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A REVIEW OF VARIOUS IRRITATING / TOXIC AGENTS: glutaraldehyde – GTA - GLUMA -- Formaldehyde (FA) – Formocresol (FC) (2·hydroxy·ethyl·methacrylate) HEMA – BENZALKONIUM CHLORIDE (zephiran · chloride)

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I currently serve as an ACTIVE CONSULTANT to the Council on Dental Therapeutics of the American Dental Association, & have served as a member of that committee since 1994. Over this past year, I have received many phone calls & personal questions from academics & practicing clinicians from across North America, as well as several countries overseas—questions specifically regarding the biological safety, acceptance & usefulness of certain commercial solutions which are sold for: cavity desensitizing, cavity disinfection, re-wetting agents to re-hydrate denatured collagen & to stabilized the demineralized dentine substrate. These GTA-HEMA-FA-FC-BAC agents are suggested to stabilize certain organic proteins & to prevent dentine hypersensitivity.

Many of the questions deal with clinical issues of the patients "feeling of pain" & their "response" to pain—due to the bi-directional fluid flow causing mechanical deformation of the dentinal-fluid in the dentinal tubule system. In addition, their questions also relate to the presence of bacteria in the tubules below the restoration interface & their relationship to dentine sensitivity.

To assist in providing valid scientific documentation for the ADA to use in their council meetings, I am submitting this document concerning the biological suitability, efficiency & biological safety, toxicity & immunogenic effect of certain commercially available solutions e.g. benzalkonium chloride (BAC), 2·hydroxy·ethyl·methacrylate (HEMA), GTA (Gluma), FC & other aldehyde containing systems either as a cavity disinfectant, or as desensitizing agents for the treatment of dentine hypersensitivity (pain) in humans—following cavity preparation.

In order to provide academic documentation of my biological & clinical research experience, this document is accompanied by my current CV, listing academic credentials, research experience & publications in the area of dentine & pulp biology of dental materials

biocompatibility. In addition to the listed publications & abstracts in the CV I have presented my research data at many US & other International Dental meetings since the early 1970's. If needed, additional information will be provided upon request.

Since the mid 1970's my research efforts have dealt primarily with the biocompatibility of dental materials in the non-human primate model, evaluation virtually the entire spectrum of clinically available restorative materials, which have been—& continue to be sold to the international dental market since the early 1900's. Since joining the UAB faculty in 1989, I have been an active participant in the development & publication of newer biological usage tests & clinical studies dealing with the evaluation of dentine primers, adhesive bonding & resin composite systems. Perhaps the most significant finding is the scientific demonstration that most acids do not irritate the vital dental pulp or cause cell necrosis.

THEORIES OF DENTINE SENSITIVITY

Until 1970, there were three classic theories to explain dentine (pain) hypersensitivity. Early in formal dental research (circa 1820-1890), two general theories of dentine sensitivity were popular & had been published in the literature of that time. The **most popular theory** was of direct neural innervation—based on the presence of small (non-myelinated) nerves that were present in the odontoblastic layer & then divide & branch to penetrate into the dentinal tubules for 50mm to 150mm—the **second sensitivity theory** considered the presence of odontoblastic processes (Tomes fibers) in the dentinal tubules as the prime stimulus to explain the mechanism of human pain.

It was not until the late 1800's when the **third theory** was proposed by Sir Alfred Gysi who had observed the pooling of fluid on the cavity floor—its stimulus caused a painful response in his patients when the fluid was mechanically deformed, dried or mechanically deformed. Many in the profession were quick to consider the fluid movement as a valid "third" theory.

In the late 1960's, the clinical research of Professor Martin Brännström provided scientific evidence that fluid (hydrodynamic) movement was the actual stimulus that caused dentine pain. Today, the hydrodynamic theory of fluid movement is now accepted scientific fact----there is no longer any scientific basis for arguments regarding the other theories.

