The Biocompatibility of Copper Enriched Amalgam

Richard A. Pasiewicz
Loyola University Chicago

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THE BIOCOMPATIBILITY OF COPPER ENRICHED AMALGAM

by

Richard A. Pasiewicz

A Thesis Submitted to the Faculty of the Graduate School of Loyola University in Partial Fulfillment of the Requirements for the Degree of Master of Science

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VITA

The author, Richard A. Pasiewicz, is the son of Virginia and Richard S. Pasiewicz. He was born June 8, 1953 in Chicago, Illinois.

He attended St. Turibius Grammar School in Chicago from 1959 to 1967. Upon graduation, he entered Brother Rice High School. He graduated in 1971 as a member of the National Honor Society and an Illinois State Scholar.

In September 1971, he entered Loyola University of Chicago. In June 1975, he received his degree of Bachelor of Science with a major in Biology.

In September 1975, he entered Loyola University's Graduate School and proceeded to work towards the Degree of Masters of Science in Oral Biology.

In September 1977, he began his dental education at Loyola University School of Dentistry.
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CHAPTER I

REVIEW OF LITERATURE

SECTION I

One of the most common materials used in the dental office is silver amalgam. It accounts for 80% of all dental restorations. Its eminent place in the profession is a result of many achievements that have occurred during the past one hundred years.

G.V. Black in his book, "Operative Dentistry," presents a review of the history of dental amalgam. Most of the facts mentioned below are only briefly reviewed.

Amalgam, a mixture of mercury with a metal has been known to chemists and has seen limited use in the arts for many years. Its application to dentistry began early in the nineteenth century. The precise date and person who first used it is uncertain, but many authors believe that M. Taveau, in 1826, is responsible for developing the first dental amalgam.

The first amalgam consisted of filings from a silver coin mixed with mercury. This mixture, "a silver paste," was very difficult to amalgamate and hardened very slowly. In 1833, the Crawcour brothers introduced their amalgam, the Royal Mineral Succedaneum, to the United States. The amalgam alloy was poor in quality and condemned by dentists
who believed that mercury would injure the patient. A controversy a-
rose regarding the use of amalgam as a filling material which lasted
from 1840-1850, a period which has become known as the "amalgam war".
The first official act of the opponents of amalgam was the formation
of a committee to study all mercury compounds used for dental fillings.
They reported that "all mercury compounds proved to be harmful to the
teeth and mouth". As a result, in 1843, the American Society of Den-
tal Surgeons issued a statement saying "the use of amalgam was declared
to be malpractice". This motion was later rescinded by the Society in
1850 and officially ended the "amalgam war".

This controversial issue stimulated some research in attempts to
study the nature and uses of silver amalgam. Three pioneers in the field
were E. Townsend, J.F. Flagg, and G.V. Black.

Townsend proposed an alloy composed of approximately equal parts of
silver and tin. This mixture amalgamated easier and hardened much quick-
er. This addition of tin to the silver amalgam is considered to be its
first improvement.

Flagg conducted a series of investigations and demonstrated that
changing the composition of the amalgam to 60% silver, 35% tin, and 5%
copper, resulted in an improved alloy.

In 1895, Black published his extensive reports on the effects of
the composition of the alloy on the properties of the finished amalgam.
His results indicated that both the composition of the alloy and the man-
ner of mixing were important factors in controlling the strength of the
amalgam and the shrinkage and expansion that occurred during the harden-
ing process. Black proposed an improved alloy, a modification of Flagg's amalgam, with 72.5% silver, 27.5% tin, and smaller quantities of copper and zinc using a mercury to alloy ratio of approximately 8:5. His work laid the foundation for our knowledge about the present amalgam systems. Since the time of Black, only minor changes have been made in the basic composition of dental amalgam.

Upon the request of the U.S. government, Souder, in the early 1920's, conducted a series of investigations on the physical properties of dental amalgam. His reports confirmed the necessity for having standard specifications. His results were later adopted by the National Bureau of Standards as the specifications for an amalgam alloy.

In 1929, Taylor, and in 1933, Paffenburger and Sweeney, published reports on dental alloys not meeting standard specifications and that were still commercially available to the profession. Through their efforts and others in the field, continued progress in amalgam research has been maintained.

Many papers have been published dealing with the setting mechanism of dental amalgam. In 1912, McBain and Joyner studied the composition and chemistry of the ternary silver-tin-mercury system. Their experiments indicated that a chemical reaction occurred in the reaction of the compound $\text{Ag}_3\text{Sn}$ to form $\text{Ag}_3\text{Hg}_4$, therefore leaving all the tin in the free state. They proposed a setting reaction for dental amalgam as:

$$\text{Ag}_3\text{Sn} + 4\text{Hg} \rightarrow \text{Ag}_3\text{Hg}_4 + \text{Sn}.$$  

From 1933-1937, Gayler conducted a series of investigations on the setting and factors affecting the setting of dental amalgam. She
attributed the setting of amalgam to a transformation that occurred with the mixing of Ag$_3$Sn with mercury to form three phases. The three phases present were 1) B$_1$, a silver-tin compound that contained less tin than Ag$_3$Sn, 2) $\gamma_1$, a compound of silver and mercury, and 3) $\gamma_2$, a tin-mercury compound. Her proposed equations for the changes that took place during the setting were:

1) \[ \text{Ag}_3\text{Sn} + \text{Hg} \rightarrow B_1 + \gamma_2 \]
2) \[ B_1 + \gamma_2 \rightarrow B_1 + \gamma_1 + \gamma_2 \]

She hypothesized that the transformation would not proceed to completion, but that the first equation would take place with the absorption of mercury during the initial stages of setting. The second equation would take place gradually with time depending on the size of the original Ag$_3$Sn particles and the rate of formation of $\gamma_1$ and $\gamma_2$ from B$_1$ which took place at mouth temperatures.

In 1938, Troiano proposed a series of reactions for the setting mechanism of dental amalgam. With them he attempted to explain the non-equilibrium manner in which the alloy was prepared. The equations were:

1) \[ \text{Ag}_3\text{Sn} + \text{Hg} \rightarrow \gamma_1 + \Delta_2 + \text{Ag}_3\text{Sn} \text{ (unreacted)} \]
2) \[ \gamma_1 + \Delta_2 + \text{Ag}_3\text{Sn} \rightarrow \gamma_1 + \gamma_2 \rightarrow \gamma_1 + \gamma_2 + B_1 \]

He agreed with Gayler that B$_1$, $\gamma_1$, and $\gamma_2$ would be present in equilibrium. But he thought that the setting mechanism of amalgam started with the formation of $\gamma_1$ and Ag$_3$Sn and proceeds to go through a stage where only $\gamma_1$ and $\gamma_2$ are present until finally B$_1$ is formed. Troiano defined $\gamma_1$ to be Ag$_3$Hg$_4$ and $\Delta_2$ as Sn$_7$Hg. It was later shown that $\Delta_2$ was $\gamma_2$. 
(Sn$_7$Hg), by Fairhurst and Ryge.

Extensive investigations were conducted from 1952 through the middle of the 1960's in efforts to understand more fully the setting of dental amalgam.

Frankel and Fankucken used x-ray diffraction on commercially available amalgam systems. They reported that upon setting of the amalgam, a very complex pattern formed in which a compound Ag$_2$Hg$_3$ was the major constituent present.

Ryge, Moffet, and Barkow determined the phases formed in the setting of amalgam and their order of appearance. The $\gamma_1$ phase, (Ag$_2$Hg$_3$), appeared 5-15 minutes from the start of trituration and reached its maximum within 24-48 hours. The $\gamma_2$ phase, a crystalline hexagonal Sn-Hg phase developed slower than the $\gamma_1$ phase. The formation of the tin-mercury phase was regulated by the $\gamma_1$ phase. They concluded that mercury reacted with Ag$_3$Sn to form a matrix of $\gamma_1$, (Ag$_2$Hg$_3$), and $\gamma_2$, (Sn$_x$Hg$_x$).

Allan, Asgar, and Peyton using a combination of photomicrographs and electron probe analysis reported that not all of each particle was attacked by mercury during amalgamation. The alloy particles, Ag$_3$Sn, were bound together in a matrix of Ag$_2$Hg$_3$ and the tin-mercury phase. Their findings confirmed those previously reported by Ryge, Moffet, and Barkow.

Fairhurst and Ryge published a report indicating that the $\gamma_2$ phase, a tin-mercury phase, consisted of a simple hexagonal crystal and could contain 5-12% mercury. They therefore designated the $\gamma_2$ phase as Sn$_7$Hg or Sn$_8$Hg.
Wing and Ryge investigated the reactions that take place between silver-tin alloys of varying composition and mercury at mouth temperatures, 37° C. Using metallographic techniques and x-ray diffraction, they observed that when the silver-tin alloys were exposed to mercury, a surface reaction occurred forming Ag$_2$Hg$_3$. They also noticed that when the ratio of Ag$_3$Sn to tin was high, zones of reactive areas appeared on the surface of the Ag$_3$Sn particles. Unreacted Ag$_3$Sn was also found. Also, at high tin concentrations, the ratio of $\gamma_1$ to tin was low, and a reaction of Ag$_3$Sn with mercury occurred forming a matrix of $\gamma_1$ and $\gamma_2$.

Wing used electron microprobe analysis to describe the nature of the $\gamma_1$ and the $\gamma_2$ phases. He observed that the $\gamma_2$ phase existed in two forms, an irregular shape and long straight crystals. He reported that when $\gamma_2$ forms in a mercury rich environment, it does so as large size crystals. These can be recrystallized as a result of deformation or while preparing the amalgam. He reported that temperatures of about 50°C would be sufficient to initiate recrystallization. He also noted that a high concentration of $\gamma_2$ was associated with internal voids, where mercury may have been trapped during condensation, and on external surfaces.

In 1962, Demaree and Taylor published a report on their development of a dental amalgam composed of spherical particle alloys. They used three commercially available alloys as controls. Their experimental group consisted of dental alloys composed of 71% silver, 26% tin, 2.5%
copper, and 0.5% zinc, with variations in particle sizes. They conducted a series of tests to observe the physical properties of the alloys. Their results indicated that the effect of increasing the particle size caused an increase in expansion during setting, lengthened the time required for a final set, and decreased the residual mercury content. They observed that the size of the particles had no significant effect on flow after hardening. Particles in the range of 15-50 microns produced maximum strength at all time intervals and that all particles under 50 microns produced high early compressive strengths. They concluded that their best experimental alloy consisted of a mixture of spherical particles of which 89.3% of the particles were in the range of 105-49 microns while 10.7% were smaller. The advantages of such a system became quite clear. With the proper mixture of particle size, one could produce an amalgam that offered easier manipulation, less flow, and a higher early compressive strength.

