Scientific Methods in Dental Research

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The concentration of the principal trace elements in enamel was established and it was considered important to examine the relationship between these and this is now being investigated.

One of the elements which was investigated was mercury both as a trace element and from the viewpoint of its inclusion in dental amalgam fillings. The initial study showed that mercury was present in sound enamel as a dietary contaminant. Where amalgam fillings were present in the mouth contamination of sound teeth resulted from the continuous migration of mercury from amalgam fillings. This study stimulated further investigation into mercury as a possible hazard to dental personnel in the dental surgery. These investigations showed that this element must be considered as a potential health hazard.

The second part of the thesis is concerned with the examination of specific problems associated with techniques employed in dental practice. One of the most significant advances in dental techniques in this century has been the introduction of the high speed air turbine handpiece for cutting hard dental tissues. The use of this handpiece however, introduced a hazard to the operator as a result of the formation of aerosols from the air necessary to drive the handpiece and the oil used for lubrication. These aerosols also produced an increase in the bacterial content of the atmosphere. In addition, debris which resulted from the cutting caused an increase in respiratory complaints amongst dentists. The noise levels of this type of handpiece were also investigated as a possible hazard to the hearing of the operator. A five-year study showed that significant hearing losses could occur. As a result of these and other studies with dental handpieces modifications were made to reduce both the noise level and the need to include oil for lubrication.

The third section is concerned with the effects on the pulp of the tooth of materials used in restoring tooth tissue. Many materials have been employed for this purpose but despite satisfactory physical properties have proved to be toxic to this tissue.

As with all research the implications and importance of the results may not yet have fully emerged. It is hoped, however, that this work represents some contribution towards the relationship between the chemistry and the biological processes of the human tooth and its supporting tissues.

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DECLARATION I declare that the work has been done and the thesis compiled by myself, and that the books and papers cited were all consulted by me personally, unless it is otherwise stated.

COLLABORATIVE WORK where material based on work undertaken in collaboration with others is included in the thesis a *further and separate statement* must be submitted clearly defining the candidate's individual contribution.

DATE: 25th August 72.

CERTIFICATION

I hereby certify that the above named candidate for the degree of D.D.Sc. has been engaged since graduation for at least one year either in scientific work bearing directly on his profession or in the practice of Dentistry.

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A Thesis

presented to

THE UNIVERSITY OF GLASGOW

for the degree of

DOCTOR OF DENTAL SCIENCE

by

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The author was appointed Lecturer in Conservative Dentistry in the University of Glasgow in 1954. From that time he initiated a programme of research in several fields of dental science and on the basis of this research presented a thesis for the degree of Ph.D. which was accepted by the University of Glasgow in 1959. In 1964 he was appointed Senior Lecturer in Conservative Dentistry and in 1965 Senior Lecturer in Periodontology of the University of Glasgow. In 1966 he was appointed to the Chair of Conservative Dentistry in the University of Manchester where he has continued his research.

Research from 1954-1966 was carried out in the Dental School, and Departments of Chemistry, Physics and Forensic Medicine of the University of Glasgow and in the Regional Department of Clinical Physics and Bioengineering of the Western Regional Hospital Board. In addition to the Biological Sciences, this research involved the disciplines of Chemistry and Physics.

Support for the research for the Ph.D. thesis was given by the Scottish Hospitals Endowment Research Trust. On the basis of the results of the first project an increased grant was given by that body in 1962 which enabled a research assistant to be appointed. From 1966 his work has continued in the University of Manchester.

In all papers presented in this thesis the author has initiated the research and where there is a joint authorship, he is the senior author. He wishes to state that, apart from the paper on Estimation of Arsenic in the Dental Tissues (Paper No. 1) and on Zinc Phosphate Cement (Paper No. 2) none of the papers has been submitted for a thesis. These papers (Nos. 1 and 2) were included in the Ph.D. thesis but have been included here as they represent the first stages of the research programme and indicate the development of methods of research.

The research carried out in this period can be divided into three main sections. The first of these relates to the trace elements in the dental tissues. The post-war development in the use of radio-active isotopes and radiochemical techniques encouraged their use in research. The availability of the Scottish Universities' Research Reactor at East Kilbride provided facilities for irradiation and the production of many of the short-lived isotopes used in this research. Radiochemical techniques were developed in the Department of Chemistry and the Department of Forensic Medicine. In particular a sensitive technique of analysis, neutron activation analysis, had shown itself to be suitable for examining the trace elements in many biological tissues. Dr. H. Smith, a chemist in the Department of Forensic Medicine, was responsible for developing many of the methods of analysis in biological tissues using this technique.

From 1950 onwards animal experiments and nutritional

studies had indicated the significance of the trace elements in the prevention and reduction of dental caries. The element which had been shown to be the most significant in caries prevention was fluorine. Numerous studies of this element had shown a positive correlation between fluoride intake, tooth fluoride content and caries resistance. Other investigations, however, had indicated that other elements possessed anti-cariogenic properties and also that the relationships between trace elements could be important and that interaction between them could be significant.

While the composition of the tooth is not the only factor in the carious process, it may play a more significant part than has previously been believed. The range of concentration of many trace elements in a single tooth had not been established due to difficulties in achieving a satisfactory method of analysis. Most previous analyses had been carried out on pooled samples of teeth to obtain the necessary amount of tooth for analysis. It is the enamel of the tooth which is first attacked by the carious process and therefore it is important to examine the chemical content of this tissue. Analyses of successive layers of enamel have shown that the chemistry of the surface enamel differs from that of the underlying layers.

The development of sensitive analytical techniques such as neutron activation analysis and atomic absorption spectrophotometry enabled analyses of individual teeth to be carried out.

Dr. Smith is the co-author of Papers Nos. 1, 3, 5, 6, 10, 11, 12 and 13 and he has published full details of his

analytical methods in specialised scientific journals. In Paper No. 6 the co-author was Mr. G. D. Paxton, a technician working with Dr. Smith. The applications of this technique were published as a chapter in a text-book on activation analysis (Paper No. 4).

The appointment of Mr. H. D. Livingston, B.Sc., a Research Assistant, in 1962 enabled the analysis of the trace elements, zinc, antimony, manganese, vanadium and molybdenum to be carried out. Of these elements it was considered that zinc, vanadium and molybdenum played an important part in increasing the resistance of the surface enamel layer to caries. Mr. Livingston carried out his work in the University Dental School and Department of Forensic Medicine. His Ph.D.thesis on the development of methods of analysis for the trace elements in the dental tissues was accepted by the University of Glasgow in 1966. Together with Dr. H. Smith he is a co-author of Paper Nos. 10, 11, 12 and 13.

As a result of the interest in the uptake of Strontium-90 by the calcified tissues of the body from the 'fall-out' of nuclear explosions, measurements of this element were made in the deciduous and permanent teeth of children in the West of Scotland. The results of this investigation were published as part of a report dealing with the uptake of Strontium-90 by the calcified tissues (Paper No. 7). The co-author of this paper was Miss J. Warren, of the Physics Department of the Western Regional Hospital Board.

The work on trace elements was continued in Manchester and Miss Valerie Myers, B.Sc., was appointed research

assistant. The trace element under investigation at this time was selenium, an element which was considered to demonstrate a direct relationship between selenium uptake and incidence of dental caries. The analytical techniques developed for the analysis of this element was successfully submitted by Miss Myers for an M.Sc. degree of Manchester University and was published with Miss Myers as co-author (Paper No. 16).

It was considered appropriate in 1969 to publish a major paper on the trace elements in the hard dental tissues (Paper No. 15) in which the range of concentration of the trace elements and also with the analytical methods best suited to these elements, were discussed.

The relationship between trace elements was considered to be important as this could influence the effects which individual trace elements could have on their concentration in enamel and also on their effect in reducing the caries resistance of enamel. In 1965 a major research project was initiated to examine the relationship between the trace elements copper and molybdenum. Previous work on animals had shown a relationship but no investigation had been carried out with regard to their dietary concentration and uptake by the hard dental tissues. This work was carried out using rats as the experimental animals. While it was possible to measure the copper content of the teeth of these animals by activation analysis, it was found that this technique could not be employed for the measurement of molybdenum. The use of atomic absorption

spectrophotometry enabled both copper and molybdenum analyses to be carried out, and this study has now been completed. The Research Assistant for this investigation, Mrs. Christine Helsby, B.Sc., has now completed her Ph.D. thesis for submission to the University of Manchester on the analytical methods used in this research (Paper No. 20). This work has been prepared for publication and is included.

One of the elements investigated in the trace element study was mercury. It was examined both as a trace element in tooth tissue and also from the viewpoint of its migration from dental amalgams in the mouth. It was shown in the initial study that this element was present in sound teeth as a contaminant from dietary and other external sources. Where amalgam fillings were present in the mouth this contamination was increased due to the continuous migration of mercury from amalgam fillings.

The results of this first study stimulated an investigation into a consideration of mercury as a possible hazard to dental personnel working with this element in the dental surgery. An investigation of dental surgery assistants handling this material showed that, by analysis of body tissues, there was a potential hazard from mercury and that precautions had to be taken when handling this material. This paper (No. 5) was published with Dr. H. Smith as the co-author. The work on mercury was continued in Manchester to determine if amalgam fillings in the mouth could cause a significant uptake of mercury in the body tissues. Analyses were carried out on body tissues from cadavers and patients with large numbers of amalgam fillings. The

results of this were incorporated in a paper relating to the use of mercury in the dental surgery. Further work was carried out on this problem and this demonstrated that a further mercury hazard was present when high-speed mechanical amalgamators were employed to mix the amalgams. The results of this paper were published (Paper No. 19) with Mr. T. C. Rowbotham, Senior Lecturer in the Department of Conservative Dentistry, University of Manchester, as co-author.

The second part of this research was concerned with the examination of specific problems associated with techniques used in dental practice.

Perhaps the most significant step forward in dentistry in this century was the introduction of the air turbine handpiece for the removal of the hard dental tissues. With this handpiece cutting speeds of up to 250,000 revolutions per minute are obtained as opposed to 5,000 revolutions per minute of the traditional handpiece. At these ultra-high speeds the cutting efficiency of dental burs is increased. The use of this handpiece, however, introduces a hazard to the operator as a result of the formation of aerosols by air necessary to drive the handpiece and the oil which is necessary to provide lubrication. These aerosols produce an increase in the bacterial content of the atmosphere and also the cutting debris causes an increasing amount of respiratory complaints which result from the use of these high speed handpieces. The dangers of oil inhalation were investigated in a study with Mr. R.G. Tilston, a physicist with the Physics Department of the Western

Regional Hospital Board, who was the co-author of Paper No. 8. This work indicated that a significant amount of oil could be inhaled by the operator from this handpiece.

A further disadvantage of this type of handpiece is that the noise levels of around 75 decibels might be a potential hazard to the hearing of the operator. A five-year study was undertaken on dental students with the assistance of Miss E. C. Knox, an audiologist to compare hearing levels before and after using these high speed handpieces. The results of this study (Paper No. 9) showed that significant hearing losses could occur. As a result of these two studies with handpieces, modifications were made which reduced both the noise level and the need to include oil for lubrication.

The third study was to examine the effects which many materials employed in restoring tooth tissue may have on the pulp of the tooth. Many materials have been employed for this purpose and despite satisfactory physical properties have proved to be toxic and harmful to this tissue. The first of these materials to be examined was zinc phosphate cement, one of the most commonly used materials in dentistry (Paper No. 2). The results of this research indicated that comparison could be made between the reactions of the pulp on the dog's tooth and that of the human tooth. Similar methods were used to examine a number of commonly used materials and were used in an assessment of these materials. With the introduction of the newer composite resin filling materials a study of their effects

was carried out in conjunction with Dr. D. C. Smith, Reader in Dental Materials, who as a materials chemist was concerned with the development of these materials and with evaluating their physical properties. Two papers on the histological examination of a new material were published (Paper No. 18) with Dr. D. C. Smith as co-author. These studies showed that the effects of this material on the dental pulp were less toxic than had been believed.

A similar histological evaluation of the butyl cyanoacrylates was carried out. Cyanoacrylates were employed as tissue adhesives and they had been introduced into dentistry because of their haemostatic and bactericidal properties. There had been little research on their effects on the dental pulp and it had been suggested that they would be particularly valuable where the pulp of the tooth had been exposed. The application of this material could produce a seal which would stimulate the formation of a bridge of dentine and produce a healing of this exposure. The results of this investigation have shown that this material does not stimulate the dental pulp to healing and is not recommended for this purpose. The co-author of this paper which is prepared for publication (Paper No. 21) is Mr. C. Hannah, a Lecturer in the Department of Conservative Dentistry, in the University of Manchester.

The nineteenth century philosopher and scientist Helmholtz stated 'All science is measurement'. While clinicians would perhaps disagree with this dictum there is little doubt that chemical and physical techniques have contributed much to the progress which both medical and dental research have made in the present century.

In its earliest days dental research was to some extent empirical and depended mainly on individual observation. To-day, however, like other biological subjects dental research has become one of inter-disciplinary co-operation between dental research worker and scientist. The extent to which this relationship has increased is presented in Paper No. 14.

Scientific Methods

Throughout the years of dental research the principal method of investigation has been that of microscopic examination of histological sections of the dental tissues. While refinements in the preparation of sections have been introduced microscopic examination still remains the basis upon which the evaluation of the effects of dental materials on the dental tissues is based. This is the method which has been employed in the studies presented in this thesis. Most of these have been carried out in animals and while the results cannot be extrapolated to determine the effects on human dental tissues they are sufficiently close to be able to assess the relative effects on vital tissues, as the cellular structures of the dog's teeth are similar to those of the human.

Studies of the composition of the dental tissues have employed many methods of analysis. These methods were directed mainly to the determination of the major elements such as calcium and phosphorus. Few attempts were made to determine the levels of the trace elements present in the dental tissues. The term 'trace element' may lead to some confusion as there is no definitive boundary of concentration and no widely accepted grouping of elements into trace and non-trace elements. It has been suggested that limits of concentration of 100 ppm. by weight or less should constitute a trace element level but in the hard dental tissues concentrations greater than this are generally considered as trace elements. One of the main

difficulties in previous methods of analysis has been in obtaining sufficient quantity of sample. The distribution of many elements varies between teeth and even within the enamel of individual teeth; therefore results may vary according to the method by which samples are taken. Where pooled tooth samples are used from different teeth only an average concentration of the elements will be obtained which is of little value when examining the relationship between teeth which are considered caries-free and those which are caries-prone and between sound and carious teeth.

The progress of the analysis of the trace elements has been linked to the development of more sensitive techniques which has resulted in more precise information regarding both their concentration and function. In selecting an analytical technique for trace elements various criteria such as sensitivity, accuracy, precision and selectivity were considered. There was also the problem of interferences which may be present in the matrix of the sample or produced by the presence of other trace elements.

The development of nuclear science led to the introduction of new analytical techniques amongst which was that of neutron activation analysis. This technique proved to be most suitable for the investigation of many of the trace elements in the dental tissues and had many advantages over other methods of analysis which were:

(a) Specificity and certainty of identity

(b) High sensitivity.

One of the problems to be overcome was the avoidance of contamination during the preparation of the sample. The tooth samples were prepared as follows:

Teeth were extracted using extraction forceps which were protected with polythene tubing to avoid contamination of the tooth crown from the metal of the forceps or the teeth were removed surgically. Immediately after extraction, teeth were washed and polished lightly in distilled water to remove any dental plaque which was present. The crown of each tooth was sectioned horizontally into occlusal, middle and gingival thirds using a carborundum disc. The enamel of the middle and gingival sections was separated into an inner and outer layer again using a carborundum disc with a micrometer gauge. As each new element was investigated the degree of contamination was also assessed to determine if this was significant. It was found to be of no significance in all the elements reported. However it was found that contamination could easily be picked up as was demonstrated during the preparation of a homogeneous enamel powder prepared in a metal mortar. This powder gave a consistent manganese content of 4.19 ppm. compared with the average value of sound enamel of 0.83 ppm.

In the study which was carried out to examine the relationship between copper and molybdenum in the hard dental tissues of the rat it was found that the high sensitivity of atomic absorption spectrophotometry made the method particularly suitable for the analysis of these trace elements. Activation analysis techniques whilst

satisfactory for the measurement of copper, did not prove sensitive enough for the measurement of molybdenum.

Radioactive isotopes were employed in several studies. Most of these were concerned with the "labelling" of materials or of compounds. In the study of aerosols from handpieces radioactive iodine (I^{132}) was employed to label the oil and to measure the uptake by the operator and patient. Autoradiographic techniques were also used to determine the location and distribution of radioactive isotopes.

The environment of the tooth in the mouth is an unusual one of hard tissues surrounded by a fluid medium of heterogenous composition. This fluid is composed of the secretions of the major and minor salivary glands together with the secretions from the gingival tissues and mucous membrane which surround the teeth. The crown of the tooth is covered with enamel which is almost totally inorganic in composition but which is permeable to fluids passing from the pulpal circulation to the oral fluids and vice versa. The specific relationship between the fluid environment which surrounds the tooth and the hard tissues has not yet been clearly established. Nor has the relationship between the structure and composition of the tooth itself and individual susceptibility to dental caries.

As with all research the implications and the importance of the results presented in this thesis may not yet have fully emerged. It is hoped, however, that this work represents some contribution which will enable dental

scientists to piece together a more complete picture of the relationships between the chemistry and the biological processes of the human tooth and its supporting tissues.

C O N T E N T S

P A P E R 1

ESTIMATION OF ARSENIC IN TEETH BY ACTIVATION ANALYSIS

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Journal of Dental Research

Vol. 39, No. 3, 514-516, May-June 1960.

ESTIMATION OF ARSENIC IN TEETH BY ACTIVATION ANALYSIS

From time to time various analytical methods have been applied to tooth substance to establish the content of so-called trace elements such as lead and tin.[1][2] Other experimental work has shown that these trace elements may play an important part in the formation and preservation of teeth.[3]-[6] As trace elements are present in very low concentrations it has been necessary in previous investigations to use pooled material from many different subjects to provide an adequate sample for analysis by chemical, spectrographic or colorimetric methods. The conclusions which may be drawn from such experiments are necessarily limited by the non-specific nature of the material.

Radioactivation analysis is a technique by which many elements may be detected and estimated with a greater sensitivity than that of conventional analytical procedures. The essential basis of the method is that the element to be determined is made radioactive by exposing the sample to bombardment by neutrons inside a neutron reactor. The radioactivity induced in this way has properties which are characteristic of the element concerned. After suitable chemical separation of the element, if necessary, the amount of the induced radioactivity, the rate of its decay and the energy of the associated nuclear radiations can be measured without much difficulty by instruments such as Geiger and Scintillation counters.

When the only stable isotope of arsenic is irradiated

with thermal neutrons an unstable isotope is produced by neutron capture. This unstable isotope decays with a half-life of 26.8 hours, with the emission of B-particles and -rays. The activation cross section is 4.0 barns and the saturation activity for a pile flux of 10^{12} neutrons per square centimetre per second is about 2×10^{12} disintegrations per minute per gram of elemental arsenic. The reaction is represented by:-

$$^{75}_{33}As + ^{1}_{0}n \rightarrow ^{76}_{33}As$$

The sensitivity of the determination is 10^{-9} gm. compared with the highest chemical sensitivity of 10^{-7} to 10^{-8} gm.

Sub-micro chemical separations are avoided as inactive carrier arsenic may be added to increase the total weight of arsenic present. In addition 'carriers' may be added to hold back other active atoms produced. The accuracy of determination is limited only by the statistical error of counting.

Experimental Methods

Longitudinal ground sections of 25 sound permanent teeth were prepared to a thickness of 400μ. Samples of these teeth containing both enamel and dentine, and weighing not more than 10 mg. were taken.

About 3-6 mgm. of the tooth sample were weighed and sealed into a polythene bag. 1 or 2 mgm. of a sample of arsenious oxide were 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 a suitable atomic pile for irradiation by low velocity neutrons. The can remained in the pile for 24 hours when the activity generated was sufficient 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. From this it was possible to calculate the count rate expressed as counts per minute per mgm. of elemental arsenic. The method of separation of the arsenic was a modification of Thomas and Collier's Gutzeit technique[7] and was carried out as follows:-

The tooth sample was removed from the bag and digested in a mixture of 3 ml. of concentrated sulphuric acid and 5 ml. of concentrated nitric acid and heated until all the nitric acid had been removed. The remaining acid with the arsenic retained in it was well washed into a 200 ml. flask. A further 2 ml. of concentrated sulphuric acid and 4 ml. of concentrated hydrochloric acid were added, together with 10 µg. 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 in a boiling water bath for 5 minutes. Ten grams of 16-22 mesh zinc

pellets were added and the reaction allowed to continue for 15 minutes. During this reaction the arsenic was evolved as arsine. Hydrogen was also evolved and, together with the arsine, was passed through a cotton wool filter impregnated with lead acetate to remove any small amounts of hydrogen sulphide liberated from the reaction mixture. The arsenic in the form of arsine was removed from the hydrogen by passing it through a trap containing 1 ml. of a 1.6% solution of mercuric chloride. Five ml. of a 0.001 N iodine solution in 40% sodium iodide were then added to the mercuric chloride to complete the solution of any solids formed. The solution was then made up to a standard volume and the activity estimated using a Geiger Müller counter. This was compared with the standard sample and the arsenic content so obtained. As the presence of antimony could be a possible source of error in the determination, decay curves were prepared from the samples of arsenic separated from the teeth. These curves showed only the presence of ^{76}As.

Results

The results of the analysis of 25 sound permanent teeth showed the arsenic content of normal teeth to lie within the range of 0.031 - 0.145 ppm. giving a mean value of arsenic for sound human teeth of 0.060 ppm.

Summary

The activation analysis method described is a quick and accurate technique for the estimation of arsenic in biological materials. After nitric/sulphuric acid

digestion of the activated sample the Gutzeit separation is used in conjunction with an estimation using a Geiger tube accepting liquid samples. The method has the advantage that very small samples can be tested without difficulty. Using this method, the arsenic content of 25 teeth was estimated and a mean value of 0.060 ppm. obtained.

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P A P E R 2

ZINC PHOSPHATE CEMENT

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The Dental Practitioner

Vol. XII, No. 9, 322-326, May 1962.

ZINC PHOSPHATE CEMENT

Zinc phosphate cement is one of the most widely used materials in conservative dentistry. Paffenbarger, Sweeney, and Schoube (1955) estimated that it is used in 40-60 per cent of all conservative restorations. It is used to protect the pulp from thermal shock or as a luting medium for crowns and inlays.

The effect of zinc phosphate cement on the dental tissues, particularly the dental pulp, has given rise to 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 and germicidal" often produced a wide range of clinical conditions from a mild inflammatory process to complete death of the pulp.

Manley (1943) and others, notably Gurley and Van Huysen (1937), Schroff (1946), and Zander and Pejko (1947), demonstrated by histopathological methods that serious pulp damage occurred after the use of these cements. It was generally agreed that the main causative factor was the acidity of the cement in the plastic state at the time of insertion into the tooth cavity.

The precise mechanism of the setting of these cements and their behaviour is not yet fully understood and the author felt that the subject justified further investigation.

Composition of Zinc Phosphate Cement

There are many commercial zinc phosphate cements, the compositions of which remain manufacturers' secrets. The chief difference in these cements is in the chemical composition of the powder. Most cement powders consist mainly of zinc oxide to which magnesium is added in various 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, to modify its colour, and to increase the smoothness while mixing (Ward and McCormick, 1915). The arsenic content is very small, usually being less than 0.0002 per cent.

The liquids are composed mainly of phosphoric acid, to which are added solutions of aluminium phosphate and, in some cases, of zinc phosphate. These metallic salts are added to the liquid to reduce the acidity and to control the rate of the setting reaction. The average water-content of the liquid is 33 ± 5 per cent and is a critical factor in the rate of the liquid-powder reaction. When exposed to the air, these liquids take up or give off water, depending on the atmospheric humidity.

Setting Reaction of Zinc Phosphate Cement

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

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 is 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, the final compound between phosphoric acid and zinc oxide is the formation of tertiary zinc phosphate, $Zn_3(PO_4)_2 4H_2O$, which is a relatively insoluble compound. In such a compound there is no unused phosphoric acid and no water.

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, $3ZnO + 2H_3PO_4 + XH_2O$, to its final state, $Zn_3(PO_4)_2 4H_2O$ (tertiary zinc phosphate).

Two such routes may be envisaged:-

1. As zinc forms only primary and tertiary phosphates, soluble primary zinc phosphate, $Zn(H_2PO_4)_2$, may be formed and, at a later stage, tertiary zinc phosphate, $Zn_3(PO_4)_2 4H_2O$, crystallizes out from this solution. This may occur after the cement has been placed into the tooth cavity.

2. The phosphoric acid converts the zinc oxide, ZnO, to tertiary zinc phosphate, $Zn_3(PO_4)_2 4H_2O$, 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. These are:
(a) Proportions of liquid and powder used; (b) Speed of

mixing powder and liquid; (c) Temperature of mixing and the presence of additional material in the powder and liquid which may affect the rate of crystallization of the final product.

When zinc phosphate cement is used clinically it must be in a plastic state irrespective of the "route" by which the final compound is reached. In this plastic state either primary zinc phosphate or phosphoric acid or both are present when the cement is placed in direct contact with the dental tissues.

It is important to emphasize the presence of free acid liquids throughout the setting process.

Consideration of pH Value of the Mixed Cement

The graph (Fig. 1), which is qualitatively correct, shows how the pH value would vary if zinc oxide was added to phosphoric acid and no precipitation of $Zn_3(PO_4)4H_2O$ assumed.

If "route" 1 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" 2, 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 would exist. In an attempt to reduce this acidity manufacturers have replaced the pure phosphoric acid of the liquid by acid in which some zinc oxide has already been dissolved. Because of the shape of the pH curve (Fig. 1) this will not

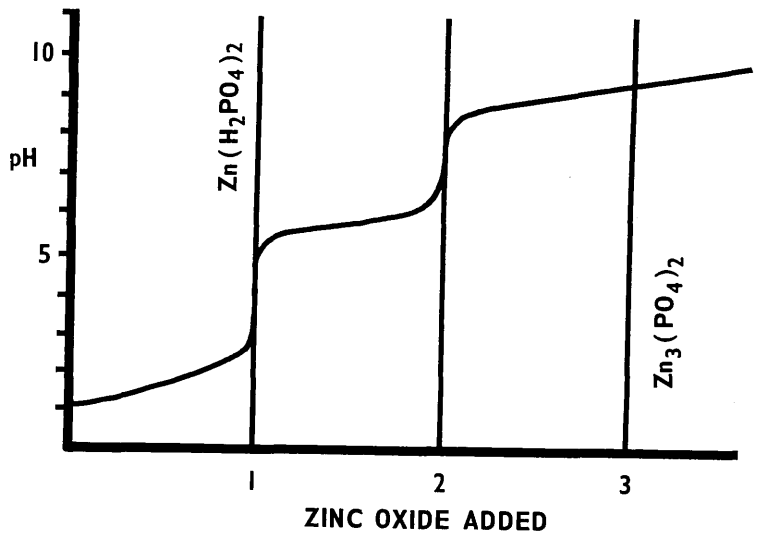

Fig. 1 Graph showing variation of pH value on adding zinc oxide to phosphoric acid. No precipitation of tertiary zinc phosphate is assumed.

effectively raise the pH value, but will reduce the acidity in isolated parts of the mix.

Measurement of the pH Value of the Mixed Cement

Many investigations have been undertaken to measure the acidity of mixed phosphate cements.

Eberly (1934) made one of the earliest determinations and concluded that the reaction was completed in 15 minutes. Other determinations were carried out by Matthews (1934), Paffenbarger, Schoonover, and Souder (1938), and Worner (1940).

It was agreed by these observers that the cement was acid when set and that a pH of 3.8-6.0 may be expected. Worner stated that "zinc phosphate cements are not likely to be irritant to the pulp because they are practically neutral when fully set". In many of these investigations, however, the first pH measurement was recorded some 15-20 minutes after mixing the cement, when the mass was certainly much less acid than immediately after mixing. The harmful effects on the pulp, clearly demonstrated histologically by Manley and others, were, therefore, attributed to acid penetration whilst in the plastic state.

In the most complete investigation into the acidity of the zinc phosphate cements, Harvey, Le Brocq, and Rakowski (1944) repeated many of these previous experiments and showed that, by measuring the pH of dilute extracts of water in contact with the set cement, a dilution error was introduced. They point out that this error caused the cement to appear much less acid than the true value at the time of its

insertion into the tooth cavity while in the plastic state. They further showed that, in the later stages of setting, tertiary zinc phosphate may be formed, giving a more alkaline pH value.

Since the acidity of the cement decreases as it sets, it is most important to measure the pH of cement at the time of its insertion into the tooth cavity, when damage to the dental pulp is most probable. By making direct measurements on the plastic and set cement, Harvey and others 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, in contradiction of Worner's statement that they became "practically neutral".

The investigations by Harvey and his associates were extensive, but they omitted to consider the importance of the time interval between mixing the cement and placing the plastic cement 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 this dilution of the residual liquid, the organic indicator powder was incorporated in untinted 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.

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 irrespective of the proportions of powder and liquid 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 colour obtained was compared against a known standard pH colour. The following organic dyes were used:-

Thymol blue	pH range	1.2-2.8
Methyl orange	" "	2.9-4.0
Congo red	" "	3.0-5.0

These papers were moistened with distilled water 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 was not considered accurate to determine intermediate 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 demonstrate by any of the colorimetric methods described above changes in acidity which may take place within the cement during setting.

It was now established that the cement mix is acid at all times, but accurate quantitative assessment of this acidity was not possible. Previous research work strongly supports the view that acid products do penetrate dentine, but this process has not yet been satisfactorily demonstrated. In recent years, the tracing of substances within the tissues of the body has become a routine procedure by the use of radiochemical methods. Essentially these methods involve the replacement of part of the normal compound with its radio-active analogue. Applying this method to the present study the normal phosphoric acid of the cement liquid was "labelled" with radio-active phosphoric acid and the

subsequent movement of the radio-active phosphorus ion was traced throughout the dental tissues, using autoradiographic techniques. The zinc phosphate salts formed by the chemical reaction between the cement liquid and the cement powder were also "labelled" with radio-active phosphorus.

In order to standardize the consistencies of the cement within the range of clinical use three standardized consistencies were adopted, viz., thin, medium, and thick. Experiments were carried out on dogs and on humans to determine the effect of each of these consistencies of cement on the dental pulp and also to determine the penetration of acid through dentine. The effect of calcium hydroxide in preventing this acid penetration and in reducing any harmful effects on the pulp was also examined.

The results of these studies with radio-active tracers showed conclusively that the acid products, phosphoric acid and primary acid phosphate, present during the setting of the cement, penetrate towards the pulp along the dentinal tubules cut during cavity preparation. The depth of this acid penetration is greatest with thin consistencies inserted into the cavity immediately after mixing. This depth becomes reduced as the powder-liquid ratio is increased, viz., as the consistency becomes thicker, and also as the time of inserting the cement after mixing into the cavity is increased. Where protoplasmic poisons, such as alcohol or phenol, were applied to dentine the acid penetration was much greater.

Comparison of the degree of irritation produced in the

pulp by differing consistencies of cement showed a similar result to that obtained with the acid penetration. This reaction is first seen in the odontoblast layer, which, with the thin consistency of cement, showed a severe disturbance, and in some cases even intrapulpal haemorrhage (Fig. 2). With medium and thick consistencies the effects were mostly confined to the odontoblast layer, but no generalized disturbance was observed. In all cases, irrespective of the consistency of cement used, the reaction of the pulp became much more marked as the dentine remaining between the cavity and the pulp was reduced in thickness.

The results of all experiments confirm the clinical use of the thickest possible mix of cement to reduce pulp reaction to a minimum.

Other experiments using calcium hydroxide showed that where this material is placed between vital dentine and zinc phosphate it completely prevents the penetration of acid through dentine and also eliminates its harmful effects on the dental pulp.

In view of these results, 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 a zinc phosphate cement lining.

Acknowledgement. - I wish to thank Dr. James Ireland, University of Glasgow Dental School, for his assistance and helpful criticism.

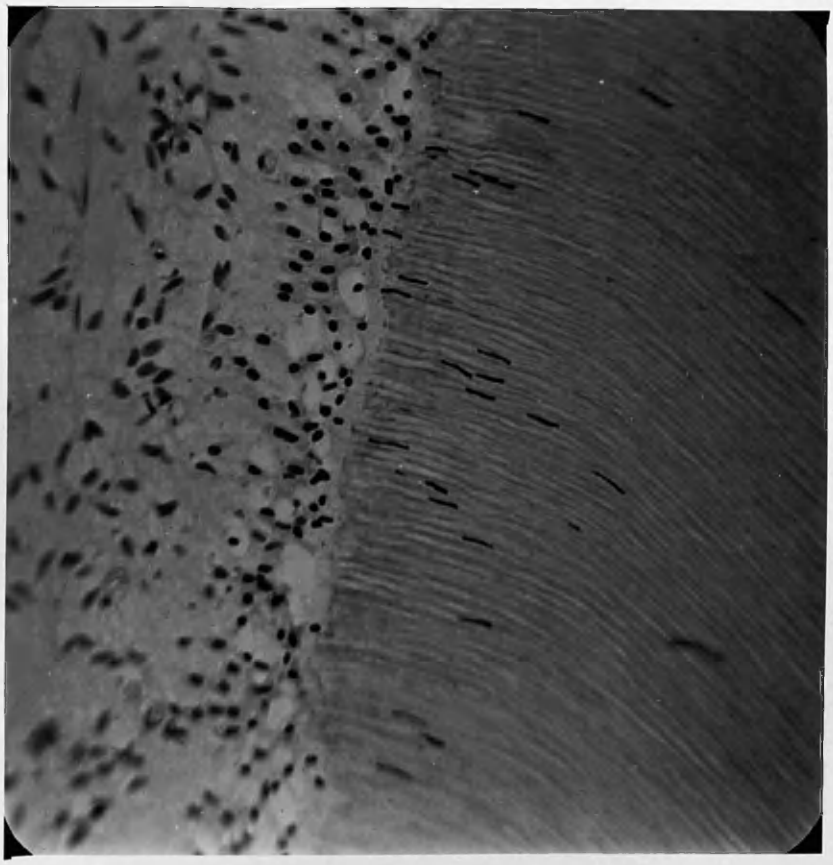

Fig. 2 - Photomicrograph of the reaction of a tooth pulp to a thin consistency of zinc phosphate cement after a period of 40 minutes. There has been a greater disturbance of the odontoblast layer and an area of haemorrhage may be seen. Many odontoblast nuclei can be seen within the dentinal tubules. (x 128).

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PAPER 3

ESTIMATION OF COPPER IN HUMAN ENAMEL BY ACTIVATION ANALYSIS

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Journal of Dental Research

Vol. 41, No. 5, 1013-1016, Sept.-Oct. 1962.

ESTIMATION OF COPPER IN HUMAN ENAMEL BY ACTIVATION ANALYSIS

The trace element copper is normally present as a constituent of human tissue, including tooth enamel.(1) The amount of copper has been previously estimated using conventional chemical or spectrographic methods of analysis.(2) It has been suggested that the presence of high concentrations of this element may produce a resistance to dental caries.(3)

The advantages of the method of activation analysis over the conventional methods of analysis have been described in a previous paper.(4) One of its great advantages is that pooled samples need not be used and more than one sample can be obtained from individual teeth.

Copper consists of a mixture of two isotopes, ^{63}Cu and ^{65}Cu, which on irradiation by thermal neutrons give the radioactive isotopes ^{64}Cu and ^{66}Cu. The most suitable isotope is ^{64}Cu which has a half life of 12.8 hours, allowing a working period of about 36 hours. The half life of ^{66}Cu is only 5.1 minutes. ^{64}Cu is suitable for activation analysis as the cross-section of ^{63}Cu for thermal neutron capture is 3.0 barns and the resulting sensitivity is of the order of 10^{-10} g.[5] ^{64}Cu emits B^- and B^+ particles on decaying and so may be detected by a Geiger or scintillation counter (0.51 MeV X-rays are emitted when a positron is stopped). In this study the samples were irradiated for 24 hours at a thermal neutron flux of 10^{12} n/cm.^{2}/sec.

Theoretically there is a possible interference from

zinc since ^{64}Zn (n,p) ^{64}Cu, but this is only found in a sample which is predominantly zinc. The neutron capture cross-section for this reaction is about 12 millibarns.

Experimental Methods

The method described is a modification of those of Smales[5] and Bowen.[6] Enamel samples weighing not more than 25 mg. were obtained from 100 sound extracted human teeth free from enamel defects. Where the age of the patient was known, this was noted. The crown of each tooth was sectioned transversally at mid-crown level using a carborundum disc. The thickness of enamel at this level was measured and then divided into an outer and inner enamel layer using a cutting disc with a micrometer gauge. When removing the inner enamel layer, care was taken to ensure that there was no contamination of the enamel with dentine.

Small sections of tooth enamel were weighed into polyethylene tubes which were sealed. At the same time a 0.2 g. of a standard copper solution (10 mg./ml.) were weighed accurately into a silica ampule which was also sealed. Both the standard and samples were packed into an aluminium irradiation can and sent for irradiation in a suitable atomic reactor (BEPO). After irradiation, the samples were removed from the polyethylene tubes and digested in 50 ml. centrifuge tubes with 10 drops of 24N nitric acid, adding extra 16N nitric acid to complete the digestion, if necessary. The standard was made up by successive dilutions to 10 litres and 1 ml. used as a working standard.

Chemical Separation

Ten mg. of copper carrier was added to each sample, together with 1 drop each of 10 per cent cobalt nitrate solution, 10 per cent manganese nitrate solution and 10 per cent ammonium dihydrogen phosphate solution. The solution was adjusted to a suitable volume (5 ml.) with water and the copper reduced to the cuprous state by adding 2 ml. of 20 per cent hydrated sodium sulphite solution. The copper was then precipitated by adding 1 ml. of 20 per cent potassium thiocyanate. Any precipitate formed during the addition of the sulphite was dissolved in nitric acid. The cuprous thiocyanate precipitate was boiled and centrifuged, rejecting the supernatant liquid. The precipitate was washed twice with hot water and dissolved in 0.5 ml. of 16N nitric acid. Two drops of the ammonium phosphate solution, 8 drops of a 10 per cent ferric nitrate solution and 3 drops of 10 per cent calcium chloride were added. The solution was neutralized carefully with 15N ammonia until a light-coloured precipitate was obtained, after which a few drops excess were added to form the copper-amine complex and to complete the precipitation of the iron as the hydroxide. The calcium was precipitated by adding 2 ml. of 1M sodium carbonate and the solution heated for a few minutes; filtered, washed, and then neutralized with 18N acetic acid until it turned a pale blue colour. It was acidified with 4 drops of 16N nitric acid and the copper reprecipitated as the thiocyanate using the method described above. The precipitate was washed twice with

hot water and then dissolved in 0.5 ml. of 16N nitric acid. This solution was neutralized with 5 per cent sodium hydroxide solution until a faint precipitate appeared. Four drops of 16N nitric acid were added and the solution heated for a few minutes. Four ml. of 2 per cent quinaldic acid solution were added and the heating continued for a few minutes more. After centrifuging, the precipitate was washed twice with hot water and once with acetone. The precipitate was slurried on to weighed trays with acetone, dried, and a comparison made between the recoveries and count rates of the samples against the standard, thus obtaining the absolute content. All reagents used were 'AnalaR' grade; and all percentages were weight/volume.

Results

The results of the analysis of 100 samples showed that small variations occurred between the copper content of the outer enamel layer and the inner enamel layer; but these differences were so small as to have no significance. The mean of the outer enamel layer was 9.5 p.p.m. with a standard deviation of 7.8 p.p.m., and that of the inner enamel layer 11.3 p.p.m. with a standard deviation of 9.0 p.p.m. Table 1 shows the copper content of teeth where the age and sex of the patient were known. The range of distribution of the copper in 100 enamel samples is shown in Figure 1.

Summary

The method of activation allows measurement of the

copper content of the outer and inner enamel layers of individual teeth. With this method the copper content of 100 teeth was estimated and a mean value of 9.5 p.p.m. of copper for the outer enamel layer and 11.3 p.p.m. for the inner enamel layer was obtained.

