Achievement for Year 1979

1. Evaluation of the microbiologic basis for mass treatment of carious lesions in the Navy and Marine Corps recruit population with the overall goal of reducing chances of pulpal destruction and pain.

A. Improvement of clinical/histologic correlation

The study of the pulpal and periapical sequelae of tooth decay demonstrated the direct correlation between the amount and penetration of bacteria towards the pulp as evaluated by the criteria used in pulp and periapical studies (p. 10). The correlation between the clinical and histologic observation were made through the forms 9ND, NDRI-3900/6(4/77) and filed (5/77). In last year's Progress Report it was stated that there is a good prognosis in pulp treatment provided bacteria have not arrived in the pulp and a minor area of necrosis has not been established.32,33 In our correlation study this year it was confirmed that deep and extensive cavities which have reached between the inner third and inner fourth of the dentin may be treated in one sitting with a high degree of predictable success.

This year's study further pursued the pulpal changes following bacterial penetration to the pulp and establishment in the necrotic area. The necrotic area was surrounded by a large amount of neutrophilic leukocytes (Fig. 1, 3120), which following the release of the lysosomes disintegrated and caused liquefaction of the involved tissue (Fig. 2, 3141). At this time, in this area, the pulp was irreversibly destroyed. Unfortunately, this condition is not predictably accompanied by pain, and it is not possible by any clinical means, except by full excavation, to determine whether or not this condition of irreversibility occurs in the pulp.

Although the coronal pulp is irreversibly changed in the area of bacterial penetration and presence, and there is a severe inflammation in the adjacent pulp, surrounded by an area of chronic inflammation somewhat further apically, the root pulp may still remain totally uninflamed. Pulpal calcifications centrally in the pulp and on the canal walls usually appear as evidence of an ongoing pathologic process.34 In considering the biologic basis for treatment at this stage, a pulpotomy would seem to be a viable possibility, provided it were possible to keep the remaining pulp in that state without further calcifications.
However, when the pulpal degeneration goes unchecked and necrosis and bacteria occur in the root canal, a totally different clinical condition is present. At that time, bacteria are present in the dentinal tubules in the predentin and the mineralized dentin (Figs. 3 & 4) some of these bacteria are live (recognized by the nuclear membrane and the interior light body) or dead (where the nuclear membrane is separated from an entirely black body). Live or dead, however, the bacteria are toxic and irritant to the remaining pulp tissue and to the perialpical tissue.

In a "Light and Electron Microscopic Study of Teeth With Carious Pulp Exposures" the results were summarized as follows:

**Tissue Changes in the Pulp**

1. Bacteria penetrate tubules of all types of dentin.
2. Bacterial penetration and necrosis are interrelated.
3. Neutrophilic leukocytes engulf bacteria as microphages.
5. During progress of necrosis of the root pulp, bacteria enter the dentinal tubules of root canal walls as far as necrosis is present.
6. Despite the presence of necrosis and severe inflammation, whole bacterial cells are not observed in the remaining vital pulp.

**Tissue Changes in the Periapical Lesion**

1. Acute and chronic inflammatory cells are present.
2. Blood vessels show varying degrees of degeneration.
3. Myelinated and non-myelinated nerve fibers are present.
4. Demyelination of nerve fibers is observed.
5. Degeneration and disintegration of inflammatory cells occur.
6. No whole bacterial cells are present in the periapical lesion.
7. Because bacteria cause pulpal and periapical diseases without being present in the lesion, it should be assumed that bacterial toxins, their metabolic end products, and degeneration products of pulp tissue cause an immune response (Manuscript Enclosed).
In a study of the "Innervation of the Inflammatory Periapical Lesions" the results were summarized as follows:

Specimens taken from involved extracted teeth with radiolucencies showed non-myelinated and myelinated nerve fibers - intact and demyelinated - in severely inflamed remaining pulp and periapical tissue. (Manuscript Enclosed).

These findings corroborate and expand the findings reported in last year's report and those reported by Lin and Langeland 1978.35
B. The viability of bacteria remaining in the dentinal tubules of hard dentin following excavation.

Evaluation of efficacy of methods of removal of carious dentin is presented.

In this study, following the removal of calculus, the application of rubber dam, the disinfection of the field of operation except the cavity with 30% H2O2 and 5% tincture of iodine, removal of the surface layer of caries down to the "leathery" dentin and culturing the surface layer, changing of instruments to new sterilized instruments and washing the surface of the "leathery" dentin with sterile saline, removal of the "leathery" dentin and culturing parts of this, again changing to new sterilized instruments and removing all soft carious dentin, washing the surface with saline, drying and inspecting that truly all clinically softened dentin was removed, applying one drop of sterile saline and with new sterilized burs taking samples from the hard dentin surface. Viable bacteria were present in all tests. Cultured on a mitis salivarius medium the following bacteria were identified: streptococcus mutans (C,D), mitis, sanguis, faecalis, and bovis. Following histologic preparation of 78 teeth, about 1/3 showed presence of bacteria in the dentinal tubules adjacent to the hard cavity floor. In such areas some dentinal tubules remain with the odontoblast process intact although changes of the tubular wall have taken place, other tubules remain open without an odontoblast process, and at last some may be more or less closed by calcification. Some of the open tubules have remnants of odontoblast processes and collagen fibers but no bacteria (Fig. 7), whereas others have bacteria in various stages of disintegration (Figs. 8 & 9).

