Histologic effects of silver nitrate on human dentin and pulp

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Ammoniacal silver nitrate has been widely used by dentists since Percy Howe[1] advocated the sterilization of infected dentin in deep caries lesions rather than mechanical removal by bur or spoon with possible exposure and injury to the pulp. Although many other drugs (thymol, phenol, creosol, creosote, benzalkonium chloride and so forth)[2-7] have also been used with the hope of sterilizing infected dentin, silver nitrate remains one of the most frequently used medicaments for cavity sterilization.

In order to determine whether the use of silver nitrate is based on rational principles, it is necessary to answer two questions: Is silver nitrate an effective sterilizing agent? Is silver nitrate damaging to pulp tissue?

REVIEW OF THE LITERATURE

Sterilizing Action of Silver Nitrate · A review of the literature reveals that the effectiveness of silver nitrate as a sterilizing agent may be questioned. Seltzer[3-5] applied silver nitrate for three minutes to teeth *in vivo* and took bacteriologic cultures from one week to a year later. He indicated that silver nitrate had only limited sterilizing action. Hardwick[6] found that silver nitrate in the concentrated

The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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1. Howe, P. R. A method of sterilizing, and at the same time impregnating with a metal, affected dentinal tissue. D. Cosmos 59:891 Sept. 1917.

2. Day, H. W. Thymol in cavity sterilization. J.A.D.A. 31:605 May 1944.

3. Seltzer, S. Comparative value of various medicaments in cavity sterilization. J.A.D.A. 28:1844 Nov. 1941.

4. Seltzer, S. Effective duration of some agents used for dentin sterilization. J. D. Res. 21:115 April 1942.

5. Seltzer, S. Effectiveness of antibacterial agents used in cavity sterilization. J. D. Res. 21:269 June 1942.

6. Hardwick, J. L. Sterilization of carious dentine. Proc. Roy. Soc. Med., Sec. Odont. 42:815 Oct. 1949.

7. Muntz, J. A.; Dorfman, A., and Stephan, R. M. In vitro studies on sterilization of carious dentin. I. Evaluation of germicides. J.A.D.A. 30:1893 Dec. 1943.

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aqueous or ammoniacal forms was effective only moderately but worked better than the other agents when tested *in vitro*.

Muntz, Dorfman and Stephan,[7] using blocks of carious dentin *in vitro*, demonstrated that the effectiveness of a saturated solution of silver nitrate as a sterilizing agent was proportional to its depth of penetration. Applications of silver nitrate for three minutes produced limited penetration and sterilization to depths of at least 0.25 mm. A ten minute application was more effective and the depth of sterilization was increased to 0.75 mm. in most cases.

Klein and Knutson,[8] and James and Parfitt[9] applied silver nitrate to the proximal and occlusal surfaces of permanent first molars *in vivo* to prevent or arrest incipient enamel caries. They could observe no protective action against caries on the treated molars when compared to the untreated molars on the opposite side. However, Schultz-Haudt, Taylor and Brudevold[10] excavated the proximal carious lesions of deciduous teeth and made the regions self-cleansing by slicing with a disk before applying a silver nitrate solution. They showed that recurrence of caries on the treated surfaces was less than on the untreated surfaces.

Penetration of Silver Nitrate • There have been many claims (empirical) that silver nitrate has a self-limiting action and stains only carious dentin but does not affect vital dentin.

Zander[11] demonstrated that sound dentin was also penetrated by silver nitrate. He showed that the penetration into sound dentin varied with different teeth and also in different regions of the same specimen. Zander and Smith,[12] studying both ground and decalcified sections of dog and human teeth which had been treated with silver nitrate before removal, found that the silver nitrate did penetrate "calcific barriers." No difference existed in the penetration of silver nitrate through the dentin over sound or degenerating pulps or in pulpless teeth. The penetration was the same when studied in ground or decalcified sections.

Zander and Burrill[13] studied the effect on penetration of varying the length of time between the application of the silver nitrate and its precipitation by eugenol. They also investigated the effect of varying the time interval between silver nitrate precipitation and extraction of the tooth. It was found that the length of time intervening between application of silver nitrate and precipitation by eugenol did not influence the depth of penetration. However, the depth of penetration increased as the time interval between precipitation and extraction increased. Eventually the silver nitrate was observed to reach the pulp. Repeated applications of silver nitrate did not vary the depth of penetration. Hardwick[6] demonstrated that silver nitrate could penetrate nearly to the pulpal surface within 15 minutes after application. He stated that he rarely observed silver particles within the pulp.

