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# PENETRATION OF $C^{14}$-LABELLED METHYL-METHACRYLATE INTO THE DENTINE

*by*

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For more than a decade methyl-methacrylate has been used as a dental filling material. This is placed into a cavity while still in a plastic stage undergoing polymerization. Thus, as early as in May 1939, *Slack* filled a cavity with methyl-methacrylate and described how the resin was polymerized by a heated plastic instrument placed right above the matrix (heat-shock), *Slack* (1943)[20].

The introduction of this material on a large scale in clinical dentistry depended, however, on the development of self-curing resins where the principle promoting polymerization is released either by fusing the monomer and polymer phase or by mixing the catalyst into the resin. Simplification of techniques led to large numbers of teeth filled with these resins before it was found that the pulp must be sufficiently protected by insulation or it would inevitably be injured by penetration of the monomer as shown by *Fischer* (1952)[5], *Bloch* (1952)[2], *Castagnola* (1950)[3], *Kramer & McLean* (1952)[8], *McLean & Kramer* (1952)[10], *Maeglin* (1952 & 1953)[11, 12], *Spreter von Kreudenstein* (1952)[19], *Müller & Maeglin* (1953)[15], *Nygaard Østby* (1955)[17], and *Hedegård* (1956)[7], among others.

Various new means and ways for protecting the pulp have been tested either *in vivo,* followed by extraction and histological examination, or by purely experimental procedures *in vitro.* In experiments of the latter type, attempts have been made to show not only how the monomer diffuses into the dentine but also what methods could be used to prevent the penetration of the monomer into the pulp of extracted teeth. Thus, *Spreter von Kreudenstein* (1952)[19] demonstrated the extent of the diffusion by adding a Sudan-dye to the monomer. He also proved that hardened phosphate cement did not give effective protection against the penetration of the monomer into the pulp cavity, whereas this cement exerted sufficient protection while still in the plastic stage.

The rate at which the resin diffused into the pulp cavity was investigated by *Spreter von Kreudenstein* (1952)[19] and *Stüben* (1952)[22]. The former conducted his experiment in the following way: A class I cavity was prepared in a molar. The tooth was cut horizontally at the level of the ceiling of the pulp chamber, and the horizontal cross-section was placed above a water surface without touching it. One drop of monomer was pipetted into the floor of the cavity. In the course of a few minutes, the monomer penetrated from the floor of the cavity through the dentine and to the cross-section. In consequence the monomer (i.g. gasses from the latter) produced a decrease in the surface tension of the water. The time for this change was registered by tensiometer-registrations ("Abreisskörper-Methode").

*Stüben* used the so-called "Schlierenmethode" (shadow-method) to follow the penetration of various fluids, including the monomer, into the dentine. In this procedure the time for complete penetration of the monomer was registered optically. Thus a cavity was prepared in the occlusal surface of a tooth which was cut horizontally. The cross-section was placed on the surface of the water in a small flask transilluminated by a strong lightbeam passing through an optical system. After passing through the water-filled flask the beam was projected onto a screen, where a homogeneous square luminous spot appeared. This corresponded to the flask, while the tooth with its cavity was silhouetted. A drop of monomer was pipetted into the cavity. After having penetrated the layer of dentine and passed into the water, the molecules of the monomer caused a change in the refraction. This appeared on the screen as a local, well-defined shadow in the picture of the flask ("das Schlierenbild").

By employing this method *Stüben* was able to prove that monomer methyl-methacrylate ("Palavit-Flüssigkeit") could penetrate a layer of dentine 1 mm thick in 1½ minutes. He was also able to confirm the results obtained by *Spreter von Kreudenstein* with hardened phosphate cement. However, in the case of unhardened cement, the observations could only be studied briefly; that is, the acid from the phosphate cement penetrated the dentine in 4 to 8 minutes to produce a shadow ("Schlieren") which prevented determination of "Schlieren" caused by the monomer.

Certain objections can be raised to the methods described for determining the extent as well as the rate at which the monomer penetrates the dentine. Thus, the molecular size of any Sudan-dye being larger then that of the monomer, the diffusion of a dye-monomer complex may not be the same as that of the monomer alone. On the other hand, the complicated light-transmission apparatus devised by *Stüben* makes reproduction difficult.

