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J DENT RES 1960 39: 781
DOI: 10.1177/00220345600390040501

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What is This?
Formation of Periodontal Tissues around Subcutaneously Transplanted Hamster Molars

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The purpose of the present investigation is to determine whether the developing tooth has a formative influence upon the tissues which surround it and, if so, how far the influence extends and how long it lasts. In vitro microdissection studies have indicated that the enamel organ of the developing tooth exerts a variety of influences inwardly upon the mesenchyme within it—the dental papilla. Its epithelium is believed to influence the initial condensation of mesenchyme to form the dental papilla, the differentiation of odontoblasts, and the general shaping of the tooth crown and roots. However, the possibility that the enamel organ might also exert influence outwardly upon the surrounding mesenchyme of the dental sac has not been experimentally tested. The transformation of the dental sac into the definitive periodontium, consisting of the periodontal ligament and alveolar bone, proceeds simultaneously with the growth and development of the roots. This suggests some kind of co-ordinating influence between periodontium development and the development of the tooth proper. In order to test whether such an influence exists, the enamel organ and dental papilla of the developing hamster molar were dissected from their dental sac before root formation, transplanted into connective tissue which ordinarily does not form periodontium, and allowed to develop. It was found that all healthy specimens recovered after 4 weeks in the host had organized periodontium around themselves; this suggests the existence of an organizing influence emanating from the developing root. The extent of the influence was found to be limited to the periodontal ligament and a relatively thin shell of alveolar bone around the roots and bone in the space between the roots.

Cells forming bone around transplants seem to have been supplied by the host. The stimulus for conversion of host cells into osteoblasts probably came from the enamel organ and its derivative—Hertwig's epithelial root sheath. This stimulus was found to be capable of influencing the development of a periodontal ligament resembling markedly the periodontal ligament which normally develops in the jaw in the presence of both prefucntional and functional formative stimuli. Because transplants developed in the absence of normal function, differences between the periodontal ligament which developed in situ and the ligaments in transplants could then be attributed to the influence of function upon structure.

Part of a thesis submitted to the Graduate College, University of Illinois, in partial fulfilment of requirements for the degree of Master of Science.

Received for publication August 28, 1959.

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Other investigators have observed bone and/or periodontal ligament around teeth transplanted into connective tissues other than the jaw. However, these writers were concerned primarily with other aspects of tooth transplantation, and, in each case, observations of elements of the periodontium were serendipitous. Willis, while testing the rat brain as a possible site for growth and development of minced embryonic rat parts, found some fully developed and some partly developed teeth in the recovered transplants of minced jaw. One fully formed tooth had bone adjacent to it, but the possibility that the bone had been transplanted with the developing tooth was not ruled out. Kostecka homoplastically transplanted embryonic dog tooth germs subcutaneously and intermuscularly into young dogs. However, it is not clear why he evaluated occasional cartilage and bone formation as signs of absorption. While investigating histologic changes in tooth germs transplanted into the tibia marrow cavities of kittens, Sutro and Pomerantz found bone around the roots of some specimens, as well as pulp tissue which had been transformed into bone in others. Villafane and Brero autotransplanted rabbit incisors without the dental sac intermuscularly and after a year recovered the transplant and reported an encapsulated specimen which had bone on some parts of the enamel surface. Fleming refers to the osteogenic activity of cells transplanted with the tooth germ into the anterior chamber of the eye. Hammer transplanted fully developed teeth to the greater omentum in dogs and monkeys, where he found the preserved part of the periodontal ligament connected to the omentum by means of scar tissue. While Bejdl and Weizenberg were showing that heteroplastic transplantation of tooth germs was possible among dogs, pigs, and rats, they also noticed and reported both periodontal ligament and adjacent bone around the roots of transplanted teeth.

Experiments reported in the literature indicate that the developing tooth can survive transplantation and continue growth and development in vivo and, to a limited extent, in vitro. However, as far as can be ascertained, no one has studied systematically the formation of periodontal tissues around teeth transplanted into sites other than the jaw. Transplantation into a connective tissue other than the jaw is to be distinguished from reimplantation in the alveolus, where healing alone may be responsible for regeneration of the periodontal ligament and where function may also contribute to the alignment of its fibers.