Zander[11] divided the silver nitrate penetration into three zones: an outer zone of heavy deposit; an intermediate zone of lighter deposit, and a deeper zone of heavy deposit. He did not attempt to explain this phenomenon.

Hardwick[6] described three zones in carious cavities treated with silver nitrate as follows: a superficial zone of black staining up to 1 mm. in depth; an inter-

8. Klein, H., and Knutson, J. W. Studies on dental caries. XIII. Effect of ammoniacal silver nitrate on caries in the first permanent molar. J.A.D.A. 29:1420 Aug. 1942.

9. James, P. M. C., and Parfitt, G. J. Clinical note on the use of silver nitrate in the prevention of fissure caries in newly erupted first permanent molars. Brit. D. J. 96:35 Jan. 19, 1954.

10. Schultz-Haudt, S.; Taylor, R. G., and Brudevold, F. Effect of topical application of silver nitrate on dentin. J. D. Res. 32:681 Oct. 1953.

11. Zander, H. A. Use of silver nitrate in the treatment of caries. J.A.D.A. 28:1260 Aug. 1941.

12. Zander, H. A., and Smith, H. W. Penetration of silver nitrate into dentin. II. J. D. Res. 24:121 June-Aug. 1945.

13. Zander, H. A., and Burrill, D. Y. Penetration of silver nitrate solution into dentin. J. D. Res. 22:85 April 1943

mediate layer of brown staining structureless material, and a deep zone with coarse particles of silver. These granules became larger the more deeply they penetrated.

Effects of Silver Nitrate on Vital Pulp • Despite the known caustic action of silver nitrate, most investigations indicate little or no damage to the pulp when the silver nitrate was applied to sound or carious dentin in even moderately deep cavities.

Zander and Burrill[13] studied the pulp reactions to silver nitrate in both dog and human teeth. They showed the histologic responses of the pulp to silver nitrate in three human teeth. One human tooth, extracted one week after application, showed a normal pulp under a shallow cavity to which silver nitrate had been applied for one minute followed by a five minute application of eugenol. Another tooth, extracted one week after the application, showed a slight hemorrhage beneath the cavity in which silver nitrate was left for 24 hours before being precipitated by eugenol. In both instances, the black granules of precipitated silver did not reach the pulp. The third tooth was examined histologically one month after silver nitrate application and showed that the precipitate reached the pulp where some reaction could be found. Examination of an untreated control tooth demonstrated that there was some reaction of the pulp in the vicinity of the odontoblasts beneath the cavity. Zander and Burrill[13] felt that this was the result of the cavity preparation and was similar to the reactions observed in the teeth treated with silver nitrate. They concluded that silver nitrate does not disturb the pulp severely.

Hardwick[6] prepared cavities in a dozen human teeth, applied silver nitrate for two minutes and extracted the teeth from 15 minutes to 15 days after application. He demonstrated a disorganization of the odontoblastic layer beneath the areas of precipitated silver. Pulpal reactions to silver nitrate varied from slight changes to complete disorganization of the odontoblastic layer, round cell infiltration and hemorrhage. In spite of these findings, he stated that only slight pulp reactions occurred after the application of silver nitrate to exposed vital dentin.

Perreault, Massler and Schour[14] applied silver nitrate in various forms to cavities prepared in incisors of the albino rat. All the pulps showed severe destruction, including abscess formation and total destruction of pulpal elements. The effects increased with the depth of the cavity preparation.

As the dentin of the rat incisor is thinner and more permeable, and the pulp is more sensitive than in the human, the reaction in the rat pulp seemed to be much more severe than the mild pulpal responses reported by other investigators on human material. In view of the widespread use of silver nitrate (in spite of its limited sterilizing action on infected dentin), it was decided that the reactions of the dental pulp to a standard, prolonged application of ammoniacal silver nitrate needed further clarification. Although silver nitrate is frequently applied in deep cavities with potential or actual small pulpal exposures, a report of a histologic investigation describing the effects of applying ammoniacal silver nitrate directly to exposed vital pulp tissue could not be found.

It was the purpose of this investigation to gather more information relative to the effects of silver nitrate on vital human pulp tissue and to describe the reactions of this drug on sound and carious dentin.

