INVESTIGATION INTO THE EFFECTS OF FILLING MATERIALS ON THE DENTAL TISSUES

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SUMMARY

This Thesis describes an investigation into the effects of zinc phosphate cement, silver nitrate and silver-tin amalgam on the dental tissues.

In the introduction a review is given of the irrational use in the past of filling materials and of previous investigations into the effects of filling materials on the vital dental tissues. The importance of the close physiological relationship between dentine and dental pulp is emphasised. The development of the use of radioactive isotopes in biological research and their suitability for further use in investigating the effects of filling materials is discussed.

Methods of detecting and measuring these radioactive materials are described with particular application of these methods to this investigation.

A description of the filling materials used in this treatise is given with the methods of preparing them for radiochemical examination. The selection of teeth, both human and animal, is also described together/
together with a standardized method for preparing the tooth cavities.

The history of zinc phosphate cement is reviewed and the complex reaction which takes place when the powder and liquid of this cement are mixed is described. It is shown that phosphoric acid and primary acid phosphate are present when the cement is in the plastic state. The importance of variations in the consistency of the plastic cement is stressed. Measurements of acidity are also given which show that this cement is strongly acid when placed in the tooth cavity, and remains acid after setting.

The results of the experimental work on this material are incorporated into a discussion. These results confirm that, a reaction of some degree will occur in the dental pulp when this cement is placed in contact with vital dentine. Radioactive tracers show that the phosphoric acid and primary acid phosphate present during the setting reaction penetrate along the dentinal tubules cut during cavity preparation. The degree of penetration of the acids and the severity of the pulpal reaction vary with the consistency and time/
time of insertion of the cement into the tooth cavity. Penetration and reaction are most severe with thin consistencies inserted immediately after mixing. A layer of calcium hydroxide paste placed between the cement and the dentine is effective in reducing the severity of the pulpal reaction. The results also show that no diffusion of arsenic takes place from zinc phosphate cement into dentine.

A chapter on silver nitrate describes the history of this material and methods of applying it to the dental tissues. The results of the investigations are given and a discussion is included.

In assessing the results of the silver nitrate experiments, the variations which may occur in the permeability of dentine are stressed. It is demonstrated that silver nitrate is taken up by the enamel of the teeth at a constant rate in relation to the duration of the application. The penetration of silver nitrate through vital dentine is also shown. The use of the medicaments, alcohol and phenol, on the cavity floor is shown to increase the permeability of the dentine to silver nitrate; and it is advised that/
that neither phenol nor alcohol should be applied to freshly cut dentine. A possible explanation is given to the varying results on dentine permeability to different dyes.

The chapter on silver-tin amalgam gives the history of this material. It describes the controversy which arose regarding the possible toxic effects due to systemic absorption of mercury. In the discussion, it is established from the results obtained that systemic absorption of mercury by the tooth pulp is most unlikely to occur.

The Appendix to the Thesis describes in detail the radiochemical methods of analysis for the presence of arsenic, silver and mercury in the dental tissues.

A bibliography appears at the end of each chapter of the treatise.
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CONTENTS

Acknowledgments

Chapter 1 Introduction
Bibliography

Chapter 2 Scheme of Study

Chapter 3 Methods of Measuring and Detecting Radioactivity

(i) Autoradiography
(ii) Quantitative Methods
(iii) Activation Analysis
(iv) Bibliography

Chapter 4 Materials and Methods

Chapter 5 Zinc Phosphate Cement

(i) History
(ii) Chemistry of the Setting Reaction
(iii) Consideration of pH Value
(iv) Experimental Work
(v) Discussion
(vi) Bibliography

Chapter 6 Silver Nitrate

(i) Experimental Work
(ii) Discussion
(iii) Bibliography
CONTENTS

Chapter 7 Silver-Tin Amalgam 168

(i) Experimental Work 174
(ii) Discussion 176
(iii) Bibliography 178

Appendix

1. Estimation of Arsenic in the Dental Tissues 180
2. Estimation of Silver in the Dental Tissues 183
3. Estimation of Mercury in the Dental Tissues 185
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INTRODUCTION

During the early part of the nineteenth century it was considered that the calcified part of the mature erupted tooth was totally composed of inert material. Unfortunately, this popular concept gave rise to many undesirable procedures relating to the indiscriminate use of filling materials in conservative dentistry, without regard to their effects on the dental tissues. In many cases degenerative changes of the pulp took place over a long period, without actually causing necrosis. Germicides, also, such as phenol, alcohol and silver nitrate, were used indiscriminately in the belief that, by their use, cavity sterilisation could be effected and the progress of caries halted before the insertion of the filling material. This practice was inadequate and empirical, when viewed in the light of later research.

Pierre Fauchard (1728),(1) one of the early teachers/
teachers of dentistry, was among the first to realise that further investigation into the histological structure and biological reaction of the tooth was necessary. He wrote:

"In order to clarify the subject of which I treat it would be necessary to explain the structure, relations and the particular mechanics of the teeth. It is on the understanding of these parts that I shall establish my theory and practice."

Unfortunately his concepts were forgotten until the early part of the present century when they were re-emphasised by Walkhoff in an introduction to his "Normal Histology of the Human Teeth", in 1901.

"Those dental surgeons who have had experience in making and examining sections of teeth are able to treat carious cavities with greater knowledge, and to appreciate and understand any complications which may arise, and therefore take more appropriate and more therapeutic measures than those unaccustomed to the science."

Advances in research, however, took place during the early part of this century particularly with regard to the response of the tooth to many types/
types of stimuli. These stimuli could be thermal, chemical, bacterial, electrical or traumatic. It was then realised that the tooth was vital and could respond readily to any stimuli mentioned above, and was not an inorganic, unresponsive organ. It then became evident that the concepts of Pierre Fauchard were beginning to bear fruit.

A great deal of research was undertaken during this period and many results were forthcoming. Fish\(^{(3)}\), basing his experiments on the pioneer work of Tomes\(^{(4)}\) and Hunter\(^{(5)}\), was one who did much to advance research into the reactions of the tooth to stimuli. He showed that injury of any nature to the dentinal fibrils in dentine caused a reaction at the pulpal ends, the amount of injury to the pulp depending on the intensity of the injury. He noted that this reaction had the effect of sealing off the damaged tubules by deposits on the surface of the pulp and the neighbouring undamaged tubules, so creating a 'dead tract' in the dentine. By other experiments he demonstrated the presence of tissue fluid circulating throughout the dentine. Fish's experiments/
experiments showed very clearly the close relationship which existed between the dentine and the pulp, and that biologically they could be considered as one tissue. This work was indeed a landmark in the advancement of dental knowledge. Subsequent investigations were carried out by Manley (6) and others, notably Palazzi (7), Fasoli (8) and Gurley and van Huysen (9), many of them connected with the reaction of the pulp to the irritant effect of certain filling materials.

One very important fact arose from these investigations, namely, that the trauma produced during cavity preparation was itself sufficient to produce changes in the dental pulp.

Many improvements in histological techniques took place and investigations continued. The use of dyes and silver nitrate demonstrated visibly the permeability and penetration of the dentinal tubules to many varied materials. Timms (10) showed that traces of the heavy metals from dental amalgams could eventually penetrate the dentinal tubules.

Recent advances in technical methods of investigation such as the electron microscope and the/
the use of radioactive isotopes as tracer elements, have again given a fresh impetus to dental research and it was mainly by the use of radioactive isotopes that this study was undertaken. The use of radioactive and stable isotopes in the study of biological problems is a comparatively recent development. Hevesy in 1923, showed the value of tracers by using the natural radioactive isotopes of lead and bismuth to study the metabolism of these elements in plants and guinea pigs respectively. However, the choice in these days was extremely limited as only a few naturally occurring isotopes were available.

The discovery of deuterium by Urey in 1931, and further advances by the Joliot-Curies in 1933, enlarged the scope of this work by increasing the number of isotopes. Supplies of isotopes were limited until after the development of the cyclotron, which again increased the number of available radioactive elements. With the greater number of radioactive and stable isotopes, the fields which could be investigated were widened. Facilities for obtaining suitable isotopes for tracer investigations in biology/
biology and medicine, including dentistry, have improved rapidly during the last 10 years. The general impetus for this work was the very rapid development of atomic energy research and the construction of atomic nuclear reactors, during and after the 1939-1945 war.

This development was important as isotopes became available for tracing elements - important in dentistry as many of the materials could be 'labelled' with radioactive isotopes, e.g. Phosphorus, Mercury and Silver.

It is now possible to follow throughout the dental tissues the course of many of the materials used in dentistry and to measure accurately, minute quantities of elements such as Arsenic and Mercury within these tissues.
BIBLIOGRAPHY


10./


CHAPTER 2

Scheme of Study
SCHEME OF STUDY

Investigations and experiments into the effects on the dental tissues of filling materials used in conservative dentistry, were undertaken in the University of Glasgow Dental School and various other University Departments; and in the Physics Department of the Western Regional Hospital Board.

The following materials were chosen for investigation because of their widespread use in conservative dentistry:

- Zinc Phosphate Cement
- Silver Nitrate
- Silver-Tin Amalgam.

Previous investigations have given much information on these materials but our knowledge of their effects and distribution in the dental tissues is still incomplete. The application of radioactive isotopes to conservative dentistry has made it possible to undertake further investigation, by incorporating radioactive tracer elements within these materials.

The/
The following investigations and experiments were carried out:-

**Zinc Phosphate Cement**

1. Investigation into the chemical nature of the setting reaction resulting from the interaction between the powder and liquid of the cement.

2. The measurement of the penetration into dentine of the acid present during the setting of the cement, with particular relation to:
   - (a) The consistency of the cement at the time of insertion into the tooth cavity.
   - (b) The time of insertion of the cement, after mixing, into the tooth cavity.
   - (c) The time which the cement remains in the tooth cavity.

3. The effectiveness of calcium hydroxide as a means of preventing the penetration of the acid products from zinc phosphate cement.

4. The effects of the cement on the dental pulp with emphasis on the consistency of the cement at the time of insertion into the tooth cavity.

5. The measurement of the amount of arsenic which may diffuse from the zinc phosphate cement into the dental tissues.
Silver Nitrate

1. Measurement of the uptake of silver nitrate by the enamel.


3. The effects of medicaments used in cavity sterilisation on the penetration of silver nitrate.

Silver/Tin Amalgam

Measurement of the amount of mercury in enamel and dentine which may result from diffusion from silver/tin amalgam.
CHAPTER 3

Methods of Measuring and Detecting Radioactivity
THE STRUCTURE OF THE ATOM

Present-day theories of atomic structure postulate that each atom of every element is composed of three fundamental particles in varying proportions.

A single atom of any chemical element contains a central nucleus which carries practically the whole mass of the atom but which only occupies a minute fraction of its total volume. The nucleus contains only protons and neutrons and is surrounded by an orbital system of one or more electrons. The Mass Number is equal to the sum of the number of protons and neutrons. It carries a positive electric charge equal to the number of protons it contains, and this value is independent of the number of neutrons. As the atom, when considered as a whole, must be electrically neutral, this positive charge on the nucleus must be balanced by the orbital electrons which will, therefore, be equal in number and in total negative charge to the number of protons in the nucleus. This number is called the Atomic Number of the atom.
Isotopes

The chemical properties of an element are determined entirely by the number of electrons in the orbital systems of its individual atoms, i.e. by the Atomic Number, and not by the Mass Number. It follows, therefore, that all the atoms present in a sample of any one element will have the same Atomic Number, but not necessarily the same Atomic Weight, i.e. all the atoms will have the same number of nuclear protons, but the number of neutrons in the nuclei may differ. These atoms, differing in the number of neutrons in their nuclei, are termed isotopes of the element. They have, in most cases, identical chemical reactions, and, excluding for the moment the question of radioactivity, they differ only in their atomic weights. These isotopes may be found either as stable isotopes or as radioactive isotopes.

Compared with stable isotopes radioactive isotopes have an excess of neutrons or protons in their nuclei. This results in the nucleus becoming unstable and these unstable atoms are said to
to be radioactive, the radioactivity continuing until the nucleus becomes stable. In order to become stable, nuclear transmutations or changes occur. These nuclear transmutations occur either by the expulsion of an Alpha-particle from the nucleus or by one of the proton-neutron interconversions.

During the course of this nuclear change a variety of rays or particles are emitted, Alpha-particles, Beta-particles and Gamma or X-rays. Once any single radioactive atom has completed its characteristic changes it ceases to be radioactive. Thus any mass of radioactive material is continually decreasing in radioactivity, or 'decaying' with the production of an inert material of a different composition.

Some isotopes have a simple decay scheme with all the nuclei decaying in the same way and emitting the same type of radiations. Other isotopes, e.g. silver, have a complicated decay scheme. In these cases different types of radiation may be obtained. All radioactive materials decay according to an exponential law. The rate of decay or disintegration/
disintegration is conveniently indicated by the 'half-life' which is the time required for the radioactivity to fall to half of its previous value. Half-lives of the useful radioactive elements in biological application, vary between a few seconds and a few years. Their usefulness lies in the fact that radioactive atoms behave like ordinary atoms but their behaviour can be traced by the radiations which they emit without disturbing the chemical systems in any way.

General Principles of Isotope Tracer Technique

Many attempts have been made throughout the whole history of biochemical investigation to find a method whereby organic compounds could be identified or 'labelled' in such a way that they could be traced within the body.

One of the most satisfactory labels used by biochemists was the benzene ring. Knoop, \(^{(1) (2)}\) made valuable contributions to our knowledge of fatty acid metabolism as a result of his studies with benzoic acid/
acid, phenylacetic, and other phenyl fatty acids. The benzine ring in such compounds is largely resistant to disruption in the animal body and the study of aromatic compounds excreted in the urine, following the administration of these substances, gave useful information about the metabolic fate of the particular acid or other labelled compound under investigation.

Other methods of investigation were available by using some of the few naturally-labelled compounds such as proteins containing copper or iodine, but the investigations which could be made with these compounds were very limited. This was also true for drugs which contained readily determinable elements such as arsenic and antimony, or protein conjugates of the azo-protein type.

None of the methods mentioned above could be applied generally and the development of the isotope tracer method opened up a new field in biological research.

Essentially, the isotope tracer method involves the labelling of a compound with one or more radio-active/
active or stable isotopes in such a way that the 'label' is firmly attached to the molecule and will not be removed by any metabolic process. The labelling is accomplished either by altering the ratio of the stable isotope present in the element or by adding a radioactive isotope. Either of these two types of isotope is called a 'tracer' as its presence can be detected throughout the investigation.

In the present work all the isotopes used were radioactive, and their presence detected and measured by the methods described below.

The Measurement and Detection of Radioactive Isotopes

The quantitative determination of radioactivity is frequently based on the ionization effects produced in gaseous media by radiation emitted from radioactive bodies. The Geiger-Müller counter is an example of the type of apparatus utilising this principle. As these counters, however, are relatively insensitive to Gamma or X-rays, scintillation counters are used to/
to overcome this difficulty. These counters take advantage of the physical effects produced when Gamma or X-rays are absorbed in a suitable solid medium. This solid medium may consist of a crystal of such substances as anthracene, calcium tungstate, solium iodide (when activated by thallium) and certain polymerised terpene derivatives. These substances are collectively termed 'phosphors' and have the property of emitting a scintillation or light flash when they absorb incident radiations from radioactive sources. These scintillations may be too small to be seen with the naked eye or they may not be of a wavelength falling within the visible spectrum. Their presence is detected by using an electronic device called a photomultiplier or an electron multiplier which responds to the reception of a single photon by emitting a large number of electrons.

The amount of the radioactive element present is measured in terms of the amount of radioactive emission produced by the disintegration of the nucleus. The unit of measurement is the curie (c), which/
which is the amount of radioactive isotope required to give $3.7 \times 10^{10}$ disintegrating nuclei per second.

A typical tracer experiment may involve the use of only a few micro-curies (uc) or millicuries (mc), millionths or thousandths of a curie respectively.

Detection

There are a number of techniques whereby the passage of radiation may be used to evoke a visual effect. The presence in the tissues of the radioactive material may be observed by the visual effects which radiations produce on a photographic emulsion.

The techniques used in this method are all based on the activation of photographic emulsion treated so as to be sensitive to various kinds of radiation. This procedure is called autoradiography.

In general, photographic visualisation of the distribution of radioactive materials may be attained in the following manner.

The tissue section containing the radioactive element is prepared by the usual histological techniques. The section is placed in close contact with/
with the photographic emulsion. All regions of the tissue containing radioactive element emit radiation which produces effects on the emulsion, similar to visible light. When the film is developed, all regions corresponding to the location of the radioactive element are darkened, so that a photographic image of the distribution of the radioactive element is obtained. The image, so produced, is called the autoradiograph.
AUTORADIOGRAPHY

Development of Autoradiographic Techniques

Autoradiography is the process by which an image is produced on a photographic emulsion by radiation from a radioactive substance. The term was first used by Lacassagne\(^{(3)}\) \((\text{vide infra})\) who called the results of this process 'autoradiographs' or 'histoautoradiographs'.

The development of autoradiographic technique goes back to the year 1896 when Henri Becquerel attended a meeting of the French Academy of Science at which Henri Poincare showed X-rays and read a paper which had been sent by Roentgen from Vienna. It was suggested at this meeting by Poincare that X-rays may arise from fluorescent radiation from the walls of the glass X-ray tubes. Becquerel\(^{(4)}\) commenced work with this idea in mind. He showed that the emulsion of a photographic plate could be blackened by uranium salts, after they had been exposed to sunlight. Further experiments showed him/
him that it was possible to obtain an image on a photographic plate with uranium salts which had been previously exposed to sunlight. At first this experience convinced him that this phenomenon was due to fluorescence but later he began to consider the possibility of the emission of rays from the uranium salts. In this way the first autoradiographs were made, but, unfortunately, were not recognised as such.

It remained for Marie Curie\(^5\) in 1898, to establish by ionisation measurements the relationship between the quantity of radiation to the quantity of the radioactive element present.

The first published application of autoradiographs, for biological research, appeared in 1904 by E.S. London\(^6\) of the Imperial Institute of Experimental Medicine in St. Petersbourg, and was made by exposing a frog to radium emanation. In this paper no details of the technique were given, however.

In 1924, Lacassagne\(^7\),\(^8\) and his co-workers at the Radium Institute in Paris, in a long series of papers, reported their studies of the distribution of polonium/
polonium and other elements in histological specimens. Their first method was to embed the tissues in a paraffin block and to place the flat surface of the block against the photographic plate which was later developed. The plate was blackened in the regions in which the polonium was located. This was an important discovery which opened up a new field of research. It was now possible to locate individual radioactive elements in the tissues.

The first autoradiograph of induced radioactivity was made by Groven, Govaerts et al \(^{(9)}\) when they demonstrated that a photographic plate could be blackened by neutron-irradiated iridium.

New methods of autoradiography were developed. Leblond et al \(^{(10)}\) mounted sections on a glass plate for apposition with a photographic plate in order to study the distribution of polonium.

One of the main disadvantages of these methods was that the stained sections and the resultant autoradiographs, had to be studied independently. Thus it was difficult to determine the precise location of the radioactive element. In order to overcome/
Apparatus for cutting ground sections. Perspex shield to prevent inhalation of dust is shown by the arrow.

Fig. 1
overcome this, Belanger\(^{(11)}\) developed the first successful method for observing simultaneously the autoradiograph and the stained tissue under the microscope.

In recent years new techniques of autoradiography have been developed which have increased the accuracy and reliability of the process. Improvements in nuclear film emulsions by commercial firms and a greater understanding of the reaction between ionising particles and film emulsions have contributed to this. These new developments, by making it possible to establish the precise location of radioactive elements within the tissues, have opened up many new fields for research including dentistry.

The autoradiographic technique used in this work is the 'Stripping-Film' technique developed by Donniach and Pelc\(^{(12)}(13)\), a description of which follows.

**Preparation of Ground Sections**

Ground sections of teeth were prepared using the apparatus shown in Fig.1. In order to prevent inhalation/
inhalation of the dust from the cutting process the section cutting was done under a perspex shield.

Tooth sections were cut with a thin diamond disc and the direction of the cutting wheel was always away from the tooth cavity containing the radioactive compounds to avoid contamination of the section. The surface of the cutting diamond was kept under a constant flow of water when preparing sections in which radioactive silver nitrate had been used. The sections which contained radioactive phosphorus were cut using xylol or alcohol to avoid leaching of the phosphorus from the specimen.