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Table 1

Copper in Inner and Outer Enamel Layers of Permanent Teeth

Age	Sex	Inner Layer	Outer Layer	Age	Sex	Inner Layer	Outer Layer	Age	Sex	Inner Layer	Outer Layer
6	M	9.46	10.50	16	F	9.56	3.24	23	M	13.10	5.83
9	M	10.00	6.61	16	M	10.50	15.00	23	M	14.70	18.80
9	M	6.41	5.87	16	M	16.80	6.84	25	M	23.40	21.90
12	M	15.40	15.00	16	F	8.10	3.08	26	M	5.53	2.130
12	F	7.65	14.10	16	M	3.95	3.34	26	F	16.00	1.83
12	F	14.60	35.70	16	F	4.65	6.44	26	M	11.10	14.20
13	M	6.70	19.10	17	F	5.28	4.64	30	M	7.88	3.62
13	M	1.75	3.91	17	F	4.45	12.00	31	F	2.19	2.79
14	M	12.50	2.15	17	F	5.28	4.64	32	F	8.85	4.25
15	F	5.39	6.35	17	M	7.65	14.10	32	F	3.55	7.00
15	F	39.70	7.00	17	M	35.70	14.60	41	F	17.10	17.90
16	F	3.39	2.61	20	M	18.70	7.91	65	F	34.00	27.80
16	F	1.61	1.59	22	F	15.20	12.90	65	F	22.80	18.10

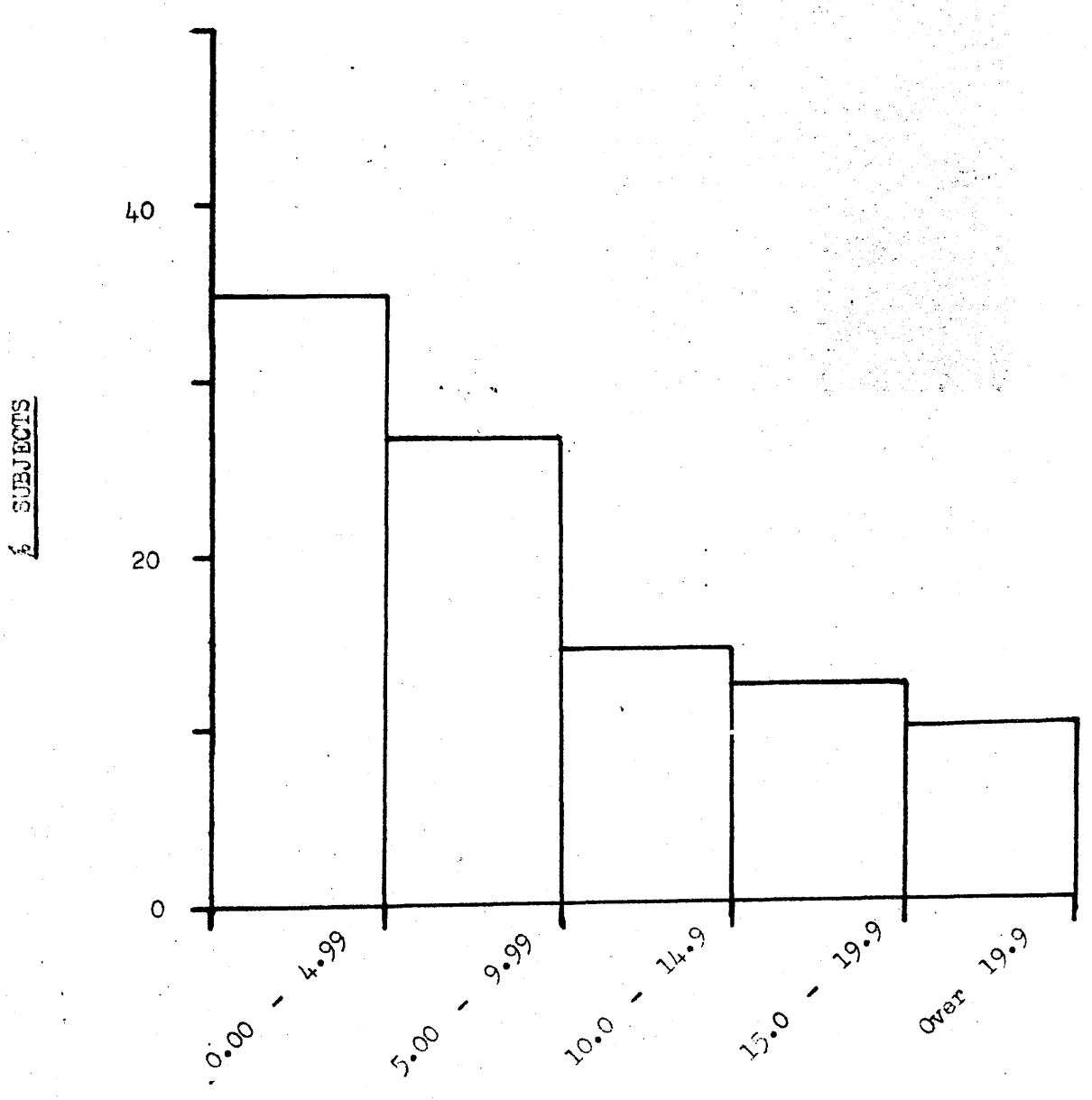
% SUBJECTS
40
20
0
0.00 - 4.99
5.00 - 9.99
10.0 - 14.9
15.0 - 19.9
Over 19.9
P.P.M. COPPER

Fig. 1.

P A P E R 4

ACTIVATION ANALYSIS IN DENTAL MEDICINE

G. S. Nixon

Chapter 21, 133-138, Activation Analysis,
Lenihan and Thomson, Academic Press,
London and New York, 1965

also published as:

U.S. Atomic Energy Commission
Publication No. 084-11, 1964.

ACTIVATION ANALYSIS IN DENTAL MEDICINE

Dental Caries

Apart from racial and dietary factors which influence dental caries, the answer to individual susceptibility to caries may lie in the composition of the hard dental tissues, with particular reference to the trace elements.

It is important, therefore, not only to establish values for these elements, but also their distribution throughout the hard tissues of the teeth, particularly enamel. Once these values are established for sound teeth, carious teeth can then be examined.

One great advantage of activation analysis has been the ability to select many samples from different areas of enamel of a single tooth. Using a micrometer sectioning technique, six samples can be obtained from different areas of enamel. This is important as it is known that the composition of the tooth enamel is not uniform throughout. As the process of caries commences on the outer surface of the enamel, it might be expected that the surface composition would be most closely related to caries resistance.

A great amount of publicity has been given to the action of fluorine in the role of preventing caries. Other elements, however, have also been suggested as being anti-cariogenic. Elements such as molybdenum, manganese, boron, strontium, lithium and vanadium.

Many laboratory studies have been carried out feeding rats and other laboratory animals on diets containing these

elements to determine their effect on dental caries. The results of these experiments have not only been conflicting, but even confusing.

Several recent investigators have indicated that interactions may exist between trace elements which may account for the conflicting results when the trace elements are administered separately. Stookey and Muller (1960) have shown that there is increased retention of fluorine when molybdenum was added to the diet. Furthermore, Kruger (1959) found the same between fluorine and boron, and fluorine and aluminium. It has also been shown that fluorine, when administered with vanadium, is more effective in reducing dental caries than fluorine alone.

These studies, together with others, would appear to show that not any single trace element is responsible for the reduction of dental caries, but that a relationship probably exists between trace elements in teeth - an interaction which may take place producing an alteration, either in the organic, or in the inorganic constituents of teeth.

In the past, the emphasis has been mainly on the alteration which takes place in the apatite structure of the inorganic portion of the tooth and the relationship which exists between it and the trace elements, but little to their relationship with the organic portion.

It is well known that fluoritic teeth are more resistant to caries and that fluoritic enamel is more resistant to decalcification. It is sometimes forgotten that, where there are high fluorine water supplies, the

enamel may be grossly hypocalcified, while the teeth are relatively resistant to attack by dental caries and to decalcification. It may be argued that, if caries is simply a decalcification process, such teeth would be more susceptible to caries - but this is not so. On close examination of these teeth, it is found that they have an unusual organic structure, which is highly resistant to acid attack.

Although the organic components of teeth are present in relatively small amounts (3%) in enamel, it is realised that they play an important part in tooth formation and in dental caries.

It is generally recognised that the protein of the enamel matrix is a type of keratin, which has been characterised as a eukeratin. With increasing age, it is believed that changes take place in the organic portion of the teeth and that there is an increased resistance to dental caries.

Trace elements are known to produce changes in other ectodermal substances, such as wool etc. As enamel is also of ectodermal origin, it may be from a comparative biochemical point of view that trace elements may cause changes in the enamel proteins and thus produce an increased resistance to caries.

Experiments are being carried out at present to examine the relationship between trace elements and their uptake in teeth. Stable isotopes of trace elements considered to produce alteration in caries have been added to the diet of rats. As rats have continuously growing incisors, their teeth form a record of the amounts of trace elements taken up by the hard dental tissues. By removing a small portion

of the incisal tip of the growing tooth *in vivo* and activating it, the amounts of the trace elements present in the dental tissues can be measured. Variations in the proportions of these trace elements in the diet can be given to the same rat.

Calculus

One other aspect of dental medicine to which activation analysis is being applied is that of calculus.

Approximately 80% of dental calculus is composed of such inorganic material as calcium, magnesium, phosphate carbonate and fluorine. However, there appears to be no full agreement about this and there has been very little published work.

There are two main types of calculus, *supragingival* - or that which occurs above the gum and which is most common - and *subgingival*, which occurs below the gum level.

Opinion as to the origin of calculus has been divided - one school of thought believing that calculus, whether found above or below the gum, is formed by saliva. The other opinion is that, while the supragingival calculus is formed from saliva, that below the gum margin is formed from a serous exudate from the blood.

Recent work by Brill (1962) using a fluorescent dye injected into the blood stream showed that the fluid in the gingival pocket did, in fact, come from the blood stream and could be a possible source of subgingival calculus.

It is proposed to examine both supra- and subgingival calculus for trace elements and to establish if any relationship exists between the trace elements in saliva and blood and those in calculus.

This study links up with our previous caries study as it is a well-known dental fact that patients who have a heavy calculus deposit have very little caries. This calculus/caries antagonism has never yet been satisfactorily explained.

Amalgam fillings as a means of assessing radiation dosage

One of the difficulties in assessing the biological effects of radiation on human beings is that no direct experimental work could be allowed. Extrapolation of effects from radiation on animals to radiation on man is, in principle, unreliable, and so information on radiation damage to man comes from limited sources:-

(a) X-radiographers of the old school who were poorly protected;

(b) the early workers on radioactive species;

(c) bomb irradiation at Hiroshima and Nagasaki;

(d) reactor incidents.

In (d) after a reactor incident, it is possible to obtain some information on total neutron dose from a study of:

(i) body ^{24}Na in blood

(ii) Au in rings, buckles and watches

(iii) the present proposal - Hg in teeth.

Suppose we consider a typical filling to contain 2 g. of Hg. Let this be exposed to a total dose of $\emptyset$ neutrons (note this is a total dose, not flux x time). Then the number of radioactive atoms of ^{197}Hg formed (N^*) will be given by $N^* - N\emptyset$ where N is the number of mercury atoms irradiated -

i.e. $N^* = \frac{2}{200} \times 6.02 \times 10^{23} \times 4.5 \times 10^{-24} \times \emptyset$

$\frac{\text{Wt. of Hg}}{\text{Atomic Wt. Hg}}$ x Avogadro's No. x (the cross-section for producing ^{197}Hg from the natural element for thermal neutrons)

$3 \times 10^{-2} \times \emptyset$

Now the rate of radioactive decay of these atoms will be

$$\frac{-dN^*}{dt} = N^* = \frac{.693}{t_{\frac{1}{2}}} \times N^*$$

where is the disintegration constant and

$= 0.693/t_{\frac{1}{2}}$ $t_{\frac{1}{2}}$ = ½-life of element.

i.e. $$\frac{-dN^*}{dt} = \frac{.693 \times N^*}{65 \times 60}$$

($t_{\frac{1}{2}}$ = 65 hours = 65 x 60 min.)

$$= \frac{.693}{65 \times 60} \times 3 \times 10^{-2} \times \emptyset$$

disintegration per minute

$$= \frac{69.3 \times 10^{-2}}{6.5 \times 6.0 \times 10^2} \times 3 \times 10^{-2} \times \emptyset$$

$2 \times 10^{-4} \times 3 \times 10^{-2} \times \emptyset$

$6 \times 10^{-6} \times \emptyset$ disintegration per min.

If we assume that the counting system is only 1/6th efficient

Counts per minute in counter = $10^{-6} \times \emptyset$

Thus for a neutron burst of 10^8 neutrons the 2 g. Hg sample should give 100 c.p.m.

Now in pulsing a reactor one might obtain a flux of 10^{16} neutrons per second per square cm. for 20 milliseconds

i.e. 2×10^{14} neutrons.

The number of neutrons expected in an "incident" would be at least equal to this.

Suppose only 10% escape the cone and a person stands 20' away from it. Then his total dose per square cm.

$$= 2 \times 10^{14} \times \frac{1}{10} \times \frac{1}{4 \times 11 \times 20 \times 30 \times 20 \times 30}$$

This comes from considering source as a point and finding neutrons per sq. cm. on the surface of a sphere of radius 20'.

$$= 2 \times 10^{14} \times \frac{1}{10} \times \frac{3 \text{ cm.} \quad 1'.}{4 \times 3 \times 6 \times 10^2 \times 6 \times 10^2}$$

$\frac{1}{2} \times 10^9$ neutrons

i.e. under these conditions a person standing 20' away might have upwards of 10^9 neutrons falling on each square cm. of his body - which in his Hg fillings would give, for a 2 g. filling 10^3 c.p.m. in a counter.

It might be argued that the cross-section chosen = 4.5 barns (1 barn = 10^{-24} cm.^{2}) is wrong and would, of course, vary with neutron energy; but Guinn (1964) in his studies of γ versus neutron distribution during pulsing of a reactor finds that the spectrum of neutrons deviates little from a thermal spectrum. Thus, the errors introduced by taking for thermal energies probably only makes a few % error in the result.

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P A P E R 5

HAZARD OF MERCURY POISONING IN THE DENTAL SURGERY

G.S. Nixon and H. Smith

J. Oral Therapeutics and Pharmacology,

Vol. 1, No. 5, 512-514, 1965.

HAZARD OF MERCURY POISONING IN THE DENTAL SURGERY

Since the introduction of silver and copper amalgams as dental filling materials at the beginning of the 18th century, the presence of mercury used in their preparation has given rise to much discussion regarding its possible hazard to health and many investigations were carried out. In the most recent reports[1,2,3] it is stated that there is no serious risk to dental personnel from volatilized mercury in the dental surgery.

In mercury intoxication, the classical symptoms, such as stomatitis, profuse salivation and tremors of the tongue, are not always evident. Different degrees of intoxication may take place, particularly if absorption of mercury occurs over a long period of time. Symptoms, such as restlessness, irritability and insomnia with fine tremors of the hands have been reported as being present in such cases.[4]

Metallic mercury is absorbed into the body mainly by inhalation of mercury vapour into the lungs. Soluble salts of mercury can also pass into the circulatory system via the digestive tract. Mercury can also be absorbed through intact skin.[5] The presence of perspiration on the skin increases the absorption by dispersing the mercury over a wider area. After passing into the circulation, mercury is rapidly taken up by the tissues. The highest concentration is found in the kidney followed by the liver, spleen, intestinal wall, heart and skeletal muscle.

Mercury volatilizes readily at room temperature and theoretically 1 cu.m. of air at 20°C can hold 30 mg. mercury.

Measurements of the amounts of air have been made in various dental surgeries and reports indicate that these concentrations may be as high as 0.20 mg. Hg./cu.m.[6] to less than 0.01 mg. Hg./cu.m.[3] In America the maximum acceptable concentration of mercury for an eight-hour five day week is 1/100th of 1 ppm.[7] When this concentration exceeds 1 ppm. mercury may become a hazard to health. In the dental surgery the mercury concentration in the air depends on a number of factors, such as the ventilation and temperature of the surgery.

The preparation of copper amalgam by heating the pellets contributes a much greater mercury hazard than the preparation of silver amalgam. In the preparation of silver amalgam the practice of mulling the amalgam in the palm of the hand has been condemned because of the dangers of contamination of the absorption from the skin. The technique recommended for the preparation of silver amalgam at present is to place the amalgam, after mixing, in a dental napkin and remove the excess mercury by wringing the napkin between the fingers. Even with this method, mercury is in direct contact with the skin.

To investigate the possible health hazards of mercury to dental surgery personnel, a preliminary investigation was carried out to measure the mercury content of hair and nails of female dental surgery assistants. These assistants who are continuously handling mercury are most likely to be exposed to any danger.

Methods

Twenty female dental surgery assistants were selected who were currently employed in a dental surgery and who had been actively engaged in surgery work for a period of not less than two years.

Samples of head hair and axillary hair, finger nails and toe nails were obtained from each assistant. As controls, similar samples were obtained from 26 female subjects not exposed to mercury. The mercury content of the hair and nail samples was estimated using a method of neutron activation analysis.(8) This method had a sensitivity of 10^{-10} gm.

The hair and nail samples, weighing about 20 mg. were sealed in aluminium or silica tubes and irradiated in a thermal flux of 10^{12} neutrons per square centimetre per second for a period of one week (B.E.P.O.) After irradiation, two radioactive isotopes of mercury 197 and mercury 203 were formed, which were suitable for measurement by activation analysis. Because of its greater ease of detection and longer half-life, mercury 203 was selected for estimation.

Results

In the control group, the mercury content of the nail samples showed a mean value of 5.10 ppm. In this group there was little difference between the mercury content of finger nails or toe nails. In the surgery assistants' group, the mean value for toe samples was 9.3 ppm. mercury, while that of finger nails was much higher at 68.76 ppm.

The mean mercury content of both axillary and head hair samples in the control group was 8.8 ppm.

In the surgery assistants group, while the mean mercury content of axillary hair was 7.88 ppm., that of the head hair was 32.25 ppm.

It can be seen, therefore, that the mercury content of both head hair and finger nails, parts of the body which are exposed to mercury, was much higher in the surgery assistants group than in the control group.

These findings suggest that chronic mercury poisoning (in some cases undiagnosed perhaps) may be a hazard to either dentist or his staff. One such case which came to notice following this investigation concerned a dentist and his surgery assistant who complained of signs and symptoms similar to chronic mercury poisoning. Both had been complaining of profuse salivation together with tremors of the hands, insomnia and irritability. The mercury content of the dentist's finger nails was 558 ppm. and the head hair 171 ppm., while the assistant showed 286 ppm. for the finger nails and 50.8 ppm. for the head hair. On investigation it was found that amalgam fillings were prepared by both dentist and assistant and that excess mercury was allowed to escape on to the floor in close proximity to an electric convector heater. When the floor covering was removed, a quantity of mercury (approximately 3 ml.) was found below the floor boards.

A further investigation is being carried out to determine the reasons for the higher mercury content in these subjects. In these cases, such factors as careless handling

of mercury during preparation of amalgam, or working in dental surgeries where ventilation and working surfaces are inadequate are being considered.

Summary

Mercury is a possible hazard to health in the dental surgery. Measurements of the mercury content of hair and nail samples were carried out on female dental surgery assistants using a sensitive method of neutron activation analysis. The results of these analyses showed that the mercury content in both finger nails and head hair in this group was much greater than in the control group, and could constitute a possible hazard to health.

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PAPER 6

ESTIMATION OF MERCURY IN HUMAN ENAMEL BY ACTIVATION ANALYSIS

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Journal of Dental Research

Vol. 44, No. 4, 654-656, 1965.

ESTIMATION OF MERCURY IN HUMAN ENAMEL BY ACTIVATION ANALYSIS

Mercury is found in all human tissues in trace amounts. The highest concentration is found in the kidneys followed by the liver and spleen and there is evidence that mercury can be stored in the bones.(1) It was the purpose of the present study to determine the mercury content of human enamel using a sensitive method of activation analysis.(2)

Since the introduction of silver amalgam as a restorative material, the presence of mercury in the tissues has given rise to much discussion because of its toxic properties. Frykholm(3) has stated that mercury vapour may be given off from dental amalgam from the time of its insertion into the tooth cavity until it is set, that is, until the mercury is bound into the stable phase of amalgam. He considers that there is a short exposure to mercury, during this setting period, of the order of 0.02-0.20 mg. Hg/m^3.

Mercury consists of a mixture of many isotopes. After irradiation, the two isotopes most suitable for measurement are $^{196}Hg\ (n, \gamma)\ ^{197}Hg$ with a half-life of 65 hours and $^{202}Hg\ (n, \gamma)\ ^{203}Hg$ with a half-life of 47 days. The thermal neutron capture for ^{197}Hg is 4.5 barns and for ^{203}Hg is 1.13 barns. Because of its longer half-life and greater ease of counting, ^{203}Hg was used in this study. This isotope emits β rays with a maximum energy of 0.208 MeV and γ rays of 0.279 MeV.

Materials and Methods

Enamel samples were obtained from forty sound extracted permanent human teeth which were free from enamel defects. None of the teeth in this group was in direct contact either approximally or occlusally with teeth containing amalgam fillings. In this way, direct contamination of the enamel with mercury from amalgam was avoided as far as possible. The age of the patient and the position of the tooth in the arch were noted in each case. The proximity of teeth containing amalgam fillings was also noted. Twelve unerupted teeth which, on clinical examination, showed no communication with the oral cavity were also used. A further series of enamel samples was taken from seven sound teeth which were in direct approximal contact with teeth containing silver amalgam fillings.

The crown of each tooth was washed in distilled water and sectioned across at midcrown level using a Carborundum disc. The enamel thickness at this level was measured and then divided into an outer and inner enamel layer by means of a cutting disc on a micrometer gauge. A fresh cutting disc was used when cutting each section to avoid contamination. Care was taken when cutting the inner enamel layer to ensure that no contamination with dentine occurred.

Method of Analysis

Twenty milligram sections of tooth enamel were weighed into aluminium or silica tubes which were sealed. The samples were irradiated with thermal neutrons for 1 week at

10^{12} neutrons per cm^2. About 1 mg. of metallic mercury was accurately weighed and sealed in a silica ampoule. This was irradiated at the same time and employed as a standard. It was diluted as necessary to give a count rate comparable with the samples and was processed exactly as the enamel sample.

After irradiation, the samples with 10 mg. carrier mercury were digested in 2 ml. of a mixture of nitric acid (16N) and sulphuric acid (36N) in ratio of one to one. Conical-bottomed flasks of 25 ml. capacity and with 6-inch necks were used for the digestion. When digestion was complete, the acid solution was transferred to a 50 ml. centrifuge tube and neutralized with sodium hydroxide solution (5N). It was made acid by adding 3 drops of nitric acid (16N) and diluted to 10 ml. After heating on a water bath for a few minutes, the mercury was precipitated as the metal by adding 2 ml. of absorbic acid solution (1 per cent w/v). This precipitate was centrifuged and washed well with water and then acetone. The last traces of acetone were removed by heating for a minute or two on a boiling water bath. Approximately 10 drops of nitric acid (16N) were added to dissolve the dry mercury which was then diluted to about 10 ml. One ml. of silver nitrate solution (per cent w/v) was added to the mercury solution and well mixed.

The silver was precipitated by adding 2 ml. of sodium iodide solution (10 per cent w/v), the resulting solution centrifuged or filtered to obtain the supernatant and the precipitate rejected. The solution was neutralized with

Table 1

Mercury in Inner and Outer Enamel Layers of Unerupted Permanent Teeth

Age	Tooth	Enamel	Mercury (ppm)
82	$\overline{87}$	Inner	1.11
		Outer	0.10
23	/$\overline{8}$	Inner	0.10
		Outer	0.10
51	/$\overline{8}$	Inner	0.10
		Outer	0.10
12	5/	Inner	0.10
		Outer	0.10
49	3/	Inner	3.46
		Outer	0.10
21	/$\overline{8}$	Inner	0.10
		Outer	0.10
41	/3	Inner	0.10
		Outer	0.10

ammonia (10 per cent v/v) to congo red end-point and 3 ml. copper ethylenediamine complex added. This complex was prepared by mixing one part of copper sulphate solution (10 per cent w/v) with 10 parts of 1:2 ethylenediamine (10 per cent w/v). The mercury was precipitated as copper ethylenediamine mercury iodide which was then washed well with water and isopropyl alcohol. The activity was detected using a scintillation counter. The precipitate was slurried on to weighed stainless steel planchets with isopropyl alcohol and a comparison made between the recoveries and the count rates of the samples with the processed standard. All reagents were "Analar" grade.

Table 2

Mercury in Inner and Outer Enamel Layers of Erupted Permanent Teeth

Age	Outer Layer (ppm)	Inner Layer (ppm)	Age	Outer Layer (ppm)	Inner Layer (ppm)
78	0.81	0.14	9	2.13	2.61
14	4.24	2.17	21	3.55	2.90
63	2.12	1.06	41	2.79	1.40
38	2.07	12.70	20	10.00	2.74
13	5.80	4.48	17	0.50	0.56
9	16.00	8.25	20	3.74	2.43
13	1.68	2.85	20	3.74	1.22
37	2.72	1.74	3	2.61	0.84
11	3.50	2.89	14	0.34	0.68
14	3.49	0.79	21	0.95	0.80
13	3.48	0.72	12	0.68	0.90
23	10.90	2.79	13	0.72	1.34
35	3.41	1.48	11	0.30	0.27
21	4.90	1.67	13	4.10	2.78

Results

The mercury content of the unerupted teeth (Table 1), with two exceptions, was less than 0.1 ppm. This figure is just within the limits of measurement of the method. In the erupted teeth (Table 2) the mean mercury content was 2.61 ppm. and the median 2.07 ppm. Small differences were found between the outer and inner layers of the enamel of the erupted teeth. The mean of the outer enamel layer was

2.79 ppm. with a median of 2.15 ppm., while the mean of the inner enamel layer was 2.34 ppm. with a median of 1.06 ppm.

The range of distribution of the mercury in the enamel of the erupted teeth is shown in Figure 1.

In erupted teeth where the enamel was in immediate contact with silver amalgam, the mercury content was much higher, ranging from 153 to 1,200 ppm. for the inner enamel layer and from 279 to 1,600 ppm. for the outer enamel layer (Table 3).

Table 3

Mercury in enamel of erupted teeth in immediate contact with silver amalgam

Age	Tooth	Inner Enamel	Outer Enamel
9	$\underline{/6}$	286	279
11	$/\overline{5}$	850	1,260
12	$\underline{/5}$	690	730
13	$\overline{47}$	656	1,290
21	$\underline{/6}$	541	1,600
20	$\overline{77}$	1,200	1,340
35	$\overline{57}$	153	341

Discussion

The mercury content of the enamel of unerupted teeth is just within the limits of measurement detection. In the two cases where the mercury content was higher it is possible that some communication existed between the tooth and the oral cavity. The higher mercury content of the

enamel of erupted teeth can be attributed to the contamination in this enamel of mercury either from mercury vapour during the setting phase of amalgam or by migration of mercury ions from existing amalgam fillings. In teeth which were in direct contact with silver amalgam fillings, the higher mercury content may be due to direct and continuous migration of mercury.

Summary

Using activation analysis, the mercury content of the inner and outer enamel layer of individual teeth was estimated. In forty sound erupted teeth, a mean value of 2.79 ppm. was obtained for the outer enamel layer and 2.34 ppm. for the inner enamel layer. In seven unerupted teeth the mercury content of the enamel was of the order of 0.1 ppm. Enamel of teeth in contact with silver amalgam fillings had mercury contents ranging from 153 to 1,600 ppm.

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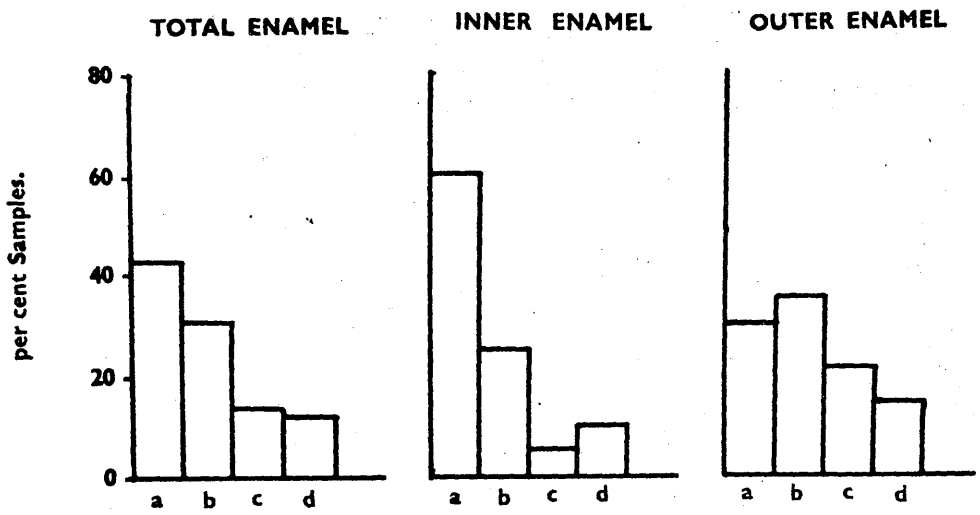

FIG. 1.—Range of mercury concentration in enamel samples: *a*, 0.00–1.49 ppm; *b*, 1.50–2.99 ppm; *c*, 3.00–4.49 ppm; *d*, over 4.50 ppm.

P A P E R 7

STRONTIUM^{90} UPTAKE IN THE DECIDUOUS AND PERMANENT TEETH OF CHILDREN IN THE WEST OF SCOTLAND

G. S. Nixon and J. G. Warren

Annual Report of Physics Department,

Western Regional Hospital Board, 1965

STRONTIUM^{90} UPTAKE IN THE DECIDUOUS AND PERMANENT TEETH OF CHILDREN IN THE WEST OF SCOTLAND

A significant uptake of the radioactive isotope strontium-90 by the mineralizing tissues has been evident since 1953. The level of the uptake is related to the concentration of this element in the diet and is a consequence of atmospheric contamination from the products of nuclear fission. Measurements of the concentration of strontium-90 in teeth have been given by Bryant, Henderson and Holgate (1960) and again by Starkey, Bryant and Henderson (1964). No attempt was made in these studies to limit the analysis of teeth to a particular geographic area. In this study it was proposed to determine the levels of strontium-90 in the deciduous and permanent teeth of children in Glasgow and its immediate environs.

Tooth Samples for Analysis

Deciduous and permanent teeth were collected from children known to have spent all their years in Glasgow. Only sound teeth were used for analysis as caries would produce decalcification of the teeth with a possible effect on the calcium to strontium-90 ratio.

The samples from each group were pooled to provide sufficient materials for analysis. Whole teeth were used for analysis and no attempt was made to separate the crown from the root portion.

Analytical Method

The method of analysis was that of Parker, Henderson and Spicer (1965). Teeth samples were weighed, then dissolved in nitric acid and an aliquot of the solution removed for calcium and stable strontium determinations. Strontium and barium carrier solutions were added to the remainder of the acid solution and the alkaline earths precipitated as oxalates. This precipitate is decomposed using nitric and perchloric acids before strontium with calcium and barium is reprecipitated as carbonate. Nitric acid separations are carried out to remove the calcium and isolate the strontium. Barium together with any radium and its associated radionuclides is removed by chromate precipitation, before finally precipitating the strontium as carbonate. After a period of about 14 days to allow the strontium-90 to come to equilibrium with its daughter yttrium-90, assay of the activity is made using a low background counting unit (Rowan and Stevenson, 1960).

Calcium is determined by a method of McIntyre (1961) and using a Zeiss spectrophotometer with flame attachment. Stable strontium is determined using the same instrument and by an additional standard method of Harrison (1958).

Results

The strontium-90 values for whole teeth are given in Table 1.

Table 1

Sample number	Age	Sample Weight	$\frac{Sr}{Ca} \times 10^3$	$^{90}Sr/Ca$ pc/g	S.D.
T3	8y (d)	14.8g	297	3.2	0.15
4	8 (p)	19.75	320	2.7	0.15
5	7y	17.25	256	3.8	0.1
6	9	10.9	183	1.35	0.07
7	10	11.0	291	1.5	0.06
8	11	6.9	201	0.95	0.1
9	6	22.9	218	2.9	0.03
10	5 (d)	2.7	292	4.5	0.4
13	4 (d)	1.86	252	2.2	0.4
14	12	7.2	354	2.0	0.1
15	5 (d)	12.9	157	3.3	0.08
16	6	24.3	196	2.85	0.05
17	7	13.4	245	3.05	0.07
18	8	11.5	273	2.1	0.07
19	9	2.3	227	1.15	0.25

Table 2

Summary of Strontium-90 Values (p Ci/g Ca) for 1963 and 1964: Glasgow Results
(M.R.C. Monitoring Report No. 11)

Age Group	1963					1964				
	No.	Min.	Max.	Median	Mean	No.	Min.	Max.	Median	Mean
Newborn	78	0.8	7.9	2.2	2.66	86	1.05	9.2	3.4 3.45	3.7
2w - 4y	84	0.8	16.05	3.5 3.55	4.25	62	2.1	21.45	6.45 6.6	8.0
5y -19y	10	1.0	2.6	1.4 1.45	1.60	12	2.2	5.65	3.9	4.0
20y and over	-	-	-	-	-	1	-	-	-	(2.15)
6 - 23m	21	1.9	12.2	5.0	5.8	19	2.4	2.45	11.8	12.1

Discussion

The results of whole teeth analysis indicate that the levels of strontium-90 in whole teeth from the West of Scotland are higher than those given by Starkey, Bryant and Henderson (1964) which ranged from 0.17 - 0.9 Sr pc/g Cu. A comparison of the levels is given with the 1963-64 tables for ^{90}Sr in bone in Glasgow (Table 2).

Even with this increase in levels of ^{90}Sr in the teeth there is no evidence available to indicate that these levels of contamination are dangerous. Structural differences between teeth and bone make it difficult to relate strontium-90 levels in bone and in teeth. The concentration of calcium in teeth is higher than that of bone and with equivalent values the amount of strontium-90 per gram of tooth is greater than the amount of strontium-90 per gram of bone by a factor of about 2.3 (Bryant, 1964). In addition as the levels of strontium-90 in food stuff vary so would the levels in bone. Whereas the concentration of strontium-90 in teeth would remain at the level originally laid down at the time of calcification.

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PAPER 8

INHALATION OF OIL PARTICLES FROM AIR TURBINE HANDPIECES

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Brit. dent. J. 119 : 114-117, 1965.

INHALATION OF OIL PARTICLES FROM AIR TURBINE HANDPIECES

The air turbine handpiece is now accepted as standard equipment in the dental surgery. By its use, tooth structure can be removed more rapidly and patient discomfort reduced to a minimum. Unfortunately, a number of side effects have resulted from the use of these handpieces, one of which has been a complaint by dentists of a greater tendency to upper respiratory infection. As a prophylactic measure, many operators now wear protective face masks.

In operation, the air turbine handpiece forms an aerosol consisting of droplets of oil and water. When cavities are being prepared in the mouth, this aerosol may contain, in addition, tooth debris and micro-organisms.

A report by Kazantzis (1961) has stated that, during cavity preparation, the amount of respirable dust is small, especially when using a water jet, and that there does not appear to be any greater risk of bacterial contamination with the use of the air turbine handpiece than with conventional handpieces. However, in a comparative study of aerosols generated by conventional and air turbine handpieces while cutting tooth structure, Madden and Hausler (1963) found that a greater concentration of aerosols occurred with the air turbine than with the conventional handpiece.

The inhalation of oils in the production of human disease was extensively studied by Pinkerton (1928). He found that different types of oil, such as mineral or vegetable, produced a different reaction within the lung.

In general, the reaction of the lung tissues to the presence of irritating oily substances is characteristically macrophagic and proliferative. The extent of the lesions and degree of fibrosis produced are mainly determined by the quantity and chemical constitution of the oil which is introduced into the parenchyma. Oils which cause the greatest reaction are the mineral oils, while in general the majority of vegetable oils, such as olive oil, may cause little residual fibrosis. Spencer (1962) has stated that the majority of vegetable oils become emulsified and are not hydrolysed by the lung lipases. They are mainly expectorated causing little or no residual damage.

One important factor in determining whether the inhaled oil reaches the lung alveoli or not is the size of the particle. It has been suggested that the maximum retention of oil particles takes place when the particle size is 0.5 to 5 μ in diameter. While particles of less than 0.5 μ in diameter tend to be exhaled as readily as they are inhaled, those of less than 100 μ in diameter are more likely to find their way into air passages, including the alveoli.

The defence mechanisms of the respiratory tract afford a certain amount of protection from inhalation of aerosols. The larger particles are stopped by the hairs of the nasal cavity, while smaller particles which pass through into the larynx and pharynx may excite the cough reflex and some will be expelled. Bland oils, however, do not excite the cough reflex and oils of this nature may flow readily from the pharynx and larynx into the dependent parts of the lungs

(Anderson, 1957). Some smaller particles of these oils may be retained in the trachea and bronchi, while others pass to the lungs. Although the quantity of oil reaching the parenchyma at any one time may be small, daily inhalation of any oil over a period of time may cause accumulation.

It was the purpose of this investigation to determine if measurable amounts of oil are inhaled from air turbine handpieces, by both operator and patient under experimental conditions and to determine also if the atmospheric concentration of these oils in the surgery was significant.

Materials and Methods

An artifical lung unit was constructed to simulate normal respiration (Figs. 1 and 2). Regulation of the respiratory rate was controlled electronically by the circuit shown in Figure 3. Air was drawn through the filter (F) by means of a respiratory bag filled with air under negative pressure supplied by pump (P). Positive pressure was supplied by nitrogen from a cylinder. Two automatic solenoid valves were incorporated into the pipeline to the artificial lung, one valve (SV1) being connected to a source of positive pressure, and the other (SV2) to a source of negative pressure. By energising the valves alternately, the artificial lung was inflated and deflated as required. The timing sequence was controlled by the transistor multivibrator (T_1, T_2). The timing impulses, the lengths of which were set accurately by means of potentiometers, (VR_1, VR_2) were fed to driver transistors (T_3, T_4) which, in turn, governed the operation of the

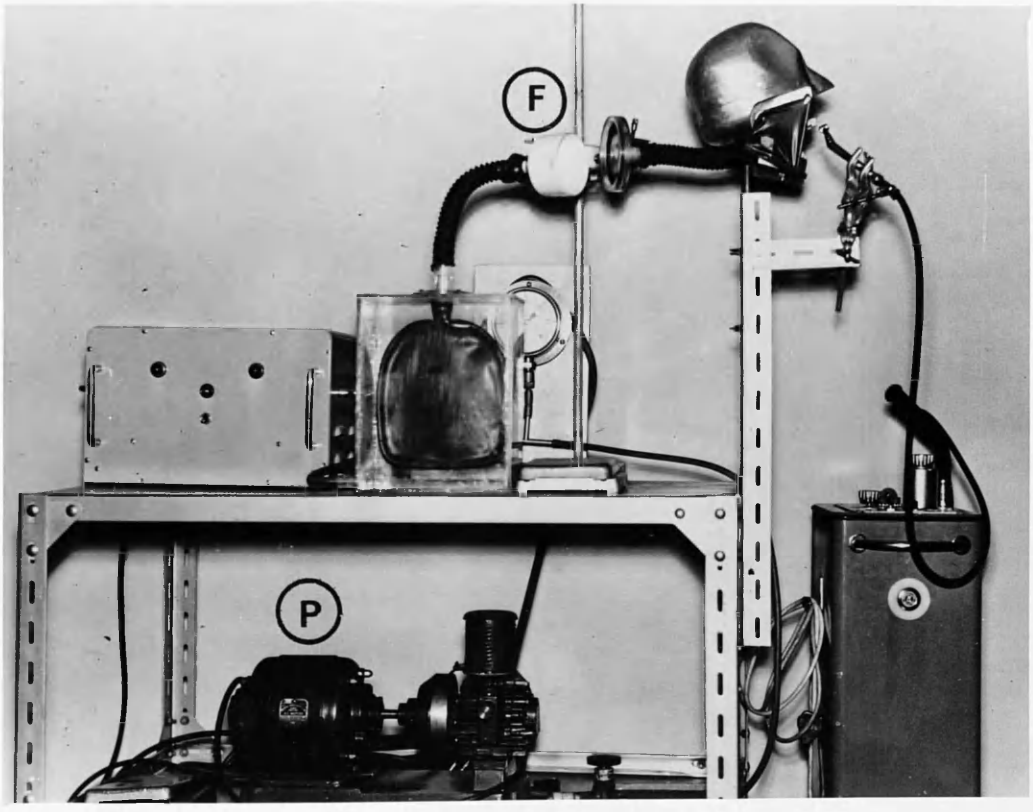


Fig. 1 Artificial lung unit.

Fig. 2 Artificial lung unit showing filter.

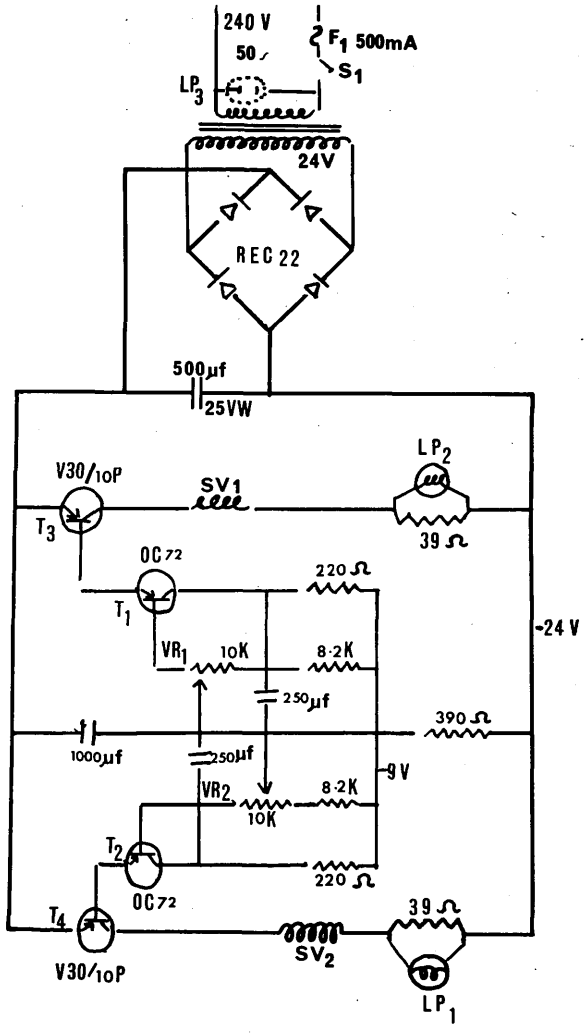


Fig. 3 Circuit for artificial lung unit.

solenoid valves. The pilot lamps (LP_1, LP_2) gave a visual indication of the timing cycle. The power supply was a basic mains-operated bridge-rectifier unit giving an output of 24V, 0.5 A. The respiratory rate was controlled at 16 inhalations per minute. The respiratory volume of the bag measured a maximum of 1.3 litres/minute at standard temperature and pressure. The filter was placed at a distance of 8 inches from the mouth of the mannikin.