Two considerations should be taken based upon this demonstration of bacteria in the hard dentin:

1. Since it is a fact that we may unintentionally leave bacteria under the cavities, and since restoration of deep cavities in general seems successful, one could think that the presence of these bacteria were without clinical importance.

2. On the other hand, it is also known that the pulp may disintegrate under deep cavities. A survey made in my clinic over the last 500 endodontically treated cases showed that 443 of these teeth had been earlier
restored, and only 57 were initial endodontic cases.

The pulp disintegration may be blamed on a number of reasons: the initial caries, the cavity preparation, remaining carious dentin, medicaments used, efficacy of liners or based used, the restorative material, and at last recurrent or secondary caries. The fact is, however, that the cumulative effect of all these factors have been pulp disintegration.

This is a condition we hope in the future will never happen to Navy or Marine Corps personnel serving at outposts, in submarines, or those being taken prisoners. It should be the pride of the Navy Dental Corps that no Navy or Marine Corps personnel should endure the conditions described under the debriefing following Vietnam. Only biologically sound therapy can prevent such suffering.
The effect on monkey pulp of carious dentin

The purification of sufficient amounts of extracts of the toxic components of human caries dentin is a time consuming procedure with a limited amount of final product.

We have as yet produced enough to show that these toxins cause severe enhanced vascular permeability and local inflammation when injected in rabbit skin.

Therefore, at the Department of Endodontics as a preliminary study, whole human carious dentin has been introduced into experimental cavities in baboon teeth. This did not produce caries in the underlying dentin, but a most severe reaction in the pulp. At short observation periods, using the criteria (pp.10-15), large numbers of odontoblasts were displaced into the involved dentinal tubules, and in the deepest areas no odontoblast remained in the pulp where there was a dense accumulation of neutrophilic leukocytes adjacent to the involved dentinal tubules. The lobes of the nuclei of the neutrophilic leukocytes could be distinguished from the odontoblast nuclei in the same dentinal tubules. Distinct circulatory changes occurred.

Since no bacteria had entered the tubules, the strong chemotactic reactions would have to be due to the toxins from the carious dentin and the disintegration products of the involved odontoblasts and the tissue fluid. With observation periods of more than 30 days, odontoblast nuclei were no longer present in the involved dentinal tubules, but a severe inflammation remained.

Thus, it is evident that it is the toxins with the disintegration products of bacteria and the content of the involved dentinal tubules - the odontoblast process and the tissue fluid - which cause the severe inflammation which leads to the subsequent necrosis when bacteria at last enter the pulp.

This response corresponds well with the response occurring in similar previous experiments in which human carious dentin was introduced in experimental cavities in human premolars (Langeland, 1957). Thus, the baboon tooth appears to be an acceptable model for this type of study.
D. Since at this stage we are isolating and gathering toxins, this part of the study is still in its initial phase.
E. Study of the effect on the root pulp of pulpotomy procedures

Implantation Tests

1. Rat skin implants and guinea pig bone implants of Hydron and collagen calcium phosphate gel.

The implantation studies were all made according to the F.D.I. Standards for Biological Evaluation of Dental Materials (Langeland and Cotton, 1979 enclosed). 37

Rat skin implants of Hydron had observation periods of 14 days (8), 30 days (12), and 90 days (12), guinea pig bone implant had observation period of 90 days (12) and 180 days (18).

Baboons 30 days (8), and longer observation periods presently planned, comprised a total of 62 specimens.

Observation period for the collagen gel implants in rats were 30 days (12), 90 days (8); in guinea pig bone implants, 30 days (20), 90 days (34), 180 days (28), and 360 days (20); in baboons (5).

At this time only short term rat, guinea pig, and baboon specimens have been examined.

An inflammatory reaction was observed at the two ends of the tube in the rat tissue and at the opening of the cup in guinea pig tissue. The material was enclosed in numerous foreign body cells.

Of the collagen calcium phosphate gel material, only four each of rat and guinea pig implants were finished in the laboratory. In the rat implants, the material was seen in macrophages, foreign body cells, and in efferent vessels.

*Figures in parenthesis indicate number of experiments.
In the guinea pig implants the material caused a more extensive foreign body cell reaction and a more extensive inflammatory response than in the rat implants.

The preliminary conclusion drawn upon these few experiments is that Hydron and collagen gel just as any other root canal sealer causes a foreign body reaction and an inflammatory response when contacting soft connective tissue. The inertness claimed by the manufacturers is not confirmed in this study.

Pulpotomies in Baboons

Forty teeth have been treated until now, but only 30 have been examined at 90 day observation period. One-half of these teeth were left open for 4 weeks before the pulpotomy procedure was carried out. The objective of this method was to copy the condition preexisting in teeth perforated to the pulp by caries.

The observations indicate a distinct difference between the teeth which were left open before treatment regardless of the materials used, including the control, the calcium hydroxide. The preliminary result indicates that the preexisting status of the pulp is more important for the end result than the materials used.