Tooth Transplantation in the Mouse

I. THE USE OF PROCION DYES AND TRITIATED PROLINE IN A STUDY OF SYNGENEIC TOOTH GERM TRANSPLANTATION [1]

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ABSTRACT Tooth germs of first molars from four- to six-day old mice were transplanted heterotopically into the connective tissue under the dorsal skin of adult syngeneic recipients. The fate of the transplants was studied by histologic staining, vital staining with procion dyes, and autoradiography. Together these methods provided the following picture of the development of the transplant. Extraction of the tooth germ severs its vascular connections and disturbs the nutritional environment of the tooth so that many cells undergo necrosis and degeneration. The resulting inflammatory response clears the transplant of necrotic tissue, leaving a fibrous pulp of low cellularity and an extensively disrupted odontoblastic layer; the enamel organ is reduced to only a few epithelial cells. In the second week following transplantation, however, the surviving cells start to proliferate and the tissues of the transplant begin to reorganize. Reorganization starts at the apical foramen and spreads to the rest of the tooth. The central pulp is penetrated by new blood vessels, its cellular content is reestablished, and its morphology returns to normal. Odontoblasts start producing dentin which at first is highly irregular and contains many cells (osteodentin). Later the dentin formation becomes more and more regular and the dentin assumes its normal tubular structure. New enamel is not formed at any time after transplantation. The cells of the enamel organ undergo squamous metaplasia and form epithelial cysts. Abortive root formation is observed but typical roots never develop. Rudiments of periodontal ligament develop rarely. The transplants are enclosed in a capsule of connective tissue.

It is concluded that the heterotopically transplanted tooth germs maintain their capability to recover, develop, and differentiate in syngeneic recipients. Although almost all of the developmental processes continue in the transplant after a period of adjustment, some of them are abortive.

The literature on tooth transplantation is enormous. A bibliography compiled in 1962 (Iványi, '62) lists over 300 publications, and since then some 300 more have appeared (Natiella et al., '70). Widely contrasting views on the antigenicity of teeth have been expressed. It has been reported that tooth xenografts survive (Flemming, '55; Cserepfalvi and Price, '68), which implies that the tooth tissues are non-antigenic. Other investigators, however, have concluded that even syngeneic grafts do not survive (Lefkowitz, '61) and that transplantation of teeth is not possible technically. Between these two extremes lie various concepts, for example, host-tissue acceptance of both syngeneic and allogeneic grafts (Haley and Costich, '69), acceptance of syngeneic grafts and rejection of allografts (Iványi and Vacek, '64), rejection of some allografts and acceptance of others according to the strength of the histocompatibility barrier (Iványi, '68;

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Weinreb et al., '68), and induction of transplantation immunity by allogeneic hard tissue (Shulman, '64; Mincer and Jennings, '70) and soft tissue of the tooth Zaleski et al., '67).

The confusion in the field of transplantation immunology stems from two main factors: first, the use of laboratory animals with nondescript genetic backgrounds, and second, the use of different criteria for evaluating acceptance or rejection of the graft. In most studies the experimental subjects were "monkeys," randomly bred dogs, rabbits, guinea pigs, or rats. Only a few investigators have used inbred strains, and to our knowledge no one has used congenic resistant lines. As for evaluation methods, many of these have involved only gross observation of the tooth graft; others have included histologic techniques, but the histologic criteria for survival or destruction of the tooth graft vary from one report to another.

The experiments described below were undertaken to reexamine the role of genetic and nongenetic factors in tooth transplantation. The subjects were mice with known genetic background, and the methods involved standard histologic techniques augmented by the use of procion dyes and tritiated proline as markers for the study of dentinogenesis in transplanted teeth. The first set of experiments focused on the morphologic characteristics of syngeneic heterotopic transplants.

MATERIALS AND METHODS

Mice. Mice used in this work were of the highly inbred strain C57BL/10ScSn (abbreviated to B10) and its congenic resistant lines B10.BY, B10.A, B10.LPa, and B10.129(21M). Breeding pairs were supplied by Dr. George D. Snell, The Jackson Laboratory, Bar Harbor, Maine, and were maintained in our colony by brother-x-sister matings. Skin grafts exchanged between individuals of the same line survived permanently.

Transplantation of tooth germs. Maxillary or mandibular first molar germs of four- to six-day old donors were carefully dissected out with 25-gauge syringe needles under a dissecting microscope and transplanted into the dorsal connective tissue of the recipient. The site for reception of the transplant was prepared by making an incision in the skin just behind the ears and opening a subcutaneous pouch by blunt dissection with iridectomy scissors. The tooth germ was inserted as far into the pouch as possible and the incision closed. The operation was performed under intraperitoneal Nembutal anesthesia (0.5 mg pentobarbital sodium per 10 gm body weight). The recipients were two- to three-month old mice. To avoid possible sex-linked histoincompatibility, only males were used.

Histologic examination. At termination of the experiment, the transplanted teeth, together with the surrounding tissue, were dissected out and fixed in 4% paraformaldehyde dissolved in 0.2 M phosphate buffer, pH 7.4. After fixation for 24 to 36 hours, the specimens were decalcified with chelating agents in dilute HCl[3] and embedded in paraffin. Serial sections of 10 μ thickness were cut and stained with hematoxylin and eosin (H and E).

Grossly the transplant usually appeared as a small bulge at the site of the operation, at least in the first postoperative days. Later, when the transplant was being surrounded by connective tissue, the bulge usually disappeared but the tooth was still palpable. About 1% of the transplants could not be found; they were probably lost either by migration or by technical failure. In some instances the grafts were lost because of infection.