A Borden air turbine handpiece on a portable machine was used throughout the experiment. The oil-drip feed was controlled at the recommended drip rate of 26 drops per minute and the water flow adjusted to 2 ml. per minute. On a count taken over the experimental period of 5 minutes running time, the oil drip rate varied from 26 ± 3 drops/minute. The handpiece operational speed was maintained by controlling the air pressure to 42.6 p.s.i.

All experiments were carried out in the same room, which had a cubic capacity of 1,500 cubic feet, and the temperature was maintained at 68°F. The apparatus was fixed in the surgery and each experiment allowed to run for a period of 5 minutes. During the actual experimental period, the respiratory volume of the bag was maintained at 1,000 ml., which is approximately twice the tidal volume. The apparatus was controlled from the outside of the room to avoid inhalation of radioactive material.

In these experiments, the handpiece was fixed in the mouth of the mannikin in a position similar to that used in preparing cavities. In other experiments the handpiece was

placed at varying distances and positions from the mouth of the mannikin to simulate the distance of a handpiece from the face of an operator during cavity preparation. The direction of the aerosol jet from the handpiece was varied. In this way, the mannikin simulated the position of the operator.

The filter was prepared by placing four layers of dental gauze in the filter funnel. It was found that greater reproducibility of results was obtained if the gauze filter was lightly coated in unlabelled oil before placing in the filter. The filter, connecting tube and mannikin were cleaned with ether after each five-minute run.

Similarly prepared gauze filters of 40 $cm.^2$ in area were placed at different levels in four positions in the surgery to measure the atmospheric dispersement and settling of the oil. Four gauze pads were used for each position.

After a continuous running time of five minutes, one pad was removed from each position and examined for labelled oil. This procedure was repeated at five-minute intervals until a total of twenty minutes continuous running time was observed.

The radioactivity of the oil in the gauze was counted by placing the folded gauze in a glass test-tube and counting in a well-type scintillation counter. The count was corrected against the standard for decay and background.

Oil Labelling Technique

A standard commercial oil which consisted of 99.7 per cent olive oil to which had been added citric acid,

propylgallate, butilated hydroxyanisole to prevent oxidation and increase the viscosity was used. This oil, when prepared, was filtered to 10 microns.

The oil was labelled with radioactive iodine (I^{132}) by a technique recommended by Veall and Vetter (1958). I^{132} has a half-life of 2.3 hours and was obtained as a daughter product of Te^{132} which has a half-life of seventy-seven hours. The Te^{132} was washed with 10 ml. N/100 ammonium hydroxide to produce 0.5 mc. I^{132}, to which was added 20 ml. of ether. To this was added 3 ml. KI (1 mg./ml.) solution, 1.5 ml. KIO_3 (2 mg./ml.) solution and 0.5 ml. 2N H_2SO_4. When this mixture was agitated gently, the liberated iodine passed into the ether layer. The coloured aqueous layer was removed with a Pasteur pipette. A chlorine solution in ether was added to the purple iodine solution until it was discoloured. Oil which had been dissolved in ether was added to the iodine monochloride solution so formed and left to stand for one and a half hours. At this stage, about 5 per cent of the I^{132} was attached to the oil. The remaining inorganic iodide was removed by washing twice with a few millilitres of an alkali iodide-thiosulphate solution (5 per cent W/V sodium thiosulphate solution, 5 per cent potassium iodide and 1 per cent sodium hydroxide in water) and the aqueous layer removed, followed by a further washing with water. The washed solution was transferred to an oven and the ether evaporated off at 60°C. The labelled oil was then diluted with 40 ml. of unlabelled oil and placed into the cleaned oil reservoir of the machine. 1 ml. of labelled oil was

retained as a standard.

Particle Size Determination

Oil was sprayed from the handpiece on a clean glass slide. The diameter of 250 particles was measured using a measuring graticule in the eye piece at a magnification of 400. The volume of these particles was calculated on the assumption that the particles remained spherical. A graph of the relationship between the volume and size was prepared.

Results

Inhalation of Oil Particles

The results of the test positions of the handpiece are presented in Table 1. These results show that the amount of oil found on the filter varied from 0.018 ml. to 0.12 ml. per hour. The highest result (0.12 ml.) appears excessive in comparison with the other figures and when the experiment was repeated at the same distance, a figure of 0.034 ml. was obtained. From the results of the settling rate of the particles, it would be expected that the concentration of the oil in the region of the mouth of the mannikin would increase with running time. The smallest concentrations occurred when the handpiece was directed away from the mouth and in all cases where the handpiece was directed towards the mouth, the amount of oil increased. Small differences were observed with variations in the distance of the handpiece from the mouth. The greatest factor in producing differences was the direction of the handpiece -

Table 1

Amounts of Oil Inhaled with Variation in Handpiece Position

Position of Handpiece	Background count	Corrected count rate	Standard count	Inhalation (ml. oil/hr.)
1st Series				
Handpiece facing mouth at 1"	50 50	350 320	107,600 88,400	.038 .039
Handpiece facing directly towards mouth at 1.5"	59	340	92,400	.045
Handpiece 3" from mouth (directed away from mouth)	36	132	87,656	.018
2nd Series				
Handpiece in mouth facing downwards	379	786	192,720	.049
Handpiece facing directly towards mouth at 2"	423	1,100	184,080	.071
Handpiece facing mouth at 6"	450	500	182,320	.033
Handpiece facing mouth at 12"	450	1,750	174,048	.12
Handpiece facing mouth at 12" (second attempt)	450	450	161,600	.034

whether facing towards or away from the mouth.

Particle Size and Setting Rate

The particle was measured and a graph showing the relationship between particle size and volume is shown in Figure 4. This shows that the majority of the particles from the air turbine are 3-8 μ in diameter.

The settling rate of the particles was calculated from Stokes formula:

$$V = -\frac{2}{9}\frac{6 - e}{\eta} g a^2$$

Where V = velocity of fall (cm./sec.)

6 = density of oil (g./cm.^{3})

e = density of air (g./cm.^{3})

η = viscosity of air (poises)

g = acceleration due to gravity (cm./sec.^{2})

a = radius of drop (cm.)

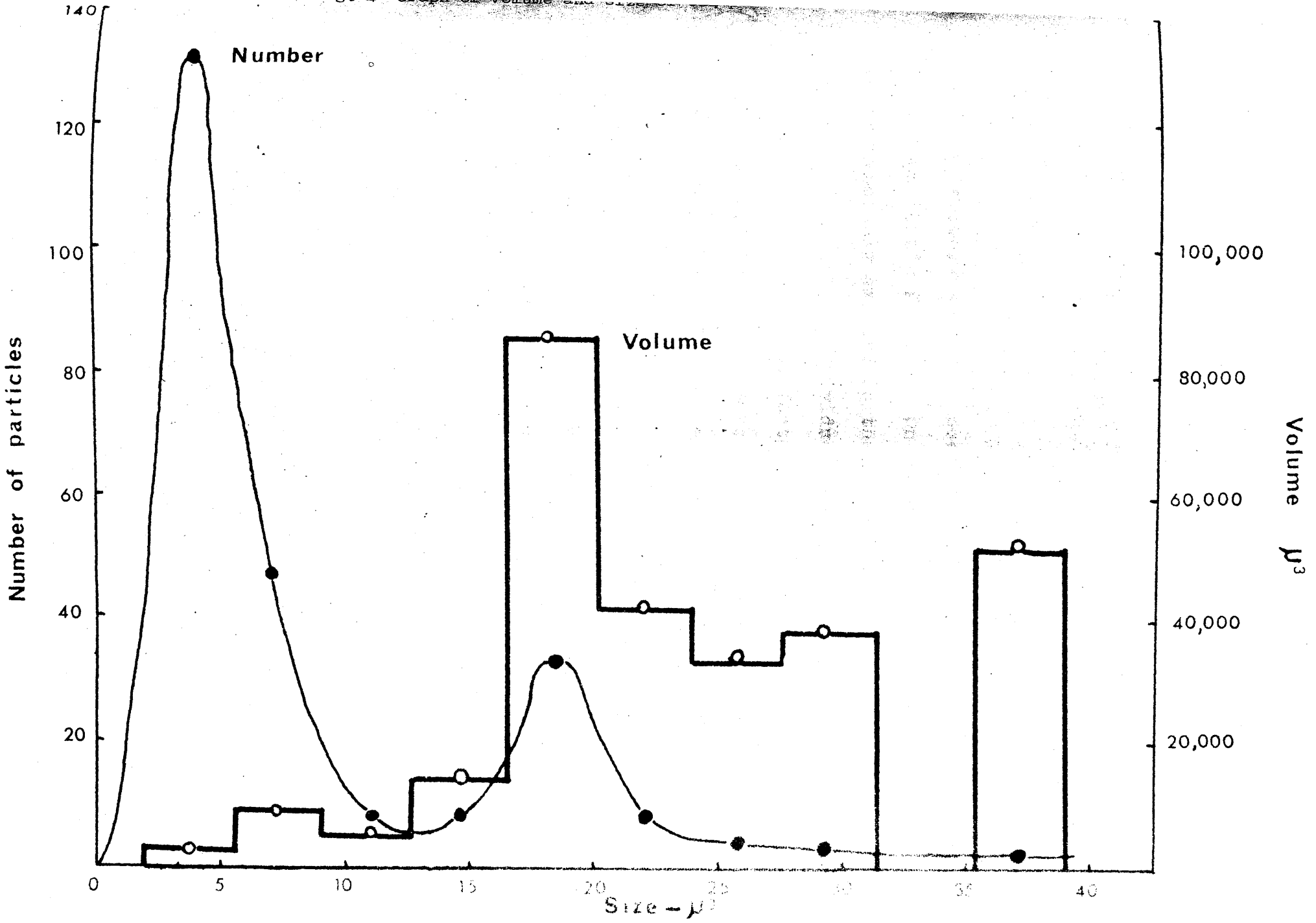
which showed that the settling rate was 0.01 cm./sec. for a drop of 1 μ in diameter. This will lead to a build-up of oil concentration as the running time increases.

Discussion

The results show that inhalation of minute amounts of oil from the air turbine handpiece does occur and that the amounts inhaled vary according to the position of the handpiece and its distance from the face.

The period of time chosen for the running of the handpiece was in excess of that used during any single operation. In any one day, however, the total running time would be in

Fig. 4


Number
Volume
Number of particles
Volume μ^3
Size – μ
140
120
100
80
60
40
20
0
5
10
15
20
25
30
35
40
100,000
80,000
60,000
40,000
20,000

excess of this. Variables, such as temperature and ventilation, were controlled by conducting experiments in a closed room. Physical factors, such as the position of the patient's head in the headrest and the trajectory of the expelled aerosols, will also affect the amount of oil inhaled.

As the time of exposure of the patient to these oils is relatively small, it does not constitute a hazard to the patient's health. On the other hand, the operator is continuously exposed to the cumulative effect of minute amounts of oil. Individual reaction to this exposure will depend on a number of factors, such as total time of exposure, position of working, ventilation of surgery and rate of oil-drip feed. Also, the defence mechanisms of the body have to be taken into consideration as wide individual variations do occur.

To reduce the possibility of inhalation of oils, the minimum oil drip feed should be employed and a face mask worn during prolonged operative procedures.

As a result of the build-up of atmospheric concentration of oil, a well-ventilated surgery is desirable.

Summary.

An artificial lung unit was constructed to allow measurement of oil 'labelled' with radioactive iodine from an air turbine handpiece. Results showed that inhalation of oil does occur and that the amounts inhaled varied with the angle and distance of handpiece. Atmospheric concentration of oils increases with time.

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PAPER 9

FIVE YEAR STUDY TO DETERMINE VARIATIONS IN HEARING DUE TO HIGH-SPEED AIR TURBINE HANDPIECES

G. S. Nixon and E. C. Knox

J. Dent. Res. Abstr. 82, 1966.

FIVE YEAR STUDY TO DETERMINE VARIATIONS IN HEARING DUE TO HIGH-SPEED AIR TURBINE HANDPIECES

It was considered by many dentists that the noise produced by high-speed air turbine handpieces produced alterations in hearing. A preliminary study was carried out in Glasgow to examine the hearing of dentists exposed to this noise. A previous study by Cantwell (1960) had analysed the noise levels of air turbine handpieces and had concluded that, on the basis of an eight-hour day exposure, neither dentists nor patients could expect loss of hearing. It was also stated in this report that temporary hearing threshold shifts may be obtained which could not be considered a hazard to hearing.

A later study by Taylor et al. (1965) in a survey of 40 dentists exposed to dull noise for an average period of 3.7 years, concluded that a significant noise-induced threshold shift was sustained particularly in the 4 kc/s and 6 kc/s frequency range. This report indicated that, with time, agradual encroachment of the upper frequencies of speech range may occur.

In the preliminary study in Glasgow seventeen dental practitioners who had been using air-turbine handpieces up to two years were examined. Seventeen controls of a similar age group were also examined.

To overcome individual variations due to age and previous noise exposure, final year dental students who had no exposure to high frequency sound were selected as subjects. At the time of testing subjects were considered

to be otologically normal as far as could be ascertained from previous medical history without clinical examination. A control group of approximately the same age was similarly tested. This procedure was carried out annually with a new group of final year students. Previous subjects and controls were recalled and hearing re-tested at annual intervals wherever possible. At the time of testing a questionnaire was filled in by each subject giving information such as approximate exposure to the drill and if any alteration or upset in hearing had been observed. Subjects were also asked the type of handpiece used. Groups were tested from 1961 - 1965.

Testing of Subjects and Controls

Subjects and controls were tested in an accoustically treated booth using a pure-tone audiometer calibrated to current British Standard Zero. British Standard Zero is based on the hearing of 600 young airmen between the ages of 18-25 who were considered to be otologically normal.

The frequencies used during testing of subjects were 500, 1000, 2000, 4000, 6000 and 8000 c.p.s. The output was attenuated in 5 db steps.

The method of testing was carried out on an agreed system and throughout the series this was carried out by six testers. Due to equipment and test procedures, variations occurred between the 1961-1965 period. Comparison of the 1961-65 audiograms was made by applying a correction to the 1965 audiogram as follows:

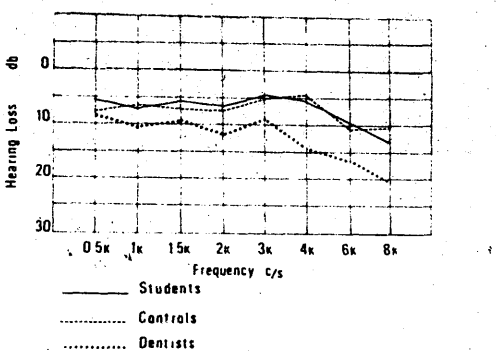

Fig 1. Comparison of hearing loss between dental group using high-speed air-turbine handpieces and control group. Greater hearing loss in dental group in higher frequencies.

Hearing loss of A in 1961 = + 5 db at 8 kc

Hearing loss of A in 1965 = +10 db at 8 kc

In the control group there was an 8 db improvement at 8 kc due to instruments and test procedures etc. during 1961-65.

Apparent deterioration in dentist group 1961-65 = + 5 db

Real deterioration (with correction) = 5 + 8 db

= 13 db.

Results

The results of the study of 17 dentists showed that on average the hearing loss in the dental group was greater than that of the control group (Fig. 1). These results also showed that a wide individual variation occurred in the dental group even when exposure to drill noise had been limited. These wide variations were considered to be the result of a previous history of ear infection or in some cases the result of exposure to noise such as gunfire.

The dental student and control group showed similar hearing losses which were well within the normal limits (Fig. 1).

The total numbers of subject tested from 1961-1965 are shown in Table 1.

The results of a group of 12 dentists examined from 1962-1965 is shown (Table 2) and compared with a control group of 9 (Table 3).

Table 1

Totals Tested

Dental Group	Pre-exposure	After exposure of:			
		1 yr.	2 yrs.	3 yrs.	4 yrs.
1961 students	28	8	4	6	13
1962 "	32	7	6	12	
1963 "	21	3	3		
1964 "	21	7			

Control Group	Total No. tested	
1961 students	30	No. tested after 4 yrs. = 6
1962 "	21	" " " 3 yrs. = 9
1963 "	23	" " " 2 yrs. = 13
1964 "	23	
1965 "	35	

Table 2

Dental Group

Hearing loss	Group of 12 1962-1965	25% who complained	75% with no complaints	Dentists with H.L. R. ear	L. ear	Both ears
No sig. H.L.	50%	33.3%	55.5%	-	-	-
Sig. H.L. at 1 freq.	33.3%	33.3%	33.3%	66.7%	-	-
Sig. H.L. at 2 freq.	8.3%	-	11.2%	-	-	16.6%
Sig. H.L. at 1 kc/s 3 kc/s	8.3%	33.3%	-	-	-	16.6%

Of 12 ears with H.L., ten were in the right ear (83.3%)

Controls - 2 subjects correct for presbycusis.

Table 3

Control Group

Hearing Loss	Group of 9 1962-1965
No sig. H.L.	77.8%
Sig. H.L. at 1 freq.	11.1%
Sig. H.L. at 2 freq.	11.1% (subject noise exposed)

Discussion

It would appear from this study that the dental group showed a more significant deterioration of hearing than the control group and that greater individual susceptibility was observed. If a threshold shift of 15 db is not considered significant the greatest hearing losses occurred at the 4, 6 and 8 kc. level. Within the dental group a greater deterioration of hearing was observed in the right than with the left.

No attempt was made in this study to correlate hearing loss with noise levels. The importance of any hearing loss in a young person is that this loss will be added to hearing loss due to age. While a hearing loss in a younger person may not appear to be a social handicap at this stage it may become so when the hearing loss due to age is added in later years.

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P A P E R 10

ESTIMATION OF MANGANESE IN HUMAN ENAMEL BY ACTIVATION ANALYSIS

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Arch. oral Biol., Vol. 11, p.247-252, 1966

ESTIMATION OF MANGANESE IN HUMAN ENAMEL BY ACTIVATION ANALYSIS

Summary

A method for the estimation of manganese in biological tissues by means of neutron activation analysis is described. Small enamel samples, weighing from 5 mg. to 25 mg. are taken from the outer and inner enamel layers of human teeth and irradiated in a reactor for two hours at a thermal flux of 10^{12} n/cm.^{2}/sec. A standard manganese sample is irradiated at the same time. The chemical recovery of the manganese is adjusted for phosphate adsorption. The results show that manganese is present in all enamel samples and varies from 0.30 - 2.01 p.p.m. There is a greater concentration of manganese in the outer enamel layer than in the inner enamel layer.

Introduction

Manganese is always present in body tissues. A 70 kg. man may have a total of 12.20 mg. Mn (Cotzias, 1958). Higher concentrations are found in liver and pancreas than in tissues, such as skeletal muscle. Little is known of the forms in which manganese exists in the body tissues.

The daily intake of manganese may vary from 2.0 - 8.0 mg., depending on the diet. Some foods, such as tea, are exceptionally rich in manganese (150-900 p.p.m.). The role of manganese in calcification is not fully understood. It has been suggested that manganese is necessary for certain enzyme activities probably required in

calcification (Hess, 1960).

The concentration of manganese in teeth has been estimated by chemical and spectrographic methods. Samsahl and Soremark (1961) have given a mean value of 0.54 p.p.m. for enamel using gamma-ray spectrometry.

Materials and Method

Enamel samples were obtained from thirteen sound extracted permanent human teeth which were considered to be free from enamel defects. The teeth were washed with de-ionised water and transversely sectioned into an occlusal, a middle and a gingival layer. The enamel thickness of the middle and gingival layers was measured microscopically and each layer then divided into an inner and outer enamel layer by means of a carborundum cutting disc with a micrometer gauge. A fresh cutting disc was used for each section to avoid contamination. Particular care was taken when cutting the inner enamel layer to ensure that no dentine was included in this section. No attempt was made to separate the occlusal enamel into inner and outer layers.

Experimental

Outline of Method

The enamel samples were digested in concentrated nitric acid, following irradiation, and the radioactive manganese separated by precipitation of MnO_2 by sodium chlorate oxidation. The activity of the sample was measured using a -spectrometer and compared with a manganese standard processed in the same way as the samples.

Preparation and Irradiation of Samples

The enamel samples (5 - 25 mgs.) were weighed into 1" square polyethylene envelopes, which were then heat-sealed. For preparation of a standard, a piece of polyethylene sheet (1" square) was thoroughly cleaned by rinsing in concentrated nitric acid, followed by two rinses in distilled water. A known weight of manganese (about 0.1 microgrammes) was evaporated from solution onto this sheet under an infra-red lamp at a sufficiently low temperature to avoid damaging the polyethylene. The standard was also enclosed in a polyethylene envelope, packed with the enamel samples in a polyethylene tube and irradiated for two hours at a thermal neutron flux of 10^{12} n/cm.^{2}/sec. in the Scottish Universities Research Reactor at East Kilbride, Glasgow.

Manganese 56

100% of naturally occurring manganese is in the form ^{55}Mn. On thermal neutron irradiation, the isotope ^{56}Mn ($t_{\frac{1}{2}}$ - 2.58 hrs.) is produced by the nuclear reaction $^{55}Mn\ (n, \gamma)^{56}Mn$. The activation cross-section is 13.3 barns.

Chemical Separation

After irradiation, samples and standard were removed from their containers and transferred to 125 ml. conical beakers containing 10 ml. of 16M. nitric acid and 1 ml. of manganese carrier solution ($MnCl_2$ - 10 mg. Mn/ml.). The beakers were then heated on a hotplate until the enamel was completely dissolved. In the case of the standard, the

polyethylene is not destroyed, but complete exchange between the radioactive and carrier manganese quickly takes place in the hot solution. The polyethylene is removed in subsequent transfer steps. One ml. of 50% (w/v) sodium chlorate solution was then added to the hot solution to precipitate the manganese as MnO_2. The solutions and suspended precipitates were transferred to 50 ml. centrifuge tubes, centrifuged and the supernatant discarded. The precipitates were thoroughly washed with water and slurried with water onto weighed planchettes. They were evaporated to dryness at 100°C under infra-red lamps and re-weighed as $MnO_2.H_2O$ (Sidgwick, 1952). The recoveries obtained are in the region of 85%.

Counting

Using a 3 in. NaI(Tl) crystal and a Laben 512 channel pulse height analyser, the γ-spectrum obtained (Fig. 1) showed the 0.845 MeV photopeak of Mn^{56} free from contamination. The degree of radiochemical purity, as determined by γ-spectra and half-life considerations, is suitable for the accurate determination of manganese.

Calculation of Results

The relative activities of samples and standard were obtained by calculating the area of the photopeak by the digital method of Covell (1959). This is a method of peak area estimation which involves summing the counts in an equal number of channels above and below the photopeak maximum and subtracting an amount representing the peak base from the total sum obtained. These areas were

corrected for decay and chemical recovery and the manganese content of the samples obtained by comparison of sample and standard activities.

Chemical Recovery

When tooth samples are subjected to the chemical separation, high recoveries are obtained. This is thought to be phosphate from the enamel being co-precipitated with the MnO_2.

It is shown in Table 1 that for samples weighing up to 40 mg., the amount of adsorbed phosphate is directly proportional to the sample weight. Above this weight, the amount of phosphate adsorbed reaches a constant value.

For samples weighing less than 40 mg., an average adsorption value of 0.092 mg. phosphate per mg. tooth sample was calculated.

The chemical recoveries obtained during an analysis were adjusted for phosphate adsorption using the above factor before the yield comparison was made. This correction is, of course, only valid for fairly constant recoveries of MnO_2. Table 2 shows the magnitude of error between the experimentally determined adjustment and the use of the above factor. It can be seen that the error introduced by using the average figure is less than the overall precision of the analysis. The latter is estimated to be better than 5% from repeat analyses on powdered tooth samples (Table 3).

Comparison of results obtained, for tooth and biological material, using this method and the full separation

Table 1

Phosphate Adsorption

Sample Size (mg.)	Phosphate Adsorbed (mg.)
10.0	1.3
12.9	1.4
19.5	2.3
29.1	2.8
26.6	3.5
38.1	3.7
56.5	4.4
63.9	4.7
73.2	4.7

Table 2

Difference in tooth manganese content as influenced by recovery correction

Adsorption Correction Method	Tooth Manganese Content (p.p.m.)					
Average figure	1.32	0.83	0.66	0.55	1.32	0.69
Measured figure	1.34	0.84	0.65	0.55	1.29	0.67
Percentage error	1.5%	1.2%	1.5%	0.0%	2.3%	3.0%

Table 3

Repeat Analyses

Analysis	Sample 1	Sample 2	Sample 3
1	0.83	0.54	3.44
2	0.89	0.42	2.31
3	-	-	2.17
4	-	-	1.74

technique of Smith (1962) showed agreement within the quoted experimental error.

Results

The results of the analyses of successive layers of enamel samples are shown in Table 4. The manganese content of the occlusal enamel ranged from 0.45 - 1.98 p.p.m. In the middle layer, the outer enamel was 0.66 - 2.01 p.p.m., while the inner enamel was 0.34 - 0.79 p.p.m. In the gingival layer, the outer enamel was 0.35 - 1.34 p.p.m., and the inner enamel 0.30 - 0.76 p.p.m.

An interesting result is seen in samples No. 4A and 4B and samples No. 10A and 10B. Each paired sample represented premolars extracted from opposite quadrants in the same mouth. The times of eruption of these teeth were similar and there is a close relationship between the manganese content of the enamel.

Repeat Analyses

In some samples, more than one analysis was performed on sections from the same layer of teeth (Table 3). In the first two samples, the results seem close enough to be within experimental error, but in sample No. 3 , the differences are too great to be experimental errors and are probably genuine variations in the manganese content of each section of enamel.

Table 4

Manganese Content of Enamel (p.p.m.)

Sample Number	Tooth	Age	Sex	Occlusal Layer	Middle Layer		Gingival Layer	
					Outer Enamel	Inner Enamel	Outer Enamel	Inner Enamel
1	$\underline{/4}$	12	M	0.85	0.66	0.55	1.32	0.69
2	$\overline{57}$	53	F	0.85	0.86	0.56	0.69	0.45
3	$/\overline{8}$	25	F	1.25	2.01	0.60	1.29	0.60
4A	$/4$	11	F	0.72	0.97	0.66	1.38	0.49
4B	$\overline{47}$	11	F	0.74	1.12	0.51	1.22	0.54
5	$/\overline{8}$	27	M	1.24	1.29	0.46	0.79	0.76
6	$\overline{87}$	26	M	1.74	0.76	0.58	0.92	0.61
7	$\overline{87}$	19	M	1.07	0.80	0.64	0.35	0.39
8	$\overline{37}$	12	M	1.10	0.76	0.71	Insufficient material	
9	$\underline{/3}$	31	M	1.61	1.34	0.79	0.90	0.56
10A	$\underline{/5}$	11	F	0.73	1.10	0.55	0.48	0.49
10B	$/\overline{5}$	11	F	0.65	0.91	0.43	0.71	0.30
11	$/\overline{8}$	18	F	0.45	0.90	0.34	1.43	0.42

Table 5

Repeat Analysis of Powdered Enamel Samples

Mn (p.p.m.)
4.10
4.06
4.11
4.26
4.09

Note: These results are higher than normal due to contamination of the enamel during the grinding process in the steel mortar. The teeth used for this powdered sample gave normal values on previous analysis when enamel sections were used.

Discussion

The present study would indicate that manganese is a constituent of enamel. It appears to be greater in concentration in the outer enamel layers than in the inner enamel layers. Manganese is deposited in enamel during calcification, which would account for the uniformity of concentration in the inner enamel layer. When calcification is complete, there may be a post-eruptive gain in manganese from food and other external sources.

ACKNOWLEDGMENTS

The authors wish to thank Professor G. Forbes and Dr. J.M.A. Lenihan for their encouragement in this work and also the Scottish Universities Research Reactor Centre, East Kilbride, for laboratory and irradiation facilities.

We are grateful to the Scottish Hospitals Endowment Research Trust for financial support in this study.

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P A P E R 11

ESTIMATION OF ANTIMONY IN HUMAN ENAMEL BY ACTIVATION ANALYSIS

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Caries Res. 1 : 327-332, 1967

ESTIMATION OF ANTIMONY IN HUMAN ENAMEL BY ACTIVATION ANALYSIS

Introduction

Antimony is generally thought to be a non-essential element for humans and is one of the elements of high toxicity. Little is known of its distribution and metabolism in human tissues. The levels at which it occurs in dry human tissues from post-morten samples have been determined for a number of organs (Molokhia, 1966). The average values found are given in Table 1. The results are from determinations on 25 samples of each organ.

Table 1

Antimony Content of Dry Human Tissue (Molokhia, 1966)

Organ	Mean antimony content (p.p.m.)
Lung	0.56
Liver	0.14
Kidney	0.17
Brain	0.11
Heart	0.09
Stomach	0.08
Spleen	0.07
Bone	0.30

Antimonial drugs are widely used in tropical countries in treatment of bilharziasis. It has been shown that pentavalent antimonials are found in higher concentrations in the plasma than in the erythrocytes (Lippincott, 1947). Trivalent antimonials can remain in the circulation bound to

erythrocytes while the antimony in the plasma falls rapidly by excretion via the kidney (Brady et al., 1945).

Experimental

Preparation and irradiation of samples

Samples of sound human permanent teeth free from enamel defects and not in contact with teeth containing restorations were obtained from dental patients in Glasgow, Scotland (10 teeth) and Alexandria, Egypt (19 teeth). Teeth were extracted using forceps the beaks of which were covered in polythene to avoid metallic contamination of enamel. The teeth were rinsed with distilled water and enamel sections prepared by sectioning with a carborundum cutting disc with a micrometer gauge taking care to avoid sample contamination. No dentine was included in the samples. The British samples were transversely sectioned into an occlusal, a middle and a gingival layer and the last two layers were sectioned into an inner and an outer enamel section. The enamel from the Egyptian patients was not divided into outer and inner layers.

The samples were weighed and irradiated in aluminium foil in BEPO, Harwell, at a thermal neutron flux of 10^{12} n/cm^2/sec. for three days. A standard of high purity antimony potassium tartrate (about 0.5 mg.) was irradiated in a silica tube at the same time as the samples. After irradiation the standard was dissolved in hydrochloric acid and diluted as required.

Chemical Separation

After irradiation, the samples and an aliquot of the

standard solution were digested in a sulphuric/nitric acid mixture containing 10 mg. of antimony carrier.

Following digestion a full radiochemical separation was used to produce the radioactive antimony in a pure form for counting. Full experimental details of the method are given by Howie 1965. An outline of the separation is given below.

The samples were digested in sulphuric/nitric acids. The clear solution was diluted with water and antimony metal was precipitated by addition of chromous chloride solution. The separated precipitate was washed and dissolved in hydrochloric acid containing hydrogen peroxide and arsenic carrier (12 mg.).

Arsenic metal was precipitated in the hot solution by addition of ammonium hypophosphite solution. Copper carrier (10 mg.) was added to the solution after cooling and precipitated as the ferrocyanide. Excess ferrocyanide was precipitated by addition of cobaltous chloride solution. The combined precipitates served as a general scavenging step. The precipitates were centrifuged and the supernates filtered. After filtration the antimony was precipitated as the initial precipitation by addition of chromous chloride solution. After washing with water the precipitates were transferred to aluminium planchettes with acetone and dried at 95°C. The activity of the precipitates was measured using an end-window Geiger counter.

As a check on precision and accuracy five samples of a homogenous biological standard (powdered kale prepared by H.J.M. Bowen of Reading University) were analysed. The

results of the analysis of 100 mg. samples, 0.054, 0.060, 0.056, 0.062, 0.065 p.p.m. compare well with determinations of other workers which give a value of 0.082 ± 0.02 p.p.m. (Bowen, 1966).

The chemical recovery of antimony was obtained by weighing the planchettes before and after transferring the final precipitate. The antimony content of each sample was calculated by comparing the count-rates of samples and standard after correction for counter dead time, chemical recovery and background radiation. The sensitivity of the method was 10^{-10} gm.

Results

The antimony content of the enamel from the teeth of Glasgow patients is shown in Table 2.

Comparison of Antimony and Arsenic Levels

As arsenic and antimony are chemically similar, the range of results has been compared with previous arsenic results (Nixon, 1960) (Fig. 1).

The results for the antimony content of the enamel from Egyptian patients are shown in Table 3. The results are grouped according to whether or not there was a history of antimony treatment for bilharzia.

Table 2

Antimony content of enamel from Glasgow patients
(p.p.m. antimony)

Tooth	Age	Sex	Occlusal	Middle		Gingival	
				Outer	Inner	Outer	Inner
$\overline{8}$/	25	F	-	0.665	0.037	0.016	0.044
5/	27	M	-	0.026	0.379	0.075	-
$\overline{8}$/	27	F	-	0.044	0.021	0.065	0.016
$\overline{8}$/	17	F	-	0.037	0.058	-	0.015
$\overline{8}$/	26	M	-	0.011	0.005	0.010	0.020
5/	61	F	-	0.019	0.048	0.074	0.243
$\overline{8}$/	33	F	0.005	0.019	0.054	0.091	0.099
$\overline{8}$/	19	F	0.139	0.464	0.030	0.282	0.121
$\overline{8}$/	33	F	0.010	0.054	0.034	0.071	0.043
$\overline{5}$/	14	F	0.011	0.019	0.095	0.028	0.060
Mean			0.041	0.136	0.078	0.079	0.073
Median			-	0.031	0.042	0.079	0.044

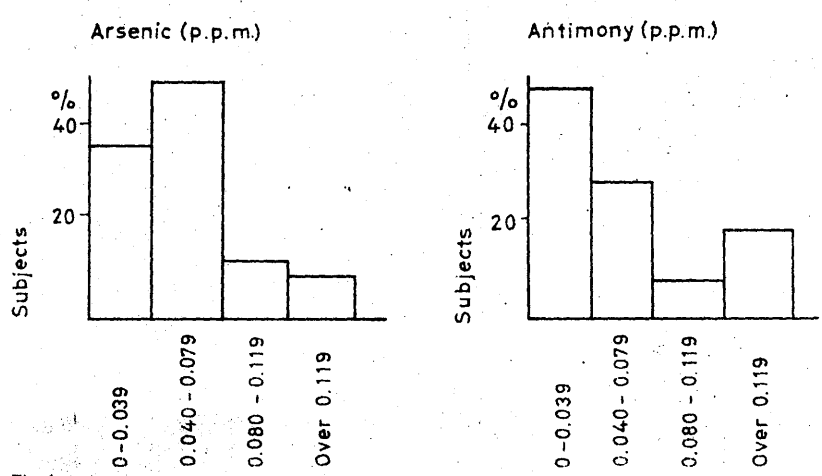
Arsenic (p.p.m.)
%
40
20
Subjects
0–0.039
0.040–0.079
0.080–0.119
Over 0.119
Antimony (p.p.m.)
%
40
20
Subjects
0–0.039
0.040–0.079
0.080–0.119
Over 0.119

Fig. 1.

Table 3

Antimony content of Enamel from Egyptian Patients
(antimony p.p.m.)

Untreated patients (no bilharzia)				Treated patients (antimony treatment for bilharzia)			
Age	Sex	Tooth	Sb level	Age	Sex	Tooth	Sb level
19	F	/5	0.055	41	M	$\overline{5}$/	0.080
15	F	6/	0.027	30	F	$\overline{6}$/	0.091
15	F	$\overline{6}$/	0.012	15	M	/5	0.194
23	F	/6	0.018	50	M	$\overline{7}$/	0.058
23	F	/$\overline{6}$	0.096	30	-	$\overline{6}$/	0.189
28	F	8/	0.023	22	M	$\overline{6}$/	0.043
20	F	/$\overline{6}$	0.039	27	M	$\overline{7}$/	0.034
22	M	/$\overline{6}$	0.022	40	F	$\overline{6}$/	0.025
50	F	$\overline{6}$/	0.027	25	M	/$\overline{8}$	0.024
45	F	6/	0.012				
Mean antimony content			0.034	Mean antimony content			0.070
Median antimony content			0.026	Median antimony content			0.058

Discussion

Antimony appears to be present in teeth, as in other body tissues, as a non-essential trace element whose presence depends on the absorption of antimony into the body from the diet or the atmosphere. No difference was found between the antimony content of outer and inner enamel layers. There would appear to be slightly more antimony present in teeth from people who have a history of antimony

injections. This difference is not very significant and further consideration must be given to factors such as the age of the subject when drugs were administered together with dosage and duration of administration. The level of antimony in enamel from untreated Egyptian subjects is slightly lower than that of enamel from Scottish subjects. This may be attributable to the higher degree of industrial contamination in Glasgow relative to Alexandria.

Summary

The antimony content of the outer and inner layers of enamel from samples of human teeth was determined by a method of neutron activation analysis. The samples, weighing from 20-25 mg., were irradiated in a reactor for three days at a thermal neutron flux of 10^{12} n/cm^2/sec. The antimony activity was determined after a radio-chemical separation by means of a Geiger counter. The samples analysed were from the teeth of Scottish patients and also from Egyptian patients some of whom had received antimony injections in treatment of bilharziasis. No difference was found in the antimony content of the outer and inner enamel layers. The mean level of antimony found was 0.04 p.p.m. and the range 0.005 - 0.67 p.p.m.

Acknowledgments

The authors wish to thank Professor G. Forbes and Dr. J.M.A. Lenihan for their encouragement in this work. We are grateful to the Scottish Hospitals Endowment Research Trust for financial support in this study.

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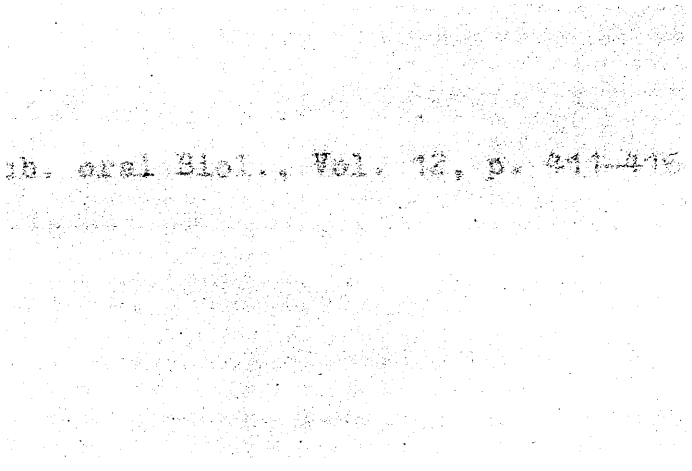


P A P E R 12

ESTIMATION OF ZINC IN HUMAN ENAMEL BY ACTIVATION ANALYSIS

G. S. Nixon, H. D. Livingston and H. Smith

Arch. oral Biol., Vol. 12, p. 411-416, 1967

ESTIMATION OF ZINC IN HUMAN ENAMEL BY ACTIVATION ANALYSIS

Summary

Chemical and physical methods are described for estimating zinc in biological tissues by means of neutron activation analysis. Small enamel samples weighing approximately 20 mg. were taken from the outer and inner layers of permanent human teeth and irradiated in a reactor thermal neutron flux of 10^{12} n/cm^2/sec. A standard zinc sample was irradiated for a similar period. The results showed that zinc was present in all enamel samples and ranged from 58-1550 p.p.m. Greater concentrations of zinc were found in the outer enamel layer than in the inner. Satisfactory agreement of results was found between the chemical and instrumental methods.

Estimation of Zinc in Human Enamel by Activation Analysis

Zinc is a constant trace element found in all human tissues and fluids. Values of 1.4 to 2.39 g. have been estimated for the whole body in a 70 kg. man (Widdowson, McCance and Spray, 1951). It appears to be an essential trace element and is a constituent of the enzyme carbonic anhydrase. The zinc content in human tissue ranges from 12 p.p.m. in the adrenal glands to higher concentrations in the skeleton. In blood, zinc is a constant constituent of plasma or serum and of erythrocytes and leucocytes. The zinc content of the skeleton has been given by Taylor (1961) as relatively high at 150-200 p.p.m. He states

that zinc is taken up and retained by the skeleton. Previous studies on the zinc content of teeth have been carried out by Cruickshank (1936). Using chemical analysis he found concentrations of zinc in enamel to be within the range 211-260 p.p.m. for permanent teeth. In dentine the concentration was 130 p.p.m. His method of separation of enamel and dentine was, however, empirical. In a later paper Cruickshank (1940) stated that a 17.1% increase of zinc was found in enamel of patients suffering from tuberculosis.

Estimations of zinc were carried out by Brudevold et al. (1963) by spectrographic and spectro-photometric methods using pooled samples of teeth. These investigators found that the concentration of zinc was much higher on the outer surface of enamel than on the sub-structure and that concentrations ranged from 430-2100 p.p.m. The concentration of the deeper layers of enamel was similar to those previously shown by Cruickshank (1936).

Soremark and Samsahl (1961) found a mean value of 276 p.p.m. for enamel using gamma-ray spectrometry but no separation of enamel into layers was attempted. They stated that zinc levels in enamel were higher than in dentine.

Isotopes

On neutron irradiation, the following isotopes of zinc are produced:-

Zn^{65}, Zn^{69}, Zn^{69m}, Zn^{71}, Zn^{71m}. In this work Zn^{69} ($t_{\frac{1}{2}}$ -

57 mins.) and Zn^{65} ($t_{\frac{1}{2}}$ - 245 days) were used. It is also possible to use Zn^{69m} ($t_{\frac{1}{2}}$ - 13.8 hours) but with decreased sensitivity. Zn^{69}, and Zn^{69m} may be detected using a Geiger counter. A scintillation counter is required for Zn^{65}.

