proline in the zone of the intermediate plexus which is reutilized. Therefore, no increased uptake of 3H proline would be seen in the autoradiographs.

The observations in this experiment support the concept of an intermediate plexus with a rearranging function in the periodontal membrane of guinea pig molars (*Sicher*, 1923). The apparent sensitivity to changes in the homeostatic state of this plexus seems to agree with *Hindle* (1967) who suggested a less mature zone of fibers in the middle of the periodontal membrane of incisors in the rat.

SUMMARY

A slowly rotating small fissure bur was introduced between the maxillary first and second molars in 24 anesthetized guinea pigs in order to mark the teeth and the interdental septum. After varying observation periods the

animals were sacrificed and the teeth and the periodontal membranes were studied histologically.

A change in the normal orientation of the principal fibers was observed after the trauma. This finding was taken to support the concept of an intermediate plexus in the middle of the periodontal membrane.

RÉSUMÉ

RÉPONSE DU PLEXUS INTERMÉDIAIRE DES MOLAIRES DU COBAYE AU TRAUMATISME EXPÉRIMENTAL

Chez 24 cobayes anesthésiés, une fraise à fissure tournant à faible vitesse a été introduite entre les premières et les deuxièmes molaires supérieures afin de repérer les dents et le septum interdentaire. Après des périodes d'observation de différentes durées, les animaux ont été sacrifiés et une étude histologique des dents et des desmodontes a été faite.

Un changement de l'orientation normale des fibres principales a été observé après le traumatisme. Cette constatation viendrait à l'appui de l'idée de l'existence d'un plexus intermédiaire à la partie moyenne du desmodonte.

ZUSAMMENFASSUNG

REAKTION DES INTERMEDIATEN PLEXUS DES MOLARZAHNES BEIM MEERSCHWEINCHEN AUF EXPERIMENTELLES TRAUMA

Ein langsam rotierender kleiner Fissurbohrer wurde zwischen dem ersten und zweiten Oberkiefermolarzahn bei 24 anästhesierten Meerschweinchen eingeführt, um die Zähne und die Zahnwurzelumwände zu markieren. Nach verschiedenen Observationsperioden wurden die Tiere getötet und die Zähne und periodontalen Membranen histologisch untersucht.

Eine Veränderung im normalen Verlauf der Hauptfasern wurde nach dem Trauma beobachtet. Dieser Befund wurde als Unterstützung des Konzepts eines intermediaten Plexus in der Mitte der periodontalen Membrane angesehen.

REFERENCES

Crumley P. J., 1964: Collagen formation in the normal and stressed periodontium. Periodontics *2:* 53.

Eccles J. D., 1961: Personal communication.

Goldman H. M., 1962: Discussion of connective tissues. J. dent. Res. *41:* 230.

Hindle M. O., 1967: The intermediate plexus of the periodontal membrane. in The Mechanisms of Tooth Support. A Symposium. Bristol: John Wright & Sons Ltd. Eds., Anderson, D. J., Eastoe, J. E., Melcher, A. H. and Picton, D. C. A.

Hunt A. M., 1959: A description of the molar teeth and investing tissues of normal guinea pigs. J. dent. Res. *38:* 216.

Johansen J. R., 1960: An experimental study on repair of the periodontal membrane in humans and guinea pigs. Thesis, University of Alabama School of Dentistry, Birmingham.

Melcher A. H., 1968: Remodelling of the periodontal ligament during eruption of the rat incisor. Arch oral Biol. *12:* 1649.

Orban B., 1962: Oral Histology and Embryology, p. 187. The C. V. Mosby Company, Saint-Louis.

Ramos H. B., Jr. & A. M. Hunt, 1967: Remodelling of the periodontal ligament of guinea pig molars. in The Mechanisms of Tooth Support. A Symposium. Bristol: John Wright & Sons Ltd. Eds., Anderson, D. J., Eastoe, J. E., Melcher, A. H. and Picton, D. C. A.

Sicher H., 1923: Bau und Funktion des Fixationsapparates der Meerschweinchen Molaren. Z. Stomat. *10:* 580.

—»— 1925: Über die Fixation und das Wachstum dauernd wachsende Zähne. Correspondenz-Blatt für Zahnärzte *3:* 332.

—»— 1953: The biology of attrition. Oral Surg. *6:* 406.

—»— 1959: Changing concepts of the supporting dental structures. Oral Surg. *12:* 31.

—»— 1942: Tooth Eruption: The axial movement of continuously growing teeth. J. dent. Res. *21:* 201.

Stallard R. E., 1963: The utilization of H^3 proline by the connective tissue elements of the periodontium. Periodontics *1:* 185.

Zwarych P. D. & M. B. Quigley, 1965: The intermediate plexus of the periodontal ligament: History and further observations. J. dent. Res. *44:* 383.

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