The final polishing of the tooth in each case was completed between two ground glass plates using pumice powder as a rough polishing paste initially, and finally employing precipitated chalk to give a fine polish. When the sections were ground to a thickness of 100-150 μ they were washed thoroughly to remove any debris left from the grinding. Water was used to wash sections containing radioactive silver nitrate, and absolute alcohol was employed for washing the radioactive phosphorus specimens.

The/
The sections were finally cleared in xylol before mounting on the prepared glass slides.

**Preparation of Glass Slides**

In permanent contact autoradiographs, especially those using the stripping-film techniques, the adhesive bond between the wet gelatine of the film and the glass slides is insufficient to prevent displacement of the image, relative to the site of radioactivity. Leblond\(^{(14)}\) and Bogoroch\(^{(15)}\) recognised this fact early and advised that the glass slides should be treated with an adhesive to ensure a good wet adhesion of the emulsion to the glass when the autoradiograph was processed. This treatment of the glass slides with an adhesive is called 'subbing' the slide and has been used in all the experiments in this thesis.

Before subbing, the slides were cleaned by being placed in the following solution:

\[
\text{Potassium bichromate} \ldots \ldots \ldots 100\text{gm.} \\
\text{Sulphuric acid} \ldots \ldots \ldots \ldots \ldots \ldots 100\text{cc.} \\
\text{Water to make} \ldots \ldots \ldots \ldots \ldots \ldots 1000\text{cc.}
\]

After cleaning, the slides were washed in water and/
Fig. 2.

The upper photograph illustrates the lines along which the emulsion and gelatine layer of the film is cut. The method of removing the strips of film from the glass plate is shown in the lower photograph.
and dipped bodily in the following solution at 70°F.

Gelatin................. 5.0gms.
Chrome alum............. 0.5gms.
Water to make........... 1000cc.

The prepared slides were placed on a rack and allowed to dry.

**Preparation of the Autoradiograph**

The photographic emulsions which were used were those of the Kodak Autoradiograph Plates A.R.10 and A.R.50. These plates consist of a thin coating of a very fine grain nuclear track emulsion carried on a gelatine layer. A Wratten Series No.1 Safelight was used throughout the preparation of the autoradiograph. The emulsion and gelatine layer of the film was first cut through with a sharp scalpel blade at a distance of $\frac{1}{4}$" from the edge of the plate *(vide Fig. 2)*. The film was cut to obtain strips of film about $1\frac{1}{2}$" wide and about 2" long. The strips of film were lifted slowly from the glass and floated in distilled water at a temperature of 23°C-25°C, in such a way that the emulsion face was downwards. The film was allowed to soak for a period of 2-3 minutes.
to allow expansion of the emulsion to take place.

The slide with the specimen facing upwards was slipped into the dish under the floating film. The film and the section were removed together by holding the slide at an angle of 30° from the horizontal so that the edge of the section touched the emulsion first. In this way the emulsion draped itself over the section, allowing the water to drain away as the slide was gradually lifted clear of the water.

The specimen was dried in a stream of cool air and placed in a light-tight box until a suitable exposure time to the radioactive material had elapsed.

The Exposure Time

The exposure time is the time required to permit a number of ionising particles from the radioactive material, contained in the specimen, to strike a unit area of the film emulsion. In practice it is impossible to predict this time accurately from theoretical considerations alone. Electronic methods of calculation may be used if the radioactivity/
radioactivity is uniformly distributed throughout the tissue section. The degree of distribution, however, is unknown until the autoradiograph is made. As all the autoradiographs prepared for this work showed a non-uniformity of distribution of the radioactive material, the electronic method could not be used. It was found, therefore, that the best method of determining the exposure time was by trial and error for each radioactive isotope used.

Factors which affect the exposure time are the radioactive strength of the isotope used and the speed of the film emulsion.

**Processing the Autoradiograph**

The autoradiograph is processed with the emulsion layer of the film in permanent contact with the specimen so that the final autoradiograph is in perfect register with the tissue section.

The sections were placed in a solution of Kodak D.19B. developer for a period of 5 minutes at a temperature of 17.5°C-18.0°C.

The autoradiographs were washed in distilled water/
water for one minute, then fixed in an acid-hardener solution until the film emulsion became transparent. In the stripping-film autoradiographs, when the film is unprotected during the microscopic examination, it is important to use acid hardener solution.

The autoradiographs were finally washed in distilled water and placed on racks to dry in a dust-free atmosphere. After the autoradiographs were dried, the sections were dehydrated by flooding in frequent changes of absolute alcohol for 30 minutes and cleared with xylol for a further 15 minutes. The sections were then mounted in D.P.X. medium and protected with cover slips.

Resolution

In autoradiography, the term 'resolution' is taken to mean the precision with which sources of radioactivity may be located. If the resolution of the autoradiograph is good, microscopic sources of radioactivity may be located and, in some cases, evaluated quantitatively. To obtain the degree of resolution necessary for quantitative measurement very/
Film emulsion demonstrating pattern of silver halide image grains. Mag. X 60.
very thin sections have to be used with a thin film emulsion. It was not possible, in this work, to measure the amount of radioactive element in the sections owing to the difficulty of obtaining ground sections less than 100 μ-150 μ in thickness.

**Interpretation**

The ultimate aim of autoradiography is the production of an image on the film. This image corresponds to the distribution of the radioactive material throughout the tissue section and is composed of developed grains of silver halide. Ionising particles from the radioactive material are responsible for the production of the image, which varies in density according to the strength of the ionising agent at any given site in the section.

In microscopic examination these silver halide grains appear as black specks distributed either as single grains at random or as patterns of single grains throughout the autoradiograph (*vide* Fig. 3).

The production of undesirable random grains, known as background grains or 'fog', occurs in each autoradiograph/
Fig. 4.

Autoradiograph showing displacement of image on film in relation to cavity.
autoradiograph. These are produced by agents other than the ionising particles and must be kept to a minimum during the preparation of the autoradiograph.

It is important to remember, when interpreting, that the density of the image is uniformly increased by the degree of background present and that, where there is only slight radioactivity, it may be impossible to differentiate between random grains forming the image and those forming the background.

Displacement of the image relative to the specimen may also occur due to lack of adhesion between the emulsion and the glass slide. In the majority of autoradiographs this can readily be recognised as shown (**vide** Fig. 4).

By using the method described above it was possible to produce successful autoradiographs on tooth sections.
Autoradiographic methods are used to determine the precise location of radioactive material within tissues which have been treated with radioactive elements, or even in individual cells but, as explained previously, the method is unsuitable for the quantitative assay of radioactive materials. The methods which are used for quantitative assay are generally based on measurements of the ionisation produced in various media by the radiations from radioactive material.

The Geiger-Muller tube is the most sensitive type of detecting apparatus available for general purposes and the most convenient generally for a variety of different types of measurement. The Geiger-Muller tube is particularly sensitive to Beta rays and may have measuring efficiency up to 100% for the rays which enter the counter, but for measuring Gamma or X-rays its efficiency is much less, being of the order of 5%. As most of the quantitative/
quantitative experimental work was done with radioactive silver which emitted Gamma rays, another form of measuring instrument, a scintillation counter, was used in lieu of the Geiger-Muller tube to increase the efficiency of the experiments. The scintillation counter has a 10% higher efficiency for the measurement of Gamma rays than the Geiger-Muller counter.

History

As mentioned in the chapter on Autoradiography, (vide supra.) Henry Becquerel found that radiations like X-rays and Cathode rays possessed the ability to produce fluorescence in a number of substances such as zinc sulphide. This property of the radioactive rays was developed further by Marie Curie and A. Debierne(16) in the study of gaseous emanations.

In 1903, it was reported by a number of observers that the fluorescence produced in these materials was not continuous but consisted of a number of light flashes which were visible under the microscope.

Rutherford, in 1904, wrote -

"In/
"In the scintillations of zinc sulphide we are actually witnessing the effect produced by the impact of single atoms of matter ....... This would offer a very convenient means of actually counting the number of particles ....... if each particle gave rise to a flash of light."

At that time, Rutherford did not think it possible to do so but, later, when he was working in collaboration with Geiger, he proved that this was actually the case. They showed that each Alpha-particle from the radioactive material gave rise to a single scintillation in the crystal and caused a single pulse in the counting unit.

Early forms of scintillation counters were crude, and involved tedious visual observations. The efficiency of this method was very low indeed because of the human error in recording, and it remained only as an interesting phenomenon until recent years when, with the advances in electronics, accurate measurements of the scintillations could be recorded.

In using scintillation counters to detect and measure Gamma rays, advantage is taken of the fact that physical effects are produced when these rays are/
are absorbed in suitable solid or liquid media. These media are composed of substances such as anthracene, calcium tungstate, thallium-activated sodium iodide and certain polymerised terpene derivatives, which are collectively termed 'phosphors'. These phosphors have the property of emitting a scintillation, or light flash, when they absorb radiation from radioactive sources.

The scintillations, however, are too small to be seen with the naked eye, or they may not be of a wavelength falling in the visible spectrum. Their presence may, however, be detected by using a photomultiplier or electron multiplier. The light which is produced by a single scintillation in the crystal is too feeble to allow a direct measurement. If this light is allowed to fall on the cathode of the photomultiplier tube it releases a number of electrons which are greatly amplified in the successive stages of the multiplier tube, so that a measurable current pulse is produced. The electrons finally emitted by photomultiplier are collected and fed into a suitable electronic counting device called a Scaler. Each/
Fig. 5.

Ecko N550 scintillation counter with specially designed perspex jig, showing relation of tooth (A) to detecting crystal (B). The lid of the lead shield has been left open to demonstrate this.
Scintillation counter connected to Panax Universal Scaler type C100. The lid of the lead shield is now closed in contrast to Fig. 5 where it is open.
Each single pulse which is emitted from the radioactive material is detected and recorded separately. The total number of pulses emitted in any given period of time may be measured.

**Equipment**

An Ecko Scintillation Counter N.550 was used (vide Fig. 5). A special perspex jig (vide Figs. 5 and 6) was constructed which enabled any tooth under examination to be placed in a constant defined position relative to the detecting crystal. The jig was placed over the crystal of the scintillation counter as shown. The counter was connected to a Panax Universal Scaler Type 100 C. (vide Fig. 6).

The efficiency of this arrangement was tested by placing in a perspex jig a tooth which had been previously immersed in a radioactive silver nitrate solution. The radioactivity recorded from the tooth was determined and expressed as counts per minute. In order to determine that the tooth could be replaced in a constant position in the perspex jig, it was removed and immediately replaced, when the/
Table 1.

<table>
<thead>
<tr>
<th>Counting Rates per Minute</th>
<th>1st Count</th>
<th>2nd Count</th>
<th>3rd Count</th>
<th>4th Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17240</td>
<td>17580</td>
<td>17408</td>
<td>17040</td>
</tr>
</tbody>
</table>
the counting rate was again determined. This was repeated a number of times and the results are shown in Table I. It can be seen from the results that this arrangement was satisfactory.

Methods of Expressing Results

The amount of radioactivity recorded from the specimen under examination is expressed in counting rates over a fixed period. The results are expressed as counts per minute or, when specimens are used which have a high radioactivity, the results may be expressed as counts per second.

The term 'count' is purely an arbitrary unit and depends on a number of variable factors such as the type of counter used and the nature and energy of the radioactive emission.

Interpretation of Results

The fundamental determination of the amount of radioactivity from any sample is based on the counting rates observed on the Scaler unit. Certain corrections must be made, however, to these observed rates/
rates before they can be taken as an accurate representation of the amount of radioactive isotope present in the sample.

Correction for Background

The instruments used for ionisation measurements are extremely sensitive and are unable to distinguish between different types of ionising radiations. They record, therefore, a background count rate even when no radioactive source is being measured. This background is partly due to cosmic radiation and radon and thoron in the air, and partly to the small amounts of natural radioactivity from the materials of which the measuring equipment is constructed. To obtain the best results, the background must be kept as low as possible.

In practice the actual counting rate from the radioactive sample cannot be determined directly but must be calculated by subtracting the independently determined background rate from the observed count rate before making any further corrections. This background rate is determined by observing the count rate/
rate without any sample present in the scintillation counter.

Correction for Decay of Radioactive Isotope

In experiments employing radioactive isotopes with short half-lives, corrections for the decay were made by using a standard decay factor which is calculated for each isotope used.

When the radioactive isotope used had a long half-life, as in the radioactive silver nitrate, a standard solution was prepared. This standard solution contained a known fraction of the original radioactive isotope. The ratio between the corrected count rate of the unknown sample and the standard sample will measure directly the ratio of the amounts of radioactive isotope in the two samples, as they will have decayed at the same rate.

Statistical Consideration of Counting

Apart from the necessary corrections to the observed count rate obtained with the radioactive sample/
sample, the accuracy of measurement which can be achieved is dependent *inter alia* on the actual value of the count rate and of the background rate. The disintegration process for a number of radioactive atoms takes place according to the laws of probability. Thus the number of counts observed in a short time interval compared with the half-life varies from one count to the next, about a mean value. The error in an observation of a number of random events such as this is represented by a function known as the Standard Deviation. This Standard Deviation is shown as equal to the square root of the total number of events recorded, i.e. the square root of the total number of counts recorded.
Activation Analysis

Radioactive isotopes can be used in analysis to determine the components present in samples in both macro and micro concentrations. The number of applications of radiochemical techniques to analytical problems is large and a wide field of application has been opened up.

The determination of the concentration of most of the elements in the periodic system can be performed by activating the required element with neutrons and separating it from the other elements present in the sample, either by chemical or electronic means. This method of analysis can be used to determine amounts of different elements as low as $10^{-7} - 10^{-10}$ gm. The chemical separations are often similar to those of a refined quantitative analysis method.

The method of activation analysis is as follows:

The sample is weighed into a suitable capsule for irradiation in the neutron flux. After irradiation, a non-radioactive carrier for the element is added to a solution of the irradiated sample. The element is isolated by chemical methods and the weight of the isolated carrier element is determined. The radioactivity of the desired element is now measured.
Electronic methods can sometimes be used to eliminate the necessity of using difficult and often time-consuming chemical separations. If the nature of the rays given off by irradiated elements in the sample are known, it is often possible to separate these rays by electronic means to measure only those of the desired sample.

The application of activation analysis to dentistry has enabled minute quantities of elements in the dental tissues to be measured.


8./


CHAPTER 4

Materials and Methods
MATERIALS AND METHODS

A zinc phosphate cement, which conformed to the specification laid down by the American Dental Association was used throughout this work. The possible effects due to the chemical variation in the composition were avoided by using one material only.

Zinc Phosphate Cement

Radioactive phosphorus was obtained in the form of orthophosphoric acid with a pH1 - pH4 from the Radiochemical Centre in Amersham. The zinc phosphate cement was prepared for radiochemical investigation by adding radioactive phosphate, in the form of phosphate ion, to the cement liquid. Radioactive phosphorus has a half-life of 14.3 days and emits Beta rays only, with an energy of 1.70 MeV. The radioactive phosphoric acid when obtained had a specific activity of up to 4mc. for a source of 0.76 - 0.81gms; this was then added to the cement liquid in amounts to give a specific activity of 3 - 25µc/
3 - 25µc for each cavity, the actual amount depending on the nature of the experiment. In this way it was possible to 'label' the phosphoric acid of the cement liquid with radioactive phosphorus. As a result, the zinc phosphate salts formed by the chemical reaction between the cement liquid and the cement powder were also labelled with radioactive phosphorus.

Measured amounts of cement liquid and weighed quantities of powder were used to produce the desired consistency of the mixed cement. The same consistencies were used for investigations by radioactive and histopathological methods.

Silver Nitrate

Radioactive silver, \(^{110}\text{Ag}\), was obtained from the Atomic Energy Research Establishment in Harwell in the form of radioactive silver nitrate solution with a specific activity up to 3mc. The radioactive silver nitrate was added to an ammoniacal silver nitrate solution to give a specific activity of 5 - 100µc for this solution. Radioactive silver has a half-life of 270 days and decays with Beta and Gamma/
Gamma rays of various energies.

Mercury

Radioactive mercury was obtained from the Atomic Energy Research Establishment in Harwell. The 'short-lived' radioactive isotope, $^{197}\text{Hg}$, was allowed to decay and the 'long-lived' radioactive isotope, $^{203}\text{Hg}$, was used for the experiments. The specific activity of $^{203}\text{Hg}$ was 20 mc. per grammé of mercury. Radioactive mercury has a half-life of 47.9 days and decays with the emission of Beta and Gamma rays of 0.208 MeV and 0.279 MeV respectively.

Selection of Teeth

Young sound teeth were used throughout this work, wherever possible. The purpose of this choice was to avoid those regressive changes which, Kronfield states, 'are on the border-line between the normal and pathological and which occur during the ageing of the pulp.'

Experiments were carried out on teeth selected from the following three groups.

Group 1/
Group 1

Human teeth were used for in vivo experiments with radioactive zinc phosphate cement and also for histopathological examination of the dental tissues. (Permission was given by the Medical Research Council to use 3uc of radioactive phosphorus in human teeth for radiochemical investigation). In the majority of cases these teeth were caries-free premolars which were scheduled for extraction for orthodontic reasons. The ages of the patients were from 10 - 14 years.

When more than one tooth was being extracted in this group, a control cavity was prepared in one of these teeth where histopathological examination was being carried out. In this control tooth, the traumatic effects of cavity preparation on the pulp could be observed.

Thirty-six teeth were used in this group and 54 cavities prepared in the teeth. As it was difficult to obtain sufficient numbers of sound teeth in this group and to persuade patients, or parents, to allow a prolonged time interval to elapse/
elapse between inserting the cement into the cavity and extracting the teeth, further experiments were carried out in teeth from the other groups (vide infra).

In cases which were selected for histopathological examination on live subjects, an attempt was made to demonstrate the reaction of the pulp to zinc phosphate cement, particularly with relation to the consistency of the cement at the time of its insertion into the cavity.

**Group 2**

Extracted human teeth were used for investigations with radioactive zinc phosphate cement, radioactive silver and radioactive mercury. Freshly extracted teeth, which had been kept in normal saline, were used wherever possible. Despite the work of Glasstone (3) on the growth of developing tooth buds in normal saline, extracted teeth must be considered non-vital, similar to these fixed in formalin. Otherwise, extracted teeth which had been fixed in formalin were used. The age of the/
Teeth of Dog aged 6 months

(a) Radiograph of lower left canine tooth.
(b) Radiograph of lower left carnassial tooth.
(c) Section through lower left carnassial tooth.

Fig. 7
Fig. 8

Teeth of Dog aged 5 years

(a) Radiograph of lower left canine tooth
(b) Radiograph of lower left carnassial tooth.
(c) Section through lower left carnassial tooth.
the patient was noted where it was known.

Forty teeth were used in this group; sixty cavities being prepared on these teeth.

Group 3

The teeth of young dogs were used in vivo for radiochemical and histopathological examination of the dental tissues. The method of selecting and preparing the animals is described as follows:

Selection and Preparation of Dogs

Dogs were used as experimental animals in the present investigation. The teeth of dogs of six months and five years old were examined to determine the best age of the animal for this purpose.

At the age of six months the roots of the permanent teeth are fully formed, but the pulp tissue still occupies a large amount of the tooth as shown in Figs. 7a, 7b and 7c. With increasing age there is a continued deposition of dentine on the walls of the pulp cavity with consequent reduction of the cellularity and vitality of the pulp tissue. Figs. 8a, 8b and 8c show teeth of a dog at the age of five years.
Fig. 9

Drawing of mandible of dog to demonstrate the position of cavities on the canine tooth.
The pulp now occupies a small slit-like cavity in the tooth. It was therefore decided to use healthy dogs from eight months - one year wherever possible, to enable the response of healthy pulp tissue to be assessed. However, before using teeth of dogs in this age group, they were examined macroscopically for enamel hypoplasia and any other defects. This ruled out the possibility of impairment of vitality of the dentine or pulp.

The dogs were anaesthetised to third stage anaesthesia with Thiopentone before cavity preparation was commenced. Cavities were prepared according to the standardised procedure on the labial surfaces of the canine teeth and on the third incisor tooth (vide Fig. 9). Contamination of the cavities by saliva was avoided by isolating the tooth with gauze placed in the labial sulcus to enable the lip to be retracted from the tooth.

In order to reduce the trauma from the mechanical preparation of the cavity to a minimum, a slowly-running bur was used and all the cutting done under a cooling jet of air. A new bur was used for each cavity.
cavity.

Fourteen dogs were used for experiments in the zinc phosphate cements. These dogs were divided into two groups:

Nine dogs were used for experiments with radioactive zinc phosphate cement and 108 cavities prepared on the teeth.