Sample preparation

The enamel samples are prepared from sound, extracted human teeth, free from enamel defects. The enamel is separated into an inner and an outer layer using a carborundum cutting disc with a micrometer gauge. The size of sample used is about 20 mg.

Analytical Method

Two methods of analysis are used in this study.

(a) Chemical Separation

The samples and a zinc standard are irradiated for 1 hour in a reactor with a thermal neutron flux of 10^{12} $n/cm^2/sec$. This irradiation produces mainly Zn^{69}. Following irradiation, the samples are digested in 16M nitric acid along with 10 mg. of both copper and zinc carrier. The zinc standard (about 1 microgram of zinc) is similarly treated. When digestion is complete, the solutions are evaporated to dryness. 1 ml. of 11M hydrochloric acid is added and the sample taken to dryness again. The residue is then dissolved in about 10 ml. of 2M hydrochloric acid and transferred to a strongly basic anion exchange resin in the chloride form. After transfer,

the column is washed with 25 ml. of 0.12M hydrochloric acid containing sodium chloride (10% w/v) 25 ml. of 2M sodium hydroxide solution containing sodium chloride (2% w/v) are then passed through the column to elute the zinc.

The zinc fraction is made slightly acid with 17.5M acetic acid and heated in a water bath. 3 ml. of quinaldic acid solution (2% w/v) is added to precipitate the zinc as the quinaldate. The precipitate is washed with water and acetone, transferred to an aluminium planchette and dried at 100°C. The activity is measured with an end window Geiger counter. Following correction for background radiation, decay and chemical recovery, the zinc content is calculated by comparison with the zinc standard.

(b) Instrumental

For this analysis the enamel samples and zinc standard are irradiated for one week in a reactor with a thermal neutron flux of 10^{12} n/cm^2/sec. (BEPO, Harwell). They are then put aside for about three months so that the short-lived activities decay. After this decay period gamma spectrometric analysis shows that the only photopeak remaining in the sample is from Zn^{65} ($t_{\frac{1}{2}}$ - 245 days). Using a pulse height analyser and a sodium iodine (Tl) crystal, the activity in samples and standards are measured over the Zn^{65} photopeak (1.12 MeV). The zinc content of the samples is calculated by comparison of the measured activity with the standard following a correction for background radiation.

Results

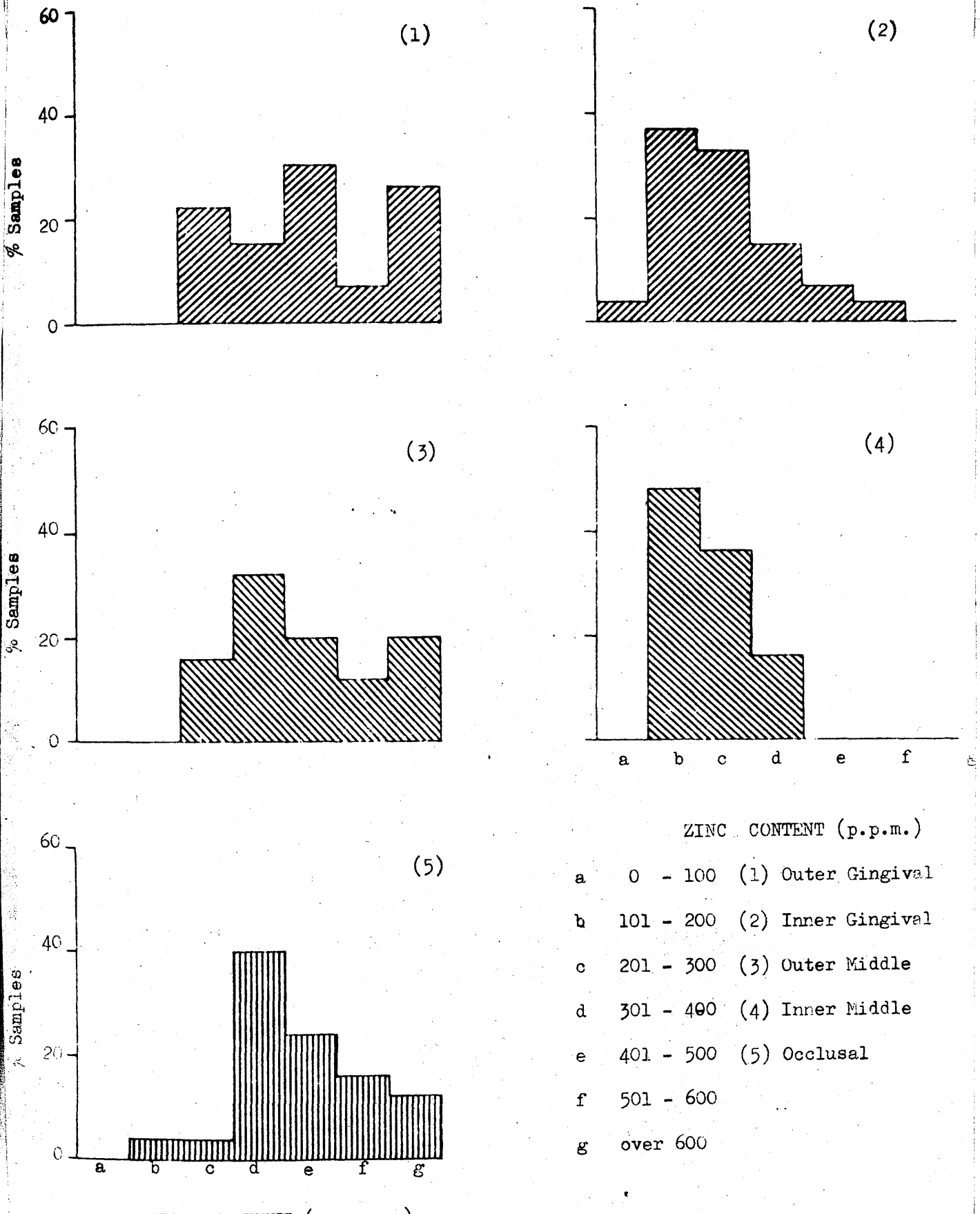
The results obtained by the two methods of analysis are shown in Tables 1 and 2, and Figure I. The agreement between the two methods is shown to be satisfactory by comparison of the mean values and the ranges of zinc content found in the different layers examined. In general, the spread of values is slightly greater for samples analysed by the instrumental method but this is not unexpected since more samples were analysed by this method.

It can be seen from the results that an occasional unusually high zinc value is found in some samples using either method of analysis. It is observed that many of these high values correspond with rather small enamel specimens. The high value may be obtained by analysing a section of surface enamel relatively richer in zinc than the more deeply cut sections. To examine this suggestion, very thin vertical sections of enamel from different levels in the enamel are analysed and the results shown in Table 3.

The pattern of these results shows a definite fall in the enamel zinc level from the outer surface inward. They do not show the very high levels in the outermost layers which may be expected. It should be noted, however, that the zinc levels of this tooth are lower than in some other teeth examined.

The elevated values in the outer sections of enamel require some other explanation such as uptake of zinc from either amalgam fillings or zinc oxide cements. Enamel sections from teeth adjacent to teeth containing amalgam

FIGURE I (ENAMEL ZINC CONTENT)



ZINC CONTENT (p.p.m.)

a	0 - 100	(1) Outer Gingival
b	101 - 200	(2) Inner Gingival
c	201 - 300	(3) Outer Middle
d	301 - 400	(4) Inner Middle
e	401 - 500	(5) Occlusal
f	501 - 600	
g	over 600	

Table 1

Enamel zinc content (ppm) - results from analysis without chemical separation

Sample Description				Layer Description				
	Sex	Age yrs.	Tooth	Occlusal	Middle		Gingival	
					Outer	Inner	Outer	Inner
1	M	24	/8	773	1070	303	1550	352
2	F	24	/8	475	362	387	925	425
3	M	22	8/	406	681	267	775	584
4	M	32	8/	359	725	344	626	440
5	M	27	/8	384	774	214	238	247
6	M	18	/8	546	213	251	421	251
7	F	31	8/	530	711	196	244	155
8	M	21	/8	762	245	163	392	58
9	F	25	/8	551	496	197	564	201
10	F	27	8/	476	301	226	217	267
11	F	21	8/	820	387	149	866	151
12	F	21	/8	177	358	167	294	159
13	F	24	/8	385	309	309	471	216
14	F	29	8/	407	339	180	332	210
15	M	23	8/	352	429	206	441	246
16	M	32	3/	322	263	208	493	145
17	M	32	/3	310	441	192	432	192
18	M	17	8/	511	492	164	455	206
19	M	17	8/	364	291	207	273	182
20	F	22	/8	344	463	183	387	234
		Mean		463	468	226	519	246
		Ranges		177-820	213-1070	149-387	217-1550	58-584

Table 2

Enamel zinc content (ppm).
Results from analysis with chemical separation

Sample Description				Layer Description				
	Sex	Age yrs.	Tooth	Occlusal	Middle		Gingival	
					Outer	Inner	Outer	Inner
A	F	25	$\overline{8}$	327	504	165	655	187
B	F	29	$\overline{8}$	437	566	125	328	192
C	F	18	$\overline{8}$	371	302	248	580	366
D	F	29	$\overline{8}$	419	-	-	479	322
E	F	27	$\overline{8}$	271	520	182	289	110
F	M	15	$\overline{8}$	362	-	-	471	131
G	F	27	$\overline{8}$	-	336	231	992	379
		Mean		365	446	190	542	241
		Ranges		271-437	302-566	125-248	289-992	110-379

Table 3

Thin section analysis from a single tooth

Layer Description	Thickness (mm.)	Weight (mg.)	Zinc content (ppm)
Outer	0.060	0.72	202
Outer	0.060	0.70	219
Outer	0.052	0.66	242
Middle	0.075	1.16	131
Inner	0.060	0.78	113
Inner	0.082	1.02	125
Inner	0.067	0.97	59

fillings were examined. No significantly high values were found in these teeth to support this hypothesis.

Discussion

The results of the present study show that the concentrations of zinc are greater in the outermost layers of enamel than in the inner layers. It would appear that a certain amount of zinc is incorporated in the developing enamel and that there may be an increased deposition after eruption. Brudevold et al. (1963) have shown that hydroxyapatite rapidly acquires zinc. Migration of zinc ions may take place from amalgam fillings similar to those of mercury (Nixon and Smith, 1965) but this has not been supported by our findings. Zinc forms complexes readily with plasma proteins and Rubini (1961) has shown with isotope studies that intravenously administered Zn^{65} concentrates in the liver, pancreas and spleen and this may be transferred to the skeleton where it is firmly fixed.

Acknowledgments

The authors wish to thank Professor G. Forbes and Dr. J.M.A. Lenihan for their support and encouragement in this work and also the Scottish Research Reactor Centre for laboratory and short irradiation facilities.

We are grateful to the Scottish Hospitals Endowment Research Trust for financial support in this study.

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P A P E R 13

TRACE ELEMENTS IN HUMAN TOOTH ENAMEL

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Nuclear Activation Techniques in the Life Sciences
Proceedings of a Symposium, Amsterdam, May, 1967
I.A.E.A., 455-462.

TRACE ELEMENTS IN HUMAN TOOTH ENAMEL

The importance of the trace elements in the prevention and reduction of dental caries has not yet been fully established. There are many indications that trace elements do play an important part in caries-resistance. The most notable trace element is of course fluorine. Molybdenum, vanadium, manganese and boron are said to possess anti-cariogenic properties.

While it is considered that the composition of the tooth is not the only factor in the carious process it may play a more significant part than has hitherto been believed. An obvious approach to the problem is to determine variations in the composition of caries-free and caries-prone teeth. This approach is not as straight forward as it would appear. Difficulties arise such as in deciding which teeth are caries-free and which are caries-prone. Despite these problems the composition of the teeth is one of the important factors under investigation at present. Analyses of successive layers of enamel have shown that the chemistry of the surface enamel differs from that of the underlying layers. Special attention is given to the surface enamel since it is the site of the initial attack by caries and also as it appears more resistant to the carious process than the deeper layers of enamel. The trace elements which are of greatest interest in considering the mechanism of dental caries are those which increase the resistance of the enamel surface.

Many methods have been employed to determine the level

of over twenty elements in teeth. In general, there is good agreement between the results obtained by activation analysis and other methods. One of the difficulties in previous methods of analysis has been in obtaining sufficient quality of sample. As the distribution of many elements varies throughout teeth and even within the enamel of an individual tooth, results may vary according to the method by which the samples are taken. Where pooled total enamel samples from different teeth have been taken an average concentration of the elements will be obtained. Where pooled samples of enamel layers have been used average variations between layers will be obtained.

The general advantages of activation analysis techniques in relation to this study may be considered as follows:

(a) Specificity and certainty of identity.

The identity of the element detected can be confirmed by decay or γ-spectra studies. This provides a check against the interference by another element in the measurement. Once the technique has been established there is seldom any difficulty with interferences.

(b) Freedom from reagent contamination.

(c) The avoidance of micro-separations.

(d) The analysis of up to fifty samples can be carried out in a single day (apart from sample preparation and irradiation).

(e) High sensitivity.

Results

The results are summarised in Table 1 and in Figure 1. The methods employed for analysis of these elements are shown in Table 2. The results show that non-essential trace elements (Sb, Hg, As) have a skew distribution and essential elements (Cu, Mn, Zn), a normal distribution. It should be noted that for convenience the figures all have a logarithmic base and as such should all have a normal distribution. However, those with narrow distribution ranges give normal distributions on a linear base while those with wide ranges give skew distributions on a linear scale.

In the enamel analysis copper is only a fair fit for this observation but with other tissues it is a much better fit [9]. This may indicate that copper is present in enamel in amounts which are not adjusted by the normal body copper level control mechanism. The other elements all seem to fit into their proper patterns without deviation.

Antimony

Antimony appears to be present in teeth as in other body tissues as a non-essential trace element where its presence depends upon the absorption of antimony into the body from the diet or the atmosphere. The mean level of antimony found was 0.04 ppm. and the range 0.005-0.67 ppm. No difference was found between the antimony content of the surface and the deeper layers of enamel.

Table 1

Trace Elements in Human Enamel.

Element	No.of samples	Maximum	Minimum	Median	Mean	Standard Deviation	Remarks
Antimony	61	0.66	0.005	0.39	0.078	0.115	-
Arsenic	75	0.63	0.003	0.050	0.070	0.085	-
Cadmium	6	-	-	-	-	-	0.03
Copper	103	39.7	1.59	7.88	10.11	7.84	-
Manganese	62	2.01	0.30	0.73	0.83	0.37	-
Mercury	59	18.1	0.14	2.13	3.22	3.62	-
Molybdenum	11	0.12	0.026	0.054	0.054	0.027	-
Vanadium	23	-	-	-	-	-	0.01
Zinc	130	992	58	339	365	182	-

Table 2

Analytical Methods Used

Element	Reference	Neutron flux used ($n/cm^2/sec.$)	Remarks
Antimony	Howie, Molokhia & Smith [1]	10^{12}	Adequate sensitivity
Arsenic	Smith [2]	10^{12}	Adequate sensitivity
Cadmium	Livingston, Smith & Stojanovic [3]	10^{12}	Inadequate sensitivity
Copper	Nixon & Smith [4]	10^{12}	Adequate sensitivity
Manganese	Smith [5]	10^{12}	Adequate sensitivity. A modified separation was used.
Mercury	Smith [6]	10^{12}	Adequate sensitivity
Molybdenum	Livingston & Smith [7]	10^{12}	Low sensitivity. A flux of 10^{14} was used but gave very high general radiation.
Vanadium	Livingston & Smith [8]	10^{12}	Inadequate sensitivity
Zinc	Livingston, Smith & Stojanovic [3]	10^{12}	Adequate sensitivity

Arsenic

The level of arsenic was similar to that of antimony and lay within the range of 0.031-0.145 ppm. giving a mean value of 0.060 ppm.

Cadmium

Little cadmium activity could be found in any of the samples. It was estimated that the amount of cadmium in teeth must be less than 0.03 ppm. As sample sizes necessary to obtain activity included all available enamel from one tooth this investigation was not continued.

Copper

Slight variations were observed between the surface or outer enamel layer and the inner enamel layer, but the difference is so small as to have no significance. The mean of the outer enamel layer was 9.5 ppm. and that of the inner enamel layer was 11.3 ppm.

Mercury

A mean value of 2.79 ppm. was obtained for the outer enamel layer and 2.34 ppm. for the inner enamel. In teeth which had not erupted into the oral cavity the mercury content of the enamel was of the order of 0.1 ppm. The enamel of teeth in contact with silver amalgam fillings had mercury contents ranging from 152-1600 ppm.

Molybdenum

Measurements of molybdenum were just within the limits of detection when 100-600 mg. of enamel sample were used. This amount approaches all the available enamel from one tooth and it was not considered worthwhile to continue with this investigation. Further investigations are being carried out using a neutron flux of 10^{14} n/cm^2/sec. which require smaller samples in the region of 10-25 mg. These,

however, have the disadvantages of a relatively high matrix activity.

Vanadium

No evidence of vanadium was found in over twenty analyses of enamel varying in amount from 100-500 mg. It was concluded that the level in enamel must be less than 0.01 ppm.

Manganese

The manganese content of occlusal enamel ranged from 0.45-1.98 ppm. In the middle layer the outer enamel was 0.66-2.01 ppm., while the inner enamel was 0.34-0.79 ppm. In the gingival layer, the outer enamel was 0.35-1.34 ppm. and the inner enamel 0.30-0.76 ppm.

The concentration of manganese is greater in the outer layer of enamel than on the inner. Manganese is deposited in enamel during calcification which would account for the uniformity of concentration in the inner enamel layer. There may be a post-eruptive gain in manganese from food and other external sources.

Zinc

The results of the analysis show that the zinc content of the outer enamel layer ranged from 213-1550 ppm. while that of the inner enamel was from 58-387 ppm. The pattern of the results shows a definite fall in the enamel zinc levels from the outer surface inward. It would appear that a certain amount of zinc is incorporated in the developing enamel and that there may be an increased deposition after

eruption. It has been shown that the hydroxyapatite of enamel rapidly acquires zinc.

Discussion

Of the elements determined only manganese and zinc were found to occur in higher concentrations in the outer enamel layers. Other elements which have been shown to occur in greater amounts in the outer enamel are fluorine, lead, iron, silver, silicon and tin [10]. In the same work it was found that sodium, manganese and carbonate follow the reverse pattern, i.e. a higher element concentration in the inner enamel.

The zinc and manganese content of inner enamel samples shows less variation than the outer enamel layer. When unerupted teeth were used the same pattern was observed indicating that the higher outer enamel levels are not only formed post-operatively. It has been shown that outer enamel fluorine content increases post-eruptively with age and dietary fluoride intake while the inner enamel remains unchanged. A similar situation has been shown to exist for zinc with respect to age increases of outer enamel zinc levels [10]. The presence of either zinc or fluorine has been shown to increase the resistance of hydroxyapatite of enamel to acid solution. It may be that zinc is as important as fluorine in increasing the caries resistance of enamel.

The wide range of values found in outer enamel samples may involve a post-eruptive gain from external sources as has been shown in several studies [11], [12], [13].

Using activation analysis techniques it has been possible to establish concentrations of many trace elements in human enamel in a single sound tooth. The great value in the methods has been the degree of sensitivity and the use of small samples. The initial carious lesion involves only a small area of surface enamel and is characterised by the production of a white spot on the enamel surface indicating a surface change involving the mineral fraction. Analyses are being carried out at present to determine changes in mineral content of this area using the techniques previously described.

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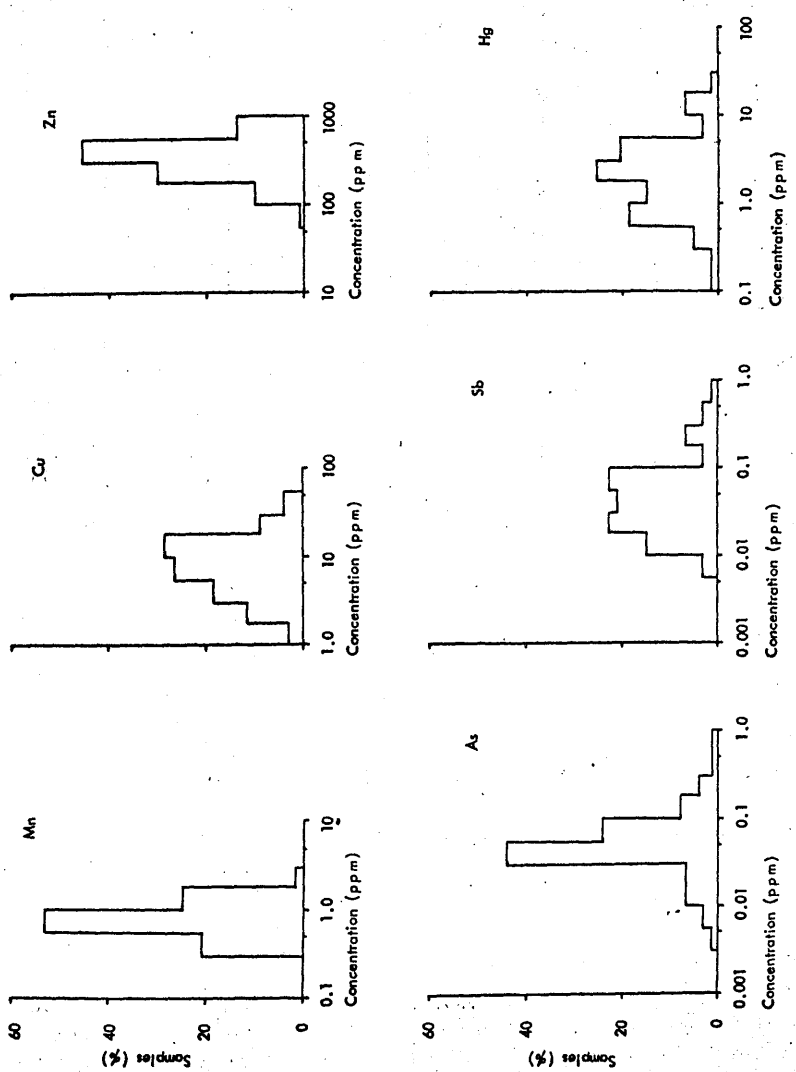

FIG. 1. Distribution of trace elements in human enamel.

P A P E R 14

PHYSICAL METHODS IN DENTISTRY

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Phys. Med. Biol.

Vol. 13, No. 2, 145-157, 1968.

PHYSICAL METHODS IN DENTISTRY

1. Introduction

Dentistry like other biological sciences has become increasingly dependent on the employment of physical methods both in research and in the clinical application of new ideas and developments. Current dental research programmes are now requiring greater inter-disciplinary co-operation between the dental research worker and the specialist in other fields.

The environment of the tooth in the mouth is an unusual one of hard tissues surrounded by a fluid medium of heterogeneous composition. This fluid is composed of the secretions of the major and minor salivary glands together with the the secretion of the fluid from the soft tissues which surround the teeth. The crown of the tooth is covered with enamel which is almost totally inorganic in composition but which is permeable to fluids passing from the pulpal circulation to the oral fluids and vice versa. The specific relationship between the fluid environment which surrounds the tooth and the hard tissues has not been clearly established.

2. Dental caries

Dental caries, which remains a major disease of civilized communities, has been the subject of many investigations from the early days of dentistry. The aetiology and mode of action of the carious process on the hard dental tissues, enamel dentine and cementum, have been

widely studied. An extensive survey of the literature on dental caries has been carried out and published by Brislin and Cox (1964). Investigations have covered a wide field of scientific disciplines each of which has contributed much specialized knowledge to this basic problem. Factors such as age, composition, diet, saliva and heredity have been studied in great detail.

The composition of a tooth may be a more important factor than has hitherto been believed. An approach to the problem is to determine variations which may exist between teeth which resist caries and those teeth which are attacked readily. Difficulties arise, however, in deciding which teeth are caries prone and which are caries resistant. Caries resistance is a relative condition. Teeth may be free from caries not because they are particularly resistant but because they may have been spared the attack by caries. They may also become carious under different environmental conditions. Some tooth structures may be more resistant than others and the resistance of some components may be altered by the chemical environment of a carious lesion.

2.1 Studies in tooth composition

Earlier studies of the composition of teeth have indicated that differences of composition of teeth do occur between the outermost surface layers of enamel and the deeper layers. The importance of the outermost enamel layer lies in the fact that this is the first site of attack by caries.

It has been considered for many years that differences between the trace-element levels in sound and carious teeth may show the value of certain elements in forming caries-resistant teeth. There has been little scientific evidence to support this belief and there is still no certainty about the mechanism of their action. The element which has been shown to have the greatest significant effect on caries prevention is fluorine. Water fluoridation studies, animal studies and nutritional studies have shown that there is a positive correlation between fluorine intake tooth fluoride and caries resistivity. Other trace elements such as molybdenum and vanadium have also been considered as increasing the caries resistance while the presence of selenium shows an increased caries proneness.

When the level of the trace element is less than 100 ppm the amount present in enamel or dentine is often below the limit of sensitivity of many techniques such as micro-analysis or colorimetry; consequently, pooled samples of enamel and dentine have been used so that a significant analysis could be made. The use of pooled samples, however, produces only average figures for a particular type of tooth section. Individual variations or variations within a particular part of the tooth are not detectable by these methods.

Neutron activation analysis has been employed by Nixon, Livingston and Smith (1967) to determine the concentration of trace elements in human dental enamel (Table 1). Activation analysis has the advantage in that the increase in sensitivity it offers for many elements allows the

Table 1

Trace elements in human tooth enamel (ppm)

Element	Maximum	Minimum	Median	Mean	Standard Deviation	Remarks
Antimony	0.66	0.005	0.39	0.078	0.115	-
Arsenic	0.63	0.003	0.050	0.070	0.085	-
Cadmium	-	-	-	-	-	< 0.03
Copper	39.7	1.59	7.88	10.11	7.84	-
Manganese	2.01	0.30	0.73	0.83	0.37	-
Mercury	18.1	0.14	2.13	3.22	3.62	-
Molybdenum	0.12	0.026	0.054	0.054	0.027	-
Vanadium	-	-	-	-	-	< 0.01
Zinc	992	58	339	365	182	-

analysis of small enamel samples from a single tooth. Where there is the combination of suitable isotope characteristics and an enamel abundance of an element it is possible to make accurate determinations on samples often as small as 1 mg.

There is an additional advantage in activation analysis in that the identity of an element can be confirmed by decay or γ-spectra studies. These provide a check against the interference by another element in the measurement. In many cases micro-separations can be avoided and instrumental methods may be employed.

One of the difficulties in activation analysis is that of sample contamination. The extraction of teeth for sampling has to be carried out with forceps, the beaks of which are protected by polythene tubing, or the teeth have to be removed surgically to avoid contamination of the enamel of the tooth crown with a metal instrument.

Elements such as zinc and manganese are present in tooth enamel in concentrations which permit small enamel samples to be used but with others such as molybdenum and vanadium, the total enamel sample from one tooth, up to 500 mg. was required. Most studies on activation analysis have been carried out using a neutron flux of 10^{12} n/cm^2/sec.

The concentrations of many of the inorganic elements of human enamel have been studied by Soremark and Samsahl (1961). The enamel samples were irradiated for about 20 hours in a neutron flux of 2×10^{12} n/cm^2/sec. Gamma-ray spectrometric analysis was carried out after chemical group

separation.

Charged particle analysis has been applied to the determination and distribution of carbon, calcium and phosphorus by Fremlin and Stubbins (1967). These are three of the principal elements of tooth enamel. Use has been made of different activating particles to produce isotopes of different half-lives to enable independent study of the three elements (Table 2).

After irradiation an autoradiographic technique was used to estimate the activity produced. It was found by this method that the total carbon content of human tooth enamel is higher at the innermost surface by a factor of 1.5, from that of the outer edge.

Electron probe analyses for phosphorus and calcium distribution in tooth enamel have been carried out by Frank, Capitant and Goni (1966). In this study highly polished ground sections of teeth were used for qualitative and quantitative electron probe microanalysis. In mature normal and carious human enamel calcium, phosphorus and chlorine could be detected on X-ray probe spectral recordings. The comparison of caries-resistant and caries-susceptible enamel showed that the superficial enamel layer does not contain more calcium and phosphorus and that there is no significant statistical difference in the distribution of these two elements in the two groups. Electron probe studies of enamel caries, before any cavity formation, confirm the loss of calcium and phosphorus in the sub-surface whereas the superficial caries layer has a normal chlorine, calcium and phosphorus content.

Hardwick and Martin (1967) have used mass spectrometric methods for the estimation of the trace element content in dental tissues. The pilot study showed that the method was feasible for the identification of many elements, but at the early stage was not truly quantitative. By the standardization against prepared matrices of known composition and by the use of a micro-densitometer for comparing line images on a photographic plate, the method could be quantitative for many elements.

2.2 Water content of enamel

Studies of the water content of enamel have been carried out by Myers (1965) by means of Line Nuclear Resonance Spectrometry. He has shown that a proton resonance signal with a line width of the order of tenths of a gauss could be obtained from powdered and hydrated enamel. An unusual feature of the signal was its ability to resist dehydrating temperatures of 200°C for one week. This property is not shared by other calcified tissues. It was inferred by Myers that the protons were part of trapped water present in ultra-structural features found only in enamel. A further study by Myers and Myrberg (1965) indicated that the water present in unheated enamel behaved in an unusual manner with regard to freezing. Again a property not found in unheated dentine, ashed enamel or in mineral apatite. The authors state that ashing procedures destroy this property by its effect on the protein or by its effect on the ultra-structural organization of the tissue. The depressed freezing part

of the water is consistent with its being in mean capillary spaces of 15 Å radius.

2.3 Public health dentistry

Activation analysis has also been used to examine the possible health hazard of mercury poisoning in the dental surgery. Mercury is used extensively in the preparation of silver amalgam fillings. An examination of dental surgery assistants by Nixon and Smith (1965) showed a significant increase in the mercury content of hair and nails

Table 2

Activation and detection of calcium, phosphorus and carbon in human dental enamel (Fremlin and Stubbins, 1967)

Element under investigation	C	P	Ca
Bombarding particle	^{2}D	^{2}D	^{1}H
Energy of particle	2.2 MeV	2.2 MeV	10 MeV
Beam current on 30 cm^2	1 μA	2 μA	0.1 μA
Irradiation time	20 min.	40 min.	30 min.
Time between end of irradiation and start of exposure	4 min.	5 d	4 h
Exposure time	40 min.	8 d	20 h

when compared with a control group. In the control group a mean value of 5.10 ppm was obtained for the nail and hair samples. In the surgery assistants group the mean value for toe nail samples was 9.3 ppm mercury, while that of finger nails was 68.76 ppm. They also reported a case of

chronic mercury poisoning in a dental surgeon with a mercury content of 558 ppm for the finger nails and 171 ppm for the head hair, while the findings in the dental surgery assistant were 285 ppm for the finger nails and 50.8 ppm for the head hair. Upon further investigation it was found that amalgam fillings were prepared by both dentist and assistant and that excess mercury was allowed to escape on to the floor in close proximity to an electric convector heater. Removal of the floor covering disclosed approximately 3 ml of mercury on the floorboards.

3. Laser radiation

The use of the laser beam has opened many potential fields of investigation in dentistry. Preliminary studies with laser beams demonstrated definite effects on carious lesions of the tooth and also on dental calculus. Sognnaes and Stern (1965) using exit energies of 5 joules to 20 joules reported a glass-like fusion of the enamel and a cratering of the dentine. They also showed that this glazed superficial enamel surface is more resistant to 'in vitro' demineralization than the adjacent unlased enamel surfaces.

In an investigation Peck and Peck (1967) demonstrated that destruction of tissue by the laser beam was dependent to a certain extent on the opacity and colour of the material. It was expected that the laser effects on tooth structure would have reflected the differences which are present in the optical properties of enamel and dentine.

Souder and Paffenbarger (1942) have given the opacity of enamel as ranging from 21 to 67% while the opacity of equal thickness of dentine were within the range of 50 to 91%. Contrary to expectation Peck's study showed that destruction and penetration were greater in enamel than in dentine. Enamel exhibiting gross cratering from 0.1 mm to 1.1 mm deep depending on the amount of energy delivered to the target area while laser impacts on dentine showed irregular craters less than 0.1 mm deep. It has been suggested that the structural and biochemical differences between enamel and dentine may account for the differences.

The laser beam has been employed by Goldman (1967) to prepare cavities on single surfaces of normal teeth 'in vitro'. Initial impacts were made with a totally internal reflecting ruby rod and a back surface mirrored rod. Pulse lengths of 0.5 and 0.7 millisecond duration with 0.4 and 0.6 millisecond pumping were employed. The use of a retromirror behind and separate from the back face of the rod increased the oscillator dimension, decreased the beam divergence and caused a more uniform pattern. Using this technique a spot size of less than 0.5 mm was obtained according to the measurement of the diameter of the hole cut in the tooth structure. The enamel and dentine of the tooth structure were removed by abrupt vaporization. Initially impact energies of 50 joules exit energy per impact were used but with refinement of focus it was possible to reduce the exit energy levels to 20 joules per impact. This study showed the possibility of employing laser energy as a cavity preparation instrument. It also

demonstrated that considerable technical refinement of instrumentation would be necessary before this method of cavity preparation would be suitable for practical clinical use.

The effects of the laser beam have been associated with increased elevation of the temperature of the tooth. It is not known also what the effects of the energy impact are on the human dental pulp.

The effects of laser beams on the dental pulp and oral mucosa were studied by Taylor, Shklar and Roeber (1965). They demonstrated on Syrian hamsters that tissue changes resulting from radiation were severe. When a 55 joule beam was employed the pulp of the incisor tooth was destroyed. This beam also produced severe changes in the mucous membrane. When a lesser beam of 35 joules was employed pulpal changes were still severe. There was also evidence of pulpal degeneration at a considerable distance from the point of radiation. Healing of both pulpal and mucosal tissue progressed slowly. The changes which take place in the mucosa and pulp appear to be consistent with trauma resulting from excessive heat. The authors stress that the use of laser radiation in dentistry presents many problems concerning the protection of the surrounding tissues.

One of the interesting applications of the laser beam in dentistry is the direct fusion of dental filling materials such as porcelain in the cavity. By this means, good adaptation of the filling material to the walls of the cavity could be achieved. There is also a potential application in the field of dental prophylaxis by removing

stains and dental plaque from the tooth surface. Any pigmented areas on the tooth surface will enhance the superficial absorption of laser light.

4. Marginal seal of filling materials

It has been assumed for many years that a satisfactory restoration of tooth possesses a hermetic seal between the tooth and the filling. This would appear to be a fundamental requirement considering the moist, contaminated environment of all dental restorations. The degree of marginal seal achieved is generally assessed clinically on an empirical basis where the history of the restoration is known. In the laboratory a variety of techniques have been employed upon extracted teeth, but some work has been done on metal models of suitable cavity shapes. Radioactive tracers have been employed to determine the degree to which the margins of restorations may be penetrated. Going, Massler and Dute (1960) employed a number of radioisotopes to determine whether ionic change and chemical reactivity would influence the degree of penetration. All fillings showed marginal leakage to radioisotopes with penetration into dentine and even into the pulp. In a later study, again using radioisotopes, Going and Massler (1961) demonstrated that certain protective cavity lining materials could reduce the penetration of the isotope into dentine and pulp. Brannstrom and Soremark (1962) showed that a varnish placed over the cavity significantly reduced marginal leakage but when subject to variations in temperature this leakage was again increased. They concluded that any seal which had

formed was broken down by temperature changes. Teeth which had been restored with amalgam were shown by Stowell, Taylor and Wainwright (1962) to leak less with saliva than with normal saline at the end of a two-week period. They considered that the first two weeks after insertion were critical and suggested that a deposit from the saliva had prevented the penetration of radioactive iodine employed as a tracer, and that this may occur with fillings in the mouth.

Air pressure was used by Pickard and Gayford (1965) as a means of assessing any leakage which may exist between silver amalgam fillings and the tooth. This method allowed a continuous and quantitative assessment of the leakage area up to a ten-week period. The method employed is shown in fig. 1. Compressed air was forced through the tooth until a pressure of 900 mm of mercury was reached. The authors postulated that any single leak path submitted to a rising air pressure from the base of a restoration would reach a critical point where the capillary pressure of water entering from without equalled the air pressure from within. The pressure required to establish this state of equilibrium, the 'critical pressure', would be constant for a leak path of constant minimal cross-sectional area in water and that this postulate would hold good for any liquid or solution.

From all studies employing different methods, it would appear that penetration between restoration and tooth is greatest immediately after the restoration is inserted and decreases after a few weeks when it may actually cease.

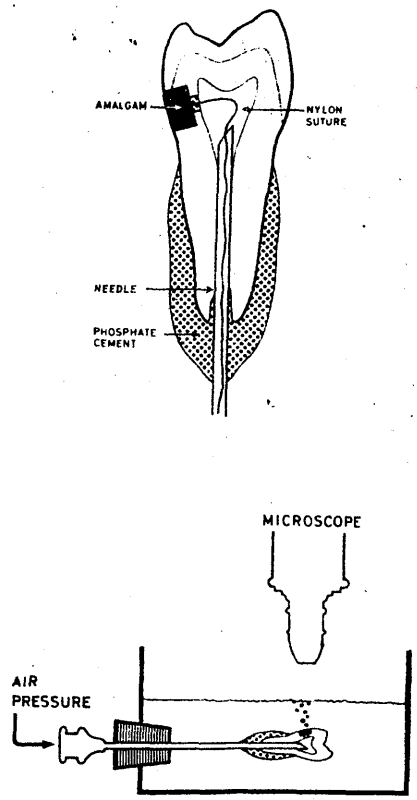

Fig. 1. Specimen of tooth under investigation with section demonstrating needle sealed into root canal through which air pressure was applied. (From British Dental Journal.)

5. High speed cutting

5.1 Air contamination

Air turbine handpieces which produce speeds up to 350,000 revolutions per minute are now standard equipment in the dental surgery. While these handpieces have increased the cutting efficiency of dental burs, they have introduced many problems. A survey of the dental profession by Grundy (1966) in the United Kingdom, has suggested that the use of this type of handpiece has some effect on the eyes and respiratory tract of the operator. This may be due to air contamination by cutting debris and also the oil used to lubricate the turbine handpiece may be emitted in aerosol form and inhaled by the operator. Grundy (1967) demonstrated by means of an air sampling technique that during certain operative procedures small enamel particles are thrown into the area of the operator's face. This is particularly so when cutting is carried out on a dry field. It was estimated that up to 59 million particles were thrown up in a half-minute sample of 5 litres of air and up to 4 mg in a two-minute sample of 24 litres. The air from the handpiece helps to project the particles towards the operator. The inhalation of oil was measured by Nixon and Tilston (1965) using oil labelled with ^{132}I and an artificial head and lung. Measurable amounts of oil, 0.018 ml to 0.034 ml per hour, were obtained which may be inhaled by the operator. The size of the oil particles from the handpiece was found to be from 3-8 μ in diameter. It has been recommended by most observers that operators using air

turbine handpieces should wear protective face masks. Both the amounts of oil and enamel debris inhaled were dependent on the position of the tooth being cut and the distance of the operator from the patient.

5.2 Noise levels

The sound levels produced by the air turbine handpiece have been considered a potential hazard to hearing. Analysis of drill noise by Cantwell, Tunturi and Sorensen (1965) concluded that with an exposure of 12 minutes combined in one working period, safe sound pressure levels could extend to 101 decibels without causing hearing loss. The overall averages for handpieces in this study were 66.0 decibels at 40 psi, 67.9 decibels at 50 psi, and 67.6 decibels at 60 psi. A further study by Taylor, Pearson and Mair (1965) in a group of dentists exposed to the air turbine drill for an average period of 3.7 years concluded that a noise-induced threshold shift was sustained. This report indicated that with time, a gradual encroachment of the upper frequencies may occur. This hearing loss was particularly noticeable in the 4 kc/sec to 6 kc/sec frequency region. A five-year study by Nixon and Knox (1966) on dental students and dentists demonstrated a more significant loss in the dental group than in the control group (fig. 2) and that greater individual susceptibility was observed in the dental group. They assumed that a 15 decibel hearing threshold shift was significant and the hearing losses in the dental group occurred at the 4 kc/sec, 6 kc/sec and 8 kc/sec level. The dental group also

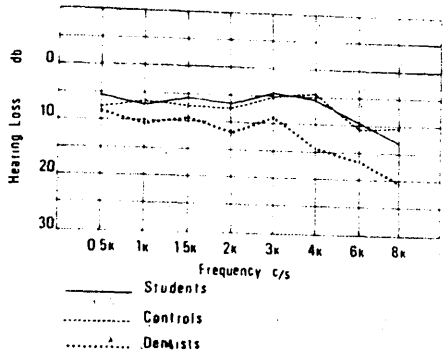

Fig. 2. Comparison of hearing loss between dental group using high-speed air-turbine handpieces and control group. Greater hearing loss in dental group in higher frequencies.