As a result of Demaree and Taylor's new spherical amalgam, further research was conducted into the structure of set spherical amalgam by Wing and Ryge. Using metallographic examinations, x-ray diffraction, and a hardness test, they observed that the spherical particles retained their original shape and that minimal amounts of the material reacted. They also noted that the original alloy was present in fully set amalgam even in the presence of excess mercury. They detected two phases in the dental amalgam, $\gamma_1$ and $\gamma_2$. Gamma one was determined to be the harder of the two phases and influenced the formation of the slower forming $\gamma_2$. 
The $\gamma_2$ phase occurred on external surfaces, in internal voids, or soft environments where excess mercury allowed the $\gamma_2$ crystals to grow in flat sheets.

A majority of the studies cited have no mention of the $B_1$ phase as proposed by Gayler and Troiano. In 1967, Johnson reported that the $B_1$ phase was present in dental amalgam that had been stored at temperatures higher than 60°C for long periods of time. He observed that a transformation of $\gamma_1$ into $B_1$ began at areas deficient in mercury. Even after heat treatment for a year, some $\gamma_1$ still was present.

The setting of dental amalgam is still not thoroughly understood and is the subject of continuing research. Amalgamation with mercury produces a cored structure composed of gamma particles. The reaction between mercury and the surface of the amalgam alloy produces a matrix that consists of $\gamma_1$, ($\text{Ag}_2\text{Hg}_3$), $\gamma_2$, ($\text{Sn}_7\text{Hg}$), and $\gamma$, ($\text{Ag}_3\text{Sn}$). The basic reaction is controlled by the diffusion of free mercury into the gamma phase. Once this occurs, the $\gamma_1$ and $\gamma_2$ phases form a layer over the unreacted particles and prevents further mercury diffusion.

Young and Wilsdorf published a report on the properties of the phases present in dental amalgam. They determined that the unreacted $\text{Ag}_3\text{Sn}$, was the strongest phase: $\gamma_1$, ($\text{Ag}_2\text{Hg}_3$), intermediate; and $\gamma_2$, ($\text{Sn}_7\text{Hg}$), the weakest phase.

The $\text{Sn}_7\text{Hg}$ is the weakest phase and accounts for about 10% of the set amalgam. It has a compressive strength of about 10,000 psi as compared to that of $\text{Ag}_3\text{Sn}$, 75,000 psi. Asgar and Sutfin conducted a
study on the fracture of dental amalgam. They reported that cracks most frequently went through voids, then the $\gamma_2$ phase, followed by the $\gamma_1$ phase. They also noted that the $\gamma$ phase was the most resistant to failure. But when no $\gamma_2$ phase or voids were present, the cracks moved along the grain boundaries of the $\gamma_1$ phase.

Corrosion of amalgam restorations is usually characterized by the formation of a brown stain, followed by blackening. Corrosion involves two reactions. The first is the anodic or oxidation reaction involving the loss of electrons from the metal to form an ion. The second, is the cathodic reaction. This involves the acceptance of the electrons from the oxidation reaction. Amalgam corrosion in the oral environment occurs when there is a difference in the oxygen concentration present on the amalgam surface. The area with the lowest concentration forms the anode. These areas are primarily where the amalgam is covered by gingiva or plaque, the surfaces of the filling facing the cavity, and the walls in the pores of the amalgam.

Schoonover and Souder in 1941, published their extensive studies on corrosion of dental amalgam. They reported that corrosion occurred when amalgam was placed in contact with gold alloys in a 1% NaCl solution and artificial saliva. They also observed that corrosion occurred when a corroded amalgam came in contact with a polished specimen. Schoonover and Souder noted that tin compounds were the major constituent of corrosion products and was deposited either within the amalgam or on the surface. Upon examining fifty freshly extracted teeth that had been filled with amalgam, they observed that corrosion occurred where adaptation of
the filling to the cavity wall was poor. Corrosion was often so severe that the amalgam had lost most of its strength.

In 1958, Swartz, Phillips, and Tanir conducted an experiment to determine the chemical nature of tarnish. They observed that tarnishing of amalgam occurred in solutions of Na₂S, NaCl, H₂O₂, air distilled water and artificial saliva. X-ray diffraction patterns of clinically tarnished amalgam restorations indicated that sulfide was the major factor in the tarnishing of dental amalgam. The specimens showed discoloration in sulfide solutions and lesser degrees in NaCl, synthetic saliva, and water. They also reported that the degree and rate of tarnishing was not influenced by the residual mercury content or by the presence or absence of zinc.

The first investigator to suggest that corrosion of an amalgam was due to a specific phase was Wagner in 1962. He indicated that the γ₂ phase was the most susceptible to chemical attack. He reported that in dental alloys having a high tin content, the γ₂ phase could form continuous networks throughout the amalgam. As a result of these networks, corrosion could penetrate deep into the amalgam. Wagner also noted that corrosion products functioned as marginal sealants.

In 1965, Jorgensen published a paper on the mechanism of marginal fracture of amalgam restorations. He reported that corrosion attacks γ₂ phase releasing mercury which then diffuses into the amalgam from the cavity site causing an expansion. He agreed with Wagner that the γ₂ phase is the most susceptible to corrosion and due to its continuity may penetrate deep into the amalgam. He reported continuous γ₂ networks even when
condensation reduced the mercury content to as low as 40%.

In 1967, Guthrow, Johnson, and Lawless reported their observations on the corrosion of individual phases of dental amalgam. Using prepared samples of $\gamma$, $\gamma_1$, and $\gamma_2$ phases in Ringer's solution and synthetic saliva, they reported that the $\gamma_2$ phase was the most severely attacked phase, usually characterized by pitting. The gamma phase exhibited little attack or deposition. The $\gamma_1$ phase only developed deposits of AgCl. Their research confirmed that the $\gamma_2$ phase was the preferred phase for corrosion.

Mueller, Greener, and Crimmins conducted a series of electrochemical experiments on amalgam corrosion. They studied a number of alloys and the effects of clinical variables on the corrosion rates. They reported that practices that resulted in formation of more $\gamma_2$ phase led to more corrosion.

Johnson and Lawless attempted to measure the corrosion potentials of dental amalgam in artificial saliva as they were subjected to an increasing tensile load. They reported that stress caused an increase in the anodic direction for the corrosion potential of $\gamma_2$, but only a slight change for dental amalgam or $\gamma$ or $\gamma_1$. They concluded that stress was not a major factor in corrosion of amalgam under oral conditions.

Mateer and Reitz attempted to describe the morphology of corrosion and identify the corrosion products. They used metallographic techniques, x-ray diffraction and chemical analysis. Ground and polished cross sections through fifty extracted teeth containing amalgam were examined. Their observations indicated that corrosion products deposited as two distinct lay-
ers in the marginal regions between the filling and the cavity walls. These corrosion products were identified as \( \text{SnO}_2 \) and \( \text{Sn}_2\text{S}_3 \). They concluded that corrosion propagated through the \( \gamma_2 \) phase and part of the \( \gamma \) phase. This occurred through the formation of new phase due to the reaction of the mercury released from the corrosion of the \( \gamma_2 \) phase. Their results were consistent with Jorgensen's hypothesis on expansion of dental amalgam.

Many investigations have been conducted in an attempt to identify the corrosion products. Swartz, Phillips, and Tanir identified sulfide to be the principle factor in the tarnish of an amalgam alloy. Corrosion products that were identified were silver sulfide, \( \text{Ag}_2\text{S} \), mercury sulfide, \( \text{HgS} \), silver chloride, \( \text{AgCl} \), and an unidentified complex, \( \text{Hg,Ag}_x\text{S}_y \). No correlation existed between the amount of mercury and tarnish. Mueller and Greener (using electronmicroscopy) reported chlorine present in the \( \gamma_2 \) areas of the amalgam. Mateer and Reitz detected the presence of sulfides, \( \text{Sn}_2\text{S}_3 \), \( \text{sic} \), and tin oxides, \( \text{SnO}_2 \).

One conclusion that can be drawn from the many papers reported is that the elimination of the gamma two phase is a necessity for the development of an improved dental amalgam with minimal corrosion.

In 1959, Eames published a report that revolutionized the concept of the mercury to alloy ratio. Up to this time, Black's 8:5 ratio was used producing an amalgam that required removal of the excess mercury to assure cohesion between the alloy particles. By reducing the mercury to alloy ratio to 1:1 and triturating the mix in a mechanical amalgamator,
Eames produced an amalgam that contained less than 50% mercury. The amalgam mixed in those proportions offered better clinical advantages in condensing and manipulating the mix and exhibited higher early compressive strength compared to the amalgams using the 8:5 ratio.

In 1963, Innes and Youdelis reported their development of a dispersed phase amalgam. The alloy, commercially known as Dispersalloy, was a blend of two different compositions. It consisted of spherical and irregular shaped particles. The spherical particles were silver copper eutectic spheres composed of 71.9% silver, and 28.1% copper. The irregular shaped particles were an alloy of Ag₃Sn composed of 73.2% silver to 26.8% tin. They used the mercury to alloy ratio of less than 1:1 that was proposed by Eames.

Their basis for the design of the new amalgam was to increase the strength of the amalgam by the addition of a strengthening dispersed phase, the silver-copper eutectic spheres, to a conventional alloy. The silver-copper spheres were utilized since they satisfied the basic requirement for a dispersion phase. Its hardness and strength was greater than the $\gamma_1$ matrix.

The introduction of Dispersalloy on the market stimulated an area for new research. In 1970, Mahler, Terkla, Van Eysden, and Reisbeck published the results of their clinical studies in which they showed amalgam restorations made using Dispersalloy had better marginal integrity and fewer fractures than those restored with other amalgam alloys. Studies done by Duperon, Neville, and Kasloff and Mahler, Terkla, and Van Eysden have attempted to correlate the clinical behavior of dental amalgam
to its mechanical properties. They have shown that cavities restored with Dispersalloy, an amalgam that has lower creep and higher compressive strength, behaved better clinically and had a better marginal integrity than conventional systems.