For human tooth dentine to be sensitive, several biological / mechanical factors must be in place. These variables are: diffusion of a fluid, which moves across or through the dentine substrate within the tubule & communicating canaliculi. The dentine substrate may be studies totally with its clinical smear layer, the smear plugs, the deeper sclerotic dentine filled with Whitlockite crystals, & organic collagen & other proteins. Finally the age of the dentine substrate (tooth) is equally important. Other factors such as

the pressure of the fluid moving across or through the dentinal complex—as well as the viscosity of the fluid; & lastly, the radius of the dentinal tubule (raised to the fourth power Pashley 1990)—a small change in dentine tubule diameter has a dynamic effect on fluid flow. Juried publications by Heyerass & Kinnsland (1992) have shown that normal pulp pressure is greater than normal atmospheric pressure. Consequently, anything that increases rapid or chronic outward fluid flow causes an immediate painful response—when anyone leaves normal ground level & flies in an airplane to high altitudes they often experience dentinal pain due to the greatly increased outward pulp fluid flow. Another scenario of dentine pain is experienced when an individual drinks a highly osmotic or hypertonic solution (sugar, fruit juice), which may rapidly dissolve & remove the biological barrier such as plaque or zones of sclerotic crystal formation. Once the tubules are open, then any thermal stimulus will easily result in fluid flow & dentine pain.

The clinician must keep in mind that human dentine is a biological & morphological variable substrate across any restoration cavity interface. Added to this scenario is the biological variability of the smear layer complex & smear plugs. It must be noted that the continual outward flow of dentinal fluid tends to decrease the inward flow or diffusion of large molecular weight molecules—such as certain of the large molecular weight factors found in bacteria. Bacteria do not rapidly diffuse into the pulp at a rate in which to either cause or result in pain—but under the proper conditions will proliferate into the tubule complex to allow increased fluid flow. Some reports have suggested that the mere "presence" of bacteria are the main cause of dental pain. However, there are no hard data to promote the presence of bacteria as a viable scientific alternative to hydrodynamic fluid movement within the dentinal tubule complex!

WHAT IS A HERMETIC or "BACTERIOMETIC" SEAL? IS IT POSSIBLE TO ALWAYS DEVELOP SUCH A SEAL IN EACH CAVITY PREPARATION?

One question central to the clinical issue of pulp response to various dental materials is the question of material toxicity versus biocompatibility;--specifically related to bacterial microleakage. We should remember that certain restorative agents i.e. FC & GTA are toxic—yet they continue to be used on a daily basis. In 1982, Brännström published data—pulp biocompatibility was basically a function of the dental materials to provide a permanent hermetic "bacteriometic" seal against bacterial microleakage & their toxic components. Separate studies by Bergenholtz et al. 1982 & Cox et al. 1987 supported those original studies of Brännström.

The term bacteriometic seal is emphasized—since the literature shows that various phenol materials (FC, eugenol, GTA) possess or present a spectrum of bactericidal to bacteriostatic activity against oral bacterial microlfora. However, these same materials are toxic to tissue culture cells & would **not** gain ISO approval under today's standards. It is essential to both clinicians & academicians—the issue of a "seal" is central to understanding the issue of microleakage of bacteria & their various products. Pashley has shown that agents such as ZnOE by itself will not provide an actual mechanical seal against the flow of

substances along the cavosurface margin. Published data (Brännström 1982, Bergenholtz et al. 1982, Cox et al. 1987, Cox 1990, Snuggs et al. 1993) demonstrate that ZnOE & other eugenolic containing materials actually provide a bacteriometic seal against bacteria & their components. That is, they simply kill bacteria. The clinician & academicians should realize that this bacteriometic seal is a function of both concentration of agent as well as the availability (time) of the agent at the material / restoration / oral environment interface. For instance, a wet (thin-soupy-mix - more liquid to powder) of ZnOE provides a greater bactericidal effect than a dry thick mix. However, because it is physically weaker, (due to the nature of the thin mix) ZnOE does not provide a long term bacteriostatic effect since bacteria do migrate along the restoration interface. On the other hand, Pashley has also shown that a commercially available dental material such as Cavit will provide a **mechanical seal**. That simply prevents their migration along the tooth-restoration interface. It is hygroscopic—it slightly expands upon setting. However, by the same token, it must be realized that Cavit provides at best—only a poor biologic seal due to its dissolution in the oral environment. Once Cavit is lost from the interface & walls of the restoration, it provides **no** biological seal. So, the clinician & academician must continue to rethink exactly just what is his / her concept of a true **bacteriometic seal** versus a true **mechanical seal** either at or along the restoration interface.

VITAL HUMAN DENTINE: A BIOLOGICAL & MORPHOLOGICAL VARIABLE SUBSTRATE

In order to discuss the issues of dentine desensitization or clinical treatment of human vital dentine, it is imperative to first present a short discussion on the morphological & physiological nature of human dentine as a vital substrate. The first portion of this document addresses the specific biological nature—both morphological & physiology of human dentine. The second portion considers the nature of human dentine sensitivity & the physiological mechanism of hydrodynamics (fluid flow) & its clinical relationship to dental pain.