Vital staining with procion dyes. The following dyes were tested for *in vivo* toxicity and for efficacy observed in marking hard tissues of normal teeth: red M-8BS, scarlet M-GS, orange M-GS, olive green M-3GS, grey M-3GS, purple H-3RS, and blue H-5GS.[4] The dyes were dissolved in distilled water and injected intraperitoneally. Toxicity was evaluated by injecting groups of five mice with the dye solutions in dosages ranging from 2 to 5 mg/10 gm body weight (b.w.) and recording the deaths each day for one week (table 1). Marked difference in tolerance to different dyes was observed: procion orange M-GS and scarlet M-GS were tolerated in doses up to 5 mg/10 gm b.w.; whereas procion purple H-3RS had some lethal effect even

[3] The Rupp and Bowman Co., Highland Park, Michigan.

[4] Colab Laboratories, Inc., Glenwood, Illinois.

TABLE 1

In vivo toxicity of different procion dyes

Procion dye	Dose mg/10 gm b.w.	No. of mice injected	No. of mice dead at days after injection				
			1	2	3	4	5
Red M-8BS	5	5	2	2	1	—	—
	4	5	1	2	0	0	0
	3	5	1	2	1	1	—
	2	5	0	0	0	0	0
Scarlet M-GS	5	5	0	0	0	0	0
	4	5	0	0	0	0	0
	3	5	0	0	0	0	0
	2	5	0	0	0	0	0
Purple H-3RS	5	5	3	2	—	—	—
	4	5	5	—	—	—	—
	3	5	1	3	1	—	—
	2	5	0	2	0	0	0
Orange M-GS	5	5	0	0	0	0	0
	4	5	1	0	0	0	0
	3	5	0	0	0	0	0
	2	5	0	0	0	0	0
Olive green M-3GS	5	5	3	0	1	0	0
	4	4	1	0	0	0	0
	3	5	0	0	0	0	0
	2	5	1	0	0	0	0
Grey M-3GS	5	5	2	0	0	0	0
	4	5	1	1	0	0	0
	3	5	0	0	0	0	0
	2	5	0	0	0	0	0
Blue H-5GS	5	5	1	1	1	1	1
	4	5	1	0	0	0	0
	3	4	0	0	0	0	0
	2	4	0	0	0	0	0

Mice were injected intraperitoneally with water solutions of the dyes. Dose range: 2 to 5 mg per 10 gm of body weight.

in the low dosage of 2 mg/10 gm b.w. The efficacy of the procion dyes was evaluated by their capacity to reveal distinct incremental zones of growth in dentin of decalcified sections of normal teeth cut at 7 to 10 μ. Groups of three to five mice one to three weeks old were injected with the individual dyes at the highest nontoxic dosage as determined in the previous experiment. One week after the injection, the mice were sacrificed and their teeth extracted, fixed, decalcified, and sectioned. Half of the sections were stained with H and E and the other half remained unstained. The unstained sections were examined by means of a standard microscope with direct or oblique illumination, and by a fluorescence microscope (GFL) equipped with an Osram HBO 200W super-pressure mercury lamp, a BG12 exciter filter, and Zeiss barrier filter inserts (47 and 50). The seven tested dyes fell into three groups according to their marking suitability. In the first group, procion red M-8BS and scarlet M-GS were considered the most suitable of those tested. Zones of dentin stained with these two dyes were visible under the standard microscope but only as pale lines with poor contrast against the background. For this reason standard microscopy was abandoned. With ultraviolet light, the two dyes yielded bright fluorescence of excellent visibility (fig. 9). Although the fluorescence had the same orange tint, the procion red M-8BS was slightly more intense and for this reason it was preferred over scarlet M-GS for subsequent experiments. In the second group,

zones stained with procion grey M-3GS and orange M-GS were visible only under the fluorescence microscope. The intensity of the fluorescence was relatively low in both cases, and the dyes were judged not suitable for transplantation experiments. In the third group, zones stained with procion blue H-5GS and purple H-3RS were not visible under the conditions described.

Mice carrying syngeneic tooth transplants were injected with procion red M-8BS at different intervals after transplantation, killed one week after the injection, and tooth sections examined with the fluorescence microscope.

Autoradiography. Tritiated proline was used as a marker of dentinogenesis in both normal and transplanted teeth. In the studies of normal dentinogenesis, groups of five-day old mice were injected subcutaneously with ^{3}H-proline,[5] 50 μCi per mouse. The mice were killed 7 and 14 days later, their first molars extracted and sectioned. The sections were covered with photographic emulsion NTB_2[6] exposed for ten days, and then developed, fixed, and stained with H and E. In the transplant studies, another group of five-day old mice were injected with ^{3}H-proline, killed 24 hours later, and the first molars extracted and transplanted into adult mice of the same strain. Thirty days after transplantation, the recipients were killed and the transplants processed histologically and autoradiographically.

OBSERVATIONS

Normal development of the first molars. As a control for the transplants, sections were made of normal first molars (mandibular and maxillary) at different ages from 0 to 24 days after birth. In another experiment, procion red M-8BS was used as a marker of dentin formation. In this experiment 10 mice were injected with the dye once a week for four weeks starting at five days after birth. The mice were killed one week after the last injection and the sections of their molars examined with the fluorescence microscope (fig. 9). The observed major events in the postnatal development of the first molar (fig. 1) were basically in agreement with what has been reported on this subject (Gaunt, '56; Cohn, '57; Hay, '61) and, therefore, will not be discussed here.

Sections also were made from extracted five-day old tooth germs to determine morphologic features before transplantation. A typical extracted tooth germ (fig. 2) consisted of enamel matrix, dentin, and undisturbed pulp with a continuous layer of odontoblasts. Of the enamel organ, usually only the inner dental epithelium (ameloblasts), stratum intermedium, and small areas of stellate reticulum were preserved. The continuity of these tissues was disturbed in some grafts. Only a small portion of the dental sac was present. Some grafts also included small pieces of alveolar bone.