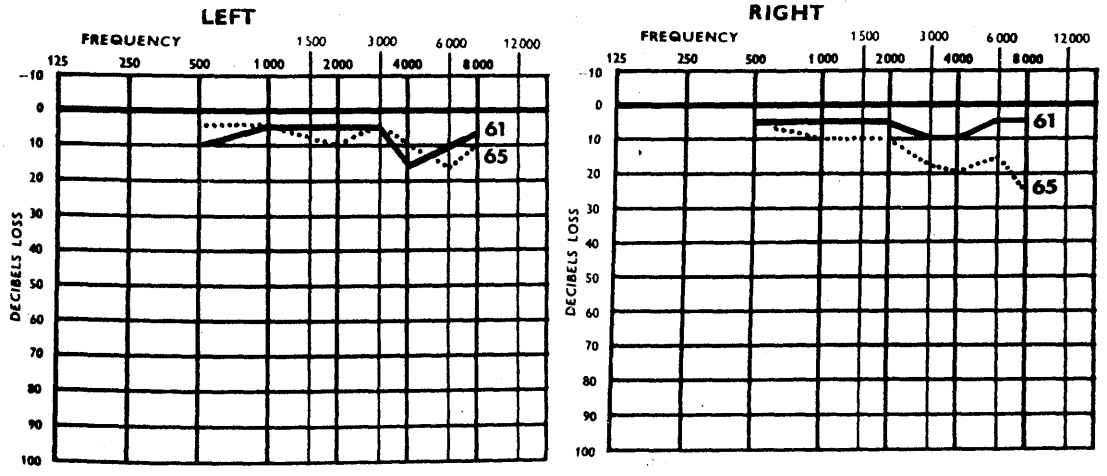

Fig. 3. Audiograms of dental subject using an air-turbine drill from 1961–1965 showing greater hearing loss in right ear.

demonstrated a greater susceptibility of hearing loss in the right ear than in the left (fig. 3); this ear being in closer proximity to the drill in right-handed dentists. This report emphasizes that any hearing loss sustained in a young person through drill noise will be added to the hearing loss due to age. Keller, Olk and Opitz (1964) found the greatest hearing loss from air turbine drills in the 30-40 years age group. They state that not every dentist is affected in the same way and recommend regular audiometric examination to protect susceptible individuals from further damage.

6. Radioactive isotopes

Radioactive isotopes have been employed in dentistry, as in other fields, for a variety of purposes. Most studies with radioactive isotopes in dentistry involve autoradiographic techniques together with microradiography and histological examination to localize the radioactive elements specifically within the tissues. Dental studies using radioactive amino acids have been widely employed to examine cellular protein metabolism, the turnover of proteins associated with cellular function and the synthesis of the organic tissue matrix. Tritiated glycine leucine and methionine have been used to trace the formation of the dentinal matrix. Methionine labelled with ^{3}T, ^{14}C and ^{35}S has also been used. A study to examine the localization and distribution of tritiated histidine in the developing incisor tooth of mice was carried out by Hwang, Tonna and Cronkite (1963). This demonstrated that ameloblasts, the enamel-

forming cells, showed a rapid turnover of histidine. The enamel matrix was rapidly and intensively labelled after one hour. The dentine-forming cells did not show the same degree of uptake, demonstrating the importance of histidine as a component of the enamel matrix.

The presence and distribution of radioactive isotopes in the hard tissues of the teeth and jaws has been studied in both human and animal subjects. Haslam, Jee, Dockum and Sears (1964) has recorded the localization of ^{226}Ra in the teeth and jaws of Beagles, and reported the concentration in microcuries per gramme of tissue. Rowland (1963) reported on the distribution and clinical effects on human patients suffering from radium poisoning. Equal amounts of residual radioactivity were found in the teeth and bone in these cases where ingestion had taken place after dental development had occurred. If ingestion of radium took place during the development of the teeth, the concentration in the teeth was occasionally five times as great as that of bone.

Significant levels of strontium-90 have been experienced since 1953. The deciduous and permanent teeth of children have been employed as a means of measuring the ^{90}Sr fall-out by Reiss (1961) Rosenthal, Gilster and Bird (1963), Starkey, Bryant and Henderson (1964) and others. Concentrations in different portions of the tooth are related to the rate of ingestion during development.

There have been numerous reports on the oral effects of ionizing radiation on the development and growth in the biological tissues including the dental tissues. Gowgeil

(1961) reports on the administration of fractionated doses of 4500, 5500 and 7500 rads to the maxillae and mandibles of young monkeys. He studied development for 3¼ years after irradiation and observed that while doses of this magnitude prevented further development of the teeth, these teeth which were partially formed tended to erupt into full occlusion. Gorlin and Meskin (1963) reported a human case whose dental development 13 years after irradiation ranged from complete absence of a tooth or tooth root to areas of hypocalcification.

Circulation studies of the dental pulp have been carried out by Meyer (1966). Radioactive solutions of sodium-24, iodine-131 and potassium-42 were adjusted to isotonicity at a pH 7.2 with an activity of 300-500 μCi/ml. Small quantities (0.01 ml) of the isotope solution were placed into cavities prepared on the canine tooth of a dog. The solution was covered with mineral oil to minimize evaporation. A 6 mm scintillation probe equipped with a collimator was placed above the cavity and the activity recorded for two-minute periods at six-minute intervals. Removal of the isotope from the cavity by pulpal circulation was indicated by an increase in blood radioactivity which tended to reach a plateau as the clearance rate diminished. In some of the experiments epinephrine or isotonic saline was infiltrated at the apex and the findings with epinephrine suggest a qualitative relationship between clearance and blood flow.

7. Study of tooth contacts

Many studies have been undertaken to examine the relationship between the contacts of tooth surfaces in the upper and in the lower teeth. This relationship is important with both the natural and artificial dentition as abnormal contacts may produce harmful sequelae. Early methods of recording these contacts was by direct leads taken from the tooth surfaces and connected directly to recording equipment outside the mouth. The presence of the wires passing between the lips introduced many difficulties in these early studies. The contact of wire with the mucous membrane could alter the response of normal function either by the stimulation of the contact reflex nervous mechanism which could alter the chewing pattern and produce an abnormal masticatory cycle or the wires themselves could displace the denture. These difficulties have been overcome by employing telemetric equipment. The development and construction of a transmitter which could be built into artificial dentures and which would operate when contact was made between opposing teeth has been described by Neill (1967). This transmitter was a modification of the "radio pill" type of transmitter. The transmitter was installed in the lower denture and sealed in such a way that no signal could be transmitted due to leaking of current through the saliva.

Tooth contacts and studies of mastication were carried out by Kavanagh and Zander (1965) using a transmitter built into a dental bridge. This bridge was made of acrylic and replaced a missing tooth. Simultaneous recording was

carried out with an electromyograph to examine the relationship between tooth contacts and muscle activity. By moving the contact points of the tooth transmitter to a succession of locations on the occlusal surface position - time studies of tooth contacts have been made (Adams and Zander 1964). The onset of tooth contact was related to associated bursts of muscle activity recorded for each contact site on the electromyograph channels, permitting a determination of the time sequence of contacts at these locations.

The sounds produced during the masticating cycle have been studied by Watt (1966). In his study a microphone was used in place of a stethoscope chestpiece and the sounds made during mastication were recorded on a tape recorder with a tape speed of 15 inches per second. The tape was played back at 3.75 inches per second through an Oscillomink paper recorder which had a paper speed of 10 cm per second. This procedure was carried out to overcome the difficulty produced by the lower frequency response of the paper recorder as compared to the oscilloscope.

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P A P E R 15

TRACE ELEMENT CONTENT OF THE HARD DENTAL TISSUES AND DENTAL PLAQUE

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Caries Res., 3 : 60-74, 1969.

TRACE ELEMENT CONTENT OF THE HARD DENTAL TISSUES AND DENTAL PLAQUE

The term "trace element" may lead to some confusion as there is no definitive boundary of concentration and no widely accepted grouping of elements into trace and non-trace elements. It has been suggested that limits of concentration of 100 ppm. by weight or less should constitute a trace element level but in the hard dental tissues concentrations greater than this are generally considered trace elements. The progress of trace element analysis has been linked to the development of more sensitive techniques which has resulted in more precise information concerning the function of trace elements.

Selection of Analytical Technique

Various criteria, such as sensitivity, accuracy and precision, and selectivity have to be considered before deciding on a particular technique. Sometimes some confusion exists between these terms. In one sense "sensitivity" is used to indicate the smallest quantity of a substance which can be detected, which really implies "lowest limit of detection". In its strict chemical sense, sensitivity implies the ability of a method to observe the difference between very small amounts of a substance.

The lower limit of detection may be expressed either as the absolute limit (i.e. the smallest detectable weight of a substance expressed in micrograms (10^{-6} gm.) nanograms

(10^{-9} gm.) or picograms (10^{-12} gm.)) or the relative limit (i.e. the lowest detectable limit of concentration expressed as a percentage, parts per million or parts per billion).

As several methods of adequate sensitivity are available for trace analysis the analyst must choose a method which is accurate for the purpose. The accuracy of a method is characterised by the absolute and relative errors which may affect the results. Sometimes this term is confused with precision, which is the reproducibility of experimental results. Systematic errors may be introduced into an analytical process at many points such as in sampling, or in the preparation of the specimen.

Before deciding on the method for trace analysis consideration must be given to any interferences which may arise. At times interferences may be present in the matrix of the sample or are produced by the presence of other trace elements, which can modify the result. The analyst must employ several methods to achieve adequate selectivity.

In deciding on a method of trace analysis the choice is often determined by practical considerations such as expense and laboratory facilities. A desirable feature in any technique is to determine as many trace elements as possible in a given analysis.

The only comprehensive techniques giving this type of information are emission spectrography and spark source mass spectroscopy. However, because of detection and recording problems associated with these comprehensive methods, they are generally less precise than single element analysis carried out under optimal conditions.

Many analytical techniques have been employed for the detection and measurement of over twenty trace elements in the dental tissues. In general there is good agreement between differing methods of analysis. One of the difficulties is in the method of sampling and in the preparation of the sample for analysis such as whether it is a wet, dry or ashed sample.

A comparison of the limits of sensitivity of several of these techniques is given in Table 1. A brief description of a few of the methods employed is given below.

Spectrophotometry and Fluorometry

Analytical techniques based on the emission, absorption and secondary fluorescence of radiation in the visible, infra-red and ultra-violet range of the spectrum have been employed for some time. A wider range of trace element analysis became available with the introduction of photoelectric detectors and improved instruments in the visible and ultra-violet range. At the same time the development of organic compounds which complex specifically with particular elements enabled additional absorption techniques to be applied to many trace element problems. Flame spectrometric techniques can be used with absorption and fluorescent methods and do not necessitate the use of complexing organic compounds. All these methods are capable of measuring below the p.p.m. level for many elements.

The sensitivity of any method of spectrophotometry depends on the response of the instrument employed. In the case of absorption spectrophotometry using organic compounds

Table 1

Comparison of Absolute Detection Limits

Element	Spark Source * Mass. Spec. (in nanograms)	Neutron * Activation (in nanograms)	** Em. Fla. Phot. (gms/ml.)	** At. Abs. Phot. (gms/ml.)
Ag	0.2	0.01	6.10^{-8}	1.10^{-8}
Al	0.02	1	3.10^{-6}	1.10^{-6}
As	0.06	0.1		
Au	0.2	0.05		
Ba	0.2	5	25.10^{-7}	1.10^{-6}
Cr	0.05	100		
Cu	0.08	0.1	1.10^{-7}	1.10^{-8}
F	0.02	100		
Fe	0.05	5000	25.10^{-7}	3.10^{-9}
Li	0.006	-	2.10^{-10}	3.10^{-8}
Mn	0.05	0.005	1.10^{-8}	1.10^{-8}
Mo	0.3	10		
Ni	0.07	5	2.10^{-7}	3.10^{-9}
Pb	0.3	1000	1.10^{-6}	3.10^{-8}
Rb	0.1	5	3.10^{-9}	1.10^{-7}
Sb	0.2	0.5		
Se	0.1	500		
Sn	0.3	50		
Sr	0.09	0.5	1.10^{-8}	3.10^{-8}
V	0.04	0.1		
Zn	0.1	10	8.10^{-5}	1.10^{-9}

* Morrison, G.H. 'Trace Analysis of Biological Materials by Mass Spectrometry and Isotope Dilution', I.A.E.A. Symposium on Nuclear Activation Techniques in the Life Sciences, Amsterdam, 1967.

**Herrman, R., and Lang, W. 'Atomic absorption and flame emission spectrometry in trace analysis of biological materials'. I.A.E.A. Symposium on Nuclear Activation Techniques in the Life Sciences, Amsterdam, 1967.

the response is also dependent on the development of the colour reaction in the specific organic compound and sometimes interference occurs in the presence of foreign substances.

Mass Spectrometry

The use of mass spectrometers in analytical problems was suggested as early as 1913 and their value since the 1940's has been mainly in the analysis of multi-component hydrocarbon mixtures in petrochemical problems. Their employment in the analysis of non-volatile materials was very limited until 1955. Since then commercial instruments have become available employing the vacuum spark for sampling solids.

As a result of the success of the vacuum spark technique as a survey method for the determination of trace composition in inorganic materials, its application to biological materials appeared promising.

The applications of mass spectrometry to the analysis of biological materials is just beginning to be recognised and preliminary studies have been carried out in the elemental analysis of dental tissues by Hardwick and Martin (1967). In contradistinction to methods of elemental analysis, mass spectrometric studies of the molecular composition of biological systems is well established particularly since the advent of high resolution mass spectrometry.

There are many problems associated with the use of this technique. One serious problem arises as a result of spectral interferences obtained in the direct sparking of

biological materials blended with the conductor. Fragmentary organic ions of differing complexity are formed in the spark and these spectral interferences may appear at many mass/charge ratios from 14 to above 200 and overlap the trace elements lines of interest. Qualitative identification is difficult and quantitative measurement may be impossible for elements all of whose isotope masses coincide with isotope masses of other elements.

Spark source mass spectrometry is ideally suited to the analysis of inorganic materials of high purity with limited interference from major elements or matrix material.

Despite the limitations of this method, however, a great deal of information can be obtained on a large number of trace elements in a given sample, often achieving detection limits which may be considerably lower than those of other trace methods.

The detection limits for mass spectrometry and activation analysis methods, as given in Table 1, represent idealised values. In the actual elemental analysis of samples, interferences by the matrix or other trace elements do not allow the achievement of these values unless chemical methods are used for the removal of these interferences.

Neutron Activation Analysis

Activation analysis has been employed for the analysis of trace element levels in many biological materials including teeth. This is a method of elemental analysis which utilises certain nuclear properties of the elements in a sample. Nuclear particles are used to produce

radioactive isotopes by activating the nuclei of the elements contained in the sample. The radioisotopes so produced can be detected and measured by their nuclear radiations. As the nuclear characteristics of each radioactive isotope are known, the amount of the element present can be determined. Some forms of analysis can be purely instrumental such as gamma ray spectrometry or they may require associated chemical separation. When chemical separations are required after irradiation to remove any interfering ions, no errors are introduced by the presence of trace contaminants in the reagents.

The general advantages of activation analysis in relation to trace elements in the teeth are:

(a) Specificity and certainty of identity.

The identify of the element detected can be confirmed by decay or gamma-spectral studies. This provides a check against the interference by another element in the measurement. Once the technique has been established there is seldom any difficulty with interferences.

(b) Freedom from reagent contamination.

(c) The avoidance of micro-separations.

(d) High sensitivity.

The limits of detection of a trace element by activation analysis is a function of many variables such as the amount of radioactivity induced in the unit mass of an element.

Although activation analysis is a very sensitive

technique, it is also subject to a large number of errors.

As in chemical analysis, errors may also be introduced into activation analysis through the sampling process. While one of the advantages of activation analysis is that non-radioactive contamination after irradiation will not affect the result, sample contamination presents a problem. Due to limitations of space during irradiation and the high sensitivity of the method smaller samples can be used, as a result of which relative contamination is greater.

Trace elements in enamel

The concentrations of many of the trace elements in enamel are shown in Table 2. Previous analyses of teeth of a semi-quantitative or qualitative nature indicated the presence of many of these elements (Lowater and Murray 1937, Leicester 1949). The concentration of individual trace elements varies greatly from ppb. - ppm. Many of these elements are consistent constituents of enamel and vary little between the erupted and unerupted tooth being incorporated in this tissue at the time of tooth development. Some elements are present in concentrations similar to those in other tissues in the body.

In the erupted teeth however, greater variations in concentration do occur. Much of this variation may be due to accumulation of elements from food, water and general environment and many of these non-essential trace elements are present merely as contaminants of enamel. Others such as Pb, Sn, and Hg may be acquired by the enamel surface from amalgam restorations or as a result of tooth brushing.

Table 2

Trace Element Levels in Enamel

Element	Range or Mean (ppm)	Method	Reference
Ag	0.0049	Ac. An.	Soremark & Lundberg (1964)
	1-10	Mass. Spec.	Hardwick & Martin (1967)
Al	10-100	Mass. Spec.	Hardwick & Martin (1967)
As	10-100	Mass. Spec.	Hardwick & Martin (1967)
	0.031-0.145	Ac. An.	Nixon & Smith (1960)
Au	0.02±0.01	Ac. An.	Soremark & Samsahl (1961)
Cr	0.0037	Ac. An.	Soremark & Lundberg (1964)
Cu	12-30	Spect.	Brudevold & Steadman (1955)
	10.11±7.84	Ac. An.	Nixon & Smith (1962)
	0.26±0.11	Ac. An.	Soremark & Samsahl (1961)
	1-10	Mass. Spec.	Hardwick & Martin (1967)
F	32-1247	Chemical	Brudevold, Gardner & Smith (1956)
			Jenkins & Speirs (1953)
Fe	338±109	Ac. An.	Soremark & Lundberg (1964)
Mn	0.54±0.08	Ac. An.	Soremark & Samsahl (1961)
	0.30-2.01	Ac. An.	Nixon, Livingston & Smith (1966)
	0.15-1.00	Ac. An.	Battisone, Feldman & Reba (1967)
	10-100	Mass. Spec.	Hardwick & Martin (1967)
Mo	0.026-0.12	Ac. An.	Nixon, Livingston & Smith (1967)
	0.032-0.46	Spect.	Healy & Ludwig (1963)
	1-10	Mass. Spec.	Hardwick & Martin (1967)
Pb	29-550	Spect.	Brudevold & Steadman (1956)
Rb	4.90±2.2	Ac. An.	Soremark & Lundberg (1964)
Sb	0.005-0.67	Ac. An.	Nixon, Livingston & Smith (1967)

(Continued)

Table 2 (Continued)

Element	Range or Mean (ppm)	Method	Reference
Se	0.43-1.60	Ac. An.	Hadjimarkos & Bonhurst (1959)
Sn	1.0-7.0	Spec.	Brudevold & Steadman (1956)
Sr	25-350	Spec.	Steadman, Brudevold & Smith (1958)
	93.5±21.9	Ac. An.	Soremark & Samsahl (1961)
	100-1000	Mass. Spec.	Hardwick & Martin (1967)
V	0.01	Ac. An.	Nixon et al. (1967)
	1.0	Mass. Spec.	Hardwick & Martin (1967)
Zn	276±106	Ac. An.	Soremark & Samsahl (1961)
	149-1550	Ac. An.	Nixon, Livingston & Smith (1966)
	182-2100	Spect.	Brudevold et al. (1963)
	100-1000	Mass. Spec.	Hardwick & Martin (1967)

The importance of the mineral composition of the surface layer of enamel has been stressed. Certain trace elements such as F, Zn, Fe and Pb are found in higher concentrations in the surface enamel than in the sub-surface (Brudevold, Steadman & Smith, 1960).

With many trace elements where the sensitivity of the method is high, it has been possible to analyse layers of enamel, while with others where the sensitivity is low, the entire enamel from a single tooth has had to be employed or samples obtained from a number of teeth.

Trace elements in dentine

The data available on the concentration and distribution of trace elements are few in comparison with those of enamel. Table 3 gives the concentrations of many trace elements in

dentine. Much of previous work has been related to the F content of dentine and this has been reported extensively, e.g. Jenkins and Speirs (1954) and Yoon, Brudevold, Gardner and Smith (1960).

Table 3

Trace Elements in Dentine

Element	Range or Mean	Method	Reference	
Ag	1-10	Mass. Spec.	Hardwick & Martin	1967
Al	10-100	Mass. Spec.	" "	
As	10-100	Mass. Spec.	" "	
Au	0.03±0.01	Ac. An.	Soremark & Samsahl	1962
Cr	-	-		
Cu	0.21±0.10	Ac. An.	Soremark & Samsahl	1962
F	up to 8810	Spectr.	Yoon et al.	1960
Fe	-	-		
Mn	0.19±0.06	Ac. An.	Soremark & Samsahl	1962
	0.17±0.51	Ac. An.	Battisone et al.	1967
Mo	1-10	Mass. Spec.	Hardwick & Martin	1967
Pb	1-10	Mass. Spec.	" "	
Rb	1-10	Mass. Spec.	" "	
Sb	1	Mass. Spec.	" "	
Se	0.35-0.43	Ac. An.	Hadjimarkos & Bonhurst	1959
Sn	1-10	Mass. Spec.	Hardwick & Martin	1967
Sr	69.8±18.0	Ac. An.	Soremark & Samsahl	1962
	100-620	Spectr.	Steadman et al.	1958
V	1-10	Mass. Spec.	Hardwick & Martin	1967
Zn	150-1400	Spectr.	Brudevold et al.	1963
	199±78.1	Ac. An.	Soremark & Samsahl	1962

The distribution of F, Pb, Zn and Sr is discussed by Brudevold et al. (1960). Most of the analyses given represent concentration on the basis of dry weights. Soremark and Samsahl (1962) give the mean concentrations of Ca, P, Cl, Na, Sr, Zn, Br, Mn, W, Cu and Au in sound dentine as determined by a multi-element technique of activation analysis. Many trace elements have been identified "semi-quantitatively" by Hardwick and Martin (1967) employing mass spectrometric method on ashed samples.

The different rates of development of enamel, dentine and cementum may account for the difference in patterns of distribution with trace elements such as F and Zn. Continuous deposition of dentine allows continued contact with tissue fluids.

Trace elements in dental plaque

Analysis of the dental plaque has been mainly confined to the calcium, phosphorus, sodium and potassium contents. The F content was measured by chemical analysis by Hardwick and Leach (1963) while Puttnam, Bradshaw and Platt (1965) determined the concentrations by X-ray spectroscopy on dried plaque. Hardwick and Martin (1967) have given approximate concentration ranges of most of the trace elements on ashed samples. The concentrations are given in Table 4.

Table 4

Trace Elements in Dental Plaque

	ppm.*	Range ppm.**
Ag	9	1 - 10
Co	6	1 - 10
Cr	10	10 - 100
Cu	35	100 - 1000
F	-	6 - 180***
Fe	92	100 - 1000
Mn	4	100 - 1000
Mo	2	10 - 100
Ni	8	10 - 100
Pb	7	10 - 100
Sn	16	10 - 100
Zn	66	100 - 1000

* Puttnam, N.A., & Bradshaw, F. & Platt, P. (1965)

** Hardwick, J.L., & Martin, C.J. (1967) (on ashed samples)

*** Hardwick, J.L., & Leach, S.N. (1963).

Fluorine

Fluorine has been studied more intensively than any other trace element and numerous comprehensive reviews have been published. Brudevold et al. (1956) gives a figure of 331 ppm. in unerupted teeth increasing to 1247 ppm. in the enamel over 50 years of age. The fluoride level decreases rapidly to the dentino-enamel junction where figures of 30-60 ppm. are to be found. Deciduous teeth

while containing less fluoride than permanent teeth show a similar decrease in the fluoride content from the outer enamel surface.

Yoon et al. (1960) report that higher concentrations of fluoride were always found in the pulpal surface of dentine than in the inner dentine. The range of fluoride in dentine varies greatly with maximal concentrations given as 8810 ppm. where the water concentration of fluoride was 5.2 ppm.

Analysis of wet samples of dental plaque by Hardwick and Leach (1963) showed a mean fluoride content of 66.9 ppm. and a range of fluoride concentrations from 6 - 180 ppm. Later reports by Dawes, Jenkins, Hardwick and Leach (1965) showed concentrations of plaque collected from 11 year old children in a low fluoride area as 26 ppm. and 47 ppm. in a higher fluoride area (2 ppm.).

Zinc

The concentrations of zinc in enamel are similar to those of fluoride. Chemical methods of analysis were employed by Cruickshank (1937) to determine concentrations of zinc in enamel. These concentrations ranged from 211 - 260 ppm. for permanent teeth. Soremark and Samsahl (1961) found a mean value of 276 ppm. using neutron activation analysis but no attempt was made to separate the enamel into layers. Brudevold et al. (1963) using spectrographic methods of analysis on erupted teeth found that concentrations ranged from 430-2100 ppm. The high concentrations found in the enamel surface in this investigation were also

present in the enamel of unerupted teeth demonstrating that most of the zinc was deposited before eruption. Activation analysis was also employed by Nixon et al. (1967) to measure concentrations of zinc in single teeth ranging from 58 - 1550 ppm. with higher concentrations found on the outer surface enamel.

Zinc concentrations in coronal dentine have been given by Brudevold et al. (1963). These ranged from 250 ppm. at the dentino-enamel junction to 1100 ppm. at the surface adjacent to the pulp in unerupted teeth. In erupted teeth the concentration range remained greater at the surface adjacent to the pulp and ranged from 150-1400 ppm. The mean concentration for dentine in permanent teeth as given by Soremark and Samsahl (1962) was 199 ppm.

The concentration of zinc in dental plaque as given by Puttnam et al. (1965) ranges from 0.047-0.100 ppm. Hardwick and Martin (1967) place zinc in the range 100-1000 ppm. for ashed samples.

Lead

Earlier spectrographic studies by Drea (1936) showed lead was consistently present in teeth. The mean lead content for deciduous teeth as given by Altshuller (1962) was 15.1 ppm. Brudevold and Steadman (1956) found relatively high concentrations of lead in the external enamel layer which rapidly decreased towards the amelo-dentinal junction, in both erupted and unerupted teeth. The concentration of lead increased with age and the increase was more marked on the external than in the internal surface

enamel. The concentration ranged 35 - 550 ppm.

Few figures are available for the concentrations of lead in dentine and dental plaque. Hardwick and Martin (1967) state that concentration in ashed dentine is between 1 - 10 ppm. and 10 - 100 ppm. in ashed dental plaque. Puttnam et al. (1965) give a mean concentration of 7 ppm. for dental plaque.

Iron

The outer surface of enamel contains a much greater concentration of iron than the sub-surface. Torrell (1957) reports concentrations ranging from 120 - 640 ppm. the concentration increasing with age. Soremark and Lundberg (1964) give a mean concentration of 338 ppm. but Steadman and Brudevold (unpublished observations 1963) state that while much lower concentrations of iron (25 - 60 ppm.) are to be found in the surface enamel of teeth from several communities in the United States, this surface concentration was often three times as high as that of the deeper enamel.

No figures are available for dentine but Hardwick and Martin (1967) state that it may be expected to be in the range 100 - 1000 ppm. ashed. The range of concentration in dental plaque is given by Puttnam et al. (1965) as 47 - 128 ppm.

Strontium

Strontium is a constant trace element in teeth. Wide variations are found in the enamel of different teeth

and the range varies from 25 - 600 ppm. in teeth from different geographic areas (Steadman et al. 1958). The distribution is even throughout enamel with no difference in the strontium concentration among different age groups or between unerupted and erupted teeth. Soremark and Samsahl (1961) give a mean value of 93.5 ppm. in the enamel of permanent teeth.

Steadman et al. (1958) give concentrations of strontium in dentine similar to those of enamel (100 - 620 ppm.). The mean concentration 69.8 ppm. given by Soremark and Samsahl (1961) is slightly lower than that of enamel.

There are few figures for the concentration of strontium in dental plaque but Hardwick and Martin (1967) give the range between 10 - 100 ppm.

Copper

Variations are found in the concentrations of copper of erupted teeth and concentration ranges of 12 - 30 ppm. with no variation with age, have been reported by Brudevold and Steadman (1955) using spectrometric methods of analysis.

Activation analytical methods by Nixon and Smith (1962) give a mean value of 9.5 ppm. for the outer enamel layer and 11.3 ppm. for the inner enamel. Lower concentrations are reported by Soremark and Samsahl (1961) who report a mean value for copper in enamel of 0.26 ppm. and 0.21 ppm. for dentine. Hardwick and Martin (1967) in a semi-quantitative estimation reported levels of the order of 10 ppm. for ashed enamel and a slightly higher concentration

for ashed dentine.

From the results obtained it would appear that while variation does occur in concentrations of copper in individual teeth, no definite pattern of distribution is present.

In plaque the range for copper is given using X-ray spectrometry by Puttnam et al. (1965) as from 17 - 59 ppm. Hardwick and Martin (1967) report a concentration of about 100 ppm. ashed.

Manganese

Relatively little data are to be found regarding concentrations of manganese in the dental tissues. Increased concentration on the outer surface with a decrease in the sub-surface layers of enamel in permanent teeth have been reported. Brudevold, Steadman and Smith (1960) gives concentrations ranging 20 ppm. - 5 ppm. from outer to inner. Nixon, Livingston and Smith (1967) report that concentrations ranging from 2.01 - 0.34 ppm. were found again with higher concentrations of manganese on the outer enamel layer. A mean value of 0.54 ppm. has been given by Soremark and Samsahl (1961) and 0.25 ppm. by Battisone, Feldman and Reba (1967).

There are few figures available for concentrations in dentine. A mean concentration of 0.19 ppm. (Soremark 1962) and 0.25 ppm. (Battisone 1967) are reported. Puttnam (1965) gives a range of 1 - 10 ppm. manganese in dental plaque.

Molybdenum

Despite the considerable interest in the role played by molybdenum in caries (Jenkins 1967) comparatively little is known about the concentrations of this element in the dental tissues, partly due to difficulties in analytical techniques. Healy and Ludwig (1963) give concentrations of 0.034 and 0.032 ppm. in permanent teeth from Napier and Hastings in New Zealand. These figures were for whole teeth with no separation of enamel and dentine. Figures given for the same areas for deciduous teeth were 0.069 ppm. for Napier and 0.46 ppm. for Hastings. Nixon et al. (1967) give the concentration as from 0.026 - 0.12 in the enamel of permanent teeth in Scotland.

No separate figures are available for dentine, although Hardwick and Martin (1967) give a semi-quantitative measurement between 1 - 10 ppm. This report also gives concentrations between 10 - 100 ppm. molybdenum in dental plaque. Puttnam et al. (1965) report a concentration between 1 - 2 ppm.

Selenium

Despite the importance of this element as an essential trace element and its role as a caries enhancing agent, few analyses are available for the dental tissues. Hadjimarkos and Bonhurst (1959) report concentrations of 1.60 - 0.43 ppm. for enamel and 0.35 - 0.43 ppm. for dentine. Concentrations of 1 - 10 ppm. for enamel, 10 - 100 ppm. for dentine and 1 - 10 ppm. for plaque are reported by Hardwick and Martin (1967).

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PAPER 16

ESTIMATION OF SELENIUM IN HUMAN DENTAL ENAMEL BY ACTIVATION ANALYSIS

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Caries Res. 4 : 179-187 (1970).

ESTIMATION OF SELENIUM IN HUMAN DENTAL ENAMEL BY ACTIVATION ANALYSIS

Selenium is an essential trace element for higher animals and is functionally related to vitamin E. Rosenfield and Beath (1945) have given a comprehensive review on the importance of selenium in nature. In biological tissue selenium levels have been found to range from 0.005 - 0.5 ppm. The dietary requirements of selenium are thought to be in the region of 0.1 - 0.15 ppm. but these levels have not been determined accurately. Surveys of seleniferous soils have shown that the majority of these contain less than 2 ppm. selenium and in no cases have levels greater than 100 ppm. been found.

Seleniferous soils have been reported in Ireland. Concentrations of selenium in County Limerick have been found to be of the order of 1 ppm. in limestone, 5-30 ppm. in shale and 500 ppm. in stream sediments.

Analysis of stream sediments in small areas of England and Wales have given maximal values ranging from 3.8 - 9 ppm. selenium compared with the normal background of 0.2 ppm. (Webb, Thornton and Fletcher, 1966).

A direct relationship between dental caries incidence and selenium uptake has been suggested by Hadjimarkos and Bonhurst (1958). Epidemiological studies in school-children in Wyoming by Tank and Storvick (1960) have corroborated this. This relationship, however, has not been substantiated by evidence in New Zealand (Cadell and Cousins, 1960). The conclusions reached in New Zealand

studies have been questioned by Hadjimarkos (1960) on the basis that the New Zealand investigation was carried out in areas which were deficient in selenium and that the threshold value of selenium intake necessary to influence caries susceptibility was not reached.

In animal studies Buttner (1963) has shown that the administration of 5 ppm. and 10 ppm. Na_2SeO_3 in drinking water during the period of tooth development increased the caries rate.

Selenium is found as a constituent of both enamel and dentine. Concentrations have been given for 0.43 - 1.60 ppm. for enamel and 0.35 - 0.43 ppm. for dentine (Hadjimarkos and Bonhurst, 1959).

Methods available for determining trace amounts of selenium

The levels of selenium which are thought to be present in normal biological tissue range from several parts per billion to less than a part per million so that only ultra-micromethods of analysis are suitable for their detection. Methods which have been employed include X-ray fluorescence, spectrophotometry, colorimetry, fluorimetry and neutron activation analysis.

The X-ray fluorescence method has not yet given sufficient sensitivity (Handley, 1960). The spectrophotometric method has been used in conjunction with isotopic dilution techniques to estimate microgramme quantities of selenium in organic matter, the limit of detection being 0.1 µg. (Blanchard and Leddicotte, 1959). The use of 3,3'-diaminobenzidine to form the piazselenol, an intense

yellow compound which can be measured colorimetrically, provides a means of determining 0.05 µg. of selenium (Cheng, 1956). The same compound has been used for a fluorimetric determination with a detection limit of 0.02 µg. and a sensitivity of 0.02 µg. in the range of 0.1 - 210 µg. selenium (Watkinson, 1960). Still greater sensitivity has been claimed using the technique of neutron activation. One group of workers claim the practical limits of detection to be as low as 0.001 µg. of selenium (Steinnes, 1967). In the present work an attempt has been made to modify existing and develop additional neutron activatinn analysis techniques for the estimation of selenium in human dental enamel.

Neutron Activation

Neutron activation analysis has been applied to the determination of selenium in a variety of matrices which include biological material (Bowen and Cawse, 1963), amino acids (Maxia and Rollier, 1967), and vegetable matter (Hadjimarkos, 1961). Both non-destructuve and radiochemical methods have been employed.

Table 1 shows the stable isotopes of selenium, their relative abundance and the radioactive nuclides produced by n, reactions. From this table it can be seen that only three nuclides Se^{75}, Se^{77m} and Se^{81} can be prepared with high specific activity. The use of Se^{77m} which is a pure gamma emitter with a half-life of only 17.5 seconds has been employed in purely instrumental methods of analysis. It

Table 1

Neutron produced isotopes of selenium

Stable Isotope	Relative Abundance (%)	Radioactive Nuclide	Activation cross section (barns)	Half Life	Activity produced by a flux of 10^{12}n/cm^2/sec. after activation for one half-life (mCi/g)	Mode of Decay (a)	Particle Energies (MeV)	Principal Energies (MeV)	Internal Conversion
^{74}Se	0.87	^{75}Se	0.23	121d	25	E.C. 100%	-	0.12-15% 0.14-54% 0.27-56% 0.28-23% 0.40-12.5%	0.6% 1.5%
^{76}Se	9.02	^{77m}Se	10	17.5s	97	I.T.	-	0.162	
^{78}Se	23.52	^{79m}Se	0.12	3.9m	2.9	I.T.	-	0.096	
		^{79}Se		6×10^4y		B	0.16		
^{80}Se	49.82	^{81m}Se	0.015	57m	1.5	I.T. 100%	-	0.10-8% 0.011-Se x-rays	92%
		^{81}Se	0.25	18m	25	B 100%	1.4		
^{82}Se	9.19	^{83m}Se	0.05	69s	0.46	B^-	3.4	1.01 2.02	
		^{83}Se	0.04	25m	0.04	B^-	1.5	0.35 2.34	

a = E.C. - Electron Capture or I.T. - Isometric Transition.

has the advantage of the high activity obtained and the rapidity of the method. One disadvantage is the very short half-life and also that many other short-lived unclides have similar gamma-ray energies.

Most methods of activation analysis involve the use of Se^{75} which has a long half-life ($t_{\frac{1}{2}}$ = 121 d) and which allows sufficient time for complete chemical separation from the other activities. One disadvantage is the length of activation time required to achieve the specific activity shown. Irradiation times of 7-14 days have been employed to reach 5 - 10 per cent of this activity thus reducing the sensitivity.

Se^{81} has also been employed for activation analysis. This gives a theoretical sensitivity equal to that of Se^{75} but requires a much shorter activation time. The disadvantages of this isotope are its relatively short half-life (18m) and also that it is a pure beta emitter, an additional difficulty is the complexity of its decay curve. Despite difficulties a sensitivity of 5×10^{-9} g. has been claimed in biological materials. Other isotopes Se^{79m} ($t_{\frac{1}{2}}$ = 3.9m) and Se^{81m} ($t_{\frac{1}{2}}$ = 57m) have also been used but the limits of detection and sensitivity of these isotopes are poor.

Methods and Materials

Preparation of Tooth Samples

Caries-free adult permanent teeth were obtained from different areas of the British Isles. It was important to

avoid contamination during extraction and wherever possible teeth were removed surgically to avoid enamel contamination with extraction forceps. Enamel was separated from dentine by removing the dentine with tungsten carbide burs. When small fragments of enamel were prepared polythene sheets were used to protect the enamel from contamination during fragmentation.

Approximately 100 mg. samples were used and these were sealed in polythene bags for short irradiations and in aluminium cans for long irradiations.

Preparation of Standards

Approximately 20 µg. of selenium were used as a standard in the form of ultra-pure (99.999%) selenium dioxide. The impurities present were cobalt (1.0 ppm.), copper (0.3 ppm.), iron (1.0 ppm.), nickel (2.0 ppm.) and lead (0.4 ppm.).

For short irradiations standards were prepared by accurately weighing several drops of a standard selenium dioxide solution onto one inch polythene squares which have been thoroughly cleaned with nitric acid and distilled water. The water was evaporated slowly under an infra-red lamp avoiding overheating of the polythene squares which when dry were folded and sealed in polythene bags.

For long irradiations selenium dioxide crystals were irradiated in a small aluminium can. A standard solution containing approximately 20 µg. of selenium per ml. was made up after irradiation and 1 ml. aliquots of this being used as a standard.

Irradiation

(i) Selenium - 81 detection.

Short irradiations of 20 minutes and one hour were carried out at the Universities of Liverpool and Manchester Research Reactor at Risley. Neutron fluxes of 0.4×10^{12} $n/cm^2/sec.$ and 1.2×10^{12} $n/cm^2/sec.$ were obtained depending on the placing of the samples in the reactor. The samples were processed immediately after removal from the reactor and counted after a separation time of approximately 1 hour.

(ii) Selenium - 75 detection.

For this isotope the enamel samples and standards were irradiated for two weeks in a reactor with a thermal neutron flux of 10^{12} $n/cm^2/sec$ (BEPO Harwell). The samples were processed and counted after a cooling period of 1 week.

Preparation of Irradiated Samples for Analysis

After irradiation, samples were transferred to 100 ml. beakers containing 1 ml. of selenium carrier solution. A minimum volume (10 - 20 drops) of concentrated nitric acid was added to each beaker which was then covered by a watch glass and placed on a hot plate. When the tooth enamel had dissolved, the solution was taken almost to dryness to remove excess nitric acid, the beakers cooled and the residue dissolved in 5 ml. of water.

Method I: Precipitation and Ion Exchange.

The radioactive solutions were transferred to 50 ml. graduated centrifuge tubes containing 10 ml. of concentrated

hydrochloric acid and made up to 20 ml. with water. Centrifuge tubes were placed in an ice bath and a stream of sulphur dioxide gas passed rapidly into each solution for two or three minutes. The dark red selenium precipitate which formed was centrifuged, washed, dissolved in a minimum of concentrated nitric acid and the solution taken almost to dryness to remove excess nitric acid. The residue was dissolved in 5 ml. of water and made 0.1N with respect to hydrochloric acid, poured onto a Dowex 50 - X8 cation exchange column (12 cm. high and 1½ cm. in diameter) in the chloride form and eluted with water. The first 25 ml. of the eluate was collected in a beaker, made 6N with respect to hydrochloric acid and selenium precipitated as before. The precipitate was suction-filtered onto a 1" Whatman filter paper using a polythene filter stick and washed with dilute hydrochloric acid, water and acetone. The filter paper containing the precipitate was carefully placed on an aluminium planchet and dried under an infra-red lamp.

Method II: Distillation and Precipitation.

The distillation apparatus consisted of a 50 ml. pear shaped distillation flask with a side arm to which a condenser was connected. The outlet from the condenser led into a 50 ml. receiver tube with a side arm which was connected to a water pump. A thin glass tube drawn to a fine point was fitted into the neck of the flask to provide an air inlet. The flask was heated by an electro-thermal bunsen and the receiver tube was cooled in an ice bath.