Histopathological examination of the dental tissues was undertaken on five dogs in whom 53 cavities had been prepared.

Six dogs were used for experiments with radioactive silver nitrate and 68 cavities prepared on the teeth.

Method of Preparation of Cavities

Local anaesthesia was used while preparing the cavities on human teeth and general anaesthesia on animals. Cavities were prepared on suitable surfaces of the crowns of the teeth according to the standardised procedure, irrespective of the group from which the teeth were chosen.

A round bur was used to penetrate the enamel and/
and the remainder of the cavity prepared by fissure and inverted cone burs. Cavities were prepared to approximately the same depth but no attempt was made to control this accurately once dentine was reached. The important factor which had to be considered, in examination of sections, was the proximity of the floor of the cavity to the pulp. This distance was measured after sections had been prepared.

To avoid contamination of the cavity by saliva, each tooth was isolated prior to cavity preparation either by means of a rubber dam or cotton wool rolls.

In the histopathological investigations it was also most important to ensure that any changes which occurred on the pulp were not due to trauma produced by cavity preparation. To reduce this trauma to a minimum, each cavity was prepared under a stream of water or air using a slowly-running bur, a new bur being used for each cavity. A section of a cavity prepared by the method described above shows normal pulp tissue (vide Fig. 28).

Examination of Tooth Sections

In/
In order to investigate the penetration of the radioactive phosphorus ($^{32}$P) and radioactive silver ($^{110}$Ag) incorporated in the filling material, ground sections were prepared in such a way that the dentinal tubules which extended from the base of the cavity to the pulp could be seen clearly. Autoradiographs were prepared on the ground sections.

The presence of radioactive compounds in the tissues was demonstrated by the presence of the image on the film emulsion. Before determining the presence of radioactive compounds in the tissues, the film covering the section was examined for background radiation. This was done by using an eyepiece with a graticule of 0.25 square millimetres and the number of background grains determined in this area. The total surface of the film covering the section was examined in this way. It was found that there were extremely dense areas which showed, without doubt, penetration of radioactive material. At the edges of these denser areas, radioactive compounds were considered to be present in any part of the section when the number of/
of grains in the area (0.25 sq.mm.) was twice that of the background.

In in vivo experiments, in addition to examination of the tissue sections, it was considered advisable to examine the dental pulp for evidence of radio-chemical compounds. In these experiments the pulp of the extracted tooth was immediately removed and examined for evidence of radioactivity by counting methods.

After extraction of the teeth, the apices were removed to facilitate the penetration of the fixing agent. Decalcified sections, stained by haemalum and eosin, were prepared for histological examination showing the area of the dentine and the pulp relating to the base of the cavity.
BIBLIOGRAPHY


CHAPTER 5

Zinc Phosphate Cement
Zinc phosphate cement is one of the most widely used materials in conservative dentistry. It has been estimated (1) that it is used in 40%-60% of all conservative restorations. It is used either as an insulating medium to protect the pulp from thermal shock or as a luting medium for crowns and inlays on account of its adhesive properties.

The effects of zinc phosphate cement on the dental tissues, particularly the dental pulp, has given rise to much discussion and confusion in the past. Much of this confusion has arisen over differences between manufacturers' claims for zinc phosphate cements and the clinical results obtained by their use. Materials claimed as 'harmless to the pulp' and 'non-irritating' often produced a wide range of clinical conditions from a mild inflammatory process to complete death of the pulp.

At first it was considered that these deleterious effects were produced by the very small amounts of arsenic present in this material. (2) However, following/
following upon the excellent work of Manley and others, it was agreed that the main causative factor was the surface acidity of the cement in the plastic state at the time of insertion into the tooth cavity.

The histo-pathological results of these investigations left no doubt whatsoever of the deleterious effects of such cements on the pulp, even within the first 24 hours.

In the following chapter the history, composition, setting reaction, acidity, consistency, setting time and mixing of zinc phosphate cement will be discussed, and the conclusions drawn will be found in the ultimate paragraph.

History

During the early part of the nineteenth century the main materials used for filling teeth were lead, tin and gold. These materials had been popular for many years but, unfortunately, they were costly and required considerable manipulative skill to ensure the best results.

In an attempt to produce a filling material which/
which was less costly and which could be manipulated readily, the plastic zinc cements were introduced. These consisted essentially of a powder and a liquid which formed a plastic mass when mixed together. This plastic mass set hard in the tooth cavity.

The first of these zinc cements was zinc oxychloride cement, prepared from zinc oxide powder and a concentrated solution of zinc chloride. Although this was widely used by certain members of the dental profession it was soon found that it fell short of the ideal owing to its solubility in oral fluids, its poor physical properties and its harmful effects on the tooth pulp.

Many attempts were made to overcome these deficiencies of zinc oxychloride cement and, in 1879, zinc phosphate cements were introduced. This was prepared as before from zinc oxide powder but phosphoric acid was used as the liquid.

However, while zinc phosphate cement showed an improvement in its physical properties, it remained unsatisfactory as a filling material as there was still evidence of deleterious effect on the pulp.
At this early date dentists were aware of this injurious pulpal effect and opinions were divided regarding the use of phosphate cement. Frequent references were to be found in the dental literature of the day. Harrower(3) in 1886, stated:-

'The injudicious use of filling materials containing phosphoric acid has already furnished evidence of the ability of that material to cause pulp trouble and more is likely to be forthcoming........' Further he adds - 'as to the necessity of protecting the pulp from the deleterious effect of phosphoric acid.............'

Despite his strong attack on this material, Harrower was one of the first to suggest its use as a 'liner', (a thin layer of cement which lines the floor of the cavity before the insertion of the filling material) rather than a filling material. It is in this capacity that its use has continued. This idea of using the cement to line the floor of a cavity, before inserting the filling material, seems rather strange owing to the close proximity of the floor of the cavity to the pulp. In this position the cements will exert their greatest effect on the pulp, in many cases only a small barrier of dentine being present between/
between them.

Others were doubtful regarding the use of the phosphate cement at all. Manley(4) quoted Miller as having written in 1891:—

"...Another feature of the phosphate cements to which I would like to draw attention is their deleterious action on the pulp. I referred to this in 1888 though I believe others had called attention to it still earlier. It seemed to me then, however, that the disastrous results following the use of oxyphosphate sometimes many months after the insertion of the filling were to be accounted for by the fact that the cements were used chiefly in doubtful cases where probably any other filling material would have brought about the same result.... After more experience I am ready to believe that such is not the case, but that many preparations at least of the phosphate cements, even where the pulp is perfectly intact, induce in the course of time a diseased condition... Although I am not prepared to say that I consider the deleterious action of the phosphate cements upon the pulp is established beyond all doubt yet the probabilities that they do have such an action are so great that corresponding precautions are certainly in place. I accordingly never fill a cavity, except it be very shallow, with phosphate cement without having first covered the bottom of it with gutta percha or some other material which serves the same purpose."

During the next half century phosphate cement
Table 2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>ZnO</th>
<th>MgO</th>
<th>SiO₂</th>
<th>R₂O₃</th>
<th>Bi₂O₃</th>
<th>Miscellaneous</th>
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<td>0.1</td>
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<td>0.07</td>
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<td>9.</td>
<td>89.5</td>
<td>9.4</td>
<td>0.3</td>
<td></td>
<td></td>
<td>BaCrO₄, 0.8</td>
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<td>9.4</td>
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<td>0.5</td>
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<tr>
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</table>

Bi₂O₃ Miscellaneous
was widely used though opinions of its value were still divided. It was only in 1936 that a serious attempt was made by Manley to show the effects of this material on the pulp.\(^{(5)}\) He established that these cements, with their high phosphoric acid content, produced harmful effects on the pulp.

As there are a number of conflicting opinions regarding the effects of zinc phosphate cement on the dental tissues, it was considered necessary to investigate this material further. It was also proposed to examine methods of reducing the harmful effects that these cements have on the dental tissues.

**Composition of Zinc Phosphate Cement**

There are many commercial zinc phosphate cements, the composition of which remain manufacturer's secrets. The chief difference in these cements, however, is in the chemical composition of the powder. Chemical analysis of 16 commercial cement powders was carried out by Paffenbarger et al\(^{(6)}\) and is shown in Table 2. Most cements consist mainly of zinc oxide to which magnesium oxide is added in various ratios/
Table 3.

<table>
<thead>
<tr>
<th>Sample</th>
<th>PO₄</th>
<th>Al</th>
<th>Zn</th>
<th>Free H₃PO₄</th>
<th>Combined H₃PO₄</th>
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</tr>
<tr>
<td>13.</td>
<td>67.2</td>
<td>3.0</td>
<td>58.7</td>
<td>10.9</td>
<td>69.6</td>
<td>13.6</td>
<td>27.4</td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>64.9</td>
<td>2.9</td>
<td>56.6</td>
<td>10.6</td>
<td>67.2</td>
<td>13.1</td>
<td>29.9</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>54.6</td>
<td>2.3</td>
<td>37.8</td>
<td>18.7</td>
<td>56.5</td>
<td>30.7</td>
<td>30.9</td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>53.4</td>
<td>2.7</td>
<td>45.5</td>
<td>9.8</td>
<td>55.3</td>
<td>12.2</td>
<td>42.0</td>
<td></td>
</tr>
</tbody>
</table>
ratios: these ratios being approximately 9 parts zinc oxide to 1 part magnesium oxide. Other ingredients such as silica, rubidium trioxide and bismuth trioxide are added to improve the physical properties of the cement, modify its colour and to increase the smoothness while mixing.\(^{(7)}\) The arsenic content is very small being usually less than 0.0002\%. It is believed that this limited amount of arsenic is insufficient to produce a detrimental effect on the pulp.

The liquids are composed mainly of phosphoric acid plus solutions of aluminium phosphate and in some cases solutions of zinc phosphate. The liquid used in this work was found, on examination, to consist of a 10 molar solution of phosphoric acid to which a base such as zinc oxide had been added to the first third part of the solution. The hydrogen ion concentration of this liquid was found to be 1.42. The chemical analysis of the liquids given in Table 3 correspond to the chemical analysis for the powder shown in Table 2. The metallic salts are added to the liquid to reduce the acidity and to assist in the setting/
setting reaction of the liquid with the powder. The average water content of the liquid is $33\% \pm 5\%$. The amount of water present is a critical factor in the liquid/powder reaction. These liquids are hygroscopic and, if exposed to the air, take up or give off water depending on the atmospheric humidity. It was important, therefore, during experiments with phosphate cement, to treat this liquid as hygroscopic and to ensure that it was not exposed to the air.

Setting Reaction of Zinc Phosphate Cement

When the powder and the liquid of the zinc phosphate cements are mixed together, a plastic mass is formed. After a period of 4-8 minutes, the cement loses its plasticity and becomes a hard mass.

The setting reaction which takes place between the powder and the liquid is very complex, and complete agreement has not been reached on the physico-chemical reactions which take place. It was most important to consider this point fully, as it is upon the final products of the reaction that the acidity of the cement depends.

Ideally/
Ideally, the final compound between phosphoric acid and zinc oxide is the formation of tertiary zinc phosphate, $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$, which is a relatively insoluble compound. In such a compound there is no unused phosphoric acid and no water. In clinical practice, however, this compound is only formed after the cement has been in the mouth.\(^{(8)}\)

It is proposed, in this work, to discuss the chemistry of the setting reaction and to suggest the mechanism which takes place when the powder and liquid of the cement are mixed.

**Chemistry of the Setting Reaction**

One of the most important factors which must be considered is the 'route' by which the cement passes from its original state, $3\text{ZnO} + 2\text{H}_3\text{PO}_4 + X\text{H}_2\text{O}$ to its final state, tertiary zinc phosphate, $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$. Two such routes may be considered.

(a) As zinc forms only primary and tertiary phosphates, primary zinc phosphate, $\text{Zn(H}_2\text{PO}_4)_2$ may be formed and, at a later date, tertiary zinc phosphate, $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ crystallises out from this solution. This may occur after the cement has been placed into the tooth cavity.

(b)
(b) The phosphoric acid converts the zinc oxide, ZnO, to $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ directly and 'in situ'.

The actual 'route' probably lies between these two reactions and is dependent upon the precise conditions which are present at the time of mixing. Factors which may affect the reaction are:

- proportions of liquid and powder used;
- speed of mixing powder and liquid;
- temperature of mixing and the presence of additional material in the powder and liquid which may affect the rate of crystallisation of the final product.

Irrespective of the 'route' by which the final result is achieved, some liquid must be present as long as the cement remains in a plastic state, primary zinc phosphate, $\text{Zn(H}_2\text{PO}_4)_2$, in route (a) and phosphoric acid, $\text{H}_3\text{PO}_4$, by route (b).

It is necessary to emphasise the presence of free liquids during the setting process as this factor is important throughout this thesis.

When zinc phosphate cement is used clinically it must be in a plastic state. In this plastic state either primary acid phosphate or phosphoric acid, or both of these liquids must be present when the cement is placed in contact with the dental tissues.
Graph showing variation of pH value when zinc oxide is added to phosphoric acid.

Fig. 10

Zn \((\text{H}_2\text{PO}_4)_2\)

Zn\(_3\)(\text{PO}_4)_2
Consideration of pH Value of the Mixed Cement

The following graph which is qualitatively correct (Fig. 10), shows how the pH value would occur if zinc oxide was added to phosphoric acid and no precipitation of $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ predicted.

If 'route' (a) is followed and primary and tertiary phosphate formed, the pH would be raised towards a value which would not be harmful to the dental tissues.

'Route' (b), on the other hand, implies that, as long as the cement remains in a plastic state, i.e. incompletely set, some unchanged acid is present and low pH values may be given. Harvey et al.\(^{(9)}\) showed that cement of the zinc phosphate type may have a high acidity with a value as low as pH 2 when inserted into the cavity. In an attempt to reduce this acidity several manufacturers have replaced the pure phosphoric acid of the liquid by acid in which as much zinc oxide as possible has already been dissolved. Because of the shape of the pH curve (vide Fig. 10) this will not raise the pH value much but will reduce the/
the acidity in isolated parts of the mix.

Measurement of the pH Value of the Mixed Cement

Previous investigations have clearly shown that the causative factor in producing harmful effects on the tooth pulp was the acidity of the dental cements while in the plastic state, at the time of insertion of the cement into the tooth cavity. Many investigations have been undertaken to measure this acidity.

Eberly\(^{10}\) made one of the earliest determinations using a quinhydrone calomel electrode method with which he measured the hydrogen ion concentration of distilled water which had been in contact with the set cement. He concluded that the reaction was completed in 15 minutes. Other determinations were carried out by Matthews,\(^{11}\) Paffenbarger, Schoonover and Souder,\(^{12}\) and Worner.\(^{13}\) These investigators all used different methods to determine the pH of water which had been in contact with the set cement.

It was agreed by these observers that the cement was acid when set and that a pH of 3.8 - 6.0 may be reached/
reached. Worner goes further and states that 'zinc phosphate cements are not likely to be irritant to the pulp because they are practically neutral when fully set'. This statement, however, is contrary to the histological evidence of Manley (14) and others, notably Gurley, (15) Zander (16) and Schroff (17) who demonstrated that harmful effects could be produced on the dental pulp, by cements, even within 24 hours. In many of these investigations, however, the first pH measurement was recorded 15-20 minutes after mixing the cement, when the reaction was certainly much less acid than immediately after mixing.

In a most complete investigation into the acidity of the zinc phosphate cements, Harvey et al (9) repeated many of the experiments of these previous investigations, and showed that, by measuring the dilute extracts of cement that had been in contact with the set cement, a dilution error was introduced. They point out that this error caused the cement to appear less acid than the true value at the time of its insertion into the tooth cavity while in the plastic state. It was also shown in this investigation that/
that, as the cement sets, tertiary zinc phosphate may be formed at a later time, which makes the pH value of the cement more alkaline thus giving a false value.

Since the acidity of the cement decreases as it sets, the most important pH to measure is the surface acidity at the time of its insertion into the tooth cavity, when it is likely to do most damage to the tooth pulp. By making direct measurements on the plastic and set cement, Harvey et al found that pH values of 1.5 - 1.6 could be found immediately after mixing, irrespective of the proportions of powder and liquid used. They also found that the cement remained acid giving pH values up to 4.8, even after being fully set thus refuting Worner's statement that they became 'practically neutral'.

While extensive investigations were undertaken by Harvey et al, they omitted to consider the importance of the time interval between mixing the cement and placing the plastic cement in the tooth cavity. Measurements of the acidity of the zinc phosphate cements were carried out to determine whether the consistency of the cement at the time of mixing/
mixing had any effect on the final acidity of the set cement, and also to determine whether changes in pH occurred between mixing the cement and placing it in the tooth cavity.

**Determination of the Surface Acidity of the Zinc Phosphate Cements**

Organic indicators were used in the present investigation to determine the surface acidity of the cement in the plastic state, and also after the setting reaction was complete.

Solutions of organic indicators - thymol blue and methyl violet - were first applied to the surface of the cement at intervals of one minute after mixing. With these indicators a pH value of 2.8 - 3.0 was obtained for the cement while still in the plastic state irrespective of the time after mix. The difficulty of this method, however, was that the residual liquid of the mixed cement was diluted with relatively large volumes of the indicator solvent. In order to avoid the dilution of the residual liquid in the mixed cement by the indicator solvent, the organic indicator powder was incorporated in the untinted cement/
cement powder. It was found that the best way to achieve a uniform distribution of the indicator through the cement powder was to prepare an alcoholic solution of the indicator and incorporate this through the cement powder. The paste thus formed was dried, reground and then mixed with the cement liquid according to the standardized procedure (vide infra).

After mixing the cement liquid with the cement powder containing the indicator, a magenta colour, which represented a pH of 1.6, was obtained. This colour persisted even after the cement had completely set. A similar colour was obtained when different proportions of powder and liquid were used. These results agreed with those of Harvey using the same indicators.

Test papers impregnated with organic indicators were used to measure the surface acidity of the cement immediately after mixing and until the cement had completely set. The test papers were moistened with distilled water and applied to the cement. The colour obtained was compared against a known standard pH colour. The following organic dyes were used:--

Thymol/
These papers were moistened and applied to the surface of the plastic cement at intervals of one minute after mixing until the setting reaction was complete. The results obtained by this method showed a pH value of 1.5 - 1.9 of the cement at the time of mixing, irrespective of the consistency. As it was difficult to compare fine changes of colour using these papers, the method could not be considered accurate to determine variations in the pH as the cement set.

The results of these experiments show that pH values less than 2.0 may be obtained while the cement is in the plastic state immediately after mixing. The cement becomes less acid when set, with pH values of 4.8 being reached. Irrespective of the time after setting, the cement always remains acid and neutrality is never achieved.

It was not possible, however, to differentiate by any of the colorimetric methods, changes in acidity which take place within the cement while still in the plastic state.
In this work it was proposed to measure the penetration through dentine of the acid products of the setting reaction by the radiochemical method of 'labelling' the cement liquid with radioactive phosphorus.

Consistency of Mixed Cement

The consistency of the zinc phosphate cements varies according to the clinical requirements of the operator. To obtain the optimum physical properties of a cement, a mix which has a thick consistency is best. However, in clinical use a cement which has a thick consistency cannot always be used as, for example, when it is required as a luting medium, for which purpose a thin consistency is necessary.

In order to standardise the consistencies of the cement, within the range of clinical use, the following quantities of powder and liquid were used throughout:

<table>
<thead>
<tr>
<th>Cement Consistency</th>
<th>Powder</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thin</td>
<td>0.7gm.</td>
<td>0.2ml.</td>
</tr>
<tr>
<td>Medium</td>
<td>0.8gm.</td>
<td>0.2ml.</td>
</tr>
<tr>
<td>Thick</td>
<td>0.9gm.</td>
<td>0.2ml.</td>
</tr>
</tbody>
</table>
Setting Time

The time taken for the zinc phosphate cement to harden or set after mixing is very important in clinical practice. It must be controlled accurately to allow sufficient working time between mixing and setting to permit its placement in the tooth. A minimum limit of 4 minutes and a maximum of 10 minutes has been specified. Many factors have been shown to affect the setting time. Factors such as:

- powder/liquid ratio
- rate of addition of powder to liquid
- temperature during and after mixing.

In clinical practice wide variations in setting time occur, as each operator uses different mixing techniques before inserting the cement into the tooth. Some prefer a cement which will set after a few minutes while others may use a cement which may take up to 15 minutes to set. This alteration in setting time may be produced by any of the factors mentioned above. It is usual, however, to control this by an adjustment of the powder/liquid ratio. The more liquid added the longer the setting time.