MATERIAL

All the teeth selected for this study were from naval personnel ranging in age from

14. Perreault, J. G.; Massler, M., and Schour, I. Reaction of odontoblasts to medicaments placed in cavity preparations in rat incisors. J.A.D.A. 52:533 May 1956.

18 to 28 years. The great majority of these teeth, 26, had been ordered extracted because of large carious exposures with severe destruction of the crown. A few teeth which were scheduled to be removed for prosthetic reasons and third molars in severe malocclusion were also utilized.

TOTAL # OF TEETH NOT SPECIFIED

METHOD

The application of Howe's ammoniacal silver nitrate (pH-10.0) was standardized.[15] The ammoniacal silver nitrate was applied either to exposed pulp tissue or carious cavities for five minutes. Ammoniacal silver nitrate (Howe's) was applied to the carious dentin of five teeth and to the caries-exposed pulps of ten teeth. The dentin floor of all cavities and the exposed pulps were covered with a layer of zinc oxide and eugenol paste. All teeth then were sealed with an additional layer consisting of zinc oxide and eugenol paste to which zinc acetate (5 per cent) had been added as an accelerator in powder form to the zinc oxide. This second layer was carefully placed so that it did not contact the dentin floor or exposed pulp. All teeth were extracted for histologic preparation from 9 to 32 days later.

An additional series of ten teeth was studied primarily to determine the effects of silver nitrate on dentin and pulp within a short period of time after application. None of these teeth contained pulp exposures after cavity preparation. The standard application of ammoniacal silver nitrate followed by eugenol precipitation was made on carious cavities in five upper third molars. Three of these teeth were extracted within five minutes after application and two after about an hour. Ammoniacal silver nitrate not followed by eugenol was applied for five minutes to cavities in one third molar and for ten minutes in two others. These teeth were extracted ten minutes later. A saturated aqueous solution of silver nitrate was applied for ten minutes to two teeth which were extracted within 20 minutes after application.

Aqueous silver nitrate was applied to the exposed pulp of one tooth. This tooth was extracted 21 days later for histologic analysis.

Complete histories were taken on each individual including the history of pain and the physical appearance (color and depth) of the dentin. When a pulpal exposure was present, the size of the exposure, quality of hemorrhage and appearance of the blood clot was recorded. Preoperative and pre-extraction roentgenograms were taken and histories of pulpal pain were noted again before the extraction. All extracted teeth were immediately fixed in 10 per cent Formalin. The first 15 specimens of long duration (extracted 9 to 32 days after application) were decalcified in 5 per cent nitric acid. The subsequent series of 11 teeth were demineralized by means of ethylene diamine tetracetic acid. All teeth were embedded in celloidin and serial sections (10 to 12 microns in thickness) were cut and stained with hematoxylin and eosin (H and E) and with Mallory's triple connective tissue stain.

Various types of controls were utilized in analyzing the effects of ammoniacal silver nitrate on human vital pulp tissue. Untreated carious and noncarious teeth were also examined histologically. In some teeth a large cavity was prepared and the dentin floor divided into two parts; one portion of the cavity floor was treated with silver nitrate and the remainder served as the untreated control.

OBSERVATIONS

Reactions of Carious and Noncarious Dentin To Silver Nitrate • The following four layers could be distinguished in dentin treated with silver nitrate (Fig. 1):

15. Gordon, S. M., and Shand, E. W. Ammoniacal silver nitrate. J.A.D.A. 20:530 March 1933.

1. A thin, superficial layer of black precipitate on the surface of the dentin. This layer consisted of free silver precipitated by the eugenol. The precipitate was on, rather than in, the dentin and indicated that the eugenol acted on the surface of the dentin only or penetrated the dentin only superficially.

2. A layer of carious dentin which stained brown. This was probably the result of the reduction of the silver nitrate by the carious matrix to brown free colloidal silver or, perhaps, a combination of the silver nitrate with the altered available protein. The deepest border of this brown staining mass was irregular, clearly outlining the advancing carious lesion into the underlying dentinal tubules and matrix.

Carious dentin which was not subjected to silver nitrate treatment stained a deep purple with hematoxylin and eosin. Carious dentin treated with silver nitrate stained a deep brown and did not take the purple stain of the H and E. Therefore it was concluded that the brown staining was a specific result of the silver nitrate.

3. An intermediate zone relatively free of silver staining. This zone may represent a zone of reduced dentinal vitality (metamorphosed or sclerotic dentin?). This zone coincides with the region in which the tubular contents have degenerated in advance of the carious lesion.