Prior to carrying out a distillation 1 ml. of sodium hold-back carrier and a few anti-bumping granules were added to the distillation flask and 10 ml. of water added to the receiver tube, so that the end of the outlet from the condenser passed below the surface of the water. The radioactive solution was transferred to the distillation flask, 10 ml. of hydrochloric acid and 0.5 ml. of liquid bromine were added and the air inlet tube placed in position. Water was passed through the outer jacket of the condenser. The vacuum water pump was then adjusted until a steady stream of air was bubbling through the liquids in the flask and the receiver tube. The solution in the flask was then heated to boiling by the electrothermal bunsen and the temperature controlled so that distillation did not become too vigorous. Some bromine distilled initially but after 5 - 10 minutes a yellow liquid, selenium tetrabromide, could be seen in the condenser. The distillation was continued until only a few ml. of liquid remained in the flask. The electrothermal bunsen was switched off and removed, the outlet disconnected from the vacuum pump and the tube removed from the receiver tube. Suplhur dioxide was passed into the distillate which had collected in the receiver tube, until the bromine colour of the solution disappeared. An equal volume of hydrochloric acid was added to make the solution approximately 6N with respect to hydrochloric acid, and more sulphur dioxide passed rapidly into the solution to precipitate selenium, which was washed and dried as in Method I. When the longer-lived isotope (Se^{75}) was being counted, the precipitate was centrifuged, washed with water,

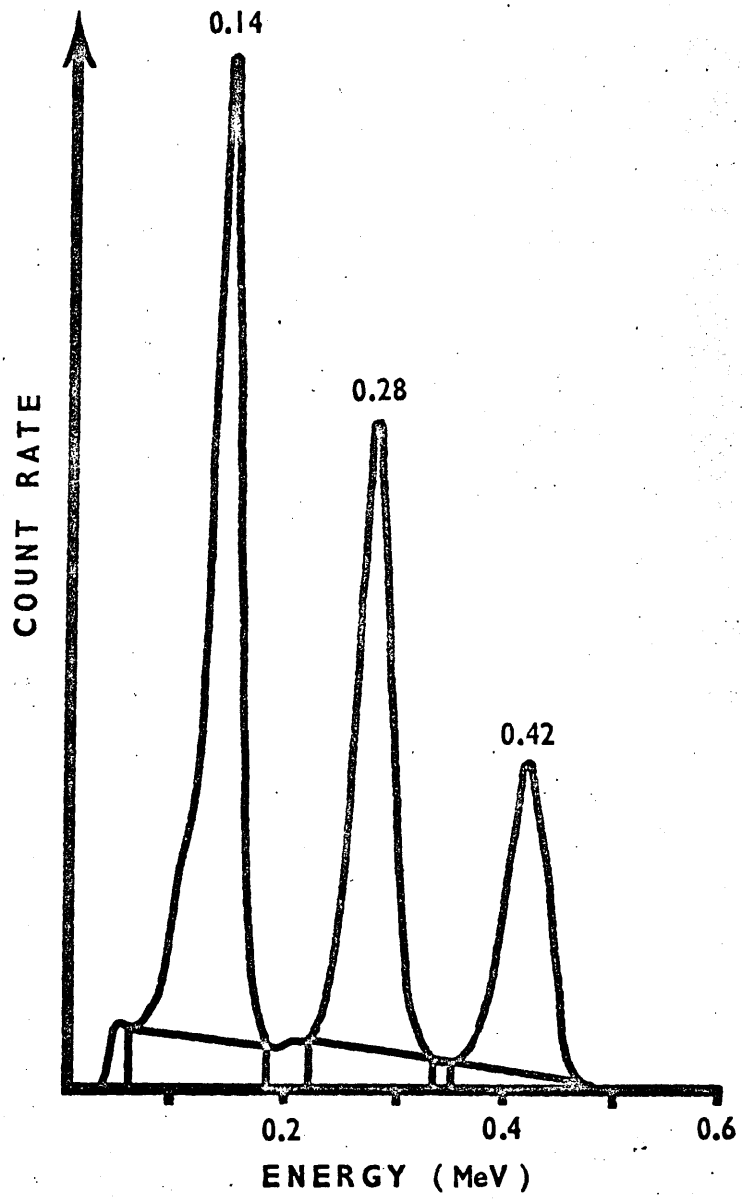

Fig. 1 Se-75 standard gamma-ray spectrum.

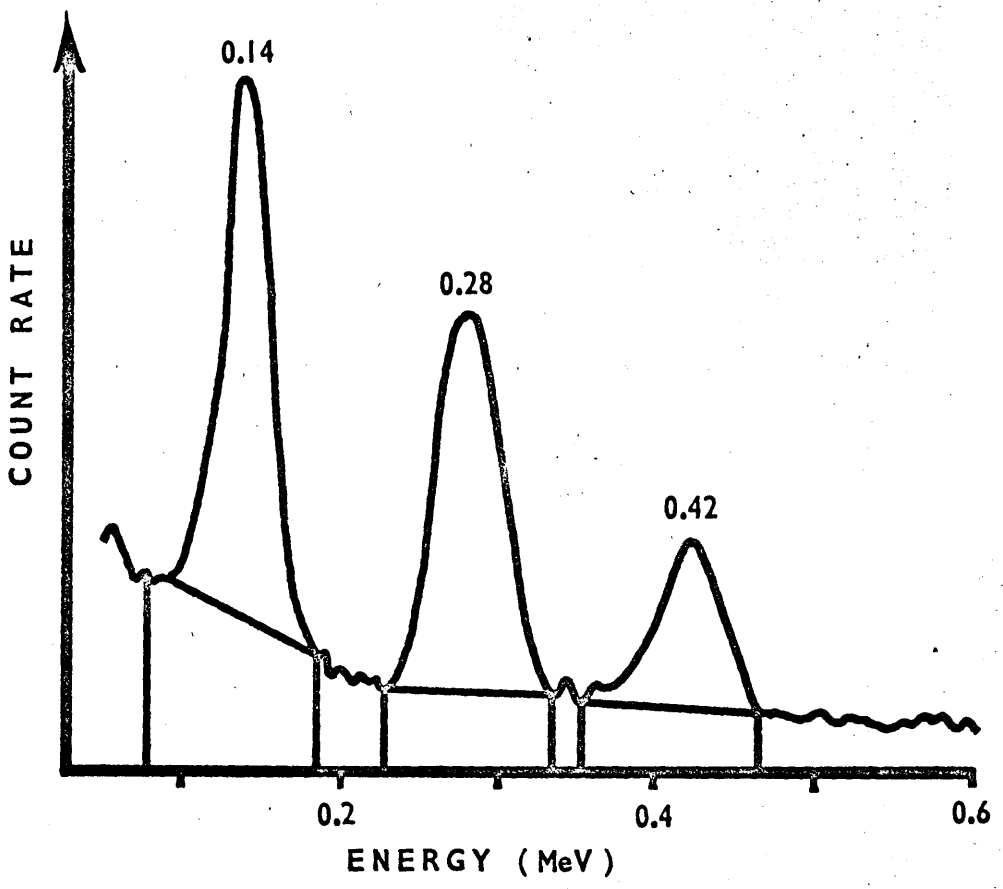

Fig. 2 Gamma-ray spectrum of selenium separated from irradiated tooth enamel by Method I.

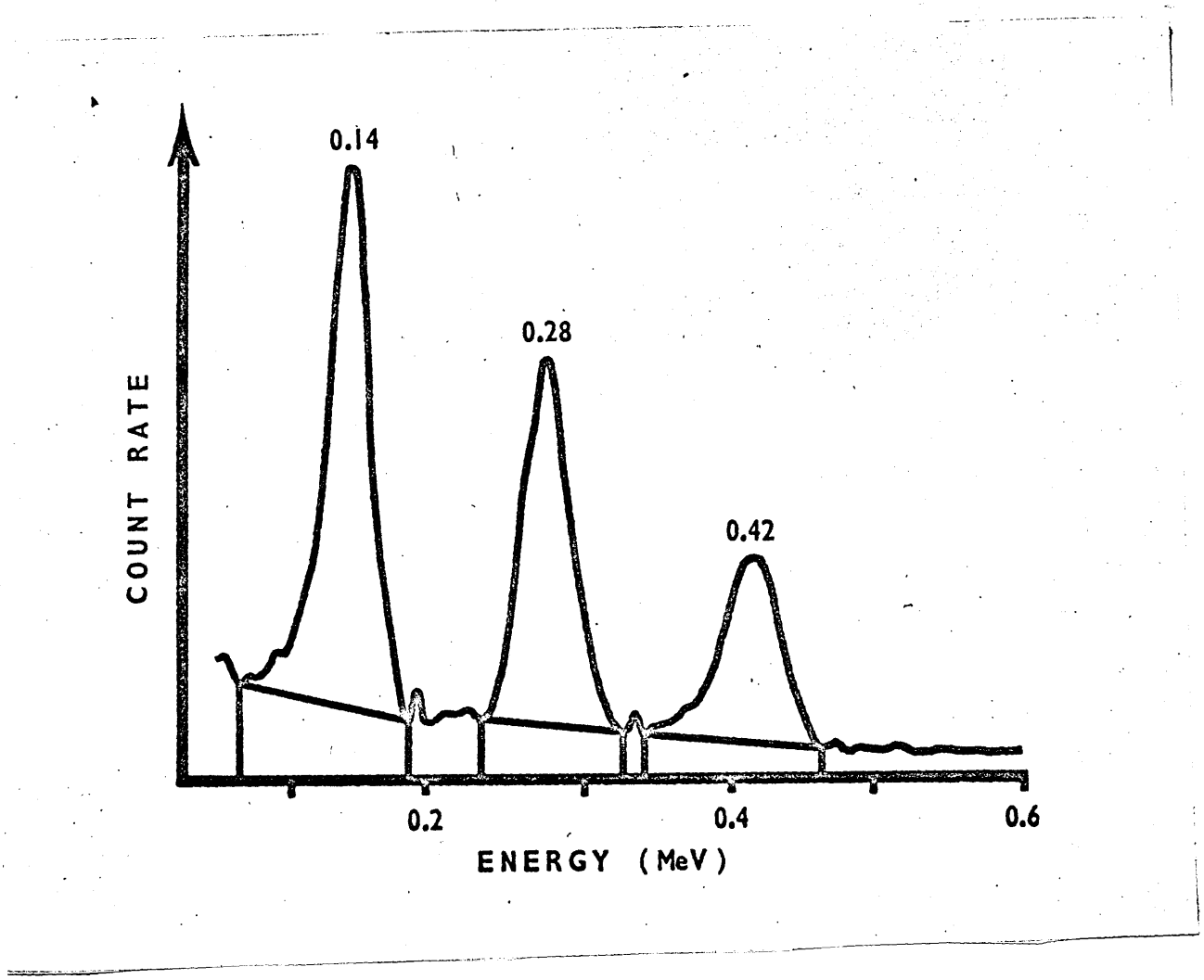

Fig. 3 Gamma-ray spectrum of selenium separated from irradiated tooth enamel by Method II.

redissolved in nitric acid and reprecipitated before finally washing and drying.

Results

The gamma spectrum of Selenium 75 obtained from selenium precipitates separated from irradiated teeth by Methods I and II are shown in Figs. 1, 2 and 3. The activity was determined from the areas of the photopeaks and the selenium content of the tooth enamel samples estimated.

The six enamel samples from Edinburgh and Manchester analysed had selenium concentrations in the range of 0.213 ppm. - 2.08 ppm. with a mean value of 0.872 ppm. (Table 2).

Table 2

Selenium Concentration in Adult Permanent Teeth using Selenium-75

Area	Se concentration (ppm.) of tooth enamel	Mean
Edinburgh	0.213 0.318 1.96	0.830
Manchester	0.274 0.388 2.08	0.914

Discussion

Both selenium-81 and selenium-75 can be used for the determination of selenium by neutron activation analysis

with a sensitivity of 0.001 μg. The use of selenium-81 is limited to cases where it is possible to achieve a high degree of chemical purity. Bowen and Cawse (1963) have employed this method successfully in the determination of selenium in biological material employing a technique similar to Method II. These workers did not report interference from silicon-31 which was found to be the major source of contamination in the present analysis of enamel. As the presence of silicon-31 did not alter the selenium-75 gamma spectrum it was possible to use this isotope in the estimation of selenium. There are, however, two main disadvantages associated with the use of selenium-75:

(a) The length of irradiation.

(b) The presence of phosphorus 32 ($t_{\frac{1}{2}}$ = 14d) due to the relatively large amount of phosphorus in enamel which renders the samples very active after an irradiation period of 2 weeks.

The selenium concentration of the anamel samples was found to fall within the range 0.213 ppm. - 2.080 ppm. with a mean value of 0.872 ppm. These values are in reasonable agreement with the range of 0.43 - 1.60 ppm. quoted by Hadjimarkos and Bonhurst (1959).

Summary

The selenium concentration of enamel from samples of human teeth was determined by a method of neutron activation analysis. The samples, weighing approximately 100 mg. were irradiated in a reactor at a thermal neutron flux of 1.2×10^{12} n/cm^2/sec. for one hour for Se^{81} and for one week

for Se^{75}. The selenium activity was measured after radiochemical separation. The samples analysed were obtained in Manchester and in Edinburgh. The mean level of selenium found in the teeth from Edinburgh was 0.830 ppm. and the range 0.213 - 1.96 ppm. In the teeth from Manchester the mean level was 0.914 ppm. and the range from 0.274 ppm. - 2.08 ppm.

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P A P E R 17

MERCURY IN THE DENTAL SURGERY

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Quarterly Dental Review,

Vol. 4, No. 3, 82-85, 1970

MERCURY IN THE DENTAL SURGERY

Since the introduction of silver and copper amalgams at the beginning of the 18th century the presence of mercury used in their preparation has given rise to much discussion regarding its possible hazard to health. Each year the number of amalgam fillings has risen and it would appear that this will continue. While dental personnel continue to work with and are exposed to mercury, it has not yet been clearly established that mercury is an occupational hazard. Reports by Grossman and Dannenberg (1949), Dalhamn (1953) and Frykholm (1957) have suggested that there is no serious risk to dental personnel from volatilized mercury in the dental surgery. However, Nixon and Smith (1965) reported from a study on mercury in dental surgery assistants that mercury must be considered as a hazard in the dental surgery and that care must be taken in its handling, a point made previously by Knapp (1963). This danger has again been emphasized in a recent paper by Cook and Yates (1969) in which they report a case of fatal mercury intoxication in a dental surgery assistant.

Early assessments of the mercury hazards have been based mainly on the determination of mercury vapour concentrations in the air of dental surgeries. Even with this type of assessment some reports are conflicting. The American Conference of Government Hygenists gave the Threshold Limit Value (TLV) of mercury as 0.1 mg/m^3. Vesterberg (1946) reports concentrations as high as 0.20 mg/m^3 while Frykholm (1957) reports concentrations as low

as 0.10 mg/m^3.

It is not generally realized that mercury vapourizes readily at room temperature, the equivalent vapour pressure being approximately 10 gm/m^3. The facts that the vapour equilibrium at room temperature is above the recommended maximum allowable concentrations and that the vapour pressure doubles if the ambient temperature rises by 10°C contribute to the ease with which mercury can be absorbed by inhalation (Bidstrup, 1964).

Joselaw et al. (1968) in a study state that many of the previous reports have a limited value as the instruments employed were capable of measuring only the vapour form of mercury. These authors state that a fine particulate mercury amalgam powder may remain suspended in the air of the surgery and produce considerably more contamination than the vapour alone. In their investigation they found that 14 per cent of surgeries showed mercury concentrations in excess of what is considered good hygienic practice. While this percentage is small, it does imply that there is a needless exposure in these cases although the relationship between mercury levels and mercury intoxication is not established.

Mercury Levels in Human Tissue

The mercury levels in human tissues have been determined by Howie and Smith (1967), some of which are shown in Table 1. Tissues such as skin, nail and hair which are exposed to general and atmospheric contamination tend

to have higher values. Two tissues which have a higher value than average are lung and kidney.

Table 1

Mercury Levels in Human Tissues (ppm.)

Tissue	Mean
Blood	0.09
Bone	0.45
Hair (head)	5.52
Heart	1.76
Kidney	9.03
Liver	3.66
Lung	2.55
Nail (finger)	7.27
Nail (toe)	2.40
Teeth	3.22

Absorption of Mercury

Metallic mercury is absorbed into the body mainly by the inhalation of mercury vapour into the lungs. Soluble salts of mercury may pass via the digestive tract into the circulating system. Mercury can also be absorbed through the skin. Perspiration of the skin increases the absorption by dispersing mercury over a wider area (Laug, Vos, Knuze and Umberger, 1947).

Sollmann (1957) states that mercurous compounds and metallic mercury are oxidized and that the mercuric salts form soluble compounds with the proteins, sodium chloride and alkalis of the blood and tissue fluids. Absorbed mercury leaves the blood rapidly and is excreted mainly in

the urine and faeces but also in bile, sweat, saliva and milk.

Although the biological effects of mercury depend on the form in which it is absorbed and on the rate of absorption, Hughes (1957) presents evidence that the principal reaction of absorbed mercury, from whatever source, is with thiols forming mercury mercaptide and that variations in distribution and effect are dependent upon this reaction.

Lipoid solubility plays an important part, although the amount of mercury present at any one site must be very small in presence of excess thiol.

The action of metallic mercury is explained on Hughes' evidence by its lipoid solubility which permits universal distribution followed by oxidation to reactive mercury salts. Hughes assumes that metallic mercury exists transiently as the metal dissolved in blood lipoids and in this form is transported to sensitive tissues where it is then oxidized to mercuric salts and fixed in the tissues.

The amount of mercury which can be absorbed without giving rise to symptoms is not known. It would appear from the available evidence that there is individual variation and also that the rate of absorption plays an important part.

Poisoning by Mercury

Mercury poisoning may be acute or chronic. The acute type, which is rarely seen, usually results from oral ingestion. Mercury poisoning of occupational origin is generally of a chronic type which occurs as a result of

absorption from the skin or from inhalation.

Signs and Symptoms

The classical signs and symptoms of mercury poisoning are gingivitis and stomatitis accompanied by excessive salivation or metallic taste. There may also be erethism and tremor. The onset of symptoms is often insidious and apart from the tremor may be ignored by the patient or attributed to other causes. Erethism, which is a particular emotional instability, is characterized by irritability, excitability and irrational outbursts of temper. There is sometimes shyness and depression. Symptoms such as these are sometimes attributed to an anxiety state, ignoring the possibility of mercury poisoning. Hypersensitivity to mercury from amalgam fillings is uncommon but by no means rare. This response often takes the form of a cutaneous reaction which may vary from a slight erythema to a severe generalized eruption and is discussed in a report by Fernstrom, Frykholm and Huldt (1962).

Mercury from Amalgam

Frykholm (1957) has stated that mercury vapour may be given off from amalgam from the time it is inserted into the tooth and until it sets, i.e. until it is bound into the stable phase of amalgam. This exposure is only for a short period and is of the order of 0.02 - 0.20 mg Hg/m^3.

Once the setting is complete it has been considered that mercury may be absorbed systemically from an amalgam

restoration either via dentine and pulp, by surface solution in saliva, or by accidental ingestion (Frykholm and Odeblad 1955).

Absorption from Dentine

Early authors such as Witzel (1899) stated that 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) gave support to this view. Appelbaum (1929) reported that sections under amalgam restorations showed a precipitate of metallic sulphide which he believed to be mercury sulphide. In discoloured dentine Massler and Barber (1953) found mercury by spectrographic analysis in concentrations of 0.5 - 5 per cent. In apparently normal dentine remote from the discoloured area smaller amounts of mercury were found.

The penetration of mercury into dentine has been investigated in vitro by Nixon (1959) using radioactive mercury. The results of analysis of dentine showed that, after a period of 40 days, this element was not found in dentine beyond a distance of 1 mm. from the original cavity margins. The concentration of mercury was greatest, as would be expected, 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 concluded in this study that systemic absorption from the

tooth pulp via the dentinal was unlikely to occur.

Migration of Mercury Ions from Amalgam

a) Teeth.

The levels of mercury in enamel of human teeth have been measured by Nixon, Paxton and Smith (1965). In unerupted teeth the mercury content was less than 0.1 ppm. In sound erupted teeth the mean mercury content was 2.61 ppm. In erupted teeth where the enamel was in immediate contact or in close proximity to silver amalgam the mercury content ranged from 279 - 1600 ppm.

The higher mercury content of the enamel of erupted teeth can be attributed to mercury either from mercury vapour during the setting phase of amalgam or by migration of mercury ions from existing amalgam fillings.

Systemic absorption of mercury from amalgam restorations has also been considered as a possible source of increased mercury contamination in the tissues. Post-morten material taken from the liver, kidney and muscle tissue of cadavers with amalgam fillings present in the mouth indicated that in none of these was the mercury concentration higher than that given for normal limits.

The results of analysis in the case of a 50-year-old woman with ten amalgam restorations present is given in Table 2.

b) Gingical Tissues.

The migration of mercury ions from amalgam restorations into the gingival tissues has also been investigated by the

Table 2

The results of analysis in the case of a 50-year-old woman with ten amalgam restorations present.

Tissue	Mercury (ppm)
Muscle	14.2
Kidney	12.5
Liver	1.21

author. The results of this study showed that the mercury content of the subgingival tissues ranged from 0.36 ppm. - 4.4 ppm. in tissues not in direct contact with amalgam restorations. In interproximal tissues in direct contact with amalgam restorations the concentration of mercury ranged from 29 ppm. - 189 ppm.

Mercury in Urine

The significance of mercury in the urine has been investigated. Storlazzi and Elkins (1941) report on urine samples collected at various times after the insertion of amalgam fillings. These observers noted increased urinary mercury excretion at the time of and about a week after their insertion. They doubted that the amount of mercury absorbed could cause mercurialism. A further study by Hoover and Goldwater (1966) confirmed that existing dental amalgams do not appear to be an important source of mercury absorption and excretion; even when freshly prepared amalgams are swallowed there is no signficant rise in mercury in urine. However, Livingston (1966) showed a

slight increase on the second day.

Handling of Mercury

Most dental personnel are careless in handling this material during dispensing of mercury and in the preparation of amalgam. As mercury is a heavy metal and of low viscosity, it is almost impossible to pour without splashing or spilling. The size of the droplets resulting from spills varies from large to microscopic, but the droplets are mostly difficult to see with the naked eye. Most surfaces are not wetted by mercury so that the drops tend to roll away and enter small holes and cracks subsequently contributing mercury content of the air "from almost impregnable positions' Biram (1957). An examination by Nicholson, Stark and Soelberg (1968) of capsules used in the mechanical preparation of amalgam showed that contamination of the operating area by mercury was almost unavoidable. Where disposable capsules were used this was reduced by 25 per cent. This point of leakage of mercury from capsules has also been stressed by Jorgensen (1970).

Further contamination of working areas occurs after trituration during the stage of expressing excess mercury from amalgam. It is at this point that some of the greatest spillage takes place. In practice, after expressing the mercury this is shaken into the pestle or a box or in many cases on to the working surface. From there it is generally swept on to the floor where, as stated above, it becomes a source of mercury vapour. This was observed in the case of mercury poisoning quoted by Nixon and Smith (1965) where

approximately 3 ml. of mercury was found on the floor beside a convector heater.

Precautions when Handling Mercury

Amalgam should not be mulled in the hand and when excess mercury is removed by means of a squeeze-cloth the mercury should not come in contact with the hands. If it is allowed to do so free mercury comes in contact with the skin and excess collects under the finger-nails. Excess mercury from the amalgam should be disposed of carefully and even when shaken into a large container care should be taken to avoid spillage. When mercury is spilled it should be swept up immediately. Excess mercury should be kept under water or in a sealed container away from heat. Waste amalgam should also be placed in a sealed container.

Mercury contaminated squeeze-cloths should not be left lying about and should also be placed in sealed containers. Smooth floors and working surfaces help to reduce those situations in which mercury can accumulate. Vacuum cleaners must not be used to clean up spilled mercury as this increases the atmospheric concentration of mercury vapour.

The use of encapsuled materials with predetermined amounts of mercury will help to reduce the possibility of mercury spillage. Adequate ventilation must be present in the surgeries to help reduce mercury vapour levels.

Mercury is a potential hazard to health in the dental surgery and should be treated as such. Reasonable precautions during handling and disposal can do much to

reduce this hazard.

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P A P E R 18

HISTOLOGICAL EXAMINATION OF T.D. 71 (I) AND (II)

G. S. Nixon and D. C. Smith

Quintessence International

Nos. 4 & 5, 15-20, 21-25, 1971.

HISTOLOGICAL EXAMINATION OF T.D. 71 (I)

The purpose of this study was to evaluate the pulpal reaction of the filling material T.D. 71.

The study was divided into two parts:

A) A short-term study of 4 days between the insertion of the filling material and the sacrifice of the dog to evaluate the immediate response.

B) A longer term study of 45 days to examine the longer term response.

Selection of Dogs

Pure-bred Beagle dogs were used throughout. These dogs were bred and maintained under strictly controlled conditions and only those dogs which were considered by the veterinary officer to be healthy were employed. The dogs selected were all between the ages of 5 months - 9 months to avoid pulpal changes which are often found in older dogs. It was considered important that the condition of the teeth should be similar as possible and all teeth were examined before preparation for hard tissue defects. Wherever possible litter mates were used to allow better comparison.

Preparation of Cavities

Cavities were prepared according to a standardised technique on the labial surfaces of the teeth. All cavities were prepared under intravenous anaesthesia. A round bur was used to penetrate the enamel and the cavity outline

completed with a fissure bur. A new bur was used for each cavity which was cut under a continuous flow of water. The cutting speed was 2,000 rpm. The depth of the cavity was varied to allow assessment at differing depths as was the position of the cavity on the tooth surface. In some of the young dogs the pulps were exposed inadvertently due to the small thickness of dentine. In every dog Zinc Oxide Eugenol cement was inserted in addition to T.D. 71 to determine the local response. After preparation the cavities were dried with a pledget of cotton to avoid desiccation of the dentine.

Extraction of Teeth and Preparation of Sections

Immediately after death the teeth were removed from the jaws by making a "V" shaped cut into the basal bone and immersed into formalin solution. By doing so a large area of the pulpal tissue was exposed which allowed rapid fixing. Sections were prepared by standard histological techniques and stained with haemotoxylin and eosin.

Histological Evaluation

The inflammatory responses were graduated according to that suggested by Baume and Fiore-Donno (1968) which were:

(a) Slight response - Indicated by a reduction or thinning of the odontoblast layer and included the first signs of inflammation as shown by haemorrhages and circulatory stasis in the sub-odontoblast region related to the floor of the cavity.

(b) Moderate response - Reduction of odontoblast

layer to one irregular layer associated with conspicuous signs of inflammation which involved localised hyperaemia or haemorrhage with presence of acute or chronic cells depending on interval.

(c) Severe response - Complete disintegration of odontoblasts. Arrest of dentine formation - acute or chronic pulpitis with presence of microabscesses.

Control Teeth

The majority of untreated teeth used as a control showed normal pulpal tissue (Fig. 1). It would appear from the examination of many untreated dogs' teeth that pulpal changes are liable to be present. Care, therefore, is necessary in interpreting the appearance of the histological appearance of the pulpal tissue. There often appears an increased vascularity of this tissue which is difficult to differentiate from the condition of hyperaemia in the human pulp. There are also present extravasated erythrocytes, a fibrin-like substance and reticulation as well as lymphocytes and plasma cells in areas of the pulp not associated with a cavity.

A) 4-Day Study

Four dogs whose ages ranged from 6-7½ months were used in this study. In each dog cavities were prepared in three of the canine teeth and in the third maxillary incisor (in the tables this tooth has been designated 12 to avoid confusion with the canine 13.

24 teeth were used for this study and 32 cavities

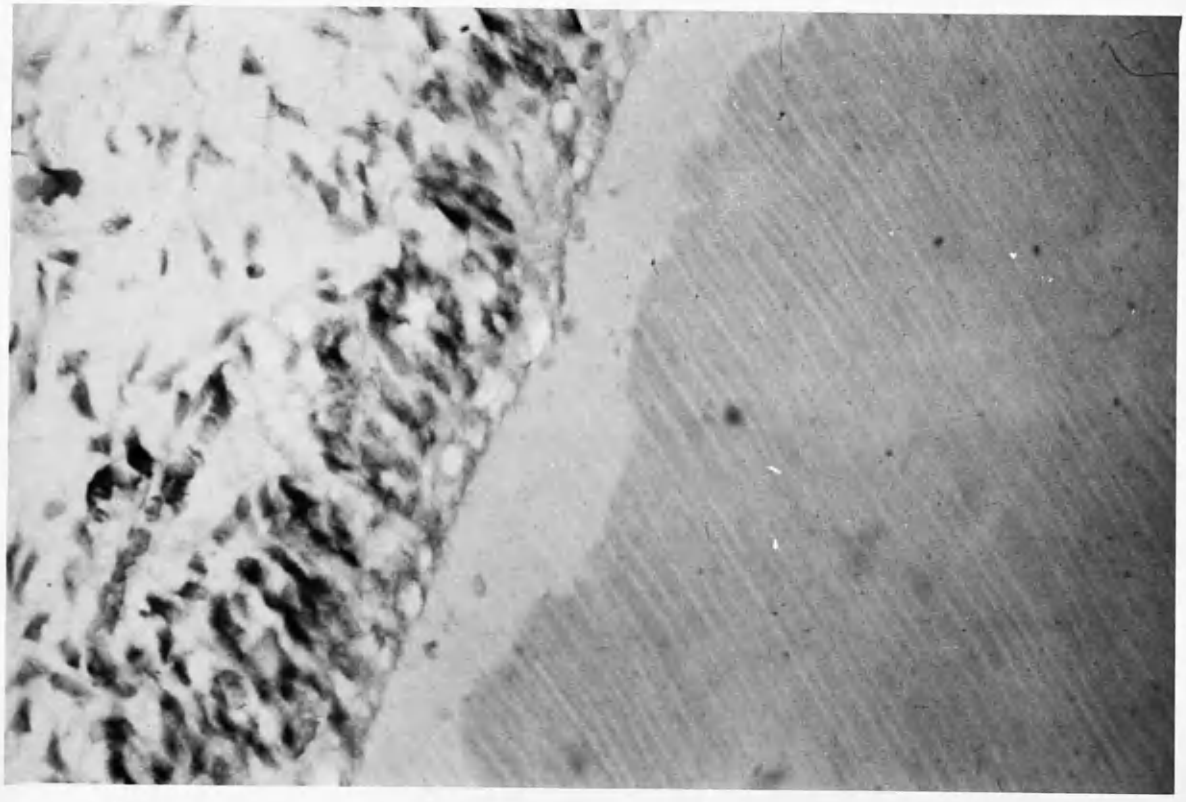

Fig. 1 Control - normal pulp.

Fig. 2 Vacuolisation of odontoblast layer.

prepared.

Preparation and Insertion of Material

The material was inserted immediately after mixing according to the instructions of the manufacturer. No variation of the mixing technique was used. Immediately after insertion the material was held in position with a celluloid matrix strip until the required time. The excess material was allowed to remain on the cavity and no attempt was made to polish the fillings. The supine position of the dogs and the accessibility of the cavities allowed the application of a matrix pressure similar to that in normal clinical use.

None of the cavities was lined prior to insertion of the material.

Results - Histological Evaluation

The results of the histological examination are shown in Tables 1 and 2 and summarized in Table 2(a). In this study the distance of the floor of the cavity from the pulp measured from zero to 1.00 mm. The early response of the pulp to both cavity preparation and insertion of either Zinc Oxide Eugenol or T.D. 71 is a vacuolization of the odontoblasts (Fig. 2). This was frequently associated with a reduction in the number of odontoblasts which corresponded to the tubules involved in cavity preparation. When this reaction was more severe there was haemorrhage into the odontoblast layer (Fig. 3). In these cases the reaction was mainly local and was confined to the area of the pulp

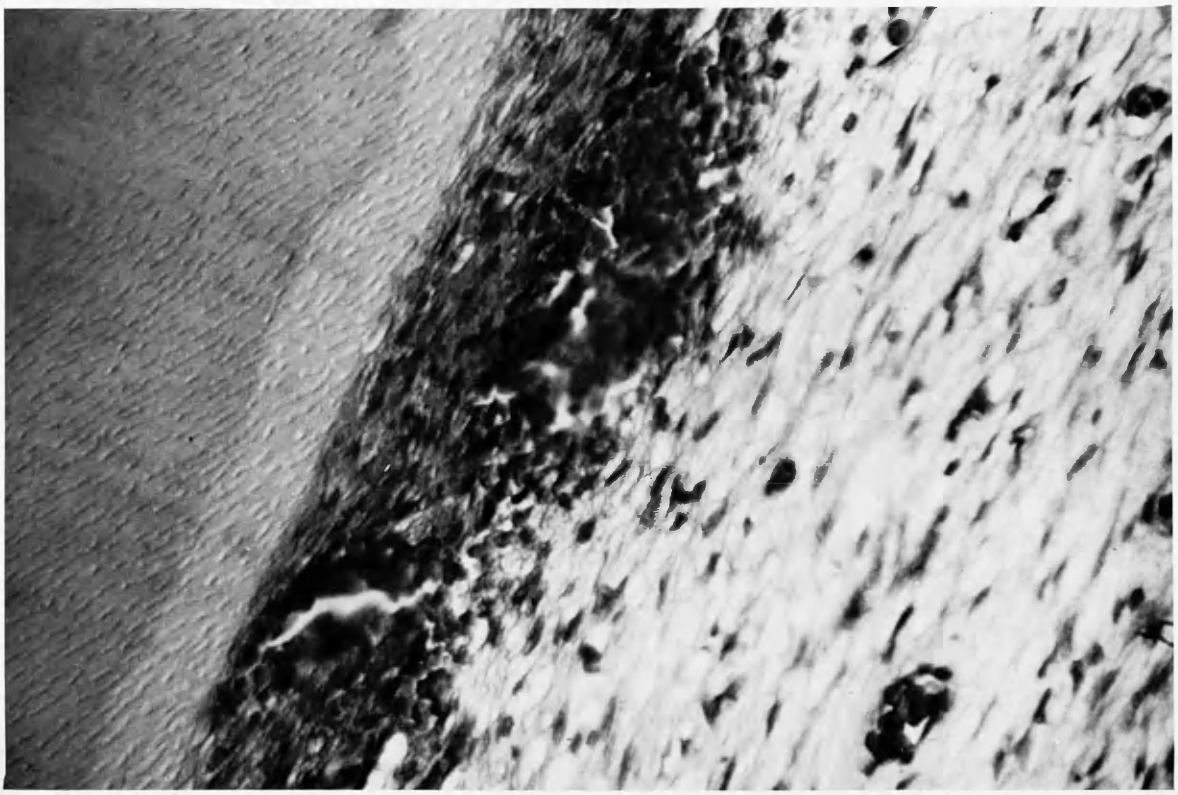

Fig. 3 Haemorrhage in odontoblast layer.

Table 1

Dog 729	Tooth	Cavity	ZnO	T.D.71	Control	Pulp Dist	Min.	Pre-dentine +	Pre-dentine -	Odont. Vac.	Odont. Red.	Haem.	Hyp.	W.B.C.	Comment
(1)	43	Inc.	X			0.84				X	X	X	X		
		Inc.		X		0.72									
(2)	13	Inc.		X		.96				X	X		X		Local response good. Slight gen. pulp. hyp.
(3)	12			X		.45	.15	X		X	X	X	X		Despite prox. to pulp response slight.
(4)	22			X		.36									
(5)	23	Inc.		X		Exp.						X	X	X	
		Gin.	X			Exp.						X	X	X	
(6)	23			X		.72									Mild response. Poor Section
Dog 7307 (7)	43	Inc.	X			.84				X	X				Pulp. reaction slight
		Gin.		X		.72									
(8)	13	Inc.	X			1.00		X					X		
		Gin.		X		0.72							X		Poor section
(9)	12			X		0.20				X	X	X		X	Moderate
(10)	22			X		.48							X	X	Moderate
(11)	23	Inc.	X			1.00									Poor section
		Gin.		X		.80				X	X		X		Slight response
(12)	23				X								X		

Table 2

Dog 731	Tooth	Cavity	ZnO	T.D.71	Control	Pulp Dist	Min.	Pre-dentine +	Pre-dentine -	Odont. Vac.	Odont. Red.	Haem.	Hyp.	W.B.C.	Comment
(13)	43	Inc.	X			.84				X					Little change in pulp.
		Gin.		X		.80									Poor specimen.
(14)	13	Inc.	X			.84									
		Gin.		X		.84				X	X				Little pulpal.
(15)	12			X		.60				X			X		
(16)	22			X		.48					X		X		Poor specimen.
(17)	23	Inc.	X			.84				X			X		Reaction slight.
		Gin.		X		.72				X	X		X		
(18)	23				X										Poor specimen.
Dog 732															
(19)	43	Inc.	X			.72							X		Little change in pulp.
		Gin.		X		.80							X		
(20)	13	Inc.	X			.48		X		X			X		Slightly less reaction.
		Gin.		X		.42							X		
(21)	12			X		.45			X				X		
(22)	22			X					X				X	X	
(23)	23	Inc.	X			.48									Expose.
		Gin.		X											
(24)	33				X								X		

Table 2a

Histological Findings - Group I - 4 Days

Dog	Age Months	Tooth	Residual Distance	Pulp Reaction
729	7½	43	.84	Moderate
		13	.96	Moderate
		12	.45	Slight
		22	.36	Section of little value
		23	Exp.	Severe
730	6	43	.72	Slight
		13	1.00	Degenerative change in pulp
		12	.20	Moderate
		22	.48	Moderate
		23	.80	Slight
731	7½	43	.80	Slight
		13	.84	Slight
		12	.60	Slight
		22	.48	Poor section
		23	.72	Slight
732	6	43	.80	Slight
		13	.48	Moderate
		12	.05	Severe
		22	Exp.	Severe
		23	.40	Slight

involved in cavity preparation. In these cases the reaction was designated as "slight". It was evident also that the effective depth of dentine, i.e. the thickness of dentine between the floor of the cavity and the pulp played an important part in the pulpal reaction. In general the greater this distance the less pulpal response. A thickness of dentine of 0.9 mm. appeared to be effective. There were however instances where distances of less than this showed a slight response (Section No. 3, Table 1). Nevertheless an overall picture of a generalised hyperaemia was evident in a number of sections in this study but as stated previously this overall increased vascularization was present in several control teeth.

A severe reaction of pulp tissue was demonstrated where there was an exposure into the pulp or the effective thickness of dentine was so thin (0.05 mm.) as to provide little protection.

HISTOLOGICAL EXAMINATION OF T.D. 71 (II)

B) 45-Day Study

Five dogs whose ages ranged from 5 months - 9 months were employed and the cavities prepared as before.

30 teeth were used for this study and 40 cavities prepared.

Preparation and Insertion of Material

The material was again prepared according to the manufacturers instructions but in this study the first part of each mix was placed in the third maxillary incisor; the second part in the maxillary canine and the third part in the mandibular canine. This technique was employed with each dog to determine if variations in pulpal response occurred with alterations of the time of insertion of T.D. 71 after mixing. Zinc Oxide Eugenol was again employed as a control filling.

Results - Histological Evaluation

The results were evaluated as before using similar criteria and these are detailed in Tables 3, 4 and 5. In the majority of filled teeth, the response of the pulp was favourable after 45 days and in none of the sections was severe damage observed. In most cases there was reduction of the odontoblast layer, and in one case aspiration of the odontoblast could clearly be demonstrated. The favourable response of the pulp could be shown by the consistent deposition of secondary dentine. This secondary dentine

Table 3

Dog 622A	Tooth	Cavity	ZnO	T.D. 71	Control	Pulp Dist	Min.	Pre-dentine +	Pre-dentine -	Odont. Vac.	Odont. Red.	Haem.	Hyp.	W.B.C.	Comment
(29)	43	Inc.		X		1.5							X		
		Gin.	X			1.9				X	X		X		
(30)	13	Inc.		X		1.3		X		X	X				Little change
		Gin.	X			1.2				X					
(31)	12			X		1.5				X	X				
(32)	22			X											
(33)	23	Inc.		X											
		Gin.	X												
(34)	23														Normal Pulp
Dog 672A															
(35)	43	Inc.	X			1.2		X			X				
		Gin.		X		1.4		X			X				Slight response
(36)	13	Inc.	X			.72		X							
		Gin.		X		1.2		X							
(37)	12			X											
(38)	22														
(39)	23	Inc.	X			.8									
		Gin.		X		.7			X		X				Slight reaction
(40)	33				X										

Table 4

Dog 760A	Tooth	ZnO	T.D.71	Depth	Min.	Control	Odont. Vac.	Odont. Red.	Hyp.	Haem.	W.B.C.	Pre-dentine +	Pre-dentine −	
(41)	43	X		1.2										Little change. No diff. in cavities.
			X	1.5										Mild reaction.
(42)	13	X		Exp.							X		X	Exp. Partial pulp abscess
			X	.72									X	
(43)	12		X											Exposed. Section no good.
(44)	22		X	.72			X	X				X		Irreg. growth of dentine. Hyperchromatic.
(45)	13	X		.36				X				X		Little difference in resp.
			X	.36								X		TD 71 resp. slightly better than ZnO.
(46)	33					X								

Table 5

Dog 868A	Tooth	Cavity	ZnO	T.D. 71	Control	Pulp Dist	Min.	Pre-dentine +	Pre-dentine -	Odont. Vac.	Odont. Red.	Haem.	Hyp.	W.B.C.	Comment
(47)	43	Inc.	X			.60			X				X		Slight hyp. Irreg.
		Gin.		X		1.10		X							Dentine. Mod. response.
(48)	13	Inc.	X			.72		X			X				Slight reaction. Sec. dentine
(49)	12	Gin.		X		1.00		X			X				
				X		1.1		X			X				Slight
(50)	22			X		.96		X			X				Sec. dentine. Slight
(51)	23	Inc.		X		1.4		X							Slight
		Gin.		X		.60		X							Slight
(52)	33				X										
Dog 869A															
(53)	43	Inc.		X		1.8		X							Slight
		Gin.	X			1.8		X							
(54)	13	Inc.		X		1.3		X							Slight
		Gin.	X												
(55)	12			X		1.2					X				Slight
(56)	22			X		.84					X				Slight
(57)	23	Inc.		X		.96									Slight
		Gin.	X			.96									Slight
(58)	33				X										

was a common feature and evidence of irregular growth could be seen (Fig. 4). Cavities with zinc oxide also showed secondary dentine similar to that of T.D. 71. In some of the teeth a mild degree of hyperaemia was demonstrable. A calcio-traumatic line was demonstrated in some sections indicative of damage to odontoblasts (Fig. 5). The thickness of dentine between the floor of the cavity and the pulp in this study ranged from 0.36 to 1.9 mm.