It seemed logical, therefore, to ascertain diffusion of the monomer into the dentine without adding chemicals. Several pilot experiments were made with a methyl-methacrylate containing the radioactive isotope $C^{14}$. At the same time the effectiveness of different "cavity-liners" was evaluated. This seems to be the first investigation of the penetration of radioactive methyl-methacrylate into the dentine [4, 1, 13, 23].

## MATERIAL AND METHODS

The radioactive methyl-methacrylate was made of radioactive methylalcohol according to the reaction:

$$CH_2 = C \left\langle \begin{matrix} CH_3 \\ COOH \end{matrix} \right. + C^*H_3OH \longrightarrow CH_2 = C \left\langle \begin{matrix} CH_3 \\ COOC^*H_3 \end{matrix} \right. + H_2O$$

(methacrylic acid) (methylalcohol) (methyl-methacrylate)

$C^* = C^{14}$

The conversion was made using 7.4 ml methylalcohol containing 1 millicurie of $C^{14}$. When the methylalcohol was converted to methyl-methacrylate, approximately 2 ml containing 0.5 millicurie of $C^{14}$ were left that could be used for experiments.*

Intact bicuspids, newly extracted for orthodontic reasons, were used. A cavity was cut on a facial surface (Fig. 1). Next, a mesiodistal section (SS') of the tooth was made from the occlusal surface so that the facial part of the tooth, where the cavity was prepared, was released from the rest of the tooth. The slope of the cross-section in relation to the axial aspect of the cavity prepared showed that the distance measured from the floor of the cavity to the cross-section was longest (1.9—2.6 mm) in the occlusal portion of the cavity and shortest (0.3—0.4 mm) in the gingival part (cfr. Fig. 1). Both the slope of the cross-section in relation to the axial aspect of the cavity and the measures described were kept as similar as possible.

The reason for making the cross-section (SS' on Fig. 1) beforehand, was to avoid contamination with radioactive substance. This could not have been done safely had the section been made *after* the cavities were filled with radioactive monomer which probably had diffused into the tooth. Since cleavage of a tooth in a plane (SS') did not give a surface sufficiently level for autoradiography, it was necessary to grind it to the smoothness required.

As mentioned before, the slope between the axial aspect of the cavity in relation to the autoradiographic section (SS') makes the thickness of the dentinal layer vary between the floor of the cavity and the surface to be autoradiographed. This should give an opportunity of determining the importance of the layer of dentine as a barrier to the diffusion of the monomer between the floor of the cavity and the pulp cavity.

After preparation of the cavities and grinding of the teeth, the cavities were lined in various ways. One cavity was lined with the material described by *Zander, Glenn & Nelson* (1950)[24], another was impregnated with zinc ferrocyanide and silver nitrate [*Gottlieb, Barron & Crook* (1956)[6]]. Impregnation of the dentinal tubuli with zinc ferrocyanide as a barrier against the

* The conversion was carried out in the Biochemical Department of the Royal Dental College by Mr. *Ingolf Crossland* to whom the authors are indebted.

penetration of the monomer into the dentine has been recommended by several authors (19, 14, 21, 9), though this method can endanger the pulp as indicated by studies by *Schmidhauser* (1952)[18]. A third cavity was lined with a thin layer of phosphate cement which hardened, and a fourth with a reactor-liner of the Swedon-Ultra type. A fifth cavity was lined with a thin

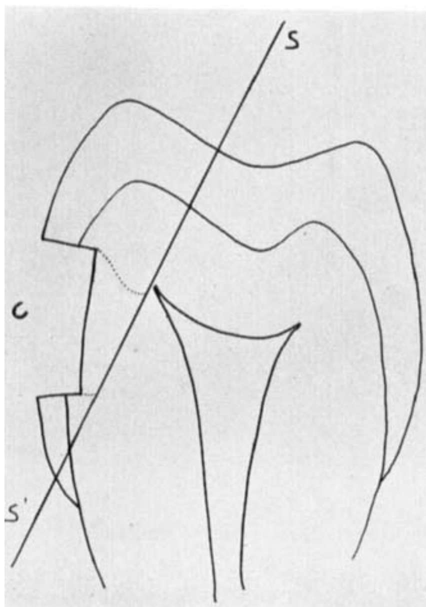


Fig. 1. Position of the experimental cavity (C) in relation to the cross-section surface to be autoradiographed (SS').