Development of syngeneic transplants as revealed by histological stains. Tooth germs from four- to six-day old mice were transplanted under the dorsal skin of adult syngeneic recipients, i.e., B10→B10, B10.BY→B10.BY, B10.A→B10.A, B10.LPa →B10.LPa and B10.129(21M)→B10.129 (21M). Since no difference in the behavior of transplants in the different strains was observed, the results were pooled. Altogether, 95 successful transplantations were performed. The transplanted teeth were excised from the recipients starting at day 3 after transplantation, then every odd day up to 29 days, and then every ten days up to 100 days. At least three or four grafts at each interval were available for sectioning. As a means of gauging developments in the transplants, the histologic changes were noted on a week-to-week basis.

First postoperative week. As a consequence of the surgical trauma, drastic changes occurred in the transplanted tooth. In the first two days the vascular system of the pulp underwent degeneration accompanied by hemorrhage and thromboses. Concurrently with the loss of vascularity, the typical triangular or spindle contour of the cells of the central pulp changed to round or oval. The odontoblastic layer became disorganized and lost its continuity. The odontoblasts were cuboidal, their nuclei pycnotic and the cytoplasm vacuolar. Dentinogenesis and amelogenesis ceased, and the predentin formed before trans-

[5] New England Nuclear, Boston, Massachusetts.
[6] Eastman Kodak Co., Rochester, New York.

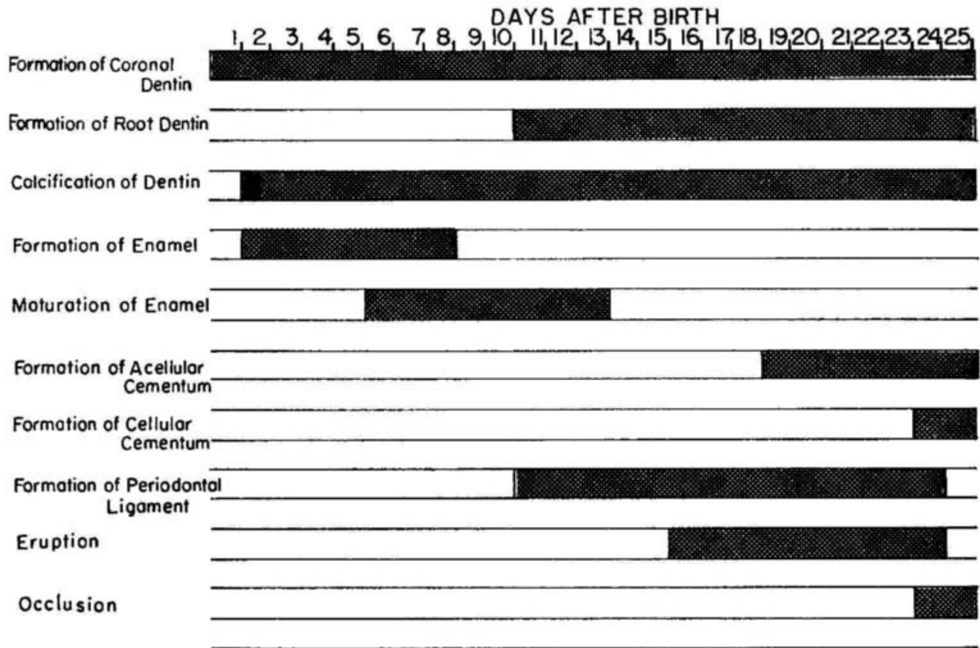

Fig. 1 Time table of events in the postnatal development of the mouse first molar (mandibular). (The dotted bars indicate activity, the empty bars indicate absence of activity.)

plantation did not calcify. The enamel organ (if present) and Hertwig's root sheath also showed signs of degeneration and necrosis. The degenerative changes in the tooth transplant were most prominent at about four to five days after the operation. At the same time, the transplant became a target of an inflammatory response. At first, round cells infiltrated the connective tissue surrounding the transplant and then invaded the pulp through the apical foramen. The necrotic cells of the central pulp were destroyed, leaving behind a network of pale ground substance and connective fibers (fig. 3). The few remaining cells were round, granular, and poorly stained. The integrity of the central pulp was lost. In some tooth germs the odontoblastic layer was limited to small strands or scattered atypical cells. In others, long segments of the layer were preserved. At the end of the week, however, signs of new organization appeared. The round-cell infiltration in the pulp subsided and revascularization developed; cells in the apex started to synthesize and deposit extracellular material.

Second week. The pulp was again cellular (fig. 4). At first, the pulp tissue was composed of a pleomorphic mixture of irregularly dispersed round, oval, triangular, and spindle-shaped cells, and a few histiocytes. Toward the end of the second week, however, the composition shifted toward normal, with a predominance of triangular and spindle-shaped cells. Deposition of dentin matrix was concentrated in the apex, almost occluding the lower part of the pulp chamber. At the beginning of the second week the pattern of matrix deposition and calcification was uneven; the matrix showed no tubules but contained many irregularly dispersed or clustered cells in small lacunae. It will be referred to as osteodentin. Toward the end of the second week, the matrix exhibited more regular deposition and the incorporation of fewer cells. Tubules appeared at this time, gradually increasing in number and becoming oriented parallel to each other.

The odontoblastic layer in the apex also underwent progressive changes. During the week the layer became more organized; the cells changed from round and irregular to

cuboidal and later columnar, and arranged themselves side by side into a continuous stratum. The nuclei of the columnar cells moved toward the eccentric position typical for normal odontoblasts. The odontoblasts of the crown, however, still produced little or no dentin, and in large segments of the pulp chamber the odontoblasts were still missing. The tooth appeared deformed by the pressure of the overlying skin.