Mahler and Asgar showed that the increase in compressive strength of Dispersalloy was not due to the dispersion hardening mechanism, but to the elimination of the $\gamma_2$ phase, $(Sn_7Hg)$. The formation of a new tin-copper phase also occurred during the process. This new phase was located surrounding the dispersed particles and had a composition between $Cu_3Sn$ and $Cu_6Sn$. The mechanism of the reaction was $Sn + Hg \rightarrow SnCu + AgHg$. The end products produced were stronger and less corrosive than the $\gamma_2$ phase. Johnson later confirmed these findings.

In attempts to eliminate the $\gamma_2$ phase, many new amalgam systems have been developed that offer better clinical advantages than the conventional alloys. In 1971, Johnson designed an amalgam that consisted of 64% silver, 26% tin, and 10% gold. He reported that upon trituration, the $\gamma_1$ and $\gamma_2$ phases formed as expected. But after four days, he was only able to detect the $\gamma_1$ phase.

Another new amalgam has become available, Sybraloy, manufactured by the Kerr Corporation. It consists of rapidly cooled spherical particles containing silver, tin, and copper. The composition of the alloy is 40% silver, 31.5% tin, and 28.5% copper.

S.S. White has introduced to the market an alloy that has better mechanical and structural characteristics over conventional or dispersion hardened amalgam alloys. The new amalgam, Tytin, is composed of
60% silver, 26% tin, and 14% copper, and is a precipitation hardened alloy.

The precipitation strengthening of Tytin involves the decomposition of one phase into a second phase that is randomly distributed throughout the original phase. This second phase provides barriers to material flow and deformation. As a result, the precipitation process strengthens the parent matrix by providing a fine distribution of the second phase particles that function as barriers to stop the material flow. Tytin provides for a much better creep and compressive behavior than other systems.

With the development of the copper enriched amalgam alloys, recent activity has centered on the function of copper in silver amalgam. Crowell first pointed out that a discrete phase of copper in silver amalgam would form a new phase. He hypothesized the phase to be Cu₃Sn. Johnson, Asgar, and Peyton conducted a study on the location of the copper phase when it reacts with other constituents of the alloy. By using electron microprobe techniques, they identified the copper phases as consisting of Cu₆Sn₅, with no silver present in the phases. Mahler, Adey, and Van Eysden utilized microprobe analysis in identifying quantitatively the copper phases of amalgam. Using three commercially available alloys, they observed the presence of tin in the ϒ₁ phase. They also observed that Cu₃Sn was found in the original alloy particles of the conventional alloy, New True Dentalloy. They noted that the dispersant alloy, Dispersalloy had little ϒ₂ and that the reactive phase was Cu₆Sn₅.

The elimination of the ϒ₂ phase in the dispersed phase amalgam is the result of two separate reactions that occur simultaneously. The end
products of the two reactions interact to produce a third reaction.

1) Alloy + Hg $\rightarrow$ Ag$_2$Hg$_3$ + Alloy

2) Ag-Cu + Hg $\rightarrow$ Ag$_2$Hg$_3$ + Ag-Cu

3) Ag$_2$Hg$_3$ + Sn + Cu + Alloy + Ag-Cu $\rightarrow$
   Ag$_2$Hg$_3$ + Cu$_6$Sn$_5$ + Alloy + Ag-Cu

Mercury reacts with both the alloy and the eutectic to form $\gamma_1$. Tin and copper are released and combine to form Cu$_6$Sn$_5$. The matrix consists of $\gamma_1$ and a little $\gamma_2$. The $\gamma_1$ and the Cu$_6$Sn$_5$ form a reactive area around the dispersed eutectic phase. The location of Cu$_6$Sn$_5$ suggests that when mercury reacts with the alloy particle, the tin is released and migrates to the dispersed phase particle. It then combines with the copper which was released by the formation of $\gamma_1$ at that location. The initial $\gamma_2$ that is found breaks down to form additional Cu$_6$Sn$_5$ and can't be detected after one week.\textsuperscript{47}

Some research has been conducted on Johnson's gold amalgam. Malhorta\textsuperscript{52} has identified the presence of $\gamma$, $\gamma_1$, $\gamma_2$, and a new phase, AuSn$_4$. He reported that after two weeks, the $\gamma_2$ phase disassociated into its components. The AuSn$_4$ was present in rings surrounding the gamma particle acting as a barrier to the diffusion of mercury. Malhorta and Lawless later reported that the thickness of the ring surrounding the gamma particle increased with an increase in the gold concentration of the alloy.\textsuperscript{53}

The reaction mechanism of Sybraloy is similar to an amalgam developed by Asgar.\textsuperscript{54} The suggested reaction for the alloy is (Ag,Sn,Cu) + Hg $\rightarrow$ $\gamma_1$ + Cu$_6$Sn$_5$ + (Ag,Sn,Cu).\textsuperscript{47}
Several papers have recently been published confirming the clinical advantages that copper enriched alloys exhibit over conventional amalgam systems.

Malhorta and Asgar have published their results of an experiment they conducted to determine the relationship between microstructure, creep, and strength in dental amalgam. Using eight commercially available alloys that included, Dispersalloy, Sybraloy, and Tytin, they reported that amalgam systems having a $\gamma_2$ phase exhibit lower compressive strength, higher tensile, and higher creep values than the systems free of $\gamma_2$.

In 1976, Eames and MacNamara conducted an investigation comparing conventional amalgam systems to the new high copper alloys. They reported that the copper enriched systems exhibited 1) 40% less dimensional change, 2) 47% less flow, 3) 14% greater early tensile strength, 4) compressive strength 27% higher, and 4) a dramatic reduction in static creep - 75% lower than the conventional alloys used.
SECTION II

When considering a material for use in a biological environment, one must consider whether the material will have any toxic effects on the environment in which it is placed. Several techniques are available to determine the amount of irritation produced by the material. Implantation in the subcutaneous connective tissue of rats is apparently the most widely used method.

With the availability and wide use of the new copper enriched silver amalgam systems, a review of the literature was undertaken to determine if any material had been published on tissue tolerance to copper enriched amalgam alloys. A majority of the papers published dealt with the tissue tolerance of endodontic materials. Two of the materials frequently cited were silver and copper amalgam.

In 1933, Dixon and Rickert published the results of their experiment dealing with tissue tolerance to foreign materials. Several endodontic materials were implanted subcutaneously and in the longissmus dorsi muscle of rabbits. They reported copper amalgam implants caused the most severe reaction, a chronic inflammatory response. Silver amalgam, implanted for five months, produced small granulomatous areas around the intramuscular implants. Eosinophils and lymphocytes were reported present in abundance.

Boulger, in 1933, reported gutta percha, a widely used endodontic material, was innocuous to rat and human tissue. Gutta percha implants
present in the muscle tissue of rats for two months, produced a fibrous capsule around the implant. No evidence of any inflammatory reaction present in the muscle was reported.

In 1959, Mitchell proposed an alternative method, subdermal implantation, to examine the amount of irritation produced by a foreign material. Twenty-two dental materials were used in the experiment. Sites of implantation were the scapula and the abdominal region. Criteria used to classify the degree of inflammation were 1) the types and relative numbers of leukocytes, 2) the vascularity of the area, 3) a description of the capsule, and 4) the relative thickness of the capsule surrounding the implant. The overall reaction was then recorded as mild, moderate, or severe. Mitchell reported copper amalgam, implanted for four and sixteen days and one month, produced the most severe reaction. The tissue response to silver amalgam implants was classified as mild and moderate. The four day specimen was classified as moderate. Its capsule contained polymorphonuclear leukocytes and some lymphocytes. The sixteen day specimen was classified as mild. Its capsule contained few inflammatory cells. Mitchell concluded the early moderate response to the silver amalgam implants was of little clinical importance.

In 1962, Feldman and Nyborg reported the effects of gutta percha and silver amalgam implants on rabbit mandible. Their results indicate the capsule surrounding the gutta percha implant was twice as thick as that surrounding the silver amalgam implant.

In 1963, Guttuso investigated the tolerance of rat connective tissue to ten endodontic materials. Three implantation sites were used:
1) the abdominal region, 2) the dorsal interscapular, and 3) the pelvic area. He reported the ventral abdominal site had a chronic inflammatory response. These implants often were lost through ulcerations. At the dorsal sites, the implant remained in the tissue causing a moderate response.

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In 1966, Sperber conducted an investigation on the tissue tolerance of rat connective tissue to dispersion strengthened silver amalgam. Subcutaneous implants of dispersion strengthened silver amalgam remained in the tissue for 2, 16, and 32 days. Compatibility of the amalgam implants was determined by the type and density of inflammatory cells present. Histological examination of the tissue specimens revealed the presence of a fibrous capsule surrounding the implants. The capsule varied according to the composition of the amalgam and the length of time the implant remained in the tissue. Sperber reported dispersion strengthened amalgam appeared to be innocuous as currently used amalgam alloys.

63

Lyons, Waterstrat, and Paffenbarger, in 1966, conducted an investigation on the use of gold-gallium alloys as a dental restorative material. Twenty silver amalgam cylinders and twenty gold-gallium cylinders were implanted subcutaneously in ten rats. Histological examination of the tissue six months postoperatively revealed minimal tissue response to silver amalgam. The gold-gallium implants caused a severe foreign body reaction.

64

In 1975, Kawahara and his associates, investigated the cytotoxicity of spherical amalgam. Three different experiments were conducted on
fibroblast cell cultures as follows: 1) experiments with spherical (mercury to alloy ratio of 4:5) and fine cut (8:5) amalgam: 2) experiments with both amalgam alloys using a mercury to alloy ratio of 8:5: and 3) experiments with spherical amalgam of various mercury to alloy ratios, (4:5, 8:5, 10:5). Spherical amalgam showed intense cytotoxicity immediately after trituration that ceased after two hours. Fine cut amalgam displayed cytotoxic effects until after 24 hours. Increasing the mercury to alloy ratios, (4:5, 8:5, 10:5), produced an increase in cytotoxicity of spherical amalgam.