Recent published data show the dynamic nature of dentine as a real vital substrate (Brännström 1966, Pashley 1985). From a morphological standpoint, vital dentine is a variable three-dimensional substrate. It is composed of an inorganic—mineral phase of approximately 70% Ca-hydroxyapatite. The mineral phase of dentine makes it harder than bone—softer than enamel—an organic phase of Type I collagen, glycoproteins, proteoglycans, phospho proteins, plasma proteins & 10% water which is adsorbed onto the surface of the mineral interstices (between the crystals). The morphology of the dentinal tubules presents a course through the entire thickness of dentine—each tubule from its origin at the enamel-dentine junction (EDJ) to its termination at the pulp wall interface. From a biological point, the reader must remember that the odontoblast process begins its course at the EDJ & builds the tubular & intertubular complex of each dentinal tubule—towards the pulp. Regarding the generalized longitudinal course of the dentinal tubules—each tubule tends to follow a wavy S-shaped path—being less pronounced (wavy S-shaped) in the

cervical root third—as opposed to the incisal edges & cusp tips where they tend to run in a straight direction. When observed in cross-section by scanning electron microscopy (SEM), a cluster (10-50) dentinal tubules form a pattern of round shaped channels in a geometric complex, which occupies less than 1% of the organic interface at or near the EDJ.

Around each tubule is a hypermineralized zone of peritubular dentine; its thickness is dependent upon the age of the individual as well as its location within the length of the entire tubule. The same organic geometric pattern or cluster of the same group of dentinal tubules occupies 45% of the dentine substrate (seen in cross section) at the pulp interface (Garberoglio et al. 1976, Pashley et al. 1985).

From a morphological consideration, each dentinal tubule is tapered shaped conduit, which measure from approx. 0.2μm at or near the DEJ, to approx. 1.2μm in the mid portion & approx. 2.5μm in diameter at the pulpal wall interface. Recent studies demonstrate a higher average tubule density on the lingual & buccal walls, & fewer (lower density) on the mesial & distal walls (Ten Cate 1994). Additional studies have also shown that the terminal branches of the dentinal tubules are more profuse in their branching in the root dentine than in the coronal dentine.

The clinical "take home" data on the human dentinal tubule complex is simply—with a greater density of tubules in the root dentine & with either the loss of both enamel, dentine & root cementum via abfraction or tooth brush abrasion—it is no wonder that patients present themselves to the clinician with immediate hypersensitivity. The question they present to the clinician---"Is it possible to just treat the dentine sensitivity to air & cold stimuli without a lengthy or expensive restorative procedure"? And if the clinician understands the morphology & physiology of the dentine tubule complex, they may answer to the patient—**YES**!

Viewing the dentine tubule complex from the EDJ—to the pulpal interface, vital dentine is a morphological variable, which presents a constantly changing substrate along each cavity wall—instrumentation of vital dentine leaves an outer contiguous smear surface layer approx. 1μm thick with debris plugs which fill the tubules for 3 to 5 μm in thickness (Pashley et al. 1988, Cox 1990). Without removal, organic portions of the smear layer will degrade to form micro channels which allow microleakage into the deeper dentine tubule complex, depending upon the nature of the cavosurface seal (Pashley 1989). When the entire smear layer complex is removed, an outward fluid flow occurs at an increased rate of flow from 70% to 91% (Pashley et al. 1991). In retrospect, academics, researchers & clinicians now understand the physiology of why the 1st, 2nd & 3rd generation systems of dentine bonding & resin composite systems did **not** completely bond into the vital dentine substrate. At best, they only provided localized or regional areas of a "bonded" interface. They were simply working against the natural physiological wetting of the vital dentine surface (Erickson 1992). Clinicians must now rethink the nature of rapid oral biofilm formation in the oral cavity & the fact that the plaque biofilm is quite inhospitable for the bonding of adhesive systems to dentine, simply due to the aqueous protein environment (Bowen 1992).

Since 1991, our profession has seen tremendous advances in the development of dentine bonding systems. These advances have come in the manner of etching the smear layer before primer application, the adhesive, the bonding resin & resin composite systems. The following (1991-1996) generation dentine bonding (4th) systems rely on the application of a acids (i.e. phosphoric, maleic, citric). Whereas, earlier generations claimed to modify the smear layer with weak acids or chelating agents such as EDTA. Frankly speaking, many of the manufacturers were simply afraid to suggest that acid etching of dentine was biologically acceptable. Some of the newer (5th bottle) adhesive systems suggest that modification via reconstitution or removal the smear layer—provides adequate adhesion onto, or more appropriately cohesively into the substrate of vital dentine. Certain systems attempt to bond to the Ca ions of the dentine surface & others attempt to bond to the hydroxyl, carboxyl, amino & amid groups of the collagen molecule (Bowen 1992).