These/
These individual variations in the control of the setting time influence the acidity of the cement both at the time of mixing and also at the time of its placement in the tooth cavity.

**Mixing Technique.**

The reaction which takes place when the powder is incorporated in the liquid can be controlled to a considerable extent by the amount of powder which is incorporated in the liquid at any one time. A standard method of mixing was therefore carried out.

The powder and liquid were mixed on a cooled glass slab, the temperature of which was kept within the range of $20^\circ C - 24^\circ C$. A weighed quantity of the powder and a measured amount of liquid were placed on the glass slab. The powder was shaped into a square of uniform thickness. The square of powder was divided into eighths and each eighth incorporated into the liquid, spatulating for ten seconds each. The mix was spatulated with a rotary movement using a light pressure together with a scraping motion which collected the cement at the end of each mix. The total/
total time taken to mix the cement was 1 minute 15 seconds.

As the liquid was hygroscopic, mixing was commenced immediately it was placed on the mixing slab.

The following investigations were carried out in the experimental section of zinc phosphate cement:

2. The measurement of the penetration into dentine of the acid present during the setting of the cement, with particular relation to:
   
   (a) The consistency of the cement at the time of insertion into the tooth cavity.
   
   (b) The time of insertion of the cement, after mixing, into the tooth cavity.
   
   (c) The time which the cement remains in the tooth cavity.

3. The effectiveness of calcium hydroxide as a means of preventing the penetration of the acid products from zinc phosphate cement.

4. The effects of the cement on the dental pulp with emphasis on the consistency of the cement at the time of insertion into the tooth cavity.

5. The measurement of the amount of arsenic which may diffuse from the zinc phosphate cement into the dental tissues.
Purpose of Experiment No. 2(a).

In this experiment the effects of variations in consistency of zinc phosphate cement on the penetration of the acid products were determined.

Method

In vivo experiments were carried out on the teeth of two dogs whose ages were eleven months and sixteen months. Twelve cavities were prepared in the teeth of each dog, three in each canine tooth. The cavities were placed in the incisal, middle and gingival zones on the labial surface of each canine tooth. All cavities were prepared according to the standardized procedure, (vide page 54), and were separated from each other by a distance of not less than 1mm.

Radioactive cement liquid was prepared by adding radioactive phosphorus, as orthophosphoric acid, to the cement liquid. This gave a specific activity of 10-15 μc. of radioactive phosphorus for the amount of cement inserted in each cavity. Zinc phosphate cement was mixed in thin, medium and thick consistencies using the powder liquid ratios previously described (vide page 76).
In this experiment the effects of each of these consistencies were observed on one tooth by placing cement of the thick consistency in the gingival cavity, the medium consistency in the middle and the thin consistency in the incisal. In a number of teeth the thick consistency was placed in the incisal cavity with the medium and thin consistencies in the middle and gingival cavities respectively.

The teeth were extracted under a general anaesthetic after 10 minutes and ground sections prepared to a thickness of 100-150 μ.

Not until all the ground sections had been prepared from each dog were autoradiographs made, using Kodak A.R. 50 film. In this way, the decay factor of the radioactive phosphorus was kept constant for each dog.

The autoradiographs were given an exposure time of 48 hours and developed in Kodak D.19 B developer for 5 minutes at a temperature of 68°F.

The maximum degree of penetration of the radioactive phosphorus was measured from the floor of the cavity along the line of the dentinal tubules.
Fig. 11. Penetration in middle cavity from cement of a medium consistency.

Fig. 11a. Penetration in incisal cavity from cement of a thin consistency.

Fig. 11b. Penetration in gingival cavity from cement of a thick consistency.

Autoradiographs demonstrating penetration and concentration of acid products from zinc phosphate cement of thin, medium and thick consistencies.
Results

The penetration of the 'labelled' acid products, primary zinc phosphate and phosphoric acid, which are present during the setting of zinc phosphate cement was demonstrated in the autoradiographs by a blackening of the film emulsion. These autoradiographs showed that the acid products penetrated from the floor of the cavity along the cut dentinal tubules towards the dental pulp. The difference in penetration of the acid products in one tooth using cement of thin, medium and thick consistencies is shown in Figs. 11(a), 11(b) and 11(c).

The maximum distance of penetration for each consistency was as follows:—

- Cement of thin consistency: 0.65mm.
- Cement of medium consistency: 0.60mm.
- Cement of thick consistency: 0.35mm.

Autoradiographs showed that the concentration of the acid products, as determined by the number of image grains on the film emulsion, was greatest with the thin consistency of cement and least with the thick.
Purpose of Experiment 2(b).

This experiment was undertaken to determine the effect of insertion time of the mixed zinc phosphate cement on the penetration of the acid products.

Method

In vivo experiments were carried out on the teeth of five dogs whose ages were from eight to fourteen months. Twelve cavities were prepared in the teeth of each dog, three on each canine tooth (vide illustration page 53). The cavities were placed in the incisal, middle and gingival zones on the labial surface of each canine tooth. All cavities were prepared according to the standardized procedure (vide page 54) and were again separated from each other by a distance of not less than 1mm.

Radioactive cement liquid was prepared by adding radioactive phosphorus, as orthophosphoric acid, to the cement liquid. This gave a specific activity of the order of 10-15 μc of radioactive phosphorus/
phosphorus for the amount of cement liquid inserted in each cavity.

Zinc phosphate cement was mixed in thin, medium and thick consistencies using the powder/liquid ratios previously described (vide page 76).

The following procedure was adopted with each consistency:-

Immediately after mixing, the gingival cavity was filled with cement; after one minute, cement of the same mix was inserted into the middle cavity and after yet a further minute into the incisal cavity. In certain teeth the order in which the cavities were filled was reversed with the incisal cavity being filled immediately after mixing.

The teeth were extracted under a general anaesthetic after 10 minutes, and ground sections were prepared to a thickness of 100-150 μ. Not until all the ground sections had been prepared from each dog were autoradiographs made, using Kodak A.R. 50 film. In this way the decay factor of the radioactive phosphorus was kept constant for each dog.
The autoradiographs were given an exposure time of 48 hours and developed in Kodak D.19B developer for 5 minutes at a temperature of 68°F.

The maximum degree of penetration of the radioactive phosphorus was measured from the floor of the cavity along the line of the dentinal tubules.
Fig. 12 (a).
Penetration immediately after mixing.

Fig. 12 (b).
Penetration one minute after mixing.

Fig. 12 (c).
Penetration two minutes after mixing.

Autoradiographs demonstrating penetration of acid products through dentine from zinc phosphate cement of a thin consistency. Mag. X 60.
Results

The presence of the 'labelled' acid products, primary zinc phosphate and phosphoric acid, was demonstrated in the autoradiographs by the blackening of the film emulsion.

The distance which these products have penetrated from the floor of the cavity along the dentinal tubules from a cement mixed to a thin consistency is shown in Figs.12(a),12(b) and12(c). In these autoradiographs, two distinct zones of concentration were demonstrated:

(a) A narrow highly concentrated zone at the margins of the cavity.

(b) A deeper more diffuse zone which extended along the line of the dentinal tubules towards the dental pulp.

The distances of penetration were as follows:

<table>
<thead>
<tr>
<th>Zone</th>
<th>Marginal</th>
<th>Deeper</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) Immediately after mixing</td>
<td>0.020mm</td>
<td>0.50mm</td>
</tr>
<tr>
<td>(II) One minute after mixing</td>
<td>0.015mm</td>
<td>0.50mm</td>
</tr>
<tr>
<td>(III) Two minutes after mixing</td>
<td>0.010mm</td>
<td>0.40mm</td>
</tr>
</tbody>
</table>

Autoradiographs demonstrating the penetration of cement mixed to a medium consistency are shown in/
Fig. 13 (a).
Penetration in gingival cavity immediately after mixing.

Fig. 13 (b).
Penetration in middle cavity one minute after mixing.

Fig. 13 (c).
Penetration in incisal cavity two minutes after mixing.

Autoradiographs showing penetration of acid products through dentine from cement of a medium consistency. Mag. X 60.
in Figs. 13 (a), 13 (b) and 13(c). The concentrated marginal zone which was demonstrated with cement of a thin consistency was not evident in the autoradiographs of medium or thick consistencies of cement.

The maximum distances of penetration with the medium consistency were:

(1) Immediately after mixing 0.9mm.
(II) One minute after mixing 0.9mm.
(III) Two minutes after mixing 0.8mm.

The concentration of the acid products as shown by the number of image grains on the film emulsion was greatest immediately after mixing and was reduced as the time interval between mixing and insertion into the tooth cavity was increased.

The results obtained with cement mixed to a thick consistency are shown in Figs. 14 (a), 14 (b) and 14 (c). The maximum distances of penetration with the thick consistency were:

(1) Immediately after mixing 0.9mm.
(II) One minute after mixing 0.8mm.
(III) Two minutes after mixing 0.8mm.

The concentration of the acid products was again/
Fig. 14 (a).
Penetration in gingival cavity immediately after mixing.

Fig. 14 (b).
Penetration in middle cavity one minute after mixing.

Fig. 14 (c).
Penetration in incisal cavity two minutes after mixing.

Autoradiographs showing penetration of acid products through dentine from zinc phosphate cement of a thick consistency. Mag. X 60.
again greatest immediately after mixing and was reduced as the time interval was increased.
Purpose of the Experiment No. 2(c)

The purpose of this experiment was to measure the penetration into dentine of the acid products of the setting reaction of zinc phosphate cement in relation to the time which the cement remained in the tooth cavity.

Method

In vivo experiments were carried out on the teeth of five dogs. Three of these dogs were also used for experiments in penetration of the acid products in relation to the consistency of cement. In each dog sixteen cavities were prepared, three in each canine tooth and two in each upper third incisor tooth. The cavities were prepared on the labial surfaces of the teeth as close to the gingival margin as possible and separated from each other by a distance of not less than 1 mm. (vide illustration on page 53). In each dog, all the cavities were prepared at one operating session.

In vitro experiments were carried out on 20 extracted premolar and anterior teeth which had been previously/
previously fixed in formalin. One cavity was prepared in each extracted tooth; on the occlusal surface of the premolar teeth and on the proximal surface of the anterior teeth.

The phosphate cement was prepared by adding radioactive phosphorus ($^{32}\text{P}$) as orthophosphoric acid, to the cement liquid to give a specific activity of 15–20 $\mu$C of $^{32}\text{P}$ for the amount of cement required to fill each tooth. The cement was mixed to a thin consistency for this experiment, using 0.7gm. of the powder and 0.2ml. of the liquid containing radioactive phosphorus. The cement was mixed according to the standardized technique and placed in the tooth cavity immediately after mixing.

In the in vivo experiments, teeth were extracted at intervals of 15 minutes, 5 days, 7 days and 12 days and ground sections prepared. Immediately after extraction, the tooth pulp was removed, placed in normal saline and examined for evidence of radioactivity by counting methods.

In the in vitro experiments, ground sections were prepared after 10 minutes, 2 days, 3 days, 7 days and/
and 12 days.

Autoradiographs were made when all ground sections had been prepared. In this way the decay factor of the radioactive phosphorus was kept constant.

Kodak A.R.50 film was used in the preparation of the autoradiographs. These were given an exposure time of 36 hours and were developed in Kodak D.19B developer for 5 minutes at 68°F.

The degree of penetration of radioactive phosphorus was measured from the floor of the cavity along the line of the dentinal tubules. The distance of the floor of the cavity to the pulp along the line of the dentinal tubules was also measured in each case.
Table 4.

Measurement of penetration of $^{32}\text{P}$ in lower cavities in 'in vivo' experiments

<table>
<thead>
<tr>
<th>Time of Extraction</th>
<th>Mins.</th>
<th>mins.</th>
<th>mins.</th>
<th>days</th>
<th>days</th>
<th>days</th>
<th>days</th>
<th>days</th>
<th>days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Depth from floor of cavity to pulp along dentinal tubules in mm.</td>
<td>1.90</td>
<td>0.96</td>
<td>1.90</td>
<td>1.68</td>
<td>1.60</td>
<td>1.90</td>
<td>1.73</td>
<td>1.80</td>
<td>2.71</td>
</tr>
<tr>
<td>Penetration of $^{32}\text{P}$ along dentinal tubules in mm.</td>
<td>0.60</td>
<td>0.52</td>
<td>0.60</td>
<td>0.12</td>
<td>0.72</td>
<td>0.36</td>
<td>0.60</td>
<td>0.24</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.24</td>
<td>0.48</td>
<td>0.24</td>
<td>0.58</td>
<td>0.72</td>
<td></td>
</tr>
</tbody>
</table>
Results

The amount of penetration of the radioactive compounds in the \textit{in vivo} experiments is shown in Table 4. Radioactivity was not found in any of the pulp tissues which were examined by counting methods. The following autoradiographs demonstrate the penetration at specific time intervals.

After a time interval of 15 minutes

Fig. 15.

The autoradiograph shown in Fig. 15 demonstrates the penetration of the radioactive compounds in a longitudinal/
longitudinal section of a canine tooth. There is a marked concentration of radioactive phosphorus at the margin of the cavity for a distance of 0.02mm. with local areas of deeper penetration for a distance of 0.05mm. which are probably due to local concentration of radioactive phosphorus. Concentration of the radioactive compounds becomes less towards the pulp following the line of the dentinal tubules and finally merging with the background at a distance of 0.5mm. from the floor of the cavity.

After 5 days

Fig. 16.
Fig. 16 demonstrates an autoradiograph of the canine tooth of a dog after five days. In this section the pulp is not shown owing to the angle at which the section was made. The marginal penetration of the radioactive compounds has increased in depth to a distance of 0.05mm. Further penetration has taken place along the line of the dentinal tubules towards the tooth pulp for a distance of 0.9mm.

There is no significant difference in the penetration of the radioactive compounds after 7 days and 12 days, therefore, an autoradiograph of 12 days only is shown below.

After 12 days

Fig. 17.
In this autoradiograph radioactive compounds have penetrated for a depth of 1.2 mm from the floor of the cavity along the line of the dentinal tubules.
<table>
<thead>
<tr>
<th>Time of Sectioning</th>
<th>Depth from floor of cavity to pulp along dentinal tubules in mm.</th>
<th>Penetration of $^{32}$P along dentinal tubules in mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 days</td>
<td>1.8</td>
<td>1.0</td>
</tr>
<tr>
<td>5 days</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>12 days</td>
<td>2.2</td>
<td>1.5</td>
</tr>
<tr>
<td>1 days</td>
<td>1.5</td>
<td>2.2</td>
</tr>
<tr>
<td>5 days</td>
<td>1.6</td>
<td>1.5</td>
</tr>
<tr>
<td>12 days</td>
<td>2.1</td>
<td>1.5</td>
</tr>
<tr>
<td>7 days</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>12 days</td>
<td>2.0</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Table 5. Measurement of penetration of $^{32}$P in lower cavities in 'in vitro' experiments.
Results

The amount of penetration of the radioactive compounds into dentine, in the in vitro experiments is shown in Table 5. The following autoradiographs of ground tooth sections demonstrate the penetration at specific time intervals from the insertion of the cement into the tooth cavity.

In these autoradiographs, the acid products of the zinc phosphate cement which have been 'labelled' with radioactive phosphorus are shown by the blackening of the film.

After 1 day

Fig. 18.
After 2 days

Fig. 19.
The autoradiograph in Fig. 19 demonstrates that only marginal penetration has taken place along the dentinal tubules for a distance of 0.5 mm. from the floor of the cavity. The penetration is more marked in the apical than in the coronal part of the cavity.

The shallow penetration in this case is probably due to the decreased circulation of the tissue fluid in the dentine as a result of the formation of secondary dentine and possible hypercalcification of the tubules.

On macroscopic examination, this tooth showed marked/
marked attrition which would account for the changes in the dentinal tubules.

After 5 days

Fig. 20.

In this autoradiograph complete penetration of the radioactive compounds has taken place down the dentinal tubules from the floor of the cavity to the pulp. As demonstrated in the previous autoradiographs, penetration has been greater in the apical part of the cavity than in the coronal part. This may be attributed to:

(a)/
(a) Gravity causing a collection of the material in the apical part of the cavity,

(b) The fact that the apical part of the cavity is nearest the pulp with the possibility of an increased size of the lumen of the dentinal tubules which would allow material to pass more easily.

After 10 days

In this autoradiograph penetration of $^{32}P$ has reached the pulp along the dentinal tubules. The penetration is more marked in the apical part of the cavity. There is no evidence of lateral spread along the amelo-dentinal junction either on the coronal or on the apical part of the cavity.
The autoradiograph which is shown in Fig. 21 demonstrates the penetration of the radioactive compounds in the proximal cavity of a lower anterior tooth. There is deep penetration in the apical part of the cavity, following the line of the primary dentinal tubules and extending to the tooth pulp.

An autoradiograph of this tooth taken on double-coated X-ray film is shown in Fig. 22. The white area indicates the presence of radioactive compounds showing that penetration has reached the pulp. The outline of the tooth has been drawn for clarity.
Conclusion

In both the *in vivo* and *in vitro* experiments, penetration of the 'labelled' acid products from the zinc phosphate cement has extended from the cavity floor to the tooth pulp, along the line of the dentinal tubules. There was no evidence of lateral spread along the amelo-dentinal junction in any of the autoradiographs.

Autoradiographs of the *in vivo* experiments showed that there were two zones of concentration after the short interval of fifteen minutes; a marked marginal zone which extends for a short distance (0.05mm.) from the edge of the cavity and a less concentrated deeper zone. As the time interval was extended, the penetration of the marginal zone increased slightly but with less concentration. There was also further penetration of the deeper, less concentrated zone. In these cases where the floor of the cavity was in close proximity to the pulp, penetration was almost complete.

The depth of penetration showed no direct relationship/
relationship to the time interval between placing the cement in the tooth cavity and extracting the tooth. In many cases only slight differences were found between five and twelve days.

In the *in vitro* experiments penetration was more marked than in the *in vivo* experiments, penetration having reached the pulp as early as 24 hours after the insertion of the cement. This penetration was more marked in the apical than in the coronal part of the cavity. The reasons for the increased penetration found in the *in vitro* experiments are considered in the discussion of the zinc phosphate cements.

In these cases where deep penetration has failed to take place, the tooth has shown some evidence of previous metabolic disturbance with the formation of dead tracts or translucent zones.
Purpose of the Experiment No. 3

The purpose of this experiment was to determine the effectiveness of calcium hydroxide in preventing the penetration through the dentine of the acid products from zinc phosphate cement.

Method

In vivo experiments were carried out on the teeth of two dogs. In each dog, twelve cavities were prepared, two on each canine tooth and two on each upper third incisor tooth. The cavities were prepared on the labial surfaces of the teeth as close to the gingival margin as possible (vide illustration page 53), and were separated from each other by a distance of not less than 1 mm. In each dog, cavities were prepared at one operating session.

In vitro experiments were carried out on fifteen extracted human teeth, which had been previously fixed in formalin. Two cavities were prepared on the labial or buccal surfaces of each tooth in the same way as in the animal experiments. In all experiments, cavities were prepared according to the standardised procedure/
Fig. 23.

Calcium Hydroxide.

Cement.

Cement.
procedure.

In each tooth, the floor of one cavity was lined with a paste of calcium hydroxide and distilled water before inserting zinc phosphate cement; and the floor of the other cavity was untreated. Both cavities were then filled with radioactive zinc phosphate cement (vide Fig.23). The cement was prepared by adding radioactive phosphorus ($^{32}$P), as orthophosphoric acid, to the cement liquid in order to give a specific activity of 15-20 uc of $^{32}$P for each cavity. The cement was mixed to a thin consistency (according to the standardised procedure) using 0.7gm. of the powder and 0.2ml. of the liquid. A fresh mix of cement was used for each cavity.

The dog's teeth were extracted at intervals of 15 minutes, 5 days, 7 days and 12 days and ground sections prepared to a uniform thickness of 100-150 $\mu$. Immediately after extraction of each tooth, the pulp was removed, placed in a normal saline and examined for evidence of radioactivity by counting methods. In the in vitro experiments, ground sections were prepared after intervals of 15 minutes, 1 day, 5 days, 7 days/
7 days and 12 days. Not until all the ground sections had been prepared from each dog were autoradiographs made. In this way the decay factor of the radioactive phosphorus was kept constant and was not a variable which had to be considered in the interpretation of the autoradiographs.