**MATERIALS AND METHODS**

Developing molars were transplanted from newborn hamsters (Cricetus auratus) into the subcutaneous tissues of young adult hamsters. Although both younger and older tooth germs were also transferred, only the procedure used for the neonatal transplantations will be described, since the technic is essentially the same for all ages.

Every effort was made to carry out transplantations aseptically. Sharp instruments were sterilized in zephiran chloride; all other instruments, as well as gauzes and towels used to dress the operating table and to protect instruments, were autoclaved, and host skins were shaved and swabbed with merthiolate before incisions.

Newborn developing teeth were easily seen with the aid of binocular loops. Upper first molars were transplanted in all cases. These teeth are unerupted and easily injured during removal; anlagen known to have been torn or pierced by instruments were discarded.
Neonatal donors were first decapitated. The maxilla and top of the head were then separated from the remainder of the head by inserting scissor points into the corners of the mouth on each side and cutting back through the base of the skull. With the maxilla facing upward, the upper head was placed on a glass slide and the elevated and rounded area of the developing first molar located. An iridectomy knife was inserted, from the buccal (lateral), just beneath the surface tissue of the rounded molar area, and the thin superficial tissue over the developing tooth was completely removed. Vertical incisions were made at the anterior, posterior, and buccal (lateral) edges of the tooth to sever adhesions and attachments to the second molar. By inserting the point of an iridectomy knife into the anterior incision and along the mesial surface of the tooth, it was possible to lift upward and distally remove the developing tooth cleanly and intact from its dental sac of connective tissue. The molar was then lifted, on the blade of the knife, and placed gently but quickly in position under the skin of the host. Silk sutures or metal clips closed host skin incisions.

Host animals were approximately 30-day-old normal healthy young adults of both sexes. Members of each series numbered from five to seven animals and were littermates. Before receiving a transplant, each host animal was anesthetized, identified by ear punches, shaved of its back fur, and an incision approximately 1 cm. long was made through the skin. With this advance preparation of the hosts, molar transplants were never more than 5 seconds from the fluids of the donor to the subcutaneous fluids of the host.

Healthy host weights at 30 days of age were found to be between 40 and 50 gm. After receiving transplants, the hosts were returned to their bank wire cages, where the regular diet of Purina pellets and water ad libitum and carrots or lettuce twice weekly was given. Animal weights at sacrifice ranged normally between 80 and 100 gm. A few hosts died or became diseased; only transplants carried by normal healthy animals were included.

After 28 days of transplant growth and development within its subcutaneous tissues, each hamster host was anesthetized, shaved, and the skin of the back incised centrally from the tail to the shoulders. After spreading the skin laterally and pinning its edges down, transplants could be readily identified. Sketch drawings were made of the general transplant outline, its color, and its vascularization. Molars, with surrounding connective tissues, were then removed and fixed in 10 per cent buffered formalin and decalcified in ethylenediamine tetra-acetic acid. Paraffin-imbedded specimens were routinely sectioned at 10 μ; a special effort was made to secure longitudinal root sections. All sections were stained with hemotoxylin and eosin.

Controls were established by comparing neonatal transplants recovered after 28 days with molars and their periodontiums which had developed normally in a 28-day-old animal.

RESULTS

The neonatal hamster molar at the time of transplantation.—A 1-day postnatal molar in situ is in the shell stage of tooth development (Fig. 1). Ameloblasts are differentiated over the cusps and are seen in earlier stages of differentiation as one passes down the slopes of the cusps either on the occlusal surface of the tooth or on the cusp slope toward the proximal of the tooth. Some dentin has been formed by odontoblasts in the central cusp, and ameloblasts here appear ready to begin enamel formation.
Bone of the jaw completely surrounds the developing molar, forming a bony crypt which is separated from the tooth by a cell-poor tissue. Osteoblasts and osteoclasts are seen along the inner surface of the crypt.

A sagittal section of a molar which was removed from the jaw of a newborn donor and prior to its transplantation is shown in Figure 2. The enamel organ and dental papilla are firmer than at younger ages; at this age they are easily freed from the dental sac. Relatively few mesenchymal cells are seen outside the enamel organ. Cells seen between the cusps belong to the stellate reticulum, and the epithelium is torn and folded back at the upper left margin of this specimen.