THE NATURE OF DENTINE SENSITIVITY IN HUMAN TEETH

The issue of human dentinal sensitivity is generally the most common complaint of patients who present to the dental office. Removal of dentine from cavity preparation to root planing will leave the underlying dentinal tubule complex exposed & the dentinal tubules altered and open to fluid flow. This allows various stimuli to affect / effect the movement of the proteins & pulpal fluids, which move through the physiologically (normal) dentinal tubule complex. These fluid movements are generally activated or stimulated by immediate placement of an air blast past the (leaky) restoration interface or placement of a cold (ice tip) source onto the tooth surface near the offending restoration—now understood to mechanically activate the mechano receptors in the odontoblastic zone, which ultimately lead to a sharp type A of pain.

As mentioned above, clinical observation of fluid flow onto the axial floor of a cavity preparation concept was first reported by Gysi in 1900. The scientific documentation was later reported by Brännström & Åstrom as the HYDRODYNAMIC theory. The physiology of this hydrodynamic phenomenon of dentinal sensitivity obeys the principles of fluid movement through capillary tubules. From a treatment perspective of the clinician any **decrease in the radius** of the dentinal tubules, or the **blockage** of the cytoplasm of the odontoblastic process within the dentinal tubule greatly reduces the rate of outward fluid flow, & dentinal sensitivity. Once the stimuli is removed, then a sharp type A pain that the patient is feeling tends to subside within a few seconds. This spontaneous pain is referred to as REVERSIBLE pain—indicative of fluid movement within the dentinal tubules. In addition this scenario is often used by clinicians as a diagnostic tool to differentiate dentinal (fluid movement) from pulpal pain. Pulpal pain is generally characterized as a low dull throbbing of a NON-REVERSIBLE type, which is generally of long duration. It is often being activated by chewing pressure onto the tooth—or the initiation by some sort of hot stimulus. It is a type of pain, which lasts for long periods, & does not subside in a short period—even with the removal of any sort of stimulus.

If given the appropriate time, nature may serve to solve dentinal hypersensitivity by

the biological formation of plaque & calculus over the oral surface of exposed dentinal tubules. In addition, formation of various "caries crystals" within dentinal tubules will sclerose (close) the tubule space by hypermineralization. This normal physiological process has been shown to stop REVERSIBLE hypersensitivity. Various therapeutic approaches have been investigated to treat the REVERSIBLE type of dentine pain.

Application of adhesive resin systems (discussed above), rinsing with various fluoride compounds, placement of certain oxalate salts to cause precipitation of hydroxyl apatite crystals, placement of a toxic agent such as GTA to harden the cytoplasmic tubule components contents, burnishing with either a slurry or fine powder of $Ca(OH)_2$ over the dentinal tubules with an orangewood stick, application of toothpaste which contain strontium or potassium oxalates or nitrates, as well as use of iontophoresis of NaF into the affected tooth have all been clinically used to block the rapid flow of fluids, proteins & cytoplasm of the odontoblastic process(es) within the dentinal tubule complex. In addition, the ability of certain hypertonic solutions to decrease nerve activity are also reported to reduce REVERSIBLE pain.

In essence, dentine hypersensitivity (pain) as perceived by most patients—an uncontrolled flow of fluid within the dentinal tubule complex of human teeth. Blockage of the fluid flow at its source of stimulus, as well as complete blockage of the hydrodynamic mechanism will also stop REVERSIBLE dentine hypersensitivity.

The following discussion deals with dental anesthetics. Amines are organic derivatives of ammonia, (NH_3), in which one or more of the hydrogen's are replaced by an aromatic or an alkyl group. When Amines react with an acid—such as hydrochloric acid, ammonium salts are produced. These ammonium salts are frequently named by placing the word hydrochloride after the name of the particular amine. An example would be thiamin hydrochloride (vitamin B_1) or procaine hydrochloride (Novocain)—a local dental anesthetic. Procaine hydrochloride itself is generally administered, rather then the specific amine(s)—the hydrochloride form is more stable in water. Amines are generally basic (pH) compounds, which react with inorganic acids to form salts. Remember that a base is a substance, which accepts protons (H^+). Such a reaction is: $NH_3 + HCl \text{ ——> } NH_4^+ + Cl^-$

Regarding a wide variety of modern dental medicines, most of their effectiveness depends either partly or else entirely on the presence of nitrogen-containing groups within their molecular structure. It should be noted that nitrogen-containing functional groups are found in more medications—than in any other type of functional groups of medicine(s). Most of the amines are organic compounds, which are derived by replacing one or more of the hydrogen atoms of ammonia with either an alkyl or aromatic group(s). Amines are classified by noting the number of (R) groups that have replaced a hydrogen in the NH_3 such as primary, secondary, tertiary or quateranium. Unlike other amine salts, quaternaries salts do not contain a hydrogen attached to the nitrogen.