Kodak A.R.50 film was used in the preparation of the autoradiographs. These were given an exposure time of 60 hours and were developed in Kodak D.19B developer for 5 minutes at a temperature of 68°F.
Fig. 24(a).

Autoradiograph of longitudinal section of canine tooth of dog through upper cavity.

Cavity lined with calcium hydroxide paste. No penetration of $^{32}\text{P}$.

Mag. X 60

Fig. 24(b).

Autoradiograph of longitudinal section through lower cavity of same tooth as above.

Penetration of $^{32}\text{P}$ as shown by blackening of film.

Mag. X 60
Results

Autoradiographs from both in vivo and in vitro experiments are shown in Figs. 24(a) and 24(b) and Figs. 25(a) and 25(b).

Fig. 24(a) shows an autoradiograph from the upper cavity of a longitudinal section of the canine tooth of a dog. The floor of the cavity of this tooth was lined with a paste of calcium hydroxide in distilled water. This autoradiograph shows that no penetration of radioactive phosphorus has taken place from the zinc phosphate cement. Fig. 24(b) demonstrates an autoradiograph of the lower cavity of the same tooth. The blackening of the film indicates that penetration has taken place along the line of the dentinal tubules. Autoradiographs of longitudinal sections of an extracted human tooth are shown in Figs. 25(a) and 25(b) which demonstrate the upper and lower cavities respectively. Fig. 25(a) demonstrates that no penetration of radioactive phosphorus has taken place in the cavity which was previously lined with calcium hydroxide paste. Fig. 25(b) shows penetration of radioactive phosphorus in the lower cavity of the same/
Fig. 25(a).

Autoradiograph of longitudinal section of lower anterior tooth through upper cavity.

Cavity lined with calcium hydroxide paste. No penetration of $^{32}$P demonstrated.

Mag. X 60

Fig. 25(b).

Autoradiograph of longitudinal section through lower cavity of same tooth as above.

Penetration of $^{32}$P shown by blackening of film.

Mag. X 60
same tooth along the line of the dentinal tubules.

An examination of the autoradiographs obtained from both *in vivo* and *in vitro* experiments showed that, in every case, no penetration of the acid products of the setting reaction had taken place in the cavity previously lined with calcium hydroxide paste, as indicated by the absence of blackening of the autoradiograph.

Penetration of radioactive phosphorus was much greater in the *in vitro* experiments than in the *in vivo* experiments. No evidence of radioactivity was found in any of the *in vivo* experiments when the pulp was examined by counting methods. In other words it was observed that, where penetration of radioactive phosphorus occurred, it was more marked in the extracted human teeth than in the dogs teeth. In many cases, penetration extended to the pulp (*vide* Fig. 26).
Autoradiograph of a premolar tooth showing complete penetration of $^{32}$P from the base of the lower cavity to the tooth pulp. The penetration is demonstrated by the shadowing shown by arrow.

Mag. X 2
Purpose of the Experiment No. 4

In this experiment, a study was made of the effects of varied consistencies of zinc phosphate cement on the dental pulp.

Method

In vivo experiments were carried out on human and animal teeth to determine, by histological examination, the reaction of the dental pulp to zinc phosphate cement.

Investigations in human teeth were undertaken on twenty sound premolar teeth which were scheduled for extraction for orthodontic reasons in patients whose ages were from eleven to fourteen years. The buccal surface of these teeth was selected for cavity preparation as it was possible to prepare more than one cavity on each tooth and also because sections could readily be prepared to show the reaction of the pulp tissue below each cavity.

Animal experiments were carried out on the teeth of five dogs aged eight months to one year. The labial surface of the canine and the third incisor teeth/
Table 6.

Duration of Experiment - 36 hours.

<table>
<thead>
<tr>
<th>Tooth</th>
<th>Position of Cavity</th>
<th>Type or Consistency</th>
<th>Time of insertion after mixing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper right canine.</td>
<td>Incisal</td>
<td>Thick</td>
<td>3 mins.</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>Thick</td>
<td>2 mins.</td>
</tr>
<tr>
<td></td>
<td>Gingival</td>
<td>Thick</td>
<td>1 min.</td>
</tr>
<tr>
<td>Upper right third incisor.</td>
<td>Single</td>
<td>Thick</td>
<td>1½ mins.</td>
</tr>
<tr>
<td>Lower right canine.</td>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper left canine.</td>
<td>Incisal</td>
<td>Thick</td>
<td>3 mins.</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>Thick</td>
<td>2 mins.</td>
</tr>
<tr>
<td></td>
<td>Gingival</td>
<td>Thick</td>
<td>1 min.</td>
</tr>
<tr>
<td>Upper left third incisor.</td>
<td>Single</td>
<td>Thick</td>
<td>3½ mins.</td>
</tr>
<tr>
<td>Lower left canine</td>
<td>Incisal</td>
<td>Thick</td>
<td>1 min.</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>thick</td>
<td>2 mins.</td>
</tr>
<tr>
<td></td>
<td>Gingival</td>
<td>Thick</td>
<td>3 mins.</td>
</tr>
</tbody>
</table>
teeth were used for cavity preparation and the cavities disposed in the incisal, middle and gingival zones of this surface.

The mixes of cements were inserted into the cavities at varying intervals after the completion of spatulation. The distribution of cavities and conditions of cement, for each dog, are shown in Tables 6, 7, 8, 9 and 10.

All cavities were prepared according to the standardized procedure (vide page 54). After preparation, each cavity was washed for five seconds with an air and water spray and finally dried with cotton wool. In some of the human teeth, a control cavity was prepared to observe the reaction of the dental pulp to the trauma of the cavity preparation alone. As the response of the pulp is strictly confined to those dentinal tubules damaged during cavity preparation, it was possible to compare the reaction of one pulp to two differing consistencies of cement. This was done by cutting two or more cavities in a single tooth. In these cases where more than one cavity was prepared, each cavity was separated/
Duration of Experiment - 11 days.

<table>
<thead>
<tr>
<th>Tooth</th>
<th>Position of Cavity</th>
<th>Type or Consistency of Cement</th>
<th>Time of Insertion after Mixing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper right canine</td>
<td>Incisal</td>
<td>Thin</td>
<td>Immed.</td>
</tr>
<tr>
<td></td>
<td>Gingival</td>
<td>Thick</td>
<td>1 min.</td>
</tr>
<tr>
<td>Upper right third incisor</td>
<td>Single</td>
<td>Thick</td>
<td>1 min.</td>
</tr>
<tr>
<td>Lower right canine</td>
<td>Incisal</td>
<td>Thick</td>
<td>Immed.</td>
</tr>
<tr>
<td></td>
<td>Gingival</td>
<td>Zinc Oxide</td>
<td></td>
</tr>
<tr>
<td>Lower right third incisor</td>
<td>Single</td>
<td>Zinc Oxide</td>
<td></td>
</tr>
<tr>
<td>Upper left canine</td>
<td>Incisal</td>
<td>Thick</td>
<td>Immed.</td>
</tr>
<tr>
<td></td>
<td>Gingival</td>
<td>Thin</td>
<td>1 min.</td>
</tr>
<tr>
<td>Upper left third incisor</td>
<td>Single</td>
<td>Thin</td>
<td>Immed.</td>
</tr>
<tr>
<td>Lower left canine</td>
<td>Incisal</td>
<td>Thin</td>
<td>Immed.</td>
</tr>
<tr>
<td></td>
<td>Gingival</td>
<td>Thick</td>
<td>Immed.</td>
</tr>
<tr>
<td>Lower left third incisor</td>
<td>Single</td>
<td>Thick</td>
<td>Immed.</td>
</tr>
</tbody>
</table>
separated by a distance of 1.5–2.0mm.

Zinc phosphate cement was mixed in thin, medium and thick consistencies using the powder/liquid ratios previously described (*vide* page 76).

Where only one cavity had been prepared, the effect of a single consistency alone was determined, whilst in others, where two or more cavities had been prepared, different consistencies were used on the same tooth.

The effectiveness of calcium hydroxide in preventing the harmful effects of zinc phosphate cement on the dental pulp was studied by placing a paste of calcium hydroxide on the pulpal floor on one of two buccal cavities of a tooth. Both cavities were then filled with the same mix of cement (*vide* Fig. 23). In this experiment, cement of a thin consistency was used as this had been found to produce the maximum irritant effect on the dental pulp.

In some dogs control cavities were filled with zinc oxide/eugenol cement to compare the response of the pulp with that of zinc phosphate cement. It has been previously demonstrated by Manley (14) and Zander/
Table 8.
Duration of Experiment - 14 days

<table>
<thead>
<tr>
<th>Tooth</th>
<th>Position of Cavity</th>
<th>Type or Consistency of Cement</th>
<th>Time of insertion after Mixing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper right canine</td>
<td>Incisal</td>
<td>Thin</td>
<td>Immed.</td>
</tr>
<tr>
<td></td>
<td>Gingival</td>
<td>Calcium Hydroxide</td>
<td></td>
</tr>
<tr>
<td>Upper right third incisor</td>
<td>Single</td>
<td>Thin</td>
<td>Immed.</td>
</tr>
<tr>
<td>Lower right canine</td>
<td>Incisal</td>
<td>Thin</td>
<td>Immed.</td>
</tr>
<tr>
<td></td>
<td>Gingival</td>
<td>Calcium Hydroxide</td>
<td>Immed.</td>
</tr>
<tr>
<td>Lower right third incisor</td>
<td>Single</td>
<td>Thin</td>
<td>Immed.</td>
</tr>
<tr>
<td>Upper left canine</td>
<td>Incisal</td>
<td>Thin</td>
<td>Immed.</td>
</tr>
<tr>
<td></td>
<td>Gingival</td>
<td>Thick</td>
<td>Immed.</td>
</tr>
<tr>
<td>Upper left third incisor</td>
<td>Single</td>
<td>Thin</td>
<td>Immed.</td>
</tr>
<tr>
<td>Lower left canine</td>
<td>Incisal</td>
<td>Thin</td>
<td>Immed.</td>
</tr>
<tr>
<td></td>
<td>Gingival</td>
<td>Zinc Oxide</td>
<td>Immed.</td>
</tr>
<tr>
<td>Lower left third incisor</td>
<td>Single</td>
<td>Zinc Oxide</td>
<td>Immed.</td>
</tr>
</tbody>
</table>
Zander (16) that zinc oxide/eugenol evokes a minimal pulp reaction. In other dogs an intact canine tooth was sectioned as a further control to study the normal pulp tissue.

In experiments on human teeth the time interval between the placement of the cement and the extraction of the tooth varied between 15-40 minutes. These teeth were extracted by forceps under local anaesthesia.

In the animal experiments this period extended from 15 minutes - 3 months before the animal was sacrificed.

Immediately after extraction, the apices of all teeth were removed to allow the fixing agent, 10% formalin, to penetrate rapidly to the pulp tissue. Sections were prepared which demonstrated the experimental cavity, together with the associated tract of cut dentinal tubules and the related pulp tissue. In this way it was possible to follow the complete course of the dentinal tubules from the cavity to the pulp.

In order to diminish the disturbing effects of acid/
Table 9.

Duration of Experiment - 21 days.

<table>
<thead>
<tr>
<th>Tooth</th>
<th>Position of Cavity</th>
<th>Type or Consistency of Cement</th>
<th>Time of insertion after Mixing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper right canine</td>
<td>Incisal</td>
<td>Medium</td>
<td>Immed.</td>
</tr>
<tr>
<td></td>
<td>Gingival</td>
<td>Thick</td>
<td>Immed.</td>
</tr>
<tr>
<td>Upper right third incisor</td>
<td>Single</td>
<td>Medium</td>
<td>Immed.</td>
</tr>
<tr>
<td>Lower right canine</td>
<td>Incisal</td>
<td>Medium</td>
<td>Immed.</td>
</tr>
<tr>
<td></td>
<td>Gingival</td>
<td>Thick</td>
<td>Immed.</td>
</tr>
<tr>
<td>Lower right third incisor</td>
<td>Single</td>
<td>Medium</td>
<td>Immed.</td>
</tr>
<tr>
<td>Upper left canine</td>
<td>Incisal</td>
<td>Thick</td>
<td>Immed.</td>
</tr>
<tr>
<td></td>
<td>Gingival</td>
<td>Zinc Oxide</td>
<td>Immed.</td>
</tr>
<tr>
<td>Upper left third incisor</td>
<td>Single</td>
<td>Thick</td>
<td>1 min.</td>
</tr>
<tr>
<td>Lower left canine</td>
<td>Incisal</td>
<td>Thin</td>
<td>3 mins.</td>
</tr>
<tr>
<td></td>
<td>Gingival</td>
<td>Thin</td>
<td>Immed.</td>
</tr>
<tr>
<td>Lower left third incisor</td>
<td>Single</td>
<td>Thick</td>
<td>1 min.</td>
</tr>
</tbody>
</table>
acid decalcification, a chelating agent, ethylenediaminetetraacetic acid with a pH of 7.0 - 7.4, was used to remove the calcium salts. The specimen was then embedded in Ester wax and sections of 7μ in thickness prepared. These were stained with haematoxylin and eosin for microscopic examination.
Table 10.

Duration of Experiment - 3 months.

<table>
<thead>
<tr>
<th>Tooth</th>
<th>Position of Cavity</th>
<th>Type or Consistency of Cement</th>
<th>Time of insertion after Mixing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper right canine</td>
<td>Incisal</td>
<td>Calcium Hydroxide + Thin</td>
<td>Immed.</td>
</tr>
<tr>
<td></td>
<td>Gingival</td>
<td>Thin</td>
<td>Immed.</td>
</tr>
<tr>
<td>Lower right canine</td>
<td>Incisal</td>
<td>Calcium Hydroxide + Thin</td>
<td>Immed.</td>
</tr>
<tr>
<td></td>
<td>Gingival</td>
<td>Thin</td>
<td>Immed.</td>
</tr>
<tr>
<td>Upper left canine</td>
<td>Incisal</td>
<td>Calcium Hydroxide + Thin</td>
<td>Immed.</td>
</tr>
<tr>
<td></td>
<td>Gingival</td>
<td>Thin</td>
<td>Immed.</td>
</tr>
<tr>
<td>Lower right canine</td>
<td>-</td>
<td>Control</td>
<td></td>
</tr>
</tbody>
</table>
Pulpo-dentinal junction showing that the reaction of the pulp is strictly confined to the pulp surface opposite the dentinal tubules opened during cavity preparation. Note the loss of the pulpo-dentinal membrane (P.D.) in the area affected. Mag. X 160

Photomicrograph showing the reaction of the pulp to the trauma of cavity preparation. The reaction is confined to the odontoblast layer which shows a slight degree of flattening together with a moderate degree of vacuole formation. Mag. X 160
Results of Experiments on Human Teeth.

Histological examination of teeth with experimental cavities showed that the reaction of the pulp to zinc phosphate cement was confined to those cells which were related to the tract of dentinal tubules cut during cavity preparation (vide Fig. 27). The odontoblast layer to either side of the affected area, or on the opposite side of the pulp cavity, was regarded as a control.

The reaction of the pulp to cavity preparation alone in the case of a freshly prepared cavity which was untreated for a period of forty minutes before the tooth was extracted is shown in Fig. 28. There was a slight flattening of the odontoblast layer and a moderate degree of vacuole formation. These features indicated a mild response of the pulp, despite precautions to minimise the traumatic effect of cavity preparations.

In vivo experiments in human teeth showed that, during a forty minute interval between insertion of the zinc phosphate and extraction of the tooth, a marked reaction of the pulp took place. This reaction was/
Fig. 29.

The reaction of the dental pulp to zinc phosphate cement of a thin consistency. Mag. X 160.
was most severe when the cement was mixed to a thin consistency.

No marked difference was observed between the reactions which followed the use of medium or thick consistencies of the cement. The changes observed in the pulp were mostly confined to the odontoblast layer. Close to the odontoblast layer areas of haemorrhage were observed in some sections, but no generalised inflammatory disturbance was seen.

The effect of a thin mix of zinc phosphate cement upon the pulp is shown in Fig. 29. In this tooth the floor of the cavity was 1.4 mm. from the pulp. A severe disturbance of the odontoblast layer was seen on the pulp surface related to the dentinal tubules exposed by cavity preparation. In place of the palissade-like layer of elongated odontoblast cells, seen elsewhere in the pulp, there was a scattered layer of small round or oval cells in a light matrix containing many fluid spaces. These shrivelled odontoblasts no longer possessed dentinal processes. The pulpo-dentinal membrane, normally a prominent feature of the junction between the odontoblast layer and/
Fig. 30.
Photomicrographs of increasing magnification, of the area marked in Fig. 30a, demonstrate the reaction of the dental pulp to a thin consistency of zinc phosphate cement. This reaction has been confined to the odontoblast layer in relation to the cavity floor.
and the predentine zone, had disappeared.

Further examples of the reaction of the pulp to thinly mixed zinc phosphate cement are seen in Figs. 30(a), (b), (c) and (d). A reaction of the odontoblast layer in relation to the floor of the cavity was again seen. The odontoblast cells had shrivelled and lost all connection with their dentinal processes. They were now separated from the predentine by vacuoles, presumably occupied by oedematous fluid which appeared to be causing pressure on the pulp tissue.

In order to compare the reaction of the same pulp to different consistencies of the cement mixture, two cavities were prepared in several teeth (vide supra). Fig. 31 shows the reaction to a thin mixture and Fig. 31(a) the reaction to a thick mixture of cement in the same tooth. Similarly, Fig. 32 shows the reaction to a cement of medium consistency and Fig. 32(a) the reaction of the same pulp to a thin consistency of cement. An unusual feature of the reaction was the presence of odontoblast nuclei within the dentinal tubules which open on to the floor and walls of the tooth cavity. A higher magnification of this section is shown in Fig. 33.
Fig. 31.

Demonstrates the effect of a thin consistency of cement after a period of 30 minutes. There is a marked disturbance of the odontoblast layer with displacement of the nuclei into the dentinal tubules.

Fig. 31(a).

Demonstrates the effect of a thick consistency of cement in the same tooth. The disturbance of the odontoblast layer is not so severe and fewer odontoblast nuclei are seen in the dentinal tubules. Mag. X 160.

Photomicrographs showing the reaction of a tooth pulp to two different consistencies of zinc phosphate cement.
Fig. 32 shows the reaction of a tooth pulp to a medium consistency of cement. The disturbance is confined to the odontoblast layer which shows a moderate degree of vacuolisation and displacement of the nuclei of the odontoblast into the dentinal tubules.

In Fig. 32a. there has been a more severe reaction of the pulp to a thin consistency of cement. There has been a greater disturbance of the odontoblast layer and an area of haemorrhage may be seen. Many odontoblast nuclei can be seen within the dentinal tubules. Mag. X 160

Photomicrographs of the reaction of a tooth pulp to thin and medium consistencies of zinc phosphate cement after a period of 40 minutes.
Higher magnification showing odontoblast nuclei within the dentinal tubules. Mag. X 400.
This phenomenon was first described by Orban in 1941(22) who suggested that the presence of the odontoblast nuclei in the dentinal tubules was due to the compressing action of the forceps during extraction. This intermittent pressure caused a pumping action on the odontoblasts. Müller in 1948,(23) however, considered that these nuclei represented a form of pulpal degeneration rather than the result of a mechanical action. The same phenomenon occurred under self-polymerising acrylic resin filling materials and, in 1952, Kramer and McLean(24) described this displacement of the odontoblast nuclei into the tubules as 'aspiration of the odontoblasts'. It was maintained by these observers that this was a specific response of the pulp to heat generated during the setting of this material. This conception was confirmed by Maeglin(25) in a later work on self-polymerising acrylic resin filling materials. The heat generated by the preparation of the cavity was considered by James and Schour(26) to be the cause. Lefkowitz(27) went further and maintained that this phenomenon could be considered as diagnostic of excessive heat.
Fig. 34.
Low power magnification showing two cavities; lower cavity filled with zinc phosphate cement of thin consistency; upper cavity floor protected with calcium hydroxide below cement of same consistency.  
Mag. X 12.

Fig. 35(a).
Reaction of pulp below zinc phosphate cement.

Fig. 35.
Reaction of pulp below upper cavity showing normal appearance of pulp tissue.  
Mag. X 170.
heat generated during cavity preparation. However, Stanley and Swerdlow\(^{28}\) in a more recent work have demonstrated that this displacement of the odontoblast nuclei may be associated with other abnormalities of the pulp such as chronic pulpitis. No mention has been made, in previous reports, of the occurrence of this phenomenon after the use of zinc phosphate cement.