Third week. By the third week the pulp was similar to that of a normal tooth. The odontoblastic layer was continuous in some transplants and interrupted in others. Dentin was deposited not only in the apex but also in other areas of the pulp chamber where the odontoblasts had reorganized. Masses of dentin at the apex in some cases reduced the apical foramen to a thin canal. In some transplants, granulation tissue surrounded the crown (fig. 5) and the whole tooth was enclosed in a connective tissue capsule. The most conspicuous event at this stage was the appearance of areas of stratified squamous epithelium (fig. 7), which spread rapidly until the whole crown was covered in some instances. This epithelium probably originated from the enamel organ. Most of the cells of the inner dental epithelium and the stratum intermedium had undergone necrosis in the first postoperative week and were removed by the inflammatory response; cells that survived became hyperplastic. In some transplants this epithelial layer underwent degeneration, and vesicles developed that had all the characteristics of dentigerous cysts (fig. 7). Young cysts were composed mostly of epithelial cells; in older cysts, fibrous connective tissue surrounded the inner epithelial layer. The inner layer was continuously shed off into the growing cavity of the cysts, which in addition to these squamous cells also contained polymorphonuclear leucocytes in some instances.

From the fourth week to the end of the experiment. Dentinogenesis continued, filling the chamber so that the pulp was reduced to small islands (fig. 8). Typical roots never formed, although abortive root formation was noted in some transplants. Rudimentary root sheath and epithelial diaphragm were often present in the early stages, and the massive formation of dentin beyond the dentino-enamel junction was occasionally observed. In some instances, definite layers of acellular and cellular cementum were deposited (fig. 6). The connective tissue around the apex was quite often organized into a structure which resembled the hammock ligament, and in some cases a rudimentary periodontal ligament also developed (fig. 6). Some transplants were surrounded by alveolar bone which had developed from pieces inadvertently included in the transplanted tooth germ. The osseous cavities were filled with bone marrow. In rare instances, bone formation had occurred in the pulp chamber.

The dentigerous cysts enlarged and sometimes encapsulated the crown of the tooth, and where secondary infection had occurred, the cyst cavity was filled with inflammatory cells.

Procion dyes as markers for study of dentinogenesis in tooth transplants. The procion dye stained hard tissues (dentin, enamel matrix, bone) selectively, but the cellular components of the transplant were also visible because of their autofluorescence. Moreover, the tissues were distinguished by variations in color, the odontoblasts appearing bright green, central pulp cells brown, and the external connective-tissue capsule yellow. This distinction greatly facilitated evaluation of the grafts. The entire enamel matrix, when present, was stained uniformly without any sign of banding. Its bright orange fluorescence of the early stages later faded. During the later post-graft periods the enamel matrix became more porous. The absence of banding in the enamel matrix was taken as evidence that no new enamel was formed after transplantation. The pretransplant enamel matrix was not lost by decalcification, indicating that it did not mature.

The staining pattern of the osteodentin was irregular and diffuse (fig. 10). The lacunae showed a green lining surrounded by orange fluorescence; apparently the cells entrapped in the osteodentin were producing extracellular material. In the second week after transplantation an orange line was observed parallel to the pulp surface of the dentin in the apex (fig. 10, L), and separated from the odonto-

blasts by a green layer of dentin (fig. 10, D). At first, the line was present only in the apex, but later it extended toward the coronal part of the graft (fig. 11, L) until it formed a continuous ring. Three weeks after transplantation the line was "embedded" in the dentin at the apex, while in the crown it lined the inner surface of the pulp chamber. Five weeks after transplantation the line was "embedded" throughout the entire layer of dentin. At the end of the experiment the line was again present on the inner surface of the pulp chamber. The presence of the line was taken as evidence of dentinogenesis at a given time and position in the transplant. During the fourth postoperative week, a second line appeared in some transplants on the host side of the apex. This line extended only to the dentino-enamel junction, and was evidently associated with cementogenesis in the rudimentary roots.

Tritiated proline as a marker for study of dentinogenesis. In both the normal and transplanted teeth, most of the isotope was concentrated in the dentin (fig. 12); enamel matrix, at least after decalcification, and cellular components of the tooth showed little or no radioactivity. Some radioactivity was noted in the tissues surrounding the transplant, probably as a result of misplacement of the tracer. In the dentin, the isotope formed a granular band which was separated from the pulp wherever a considerable amount of new dentin was formed after the injection and transplantation (fig. 13), and extended onto the pulpal surface wherever little dentin formed.

DISCUSSION

Fate of syngeneic tooth transplants. The results of our studies indicate that syngeneic, heterotopically transplanted tooth germs do resume development after an interval of adjustment. During this lag period the host tissues respond to the graft by an inflammatory reaction resembling the cellular response to an allograft. The inflammatory process can easily be confused with the rejection phenomenon, and apparently this happened in some of the studies reported by other investigators. The fact that there is complete recovery from the degenerative changes in syngeneically transplanted tooth germs indicates that the inflammatory response is nonspecific and distinct from the allograft reaction.

We found few reports in the literature dealing with syngeneic tooth transplantation, apparently because few investigators have used inbred strains of laboratory animals. An exception is the large series reported by Iványi (Iványi and Vacek, '64; Iványi, '65), who transplanted tooth germs in newborn and adult rats of an inbred strain AVN and observed the grafts histologically up to 50 days after transplantation. She found that all the grafts were accepted and developed roots with cementum and a periodontal ligament. She also observed early degenerative changes in the upper part of the pulp and in the enamel organ. She did not mention the fate of the enamel and dentin and did not describe the development of the different tissues of the tooth transplant. With this reservation, our results are in good agreement with hers.

Another set of syngeneic tooth transplants was performed by Lefkowitz ('61), in an inbred strain of Sherman albino rats. The number of transplants was relatively small, and the period of study rather brief. Lefkowitz observed that despite the alleged isogenicity of the hosts and donors, the transplanted tooth germs were destroyed by a process which he termed a typical allograft reaction. Since the recipients were all males, sex-linked histoincompatibility should not have been responsible for the rejection. The most likely explanation of these results is that the rats were not inbred and the grafts were actually allogeneic.