DOES (BAC) (zephiran chloride) SYSTEM HAVE THE CAPACITY TO STOP OR IMPEDE FLUID FLOW WHEN APPLIED TO HUMAN DENTINE? IS BAC TOXIC?

Quaternary chloride (BAC) salts have both a detergent (surfactant) effect as well as a mild antimicrobial action. In the early 1900's, BAC salts were used to "cold sterilize" instruments following their contact with blood from patients. For many years BAC was the solution of choice for disinfection of surgical instruments following their washing & cleansing to remove gross deposits of blood & other operatory debris. Its detergent action destroys the cell membrane that coats & protects the various bacterial microorganisms and or certain of its cell "machinery". From a historical (medical) perspective, BAC salts were also used as antiseptics on wounds. Generally, a 0.1% solution of BAC salts, were used in early dentistry as a means for cold sterilization of restorative, periodontal & oral surgery instruments. It should be noted that a weaker 0.01%—0.02% solution of BAC was, & continues to be used as a wet dressing on denuded areas of human wounds—gross cuts & on abrasions of the skin where large parts of tissues are removed. A weaker 0.005% BAC solution has been used as an irrigant into the ureters & bladder of humans, generally without damage to the immediate tissues (Ohta et al. 1996). To date, there are no reported data, which document that BAC has a hardening or fixation effect on the proteins of cells (such as the processes of odontoblasts or the pulpal fluid which is found in the tubule complex). However, considering that BAC is a superior antimicrobial, a recent study by Settembrini et al. (1991) has shown that an acid etchant containing BAC did not exhibit significantly larger zones of microbial inhibition when compared to other etchants used in their study. They questioned the necessity of placing BAC as a disinfectant within etchant materials, or weather it is even necessary to treat dentine surfaces with separate antimicrobials prior to the placing of definitive restoratives.

Regarding BAC as a skin irritant, recent studies report that BAC is an irritant to various mucosal tissues in certain concentrations (Park & Eun 1995; Rudzki et al. 1995), & may stimulate variable patterns of epidermal cytokine production—known as nasal stuffiness (Berg et al. 1995; Nakahori, et al. 1996; Engel et al. 1996; Holliday et al. 1996; Hguyen et al. 1996; Gonzalo et al. 1996; Graf et al 1995); Hallen & Graf 1995; Mietz et al. 1994; Baudouin et al. 1994; de Jong et al. 1994; Fuchs et al. 1993; Bjerknes & Steinavag 1993). In addition regarding the use of BAC in various nasal sprays, published data show that nasal sprays, which contain BAC also cause an increased nasal reactivity. And individuals who are engaged in personal medical care, BAC is an occupational allergen (Rustemeyer et al. 1994; Bernstein et al. 1994). More specifically, BAC induces mucosal swelling, which may explain the fact that presence of BAC as a preservative in decongestants sprays causes a prolonged rhinitis (Graf & Hallen 1996). Berg et al. (1994) recently reported that BAC is a well-documented toxic substance in several respects. In fact they suggest—it is unfortunate to use BAC as an additive in certain commercial decongestant preparations.

Takeuchi et al. (1994) showed that BAC effectively blocks neuromuscular transmission, acting as an acetyl choline receptor antagonist at smaller

concentrations. Today, BAC is still used as a medical disinfectant. However at the same time, BAC is a known allergen or irritant to many soft tissues when used in elevated concentrations.

Regarding the action of BAC as a solution, which is reported to "shut" down the outward flow of either pulp or dentinal fluids through the dentinal tubule complex—there are no published studies, which document the capacity of BAC to reduce or stop outward fluid flow. In that capacity, solutions, which contain BAC are reported to provide a mechanism to disinfect the dentinal substrate. However, in that context, there are no biological studies to demonstrate that BAC is more efficacious at disinfection of human dentine than GTA containing compounds. Again, there are no data to demonstrate that BAC contributes to a reduction of the hydrodynamic mechanism of pain, which patients report—in fact on the other side of the issue, there are published data which report that BAC actually serves to increase the permeability of various dextrans (FD-4 & FD-10) through the coroneal epithelium by 28.8 & 37.1 times (Sasaki et al. 1995). Consequently, it appears that BAC may cause an increase in the opening of dentinal tubules—resulting in increased fluid flow & an increased patients response to dental pain. This hypothesis remains to be tested.