4. A deeper zone containing large black globules of reduced silver particles. These were observed deep within the vital dentinal tubules and within the pulp cells.

Black silver particles could be found within the pulpal cells of all five non-exposed specimens which were extracted from 17 to 32 days after the standard application of silver nitrate. However, in teeth extracted within an hour after application of silver nitrate, there was usually (in 8 out of 10 specimens) only a superficial penetration of silver particles within the dentinal tubules. This confirms the findings of Zander and Burrill[13] that silver nitrate or silver particles continue to penetrate through the dentinal tubules long after being precipitated by eugenol. It is evident that the depth of penetration by silver increases with survival time. Presumably, all teeth treated *in vivo* would eventually show silver particles within the pulp.

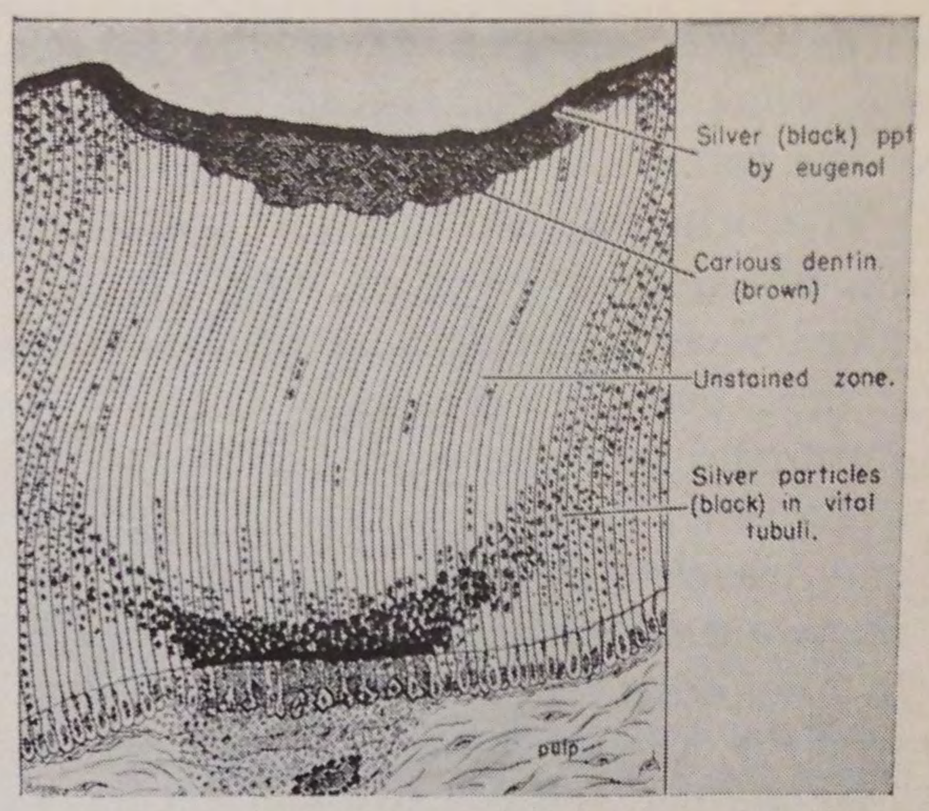

Fig. 1 • Reactions of human dentin and pulp to silver nitrate

In 8 of the 10 specimens extracted within one hour after the application of silver nitrate, penetration was only superficial. However, in two specimens, both extracted five minutes after the application of silver nitrate (for 5 and 10 minutes respectively), silver particles were observed to penetrate the entire thickness of dentin and enter the pulp tissue itself. The reason for the much greater permeability of the dentin in these two instances is not known.

The silver particles penetrated the carious dentin and into the pulp of all 11 teeth exposed by caries. In these instances the precipitate occurred only in the region where vital tubules still persisted and on the surface of the blood clot immediately under the exposure. The black precipitate within the vital tubules and on the blood clot resembled the black precipitate observed on the surface caused by the eugenol. It was obvious that the

silver nitrate must have been reduced by the protoplasmic contents of the tubules or the cells.

The penetration of the silver particles was not limited to primary dentin. Reduced silver particles were also observed to cross over into secondary dentin and eventually into the pulp. In every case where silver was observed in the pulp, it had traveled down the protoplasm of the odontoblastic process to the cell body in the odontoblastic layer. The silver particles tended to concentrate at the junction between primary and secondary dentin and at the pulpal dentinal membrane; that is, at junctions where high concentrations of protoplasm occur.