In 1976, Flander, James, Burch, and Dockem compared the response of zinc-free amalgam and Cavit, a zinc oxide eugenol used for root canal fillings. Four specimens per animal were implanted in the connective tissue of a rat. Criteria used for determining the inflammatory response were the relative thickness of the capsule, the cellularity and vascularity of the capsule, and the relative number and types of inflammatory cells present. All amalgam implants were surrounded by a connective tissue capsule containing fibroblasts and few blood vessels. Capsules surrounding the cavit implants were four times thicker than that of the amalgam implants and contained macrophages, lymphocytes, plasma cells, and giant cells.

Martin and his associates, in 1976, investigated the compatibility of zinc-containing amalgam. Implants of zinc, zinc-free amalgam, and 0.014-gauge stainless steel surgical wire, were implanted between the subdermal and supramuscular tissues on the backs of adult rats. His-
A histological examination of the tissue specimens revealed the amount of inflammation steadily decreased for all materials as a function of time. No significantly different tissue response was observed between the zinc and the zinc-free amalgam.

In 1976, Nagem and his associates conducted an investigation on tissue reaction to dispersion strengthened amalgam. Three amalgam alloys, Fine Cut, Spheraloy, and Dispersalloy, were used in the experiment. Three cylinders, 10 mm long by 2 mm in diameter, of the same amalgam were implanted in the subdermal connective tissue of each of 18 rats. Tissue specimens were taken 2, 16, and 32 days after implantation. They concluded dental amalgam implanted in rat connective tissue is toxic to the tissue for the first 16 days. After 32 days, the implanted amalgam is tolerated by the tissue.
CHAPTER II

Materials and Methods

Four dental amalgam alloys were used in this study. Three of the alloys were copper enriched silver amalgam systems. They are Sybraloy and Tytin, both spherical particle alloys, and Dispersalloy, a dispersion strengthened alloy. The fourth amalgam used was New True Dentalloy, a conventional fine cut amalgam alloy. The compositions, the mercury to alloy ratios, and trituratation times of the four alloys are listed in Table 1. Sybraloy, Tytin, Dispersalloy and New True Dentalloy were the experimental group of alloys. A cobalt-chrome alloy, L.G. Alloy,* was used as a control.

Amalgam alloys were triturated according to the manufacturer's instructions. Uniform implants, 5.6 mm in diameter by 1.6 mm thick, (7/32 inch by 1/16 inch), were made by condensing the amalgam in a type 304 stainless steel die designed by Richard S. Pasiewicz.** (See figures 1 and 2).

Cobalt-chrome implants were made by the lost wax technique. Uniformity of the cobalt-chrome implants was obtained by placing hot wax in the stainless steel die. Cobalt-chrome implants were polished on 240


** Richard S. Pasiewicz is a wood patternmaker currently employed by the General Motors Corporation.
Table I. Percentage Composition of Dental Amalgam Alloys and Manufacturer's Data

<table>
<thead>
<tr>
<th>Alloy</th>
<th>Ag</th>
<th>Sn</th>
<th>Hg</th>
<th>Cu</th>
<th>Zn</th>
<th>Hg:Alloy ratio</th>
<th>Trituration time of double spill capsule* (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New True Dentalloy†</td>
<td>71.9</td>
<td>23.8</td>
<td>50</td>
<td>4.2</td>
<td>0.9</td>
<td>1:1</td>
<td>15</td>
</tr>
<tr>
<td>High Copper</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dispersalloy‡</td>
<td>73.2</td>
<td>26.8</td>
<td>50</td>
<td>12</td>
<td>-</td>
<td>1:1</td>
<td>30</td>
</tr>
<tr>
<td>Sybraloy§</td>
<td>40.0</td>
<td>31.5</td>
<td>45</td>
<td>29</td>
<td>-</td>
<td>0.81:1</td>
<td>30</td>
</tr>
<tr>
<td>Tytin†</td>
<td>60.0</td>
<td>26.0</td>
<td>43.5</td>
<td>12</td>
<td>-</td>
<td>0.76:1</td>
<td>9</td>
</tr>
</tbody>
</table>

* Wig-1-bug 5 AR Amalgamator  
† S.S. White, Philadelphia, Pa., 19102  
‡ Johnson & Johnson, East Windsor, NJ., 08520  
§ Kerr, Romulus, MI., 48174
Fig. 1. Type 304 stainless steel die designed by Richard S. Pasiewicz.

Fig. 2. Type 304 stainless steel die with amalgam discs ready for implantation.
and 400 grit paper. The cobalt-chrome implants were cleaned in a detergent, Alconox.*

Thirty six adult male white rats (250gm) were used for the amalgam implants. Nine animals were used for each time period of 7, 21, and 60 days. Two animals were used for the cobalt-chrome group.

The rats were anesthetized with ethyl ether. The dorsal surface of the rat was shaved. The surgical area, located approximately 8 mm from the base of the neck in the lower dorsal caudal thoracic region, was then cleansed with Septisol*, a hexachlorophene surgical scrub. A midline incision was made with a number 15 scalpel blade through the cutaneous layer. Blunt dissection through the connective tissue exposed the implantation site. The fascia of the longissmus dorsi muscle was then cut. Blunt dissection of the muscle formed a small pocket for the implant. Implants were inserted in the pocket with rubber tipped cotton forceps. (See figure 3). The muscle incision was closed with one suture using a number 4 tapered needle and 4.0 silk. The same procedure then was repeated to implant another disc on the right side of the vertebral column in the right longissmus dorsi muscle. The cutaneous incision was then sutured with a number 4 cutting needle and 4.0 silk. Seven days later, the animal was lightly anesthesized with ethyl ether, and the sutures removed.


** Vistal Laboratories, a division of W.R. Grace & Co., St. Louis, Mo.
Fig. 3. Implantation of amalgam disc in the longissimus dorsi muscle of the rat.
The rats were sacrificed at 7, 21, and 60 days. Tissue specimens were removed and fixed in formalin for 24 hours. Implants were then removed and the tissue was replaced in formalin for 4 or 5 days. The tissue specimens were dehydrated with graduated strengths of ethyl alcohol; alcohol was removed with xylene; and embedded in paraffin. Six micron sections were cut from the tissue blocks and stained with hematoxylin and eosin, Chromotrope 2R, and the Prussian Blue Reaction for iron. Some sections were also stained with Uzman's method for Copper. The procedures are listed in the appendix.

Criteria used for evaluating the tissue response were the relative thickness of the capsule, and the relative number and types of inflammatory cells present.
CHAPTER III

Results

Histologically prepared tissue surrounding the implanted alloys was examined for the presence of foreign body giant cells, polymorphonuclear leukocytes, eosinophils, and the predominate inflammatory cell type. In addition, capsule thickness was measured at six different sites as indicated in Figure 4.

![Figure 4. Approximate location of capsule where thickness measurements were made.](image)

On the basis of the above criteria, each tissue section was assigned a number ranging from 1, which indicated a well accepted tissue response to the alloy, to 4, an active inflammatory reaction. Slide identification labels were covered and random selection of the slides was used to insure impartially in examining the slides.
Seven Day Results

Presented in Table II are the results of the seven day tissue reactions.

Table III represents the evaluation of the overall inflammatory response. (Analysis of variance using the between-within method was used to analyze the data. (See Appendix) Analysis of the data using a table of F at P =0.05 confidence levels indicated that a significant difference between the control alloy, cobalt-chrome, (Figure 5) and the experimental alloys does exist. The cobalt-chrome alloy induced the least reaction after seven days implantation. Surrounding the cobalt-chrome implant was a thin immature fibrous capsule composed primarily of chronic inflammatory cells and fibroblasts. No foreign body giant cells were observed. One interesting observation was the presence of an extremely high number of eosinophils in the capsule of the cobalt-chrome seven day specimens as compared to the occasional appearance of eosinophils in the other seven day specimens. Also observed in some sections of the cobalt chrome seven day tissue specimens was the presence of an eosinophilic, acellular, sharply demarcated area adjacent to the implant referred to as a psuedomembrane.

Analysis of the data obtained from the experimental alloys: New True Dentalloy, Tytin, Sybraloy, and Dispersalloy, (Figures 6-9), was done to determine if any significant difference at (P = 0.05) in tissue reaction to the alloys occurred. No significant difference was observed between the experimental alloys after seven days implantation.
Table II(a). Observations of Tissue Specimens Following Implantation of Cobalt-Chrome and Amalgam for Seven Days in the Rat.

**Observations of 7-Day Tissue Specimens**

<table>
<thead>
<tr>
<th>Alloy</th>
<th>L.G. Alloy Co-Chrome</th>
<th>New True Dentalloy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal</td>
<td>41</td>
<td>42</td>
</tr>
<tr>
<td>Section</td>
<td>A B</td>
<td>A B</td>
</tr>
<tr>
<td>FBG</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>PMN</td>
<td>0 0</td>
<td>0 +</td>
</tr>
<tr>
<td>EOS</td>
<td>+ num. +</td>
<td>+ num. +</td>
</tr>
<tr>
<td>Cell Type</td>
<td>C-F C</td>
<td>C-F</td>
</tr>
<tr>
<td>X Capsule</td>
<td>.095 .146 .105 .135</td>
<td>.18*</td>
</tr>
<tr>
<td>Rx</td>
<td>2-3</td>
<td>3 2 2</td>
</tr>
</tbody>
</table>

FBG: Foreign Body Giant Cells  
PMN: Polymorphonuclear Leukocytes  
EOS: Eosinophils

**KEY:**  
* - Inflammatory Zone  
f - Fragmented  
C - Chronic  
M - Mononuclear  
F - Fibroblast  
A - Acute  
F(C) - Some Chronic
Table II(b). Observations of Tissue Specimens Following Implantation of Cobalt-Chrome and Amalgam for Seven Days in the Rat.

**Observations of 7-Day Tissue Specimens**

<table>
<thead>
<tr>
<th>Alloy</th>
<th>Tytin</th>
<th>Sybraloy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>Section</td>
<td>A</td>
<td>B</td>
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<tr>
<td>FBG</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PMN</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EOS</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cell Type</td>
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<td>C</td>
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<td>Χ Capsule</td>
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</tr>
<tr>
<td>Rx</td>
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<td>4</td>
</tr>
</tbody>
</table>

FBG: Foreign Body Giant Cells  
PMN: Polymorphonuclear Leukocytes  
EOS: Eosinophils  

**KEY:**  
* - Inflammatory Zone  
f - Fragmented  
C - Chronic  
M - Mononuclear  
F - Fibroblast  
A - Acute  
F(C) - Some Chronic
Table II(c). Observations of Tissue Specimens Following Implantation of Cobalt-Chrome and Amalgam for Seven Days in the Rat.