**Neonatal molar transplants after 28 days in the host.**—Twenty-three neonatal molars were transplanted. Of these, 12, or 52.2 per cent, were healthy developed specimens when recovered. Gross observations of transplants were made at the time of removal from the host. Transplants which exhibited growth and development showed extensive vascularization from the host (Fig. 3). Large blood vessels supply the graft. Moreover, a capillary network can be seen spreading over its surface. Cusp outlines can also be determined. All healthy developed molars recovered were similarly vascularized and encapsulated.

The histologic appearance of alveolar bone and periodontal ligament formed in recovered specimens was similar for donors of all ages. Striking resemblances and some differences were seen between periodontal tissue developed in transplanted teeth and in teeth of similar age developed in situ. Not all transplanted teeth were recovered after 28 days. Five of 23 neonatal transplants not recovered were presumed to have been resorbed. Four transplants were recovered grossly as unvascularized, small, white, hard masses. They were microscopically dead tissues, which might have been resorbed with additional time. Two recovered transplants showed an unusual situation; grossly, they were encapsulated and vascularized, but microscopically the tissues showed little additional growth and development beyond that shown at the time of transfer. No roots were formed. Even so, bone and periodontal ligament were formed, but in these cases the periodontal tissues were found within the pulp tissue.

Figure 4 shows the histological appearance of a neonatal molar after 28 days in the host. The size and morphology of the molar were relatively normal; very little enamel formed, however. Crown dentin, not covered by enamel, was covered by cementum. Adjacent fibrous connective tissues appeared to be typical periodontal ligament; its fibers are seen to be imbedded in the cementum. Bone was formed only in relation to the cementum covering dentin of the tooth root and not in relation to the cementum and periodontal ligament over the enamel-free dentin of the tooth crown. Some cusps showed tips of dentin resembling osteodentin, in contrast to the normal tubular dentin seen above it. Roots were of normal length and contained well-vascularized pulp tissue with odontoblasts at the periphery. Pulp tissue in transplants was generally younger in appearance than in the control; it contained more cells and was more highly vascularized. Predentin was somewhat more irregular, and some areas of interglobular dentin were seen. Periodontal ligament and alveolar bone surrounded the roots; cellular cementum was heavily deposited on the sides of the roots near their apices.

Recovered transplants showed typically oriented periodontal ligament fiber groups; alveolar crest and horizontal, oblique, and apical groups could be identified. A thin
Fig. 1.—Neonatal molar in situ, sagittal section. (Orig. mag. ×62.)

Fig. 2.—Neonatal molar removed from the jaw, sagittal section. (Orig. mag. ×80.)
Fig. 3.—Transplant lying on the undersurface of the host skin after 28 days within the host. (Orig. mag. ×24.)
shell of bone surrounded the outside root surfaces, while the interradicular bone was more extensive and filled the space between the roots.

When the periodontium of the transplant (Fig. 6), was compared with the periodontium of a molar of comparable age which developed and functioned in the jaw of a control animal (Fig. 7) several points of similarity and of difference were noted.

1. Fiber orientation of the periodontal ligament between the tooth and the bone was similar in the transplant and the control.

![Fig. 4.—Neonatal transplant after 28 days in the host. (Orig. mag. ×80.)](image)

2. In contrast to the random fiber arrangement of the transplant ligament, the fibers of the control ligament were grouped into bundles which had interstitial spaces between the bundles.

3. The control periodontal ligament was wider; this is a constant finding when comparing transplant ligament width with a control periodontal ligament which developed and functioned in situ.

4. The rather delicate trabeculation of alveolar bone surrounding the transplant contrasted with the more compact alveolus of the functional control bone, with its resting lines which define prior apposition and resorption of bone. Greater cellular
activity was apparent along the alveolar bone of the control. Osteoblasts lined the osseous border of the control ligament, and an osteoclast could be seen near the upper edge of the vessel which perforates the bone.