DOES HEMA HAVE THE CAPACITY TO STOP OR IMPEDE FLUID FLOW WHEN APPLIED TO HUMAN DENTINE IN AN *IN VIVO* SITUATION? ARE THEY TOXIC?

Clinical dentistry continues to receive information that commercial formulations of HEMA are none to minimal cytotoxicity following its clinical use for desensitization. Some non-scientific reports persist that HEMA reduces & stops the flow of fluids through etched dentine. The HEMA compound is an agent, which presents with both hydrophilic & hydrophobic groups. These HEMA agents promote the diffusivity of certain dental methacrylates into vital dentine following acid etching to remove the smear layer, the smear plugs as well as certain calcium-hydroxyapatite crystals from the intertubular & peritubular dentine substrate. In addition, HEMA also possesses the capacity to carry agents into epithelial tissues. For this purpose, HEMA is used in many commercially available dentine adhesive systems to "carry" other compounds into the vital dentine substrate. It is interesting to note that since HEMA is a low weight molecule, it penetrates or diffuses through the underlying remaining dentine at a rate—faster than the other adhesive components, which contain a higher weight molecule(s). A study by Carvalho et al. (1996) demonstrated that when acid demineralized & air dried human dentine was immersed in 100% HEMA, the dry dentine would-not immediately re-expand to the original volume. Only when water was mixed with HEMA, was there some re-expansion to near the original shape. Consequently, HEMA may only effectively diffuse into dentine if water is present, or if water is present in the solution at a 50% concentration.

Depending on the age, the depth & degree of mineralization of the dentinal tubule complex, HEMA will diffuse through the dentine substrate & tubule complex at different rates. However, there are no reported data, which documents that HEMA by itself does slow or stop the

outward flow of fluids through acid etched dentine. Generally, the inward flow (diffusion) of HEMA occurs at a rate greater or faster than the outward flow of etched dentine. Published data (Hume 1985) has shown that vital dentine permits the diffusion of HEMA & other substances (i.e. eugenol) into or towards the dental pulp.

In this manner, recent reports in the literature by Bouillaguet et al 1996 & Camps et al 1997 have demonstrated that HEMA alone—especially in ascending concentrations—cytotoxic to various mammalian cell fibroblast systems when tested *in vitro*. More specifically, Bouillaguet et al. 1996 measured 10-ascending concentrations of a HEMA solution against its cytotoxic effect on BALB/c mouse fibroblasts in a dentin/cell tissue culture test. Six replicates of HEMA were challenged against the *in vitro* cells for each of the HEMA concentrations. The negative controls for this test were those of a phosphate buffered saline that was added to the cell culture medium—in place of the pure HEMA solution. The mouse cells were incubated for either 12 to 24-hours & then assessed for succinyl dehydrogenase (SDH) activity in the mitochondria of the fibroblast cells. The cytotoxicity data revealed that with increasing times & concentrations of HEMA exposure—a 0.01 & 1mmol/L of HEMA presented only minimal reduction of mitochondrial SDH activity. However, when an increase of HEMA was adjusted to greater than 1mmol/L there was a significant reduction in SDH of the mitochondrial activity in the fibroblasts. They showed that HEMA was readily driven through the dentine disk chamber within 3-minutes. When a thin disk of dentine was used—the HEMA driving force to the cells was even faster—when the human dentine disk was thicker, the HEMA driving force was less. In addition, with backpressure—resembling *in vivo* positive fluid flow from the dental pulp into the dentine diffusion system—the diffusion of HEMA decreased at a five-fold level. Bouillaguet et al. reported that depending on the concentration of HEMA released through the dentine disk, the dilution factor was independent of the driving concentration of HEMA. At no point in their study, did they demonstrate a drop/reduction in the *in vitro* outward fluid flow from the chamber to the dentine disk.

These data suggests that HEMA alone has no effect on reducing the outward flow of fluid from the pulp. From a clinical standpoint, it suggests there is no mechanism of HEMA alone which may slow or stop outward fluid filtration—from a patients perspective of dentine hypersensitivity—there is little evidence of reduction of dentine sensitivity, let alone its use as a as a diagnostic means to localize dentine pain. In addition, this study also suggests that HEMA alone may act as a local mechanism of cytotoxicity—depending upon its concentration as well as its local availability to the underlying (affected) fibroblasts & or odontoblast cells.