Nonacceptance of syngeneic tooth transplants was also reported by Grewe and Felts ('68) and by Mincer and Jennings ('70). In both cases the grafts were mouse incisors, transplanted in the former case into mandibular incisor sockets of newborn animals, and in the latter case subcutaneously in adult mice. In both cases the transplants failed to grow and ultimately their soft tissues were completely destroyed. Whether this "rejection" was related to the nature of the grafts or to technical problems is not clear. A limited number of successful syngeneic grafts was

reported by Yoshioka and Gonzales ('59), Fleming ('52; '53; '55), Coburn and Henríques ('64) and by Haley and Costich ('69).

Unlike syngeneic transplantation, autotransplantation of teeth is well documented in the literature (Iványiová, '63; Natiella et al., '70). Autotransplantation is not strictly comparable to the type of syngeneic transplantation reported here because it is usually performed between adult animals. A detailed description of tooth autotransplants has been published recently by Weinreb et al. ('67). These authors transplanted maxillary molars from ten-day old rats into the dorsal subcutaneous connective tissue and followed the grafts up to six months after transplantation. Their criteria for continued growth of the transplants included the presence of the calciotraumatic line, postoperative dentin formation, and the presence of odontoblasts. Their results are similar to ours in that all transplants continued to grow after a period of adjustment during which temporary degenerative changes accompanied by an inflammatory reaction were observed. Even after six months the transplants were still in perfect condition.

In summary, the studies reported here and the few reported in the literature lead to the conclusion that syngeneic tooth germ transplants are generally accepted. Developmental disturbances of the transplant (incomplete root formation, absence of amelogenesis) can be explained as an effect of the transplantation into a heterotopical (non-physiologic) site.

Markers of dentinogenesis. Two markers of dentin-formation were used in the present study, procion dyes and H^3-proline. Procion dyes are monochloro-s-triazinyl (procion H) or dichloro-s-triazinyl (procion M) derivatives of cyanuric chloride (2,4,6-trichloro-s-triazine). The one unsubstituted chlorine atom (two atoms in procion M) can react with functional groups of proteins and form stable covalent bonds with the matrix of bones and teeth (Goland and Grand, '68). When injected into an animal (Seiton and Engel, '70), the dye is deposited within three to six hours at sites of growth in bones and teeth. The uptake of the dye continues for two to three days, until the concentration of the dye in the serum drops to a low level. When staining ceases, an unstained material is again deposited and thus a stained band is formed in the incremental zone of growth. Since the band becomes an integral part of the tissue, it persists even after decalcification. The width of the band depends primarily upon the rate of growth, providing that the rate of clearance of the dye from the serum of different animals is the same. Thus, both the width of the stained band and the width of the unstained adjacent material are good indicators of the growth of the tissue in the interval between the injection of the dye and killing of the animal. Of the seven dyes tested in this study only two were good markers of dentin-formation in normal teeth, i.e., procion red M-8BS and procion scarlet M-GS. Procion red H-8BS was used successfully by Sherman ('69) as a marker of cementogenesis in replantation studies. Procion red M-8BS and scarlet M-GS were used by Goland and Grant ('68) with good results for staining teeth and bones *in situ*. Good staining of dentin was also reported for procion blue H-5GS (Prescott et al., '68) and olive green M-3GS (Seiton and Engle, '70). Why these dyes gave such poor results in our tests is not clear.

The second marker, ^{3}H-proline, is incorporated into the collagen of the dentin and eukeratin of the enamel. According to Anderson ('67) the total tritium activity in the serum of an animal injected with ^{3}H-proline reaches its peak from four to six hours after intraperitoneal administration of the isotope, and then declines gradually until a plateau is reached at about 20 days. The isotope is picked up first by cells, beginning 15 minutes after injection, and then, after about four hours, is transferred into extracellular structures. There is essentially no change in the amount of isotope in the dentin at 60 days after injection, whereas the cellular content is decreased sharply at that time. The autoradiographs of tooth transplants labeled with ^{3}H-proline show concentration of the tracer in the dentin where it forms a granular band running parallel to the surface of the tooth. In the experiment described in this paper, the availability of the tracer was terminated by the grafting, and the

effects in the dentin did not last longer than 24 hours. Consequently, the tracer band was relatively narrow and clearly separated the portions of dentin formed before and after the operation. The enamel matrix, as well as the cellular tissues of the transplant, showed only low and rather diffuse radioactivity.

Thus, both procion dyes and tritiated proline proved to be excellent markers of dentinogenesis in transplanted teeth. We judged procion dyes as slightly better markers for the following reasons. They are less expensive, their use is much simpler and less time-consuming, and they are less demanding technically. Also, the band formed by procion dyes in dentin is sharper than the band formed by the radioactive tracer.