Conclusions

After 45 days the T.D. 71 restorations exhibited a fairly smooth intact surface with good aesthetic appearance. The marginal adaptation was excellent with little sign of surface contraction.

As with any material the effective depth of dentine remaining between the floor of the cavity and the pulp plays an important part in determining the pulpal reaction. The trauma of cavity preparation is also a contributory cause.

The findings of this study have shown that T.D. 71 produced a slight to moderate effect on the dental pulp in dogs teeth and that where the effective thickness of dentine between the pulp and the cavity was greater than 0.8 mm. this effect was minimal. Even in some cavities where the effective dentine thickness was less than this the response was slight. Only in three cavities was a severe response demonstrated and in these additional contributory factors may have been present.

The results of the first study were reinforced by the longer study of 45 days. The response of the pulp over

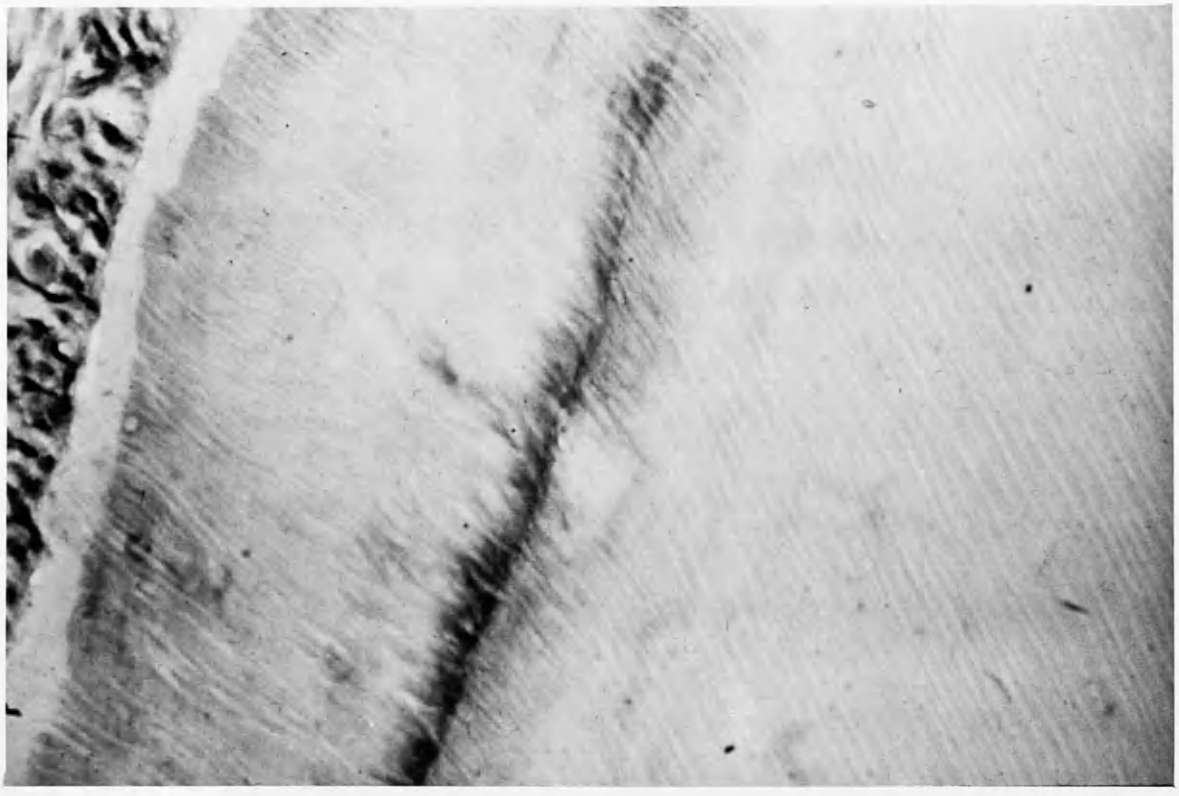

Fig. 4 Secondary dentine showing relationship to cavity floor.

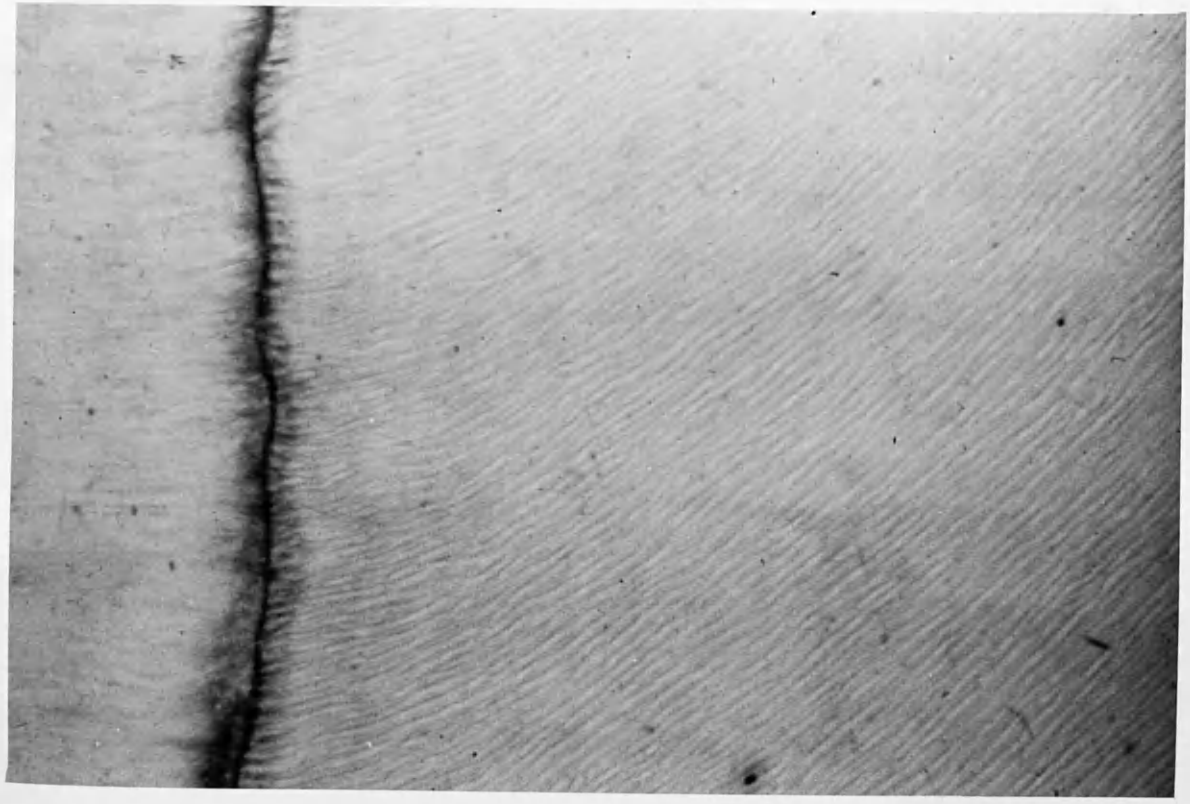

Fig. 5 Calciotraumatic line.

this period was again slight with secondary dentine being laid down and no localized or generalised severe response demonstrable. The time of insertion after mixing did not produce any demonstrable difference on histological examination.

Summary

1. The pulpal reaction produced by T.D. 71 is slight to moderate and the material is well-tolerated in the dogs teeth.
2. While it might be unnecessary to line shallow cavities it would be advisable to line deeper cavities with a material such as a calcium hydroxide lining.

P A P E R 19

MERCURY HAZARDS ASSOCIATED WITH HIGH SPEED MECHANICAL AMALGAMATORS

G. S. Nixon and T. C. Rowbotham

British Dental Journal,

Vol. 131, pp. 308-311, 1971.

MERCURY HAZARDS ASSOCIATED WITH HIGH SPEED MECHANICAL AMALGAMATORS

The possibility of leakage of mercury from capsules during high-speed amalgamation is investigated. Different types of capsules are examined and it is shown that mercury losses do occur, contributing to the hazard of mercury in the dental surgery.

The paper by Cook and Yates (1969) describing a case of mercury poisoning which resulted in the death of a dental surgery assistant has increased the awareness of the dental profession to the hazard of mercury in the dental surgery. This problem has been discussed further by Nixon (1970) and Gronka et al. (1970).

The hazard to health from mercury in dentistry is mainly three-fold:

(1) Systemic absorption of mercury through direct contact with the skin.

(2) Inhalation of mercury vapour given off at room temperature.

(3) Inhalation of airborne particles of mercury or mercury contaminated particles.

Whilst absorption by the skin can be eliminated by avoiding direct handling or touching either liquid mercury or freshly mixed amalgam, the second and third hazards pose a more difficult problem. Automatic mixing using low mercury ratios avoids the necessity for squeezing out excess mercury through a cloth and eliminates contact with the fingers (Fig. 1). Prepared pre-proportioned

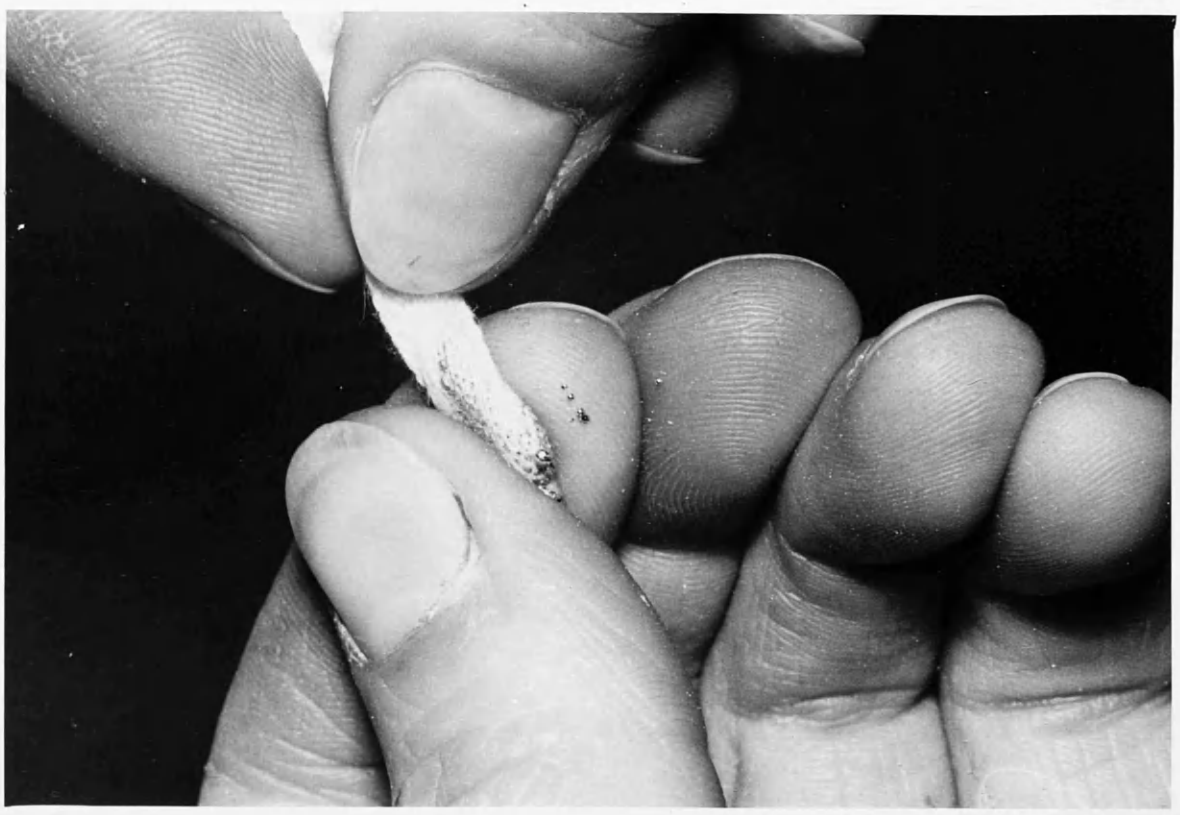

Fig. 1 Excess mercury squeezed from amalgam.

encapsulated mercury and alloy have the additional advantage of eliminating accidental spillage of mercury which lodges in inaccessible places such as cracks and crevices on the floor, around skirting boards and under the bases of dental chairs and cabinets. In these situations mercury is almost undetectable, is continuously present and is accumulating with the passage of time.

Although automatic mixing eliminates skin contact there is an additional hazard of possible leakage from the capsule while mixing, a possibility which has been suggested by Jørgensen (1970). In these cases mercury could be dissipated from the capsule through the air. These fine droplets would then be scattered over a large area which would greatly increase the surface area of mercury from which vaporisation could take place.

Numerous capsules are available for the mechanical preparation of amalgam. There are the pre-proportioned encapsulated type with known quantities of alloy and mercury. Such capsules are intended to be used once but because of their relatively high cost many dentists re-use them a number of times by re-filling with a measured amount of mercury and alloy. The other main types of capsules are those which are intended for re-use and into which weighed amounts of alloy and mercury are placed. Amalgamation is completed with or without a pestle in the capsule.

While all capsules have some form of sealing mechanism there is the possibility that this seal may not be sufficiently effective and could permit the escape of mercury.

This investigation was undertaken to examine the possibility of such a hazard occurring.

Materials and Methods

Leakage of Mercury from Capsules

The efficacy of the seal to mercury in an unused capsule of the pre-proportioned encapsulated type (Amalcap[1]) was determined by drilling a small hole in the round end of the capsule and removing the alloy. Mercury was added to give a total weight of mercury of approximately 1 g (including the weight of mercury in the capsule envelope). The hole was completely re-sealed with a cellulose cement and after 'squeezing' the mercury from the envelope, the capsule was 'mixed' for 10 seconds in a Silamat[1] machine. Five unused capsules were treated in this way and any mercury loss was recorded (Table 1).

Table 1

Alloy removed from unused Amalcap capsule. Mercury added to give approximately 1 g. Capsule resealed with cellulose cement

	Before	After	Loss (g)
1	2.2061	1.4460	.7601
2	2.2146	1.4291	.7855
3	2.1942	1.5041	.6901
4	2.1983	1.8814	.3169
5	2.2554	1.6519	.6035

Amalcap capsules which had previously been used on one

1. Vivadent, Schaan, Leichtenstein.

occasion were similarly treated and the mercury loss was again measured (Table 2). The mercury loss from S.S. White[1] and W.S.[2] capsules intended for re-use was also measured by 'mixing' 1 g. of mercury in a Silamat and W.S. amalgamator respectively for 10 seconds (Tables 3 and 4). Each capsule was filled and 'mixed' on 10 occasions.

Table 2

Mercury Losses from 10 Amalcap Capsules previously used once and re-filled with 1 g mercury only

Sample	1	2	3	4	5
Loss Grammes	.7916	.8075	.8528	.5420	.0054
Sample	6	7	8	9	10
Loss grammes	.0326	.2681	.6494	.6373	.0369

Table 3

Maximum Mercury Loss from 5 S.S. White capsules filled 1 g. mercury and each 'mixed' 10 times

Sample	1	2	3	4	5
Loss Grammes	.0005	.0003	.0001	.0004	.0002

Table 4

Maximum Mercury Loss from 4 W.S. Capsules filled 1 g. mercury and each 'mixed' 10 times

Sample	1	2	3	4
Loss Grammes	.0629	.0108	.0155	.0201

1. S.S. White Dental Mfg. Co. (GB) Ltd.
2. Walter Schnitt, W. Germany.

Loss with Alloy/Mercury

It was appreciated that, while mercury loss could occur when mercury was used alone, the preparation of amalgam in clinical practice could produce different results. Therefore 10 new encapsulated amalgam capsules (Amalcap) were weighed before and after mixing for 10 seconds in a Silamat machine and any loss recorded (Table 5).

Table 5

Loss from new Amalcap Capsules with Alloy

Sample	1	2	3	4	5
Loss grammes	nil	nil	nil	.0001	nil
Sample	6	7	8	9	10
Loss grammes	nil	.0001	nil	nil	nil

Losses due to the alteration of alloy/mercury ratios were determined using both Amalcap capsules and W.S. capsules. As the present trend is towards the use of lower mercury ratios which eliminates the need to remove excess mercury from the amalgam a 5 parts alloy : 4 parts mercury weight ratio was employed. Some practitioners however, prefer a softer mix and the experiment was repeated using 5 parts alloy : 6 parts mercury to determine if a greater loss occurred with the higher mercury ratio. In both experiments a pre-amalgamated alloy was used. The results of these are given in Table 6 and Table 7. The effects of an alloy with a longer amalgamating time was determined using a ratio of 7 parts alloy : 8 parts

mercury and mixing in a W.S. capsule and amalgamator (Table 8).

Measurement of Mercury Vapour

The concentration of mercury vapour in the air and floor around the Silamat mixer was determined using a Hanovia mercury vapour detector No. E 3472. The mixer was covered in a polythene cover of 0.2 m^3 capacity and the mercury vapour concentration measured under this cover. The results are given in Table 9.

Results

The mercury loss from unused pre-proportioned encapsulated capsules ranged from 0.3169 to 0.7855 g (Table 1). When the capsules had been used on a previous occasion these losses were from 0.0054 to 0.8528 g (Table 2). With mercury alone in the S.S. White capsule the losses were from 0.0001 to 0.0005 g (Table 3) and from 0.0108 to 0.0629 g with the W.S. capsules (Table 4). When the standard alloy/mercury Amalcap capsule was used the losses were smaller with the maximum loss in Amalcap of 0.0001 g in two capsules (Table 5). When the alloy/mercury ratio was altered the losses with the used Amalcap capsules were 0.0007 to 0.0053 g with a 5:4 ratio and 0.0004 to 0.0079 g for a 5:6 ratio (Table 6). With alteration of alloy/mercury ratios in the W.S. capsules the losses were from nil to 0.0007 g (Table 7). When the slower amalgamating alloy was used in the 7:8 ratio the losses were from 0.0003 to 0.0419 g (Table 8).

Table 6

Losses from used Amalcap Capsules - Re-filled with Alloy and Mercury

(A) 5 alloy : 4 mercury										
Sample	1	2	3	4	5	6	7	8	9	10
Loss grammes	.0022	.0021	.0027	.0050	.0053	.0007	.0021	.0012	.0008	.0024
(B) 5 alloy : 6 mercury										
Sample	1	2	3	4	5	6	7	8	9	10
Loss grammes	.0012	.0012	.0079	.0004	.0009	.0004	.0007	.0030	.0006	.0006

Table 7

Losses from W.S. Capsules filled with Alloy and Mercury

(A) 5 alloy : 4 mercury										
Sample	1	2	3	4	5	6	7	8	9	10
Loss grammes	nil	.0002	nil	.0001	nil	.0004	nil	nil	nil	.0001
(B) 5 alloy : 6 mercury										
Sample	1	2	3	4	5	6	7	8	9	10
Loss grammes	.0002	.0001	nil	.0001	.0002	nil	.0001	nil	nil	.0007

Table 8

Losses with Slower Amalgamating Alloy in W.S. Capsules using 7 parts Alloy : 8 parts Mercury

Sample	1	2	3	4	5	6	7	8	9	10
Loss grammes	.0273	.0154	.0065	.0198	.0005	.0003	.0029	.0419	.0009	.0004

The mercury vapour concentrations are shown in Table 9. In the open clinic a reading of 10 $\mu g/Hg/m^3$ was obtained with increases up to 60 $\mu g/Hg/m^3$ around the mixer. When the mixer was covered the readings were over the maximum scale reading, i.e. 200 $\mu g/Hg/m^3$.

Table 9

Mercury Vapour Measurements

In open clinic	10 $\mu g/Hg/m^3$
Floor cracks near mixer	20-30 $\mu g/Hg/m^3$
Rubber pads at base of mixer	40-60 $\mu g/Hg/m^3$
Inside mixer	30-40 $\mu g/Hg/m^3$
Silamat mixer inside polythene cover with Amalcap capsule in position	110 $\mu g/Hg/m^3$
Silamat mixer inside polythene cover with mercury in capsule	140 $\mu g/Hg/m^3$
Silamat mixer inside polythene cover after mixing	200 $\mu g/Hg/m^3$

Discussion

The results of these experiments show clearly that the danger of mercury spillage during mechanical amalgamation is real. The seal of the capsule, particularly of capsules not intended for re-use, is not as complete as it appears. This is shown in cross-section, when passage of mercury between the capsule wall and cap can be demonstrated (Fig. 2).

Using a stroboscope, triggered to give a single flash and a Silamat mixing machine which vibrates at 4,500 vibrations per minute, the ejection of mercury from a capsule

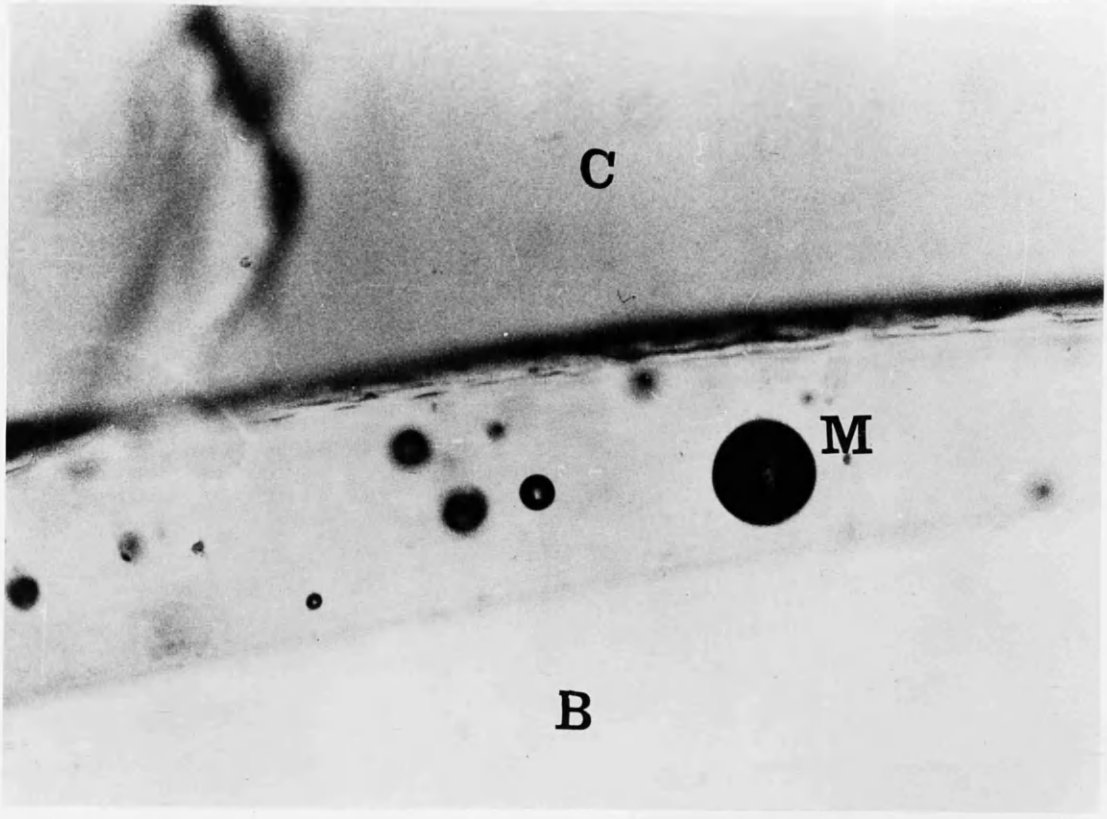

Fig. 2 Cross-section demonstrating mercury (M) between cap (C) and body (B) of used Amalcap capsule. X30.

can be shown clearly (Fig. 3).

When mercury is mixed with alloy, as in clinical practice, the loss is fortunately not so great, due to the rapid amalgamation which takes place particularly when pre-amalgamated alloy is used. Even when the mercury/alloy ratio was increased there was no significant increase in loss. There was a greater loss, however, when a slow amalgamating alloy was used due perhaps to the longer wetting time between the mercury and the surface of silver-tin alloy, leaving more free mercury available for ejection from the capsule in the early stages of mixing.

It is unwise to re-use Amalcap type capsules which are intended for use once only. Even though the loss of mercury was between 5 mg and 0.8 mg for a mix with pre-amalgamated alloy this could result in a loss of as much as 0.5 g of mercury during a week if 20 mixes a day were carried out. If an alloy with a longer mixing time were used the loss could be over 4 g for a week or 184 g for the year.

Gronka et al. (1970) drew attention to the dangers of mercury poisoning in dental surgeries and to the dangers of spilled mercury. They also stated that the smaller the droplets the greater the danger and that methods of cleaning of the surgery affect the problem; floor mops used in the surgery should not be used elsewhere as mercury is likely to be dispersed. They point out the danger of air conditioning and state that carpets and rugs should be avoided in surgeries as they quickly became contaminated.

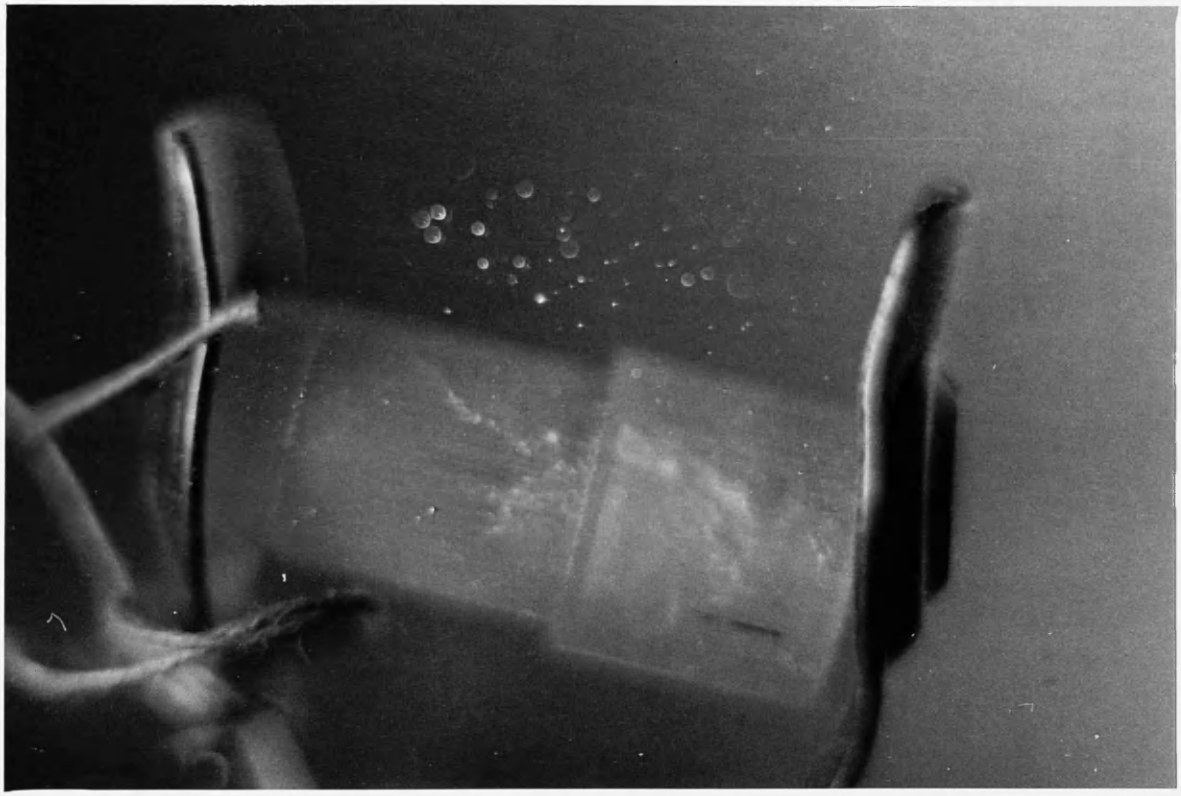

Fig. 3 Mercury globules being thrown from used Amalcap capsule in Silamat mixer.

It must be stressed that ventilation of dental surgeries is all important as evidenced by the trials with the mercury vapour meter. Where there is a free current of air the level of concentration of mercury is not particularly high even when spilled mercury is found. When the surgery is enclosed, the level of mercury may be increased and become a hazard to health. At present the maximum allowable concentration of mercury is 100 $\mu g/m^3$ for a 40-hour week exposure, but some authorities consider this figure too high and a figure of 50 $\mu g/m^3$ has been suggested. Particular attention should be paid to floors and surroundings which should be free from cracks or crevices. An examination of all amalgamators in the clinic showed, in each case, mercury lodged in inaccessible areas.

All waste amalgam should be kept under water or in sealed containers which prevents mercury vapour from being given off. Mercury should not be handled and leak proof capsules must be used for mechanical amalgamation. Consideration should be given in the design of amalgamators to sealing the outer cover and producing smooth rounded surfaces which can be readily cleaned.

ACKNOWLEDGEMENTS

We wish to thank Mr. R. T. Bagley, Safety Officer in the University of Manchester, for his assistance in measuring the mercury vapour concentrations.

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P A P E R 20

UPTAKE OF COPPER AND MOLYBDENUM IN THE HARD DENTAL TISSUES OF THE RAT

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Unpublished 1972

UPTAKE OF COPPER AND MOLYBDENUM IN THE HARD DENTAL TISSUES OF THE RAT

Copper and Molybdenum

Copper and molybdenum are both considered to be essential trace elements in animal nutrition. They are necessary in the diet for growth and health and their absence causes a deficiency state to develop.

The presence of copper in plants and animal tissue was not considered to be of importance in the early 1900's and it was not until later that its nutritional value became apparent. Copper has been shown to be essential in the growth of rats, and that deficiency of this element in the diet caused certain diseases in cattle and sheep.

Dick (1956) carried out a number of investigations in Australia into chronic copper poisoning in sheep, a condition which is treated by administration of molybdenum and sulphate. These investigations were of importance as they demonstrated that the retention of copper was influenced not only by molybdenum but also by inorganic sulphate in the diet.

With improvements in analytical techniques the exact distribution of copper in animal tissues and plants was established. It was found that copper is important in pigmentation, keratinization of wool, bone formation, reproduction, myelination of the spinal cord and also hematopoiesis. In the human body, copper occurs at low levels in the endocrine glands while higher levels are

found in the liver, heart, kidneys, hair and brain. Brudevold and Steadman (1955) using a spectrometric technique showed that the concentration of copper in erupted human teeth was in the range of 12-30 ppm. independent of age. Neutron activation analysis used by Nixon and Smith (1962) on permanent human teeth gave a mean value of 9.5 ppm. for the outer enamel layer and 11.3 ppm. for the inner enamel layer.

Molybdenum is an essential nutrient for animal tissue and a number of plants, such as axotobacter and aspergillus niger. Areas where the soil has a deficient molybdenum content give poor crops, but when the soil is treated with molybdenum salts the crops thrive.

The disease "teart" which occurs in grazing animals gave the first evidence of the metabolic importance of molybdenum. "Teart" indicated by severe diarrhea, is caused by excessive levels of molybdenum in the herbage and is treated with supplementary copper sulphate. Because copper poisoning in sheep can be treated by molybdenum and sulphate therapy, it was proposed that there was a relationship between molybdenum, copper and sulphate. Investigations have shown that a copper-molybdenum-inorganic sulphate interrelationship occurs in a number of animal species.

Evidence has shown that molybdenum has an effect on caries, for example in New Zealand high molybdenum levels in soils and drinking water reduced the occurrence of caries. Two adjacent cities, Hastings and Napier, were studied, both having the same dietary habits and the same fluoride levels in their drinking waters, but differing rates for the

occurrence of caries. Napier vegetables contained more molybdenum than those of Hastings and it was suggested that this was responsible for the low incidence of caries at Napier. This subject has been fully reviewed by Jenkins (1967).

Molybdenum is not concentrated in any particular organ in the human body, although higher levels are found in the liver and kidneys than in any other body organs. The levels of molybdenum found in the liver, kidney, bones and skin can be altered by an increase or decrease in the dietary molybdenum level. The body requires molybdenum to form the enzyme xanthine oxidase which converts xanthine to uric acid. The concentration of the enzyme decreases if molybdenum deficiency occurs.

Plants absorb nitrogen as nitrates which are converted to nitrites before they can take part in amino acid synthesis. This conversion of nitrate to nitrite is carried out by the enzyme nitrate reductase of which molybdenum is a constituent. In molybdenum deficiency, the nitrate accumulates in the plant as it is no longer converted to the nitrite, this high nitrate level can make the plant toxic to farm animals.

Although great interest has been shown in the important role played by molybdenum in the prevention of caries, little is known of its concentration in dental tissue due to analytical difficulties. Healy and Ludwig (1963) showed that whole permanent teeth from Napier and Hastings contained 0.034 ppm. and 0.032 ppm. molybdenum respectively, deciduous teeth from Napier contained 0.069 ppm. and from

Hastings 0.046 ppm. The enamel from permanent teeth in Scotland was shown to contain 0.026 - 0.12 ppm. by Nixon (1967).

Physiological Relationships between Molybdenum and Copper

Pastures which contain a high concentration of molybdenum and which cause the disease in cattle known as 'teart', occur in a number of areas, for example in Somerset. Although teartness had been known for over a hundred years a cure was not found until the 1930's. Only ruminants suffer from 'teart' disease, which is characterised by severe diarrhea and loss of condition. If cattle are left on 'teart' pasture for any length of time they may be permanently affected. They may even 'scour' themselves to death.

Muir (1936) suggested that the possible causes of teartness were:

(1) Bacteria - on removal of the cattle from teart land they cease to 'scour', but if bacteria were the cause the scouring would persist.

(2) Parasites - no abnormal parasites were found in the affected animals.

(3) Particular herbs - herbs which flourish on teart land are also found in non-teart areas.

(4) Water supply - frequently teart and non-teart areas have a common water supply.

(5) Unknown constituent in the herbage - which appeared to be the most likely cause.

Ferguson (1943) analysed herbage from teart and non-teart areas by a spectrographic technique. The only difference found between teart and non-teart herbage was in the molybdenum content, which was found to be greater in the teart regions. When land was treated with sodium molybdate cattle quickly developed symptoms of teart again indicating that molybdenum was responsible.

The most satisfactory method of healing this condition was by copper sulphate either by feeding or drenching.

Further evidence of the relationship between molybdenum and copper

The effect of molybdenum on copper retention in sheep was found by Dick (1952) to be greater in sheep fed chaffed lucerne hay than those fed chaffed oaten hay. However, when potassium sulphate was added to chaffed oaten hay to give the same sulphate level as lucerne hay, the effects of both diets were the same. It was concluded from these studies that the effect of molybdenum on copper retention in the liver depended on the sulphate level of the diet (Dick 1953).

Further experiments were carried out on sheep fed on a diet of a known copper intake and varying amounts of molybdenum in the form of ammonium molybdate. As the molybdenum content of the diet increased the liver copper content fell. The fall in the copper content was initially steep and then levelled out as the molybdenum content approached 30 mgm. per day. The next step was to determine

if the amount of liver copper, for a particular intake of copper and molybdenum, was related to the sulphate intake (Dick 1954). Sheep were fed diets adjusted to a known sulphate and copper intake, and at each molybdenum level additional amounts of sulphate, in the form of potassium sulphate solution, were added. As the sulphate intake was increased the liver copper content fell, at all molybdenum levels. It was shown graphically that the change in the liver copper content and the logarithm of the sulphate intake was linear.

Hence, molybdenum exerts a limiting effect on copper retention in sheep only in the presence of sulphate. Neither molybdenum nor sulphate alone interfere with copper retention and the effectiveness of either increases to a maximum as the intake of the other is increased.

These studies showed that an animal's copper status depends not only on the copper intake but also on the molybdenum and sulphate intakes. Therefore, high liver copper levels in sheep, which cause chronic copper poisoning, occur with moderate copper intakes and very low dietary levels of molybdenum. Whilst copper deficiency, due to loss of the animal's copper reserves can occur on normal copper and high molybdenum and sulphate intakes (Wynne 1956).

Although there is a large amount of evidence for the molybdenum-copper-sulphate interrelationship, no definite mechanism has yet been proposed.

Dick (1956) proposed that sulphate interferes with the transport of molybdenum across the cell membranes, if

the sulphate concentration is sufficiently high this transport is prevented. The molybdenum blocked at the membrane impedes the transport of copper across the membrane and if the concentration of blocked molybdenum is sufficiently high the copper transport is prevented.

Animals fed on a low sulphate diet show a ready absorption of molybdenum from the intestinal tract into the blood stream and tissues. The molybdenum in the blood passes through the ultra-filter of the kidney glomerulus, the ultra-filtrate of which passes to the kidney tubule, where most of the molybdenum is re-absorbed and returned to the blood. As the sulphate level of the diet increases, the sulphate concentration in the ultra filtrate of the kidney glomerulus will be higher and will prevent the re-absorption of molybdenum. This will cause higher levels of molybdenum in the urine but lower levels in the blood, a point which was observed in sheep fed on high sulphate levels.

Absorption of Copper

For animals on a diet of high levels of molybdenum and sulphate the amount of copper absorbed from the alimentary tract and accumulated in the liver will be insufficient to compensate for normal losses of copper by excretion. Hence, the animal's copper reserves will be depleted and a copper deficiency will occur. The blood copper level will be unaffected, unless the level of molybdenum in the diet is sufficiently high so that, in spite of a high sulphate

intake, some molybdenum is absorbed to establish a high concentration of molybdenum at membranes where copper is normally excreted. When this occurs both copper absorption and excretion are impeded. The copper deficiency at sites where it normally functions stimulates the mobilization of copper in the blood. When the copper level in the blood reaches a sufficiently high level any further mobilization of stored copper is prevented by a mass-action effect. At this stage, the level of copper in the liver will cease to fall but physical signs of copper deficiency will develop. This is observed in practice.

Most studies of the molybdenum-copper interrelationships were concerned with ruminants. Mills (1960) studied the interaction in non-ruminant animals, for example the rat. Ruminants fed on a diet supplemented with molybdenum showed reduction in growth, failure in haemoglobin synthesis, and skeletal abnormalities. As these symptoms disappear when dietary copper increases they must result from some interference in the copper metabolism.

Mills' studies indicated marked differences of the effect of the copper-molybdenum interrelationship between ruminants and non-ruminants. In the rat, molybdenum increases copper accumulation in the liver and prevents its use in the body tissues, whilst in sheep molybdenum causes copper depletion. Differences also appear with dietary sulphate, in sheep on a high molybdenum and sulphate diet there is a decrease in the liver copper level, whereas in the rat sulphate prevents accumulation of copper in the liver, caused by high levels of molybdenum. These

differences are explained by the different action of sulphate in the two species. In ruminants, sulphate is reduced to sulphide by the micro-organisms of the reticulo-rumen, but in the rat limited sulphate reduction occurs only in the caecum and colon.

Further studies have shown the molybdenum in the diet can affect the activity of a number of enzyme systems in the liver and kidney which are concerned in the metabolism of sulphur compounds.

In the rat's liver, the activity of the sulphide oxidising system is reduced by molybdate, which suggests that the detoxication of sulphide may be restricted in tissues of high molybdenum content. High levels of molybdenum in the liver also influence the activity of alkaline phosphatase, the activity of which increases in the liver but falls in the kidney.

Similar investigations were carried out into the effects of molybdenum on sulphide metabolism in sheep. Sheep fed molybdate and sulphate had higher sulphide levels in the rumen than those fed only sulphate. The presence of molybdenum and sulphate in the diet was also responsible for a reduction of soluble copper in the aqueous phases of the rumen and abomasum. It has been suggested that this reduction was due to the precipitation of insoluble cupric sulphide. Mills (1960) considered this suggestion was unlikely. Although there is a reduction in the copper level in both the rumen and abomasum, any appreciable quantity of sulphide only occurs in the abomasum if the rumen content exceeds 0.4 μ mole/ml., at other times the sulphide level

is negligible and is constant, irrespective of the presence of molybdenum. Hence, this low sulphide level makes the precipitation of cupric sulphide unlikely. Therefore, the relationship between soluble copper level and sulphide level is not clear, but the changes in the sulphide content may be due to different products from the sulphur metabolism, for example sulphur amino acids or mercaptans, which may react with copper to form insoluble products.

The metabolic changes occurring in rats fed on molybdenum are probably caused by failure of copper utilization in the tissues and there is no evidence that molybdenum interferes with the copper intake in the digestive tract. In the case of ruminants fed on molybdenum and sulphate diets, the deficiency of copper in the tissues and the low levels of copper in the aqueous phases of the rumen and abomasum may be due to changes in the digestive tract which restrict copper absorption.

The increased sulphide level in the rumen may be due to the effect of molybdenum on sulphur metabolism in the digestive tract.

The mechanisms suggested by Dick and Mills for the copper-molybdenum interrelationship did not consider the possibilities that molybdenum may inhibit the activity of copper containing enzymes or may be responsible for the fixing of copper in an unavailable form in the tissues. Scaife (1956) prepared copper containing proteins with several properties of true enzymes from sheep's hide, and showed the molybdenum had an inhibiting action on a number of these proteins.

Rat Experiments

Rats used in the experiments were albino rats of the Harvard strain. The rats were mated in the ratio 2 female to 1 male, and were maintained on a normal stock diet and tap-water. The females were separated and caged individually after ten days.

When the litters were 16 days old, the females and litters were put on a cariogenic diet with deionized water. At 20 days old the pups were weaned and randomly distributed between the experimental groups. During the period of the experiment, the experimental animals were fed on a cariogenic diet and deionized water containing varying concentrations of molybdenum, copper and sulphate.