Observations of 7-Day Tissue Specimens

<table>
<thead>
<tr>
<th>Alloy</th>
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<tbody>
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<td>Animal</td>
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<td>Section</td>
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<td>FBG</td>
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<td>PMN</td>
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FBG: Foreign Body Giant Cells
PMN: Polymorphonuclear Leukocytes
EOS: Eosinophils

KEY: * - Inflammatory Zone
      f - Fragmented
      C - Chronic
      M - Mononuclear
      F - Fibroblast
      A - Acute
      F(C) - Some Chronic
Table III. Summary and Statistical Analysis of Tissue reaction following Implantation of Cobalt-Chrome and Amalgam Alloys for Seven Days in the Rat.

### 7-Day Results

<table>
<thead>
<tr>
<th>Alloy</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
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<td>2</td>
<td>4</td>
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<tr>
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<td>3.5</td>
<td>4</td>
<td></td>
</tr>
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<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>3.5</td>
<td>2</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ \bar{x} = 2.38, 3.25, 3.41, 3.33, 3.81 \]

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Degrees of Freedom</th>
<th>F</th>
<th>P = 0.05</th>
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<tr>
<td>AB</td>
<td>1, 8</td>
<td>9.68</td>
<td>5.32</td>
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<tr>
<td>AC</td>
<td>1, 8</td>
<td>11.34</td>
<td>5.32</td>
</tr>
<tr>
<td>AD</td>
<td>1, 8</td>
<td>11.58</td>
<td>5.32</td>
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<tr>
<td>AE</td>
<td>1, 10</td>
<td>32.41</td>
<td>4.96</td>
</tr>
<tr>
<td>BC</td>
<td>1, 10</td>
<td>.38</td>
<td>4.96</td>
</tr>
<tr>
<td>BD</td>
<td>1, 10</td>
<td>.11</td>
<td>4.96</td>
</tr>
<tr>
<td>BE</td>
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<td>4.75</td>
</tr>
<tr>
<td>CD</td>
<td>1, 10</td>
<td>.00</td>
<td>4.96</td>
</tr>
<tr>
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</tr>
<tr>
<td>DE</td>
<td>1, 12</td>
<td>.12</td>
<td>4.75</td>
</tr>
</tbody>
</table>

A: L.G. Alloy, Cobalt-Chrome  
B: New True Dentalloy  
C: Tytin  
D: Sybraloy  
E: Dispersalloy
Table IV. Summary and Statistical Analysis of Capsule Thickness of Tissue Specimens Implanted with Cobalt-Chrome and Amalgam Alloys for Seven Days in the Rat. (mm)

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>.09</td>
<td>.18</td>
<td>.26</td>
<td>.23</td>
<td>.03</td>
</tr>
<tr>
<td></td>
<td>.15</td>
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</tr>
<tr>
<td></td>
<td>.11</td>
<td>.20</td>
<td>.10</td>
<td>.20</td>
<td>.16</td>
</tr>
<tr>
<td></td>
<td>.13</td>
<td>.33</td>
<td>.14</td>
<td>.36</td>
<td>.23</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>.14</td>
<td>.09</td>
<td>.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.25</td>
<td></td>
<td>.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.14</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.312</td>
</tr>
</tbody>
</table>

\[ \bar{X} = .12 \quad .25 \quad .21 \quad .23 \quad .10 \]

A: L.G. Alloy, Cobalt-Chrome
B: New True Dentalloy
C: Tytin
D: Sybraloy
E: Dispersalloy
Figure 5. Capsule surrounding the cobalt-chrome 7 day implant. H & E, 300X.

Figure 6. Tissue response to the New True Dentalloy implanted for 7 days. H & E, 100X.
Figure 7. Tissue response to Tytin disc implanted for 7 days. H & E, 100X.

Figure 8. Tissue response to Sybraloy disc implanted for 7 days. H & E, 100X.
Figure 9. Tissue response to Dispersalloy disc implanted for 7 days. Note the thickness of the capsule and the increased cellularity. H & E, 100X.
Figure 10. Tissue section of area surrounding Sybraloy disc implanted for 7 days. Note A. implant site, B. surgical trauma, C. inflammatory response to implant, and D. muscle fibers present near center of implant site. H & E, 40X.
Acute and chronic inflammatory cells were observed in tissue sections surrounding New True Dentalloy and Dispersalloy. In tissue sections of Tytin and Sybraloy, chronic inflammatory cells and fibroblasts were the predominate cell types present.

Presented in Table IV are the measurements of the capsule thickness of the tissue specimens implanted with cobalt-chrome and the amalgam alloys for seven days. Analysis of the data obtained on the capsule thickness surrounding the alloys indicated that no significant difference at \( P = 0.05 \) was observed between the five alloys used.

Histological examination of the seven day tissue sections indicated that areas of mechanical and surgical trauma existed. Mechanical trauma was prevalent around the ends of the discs where the continuous movement of the muscle around the ends of the implant induced an inflammatory cell infiltration. Areas of surgical trauma were easily identified by the massive result of surgery. Capillary proliferation was common to all tissue specimens.

A prominent observation of many of the seven day tissue sections was the presence of an isolated muscle layer separated from the bulk of the muscle tissue by an inflammatory infiltration. (Figure 10.)

**Twenty-One Day Results**

Presented in Table V are the data observed for the tissue sections surrounding the 21 day implants. Examination of the 21 day tissue sections indicated that the pathologic process was subsiding. Sections of tissue prepared from the 21 day implants were readily identified by
the presence of a well organized connective tissue capsule.

The tissue surrounding the cobalt-chrome alloy was the most easily identified of the 21 day specimens. (Figure 11) Characteristic features that were observed in the 21 day tissue sections were: a light staining cellular fibrous connective tissue capsule, the presence of a large number of eosinophils, and hemosiderin. The predominate cell types surrounding the cobalt-chrome alloy were fibroblasts and chronic inflammatory cells.

Examination of the tissue following 21 days implantation of the amalgam alloys indicated that the fibroblasts were the predominate cell type. New True Dentalloy, Tytin, and Dispersalloy showed similar results. Chronic inflammatory cells predominated in the tissues implanted with Sybraloy.

Table VI shows the overall reaction of the tissue surrounding the alloys implanted for 21 days. Analysis of the data indicated there is no statistically significant difference between the tissue responses to the alloys implanted for 21 days (P = 0.05).

No statistically significant difference (P = 0.05) was found for capsule thickness surrounding the alloys implanted for 21 days (Table VII). Comparison of the capsule thickness of the alloys implanted for 7 days and the alloys implanted for 21 days indicated that there was a statistically significant difference (P= 0.05) between them. The data is presented in Table VIII. (See Appendix)
Table V(a). Observations of Tissue Specimens Following Implantation of Cobalt-Chrome and Amalgam Alloys for Twenty-One Days in the Rat.

### Observations of 21-Day Tissue Specimens

<table>
<thead>
<tr>
<th>Alloy</th>
<th>L.G. Alloy Co-Chrome</th>
<th>New True Dentalloy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal</td>
<td>30 31</td>
<td>24 25 26</td>
</tr>
<tr>
<td>Section</td>
<td>A B A B</td>
<td>A B A B A B</td>
</tr>
<tr>
<td>FBG</td>
<td>0 0 0 0</td>
<td>0 0 0 0 0 0</td>
</tr>
<tr>
<td>PMN</td>
<td>0 0 0 0</td>
<td>+ 0 0 0 0 0</td>
</tr>
<tr>
<td>EOS</td>
<td>+ num. + + + + +</td>
<td>+ + + 0 + +</td>
</tr>
<tr>
<td>Cell Type</td>
<td>F F F C</td>
<td>C-F F F F F-M F</td>
</tr>
<tr>
<td>X Capsule</td>
<td>.056 .093 .048 .072</td>
<td>.125 .033* .023 .06* .074* .03*</td>
</tr>
<tr>
<td>Thickness</td>
<td>Rx</td>
<td></td>
</tr>
</tbody>
</table>

**Key:**
- A - Acute Inflammatory Cells
- C - Chronic Inflammatory Cells
- M - Mononuclear
- F - Fibroblasts
- * - Inflammatory Zone
- f - Fragmented Capsule

FBG: Foreign Body Giant Cells
PMN: Polymorphonuclear Leukocytes
EOS: Eosinophils
Table V(b). Observations of Tissue Specimens Following Implantation of Cobalt-Chrome and Amalgam Alloys for Twenty-One Days in the Rat.

Observations of 21-Day Tissue Specimens

<table>
<thead>
<tr>
<th>Alloy</th>
<th>Tytin</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<td></td>
<td></td>
</tr>
<tr>
<td>Animal</td>
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<td>11</td>
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<td></td>
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<tr>
<td>Section</td>
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<td>B</td>
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<td>+</td>
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<td>EOS</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Cell Type</td>
<td>F</td>
<td>M-F</td>
<td></td>
<td>C-F</td>
<td>F</td>
<td>C</td>
<td>F</td>
</tr>
<tr>
<td>C-Capsule Thickness</td>
<td>.055</td>
<td>.046</td>
<td>.058*</td>
<td>.085</td>
<td>.04</td>
<td>.082</td>
<td>.096</td>
</tr>
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<td>2</td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

FBG: Foreign Body Giant Cells
PMN: Polymorphonuclear Leukocytes
EOS: Eosinophils

KEY: A - Acute Inflammatory Cells
      C - Chronic Inflammatory Cells
      M - Mononuclear
      F - Fibroblasts
      * - Inflammatory Zone
      f - Fragmented Capsule
Table V(c). Observations of Tissue Specimens Following Implantation of Cobalt-Chrome and Amalgam Alloys for Twenty-One Days in the Rat.