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PLATE 1

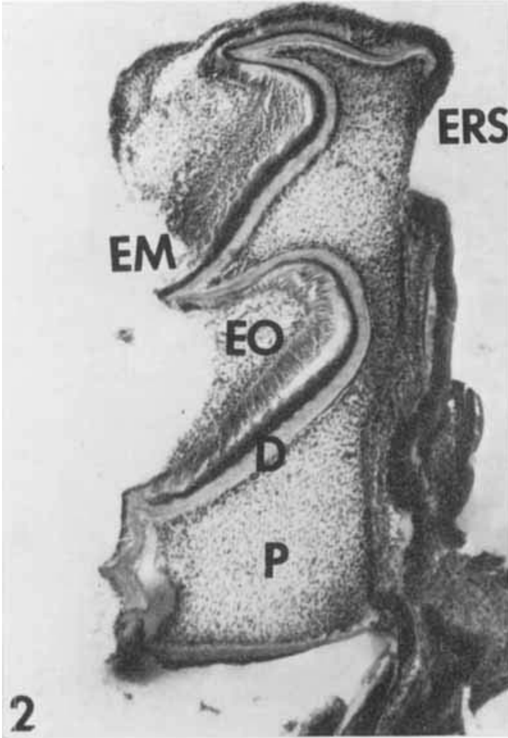
EXPLANATION OF FIGURES

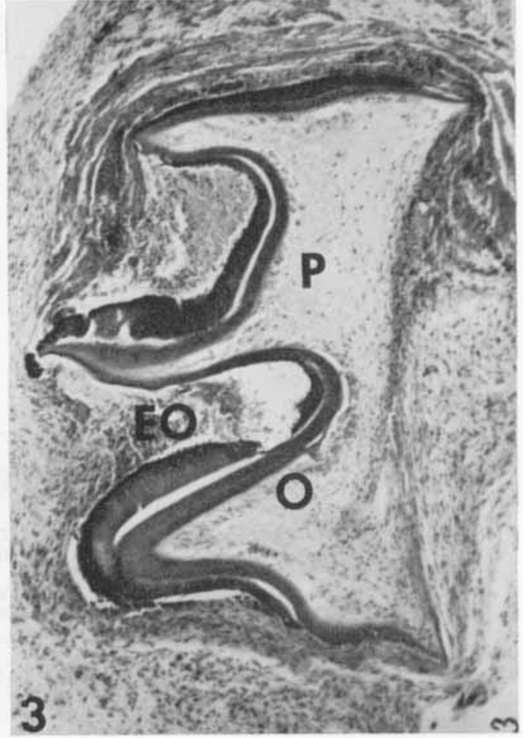
2 Mandibular first molar dissected from a five-day old mouse. P, pulp; D, dentin; EM, enamel matrix; EO, remnants of the enamel organ; ERS, epithelial root sheath. H and E. × 40.

3 First molar transplant five days postoperatively. Notice the reduced cellularity of the pulp (P), degeneration of the enamel organ (EO) and disruption of the odontoblastic layer (O). H and E. × 40.

4 Apical area of a first molar transplant 12 days postoperatively. Notice the cellularity of the pulp (P), organization of odontoblasts (O) and mass of osteodentin (Os). H and E. × 100.

5 First molar transplant 17 days postoperatively. Notice the formation of a connective tissue capsule (C) around the graft, and granulation tissue (G) around the crown. H and E. × 40.


ERS
EM
EO
D
P
2


P
EO
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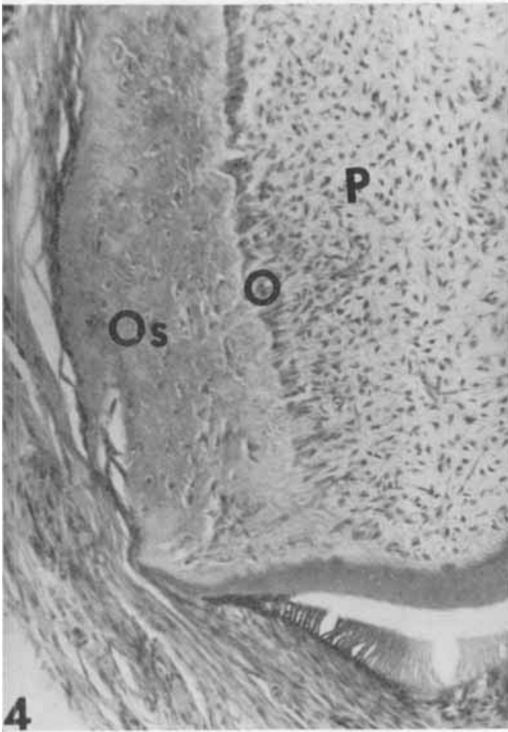
3