Effects of Ammoniacal Silver Nitrate on Nonexposed Pulps • Black silver particles could be found within the pulp in all five of the nonexposed specimens extracted from 17 to 32 days after the application of the silver nitrate. The pulps beneath carious dentin to which ammoniacal silver nitrate was applied revealed inflammatory changes under the area of application. There was destruction and atrophy of the odontoblastic layer beneath the area of application of silver nitrate and many of the degenerating odontoblasts contained silver particles. In regions where the accumulation of silver particles was dense, hemorrhage and edema could be observed within the pulp. Similar changes were absent from carious teeth not treated with silver nitrate.

A history of pain could not be elicited from any of the subjects at any time after the application of silver nitrate. The inflammation did not extend into the deeper portions of pulp. The pulp below the zone of disturbance was normal in all instances.

Figure 2 is a decalcified section (Mallory) of upper right second bicuspid from a patient 18 years old. The tooth was extracted 17 days after the application of ammoniacal silver nitrate to the carious dentin. No exposure was noted clinically or microscopically. The pulp under the area of silver nitrate application was destroyed. Silver particles can be observed penetrating into the pulp. This section shows the penetration of silver particles through a dentinal tubule to the pulpodentinal junction. Silver particles may be seen within a degenerating pulpal cell, and deposited around capillaries (Fig. 3).

Eight teeth with deep carious lesions were examined histologically without being treated with silver nitrate. These untreated controls showed little or no evidence of the pulpal disturbances which invariably were present in the teeth treated with silver nitrate.

Effects of Ammoniacal Silver Nitrate on Exposed Pulp Tissue • Ten teeth with clinically visible pulp exposures caused by caries were treated with ammoniacal silver nitrate (Howe's) for five minutes followed by the application of eugenol for five minutes. The carious and noncarious dentin in these teeth showed the same zoning as previously described. One tooth was treated with saturated aqueous silver nitrate. This showed a picture similar to that seen in the teeth treated with ammoniacal silver nitrate.

In the region of the exposure the following reactions were observed:

1. A large superficial hemorrhage always was present in the portion immediately under the exposure. The surface of the blood clot contained large black globules of free silver. The appearance of these black globules was the same as those seen within the vital protoplasm of tubules or cells. The deeper portions of the blood clot contained no black precipitate of silver.

2. A broad band of inflamed pulp lay immediately under the blood clot. This band of inflammation also was seen in the untreated exposed pulps covered with zinc oxide and eugenol for an equivalent period of time. However, the inflammatory band seemed to be wider in teeth

Fig. 2 • Mallory-stained section magnified 650X, nonexposure. Penetration of silver may be seen approaching the pulp. Silver particles may be observed within degenerating pulpal cell

that were treated with silver nitrate.

3. The pulp subjacent to the band of inflammation was normal.

Reaction in Exposed and Nonexposed Teeth • In a number of sections it was apparent that the pulp under a thin layer of dentin treated with silver nitrate was more severely inflamed than the pulp beneath an actual exposure treated with silver nitrate. Apparently, the blood clot protected the pulp against the action of the silver nitrate more effectively than did the thin layer of dentin. The silver nitrate was precipitated more completely by the blood than by the odontoblastic processes in the dentin. This may explain the relatively slight amount of pulpal injury observed under pulp exposures. Only three of the 11 teeth in this series had a history of transient thermal sensitivity during the period between silver nitrate application and extraction. Pain was severe in only one instance.

Untreated Controls • Eleven teeth which had clinically visible pulp exposures were used as controls. These were not treated