**Observations of 21-Day Tissue Specimens**

<table>
<thead>
<tr>
<th>Alloy</th>
<th>Dispersalloy</th>
</tr>
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<tbody>
<tr>
<td>Animal</td>
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<tr>
<td>Section</td>
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</tr>
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</tr>
<tr>
<td>PMN</td>
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<td>$\chi$ Capsule</td>
<td>f</td>
</tr>
<tr>
<td>Rx</td>
<td>2</td>
</tr>
</tbody>
</table>

**FBG:** Foreign Body Giant Cells  
**PMN:** Polymorphonuclear Leukocytes  
**EOS:** Eosinophils

**KEY:**  
A - Acute Inflammatory Cells  
C - Chronic Inflammatory Cells  
M - Mononuclear  
F - Fibroblasts  
* - Inflammatory Zone  
f - Fragmented Capsule
Table VI. Summary and Statistical Analysis of Tissue Reaction following Implantation of Cobalt-Chrome and Amalgam Alloys for Twenty-One Days in the Rat.

21 Day Results

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2.5</td>
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</tr>
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</tr>
<tr>
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<td>2</td>
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<td>1.5</td>
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<td>2</td>
<td>2</td>
<td></td>
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\bar{x} \quad 2.13 \quad 2.41 \quad 2 \quad 2.6 \quad 2.13

<table>
<thead>
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<td>ED</td>
<td>1, 8</td>
<td>2.63</td>
<td>5.32</td>
</tr>
</tbody>
</table>

A: L.G. Alloy, Cobalt-Chrome  
B: New True Dentalloy  
C: Tytin  
D: Sybraloy  
E: Dispersalloy
Table VII. Summary and Statistical Analysis of Capsule Thickness of Tissue Specimens Implanted with Cobalt-Chrome and Amalgam Alloys for Twenty-One Days in the Rat. (mm)

<table>
<thead>
<tr>
<th>21 Day Capsule Thickness</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>.06</td>
<td>.12</td>
<td>.05</td>
<td>.08</td>
<td>.03</td>
</tr>
<tr>
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<td>.09</td>
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<td>.56</td>
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<td></td>
<td>.05</td>
<td>.02</td>
<td>.05</td>
<td>.08</td>
<td>.02</td>
</tr>
<tr>
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<td>.07</td>
<td>.06</td>
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<td>.02</td>
<td>.03</td>
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<td></td>
<td>.07</td>
<td>.06</td>
<td>.05</td>
<td>.08</td>
<td>.16</td>
</tr>
</tbody>
</table>

Comparisons          | Degrees of Freedom | F   | P = 0.05 |
----------------------|--------------------|-----|----------|
AB                    | 1, 8               | .00 | 5.32     |
AC                    | 1, 5               | .00 | 6.61     |
AD                    | 1, 6               | .00 | 5.99     |
AE                    | 1, 6               | .57 | 5.99     |
BC                    | 1, 7               | .00 | 5.99     |
BD                    | 1, 8               | 1.0 | 5.32     |
BE                    | 1, 8               | .71 | 5.32     |
CD                    | 1, 5               | .00 | 6.61     |
CE                    | 1, 5               | 1.0 | 6.61     |
ED                    | 1, 6               | .33 | 5.99     |

A: L.G. Alloy, Cobalt-Chrome  
B: New True Dentalloy  
C: Tytin  
D: Sybraloy  
E: Dispersalloy
Table VIII. Statistical Analysis of Capsule Thickness of Tissue Specimens Implanted with Cobalt-Chrome and Amalgam Alloys for Seven and Twenty-One Days in the Rat. (mm)

<table>
<thead>
<tr>
<th></th>
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<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seven days</td>
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<td>.21</td>
<td>.23</td>
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</tr>
<tr>
<td>21 days</td>
<td>.07</td>
<td>.06</td>
<td>.05</td>
<td>.08</td>
<td>.16</td>
</tr>
<tr>
<td>T</td>
<td>4.41</td>
<td>5.77</td>
<td>4.44</td>
<td>5.0</td>
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<tr>
<td>Degrees of Freedom</td>
<td>7</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>± at P = 0.05</td>
<td>+2.36</td>
<td>+2.26</td>
<td>+2.31</td>
<td>+2.31</td>
<td>+2.14</td>
</tr>
</tbody>
</table>

A: L.G. Alloy-Cobalt-Chrome  
B: New True Dentalloy  
C: Tytin  
D: Sybraloy  
E: Dispersalloy
Figure 11. Tissue response to cobalt-chrome disc implanted for 21 days. Generally, other tissue sections of areas surrounding New True Dentalloy, Tytin, Sybraloy, and Dispersalloy discs implanted for 21 days indicated that the pathologic process was subsiding. H & E, 100X.
Table IX. Observations of Tissue Specimens Following Implantation of Cobalt-Chrome and Amalgam for Sixty Days in the Rat.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
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<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>3.5</td>
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<td>-</td>
<td></td>
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</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Table X. Summary of Capsule Thickness of Tissue Specimens Implanted with Cobalt-Chrome and Amalgam for Sixty Days in the Rat. (mm)

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>.04</td>
<td>.1</td>
<td>-</td>
<td></td>
<td>.01</td>
</tr>
<tr>
<td>.06</td>
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<td></td>
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<tr>
<td>.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A: L.G. Alloy, Cobalt-Chrome  
B: New True Dentalloy  
C: Tytin  
D: Sybraloy  
E: Dispersalloy
Figure 12. Capsule surrounding New True Dentalloy disc implanted for 60 days. H & E, 100X.

Figure 13. Capsule surrounding Tytin disc implanted for 60 days. H & E, 100X.
Figure 14. Fragment of capsule surrounding Sybralloy disc implanted for 60 days. H & E, 100X.

Figure 15. Capsule surrounding Dispersalloy disc implanted for 60 days. Note decrease in cellularity of capsule and lack of inflammatory cells present. H & E, 100X.
Figure 16. Capsule surrounding cobalt-chrome disc implanted for 60 days. Note the thickness and cellularity of the capsule, the presence of large numbers of eosinophils, (arrow), and pigmentation. Chromotrope 2R, 120X.

Figure 17. Eosinophils in capsule (arrow) surrounding cobalt-chrome disc implanted for 60 days. Chromotrope 2R, 240X.
Sixty Day Results

Table IX shows the results of the tissue reactions to the alloys implanted for 60 days. Analysis of the 60 day tissue sections was complicated by mechanical damage to the tissue. The animals implanted for 60 days were the first to be subjected to the experimental procedures. A good deal of damage was done to the tissue while preparing them for histological examination. As a result, complete statistical data was not collected on the mutilated tissues. However, careful qualitative data was collected. It was found that the capsules surrounding the alloys were of a low order of reactivity reflected by mature fibrous connective tissue containing few chronic inflammatory cells and mature fibroblasts containing small, dark staining, spindle shaped nuclei. (Figures 12 - 15)

Theoretically, after 60 days the effects of surgical trauma would be eliminated. Any inflammatory response present after 60 days would be the result of either mechanical trauma or the incompatibility of the alloy to the tissue. Examination of the tissue surrounding the alloys for 60 days indicated that the pathologic process had subsided and resolution had occurred.

Histological comparison of the alloys indicated that once again the cobalt-chrome alloy was most easily identified. Examination for the Prussian Blue Reaction for iron was positive. Examination of the tissue surrounding the other alloys indicated that mononuclear cells and fibroblasts were the predominate cells present.
Microscopic slides of the tissue sections containing chips of amalgam were selected for special staining. Uzman's Method for Copper was used to determine if any copper could be identified in the tissue surrounding the implants. The results of the staining of the slides for copper were negative. A black granular precipitate only appeared where an amalgam chip was present.
CHAPTER IV

DISCUSSION

Several important questions were raised concerning a suitable experimental design for this investigation. Where would the implant be inserted? What would the shape of the implant be? Would the implants be sterilized? Would aseptic technique be utilized during surgery? What alloys would be used for the control group?

Rabbits and rats are most frequently used as experimental animals for determining the tissue tolerance to foreign materials. A variety of implantation sites in the rabbit have been reported. The most common sites are the longissmus dorsi muscle, the mandible, and the sacrospinalis muscle. Reported implantation sites of the rat are the subcutaneous connective tissue, and the abdominal and pelvic areas and the dorsal interscapular muscle. Rats were the preferred choice for our experimental animals since they are easy to care for, require little space for storage, and are not very susceptible to infection.

The longissmus dorsi muscle of the rat was chosen as the implantation site. The advantage of using the site were: 1) The location of the longissmus dorsi muscle offered an easily accessible area, therefore, surgical procedures could be kept to a minimum. 2) The site provided an area where the rat could not remove his own sutures. 3) Implantation of an amalgam disc in the muscle provides an in vivo environment that accurately determines the toxicity of a foreign ma-
terial to the hosts tissue. If a material is biocompatible, it could be implanted anywhere in the body and not evoke a severe inflammatory response.

Ideally the best environment for determining the biocompatibility of an amalgam is the dental pulp of a human tooth. Several investigations have been conducted on the effects of filling materials on the pulp. These studies have utilized cavities in the teeth of experimental animals or man. Several problems are associated with using teeth. The availability of a certain number of teeth at a specified time and age for a sample group is one of the problems that confronts researchers. Mitchell reported that due to variations in teeth morphology, controlling the depth of the cavity in relation to the location of the dental pulp is a problem. Heat and trauma and packing pressures are other factors that influence the response of the pulp. Moller reported the distance between the pulp and the floor of the cavity is the decisive factor in determining the response of the pulp. Granath and Moller reported factors associated with amalgam-marginal leakage, expansion and contraction may influence the response of the pulp. The simple procedure of implanting an amalgam alloy disc in the longissmus dorsi muscle eliminates the effects of cavity preparation on the pulp and determines the irritational qualities of the dental materials.

Reports have indicated that wide variety of sizes and shapes of implants have been used for testing purposes. Cylinders have been
used by Sperber, Lyons, et al., and Nagem. Sandrik, et al., and Kaminski, et al., have utilized the disc for implantation.

In 1970, Wood and his associates implanted type 304 stainless steel discs and cylinders in adult rabbits. Three implantation sites were used: the anterior calvarium, the submasseteric area in the left mandible, and the body of the left sacrospinalis muscle. Tissue areas around the cylinders and discs were histologically compared. They reported areas surrounding the cylinder implants showed a greater reaction toward the ends than in the middle portion. This "clubbing" effect was the result of mechanical trauma. Areas around the discs showed many areas of reaction around their periphery. Wood concluded discs are better implant shapes than cylinders. As a result, discs were chosen for the implant shape in this experiment.