In the present investigation, the number of odontoblast cells which had been displaced into the dentinal tubules was greatest when a thin consistency of zinc phosphate cement was used. This was regarded as an indication that the thin consistency of cement produced a greater disturbance in the pulp than either the medium or thick consistencies.

The results of experiments to determine the effect of calcium hydroxide paste when placed below zinc phosphate cement are shown in Figs. 34 and 35. These photomicrographs demonstrate two cavities prepared in one tooth which was extracted thirty minutes after the placement of the cement. In the upper cavity (Fig. 35), in which calcium hydroxide had been placed, the response of the pulp was similar to that obtained by cavity preparation/
Fig. 36.
Photomicrograph demonstrating normal dentine and pulp in dog aged 13 months.
preparation alone. In the lower cavity (Fig. 35a) which was filled with zinc phosphate cement alone there was a slightly greater reaction of the pulp. This was confined to the odontoblast layer which showed vacuolisation and displacement of the odontoblast nuclei into the dentinal tubules.

Results of Experiments on Dogs' Teeth

The normal appearance of the dental pulp and dentine in a dog aged 13 months is shown in Fig. 36. This photomicrograph demonstrates the regular arrangement of the odontoblast cells at the periphery of the pulp, beneath which lies the cellular pulp matrix. Numerous blood vessels are present but there is no evidence of dilation of the blood vessels or haemorrhage. This histological picture is seen at the end of a period of rapid deposition of primary dentine and before regressive physiological changes, associated with increase in age, take place in these dental tissues.

A startling difference was found in the reaction of dogs' teeth and that of human teeth in this experiment.
Fig. 37.
Complete death of dental pulp in response to a thin consistency of zinc phosphate cement. Mag. X 70.

Fig. 38.
Hyperaemia of the pulp in response to a cement of medium consistency. Mag. X 70.

Fig. 39.
Haemorrhage into odontoblast layer of the pulp in response to a cement of a thick consistency. Mag. X 220.
Experimental results on dogs' teeth showed that the reaction of the pulp to zinc phosphate cement was more severe than in human experiments. In many cases where a thin consistency of cement had been used, there was complete death of the pulp (Fig. 37). Where a mix of medium or thick consistency was used, a generalised hyperaemia took place (Fig. 38). In less severe cases, haemorrhages were found in the odontoblast layer. (Fig. 39).

The general response of the pulp to the zinc phosphate cement masked the local reaction in that part of the pulp which was related to the floor of the cavity and it was not possible to assess the effect of variations in the time of placing the mixed cement in the tooth cavity. However, the results showed generally that the thicker the mix of cement when placed in the tooth cavity, the less severe the response of the pulp. These results agreed with those obtained in the human experiments.

Cavities which had been filled with zinc oxide and eugenol produced only a slight reaction of the pulp even/
Fig. 40(a). Reaction of the pulp to a thin consistency of cement in the same tooth.

Mag. X 70.

Fig. 40(b). Odontoblast layer intact under zinc oxide and eugenol filling. General reaction of the pulp.
even when these cavities were deep. The changes which occurred were confined to the odontoblast layer of the pulp in which the odontoblasts were disrupted with a slight degree of vacuole formation. Where zinc oxide and eugenol had been used alone, the pulp underlying the odontoblasts was normal and showed no inflammatory changes. The histological picture was relatively similar to that of the response of the pulp to cavity preparation alone.

Photomicrographs comparing the effects of zinc oxide/eugenol and of zinc phosphate cement on one tooth are shown in Fig. 40 and Fig. 40(a).

Fig. 40 shows the reaction of a pulp to zinc oxide and eugenol. The odontoblast layer of cells does not show any signs of severe damage although the underlying pulp tissue has been disrupted by a more generalised reaction. This reaction must be considered as the response of the pulp to the zinc phosphate cement filling of thin consistency in the other cavity (Fig. 40a) as similar results were obtained with zinc phosphate cement alone. This photomicrograph demonstrates the severe reaction of/
Response of dental pulp to a thin consistency of zinc phosphate cement.

Fig. 41(a). Response of pulp in same tooth to calcium hydroxide below cement filling. Mag. X 220.
of the pulp to the zinc phosphate cement. Vacuoles of fluid have appeared within and between the odontoblast cells and a marked hyperaemia has disrupted the deeper pulp tissue.

The effect of calcium hydroxide paste on the floor of the cavity below zinc phosphate cement is shown in Fig. 41. This higher magnification of the pulp surface shows the odontoblast layer which retains its regular columnar appearance with only an occasional vacuole present.

The response of the pulp to zinc phosphate cement alone in the same tooth is demonstrated in Fig. 41(a). The number of vacuoles formed between the odontoblast cells is greatly increased and the normal appearance of the layer of columnar cells has disappeared indicating that a more severe reaction has taken place.
The purpose of this experiment was to measure the amount of arsenic present in the dental tissues as a result of diffusion from zinc phosphate cement.

Method

The arsenic content of sound permanent teeth was first determined to establish the normal limits for this element before determining the arsenic content of teeth which had been previously filled with zinc phosphate cement. Samples, weighing not more than 20 mg, were taken from 26 sound permanent teeth previously fixed in formalin.

The arsenic content of four different cement powders was also determined as the teeth which were used for the experiment had been filled in general dental practice and the type of zinc phosphate cement used was therefore unknown.

Fifty extracted teeth, previously fixed in formalin and containing zinc phosphate linings, were selected for analysis. The time which the cement had been in the cavity was unknown. Longitudinal ground sections of/
Fig. 42.

Sections of teeth taken for analysis from area (A) below cement filling (C).
of the teeth were prepared to a thickness of 300-400μ and samples weighing not more than 20 mg, taken from the areas in close proximity to the cavity as shown in Fig. 42.

The method of determination of arsenic was the activation method of Lenihan and Smith (21), which is described in Appendix 1.

The sensitivity of this method is $5 \times 10^{-9}$ gm. compared with the lowest chemical sensitivity of $10^{-7} - 10^{-8}$ gm. The accuracy of the determination is limited only by the statistical error of counting which is 1% for 10,000 counts.
### Table 11.

<table>
<thead>
<tr>
<th>Arsenic Content of Sound Permanent Teeth in parts per million (p.p.m.)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.076</td>
<td>0.093</td>
<td>0.047</td>
</tr>
<tr>
<td>0.078</td>
<td>0.069</td>
<td>0.050</td>
</tr>
<tr>
<td>0.102</td>
<td>0.031</td>
<td>0.037</td>
</tr>
<tr>
<td>0.108</td>
<td>0.032</td>
<td>0.034</td>
</tr>
<tr>
<td>0.052</td>
<td>0.043</td>
<td>0.047</td>
</tr>
<tr>
<td>0.055</td>
<td>0.145</td>
<td>0.050</td>
</tr>
<tr>
<td>0.057</td>
<td>0.043</td>
<td>0.055</td>
</tr>
<tr>
<td>0.087</td>
<td>0.037</td>
<td>0.098</td>
</tr>
<tr>
<td>0.054</td>
<td>0.034</td>
<td></td>
</tr>
</tbody>
</table>

### Table 12.

<p>| Arsenic Content of Zinc Phosphate Cement Powder in parts per million (p.p.m.) |
|---------------------------------|----------------|
| Cement No. 1                   | .287          |
| Cement No. 2                   | .233          |
| Cement No. 3                   | .310          |
| Cement No. 4                   | .310          |</p>
<table>
<thead>
<tr>
<th>Arsenic Content of Teeth previously filled with Zinc Phosphate Cement Powder in parts per million (p.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.012</td>
</tr>
<tr>
<td>0.074</td>
</tr>
<tr>
<td>0.066</td>
</tr>
<tr>
<td>0.090</td>
</tr>
<tr>
<td>0.075</td>
</tr>
<tr>
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</tr>
<tr>
<td>0.026</td>
</tr>
<tr>
<td>0.008</td>
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<tr>
<td>0.034</td>
</tr>
<tr>
<td>0.183</td>
</tr>
<tr>
<td>0.087</td>
</tr>
<tr>
<td>0.106</td>
</tr>
<tr>
<td>0.034</td>
</tr>
<tr>
<td>0.262</td>
</tr>
<tr>
<td>0.035</td>
</tr>
<tr>
<td>0.017</td>
</tr>
</tbody>
</table>
Results

The results of the analysis of 25 sound permanent teeth are shown in Table 11. These show the arsenic content of normal teeth to lie within the range of 0.031 - 0.145 p.p.m.

The arsenic content of the four cement powders is shown in Table 12. This lies between 0.233 - 0.310 p.p.m.

The results of analysis of teeth previously filled with zinc phosphate cement, showed values between 0.0008 - 0.295 p.p.m. (vide Table 13).

The mean value of arsenic for sound human teeth is 0.060 p.p.m.; and 0.065 p.p.m. for those teeth which had been previously filled with zinc phosphate cement.
Discussion

As was stated in the introduction to this study, the dental tissues, dentine and pulp, must be considered physiologically to be a single tissue. Any stimulus, thermal, chemical, mechanical or bacterial, applied to dentine produces a response in the dental pulp and the degree of this response depends on the intensity and duration of the applied stimulus.

Before assessing the experimental results, it is essential to appreciate fully the fundamental difference which exists between the response of the dental pulp in an intact healthy tooth used for experiments and in one affected by a pathological process such as caries.

Once the carious process is established in a tooth, the lesion progresses continuously giving rise to a minor stimulation of the pulp. This evokes a protective response by the formation of additional calcific deposits which can be recognised histologically. These seal off the affected area of dentinal tubules, both laterally and pulpally, by the formation/
formation of dead tracts and secondary dentine. The protective barriers reduce the permeability of the dentine between the affected carious area and the pulp. In the experiments carried out, the stimulus is immediate and comparatively severe and does not permit this defensive reaction to take place and the damage to the pulp is correspondingly greater.

In cavity preparation, it is necessary to remove sound enamel and dentine in addition to the carious area of these tissues in order to retain the filling material and to preclude further caries. As a result of this mechanical preparation, in each cavity the pulp underlying the carious area is protected to some degree by secondary dentine, whilst in the remainder, where fresh dentinal tubules have been opened, no such barrier exists.

To reiterate: caries produces protective barriers within the living tissues of pulp and dentine; protective reaction is not possible when a caries-free tooth has a cavity prepared within it. Therefore penetration to the pulp from the dentine is easier in a healthy tooth than in one where caries has excited
a protective response.

The dentinal tubules which are freshly cut during cavity preparation will permit the penetration of any irritant and the conditions will be similar to those which exist in the experimental teeth. The degree of reaction of a dental pulp will also be influenced by two important factors.

(a) The condition of the dentinal tubules which are wider in the young than in the old. These become narrower with increasing age, due to progressive calcification of the walls of the tubules.

(b) The amount of dentine remaining between the floor of the cavity and the dental pulp. The smaller the amount of dentine the greater the response of the pulp.

It has been stressed by many previous workers and must be stressed again that it is dangerous to open large areas of sound dentine during cavity preparation, especially in young children.

The results of the experimental work on zinc phosphate cement have confirmed previous research work on this material in that, when this cement is placed in contact with vital dentine, some degree of reaction may be expected in the dental pulp.

Previous/
Previous investigators who reported this reaction, correctly deduced from the evidence that it was due to the acidity of this cement. In these investigations, however, no quantitative assessment was made of either consistency of cement or of the time between mixing and insertion into the tooth cavity.

In this study it has been shown conclusively by radioactive tracer methods that the acid products, phosphoric acid and primary acid phosphate, present during the setting of this cement, penetrate along the cut dentinal tubules towards the pulp. The degree of penetration and the severity of the pulpal reaction vary with the consistency and the time of insertion of the cement into the tooth cavity. Penetration and reaction are most severe with thin consistencies, inserted immediately after mixing. A progressive reduction of the severity of the reaction is observed as the consistency increases and as the time after mixing is increased. This relationship between reaction and consistency and time of insertion has long been recognised clinically but has not been previously demonstrated experimentally. Comparison of/
of the degree of irritation produced in the pulp by differing consistencies of cement fully confirms the use of the thickest possible mix of cement to reduce pulp reaction to a minimum.

The distance which the acid products penetrated along the cut dentinal tubules increased with the period for which the cement remained in the tooth cavity. In none of the in vivo experiments was complete penetration to the dental pulp demonstrated. The complete penetration demonstrated in extracted teeth, previously fixed in formalin or saline, must be considered as being due to the increased permeability of the dentine which occurs as a result of death of the protoplasm of the dentinal fibril. This increased permeability of tissues was observed by Osterhout(29) who stated:

"Death (of protoplasm) is accompanied by an increase in permeability - it is a matter of common observation that cells may resist the penetration of certain dyes as long as they are alive, but absorb them readily as they are killed."

This observation was confirmed more recently by Amler(30) and Bodecker.(31)
Histological examination of the dental pulp and examination with radioactive tracers showed that, when calcium hydroxide paste is placed between the vital dentine and the zinc phosphate cement, it is effective in preventing penetration of acid through dentine and in reducing its effect on the dental pulp. In view of these results obtained experimentally it is recommended that, where large numbers of fresh dentinal tubules are opened during cavity preparation especially in young teeth, calcium hydroxide paste should be placed below the zinc phosphate cement lining.

The results of the arsenic analysis showed that there is no significant difference in the arsenic content of sound teeth and in those which had been previously filled with zinc phosphate cement.

The experiment showed conclusively that there was no diffusion of arsenic from zinc phosphate cement into the dental tissues. This finding is in contrast to all that has been previously believed to the contrary.
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17./


CHAPTER 6

Silver Nitrate
Silver Nitrate is one of the oldest drugs used in conservative dentistry in the treatment of dental caries. Its value in this respect was recognised as early as 1846 and, since then, it has been in continuous use. However, its use in this connection was purely empirical until the late nineteenth century when articles discussing its mode of action appeared in dental literature. (1)

Very little scientific study was devoted to dental applications of silver nitrate until 1902 when Szabo (2) measured the penetration of an aqueous solution into dentine. Miller (3), in 1905, in part of his great study of dental caries, stated that coagulation of protoplasm occurred within the dentinal tubules after they had been impregnated with silver nitrate. He further stated that this coagulation protected the dentine against further acid decalcification which was the keystone to his theory of dental caries.

At this time, silver nitrate was applied either as an aqueous solution or as solid crystals. After application/
application in either form, strong artificial light or sunlight was used to reduce the nitrate to the metallic silver.

A great improvement in technique took place, in 1917, with the introduction of a new solution of silver nitrate by Howe (4). This was an ammoniacal solution of silver nitrate and the reducing agent which he recommended was a 25% solution of formalin in water. The directions for the applications of these two solutions were as follows:-

"Any applicator will answer the purpose for conveying the liquids to the cavity. Broaches wrapped in cotton wool will serve the purpose. In the clinic here we use two pieces of glass tubing - one tube we keep for the ammoniacal silver solution and the other for the formalin. A tube of solution 1 is taken, the capillary portion filled, the finger placed over the end, and this is conveyed to the cavity. By momentary raising the finger a small drop of silver solution is allowed to flow into the cavity. A small drop of solution 2 is now flowed in, the solution darkens, silver is reduced and is deposited upon the surface. After a few moments, absorb this solution and repeat the process, in order that more silver may be reduced and deposited."

Many enthusiastic claims followed the introduction of this solution as a sterilizing agent. Howe wrote:-
wrote:—

"It is effective in sterilisation of the disintegrating dentine overlying the pulp and in large cavities of carious first molars."

Prime (5) was also enthusiastic and reported that

"it will definitely neutralise, sterilise, harden and metallise deep carious tissue over the pulp."

G.V. Black (6), however, advised caution stating:—

"A word of caution should be said about the use of silver nitrate, and it is an important one. It must not be used if the decay has approached near the pulp of the tooth. There is nothing else that will cause so severe a toothache as silver nitrate used over a pulp that is nearly exposed by decay."

While it was generally agreed that silver nitrate would penetrate carious dentine, a great deal of controversy has existed regarding its penetration into sound dentine. Richards (7), Booth (8) and Ireland (9) have reported that silver nitrate would penetrate to the full depth of decalcified tissues but this penetration ceased once sound dentine was reached.

The results of a more recent work by Zander (10), in 1943, showed that silver nitrate would not only penetrate/
penetrate sound dentine, but also many forms of irregular dentine.

Silver nitrate has been widely used in the belief that it was 'self-limiting' in its action. This concept was based on the fact that silver proteinate was formed by the interaction of the silver nitrate with the protein of the dentinal fibril, contained within each dentinal tubule, thus forming a barrier to further penetration. It is still the view of many of the dental profession that further penetration will not take place beyond this barrier.

In this investigation, the penetration and uptake of silver nitrate, by the dental tissues, was studied by radiochemical methods of investigation using a radioactive isotope of silver.

There are a number of isotopic forms of silver, but for the present series of experiments the isotope Ag. was used. This isotope has a half-life of 270 days and decays with the emission of Beta and Gamma rays of various energies. It may be conveniently detected by the Gamma rays, which have energies of 0.66, 0.89, 0.94 and 1.39 MeV.
The following investigations were carried out with silver nitrate:

1. Measurement of the uptake of silver nitrate by the enamel.


3. The effects of medicaments used in cavity sterilisation on the penetration of silver nitrate.
Purpose of the Experiment No. 1

In this part of the study, experiments were carried out to determine:

1. The rate of uptake of silver by the enamel of the teeth in relation to the time of immersion in a solution of silver nitrate.

2. The total amount of silver taken up by the tooth enamel after specific time intervals.

Method I.

Rate of Uptake of Silver

A radioactive silver nitrate solution was prepared by adding 1 ml. of radioactive silver, $^{110}$Ag, (containing about 0.1 Ag.), to 1 ml. of an ammoniacal silver nitrate solution. This radioactive silver nitrate solution had a specific activity of the order of 100 uc. per ml. which gave counting rates up to 20,000 counts per minute for each tooth. The ammoniacal silver nitrate solution was prepared from the following quantities as in Howe's formula:

Silver nitrate ...... 70.4 gm.
Distilled water ...... 24.5 ml.
Strong ammonia ...... 68.0 ml.

The/
The silver nitrate was dissolved in the water and the solution heated slightly; the ammonia was then added dropwise until the precipitate formed by the addition of ammonia had re-dissolved.

Freshly extracted sound teeth, which had been kept in 10% formalin were used for this investigation. The teeth were washed in water to remove the formalin.

The ages of the patients from whom the teeth had been extracted were unknown. As this investigation was undertaken to measure the uptake by the crown of the tooth only, the tooth root was coated with a cellulose acetate varnish solution to prevent absorption of silver nitrate by the root.

Each tooth was placed in the radioactive silver nitrate solution for a period of five minutes and then washed according to a standardized procedure. This consisted of washing the tooth in running water for a period of one minute, after which the tooth surface was dried by rolling it gently on clean dry filter paper. The dried tooth was placed in the guide of the perspex jig in the counter (vide Fig. 5) and its radioactivity determined in the scintillation counter. The/
The tooth was removed from the counter and replaced in the silver nitrate solution for a further five minutes. This process was repeated at five minute intervals until the tooth had been immersed in the radioactive silver nitrate solution for a period of one hour.

Graphs were prepared from the results obtained to show the rate of uptake against the time of immersion (vide page 145).

**Method 2.**

**Total Silver Uptake.**

In the second part of this investigation, the radioactive silver nitrate solution was prepared as in the previous experiment (vide supra). The amount of silver in this solution was measured, and the count rate (per minute) determined, per mg. of silver (vide Appendix 2). As this investigation was designed to measure the uptake of silver by the crown of the tooth only, the root portion of each tooth was sealed with a cellulose acetate varnish. After the tooth had been immersed in the silver nitrate solution, the root portion/
portion was removed. This procedure was carried out in all subsequent experiments in this section of the investigation.

**Experiment (a)**

In this experiment seven sound premolar teeth, which had been previously fixed in 10% formalin, were used. The ages of the patients from whom the teeth had been extracted were unknown.

The first tooth was immersed in the radioactive silver nitrate solution for a period of five minutes. The time of immersion was progressively increased by five minute intervals for each tooth, until the last tooth was immersed for a period of thirty-five minutes. After immersion, each tooth was washed in running water for one minute and dried by gently rolling on a clean dry filter paper. The amount of silver taken up by the enamel of each tooth was determined by the method described in Appendix 2. and the results are shown in Table 15.

**Experiment (b)**

Six sound premolar teeth, of unknown age, but approximately/
approximately the same surface area were used for the second experiment. These teeth had been previously fixed in 10% formalin.