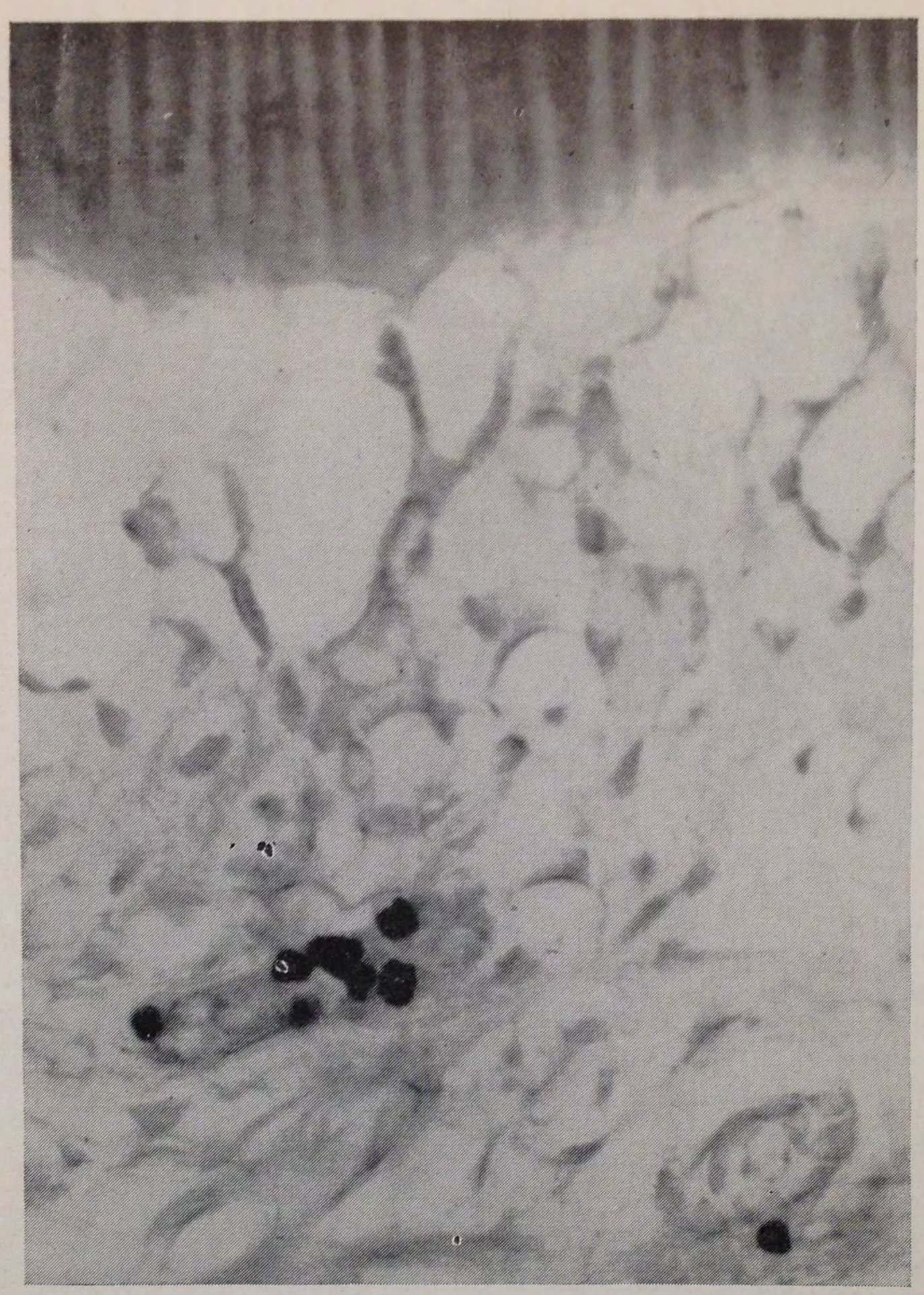

Fig. 3 • Section of pulp magnified 650×. Silver particles are evident around blood vessels

with silver nitrate but were sealed with zinc oxide and eugenol and extracted from 1 to 30 days later for histologic examination. The pulpal reactions were similar to the teeth treated with silver nitrate with the exception that the zone of inflammation subjacent to the hemorrhage was much narrower.

DISCUSSION

Although silver nitrate penetrated to the pulp in only 2 of 10 teeth which were extracted within an hour after application, it reached the pulp in all the teeth without pulp exposures which were extracted 17 to 32 days later. It appears clear that the black precipitates of silver particles eventually reach the pulp regardless of the length of time of application. This confirms previous findings by Zander and Burrill.[13] The silver particles may reach the pulp almost immediately or only after some longer time, depending on the permeability of the dentin. Hardwick was able to discern penetration to the pulp within 15 minutes, but Zander and Burrill[13] showed that the precipitate usually did not reach the pulp within one week. They showed that the silver particles did

reach the pulp after one month in all instances.