DISCUSSION

Survival, growth, and development of transplants.—Homologous grafts have always been subject to immunological problems. These problems were expected, since random breeding was employed in our colony rather than planned brother-sister matings. Although infection may have caused the loss of a few of our transplants, genetic differences between donor and host were probably responsible for the loss of most of the transplants not recovered. Syrian hamsters, however, appear unique because they will often accept homografts of malignant tissue and occasionally even heterografts of malignant tissue as well as normal tissue. Billingham and Hellemann recently tested four different closed colonies, employing random mating, for reactions to intracolony

![Image](https://example.com/image.jpg)

**Fig. 5.** Twenty-eight-day postnatal molar in situ, sagittal section. (Orig. mag. ×80.)
and intercolony skin homografts. Intercolony survivals were 41, 17, 0, and 0 per cent for a 100-day period. The breakdown of grafts in animals rejecting intracolony homografts was complete in 3 weeks, while the median survival time of different combinations of intercolony grafts where most transplants were rejected was 10.5, 9.9, 8.8, 11.2, 19.3, and 10.5 days. It is believed, therefore, that the 28-day transplantation period used in this investigation was adequate to distinguish transplants accepted from those rejected by the host.

Vascularization of transplanted tissues is essential to their survival. In all cases it is obvious that there is an interval between transplantation and vascularization of the transplanted tissues. During this interval, transplanted tissues must acquire at least maintenance nutrition through diffusion from host subcutaneous tissue fluid. Converse, Ballantyne, and Woisky showed, by grafting rabbit skin to the chorio-allantoic membrane of chicks, that transplants gained considerable weight in the early hours after transfer and before becoming vascularized. Transplant weight increased steadily from 10 per cent more after 1 hour to 52 per cent more after 20 hours. Converse calls this
initial period, during which graft vessels fill with fluid and cells from the host, the “period of plasmatic circulation.” Subsequent vascularization by the host is currently being studied in our tooth transplants.

A molar which is transplanted at birth and recovered as a fully developed tooth in 28 days obviously passes through the same developmental stages as one which develops in situ. At birth, initiation and proliferation have taken place, and morphodifferentiation and histodifferentiation of ameloblasts and odontoblasts have occurred. The size and shape of the tooth crown are established, and crown morphology is made permanent by the beginning of dentin formation at the dento-enamel junction. Following transplantation, apposition and calcification of enamel and dentin occurred, as well as the formation of roots and periodontium—processes which are normally associated with the eruption of the tooth. Hertwig’s sheath also survives transplantation (Fig. 8).

Organization of transplants.—Figure 9 is a diagrammatic representation of the experiment. The developing germ and its adjacent tissues—the lower center figure—forms the fully developed molar and its periodontium—the figure on the right. It is seen that the ectodermal derivative—the enamel organ—A forms the enamel A’ of the fully developed tooth. Mesodermal elements—the dental papilla—B formed dentin B’, and the dental pulp B’. Connective tissue C adjacent to the tooth forms the periodontal ligament, C’, and adjacent bone, D, forms the alveolar bone, D’, which supports the developed tooth.

However, when the developing tooth is transplanted to the new connective tissue, as shown in the lower center to upper center figures, only A, B, a very small amount of C, and none of D are transferred to the new environment, E. The transplant develops into the mature tooth and its periodontium at the left. A develops into A’, B to B’, C may possibly proliferate to C’, but bone was not transplanted, and yet a shell of bone formed around the roots of all healthy recovered transplants.

We have seen that if the transplant survived and developed, it formed periodontal ligament and alveolar bone around its roots. This seems to indicate that the morphologically complete dental organ consists of the enamel, dentin, pulp, cementum, periodontal ligament, and a shell of alveolar bone surrounding the roots. Teleologically speaking, transplanted teeth obviously considered themselves incomplete without this bone and hence caused it to be formed. No healthy developing molar failed to produce it.

The formation of periodontium around transplanted enamel organs may be analogized to the induction of the lens by the optic cup. Like the developing tooth, the forming eye is composed of structurally discrete parts which originate from different embryonic sources but are brought together during development to form a complete organ. Spemann10 points out that Lewis found that when the early rudiment—the optic vesicle—of the frog eye (Rana sylvatica and palustris) was exposed, severed, and pushed backward under the epidermis of the trunk, a lens formed above the trunk from the epidermis of the host. Moreover, after the eye was fully formed, removal of the lens in Urodèle resulted in the formation of a new lens from the border of the iris (Wolffian lens regeneration).11 In both cases, lens was formed from tissue, epidermis, or iris, which would not normally have formed lens, and under the influence of a stimulus provided by the optic cup. The eye was “incomplete” without lens and hence proceeded to acquire one. Similarly, the transplanted germ was an incomplete
Fig. 8.—Apical area of neonatal transplant after 28 days in the host. (Orig. mag. ×135.)
developmental unit and completed itself during its development by producing periodontal ligament and alveolar bone.