Each tooth was immersed in the radioactive silver nitrate solution for a period of five minutes and then washed and dried according to the procedure followed in the previous experiment. The amount of silver was again determined by the method described in the Appendix and the results are shown in Table 16.

Experiment (c)

As the results obtained in the previous experiment had shown wide variations in the amount of silver taken up by the enamel of the crown of the tooth, six premolar teeth from the age group of 12-14 years were used. These teeth had been previously fixed in 10% formalin.

In an attempt to determine if wide variations occurred in the surface area of these teeth, measurements were made of the bucco-lingual and mesio-distal surfaces of each tooth. An impression of the crown of each tooth was also taken in a rubber-base impression material and the volume of water required to fill this impression, was weighed.

Each tooth was immersed in the radioactive silver nitrate solution for a period of five minutes and the same/
same procedure of washing and drying each tooth carried out. The amount of silver in each tooth was determined and the results given in Table 17.
Table 14.

<table>
<thead>
<tr>
<th>Total Time of Immersion in</th>
<th>Counts per Minute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag. No. 3 in Minutes.</td>
<td>Teeth 1</td>
</tr>
<tr>
<td>5</td>
<td>6600</td>
</tr>
<tr>
<td>10</td>
<td>6825</td>
</tr>
<tr>
<td>15</td>
<td>6435</td>
</tr>
<tr>
<td>20</td>
<td>7902</td>
</tr>
<tr>
<td>25</td>
<td>8621</td>
</tr>
<tr>
<td>30</td>
<td>9100</td>
</tr>
<tr>
<td>35</td>
<td>9985</td>
</tr>
<tr>
<td>40</td>
<td>10350</td>
</tr>
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<td>45</td>
<td>10890</td>
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<td>50</td>
<td>11600</td>
</tr>
<tr>
<td>55</td>
<td>11432</td>
</tr>
<tr>
<td>60</td>
<td>11868</td>
</tr>
</tbody>
</table>
Fig. 43.

Fig. 44.
Results - 1.

The numerical results of the counting rate per minute, of five teeth, are given in Table 14. A curve of the counting rate per minute against the time of immersion in the radioactive silver, was plotted for each tooth. Two of these curves are illustrated in Fig. 43 and Fig. 44. The standard errors of the counting rate determinations (vide page 40) are also shown. In all cases, a straight line was best fitted to the plotted points of the graph.

In the specimens investigated, the uptake of silver nitrate reached a constant value after a time which varied from 0 - 5 minutes.

These results establish that human enamel is penetrated by silver nitrate. The rate of penetration is constant and after a period of about 50 minutes the crown becomes saturated and no further absorption of silver nitrate will occur.
Table 15.

<table>
<thead>
<tr>
<th>Time of Immersion in Minutes</th>
<th>Counts per Minute</th>
<th>Amount of Silver (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>46.5</td>
<td>0.186</td>
</tr>
<tr>
<td>10</td>
<td>23.3</td>
<td>0.093</td>
</tr>
<tr>
<td>15</td>
<td>46.3</td>
<td>0.185</td>
</tr>
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<td>20</td>
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<td>25</td>
<td>172.3</td>
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<td>30</td>
<td>34.3</td>
<td>0.137</td>
</tr>
<tr>
<td>35</td>
<td>154.9</td>
<td>0.618</td>
</tr>
</tbody>
</table>

Table 16.

<table>
<thead>
<tr>
<th>Counts per Minute</th>
<th>Amount of Silver (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.3</td>
<td>0.021</td>
</tr>
<tr>
<td>26.1</td>
<td>0.104</td>
</tr>
<tr>
<td>85.7</td>
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<tr>
<td>38.9</td>
<td>0.156</td>
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<tr>
<td>11.3</td>
<td>0.045</td>
</tr>
<tr>
<td>30.9</td>
<td>0.124</td>
</tr>
</tbody>
</table>
Results - 2.

The amounts of silver taken up by the enamel of the crown of the teeth, in relation to the time of their immersion in an ammoniacal silver nitrate solution, are shown in Table 15. These results showed that, where the age of the patient was unknown, there was no direct relationship between the amount of silver taken up by the enamel of the tooth and the time of its immersion in the silver nitrate solution.

The results of the second experiment are shown in Table 16. In this experiment the amount of silver taken up by the enamel of the teeth ranged from 0.021mg. - 0.343mg. when each of these teeth was immersed in the ammoniacal silver nitrate solution for an equal period of 5 minutes.

When this experiment was repeated using teeth of a known age group (12-14 years) the results did not show as wide a variation in the amount of silver absorbed as in the previous experiment (vide Table 16). These results were within the range of 0.042-0.142mg. of silver.
Table 17.

<table>
<thead>
<tr>
<th>Tooth</th>
<th>Weight of Water in Impression (gms.)</th>
<th>* Surface Measurements (mm.)</th>
<th>Counts per Minute</th>
<th>Amount of Silver (mgm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Premolar</td>
<td>0.270</td>
<td>M/D 7.0</td>
<td>11.5</td>
<td>0.046</td>
</tr>
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<td></td>
<td></td>
<td>B/L 7.5</td>
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</tr>
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<td></td>
<td></td>
<td>B. 8.5</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>L. 7.0</td>
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</tr>
<tr>
<td>1st Premolar</td>
<td>0.193</td>
<td>M/D 7.0</td>
<td>15.9</td>
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<tr>
<td></td>
<td></td>
<td>B/L 7.5</td>
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</tr>
<tr>
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<td>B. 8.5</td>
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<tr>
<td></td>
<td></td>
<td>L. 7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st Premolar</td>
<td>0.250</td>
<td>M/D 6.75</td>
<td>20.9</td>
<td>0.084</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B/L 8.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. 8.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. 7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd Premolar</td>
<td>0.267</td>
<td>M/D 7.0</td>
<td>14.5</td>
<td>0.058</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B/L 8.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. 9.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. 7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd Premolar</td>
<td>0.300</td>
<td>M/D 7.0</td>
<td>38.1</td>
<td>0.142</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B/L 7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. 10.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. 5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd Premolar</td>
<td>0.200</td>
<td>M/D 7.0</td>
<td>10.6</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B/L 7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. 9.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. 6.25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* B/L: Bucco-lingual width.
M/D: Mesio-distal width.
B: Tip of Buccal Cusp to Gingival Margin.
L: Tip of Lingual Cusp to Gingival Margin.
The surface measurements of the six premolar teeth used in this experiment, are shown in Table 17. It was observed that the tooth which showed the greatest uptake of silver (0.142mg.) had also the largest measurement from the tip of the buccal cusp to the gingival margin.
Purpose of the Experiment No. 2

It was the purpose of this part of the study to determine if the precipitation of silver from a silver nitrate solution would prevent further penetration of silver nitrate through dentine. The precipitant selected was eugenol.

Method

In vivo experiments were carried out in the teeth of two dogs whose ages were nine months and fifteen months.

Radioactive ammoniacal silver nitrate was prepared as in previous experiments with enamel (vide page 140). In the present experiments, the specific activity of the amount of radioactive silver required for each cavity was approximately 8-10 μc.

In one of the dogs, three cavities were prepared in each canine tooth, according to the standardized procedure, in the incisal, middle and gingival zones (vide Fig. 9). The radioactive silver nitrate solution was placed in each of the three cavities. After a period of 5 minutes, the silver in the incisal cavity/
cavity was precipitated with eugenol; after 10 minutes the middle cavity, and after 15 minutes the gingival cavity. In two of the teeth the order of precipitation was reversed, that is, five minutes in the gingival cavity; 10 minutes in the middle cavity and 15 minutes in the incisal cavity.

The first experiment showed that the penetration of silver nitrate was always greatest in the incisal cavity irrespective of the time of precipitation of the silver. Further experiments in the teeth of the second dog only were confined to the middle and gingival zones of each tooth. In this dog, two cavities were prepared in each canine tooth and radioactive silver nitrate placed in the cavities. The silver in one cavity was precipitated with eugenol after a period of 5 minutes while the silver nitrate in the remaining cavity was not treated.

In both dogs, the cavities were dried with a pledglet of cotton wool following the application of the silver nitrate, and sealed with gutta percha. The teeth were extracted under a general anaesthetic after periods of 10 minutes, 4 days, 7 days and 11 days. Immediately/
Immediately following extraction, the pulp of each tooth was removed, placed in normal saline and examined for evidence of radioactivity by counting methods.

In addition autoradiographs were prepared on ground sections 100μ-150μ in thickness. Kodak A.R.50 film was used for the autoradiographs which were given an exposure time of 17 hours. These were developed in Kodak D.19 B. developer for five minutes at a temperature of 68°F.

The maximum depth of penetration of silver nitrate was measured in each cavity along the line of the dentinal tubules which passed from the floor of the cavity to the dental pulp.
Fig. 45(a). Autoradiograph of penetration of silver nitrate through dentine in the incisal cavity of a canine tooth. Silver nitrate has penetrated to a maximum distance of 0.7 mm. along the dentinal tubules. Silver was precipitated 5 minutes after application of silver nitrate.

Fig. 45(b). Penetration of silver nitrate has taken place to a maximum distance of 0.18 mm. Silver was precipitated 10 minutes after the application of silver nitrate.

Fig. 45(c). Penetration of silver nitrate in gingival cavity to a maximum distance of 0.18 mm. Silver precipitated 15 minutes after application of silver nitrate. Mag. X 60.
Fig. 46(a). Autoradiograph of penetration of silver nitrate along the dentinal tubules to a distance of 1.0mm. The silver was precipitated 15 minutes after the application of silver nitrate solution.

Fig. 46(b). Silver penetration after 10 minutes. Penetration of silver to a distance of 0.24mm.

Fig. 46(c). Silver penetration 5 minutes after application of silver nitrate solution. Penetration to a distance of 0.24mm. Mag. X 60.
Results

Autoradiographs showing the penetration of silver nitrate in a canine tooth, extracted after 10 minutes are shown in Figs. 45(a), (b) and (c).

Fig. 45(a) shows a cavity in which the silver was precipitated after 5 minutes. The maximum depth of silver penetration was 0.7 mm. In Figs. 45(b) and 45(c) the silver remained unprecipitated for 10 minutes and 15 minutes respectively. Equal penetration to a depth of 0.18 mm occurred in these cavities.

Autoradiographs of a tooth in the same dog, 11 days after the application of silver nitrate, are shown in Fig. 46(a), (b) and (c). In this tooth, the silver was precipitated firstly in the gingival cavity followed by the middle and incisal cavities. An overall deeper penetration of silver nitrate has taken place in this tooth in all cavities. The greatest penetration has again occurred in the incisal cavity, the silver nitrate penetrating to a depth of 1.0 mm. In the middle and gingival cavities penetration has been equal to a maximum depth of/
Autoradiographs showing penetration of silver nitrate after a period of 10 minutes. Silver precipitated with eugenol in upper cavity (Fig. 47a). Mag X 60.
Autoradiographs showing penetration of silver nitrate after a period of 11 days. Silver precipitated with eugenol in upper cavity (Fig. 48a). These autoradiographs also demonstrate the penetration of radioactive silver beyond stained area. Mag. X 110.

Autoradiograph showing penetration of silver nitrate backwards along dentinal tubules cut during cavity preparation. Mag. X 60.
of 0.24 mm.

Autoradiographs of the second experiment are shown in Figs. 47(a) and (b). These figures demonstrate the penetration of silver nitrate after 10 minutes. The silver in the upper cavity was precipitated with eugenol while the lower cavity was untouched. The maximum penetration in the upper cavity was 0.15 mm. and in the lower cavity 0.16 mm. A slight overall increase in depth of penetration had occurred in the lower cavity and in both autoradiographs the penetration was greatest in the apical part of each cavity.

Further autoradiographs, prepared from teeth extracted after 11 days, are shown in Figs. 48(a) and (b). As in the previous tooth the silver in the upper cavity was precipitated first. Maximum penetration of silver nitrate to a distance of 0.27 mm. had occurred in the upper cavity whilst in the lower this distance was 0.25 mm.

In all sections it was observed that silver nitrate also penetrated backwards along the line of the cut dentinal tubules as shown in Fig. 49.

Pulp Examination/
Pulp Examination

No evidence of radioactivity was found from the examination of the dental pulp in all the extracted teeth.
Purpose of the Experiment No. 3

It was the purpose of this part of the study to determine if prior application of the medicaments alcohol and phenol, affected the depth of penetration of a silver nitrate solution through dentine.

Method

In vivo experiments were carried out on the canine and upper third incisor teeth of three dogs whose ages were eight months, ten months and fourteen months.

In each dog, three cavities were prepared on the canine teeth and two on the upper third incisor teeth according to the standardized procedure. These cavities were disposed in the incisal, middle and gingival zones of the labial surfaces of each tooth (vide illustration, page 53).

Radioactive ammoniacal silver nitrate solution was prepared as for the experiments with the enamel of the teeth (vide page 140). This solution gave a specific activity of 8–10 μc of radioactive silver required for each cavity.
In order to observe the effects of alcohol and phenol on the penetration of silver nitrate through dentine, a pledglet of cotton wool which had been saturated in absolute alcohol was applied to one of the cavities for a period of one minute. In the same tooth, the procedure was repeated using phenol in one of the remaining cavities. As a control, the third cavity was untouched. In teeth where only two cavities had been prepared, either phenol or alcohol was applied to one of these cavities for a period of one minute and the remaining cavity left untouched, as a control. Radioactive silver nitrate was then placed in each cavity for a period of two minutes before precipitating the silver with eugenol. The cavities were then sealed with gutta percha.

The treated teeth were extracted from the three dogs, under a general anaesthetic, after periods of 3 days, 7 days and 10 days. Autoradiographs were prepared on ground sections of 100-150μ in thickness, using Kodak A.R.50 film. These were given an exposure time of 36 hours and were developed for 6 minutes in Kodak D.19 B. developer.
The maximum penetration of silver nitrate was measured along the line of the dentinal tubules from the floor of the cavity to the pulp.
Table 18.

<table>
<thead>
<tr>
<th>Duration of Experiment</th>
<th>Position of Cavity</th>
<th>Distance of Penetration of Silver Nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incisal</td>
<td>3 days Middle Gingival</td>
</tr>
<tr>
<td>3 days</td>
<td>Middle</td>
<td>3 days Middle Gingival</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>7 days Middle Gingival</td>
</tr>
<tr>
<td>7 days</td>
<td>Middle</td>
<td>7 days Middle Gingival</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>10 days Middle Gingival</td>
</tr>
<tr>
<td>10 days</td>
<td>Middle</td>
<td>10 days Middle Gingival</td>
</tr>
</tbody>
</table>

- [ ] Alcohol
- [ ] Phenol
- [ ] Control
Fig. 50. Cavity previously treated with absolute alcohol.

Fig. 50a. Control cavity

Autoradiographs demonstrating the penetration of silver nitrate after 3 days. Mag. X 70.
Fig. 51. Control cavity

Fig. 51a. Cavity previously treated with phenol

Autoradiographs demonstrating the penetration of silver nitrate after 3 days. Mag X 60.
Results

The penetration of silver nitrate was increased by the prior application of either alcohol or phenol to the tissues of the cavity floor. This fact was demonstrated in every tooth by examination of the autoradiographs. Table 18 demonstrates the distances of penetration in the teeth treated with ammoniacal silver nitrate for periods of 3 days, 7 days and 10 days.

Autoradiographs are shown in Figs. 50 and 50(a) which demonstrate the distance to which the silver nitrate has penetrated in 3 days. In the upper cavity (Fig. 50) which had been previously treated with absolute alcohol, silver nitrate has penetrated to a distance of 0.42mm. In the lower untreated control cavity (Fig. 50a.), the penetration was 0.18mm.

The effect of phenol after a similar period of 3 days is shown in Figs. 51 and 51(a). In this tooth, the upper cavity was used as a control and silver nitrate has penetrated to a depth of 0.08mm. (Fig. 51a.). Increased penetration has taken place to a depth of 0.45mm in the cavity treated with phenol (Fig. 51a).
Fig. 52a. Control cavity

Fig. 52b. Cavity previously treated with alcohol

Fig. 52c. Cavity previously treated with phenol

Autoradiographs demonstrating the penetration of ammoniacal silver nitrate after a period of 10 days. Mag. X 60.
The effects of both alcohol and phenol, in one tooth, after an interval of 10 days, are shown in Figs. 52(a), 52(b), 52(c). Silver nitrate has penetrated to a depth of 0.15mm. in each of the cavities previously treated with alcohol and phenol, and to a depth of 0.08mm. in the control cavity.
In assessing results from experiments to determine the penetration of various substances through teeth, it is important to note that the permeability of the dental tissues is a variable factor, and it is wrong to assume that all teeth are similar in this respect.

The young dental tissues are very permeable and, as Bodecker (11) has shown, permeability decreases with advancing age and with the process of attrition. Additional layers of secondary dentine may be formed which contain fewer tubules than primary dentine. Also, any protective calcification of the tubules in primary dentine will effectively diminish the pathways available and so reduce the permeability of both dentine and the covering enamel. The changes which take place in dentine can be readily demonstrated histologically, but it is more difficult to demonstrate changes in enamel. This led to a widespread belief that enamel, once formed, remains virtually unchanged throughout life. Whilst this is true to a greater degree than in the case of dentine, experiments/
experiments have shown that changes in enamel do take place.

Dental literature contains many references to the use of metallic salts applied to enamel in the belief that their application would exert an inhibitory effect upon caries. By far the most widely used of these salts is silver nitrate, either in ammoniacal or aqueous solution.

Experiments in the present investigation showed that intact enamel of extracted teeth absorbed ammoniacal silver nitrate at a constant rate in relation to the duration of application. No further uptake was observed after a period of fifty minutes. The amounts of silver taken up by the enamel were found to vary with the age of the tooth and with the total surface area of the crown. No experiment was conducted specifically to determine the relationship between uptake and age, but the general conclusion drawn from the results was that the uptake of silver nitrate by enamel was less in young teeth. It has already been established by Wainwright (12) that these silver salts are concentrated in fissures, enamel lamellae,
lamellae, cuticle, prism sheaths and enamel defects.

Silver nitrate has been generally used in the belief that it was a 'self-limiting' drug. Its penetration into vital dentine was limited by the occlusion of the dentinal tubules as the result of the formation of a precipitate of silver proteinate formed by reaction between the silver nitrate and the protein of the dentinal fibril. In the present work it has been demonstrated with radioactive silver that penetration of silver takes place beyond this area and, therefore, the term 'self-limiting' is not strictly accurate. Silver nitrate penetration was confined to those dentinal tubules which were cut during cavity preparation. It was observed also that penetration occurred both in a pulpal and a peripheral direction where a dentinal tubule was cut at an intermediate point in its course from the amelo-dentinal junction to the pulpo-dentinal junction. Silver nitrate penetrates rapidly through vital dentine to a considerable depth but in none of the present experiments did it reach the pulp.

It was found that the application of a precipitant such/
such as eugenol to the silver nitrate solution did not affect the depth of penetration of the silver.

It has long been considered desirable that the permeability of the non-carious dentine exposed during cavity preparation should be reduced to lessen the response of the pulp to external stimuli. G.V. Black, upon whose work all modern cavity techniques are based, laid down a series of progressive stages for the mechanical preparation of the cavity. The final stage of cavity preparation specified the 'toilet of the cavity' prior to filling. Alcohol and phenol have been and are still employed for this purpose in the belief that the dentine is sterilized and a coagulum formed within the dentinal tubules which prevents the penetration of irritants along the cut dentinal tubules. In vivo studies on dogs' teeth of the effects of alcohol and phenol on the exposed dentine of the cavity floor, showed without doubt that these drugs do not decrease permeability but actually increase permeability. Coagulation of the protoplasm within the dentinal tubules by the application of these drugs produces an increase in permeability of the dentine in vital/
vital teeth, similar to that obtained in the extracted teeth previously fixed in formalin.

In view of these results it is advised that neither phenol nor alcohol should be used for the toilet of the cavity where vital dentine is cut during cavity preparation.

(It is considered relevant at this stage to discuss the conflicting results which have been obtained from previous investigations, using different dyes, into the permeability of dentine. However, recent work on the permeability of cartilage to dyes has offered a possible solution to this question.⁷)

These experiments suggest that the chondroitin sulphate of cartilage is a polyanion of high molecular weight. In this chondroitin sulphate all the anionic groups of the polysaccharide component are free and are associated with simple inorganic cations. The results of these experiments show that chondroitin sulphate behaves like a membrane with a high anionic charge and such a membrane is selectively impermeable to ions carrying the same charge and freely permeable to ions of the opposite charge. This means that cartilage/
cartilage will be impermeable to anionic dyes such as Congo Red and permeable to cationic dyes such as Methylene Blue. As the ground substance of dentine also contains chondroitin sulphate, though in a lesser amount, it may be postulated that permeability of dentine to dyes may be regulated by a similar mechanism.