A recent article (Camps et al. 1997) employed an extracted tooth model system to test the diffusibility of various HEMA containing agents in extracted human 3rd molars preserved by cryopreservation. Roots were removed & a standard occlusal cavity prepared in a uniform manner, the thickness of dentine remaining measured & the enamel & dentine of the occlusal surfaces etched with a 37% H_3PO_4 for 30-secs. The

measurement of fluid flow through the treated dentine substrate was then measured, & the teeth prepared for measurement of fluid movement as calculated by movement of an air bubble displacement, measured in millimeters along a glass tube. The cavities were then restored with Scotchbond Multipurpose Plus or Optibond adhesive systems. Control cavities were filled with Cavit & measured as a standard fluid flow. For cytotoxicity testing, L·929 mammalian fibroblasts were grown in the chamber, and SDH assays measured for mitochondrial activity, using Phenol as a control agent. Their results showed that Phenol produced the highest toxicity of SDH activity in the cells. There was a direct correlation of fluid flow of dentine & the cytotoxicity of the 2-dentine adhesive systems. The lack of differences between the 2-dentine adhesive systems was suggested as due to the water-soluble nature of its HEMA components—these are corroborated by cytotoxic data (Hanks et al. 1991, 1994 & Bouillaguet et al. 1996). This study did not support the effect of HEMA alone as a clinical means to reduce the fluid flow through etched human dentine. A study by Hamid et al. (1996) reported that high concentrations of H_3PO_4 etching of vital human dentine caused a paradoxical initial reduction of HEMA diffusion through the dentine to the pulp. This may have been due to the collapse of the unsupported collagen layer.

A study by Nakabayashi & Takarada (1992) has shown that pretreatment of demineralized human dentine with HEMA—prior to adhesive resin application increased the diffusivity of the resin system—especially when ferric chloride was omitted from the acidic pretreatment solution. Thus, pretreatment of vital human dentine with HEMA enhanced the impregnation of both the hydrophilic & hydrophobic monomers **into** the dentinal substrate. The addition, HEMA was reported to have improved the bond strength of the resin composite to dentine. Additionally, Nakabayashi has shown that hydroxyapatite crystals along the acid demineralized front actually resist post HCl demineralization following adhesive resin impregnation. This fact supports the thesis that HEMA enhanced the diffusivity & penetration of the hydrophilic system into the vital intertubular substrate.

These various studies report that HEMA alone or in combination with certain water soluble adhesive systems may permit various degrees of cytotoxicity to the underlying cells (cultured). In addition, the data also show that increasing concentrations of HEMA will allow &/or permit increased levels of HEMA to interact with the fibroblast cells—causing a reduced succinic dehydrogenase activity within the specific cell systems.

On the other hand, there are no published data to date, which support the generalized comments by some clinicians that HEMA by itself will diminish or reduce the outward flow of pulpal fluids through the human dentinal tubule complex when tested *in vitro*.

What we know—HEMA does increase the diffusability of hydrophilic solutions when combined with water. It remains to be scientifically documented that HEMA alone or in concert with water will reduce the outward flow of pulpal fluids to reduce dentinal sensitivity. It still remains for longitudinal clinical studies to be carried out by independent research units—to document the efficacy of HEMA as a dentine-desensitizing agent.

DO GTA SYSTEMS (i.e. Gluma) HAVE THE CAPACITY TO STOP OR IMPEDE FLUID FLOW WHEN APPLIED *IN VIVO* TO HUMAN DENTINE? IS GTA, FC or HEMA TOXIC?

The class of aldehydes (e.g. formaldehyde-FA) are generally a colorless gas with a very sharp acidic & sometimes offensive odor. Aldehydes have been used in the laboratory as a water solution containing about 40% FA. The 40% solution of FA is commonly known as formalin—used in early medicine as an effective germicidal agent for disinfection of excreta, surgical theaters, as well as treating infected clothing. Aldehydes harden proteins, rendering them very insoluble in water. In high concentrations, formalin is used to preserve biological specimens. It is important to note that high concentrations of FA or GTA should **not** be placed on vital tissues; as it they cause local irritation & will even result in systemic antigenic loading. FA & its oxidation product, formic acid, are primarily responsible for the systemic toxicity of methyl alcohol.