Four distinct layers were found in the dentin treated with silver nitrate (ammoniacal or plain) and reduced by eugenol:

1. A very narrow, superficial layer of free silver precipitated on the surface of the dentin by eugenol. It is clear that the eugenol did not limit the penetration of the silver nitrate and is responsible only for the superficial precipitation of free silver on the surface of the dentin. The black "silver mirror layer" seen by the clinician after reduction by eugenol was not within the dentin but lay on the surface of the dentin.

The silver nitrate penetrated quickly and deeply into the dentinal tubules almost immediately after its application with accompanying injury to the odontoblastic processes and degenerative changes in the cell bodies of the odontoblasts. This occurred whether eugenol was used or not. Silver nitrate is not a self-limiting drug but its limitation is caused by living protoplasm. The silver nitrate was not limited to the carious layer.

2. Layer of dark brown carious dentin. The layer of carious dentin was stained a deep brown with the silver nitrate. At present, the best explanation for the formation of this brown stain is that the carious process alters (depolymerizes or denatures) the protein of the dentin matrix. This depolymerized or denatured protein either combines with the silver to form a dark brown silver proteinate or reduces the silver ions to colloidal silver which also appears brown.

3. The silver-free zone. Between the carious dentin and the black precipitate of silver deep within the vital tubules, there was an unstained region relatively free of silver particles. This silver-free zone may represent a portion of degenerating tubular material. The dentinal tubules undergoing protective metamorphosis or becoming sclerotic in advance of the carious lesion, may present a modified type of protein which does not react with the silver nitrate. It is also possible of course that the silver ions may have reacted with the phosphate or chloride ions in this region to produce a white, nonvisible precipitate.

4. Zone of black, precipitated silver particles. Black particles of free silver were present within the vital tubules of the sound dentin well in advance of the carious lesion. They tended to concentrate at the boundary between the primary and secondary dentin. This corroborates the findings of Zander and Smith.

It is not likely that the Formalin used in fixation was responsible for the reduction of the silver complex ion to free silver. The reduced silver tended to accumulate rather specifically in regions rich in vital protoplasm.

Reactions of Pulp Tissue Beneath Dentin Treated With Silver Nitrate • Contrary to previous reports[6, 13] this study revealed disruption of the odontoblasts with increasing degrees of inflammation and edema of the pulp as the silver nitrate came closer to the pulp. If sufficient time were allowed to elapse between application and extraction, black silver particles always were found within the pulp. This disturbance of the pulp always was limited to the region in which the silver had penetrated into the pulp. The pulp under untreated spots always was normal.

Reactions of Exposed Vital Pulp Tissue to Silver Nitrate • When silver nitrate was applied over actual pulp exposures, free silver was precipitated by the hemorrhagic mass under the exposure. This effectively prevented the further penetration by the silver nitrate as evidenced by normal pulp tissue under the inflammatory zone normally found surrounding the region of hemorrhage. The failure to injure the pulp severely when silver nitrate has been applied directly to exposed pulp tissue is thus explicable. In these instances the silver nitrate was

reduced immediately and rendered innocuous by the blood clot.

It is interesting and clinically significant to note that more pulpal damage is caused by the application of silver nitrate to sound dentin than to carious exposures. Sound dentin apparently does not precipitate as much of the silver ion as does the blood, and so permits the silver nitrate to penetrate more deeply and thus to irritate the pulp more severely.

SUMMARY AND CONCLUSIONS

Twenty-six carious human teeth with and without pulpal exposures were subjected to a standardized series of applications with silver nitrate, ammoniacal and plain. Histologic examination revealed the following:

1. Ammoniacal silver nitrate was not self-limiting. It penetrated through sound tubular dentin, both primary and secondary, as well as through carious dentin.
2. Silver nitrate stained the sound and the carious dentin differentially. Carious dentin stained a deep brown. The degenerating tubules under the carious region did not stain at all. The contents of the deeper, vital dentinal tubules precipitated the silver ions as black particles of free silver.
3. Damage to the pulp occurred under sound dentin which was subjected to silver nitrate applications. The black particles of silver eventually reached the pulp.
4. There was a strong tendency by the pulp to localize the injury caused by the silver nitrate.
5. There was considerably less damage under silver nitrate applied directly to exposed pulp than when the solution was applied to sound dentin. This was probably because the blood of the underlying hemorrhagic region completely precipitated the silver nitrate, limiting its action and preventing its further penetration.
6. In view of the limited sterilizing action by silver nitrate and its potentially injurious action to the contents of vital dentinal tubules and the odontoblasts as well as the pulp itself, the value of its continued use on an empirical basis is questioned.