We may now see the formation of periodontal tissues around transplanted molars as the efforts of a morphogenic field to complete itself. The development of other morphogenic fields, when transplanted, has been shown to be similar in pattern to the development in situ. This suggests that the development of the transplanted molar is also the normal developmental pattern. Developing 6-day postnatal hamster molars show a shell of alveolar bone which is not completely attached to the jaw in some areas. This appears to support the contention that the mode of development of the transplant and the molar in situ are similar.

The source of cells which form bone.—Most workers agree, perhaps because of lack of other evidence, with Tomes' statement as reported by Mummery, that "the follicle is in fact formed by a condensation of the connective tissue in the neighborhood of the tooth germ." It now appears clear that the cementum, periodontal ligament, and alveolar bone shell are the derivatives of the dental sac.

The cells which form alveolar bone around transplants could have come either from transplanted tissues, the enamel organ, dental papilla, adhering connective tissue cells, or the new environment which lacked the bony crypt of the jaw. It seems quite acceptable that the enamel organ, A, should again form enamel, A', and that the dental papilla, B, should form the dentin and pulp tissue, B'. However, according to Figure 9, several possibilities exist as to the cell origins for the periodontal ligament, C', and especially for the shell of alveolar bone, D'. Only a small part of C and none of D were transferred. Moreover, the new environment, E, must be evaluated as to its possible contribution.

The enamel organ, A, is a specialized epithelial derivative of ectoderm. The possibility that such specialized epithelial cells might be converted to cells forming the

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Fig. 9.—Diagram of the experiment. Figure on left shows transplant after development in host; figure on right shows fully developed molar and its periodontium in situ. A = enamel organ, A' = enamel of fully developed tooth; B = dental papilla, B' = dental pulp; C = connective tissue, C' = periodontal ligament; D = adjacent bone, D' = alveolar bone; E = new environment.
Formation of periodontal tissues

mesodermal connective tissue—bone—seems remote. The enamel organ is therefore eliminated. Connective tissue cells from the dental papilla, \( B \), could have proliferated and migrated out of the inferior lateral corners and surrounded the tooth, as originally proposed by Mummery\(^{12} \) and Hopewell-Smith.\(^{13} \) If this were true, then these cells of the papilla would be expressing a potential greater than their normal prospective significance of forming odontoblasts and miscellaneous pulp cells. The possibility that the adhering cells, \( C \), could proliferate and be converted into the bony shell is granted. It is also possible, however, that the cells of the subcutaneous connective tissue environment which were not intended to form periodontal ligament and alveolar bone were converted, under the stimulus of the developing tooth, into cells which formed these tissues. This hypothesis implies the conversion or modulation of cells into osteoblasts.

The literature of experiments related to heteroplastic bone formation indicates that a number of different connective tissue cell types have been shown to be able to be modulated to osteoblasts to form bone. The controversy still exists as to whether the fibroblast can be converted into an osteoblast; the opponents maintain that an undifferentiated mesenchymal cell is a more likely candidate for conversion to an osteoblast than is the more differentiated fibroblast. Under any circumstances, there were ample cells which, although outside normal skeletal areas, became capable of forming bone.

**Stimulation for bone induction.**—In placing the location of the osteogenic stimulus capable of influencing the conversion of connective tissue cells to osteoblasts, we may consider that, since bone is formed around the roots of developing teeth in situ, the stimulus may originate in either the developing tooth (intrinsic) or from outside the developing tooth (extrinsic). This assumes only one stimulus originating from one location. The transplanted developing tooth formed periodontal ligament and alveolar bone in a connective tissue site different from the normal one—one which does not ordinarily form bone. Therefore, the stimulus must be located in the transplanted tissues. From which transplanted tissue does the stimulus come, the enamel organ or the dental papilla?