P
O
Os
4

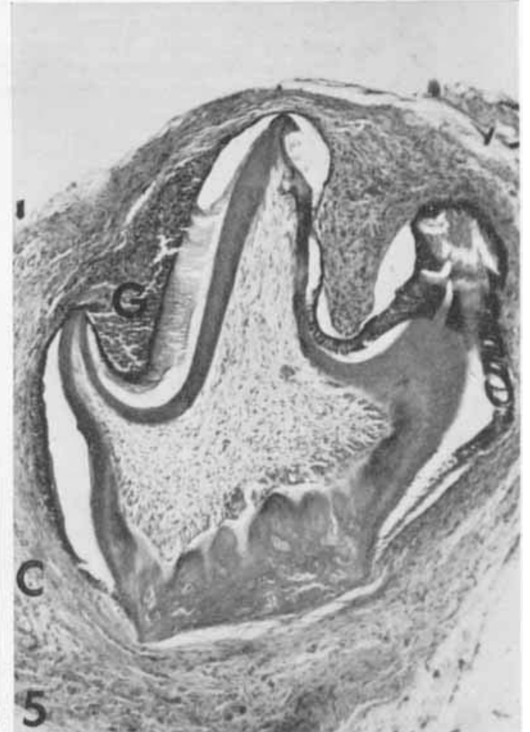

G
C
5

PLATE 2

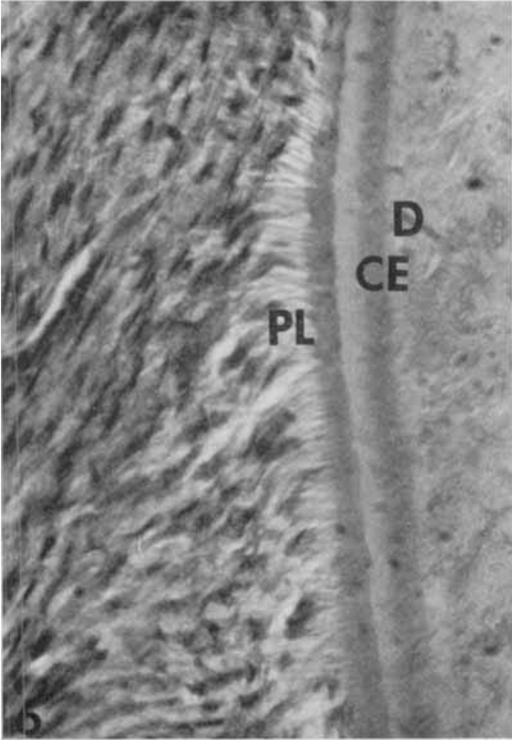
EXPLANATION OF FIGURES

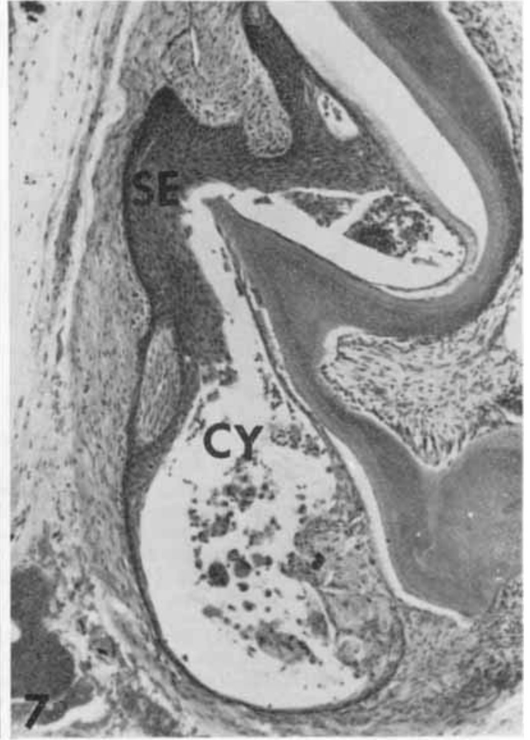
6 Root area of a first molar transplant 21 days postoperatively. PL, periodontal ligament; CE, cementum; D, dentin. H and E. × 200.

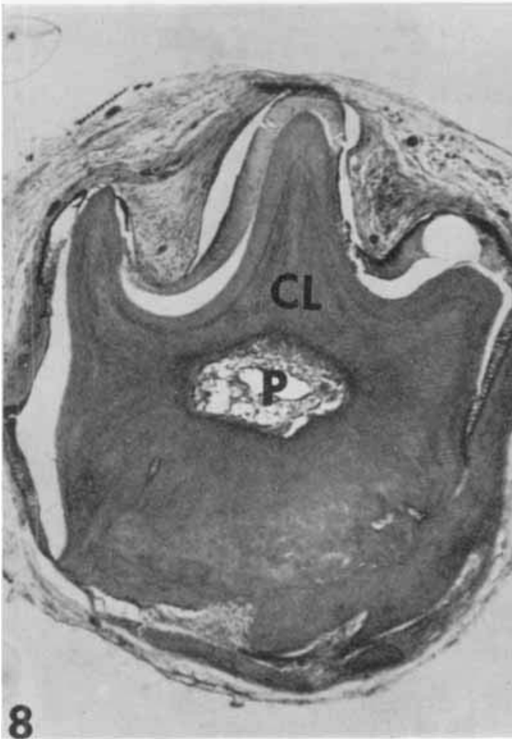
7 Coronal area of a first molar transplant 25 days postoperatively. SE, squamous epithelium; CY, epithelial cyst containing degenerated squamous cells. H and E. × 100.

8 First molar transplant 90 days postoperatively. Notice the reduction of the pulp chamber (P). A calciotraumatic line (CL) separates pre- and postoperatively formed dentin. H and E. × 40.

9 First maxillary molar with three incremental growth zones (1–3) in the dentin resulting from three weekly intraperitoneal injections of procion red M-8BS. The mouse was sacrified seven days after the last injection. Photomicrograph of unstained decalcified section was taken with the fluorescence microscope. P, pulp. × 100.