Several techniques have been utilized for sterilization of non amalgam implants. Sandrik and Wragg reported implants sterilized in ethylene oxide did not show signs of artifact corrosion or contamination. This method was utilized by Wood, et al., and Kaminski, et al.,. Sterilization of amalgam implants have been reported by Feldman and Nyborg. They placed the amalgam implants in a solution of quaternary ammonium compound for 2 hours and then rinsed the implants in 0.9% sterile NaCl. A majority of the papers do not mention any procedures used for the sterilization of amalgam implants. Luks reported:

amalgam quickly hardens and has an ultimate expansion which gives the desired effect of a rapidly
applied hermetic seal. Amalgam is also a heavy metal requiring no special sterilization, and is well tolerated in situ.

In this masters thesis investigation, amalgam implants were not sterilized. Autoclaving or rinsing the implants in ethylene oxide was avoided in order to eliminate the risk of an additional variable that could affect the response of the tissue. In light of the evidence presented, sterilization of amalgam implants was deemed unnecessary.

Aseptic technique was not utilized during surgery. Ingles and Griffith reported:

rats are less susceptible to postoperative infection than many other animals. As a matter of fact, if all aseptic precautions are disregarded, but the operation is carefully performed and the animal is kept in a dry cage, many of the animals will remain free from infection after most procedures.

Of all the 49 rats that underwent the surgical procedures, only one animal had developed an infection. This particular rat had its sutures eaten by another rat when he was returned to the transporting cage. The incision was reclosed. The next day, the animal had developed an infection. Inflammatory exudate was clearly visible. The animal was sacrificed and the tissue specimens were not included in the results.

The choice to use a cobalt-chrome alloy as a control for the experiment had been substantiated by several researchers. In 1974, Desai and Sinkford conducted an investigation to determine the changes in soft and hard tissues surrounding isolated intraosseous implants. Three gold alloys, two stainless steel alloys, and two
cobalt-chrome alloys were the materials used in the experiment. Screws made from the above materials were implanted in the femur and the connective tissue of rats for 1-5 weeks. They reported the capsule around the implant differed accordingly with the type of the metal and the duration of implantation. On the basis of the amount of new bone formation around the implants, the cobalt-chrome and stainless steel alloys were found to be more suitable for implantation than the other materials tested. 

In 1976, Subhyiah and Grant implanted cylinders of a cobalt-chrome alloy in troughs formed in the vertex of the skulls of albino rats. Tissue reactions were observed at 4, 7, 14, 30, and 180 days. They reported cobalt-chrome implants did not interfere with the mechanism of healing.

Silver amalgam has been demonstrated to be well tolerated by rat tissue. Several investigations have used New True Dentalloy, an amalgam that generally is considered innocuous, for implantation in tissue to determine the irritational qualities of the materials. Reports have indicated that New True Dentalloy evoked a mild inflammatory response at 2 days characterized by a slight to mild polymorphonuclear leukocytic infiltration. An inflammatory response present after 2 or 7 days must take into account surgical and mechanical trauma and the possibilities of chemical irritation to the tissue. Three of the amalgam alloys used in this investigation, Sybraloy, Tytin, and Dispersalloy, have an increase in copper concen-
tration from the conventional 4%. Several authors\textsuperscript{57,59} have reported copper amalgam pellets evoked a severe inflammatory response for all time periods tested. In light of these reports it was interesting to see the results of our implantation of the copper-enriched amalgam alloys in rat muscle.

Up to this time, only two other authors\textsuperscript{62,67} have reported testing the biocompatibility of Dispersalloy. No reports have indicated the use of Tytin and Sybraloy. It is this author's contention that this investigation is the first attempt to histologically compare the tissue response to the copper enriched amalgam alloys—Sybraloy, Tytin, and Dispersalloy.

Examination of the slides of the seven day tissue specimens implanted with the alloys used in this experiment indicated a variety of inflammatory cells and conditions. Statistical analysis of the data was done to determine whether there was any significant difference in the reaction of the tissue to the amalgam alloys. The results indicated that there was no significant difference (P = 0.05) between the response of the tissue to New True Dentalloy, Tytin, Sybraloy, and Dispersalloy. That is, no significant difference was found between the amalgam alloys studied; however, histological observations of the tissue tended to indicate otherwise. From the majority of the slides examined, however, a general trend developed: the tissue reaction produced by Sybraloy and Dispersalloy appeared more active than that elicited by Tytin, cobalt-chrome, and New True Dentalloy. The histologic reaction due to implantation of Sybraloy often could not be distinguished
from that produced by the implantation of Dispersalloy. Even though this statement contradicts the statistical analysis done, which indicated there was no statistical difference between the amalgam alloys used in this study, the underlying question that remains to be answered is how does one derive objective data from subjective data? To try to facilitate this difficult task, a graded system based on the presence of foreign body giant cells, polymorphonuclear lymphocytes, eosinophils, the predominate cell type, and capsule thickness was used. A value was then assigned to the section ranging from +1, a well accepted reaction, to +4, a reactive inflammatory response. The results obtained on this basis were statistically evaluated to determine if any significant differences between the tissue reaction to the alloys existed.

The results of the examinations of the seven day tissue sections from animals implanted with cobalt-chrome, New True Dentalloy, Tytin, Sybraloy, and Dispersalloy posed some interesting results. Statistical analysis of the overall reaction of the tissue indicated that there was a significant difference between the control alloy, cobalt-chrome, and the other amalgam alloys. The mean reaction index, which is the mean value of the tissue reaction based on the +1 - +4 scale, of cobalt-chrome was 2.38 compared to 3.25, 3.41, 3.33, and 3.81, respectively. The fact that there was a significant difference and the comparatively low index value indicates that cobalt-chrome is well tolerated by the tissue. These findings correspond to those reported by Desai and Sinkford\textsuperscript{78} and Subhyiah and Grant\textsuperscript{79} that cobalt-chrome does not interfere
with the mechanism of healing.

One interesting observation that appeared only with the seven day cobalt-chrome tissue sections was the pressure of an eosinophilic, acellular, sharply demarcated area adjacent to the implant referred to as a psuedomembrane. Though the relationship to cobalt-chrome is not known, the presence of a psuedomembrane has been reported to be indication of a well tolerated reaction to a foreign body. No attempts were done in this experiment to identify the composition of the psuedomembrane. Others describe the presence of a psuedomembrane as an inflammatory reaction on the surface of an organ or tissue that is characterized by the formation of a layer of exudate containing precipitated fibrin, necrotic cells, and neutrophils. The psuedomembrane appeared to resemble the intracellular pink translucent material consistent with amyloid.

Macroscopic examination of the seven day tissue section implanted with Dispersalloy indicated that the tissue surrounding the implants was often soft and dark, (Figure 18), compared to the cobalt-chrome control, (Figure 19). This type of appearance was unique to Dispersalloy. Upon removing the implants, the soft tissue adhered to the disc, (Figure 20). As a result, few capsules remained intact. Two possibilities for the cause of this reaction were considered: 1) the copper content and 2) the Sn$_7$Hg phase. The irritating effects of the copper content was presumed to be negligible. Sperber reported that due to the heterogenous distribution of the silver-copper eutectic phases in the silver-tin matrix, the majority of the copper present in the amalgam is
Table XI. Statistical Analysis of Tissue Reaction Following Implantation of "Aged" Dispersalloy and Original Dispersalloy for Seven Days in the Rat.

<table>
<thead>
<tr>
<th>&quot;Aged&quot; Dispersalloy Reaction</th>
<th>Capsule Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>.14</td>
</tr>
<tr>
<td>2.5</td>
<td>.07</td>
</tr>
<tr>
<td>3</td>
<td>.16</td>
</tr>
<tr>
<td>2.5</td>
<td>.17</td>
</tr>
</tbody>
</table>

\[ \bar{X} = 2.6 \quad \bar{X} = .14 \]

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</tr>
</thead>
<tbody>
<tr>
<td>F Value</td>
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</tr>
<tr>
<td>P = 0.05</td>
<td>4.96</td>
<td>4.84</td>
</tr>
</tbody>
</table>
Figure 18. Muscle section that had been implanted with Dispersalloy for 7 days. Note the dark soft gelatinous material surrounding the implant site.

Figure 19. Muscle section that had been implanted with cobalt-chrome for 7 days.
Figure 20. Dispersalloy implants that had been removed from the 7 day tissue specimen shown in Figure 17.

Figure 21. Muscle section that had been implanted with Dispersalloy discs incubated at 37°C for 7 days before implantation. The discs remained in the tissue for 7 days.
incorporated within the amalgam rather than on the surface of the disc. That left the possibility of the reaction being caused by the Sn-Hg phase. Asgar\textsuperscript{80} reported the $\gamma_2$, (Sn$_7$Hg), phase is eliminated within one week at body temperatures, (37°C), and results in the formation of the Sn-Cu phases. In attempts to determine whether the $\gamma_2$ phase was responsible for this difference, two more rats were implanted with Dispersalloy implants that had been incubated at 37°C for one week. The results of these implantations proved inconclusive. Three of the tissue sections of the "aged" Dispersalloy exhibited the same soft and dark tissue that was present in the original 7 days Dispersalloy implants, (Figure 21). Microscopic examination of the tissue sections indicated that chronic inflammatory cells were the predominant cell type present. Statistical analysis of the data presented in Table XI indicates that there was a significant difference in the reaction of the tissue implanted with the "aged" Dispersalloy as compared to the tissue implanted with the original seven day Dispersalloy implants. It is also interesting to note that there was no significant difference between the thickness of the capsule found surrounding the "aged" and the original Dispersalloy implants.

Another interesting phenomenon observed of the 7 day tissue sections was the appearance of muscle fibers next to the implant, (Figure 9). The presence of these muscle fibers are the result of the surgical procedure. Instead of using the scalpel to form the pocket, blunt dissection with scissors was used. As a result, muscle fibers were sepa-
rated. These muscle fibers observed on the microscopic slides represent the split ends of the muscle fibers that have not yet fully degenerated.