The enamel organ is the epithelial portion of the developing tooth. Component parts of the enamel organ are the outer enamel epithelium, the stellate reticulum, stratum, intermedium, and inner enamel epithelium. After formation of the crown of the tooth, the inner and outer enamel epithelium are believed to join in the formation of Hertwig’s sheath, which guides and serves as a mold for root formation.\(^{14} \), \(^{15} \) As a developing unit, the enamel organ has been reported to exert a dominating influence over the condensation of adjacent connective tissues which form the dental papilla and dental sac.\(^{15} \) The inner enamel epithelium differentiates into tall columnar cells—the ameloblasts—which have been shown to influence the adjacent connective tissue cells of the dental papilla to differentiate into odontoblasts.\(^{14} \) Moreover, after enamel formation is complete, the columnar ameloblasts join the stratum intermedium and outer enamel epithelium to form a flattened squamous epithelial layer. This reduced enamel epithelium is reported to have the capacity to induce atrophy of the connective tissues separating it from the oral epithelium.\(^{10} \) Since in four instances the influence and control of the epithelium of the enamel organ upon surrounding connective tissues seems certain, it may be reasonable to suggest that the epithelial derivative—Hertwig’s sheath—is also able to influence the surrounding connective tissues to form perio-
dental ligament and alveolar bone. The fact that bone forms normally only when roots are formed and only in relation to these roots seems also to point to the root sheath as the origin of the stimulus.

Lefkowitz, Bodecker, and Shipiro\textsuperscript{16} experimentally removed the dental papilla, including odontoblasts, from developing mandibular cat canines in situ. They found that the various mesodermal tissues which filled the space created by the removal of the papilla assumed an appearance closely resembling a normal periodontium. Structures similar to cementum, periodontal ligament, and alveolar bone were observed in the pulp area. Therefore, if the papilla can be removed from developing teeth in situ and if periodontal tissues are formed both in replacing connective tissues and around the root, the stimulus for periodontal tissue formation must be the enamel organ and its derivative—Hertwig’s sheath.

The completely intact tooth germ is not ordinarily subjected to pulpal changes resulting in structures characteristic of the periodontium. However, Santone\textsuperscript{17} punctured developing guinea-pig tooth germs with a sterile needle and observed the responses of the tooth in healing. If the germ was punctured before dentin had been formed (bell stage), fibrous connective tissues typical of periodontal ligament filled the hole caused by the injury. In our experiment, two transplanted newborn molars which survived but did not develop roots had a demonstrable break in the inner enamel epithelium. In these specimens the dental papilla became more fibrous, with islands of bone both free and attached to the dentin surfaces. The free islands of bone are parallel to the predentin, and tissue resembling periodontal ligament is seen between the bone and the predentin. In the Lefkowitz case, total replacement of the papilla was made by tissues which resembled periodontium. In Santone’s case, partial revision of the pulp to tissue resembling periodontium was effected by a small penetrating injury, while, in the case of the transplanted molars, total pulpal revision appeared due to a tear in the inner enamel epithelium. It seems possible that the various invasions of the papilla permitted the entrance of the postulated stimulus into the pulpal areas, which were then revised to tissues resembling periodontium under the influence of the stimulus.

The proposal that a proliferating epithelium may stimulate bone formation is not new. Efforts to recognize and identify a substance capable of converting undifferentiated mesenchymal cells and/or fibroblasts to osteoblasts in extra-skeletal areas has aroused great interest since Huggins\textsuperscript{18} demonstrated the ability of transplanted urinary bladder epithelium of dogs to stimulate adjacent connective tissues to form bone. Several investigators have subsequently confirmed Huggins’ original finding. This is clearly bone induction. However, the nature of the stimulus and the mechanism through which it operates are unknown.

No author has denied the existence of a substance capable of inducing bone formation. However, to date, no author has made positive identification of an active ingredient in an acid alcohol of any other extract which is osteogenic when injected.