The rats were maintained on the experimental diet for 25 days and killed on the 45th day of life. The heads were removed and kept in the refrigerator until defleshing was carried out, after which the teeth were extracted for analysis. During the experiment the fluid intake of the rats was recorded daily and their weights weekly.

Cariogenic Diet

During the period of the experiment, the rats were maintained on the following cariogenic diet and chemical salt mixture from which all copper and sulphate had been removed.

Cariogenic diet:

Icing sugar	72%
Casein	20%
Chemical salts (copper and sulphate free salts)	4% (see below)
Vitamins in oil	2%
Vitamins in water	2%

Chemical salt mixture

Calcium carbonate	($CaCO_3$)	160 gm.
Potassium hydrogen orthophosphate	(K_2HPO_4)	286 gm.
Magnesium chloride	($MgCl_2$)	15 gm.
Sodium chloride	(NaCl)	134 gm.
Ferric citrate	($C_6H_5O_7Fe.5H_2O$)	11 gm.
Potassium iodide	(KI)	0.3 gm.
Zinc chloride	($ZnCl_2$)	1.3 gm.
Cobaltous chloride	($CoCl_2$)	0.1 gm.

Experimental Diets

A series of experiments were undertaken on rats in which the deionized water contained varying concentrations of molybdenum, molybdenum and sulphate, molybdenum and copper or molybdenum, copper and sulphate. The experiments were not carried out together, but over a period of months. This was necessary due to the large number of rats required. Because of this, the molybdenum, copper and sulphate experiment had to be carried out in two parts, part one consisting of groups 1 to 6 and part two groups 7 to 12.

The following table gives a concise summary of the experiments.

Level of Mo in drinking water (ppm)	Level of Cu in drinking water (ppm)	Level of SO_4 in drinking water (ppm)	Mo conc. in in sample (ppm)	Cu conc. in in sample (ppm)
0	0	0	Below detection limit	1.16
0.1	0	0	0.145	1.28
0.1	0	0	0.160	1.84
0.1	0	0	0.37	0.78
0.1	20	0	0.12	3.52
0.1	40	0	Below detection limit	4.57
0.1	0	20	0.40	1.75
0.1	0	40	Below detection limit	1.02
0.1	20	20	0.13	2.71
0.1	40	20	0.16	4.47
0.1	20	40	0.13	4.56
0.1	40	40	0.14	5.75
1.0	0	0	2.96	1.40
1.0	0	0	1.65	1.70
1.0	0	0	8.82	1.24
1.0	20	0	0.60	3.30
1.0	40	0	1.65	5.20
1.0	0	20	4.08	1.02
1.0	0	40	5.92	1.91
1.0	20	20	0.67	1.46
1.0	40	20	0.79	2.14
1.0	20	40	4.19	2.80
1.0	40	40	3.06	3.31
10.0	0	0	33.24	1.74
10.0	0	0	13.06	1.98
10.0	0	0	21.27	2.32
10.0	20	0	12.94	3.45
10.0	40	0	11.6	5.70
10.0	0	20	12.85	1.35
10.0	0	40	31.23	1.34
10.0	20	20	20.15	2.66
10.0	40	20	17.37	3.60
10.0	20	40	25.75	3.60
10.0	40	40	25.46	6.95

Mo conc. in sample (ppm)	Cu conc. sampl (ppm)			Mo conc. in in sample (ppm)	Cu conc. in in sample (ppm)		Mo conc. in sample (ppm)	Cu conc. in sample (ppm)		
Below detection limit	1.16	Mo expt GROUP 4		Below detection limit	1.16		Below detection limit	1.16		
0.225	1.3	Mo expt GROUP 1 Mo + Cu expt GROUP 1 Mo + SO_4 expt GROUP 1	Mean Value	0.225	1.3		0.225	1.3		
0.10	3.52 4.57	Mo + Cu expt GROUP 4 Mo + Cu expt GROUP 7		0.12 Below detection limit	3.52 4.57	Cu in drinking water appears to have no effect on Mo content	Mean Mo Value	0.10	3.52 4.57	
0.24	1.39	Mo + SO_4 expt GROUP 4 Mo + SO_4 expt GROUP 7		0.40 Below detection limit	1.75 1.02	SO_4 in drinking water appears to have no effect on Mo content.	Mean Mo Value	0.24	1.75 1.02	SO_4 in drinking water appears to have no effect on Cu content. — Mean Cu Value
0.14	2.71 4.47 4.56 5.75	Mo,Cu + SO_4 expt GROUP 1 (1) Mo,Cu + SO_4 expt GROUP 2 (1) Mo,Cu + SO_4 expt GROUP 3 (1) Mo,Cu + SO_4 expt GROUP 4 (1)		0.13 0.16 0.13 0.14	2.71 4.47 4.56 5.75	Cu + SO_4 in drinking water appears to have no effect on Mo content.	Mean Mo Value	0.14	2.71 4.47 4.56 5.75	
4.48	1.4	Mo expt GROUP 2 Mo + Cu expt GROUP 2 Mo + SO_4 expt GROUP 2	Mean Value	4.48	1.4		4.48	1.4		
1.13	3.30 5.20	Mo + Cu expt GROUP 5 Mo + Cu expt GROUP 8		0.60 1.65	3.30 5.20	Cu in drkg. water appears to have no effect-Mo cont.	Mean Mo Value	1.13	3.30 5.20	
5.0	1.47	Mo + SO_4 expt GROUP 5 Mo + SO_4 expt GROUP 8		4.08 5.92	1.02 1.91	SO_4 in drkg. water appears to have no effect-Mo cont.	Mean Mo Value	5.0	1.02 1.91	SO_4 in drinking water appears to have no effect on Cu content. — Mean Cu Value
2.18	1.46 2.14 2.80 3.31	Mo,Cu + SO_4 expt GROUP 5 (1) Mo,Cu + SO_4 expt GROUP 6 (1) Mo,Cu + SO_4 expt GROUP 7 (2) Mo,Cu + SO_4 expt GROUP 8 (2)		0.67 0.79 4.19 3.06	1.46 2.14 2.80 3.31	Cu + SO_4 in drinking water appears to have no effect on Mo content.	Mean Mo Value	2.18	1.46 2.14 2.80 3.31	
22.52	2.01	Mo expt GROUP 3 Mo + Cu expt GROUP 3 Mo + SO_4 expt GROUP 3	Mean Value	22.52	2.01		22.52	2.01		
12.27	3.45 5.70	Mo + Cu expt GROUP 6 Mo + Cu expt GROUP 9		12.94 11.6	3.45 5.70	Cu in drkg. water appears to have no effect-Mo cont.	Mean Mo Value	12.27	3.45 5.70	
27.04	1.35	Mo + SO_4 expt GROUP 6 Mo + SO_4 expt GROUP 9		12.85 31.23	1.35 1.34		Mean Mo Value	27.04	1.35 1.35	SO_4 in drinking water appears to have no effect on Cu content. — Mean Cu Value
22.18	2.66 3.60 3.60 6.95	Mo,Cu,SO_4 expt GROUP 9 Mo,Cu,SO_4 expt GROUP 10 Mo,Cu,SO_4 expt GROUP 11 Mo,Cu,SO_4 expt GROUP 12		20.15 17.37 25.75 25.46	2.66 3.60 3.60 6.95	Cu + SO_4 in drinking water appears to have no effect on Mo content.	Mean Mo Value	22.18	2.66 3.60 3.60 6.95	

Detection limit for Mo is 0.08 p.p.m. Mo

Analysis of Rats Teeth

Preparation of Standards

Analysis of rat's teeth by Mansell(1960) showed that the following levels of trace elements were present, 144 ppm. Fe, 24.0 ppm. Zn, 1.79 ppm. Mn, 1.3 ppm. Co and 0.5 ppm. Ni. In the present study 1 gm. of tooth sample was dissolved to give 50 ml. of solution. All the standards were prepared in 100 ml. quantities, and therefore contained twice the above amounts of trace elements, that is 300 µgm. Fe, 50 µgm. Zn, 4.0 µgm. Mn, 3.0 µgm. Co and 1.0 µgm. Ni. The standards also contained calcium and phosphorus, the levels of which were equivalent to the range of concentrations expected in the teeth.
From evidence obtained in the preliminary analysis of rat's teeth, all molybdenum standards were prepared containing 20 µgm. of copper whilst all copper standards contained 1.0 µgm. of molybdenum.

Appropriate copper and molybdenum standards were prepared separately by suitable dilutions of either the copper or molybdenum stock solutions. All copper and molybdenum standards contained 3 ml. of perchloric acid (62%), 5 ml. of the iron and zinc stock solution, 1 ml. of the manganese, cobalt and nickel stock solution, 20 ml. of the calcium stock solution and 0.6 ml. of orthophosphoric acid. Copper standards also contained 1.0 ml. of the 1.0 ppm. molybdenum stock solution, whilst molybdenum standards also contained 1 ml. of the copper stock solution.

Separate copper and molybdenum blank solutions were

prepared, containing all the elements present in the standards with the exception of copper and molybdenum where appropriate.

A known weight of tooth sample was digested in 3 ml. of perchloric acid (62%) in a Kjeldahl flask. Digestion was considered to be complete when no sample remained in the acid medium and all charred organic material was oxidized to give a clear colourless solution. The solution was transferred to a 50 ml. graduated flask and the Kjeldahl flask repeatedly washed with deionized water; the washings also being transferred to the graduated flask. The solution in the graduated flask was allowed to cool after which it was made up to the graduation mark with deionized water. The sample blank containing 3 ml. of perchloric acid (62%) was treated in the same manner.

The pH of the sample solution was checked to ensure it fell within the extraction pH range of 1.1 to 1.6.

40 ml. of the sample solution was transferred to a stoppered conical flask, followed by the addition of a 5% w/v aqueous solution of ammonium pyrrolidine dithiocarbamate and 4 ml. of methyl isobutyl ketone. The resulting solution was shaken for 3 minutes and then transferred to a separating funnel where the two phases were allowed to separate for 4 minutes. The lower aqueous phase was discarded and the organic phase aspirated into an air-acetylene flame to analyse for copper and into a nitrous oxide-acetylene flame to analyse for molybdenum. The sample blank was extracted by the same method.

Suitable copper and molybdenum standards and blanks

were extracted by the above procedure and aspirated to obtain a calibration graph.

Calculation

Weight of sample = W gms.

Concentration of aqueous sample solution from calibration graph = C ppm.

Volume of aqueous solution of sample = 50 ml.

Concentration of element in sample = $\frac{50C}{W}$ ppm.

The fluid intake of different groups of rats differed; this would affect the levels of the element in the sample, hence all fluid intakes were adjusted to 1000 ml. to enable a direct comparison to be carried out.

Fluid intake of rate = f ml.

Adjusted concentration of element in sample

$$= (\frac{50C}{W}) \frac{1000}{f} \text{ ppm.}$$

$$= (\frac{5C}{fW}) \times 10^4 \text{ ppm.}$$

Adjusted concentration of element in sample

$$= 10^4 (\frac{5C}{fW}) \text{ ppm.}$$

Discussion of Results

1. Drinking water without any added trace elements

The copper content of teeth from rats fed on a cariogenic diet and deionized water was 1.16 ppm., whilst the molybdenum content was below the detection limit of the analytical method (below 0.08 ppm. molybdenum).

2. Drinking water with added molybdenum

Rats fed 0.1 ppm., 1.0 ppm., and 10.0 ppm. molybdenum in the drinking water had corresponding mean levels of molybdenum in the teeth of 0.225 ppm., 4.48 ppm., and 22.52 ppm. These results indicate that the molybdenum content in the teeth increased as the molybdenum concentration of the drinking water increased. The increase is exactly linear when comparing 0.1 ppm. and 10.0 ppm. levels.

When the concentration of molybdenum in the drinking water is compared with the molybdenum concentration in teeth the two values are of the same magnitude, for example, a molybdenum concentration of less than unity results in a concentration in teeth of less than unity. Examination of other concentrations supports this relationship.

In the case of the 1.0 ppm. level, the molybdenum content of the teeth is double that of the expected value, although of the expected magnitude. This may be due in some part to the sensitivities of different litters to molybdenum uptake. Change in sensitivity between litters is clearly indicated by the large divergence in the molybdenum content of the group 2 sample in the molybdenum and sulphate experiment. If this high result of 8.82 ppm. molybdenum is neglected and a mean value taken of the other two results (mean value 2.30 ppm. molybdenum), a completely linear relationship between molybdenum in drinking water, and absorption in the teeth can be shown. By this modification of the results drinking water concentrations of 0.1 ppm., 1.0 ppm., and 10.0 ppm. molybdenum correspond to tooth concentrations of 0.225 ppm., 2.30 ppm.

and 22.52 ppm.

By whichever method the results are evaluated it is evident that molybdenum absorption increases as the molybdenum concentration of the drinking water is increased. It was also noted that as the molybdenum concentration of the drinking water increased the copper content of the teeth remained unchanged.

3. Drinking water with added molybdenum and copper

As the concentration of copper in the drinking water increased over the range from zero to 40.0 ppm. there were corresponding increases in the copper absorption in the teeth. Increase in the copper content of the drinking water from zero to 20.0 ppm. gave rise to an increase in the mean concentration band of copper from 1.16 ppm. - 1.70 ppm. to 2.83 ppm. - 3.52 ppm. Similarly, as the level of copper in the drinking water was increased from 20.0 ppm. to 40.0 ppm., the copper in the teeth increased from a mean concentration band of 2.83 ppm. - 3.52 ppm. to 4.25 ppm. - 5.20 ppm. copper. Therefore, as the copper level in the drinking water increased by a factor of 2, the increase in copper in the teeth was by a factor which lay between 1.3 and 1.6.

Increases in the copper absorption by the teeth due to increased copper levels in the drinking water are relatively smaller than similarly resulting increases in molybdenum absorption. Also, at any given molybdenum concentration of the drinking water the uptake of molybdenum in the teeth was of the same order, that is at the 10.0 ppm.

level of concentration of molybdenum in the teeth was 22.50 ppm. However, the copper concentration of the teeth was significantly lower than the level of copper in the drinking water, that is at 40.0 ppm. copper level the copper content of the teeth was 5.20 ppm. Therefore, it appears that the behaviour of the teeth relative to molybdenum and copper is to concentrate molybdenum to a marked extent and copper to a lesser extent.

There was no significant change in the molybdenum content of the teeth as the copper concentration of the drinking water was increased. For example, at the 10.0 ppm. molybdenum level in the drinking water the molybdenum concentration of the teeth was 13.06 ppm., 12.94 ppm., and 11.60 ppm. as the copper concentration of the drinking water increased from zero to 40.0 ppm.

4. Drinking water with added molybdenum and sulphate

The presence of sulphate in the drinking water produced a behaviour similar to that of copper, in that it produced no effect on the molybdenum absorption in the teeth. For example, where the level of molybdenum in the drinking water was 1.0 ppm., the molybdenum concentration of the teeth was 5.92 ppm. and 4.08 ppm. at 20.0 ppm. sulphate and 40.0 ppm. sulphate respectively. Similarly, an increase in the sulphate concentration of the drinking water caused no change in the copper content of the teeth, which fell within the range of 1.02 ppm. to 1.91 ppm. copper compared with the range 1.16 to 1.70 ppm. copper for teeth which had not received any sulphate in the drinking water.

Therefore, the presence of sulphate in the drinking water does not affect the molybdenum absorption nor the copper content of the teeth.

5. Drinking water with molybdenum, copper and sulphate

The presence of both copper and sulphate in the drinking water showed no apparent influence on the molybdenum absorption in the teeth. This can be demonstrated with the following molybdenum concentrations in the teeth of 25.75 ppm., and 25.46 ppm. which were observed with drinking water containing 10.0 ppm. molybdenum, 20.0 ppm. copper and 40.0 ppm. sulphate and with the copper level increased to 40.0 ppm. respectively.

The enhancement in copper content of the teeth produced by an increased copper concentration in the drinking water was found to increase further with the additional presence of sulphate. For example, where the drinking water contained 0.1 ppm. molybdenum, 20.0 ppm. copper and 20.0 ppm. sulphate the copper content of the teeth was 2.76 ppm., when the sulphate level was increased to 40.0 ppm. there was an increase in the copper concentration to 4.56 ppm.

6. General Observations

Molybdenum absorption in teeth from different litters varied considerably for the same concentration of molybdenum in the drinking water. For example, with 10.0 ppm. molybdenum in the drinking water, rats bred for the molybdenum experiment had a molybdenum concentration in

the teeth of 33.24 ppm. whilst those bred for the molybdenum and copper experiment had a molybdenum concentration of 13.06 ppm. Variations in the copper concentration of teeth from different litters were not observed with the concentrations always falling in the range of 1.16 ppm. - 1.70 ppm.

Hence it appears that the absolute value for the molybdenum absorption of the teeth is dependent on the level of dietary molybdenum with a variation occurring between litters. The molybdenum absorption is independent of dietary copper and sulphate.

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Molybdenum concentration in deionized water (p.p.m.)	Copper concentration in deionized water (p.p.m.)	Sulphate concentration in deionized water (p.p.m.)	Total fluid intake of group of rats (mls)	Number of rats in group	Group No.	
0	0	0	2892	12	1	
0.1	0	0	2792	12	2	Molybdenum Experiment
1.0	0	0	2555	11	3	
10.0	0	0	2556	12	4	
0.1	0	0	1122	5	1	
0.1	0	20	858	4	2	
0.1	0	40	893	5	3	
1.0	0	0	832	4	4	
1.0	0	20	1012	5	5	Molybdenum and Sulphate Experiment
1.0	0	40	847	5	6	
10.0	0	0	786	4	7	
10.0	0	20	743	4	8	
10.0	0	40	669	4	9	
0.1	0	0	985	5	1	
0.1	20	0	1149	5	2	Molybdenum and Copper Experiment
0.1	40	0	1005	5	3	
1.0	0	0	1022	5	4	
1.0	20	0	1234	5	5	
1.0	40	0	1001	5	6	
10.0	0	0	816	4	7	
10.0	20	0	910	4	8	
10.0	40	0	885	4	9	
0.1	20	20	1198	5	1	
0.1	40	20	1090	5	2	
0.1	20	40	1211	5	3	Part 1. Molybdenum, Copper and Sulphate Experiment
0.1	40	40	1173	5	4	
1.0	20	20	2776	7	5	
1.0	40	20	2231	6	6	
1.0	20	40	1445	6	7	
1.0	40	40	1567	6	8	
10.0	20	20	1537	6	9	
10.0	40	20	1718	6	10	Part 2. Molybdenum, Copper and Sulphate Experiment
10.0	20	40	1265	5	11	
10.0	40	40	1274	5	12	

The molybdenum was supplied as sodium molybdate dihydrate ($Na_2MoO_4.2H_2O$), the copper as cuprous chloride dihydrate ($CuCl_2.2H_2O$) and the sulphate as sodium sulphate pentahydrate ($Na_2SO_4.10H_2O$).

PAPER 21

n-BUTYL CYANOACRYLATE AS A PULP CAPPING AGENT

G. S. Nixon and C. McD. Hannah

n-BUTYL CYANOACRYLATE AS A PULP CAPPING AGENT

n-Butyl cyanoacrylate was employed as an adhesive and haemostatic pulp capping agent. This was compared with calcium hydroxide and zinc oxide and eugenol. The results of this study showed that n-butyl cyanoacrylate and zinc oxide and eugenol failed to produce satisfactory dentine barriers whereas calcium hydroxide was most satisfactory in this respect.

For more than two centuries clinicians have attempted to preserve the vitality of the exposed dental pulp by pulp capping. Many of the materials and medicaments used in efforts to promote pulp healing have been documented by Glass and Zander (1949).

Since then favourable pulpal responses have been observed beneath calcium hydroxide dressings (Berman and Massler 1958, Glass and Zander 1949, Nyborg 1958), and for many years it has been the capping material of choice.

Recently alkyl-2-cyanoacrylates have been used successfully as biological tissue adhesives in a wide variety of surgical procedures (Bhaskar and Cutright 1969, Frisch and Bhaskar 1967, Healey et al. 1961, Mather and Terry 1963). In dentistry they have been used without success for the re-plantation of teeth (Huebsch 1967). Their use in a compositive form as enamel fissure sealants has produced conflicting reports (Cueto and Buonocore 1967, Parkhouse and Winter 1971).

The cyanoacrylate monomers function as haemostatic and

bacteriostatic agents and are claimed to be well tolerated by biological tissues, thereby permitting normal healing. These properties and the ease of application could favour their use as pulp capping agents if pulpal responses were favourable.

Evaluation of this potential use have been made on vital pulp tissue of swine (Bhaskar et al. 1969) and of human teeth (Berkman et al. 1971), using isobutyl cyanoacrylate monomer as an adhesive capping agent. These studies have indicated that reparative dentine bridges could be formed immediately beneath the dressing.

The time interval between pulp capping and reparative dentine bridge formation was prolonged with cyanoacrylates when compared with calcium hydroxide as a capping material.

The characteristic zone of necrosis which is found between a calcium hydroxide dressing and the reparative dentine bridge did not appear beneath cyanoacrylate dressings. It was considered that the haemostatic effect of the cyanoacrylate was advantageous.

The purpose of the present work was to evaluate the use of n-butyl-cyanoacrylate as a pulp capping agent, to assess the reparative response of the pulp to this material, and to compare it with calcium hydroxide and zinc oxide. The techniques used were similar to those described by Nyborg (1955).

Materials and Methods

Thirteen Beagle dogs and bitches aged between 6½ months and 17 months were anaesthetised using 25 mg. Thiopentone sodium per Kg. body weight.

Cervical access cavities were cut in the buccal surfaces of the maxillary and mandibular canines of five dogs and in the canine teeth and third maxillary incisor teeth in the remaining eight dogs.

The cavities were cut with a No. 5 round steel bur followed by a No. 5 cylindrical fissure bur to about 2½ mm. depth. The speed of bur rotation was limited to approximately 10,000 r.p.m. and water spray coolant was used throughout access cavity preparation.

The pulps of the 68 teeth were exposed through the axial walls of the access cavities using sterile No. ½ round burs rotating at approximately 2,000 r.p.m. without water coolant.

Twenty-two exposures were capped with calcium hydroxide*, twelve with slow setting zinc oxide and eugenol cement and thirty-four were coated with n-butyl cyanoacrylate.** These materials were placed over the exposed pulpal tissues with a flat plastic instrument. The calcium hydroxide and cyanoacrylate dressings were covered and protected with zinc oxide and eugenol cement. In each dog untreated teeth were used as controls.

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* Calxyl, Otto and Co., 37 Metzlerstrasse, Frankfurt.

** Cyanodont, M.T. Gendrault, Pharmacien, 29 rue des petites-ecuries, Paris 10e.

The treated and control teeth were excised together with the surrounding alveolar bone at post operative intervals of four and fourteen weeks. The apical one third of the roots was removed and the specimens fixed in 10% formalin. After decalcification the teeth were embedded in paraffin, sections cut at 7 μ in bucco-lingual section through the pulpal exposure and stained with haematoxylin and eosin.

Results

Of the 68 treated exposed pulps three were lost as a result of dressings washing out; two were not sectioned through the exposures and four were lost as a result of damage to, or loss of the pulp during excision or root sectioning. Of these nine specimens, five contained calcium hydroxide dressings, two zinc oxide and eugenol and two cyanoacrylate dressings.

Histological Observations

Microscopic examination of the serial sections showed that each specimen could be allocated to one of five subgroups contained within two major groups. The two major groups were satisfactory and unsatisfactory pulpal responses.

Satisfactory - The two subgroups of the satisfactory response group were:

a) Completed bridge: in which a secondary dentine barrier was fully formed and closed over a pulp which

appeared normal throughout the serial sections and

b) Partially completed bridge: in which closure of a secondary dentine barrier approached completion. In the absence of any inflammation of the pulp, the prognosis for completion of the barrier was considered to be good.

Unsatisfactory - The subgroups of the unsatisfactory response group were:

c) Incomplete bridge: in which bridge formation was well established but the prognosis for completion of the barrier was considered to be poor on account of associated pulpal inflammation.

d) Poor barriers: where the degree of secondary dentine formation was minimal and where severe local or generalised inflammation of the pulp was apparent.

e) No barriers: where there was no evidence of any attempt at formation of a secondary dentine barrier.

The results are summarised in Tables 1 and 2.

Zinc Oxide and Eugenol Dressings

In nine of the ten remaining teeth treated with zinc oxide and eugenol there was little evidence of dentine bridging. In three of these cases aggregation of irregular discrete areas of mineralising matrix produced some form of barrier, but there was no apposition of secondary dentine from, or continuity with the cavity margins. Calcio-traumatic lines were faint and in general there was no evidence of increased deposition of secondary dentine or of an increased width of the pre-dentine layer adjacent to the

Table 1

Satisfactory Responses

Barrier	Weeks	Zinc Oxide/ Eugenol	Calcium Hydroxide	Butyl Cyanoacrylate
Complete	4		8	NONE
	14	NONE	1	NONE
Almost Complete	4		2	1
	14	1	NONE	NONE
TOTAL		1	11	1

Table 2

Unsatisfactory Responses

Barrier	Weeks	Zinc Oxide/ Eugenol	Calcium Hydroxide	Butyl Cyanoacrylate
Estab-lished	4		1	4
	14	3	2	2
Poor	4		NONE	3
	14	3	3	12
No barrier	4		NONE	4
	14	3	NONE	6
TOTAL		9	6	31

margins of the cavity. Fractured spurs of mineralised dentine remained attached to the margins of four exposure cavities (Fig. 1), with their free ends projecting into the pulp tissues.

Neither odontoblasts nor predentine layers were present over these projections, and the location of the calcio-traumatic lines indicated that these spurs were probably composed of non-mineralised predentine at the time of operative exposure.

The pulp tissues of nine teeth were diffusely infiltrated with polymorphonuclear leucocytes, which were found in greatest numbers adjacent to the exposures almost obliterating the normal cellular architecture (Fig. 2). Other signs of pulpal reaction were evident such as dilation and congestion of the blood vessels with extravasation of red corpuscles. The formation of vacuoles and disintegration of the cellular elements of the pulp were evident in all sections in this group.

An unusual form of closure was noted in three instances in which the exposure had been almost occluded by the mineralisation of organised material lying within the exposure cavity superficial to the grossly inflamed pulp (Fig. 3).

Calcium Hydroxide Dressings

All remaining seventeen pulp exposures which had been capped with calcium hydroxide produced some degree of repair by deposition of secondary dentine.

Fig. 1 Attached fractured spurs of dentine project into the pulp. Large numbers of polymorphs are evident. Vacuole formation and disintegration of the cellular elements are apparent. x 55.

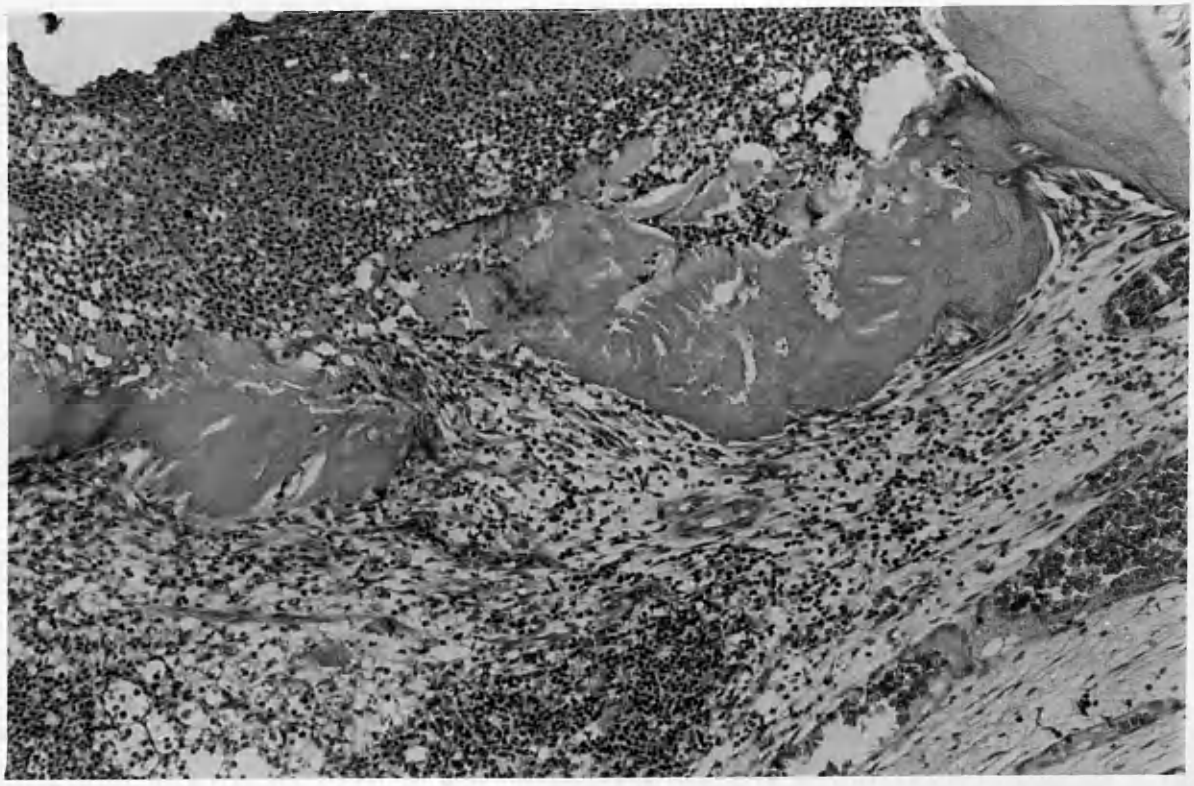

Fig. 2 Large numbers of polymorphs are present between the walls of the exposure cavity (top) and in the pulp. x 110.

Fig. 3 Partial occlusion of the exposure cavity by a mass of partly mineralised material. x 62.

Satisfactory - The reaction of the pulp was considered satisfactory in eleven teeth. In eight of these the barriers were completed by four weeks. In all cases the reparative dentine bridges were thickest at the point of attachment to the dentine wall of the pulp chamber adjacent to the margins of the exposure cavities (Fig. 4). Where closure was incomplete the bridges appeared to be formed by the progressive closure of a diaphragm from the peripheral points of attachment towards the centre. Six of these barriers incorporated attached mineralised spurs of dentine.

The secondary dentine and predentine layers were considerably increased in thickness in the region of the points of attachment and a wide predentine layer was present over the mineralised secondary dentine of each bridge. Odontoblasts were present and continuous over all bridges. In most cases calcification had commenced in discrete foci which increased in size to form irregular fused mileralised areas lying within non-mineralised matrix.

All pulps were slightly hyperaemic with many dilated and congested blood vessels. There were no inflammatory cells in the pulp tissues and there was no evidence of increase in number of capillaries or fibroblasts.

Unsatisfactory Response - Established dentine bridges were present in three of the remaining six teeth containing calcium hydroxide dressings, and in the other three barriers were present but poorly formed. These barriers had formed in a similar manner to those of the satisfactory calcium hydroxide barriers.

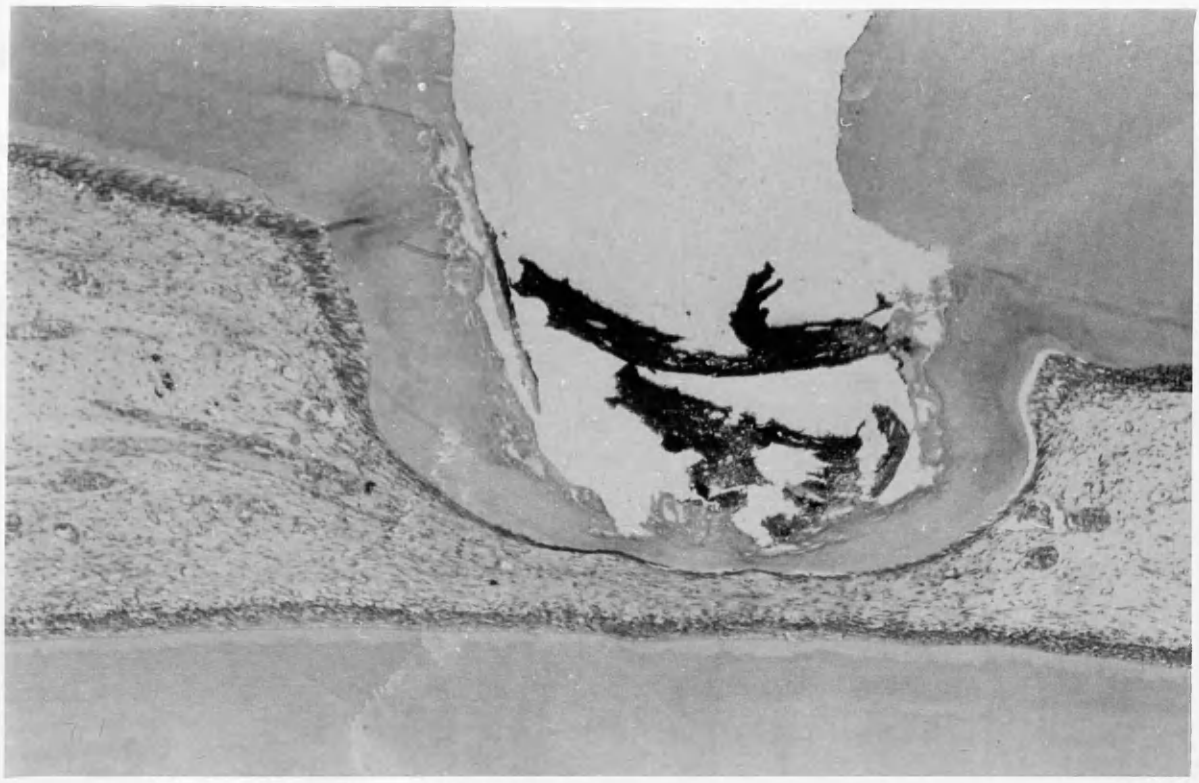

Fig. 4 Reparative dentine barrier beneath calcium hydroxide is closed in 4 weeks. Debris of the necrotic zone lies within the concavity of the barrier. x 55.

Fig. 5 The only satisfactory barrier found beneath a cyanoacrylate dressing. x 43

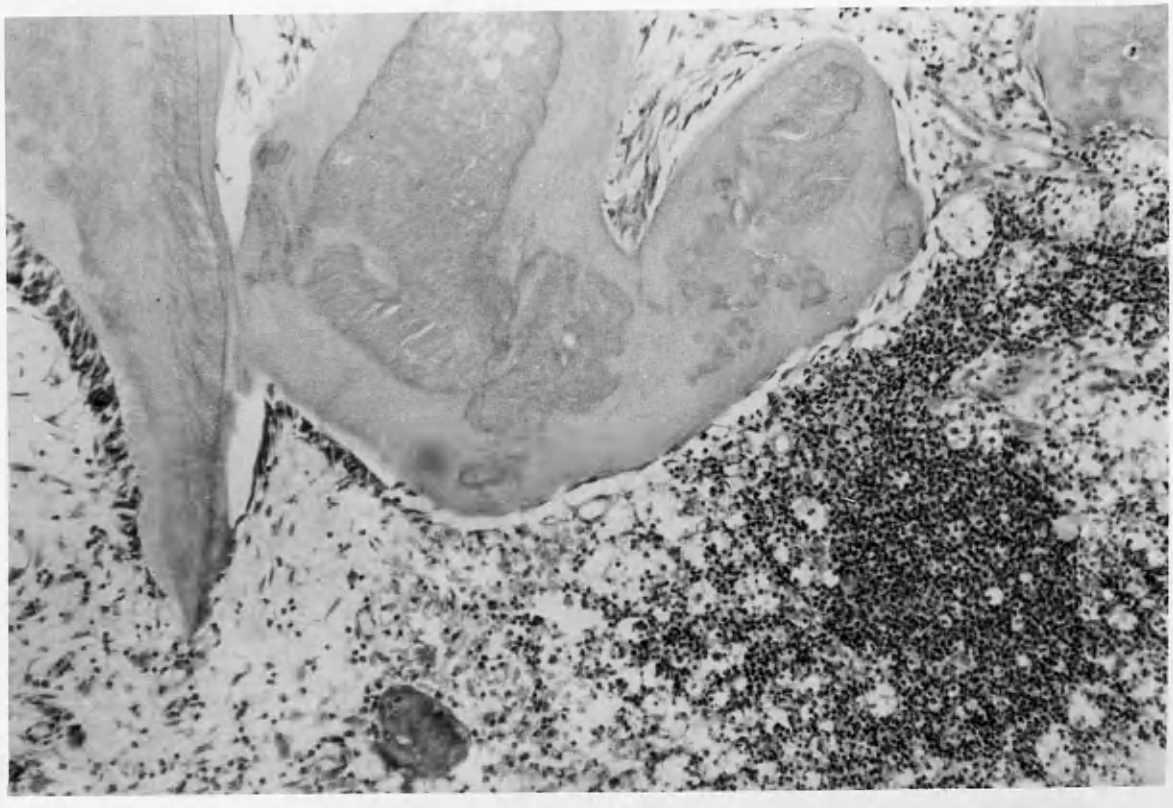

Fig. 6 Increased thickness of spur which has occurred after operative exposure. Many polymorphs are present throughout the pulp. x 215.

In all six teeth abscesses were present either within the exposure cavity superficial to the incomplete bridge or adjacent to the projecting free ends of the forming barrier.

In three cases the remainder of the pulp was hyperaemic without inflammatory cells and in three teeth there was a diffuse infiltration of polymorphs throughout the pulp with dilated, congested vessels. Where the pulp was diffusely inflamed, odontoblasts and predentine were absent for a short distance from the points of attachment of the bridges, but the exposure cavities were occluded by mineralised tissue lying between the walls of the cavity.

Cyanoacrylate Dressings

No completed barriers were formed beneath any of the thirty two cyanoacrylate dressings throughout the period of the study.

One partially completed bridge, for which the prognosis was considered to be favourable, was formed in the four week period. This bridge was composed in part of an attached fractured spur of mineralised dentine. The location of the calcio-traumatic line indicated that this spur had been greatly thickened after operative exposure. Odontoblasts and a wide predentine layer were continuous over the mineralised part of this spur. In addition many areas of partially mineralised matrix had fused to occlude and bridge the exposure (Fig. 5).

A localised micro-abscess was present in the exposure and there was a mild diffuse infiltration of polymorphs

throughout the pulp tissues. There was a localised increase in the number of capillaries, many of which were empty.

Established incomplete barriers with a poor prognosis for completion were present in four teeth after four weeks and in two teeth after fourteen weeks. In all six teeth the type of bridge formation was similar to that described above. Numerous polymorphs surrounded both the thickened fractured spurs of dentine and the irregular partially mineralised areas (Fig. 6). The pulp tissues were hyperaemic with widely dilated congested blood vessels and areas of vacuolisation, haemorrhage and disintegration. In fifteen teeth containing cyanoacrylate dressings partially formed bridges were present.

In all cases the pulps contained masses of polymorphs to the extent of almost complete obliteration of the normal pulp tissue. Odontoblasts and predentine were absent from the vicinity of the margins of the exposure cavities and from any attached fractured spurs of dentine.

In contrast to the calcium hydroxide dressings, many teeth with cyanoacrylate dressings showed no bridge formation whatsoever. There was no increased deposition of secondary dentine or predentine after fourteen weeks in six teeth and after four weeks in four teeth. The pulp tissues of seven of these were densely infiltrated with polymorphs and there was widespread disintegration of the tissues. In two teeth abscesses were localised to the region of the exposure cavities.

Discussion

Throughout the fourteen week period of the study, completed secondary dentine bridges were found beneath calcium hydroxide dressings only. Closure was completed in most cases within four weeks, a similar time interval to that found by Glass and Zander (1949). The response beneath calcium hydroxide was satisfactory in sixty-five per cent of the cases. This observation is similar to the seventy per cent success report by Nyborg (1955) using a similar calcium hydroxide preparation.

The characteristic zones of necrosis were apparent on the concave surfaces of the formed bridges.

The responses of the pulpal tissue beneath the zinc oxide and eugenol dressings were unfavourable. No reparative dentine barriers had been completed, and with one exception only there was severe inflammation of the pulp tissue throughout. It was considered that completion of the dentine barrier could have occurred in one case only, a finding which is again similar to that of Glass and Zander (1949).

The general histological picture beneath thirty one cyanoacrylate dressings was of massive infiltration of inflammatory cells with absent or poorly formed reparative dentine bridges. It was apparent also from one isolated case that bridging could occur beneath a cyanoacrylate dressing under favourable circumstances in a similar time interval to those formed beneath calcium hydroxide dressings.

The overall failure rate beneath cyanoacrylate

dressings was ninety-seven per cent. The unfavourable pulpal reaction may possibly be related to the low pH of this material immediately before polymerisation. The cyanoacrylate used in the present work is stabilised with sulphurous anhydride. It is also possible that the destruction of potentially infected superficial pulp tissues in the necrotic zones beneath calcium hydroxide is a prerequisite for successful bridging.

Bhaskar (1969) noted that complete bridging was not observed with isobutyl cyanoacrylate after eight weeks and that pulp inflammation appeared less severe than that beneath calcium hydroxide.

It is possible that freshly prepared cyanoacrylate monomer without a stabiliser and with a shelf life of about ten days may elicit a mild inflammatory response. Bhaskar does not indicate the source of his material.

Whilst a pulp capping material with adhesive and haemostatic properties is desirable, the pulpal reaction to the capping agent is more important.

ACKNOWLEDGEMENTS

The authors are indebted to Geigy Pharmaceuticals Ltd. and to Mr. W. A. Bradley, Deputy Head of the Toxicology Division, in particular, for providing facilities and assistance for this study.