D
CE
PL
6


SE
CY
7


CL
P
8

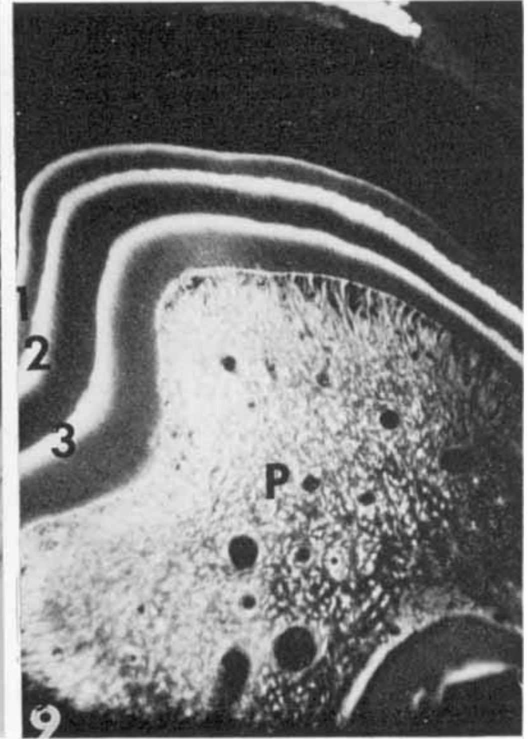

1
2
3
P
9

PLATE 3

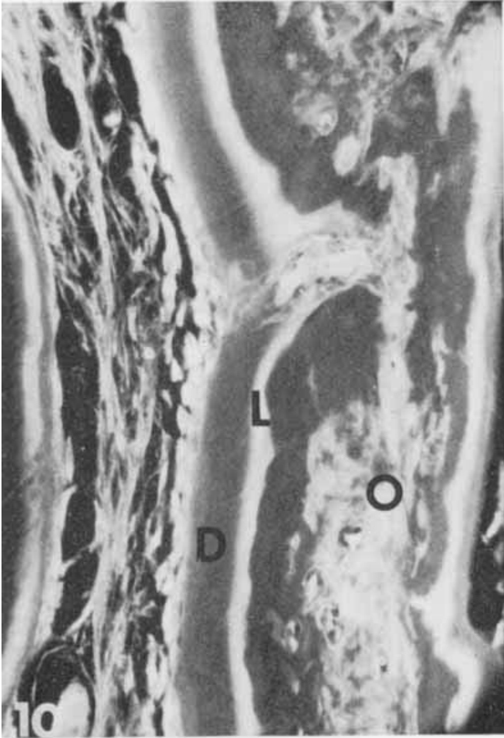
EXPLANATION OF FIGURES

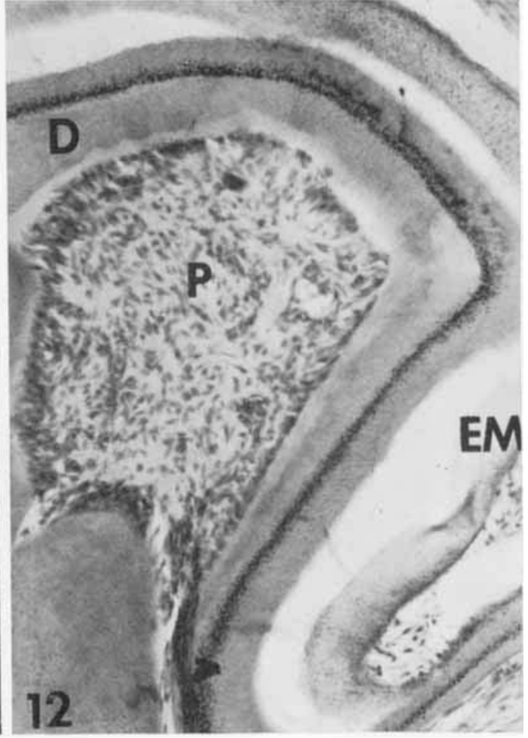
10 Apical area of a first molar transplant 14 days postoperatively. The recipient was injected with procion red M-8BS seven days prior to sacrifice. Photomicrograph of an unstained section was taken with the fluorescence microscope. Notice the irregular staining pattern of the osteodentin (O) and the straight line (L) in the dentin due to the procion dye. × 150.

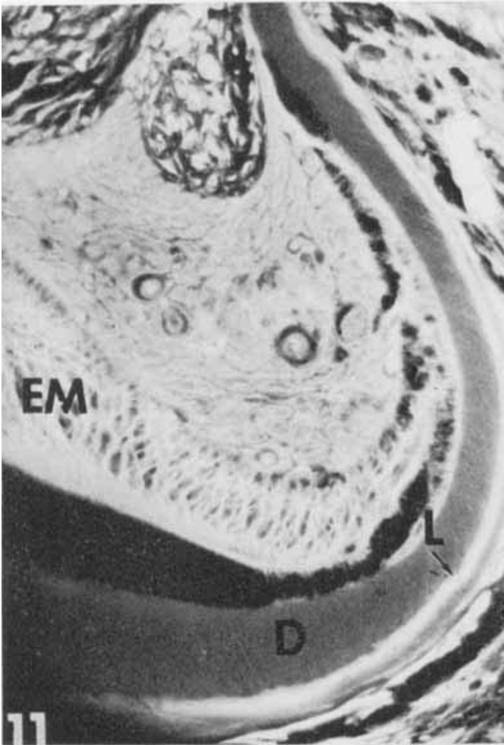
11 Coronal area of a first molar transplant 19 days postoperatively. The recipient was injected with procion red M-8BS seven days prior to sacrifice. Photomicrograph of an unstained section was taken with the fluorescence microscope. D, dentin; EM, enamel matrix; P, pulp; L, line due to the procion dye. × 100.

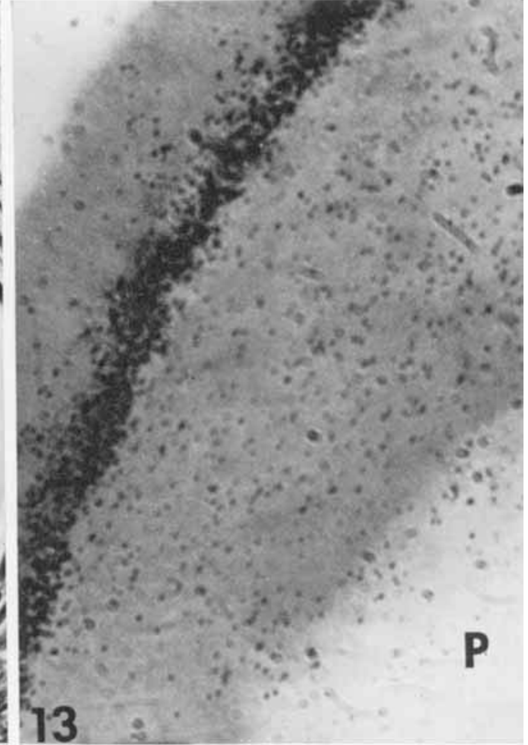
12 Autoradiograph of the coronal area of a first molar transplant, 30 days after transplantation. Notice the heavy labeling of the dentin (D) and the diffuse pattern of labeling of the enamel matrix (EM). The pulp (P) has almost no label at all. The donor was injected with ^{3}H-proline 24 hours prior to sacrifice. H and E. × 100.

13 Higher magnification of area in figure 12. Notice the displacement of the label throughout the newly formed dentin. P, pulpal surface of the dentin. H and E. × 200.


L
O
D
10


D
P
EM
12


EM
L
D
11


P
13