It is possible that the enamel organ and Hertwig’s sheath of the developing tooth produce such a substance. In this respect, it seems that the developing tooth falls into Urist and McLean’s\textsuperscript{19} group of tissues which form new bone by stimulating host connective cells to be converted into osteoblasts. However, additional evidence should be obtained to establish conclusively that no transplanted cells were precursors to perio-
dental tissues formed. Without doubt, a certain few cells adhered to the outside of the outer enamel epithelium and were transplanted. It seems improbable that these few cells could have been responsible for the extensive formation of periodontium routinely seen around transplanted teeth after 28 days in the host subcutaneous tissues. More likely, the cells forming the periodontium were host cells, which, under the influence of the enamel organ and Hertwig’s sheath, were converted to cells capable of forming the various elements of the periodontium.

Stimulation of function.—When comparing periodontal ligament formed by transplants and by molars developing in situ, one notes the striking similarity in the appearance of the fibrous elements and also that similar cells—fibroblasts, cementoblasts, osteoblasts, and osteoclasts—are seen in both. Similar structures have similar positions. Blood vessels have the same relationship to bone of the alveolus and to the periodontal ligament. The arrangement of fibers in the periodontal ligament of the transplanted molars is of particular interest. Gingival fibers, alveolar crest, and horizontal, oblique, and apical fibers are found in identical root areas as those in the control molar which developed its periodontium in situ. This is also true of periodontal ligament developed from teeth transplanted both prenatally and postnatally.

However, some differences also exist between periodontal ligament developed in the absence of function, the transplants, and the controls which were exposed to functional influences during development. Fewer blood vessels were found related to the periodontal ligament of transplanted molars. Fiber-bundle definition is less clear in periodontal ligaments which have never been functional. Moreover, there is less grouping of fibers into bundles and consequently indefinite interstitial spaces (Fig. 7). Although comparative central sections have not been subjected to precise measurement, it is obvious that the periodontal ligament of transplanted molars is narrower than in the molar which develops with function in situ. Some transplanted molar periodontal ligaments show only a few fiber bundles between the middle of the root and the apex. A few long fibers are seen running parallel to the root surface in these areas.

These similarities and differences between functionless and functioning periodontal ligaments seem best understood on a biological basis. Wilhelm Roux, as quoted by Russell in his book Form and Function,²⁰ first established the concept that there were two periods of development. The first period was under the control of genetic factors inherent in the germ plasm, and the second—the further differentiation and maintenance of form—was controlled by functional activity. This concept appears directly applicable to this study, since transplantation ostensibly separated the development related to genetic factors from that related to function, by providing for development in the absence of function. Within the first period, Roux includes all growth, development, and maintenance of an organ that take place without active function. Then, with function, there is an additional formative activity which acts partly by producing new form and partly by maintaining that which is already formed. This he calls the “finer functional differentiation of the organ.”

Subsequent examples of the effect of function upon structure, which have placed more emphasis on the genetic factors operative during the first phase of development, have been shown. The structure of muscle, for example, has been shown to be completely developed without function. Hamburger, quoted by Weiss,²¹ prevented the innervation of developing limbs and found normal muscular development despite the
elimination of all function. However, he also found that, unless the muscle was innervated and functioned before a certain critical stage, some of the earlier differentiations were eliminated by regressions in structure. It was apparent that muscle could develop without innervation and function but, once developed, required them if it were to be maintained.

By culturing explanted fowl limb buds in vitro, Fell and Canti\textsuperscript{22} showed that bones and joints could also form typically without movement. However, fusion of the bones took place later because of the lack of movement. Again normal structural features were not maintained without function.

It is also known that the internal trabecular pattern of the long bones of the embryo is structurally preadapted for function before any body weight has been placed upon them. In this case, however, Weiss\textsuperscript{21} states that function itself can mold such patterns directly, particularly after injury, during later stages of life.

It seems probable that fiber orientation of the periodontal ligament is similarly developmentally preadapted and does not require function in the formation and orientation of its fibers. This assumption is substantiated by the fact that transplanted molars developed typically oriented principal fibers without the influence of function. One might argue that a certain amount of movement of the transplant beneath the host skin might serve as adequate function. While this possibility exists—and if it were true—the function required to affect the orientation of fibers would be far less than masticatory forces. Moreover, transplants were grossly oriented in all directions within the host, and yet the fiber orientation was similar in all.