The conclusions obtained in this study apply only to cavities which have been prepared for experimental purposes in sound teeth.


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   D. Items Int. 50:894.


    and Cytochem. 5:28
CHAPTER 7

Silver-Tin Amalgam

At this time amalgam alloy was structured commercially and it was the

...
Amalgam is the most commonly used restorative material in dentistry. A recent survey showed that amalgams comprised 80% of all filling materials.\(^{(1)}\)

Amalgam was first used in Europe as a restorative material at the beginning of the 19th century and later, in the year 1833, in America under the proprietary name of Royal Mineral Succedaneum. At this time gold was the principal material in use and restorations were costly. Amalgam soon fell into the hands of charlatans and many others who saw in its ease of manipulation a rapid means of making money. As a result of careless use, amalgam fell into disfavour with the more ethical members of the dental profession. The pain and the abscesses, which were often sequelae to the use of amalgam, were attributed to an excess of mercury. At this time amalgam alloy was not manufactured commercially and it was the custom for each operator to compound his own from the filings of silver coinage. A controversy arose within the profession/
profession regarding the use of amalgam. The American Society of Dental Surgeons demonstrated their radical attitude on this matter by preparing the following resolution in 1845:

'Resolved, that any member of the Society who shall hereafter refuse to sign a certificate pledging himself not to use any amalgam, and moreover protesting against its use, under any circumstances, in dental practice, shall be expelled from the Society.'

In the latter part of the 19th century amalgams were introduced, made from pure silver and pure tin, which were much superior in their physical properties, and their popularity increased. These improved amalgams were ultimately accepted by the profession due to the investigation and instruction in their manipulation by Black (1895). However, controversy still existed and from time to time articles were published on cases of intoxication by mercury from amalgams.

As a practical step towards defining the toxic effects of silver amalgam from both medical and dental points of view, a committee was appointed by the Medical/
Medical Department of the Charitie Hospital in Berlin. In 1930, a complete report on the committee's findings was published by Harndt(3) which stated that there was no reason whatsoever on these grounds to discontinue the use of silver amalgam.

The discussion of the toxicity of dental amalgam is by no means completely settled. Publications have appeared in the interval and, as recently as 1950, silver amalgam was condemned as being a possible cause of acrodynia.(4)

Mercury may be absorbed systemically from an amalgam restoration either via dentine and dental pulp, by surface solution in saliva or accidental ingestion.(5) Early authors such as Witzel (1899)(6) stated that the darkening of the dentine beneath amalgam fillings was due to precipitation of sulphides caused by diffusion of mercury or other metals present in the amalgams. Later work by Timms (1924)(7) gave support to this view. Further work by Appelbaum(8) on thin sections of tissue under amalgam restorations showed a precipitate of metallic sulphide which he believed to be mercury sulphide. In discoloured dentine/
dentine under amalgam fillings, Massler and Barber,\(^{(9)}\) in 1953, found mercury by spectrographic-analysis in a concentration of 0.5-5%. In apparently normal dentine remote from the discoloured area smaller amounts of mercury were found.

Whilst the above works established the presence of mercury not only subjacent to, but remote from, the actual areas of filling, they did not adequately define the amounts present.

It was the purpose of this section of the thesis to investigate only absorption by the dental tissues and to establish a satisfactory method for the determination of minute amounts of mercury in the dental tissues and to determine the amount of mercury which may have diffused from silver/tin amalgam into these tissues.
Composition and Manipulation of Dental Amalgam

Silver and tin, with smaller amounts of copper and zinc, are the constituents of a dental amalgam alloy. The composition of these alloys is defined in the Specification No. 1 of the American Dental Association (10) as follows:

<table>
<thead>
<tr>
<th>Element</th>
<th>Minimum/Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silver</td>
<td>65% minimum</td>
</tr>
<tr>
<td>Tin</td>
<td>25% minimum</td>
</tr>
<tr>
<td>Copper</td>
<td>6% maximum</td>
</tr>
<tr>
<td>Zinc</td>
<td>2% maximum</td>
</tr>
</tbody>
</table>

The amalgam is prepared by triturating the alloy with mercury, in a ratio of five parts alloy to eight parts of mercury by weight, which represents a slight excess of mercury and without which an amalgam cannot be satisfactorily manipulated. The precise ratio of alloy to mercury varies slightly with the size of the particles of the alloy. Immediately after mixing, the excess mercury in the amalgam is removed by wringing in a dry sterile napkin before it is packed in the tooth cavity.

The amalgam filling is built by condensing small portions of the amalgam progressively from the base to the surface of the cavity, either by hand or by mechanical/
mechanical pluggers. This further condensation of the amalgam within the cavity, removes more mercury which appears at the surface of the filling as a 'sludge' of mercury and alloy.

Thus the exposed margins of the filling have a greater mercury content than the centre. Analyses of set amalgams have confirmed this statement.\(^{(11)}\)

It has also been shown that there is a direct relationship between strength and amount of mercury remaining in the filling, particularly when the amount of mercury in the amalgam filling is reduced below 55%.\(^{(12)}\),\(^{(13)}\),\(^{(1)}\) Most of the excess mercury expressed from the amalgam appears at the exposed surface of the filling owing to the rigidity of the confining cavity walls. Free mercury may be present on the other surfaces of the filling in close contact with the walls of the tooth cavity, and it is this mercury which may be forced into the dentinal tubules during mechanical condensation. Alternatively the mercury may diffuse from the set amalgam into the dentinal tubules throughout the life of the restoration.
Fig. 53.

Drawing of the occlusal surface of a premolar tooth illustrating the limits of cavity preparation on this surface.
Purpose of the Experiment

The purpose of this experiment was to measure the amount of mercury which diffuses into the dental tissues, enamel and dentine, from silver/tin amalgam restorations.

Method

*In vitro* experiments were carried out on ten sound extracted premolar and molar teeth which had previously been fixed in formalin. The ages of the patients from whom the teeth had been extracted were unknown (*vide infra*).

Cavities were prepared on the occlusal surfaces of the teeth and an attempt was made to keep the size of the prepared cavities within the limits of 10mm. in length by 5mm. in width (*vide Fig. 53*).

A mix of amalgam was made using mercury prepared by adding 10 uc of radioactive mercury, $^{203}\text{Hg.}$ to pure inactive mercury. Five parts of the alloy were added to eight parts of the radioactive mercury, by weight. The amalgam was then packed into the prepared cavities using hand instruments for condensation.
The shaded area in the drawing of a longitudinal section of a premolar tooth demonstrated the amount of enamel and dentine removed from the original cavity surface.
**TABLE 19**

<table>
<thead>
<tr>
<th>Time Interval in days</th>
<th>Treatment of Cavity Walls</th>
<th>Amount of Mercury</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Wash only</td>
<td>0.42 mg.</td>
</tr>
<tr>
<td>4</td>
<td>2mm. removed</td>
<td>nil.</td>
</tr>
<tr>
<td>14</td>
<td>Wash only</td>
<td>1.60 mg.</td>
</tr>
<tr>
<td>14</td>
<td>0.5mm. removed</td>
<td>0.44 mg.</td>
</tr>
<tr>
<td>14</td>
<td>1.0mm. removed</td>
<td>0.34 mg.</td>
</tr>
<tr>
<td>14</td>
<td>2.0mm. removed</td>
<td>nil.</td>
</tr>
<tr>
<td>40</td>
<td>1.5mm. removed</td>
<td>nil.</td>
</tr>
<tr>
<td>40</td>
<td>1.0mm. removed</td>
<td>0.026 mg.</td>
</tr>
<tr>
<td>40</td>
<td>2.0mm. removed</td>
<td>nil.</td>
</tr>
<tr>
<td>40</td>
<td>2.0mm. removed</td>
<td>nil.</td>
</tr>
</tbody>
</table>

* The amount of mercury remaining in the tooth tissues.
condensation.

After intervals of 4 days, 6 days, 14 days, 17 days and 40 days, the amalgam fillings were removed in toto from the cavities.

In two of the teeth, the cavities were scrubbed with a bristle brush under running water for a period of 2 minutes after the filling was removed. In the remaining teeth, enamel and dentine surrounding the cavity were removed with carborundum stones for varying distances up to 1.5mm. (vide Fig.54). The amount of mercury present in the teeth was determined by the method described in Appendix 3.

Results

The results of the analyses of the amounts of mercury found in the teeth are given in Table 19.
Discussion

The results of the analyses of the mercury content of the teeth show that, after a period of 40 days, this element was not found in the dentine beyond a distance of 1mm. from the original cavity margins. The concentration of mercury was greatest in the dentine which was in immediate contact with the filling and, as the distance from the original cavity margins increased, the amounts of mercury found in dentine diminished.

As the quantity of mercury found was so small it was almost certain that systemic absorption from the tooth pulp by the dentinal tubules was unlikely to occur.

Dentine is a calcified structure containing many tubules and it is probable that the mercury found in this tissue was located in these tubules. Accordingly the uptake of mercury would depend, amongst other factors, upon the surface area of the dentinal tubules exposed to the amalgam. This surface area is dependent on the size of the lumen of the dentinal tubules which are wider in young persons/
persons and which gradually decrease in size with increasing age.

It is possible, therefore, that greater depth of penetration of mercury will take place in young teeth than in old.


8./


APPENDIX
APPENDIX 1

Estimation of Arsenic in Biological Material by Activation Analysis.

About 3 to 6 mgm. of the sample to be analysed was weighed into a polythene bag which was then sealed. 1 or 2 mgm. of a sample of arsenious oxide was also prepared in this way for use as a standard. The weights were accurate to 1/100 of a milligram.

The polythene bags containing the samples were placed in a standard aluminium irradiation can and sent to Harwell and irradiated by low velocity neutrons. The can remained in the pile for one day when the activity generated was enough to be used for analysis. The standard sample was removed, dissolved in sodium hydroxide solution and made up to 1 litre. One ml. of this solution was taken and made up to 100 ml. One ml. of this solution was taken as the standard sample and the count rate of this sample expressed as counts per minute per mgm. of elemental arsenic obtained.

The tooth sample was removed from the bag and destroyed in a mixture of 3 ml. of concentrated sulphuric/
sulphuric acid and 5 ml. of concentrated nitric acid by heating until all the nitric acid had been removed. After this, the remaining acid with the arsenic retained in it was washed well into a 200 ml. flask. A further 2 ml. of concentrated sulphuric acid and 4 ml. of concentrated hydrochloric acid was added, together with 10 ug of inactive arsenic as a carrier, followed by 5 ml. of a 15% solution of sodium iodide and 0.4 ml. of a 40% solution of stannous chloride in 50% hydrochloric acid. The solution in the flask was then diluted to about 150 ml. and the flask placed on a boiling water bath for 5 minutes. 10 grams of 16-22 mesh zinc pellets were added and the reaction allowed to continue for 15 minutes. During the reaction the arsenic was evolved as arsine. Hydrogen was also evolved in this reaction and, together with the arsine, was passed through a lead acetate on cotton wool filter. The arsine was absorbed in 1 ml. of a 1.6% solution of mercuric chloride. 5 ml. of a 0.001 N iodine solution in 40% sodium iodide were then added to help the solution of any solids formed. The solution was then made up to a standard volume and the/
the activity estimated using a Geiger Muller counter. This was compared with the standard sample and the arsenic content so obtained.
Method of Analysis to Determine the Amount of Silver in the Dental Tissues

Standardization of Radioactive Silver Nitrate Solution

The radioactive silver nitrate solution was prepared as described in page 140. 0.1 ml. of the solution was made up to 100 ml. with distilled water. The radioactivity of 10 ml. of this solution was measured in a liquid counter and a further 50 ml. of the diluted solution used to determine the amount of silver which it contained. The silver was precipitated as silver chloride, using 3 ml. of a saturated solution of sodium chloride. This suspension was centrifuged, the residue made into a 'slurry' with water and filtered through a sintered glass filter. The residue was washed in very dilute nitric acid then weighed. The result of standardizing this solution was expressed as mgms. of silver, per counts per minute.

Dissolution of the Tooth

The/
The crown of the tooth was placed in a flask containing 10ml. concentrated sulphuric acid to which was added 1 mg. of silver oxide, $\text{Ag}_2\text{O}$, as a carrier for the active silver. This solution was heated gently under reflux until the tooth had dissolved, the dissolution being completed in one to two hours. The volume of the solution was made up to 100ml. with distilled water and the slight residue, which remained after dissolution and dilution of the solution, was removed by centrifuging. The residue was washed further with 2ml. of dilute sulphuric acid and the resultant filtrate added to the original filtrate.

Recovery of Silver

3ml. of a saturated solution of sodium chloride were added to the filtrate to precipitate the silver salt. The solution was again centrifuged and the precipitated silver chloride collected. This was dissolved in 2ml. of concentrated ammonia and made up to 10ml. with water. The radioactivity of this solution was determined in a liquid counter.
APPENDIX 3.

Method of Analysis to Determine the Amount of Mercury in the Dental Tissues

Isotopic Dilution

This method of analysis is very sensitive for determining minute quantities of mercury in teeth. A quantity of radioactive mercury was made into a silver/tin amalgam and a portion weighed out for conversion into a standard source for calibration purposes. As the quantity of mercury which is recovered from the dental tissues is small, it is difficult to recover it completely, due to solubility and loss by adsorption on the walls of the glass vessels. A known weight of inactive mercury, in this case 30mg, was added during the recovery process to overcome this difficulty. The inactive mercury mixes with the active mercury to give a quantity of mercury which is readily determinable and whose specific activity can be counted.

The weight of the mercury which diffuses into the tooth may be calculated when the initial activity of the/
the recovered mercury is known.

Let the initial activity of the mercury determined from the standard source be \( A \) c.p.m./mg. and the quantity of mercury which has diffused from the amalgam into the tooth \( X \) mg. This mercury will have a total activity of \(XA\) c.p.m.

Let the weight of mercury which was added as carrier be \( Y \) mg.

and the specific activity of the mercury recovered be \( C \) c.p.m./mg.

The activity of the mercury recovered

\[
C = \frac{XA}{X+Y}
\]

The weight of mercury \( X \) which has diffused into the tissues can be determined as \( A, Y, \) and \( C \) are known.

**Method**

In this method, pure radioactive mercury (10mg.) was diluted with pure inactive mercury to one gramme. A small quantity (30mg.) of this diluted mercury was weighed out and set aside for further experiment and the remainder was used for the preparation of amalgam.

Pure inactive mercury (30mg.) which was used as a carrier was heated with concentrated sulphuric acid (3ml.) till all the reaction had ceased. The solution/
solution was cooled and any remaining insoluble salt was dissolved by the dropwise addition of hydrochloric acid.

The tooth was dissolved in (50ml.) concentrated sulphuric acid. (Sulphuric acid was chosen because there is less complexing and hence less chance of mercury loss than with hydrochloric acid, with which the mercury does not react. Nitric acid was not used because of its reaction with the copper.) The carrier solution containing the inactive mercury was now added to the sulphuric acid.

The flask containing the tooth was heated under a reflux condenser on a hot plate with medium heat until all the tooth was dissolved. The solution was then heated to boiling point, to ensure complete dissolution of the mercury. After cooling, this solution was diluted with 800 ml. water.

Finely divided copper (3-4gm.) was added to the solution and the flask stoppered. Before adding it to the solution, the copper was previously heated under carbon dioxide to remove organic materials used as a flux in its preparation. The mixture was swilled/
Apparatus used to distil mercury under vacuum.

Fig. 1.
swilled frequently for 2-3 hours and left overnight. It was then filtered through a Gooch crucible containing asbestos and all the copper transferred to the filter where it was washed with water, alcohol and finally with ether.

When dry, the copper was introduced in the apparatus shown in Fig. 1 which was then sealed. The flask was evacuated and the mercury distilled off under vacuum by heating vigorously. The U-tube was cut off from the flask and the mercury dissolved in concentrated nitric acid. This was precipitated and counted as mercury zinc thiocyanate. Mercury zinc thiocyanate was chosen because of its ease of formation and because it readily forms a uniform layer on the tray for counting.

Procedure for the Preparation of Mercury Zinc Thiocyanate Precipitate

The mercury zinc thiocyanate was prepared by dissolving the mercury from the distillation in (2-3ml.) of concentrated nitric acid. This was heated and boiled gently to ensure that the mercury was in the divalent state. The solution was diluted with/
with water (5ml.) and almost neutralised with sodium hydroxide. A reagent was prepared by dissolving 3.9gm. zinc sulphate in 100 ml. water, and 2.5ml. of this reagent was added to the solution containing the mercury.

A precipitate gradually separated and was allowed to stand for one hour. The mixture was centrifuged, washed twice with 5ml. portions of a washing liquid (5ml. of reagent to 450 ml. water), then washed with small amounts of water before being made into a slurry with acetone and finally transferred to a counting tray.

Counting the Radioactivity of the Sample

The radioactivity of the mercury zinc thiocyanate was counted by placing the tray at a known distance from the window of the Geiger-Muller tube. To obtain reproducibility of results the tray containing the reagent must be placed in a constant geometrical arrangement in relation to the window of the counter. It was found that the count observed for 10mg. of material on the counting tray was not necessarily one half/
Fig. 2.

Unit of thickness corresponds to the point (A) on the curve and is taken as 10 mg./sq.cm.

Counts per minute for unit thickness for a sample of thickness T (marked by the point B on the curve) is calculated by:

\[ \text{Counts per minute} \times \frac{CA}{TB} \]
half the number of counts observed from 20g. of the same material when placed on a tray of similar area. This was due to the fact that, as the layer of the material on the tray becomes thicker, Beta-particles which are emitted from material at the bottom of the tray begin to be absorbed by the material of the source. This is known as self-absorption.

As the samples obtained in the determination contained different weights of mercury zinc thiocyanate it was necessary to relate them to a standard sample, i.e. a unit of thickness at which the activity is known. In this case the unit of thickness chosen was 10mg. This was done by preparing a self-absorption curve (Fig. 2) for different weights of mercury zinc thiocyanate. These weights were prepared by dissolving a quantity of radioactive mercury in concentrated nitric acid and precipitating the mercury as mercury zinc thiocyanate. A number of trays were weighed and increasing quantities of the precipitate added to each of the trays which were again weighed. The intensity of activity of material on each tray was determined. A graph of c.p.m./mg./
c.p.m./mg. against mg./sq.cm. was drawn and from this graph the number of counts per minute of any sample could be referred to unit of thickness.
CALCULATION of RESULTS

Standard
Wt. of Mercury used for precipitation............ 30.0mg.
Wt. of precipitate.................................. 56.8mg.
Wt. of Mercury in precipitate...................... 22.9mg.
% Mercury recovered................................ 76.3%
Background count................................. 13.6 ± 0.43 c.p.m.
Count recorded for precipitate.............. 5193 ± 51 c.p.m.
Actual count precipitate...................... 5179.4 ± 51 c.p.m.
Area of tray........................................ 1.90 sq. cm.
mg/sq. cm........................................... 29.9 mg/sq. cm.

At Unit Thickness
c.p.m. = 5179.4 ± 51 x \frac{380}{234} = 8420 ± 82.7 c.p.m.

Count corresponding to 10.0%... 11,050 ± 109 c.p.m.

Sample
Wt. of Mercury added to tooth...................... 30.80mg.
Wt. of precipitate.................................. 18.10mg.
Wt. of mercury in precipitate...................... 7.3 mg.
% Mercury recovered................................. 23.7%
Background count................................. 14.9 ± 0.27 c.p.m.
Count/
Count recorded for precipitate..... 52.6 ± 0.61 c.p.m.
Actual count of precipitate........ 37.7 ± 0.67 c.p.m.
Area of tray.......................... 1.90 sq. cm.
mg/sq. cm............................ 18.1 mg/sq. cm.

At Unit Thickness
c.p.m. = 37.7 ± 0.67 x \frac{380}{392} = 36.6 ± 0.65 c.p.m.

Count corresponding to 100%..... 154.5 ± 2.74 c.p.m.

The equation \frac{XA}{X + Y} = C c.p.m./mg. may be
used to calculate the weight of mercury which had
diffused into the tooth by inserting the appropriate
values from the above results where

X = weight of mercury in tooth;
A = initial specific activity of mercury;
Y = weight of mercury added as carrier;
C = specific activity of the recovered mercury.

Weight of mercury found in tooth = 0.42 ± 0.01 mg.