The results observed of the 21 day tissue sections of the alloys used indicated the pathologic process had subsided and the alloys were tolerated by the tissue. Statistical analysis of the data regarding the overall reaction of the 21 day implants indicated that there was no significant difference between the reaction of the tissue to the alloys used. Therefore at 21 days all the alloys were well accepted by the tissue. These findings confirm those previously reported by Nagem$^{67}$ and Sperber.$^{62}$

In several investigations$^{59-62}$ the relative thickness of the capsule had been used as a measure of the degree of irritation. In our experiment, the capsule thickness was measured at six different sites and a mean value was obtained for each alloy at each time period. Statistical analysis of the data indicated that there was no significant differences ($P = 0.05$) in the capsule thickness between the alloys implanted for seven days. At 21 days, no significant difference occurred between the alloys. This is quite surprising due to the fact that in this study there is a significant difference between the response of the tissue to the cobalt-chrome implants compared to the other alloys. Since this significant difference between cobalt-chrome and the other alloys is not manifested in the statistical analysis of the capsule thickness, it is felt, therefore, that capsule thickness measurement as a criteria for evaluating a foreign body reaction is not
valid.

One of the problems confronting us in this investigation was obtaining accurate values from the tissue sections for capsule measurement. In this study, for the seven days alloys, a capsule was often absent, but an inflammatory zone was prevalent, fragments of capsules were the only remnants available to take any measurements.

Another problem encountered in this investigation was the large number of data points lost due to improper handling of the tissue specimens. This was evident in the 60 day results. Inexperienced technical personnel prepared slides that were poorly processed, stained, and cut. As a result, careful qualitative rather than quantitative analysis of these sections was done.

In examination of the 60 day sections, the cobalt-chrome had a very thick cellular capsule compared to the other alloys. A probable cause for the unusual appearance of the capsule, could lie in the preparation of the cobalt-chrome disc. After casting of the alloy was completed, the bur remained to be removed. After removal, the rough edges on the disc were then smoothed down to obtain a round disc. The possibility that a small knick or an unrounded part of the disc remained when implanted in the tissue is plausible. This unevenness coupled with mechanical trauma could explain the thick and cellular capsules of the 60 day specimens.

Another interesting aspect observed in the 60 day cobalt-chrome tissue sections was the presence of large numbers of eosinophils, (Figure 17). Hurley\textsuperscript{82} reported eosinophils have many properties sim-
ilar to neutrophils. They react chemotactically to the same substances that attract neutrophils and predominate in acute allergic reactions. It seems likely that eosinophils have a specific role in response to injury, but still remains unknown. Kaminski and Wood have reported that eosinophils in tissues surrounding the implants indicate some degree of incompatibility. The abundance of eosinophils present in the cobalt-chrome 60 day capsule and the thickness of the capsule may indicate some degree of incompatibility. The eosinophils were primarily concentrated in areas where hemosiderin was prevalent. No evidence of corrosion products were observed.

Large amounts of hemosiderin was observed in the tissue sections seen in this study. Hemosiderin is an endogenous pigment derived from hemoglobin. The color varies from golden-yellow to brown, is granular or crystalline, and contains iron. The appearance of hemosiderin in the sections indicates that a local excess of iron was present. The cause of the hemosiderin deposits is internal hemorrhage produced by surgery. Accumulations of hemosiderin generally does not damage the cell or impair its functions.

The results of this investigation indicates that the increase in the copper content of silver amalgam from the conventional 4% to 12%, and 29% in Dispersalloy, Tytin, and Sybraloy, respectively, does not increase the tissue response to the alloys. The silver copper amalgam alloys appear to be as innocuous as the silver tin amalgam. The initial inflammatory response present in the 7 day tissue specimens represents surgical and mechanical trauma and chemical injury. The tissue sections
of the 21 day period indicated that the pathologic process was subsiding. All experimental silver copper alloys induced similar tissue responses. At 60 days, the experimental alloys – New True Dentalloy, Tytin, Sybraloy, and Dispersalloy were well tolerated by the tissue. The increase in the copper content of the alloys did not induce a more severe inflammatory response. The results of this investigation agree with findings of Sperber\textsuperscript{62} and Nagem\textsuperscript{67} mainly that the silver copper amalgam alloys appear to be innocuous as the silver tin amalgam, New True Dentalloy.
SUMMARY

Adult white rats (250 g) were implanted with discs of three copper enriched amalgam alloys, a conventional amalgam, and a cobalt-chrome alloy. The implantation site was the longissimus dorsi muscle. The rats were sacrificed at 7, 21, and 60 days. Histo-logic examination of the tissue specimens indicated that the 7 day specimens exhibited the most tissue response of the time periods. No significant difference (P=0.05) was observed between the response of the tissue to New True Dentalloy, Tytin, Sybraloy and Dispersalloy implanted for 7 days. No significant difference (P=0.05) occurred between the cobalt-chrome alloy, and the amalgam alloys implanted for 21 days. Examination of the tissues of the 21 and 60 day periods indicated that the copper enriched amalgam alloys were well tolerated by the tissue and appeared as innocuous as the conventional amalgam.
BIBLIOGRAPHY


HEMATOXYLIN AND EOSIN STAIN

Solutions

ACID ALCOHOL

Alcohol, 70% . . . . . . . . . .

Hydrochloric acid, concentrated . . . 10.0 ml

Staining Procedure

1. Deparaffinize and hydrate to water.
2. Hematoxylin stain for 4 minutes.
3. Rinse in tap water for 3 minutes.
4. Dip in acid alcohol 2-5 times.
5. Rinse with distilled water, 6 dips.
6. Rinse with tap water for 10 minutes.
7. Dip in 75% alcohol for 1-2 minutes, (5 dips).
8. Dip in 95% alcohol for 1-2 minutes, (5 dips).
9. Stain with eosin for 1-2 minutes.
10. Dehydrate in 95% and absolute alcohols until excess eosin is removed, 3 changes of 3 minutes each, and 2 changes of 3 minutes each, respectively.
11. Xylene, 3 changes of 2 minutes each.
CHROMOTROPE 2R STAIN

Solutions
Melt 1 gm of phenol crystals and add 0.5 gm of Chromotrope 2R.
Mix well and dissolve the mixture in 100 ml of distilled water.

Staining Procedure
1. Stain in hematoxylin as usual.
2. Wash in tap water.
3. Stain in Chromotrope solution for 1/2 - 1 hour.
4. Rinse in tap water, dehydrate, clear and mount.

Results:
Eosinophils: red, nuclei: blue.
Prussian Blue Reaction

Staining Solution:

0.4 gm potassium ferrocyanide

40 ml 0.06N hydrochloric acid (1 ml conc. HCl in 199 ml H₂O)

Counterstaining Solution:

0.5 gm basic fuchsin

100 ml 1.0% acetic acid (v/v)

or

0.1 gm safranin 0

100 ml 1.0% acetic acid (v/v)

Staining Schedule:

1. Deparaffinize and hydrate sections through distilled water.

2. Flood slide with PBR solution for 1/2 to one minute. Do not heat.

3. Rinse in distilled water.

4. Counterstain in basic fuchsin for a few seconds and differentiate in 95% ethyl alcohol. Or counterstain in safranin 0 for two to five minutes. Omit counterstaining for critical evaluation.

5. Dehydrate, clear and mount in Permount.
Fixation. 10% buffered neutral formalin.

Technique. Cut paraffin sections at 6 microns.

Solutions

Rubeanic Acid (Dithiooxamide) Solution

Dithiooxamide ... 0.1 gm

Alcohol, 70% .... 100.0 ml

Staining Procedure

1. Deparaffinize in xylene, two changes.
2. Equal parts of xylene-absolute alcohol, two changes.
3. Absolute alcohol, two changes.
4. Rubeanic acid solution for 20 minutes.
5. Add solid sodium acetate (0.2 gm/100 ml) to the staining jar and allow to settle to the bottom - leave slides for 24 hours.
6. 70% alcohol, two changes of 1 - 1/2 hours each.
7. Absolute alcohol, 24 hours.
8. Clear in xylene, two changes.
9. Mount with Permount or Histoclad.

Results

Copper - fine granular black precipitate.
Example of Analysis of Data using the Between-Within Method

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3</td>
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<tr>
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<tr>
<td>1</td>
<td>3.5</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>2.5</td>
<td>3.5</td>
</tr>
</tbody>
</table>

\[
\begin{align*}
\chi^2 & = 12.5 \quad 16 \\
\chi^2 & = 36.25 \quad 51.5 \\
(\chi^2) & = 156.25 \quad 256
\end{align*}
\]

Total SS = \((\chi_A^2 + \chi_B^2) - (\chi_A + \chi_B)^2\)

\[
(28.5)^2 \quad 10
\]

= 6.53

Between SS = \(\frac{(\chi_A^2)}{N_A} + \frac{(\chi_B^2)}{N_B} - \frac{(\chi_A + \chi_B)^2}{N}\)

<table>
<thead>
<tr>
<th>Variance Source</th>
<th>Degrees of Freedom</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>1*</td>
<td>1.23</td>
<td>1.23</td>
<td>1.86</td>
</tr>
<tr>
<td>Within</td>
<td>8*</td>
<td>5.30</td>
<td>.66</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>9*</td>
<td>6.53</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total DF = \((N_A + N_B) - 1\)

Between DF = \((N-1)\)

Within DF = Total - Between

P = 0.05 \quad F = 5.32 \quad No sign. dif.
Example of Analysis of Data using a T-Test

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2.5</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

\[ \bar{X} = \frac{\sum X}{N} \]

\[ \bar{X}_A = 3.25 \]

\[ \bar{X}_B = 2.87 \]

\[ N = 8 \]

\[ \mu = 3.06 \]

\[ S^2 = \frac{(\bar{X}_A - \bar{X}_B)^2}{N - 1} = 1.40 \]

\[ = S^2 = 1.18 \]

\[ T = \frac{\bar{X}_A - \bar{X}_B}{S/\sqrt{N}} = \frac{3.25 - 3.06}{1.18/\sqrt{10}} = .51 \]

\[ DF = N - 1; \quad 8 - 1 = 7 \]

\[ DF 7 \text{ at } P = 0.05 \quad t = 2.36 \]

\[ .51 < 2.36 - \text{therefore no significant difference} \]
APPROVAL SHEET

The thesis submitted by Richard A. Pasiewicz has been read and approved by the following committee:

James L. Sandrik, Ph.D., Director
Associate Professor, Chairman Dental Materials, Loyola

Norman K. Wood, D.D.S., Ph.D.
Professor, Chairman Oral Diagnosis, Loyola

Gerald R. Guine, D.D.S., M.P.H.
Associate Professor, Preventive Dentistry and Community Health

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science in Oral Biology.

Date April 20, 1979

Director's Signature