On the other hand, GTA is superior to FA as a sterilizing agent—gradually replacing it in many dental material systems. It is an anti microbial against many microorganisms, including spores & viruses. In addition, GTA does not have the same disagreeable odor as FA, & it is much less irritational to the eyes & skin.

GTA has two aldehyde groups at the end of carbon chains, either of which may react with amines of collagen as well as the functional groups of proteins of microorganisms. And, since its molecular weight is high (30.03MW), with low volatility, its irritational potential to tissues is very low. GTA solutions, usually 2% with water, are used as disinfecting & sterilizing solutions as their buffered & alkaline forms are capable of killing spores.

More recently, GTA has replaced FC as the accepted therapeutic medicament of choice in clinical treatment of pulpotomy procedures in both primary teeth as well as in permanent molars of young patients needing immediate pulp therapy to save teeth for maintenance of arch space (Ranly & Garcia Godoy 1991). In addition, it has also been suggested as an intracanal medicament for certain endodontic procedures due of its excellent antibacterial qualities. In light of this information, GTA has received an ADA “accepted agent” for use in pediatric, endodontic & restorative applications for clinical treatment of various dentine & pulp pathologies.

Until 1984, molecular adhesives such as isocyanate, carboxylic acid chloride & certain anhydrides were suggested as potential collagen binding molecules. However, at that time, their reported bond strengths to dentine were quite low. In the mid 1980’s, studies evaluating the Gluma® collagen bonding mechanism reported the promotion of adhesive bond strengths between dentine & adhesive resin composites which minimized contraction gap formation (Asmussen & Munskgaard 1984, Elaides et· al. 1985). They reported that the enhanced dentine bonding mechanism was due to the chemical reaction between GTA & the amino groups of the collagen of dentine. And the addition of 2-hydroxy·ethyl·meth·acrylate (HEMA) to GTA increased restorative resin composite bond strengths, as well as enhancing the penetration of the Gluma

system into vital dentine (Asmussen & Munskgaard 1983, 1984). With these data, Gluma was touted to offer the clinical efficacy of enhancing dentine bond strengths for adhesive bonding (Odén & Ølio 1986).

SEM & TE microscopy have demonstrated that dentine bonding is enhanced by the creation of a diffused zone of the primer system into the pretreated vital dentine. This zone has been reported as a dentine-resin reinforced "hybrid" zone, which supposedly enhances the dentine bonding system.

GTA has received the accepted ADA clinical alternative to FC in pulpotomy of primary teeth (Feigal et al. 1990). It offers the positive anti microbial characteristic of FC, without inducing less desirable side effects by cross-linking tissue proteins due to its two active sites (Russell 1976). In addition, GTA demonstrates superior tissue fixation with minimal immunogenicity (Ranly & Lazzari 1983, Ranly et al. 1985). Perhaps more importantly, it diffuses minimally into pulp tissue, with no evidence of periapical inflammation (Dankert et al. 1976). It also presents lesser systemic distribution than FC with excellent clinical results (Myers et al. 1986, Garcia-Godoy 1986, Fuks, et al 1986). GTA shows less apical damage, less necrosis in specimens than FC & is a better tissue treatment than FA (Ranly & Lazzari 1983, Alacam 1989). In addition, GTA are less antigenic than FA (Ranly et al. 1991).

In the mid 1980's, several *in·vivo* usage studies were published on the histopathological response of the Gluma system. They compared Gluma to control dental restorative materials, as well as to other commercially available adhesive resin composite systems (Horsted & Simonsen 1986, Horsted 1987). These publications reported an absence of pulp inflammation from placement of Gluma in non-exposed cavities; they also suggested that Gluma offered a protection & seal against microleakage of bacteria & their toxic by-products when applied to vital prepared dentine. Again, the clinical relevance of this point should be reinforced by the published data (Asmussen & Munksgaard 1984) indicated bonding of the aldehyde molecule to the collagen molecule. Another published report challenged infected class·V cavities in non-human primates with clinical restorations using Gluma & Scotchbond 2 adhesive resin systems (Felton et al. 1989). They reported the presence of bacteria in underlying dentine below infected cavities, which were restored without Gluma or Scotchbond 2, along with an associated pulp response. Neither Gluma or the Scotchbond 2 adhesive systems were associated with pulp inflammation. In addition, no bacteria were detected in any of the Class·V cavities pretreated with the Gluma system, reinforcing the thesis that Gluma imparts an antimicrobial effect to the substrate of vital dentine due to its cross linking to the vital collagen substrate.

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