How, then, are the differences in the non-functional and functioning periodontal ligament to be accounted for? Preissecker\textsuperscript{23} removed the upper or lower molars on one side in a group of rats, to determine the effect of lack of function upon the periodontal ligament of the unopposed remaining molars. When these were later compared with molars on the opposite side of the mouth where function remained, he found their periodontal ligament width to be approximately one-third less than in those molars which had been in occlusion. Function, then, was responsible for a thickening of the periodontal ligament.

Gottlieb\textsuperscript{24} called the minimum width of the periodontal ligament between the root and the alveolar bone, as represented by the unerupted and imbedded tooth, the "biological periodontal width." He referred to the larger functional width as the "physiological periodontal width." It seems that the biological periodontal width is represented by the periodontal ligament formed around transplanted molars. In order to characterize this ligament further, the term "biological prefunctional" is suggested—"biological" to indicate the minimum width and "prefunctional" to indicate the predadaptation of structure to its future functional requirements.

Teeth which are subjected to normal function have strong, well-developed fiber bundles (Boyle).\textsuperscript{25} Non-functioning teeth lack this definite fiber-bundle formation. It seems probable, therefore, that the lack of prominent fiber bundles in the periodontal ligaments of the transplanted teeth represents the lack of functional influence in modifying the structure of the ligament. Boyle also states that the periodontal ligaments of unopposed and imbedded teeth are sometimes replaced by fat tissue. Apparently, function is as important in the maintenance of the periodontal ligament as it was in the preservation of muscle, joints, and bone.
In summary, the periodontal ligament development is believed to be essentially prefunctional and preadapted in its structure and principal fiber orientation. Function modifies the biological prefunctional ligament by increasing its width and grouping its fibers into more definite bundles. These modifications are believed to be functional adaptations important in the maintenance of the tissue. This concept seems to fit well with Roux's original and Weiss's later suggestion that the development of most organs passes through a prefunctional formative phase and that subsequent functional activity may modify these structures slightly in the conservation or maintenance phase. Each phase, they state, is under the prevailing control of a different set of factors, the first phase controlled by genetic considerations and the second by functional factors.

**SUMMARY AND CONCLUSIONS**

1. Developing hamster molars prior to periodontal tissue formation were transplanted subcutaneously into 30-day hamsters to determine their capacity to develop periodontal ligament and alveolar bone in a connective tissue environment other than the jaw. Tissues transferred included the enamel organ, dental papilla, and a certain number of cells which cling to the external surface of the external enamel epithelium; no bone was transplanted.

2. If the transplant survived and was vascularized by the host, it formed typical periodontal tissues and a shell of alveolar bone as integral parts of the complete dental organ.

3. The development of the dental organ, the tooth, its periodontal ligament, and a shell of alveolar bone appears to represent a morphogenetic organ field. Therefore, biological principals such as "wholeness," organizer activity, and the competence of tissue, should be applicable to the developing tooth and its periodontium.

4. The original jaw bony crypt surrounding the tooth germ was not transplanted. Therefore, the source of cells which formed bone was provided either by one of the transplanted tissues, the enamel organ, dental papilla, or adhering cells, or by the tissues of the new connective tissue environment.

   a) The enamel is presumed not to be the source of cells forming the connective tissue periodontium, since it is entirely epithelial.

   b) If the cells forming the periodontium came from the dental papilla, then proliferation and migration occurred, and these cells realized a potential greater than their normal prospective significance.

   c) Mesenchymal cells adhering to the outer enamel epithelium may have proliferated.

   d) If the new environment supplied the cells which formed the periodontium, then cells not ordinarily forming bone were modulated or differentiated into osteoblasts.

5. The source of the stimulus for the formation of periodontal tissues obviously resides in the transplanted tissues. It is proposed that, of these tissues, the enamel organ and its derivative—Hertwig's epithelial root sheath—are the most likely source of the stimulus.

6. A typically functional orientation of periodontal fibers is found in recovered transplanted teeth which have not functioned as in mastication. Basic fiber orientation appears to be developmentally prefunctional and preadapted, while the grouping of fibers into bundles and the increase in fiber detail seem to represent modifications of structure to function associated with the maintenance of the periodontal ligament.
REFERENCES

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