layer of silver amalgam which hardened. Two cavities were not lined at all. One of these was prepared 24 hours before the experiment and kept dry in contrast to the others which were prepared just before the experiment to avoid dehydration. In addition, a cavity was prepared in a carious tooth where transparent dentine in the floor of the cavity was clearly to be seen after excavation. This cavity was not lined at all. Finally, a tooth was prepared in exactly the same way as the others with the exception that its cavity was not filled with radioactive substance. This tooth served as a control.

Since the monomer was extremely volatile, the prepared teeth, the equipment necessary for all filling procedures, and radioactive monomer were placed in a closed box to prevent inhalation of radioactive vapours or contamination of the laboratory. All fillings were made in this glass box (Fig. 2). A micro-pipette was used to flood the floors of the cavities with the radioactive monomer, after which a thin layer of polymer powder was

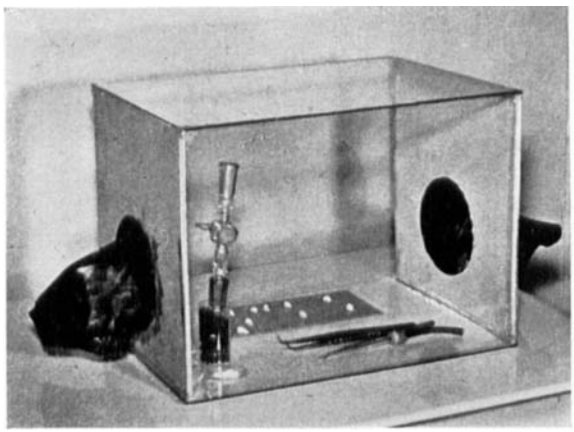

Fig. 2. Glass box with rubber gloves used when filling cavities with radioactive monomer.

sprinkled in such a manner that the floors of the cavities were covered with layers of self-curing acrylics a.m. *Nealon* (1952)[16]. After 24 hours when complete polymerization should have occurred, the teeth were removed from the closed case. All radioactive material was carefully ground out of the cavities under running water. Autoradiographs were made by placing the teeth with the polished surface (SS') down on nuclear track plates*. The exposure times were 7, 14, and 21 days respectively, which gave blackenings satisfactory for microscopic examination.

## RESULTS

To express the uptake of the monomer methyl-methacrylate in the dentine, the following terms were used to describe the

* N.T.B.-plates, 25 $\mu$; made by Research Laboratories Eastman Kodak, Rochester, N.Y., U.S.A.

different degrees of blackening in the autoradiographs: *none*, *vague*, and *distinct*. The different degrees of blackening and the corresponding exposure-times for the nuclear track plates are listed in Table 1. Table 1 shows that of the liners tested only *Zander*'s provided a safe barrier against penetration of the monomer. In this tooth (cavity no. 3) the autoradiograph was

**Table 1**

*Comparison of Cavity Liners in Preventing the Penetration of $C^{14}$-labelled Methyl-methacrylate into the Dentine of Extracted Teeth.*

| Cavity no | | Cavity Liner | Time of Exposure | Blackening on Autoradiograph |
|---|---|---|---|---|
| 1 | dehydrated | none | $14^d$ | distinct |
| 2 | newly extracted | none | $7^d$ | distinct |
| 3 | » | *Zander*'s liner | $14^d$ | none |
| 4 | » | phosphate cement (hardened) | $7^d$ | vague |
| 5 | » | reactor-liner | $21^d$ | vague |
| 6 | » | zinc ferrocyanide + silver nitrate | $21^d$ | vague |
| 7 | » | silver amalgam | $14^d$ | distinct |
| 8 | » | none* | $21^d$ | distinct |
| 9 | » | control** | $14^d$ | none |

* Transparent dentine from caries.

** Cavity not filled with radioactive monomer.

negative after 14 days of exposure. Zinc ferrocyanide plus silver nitrate did not completely prevent the passage of the monomer into the dentinal tubuli (cavity no. 6). The catalyst-lined cavity (no. 5) prevented the penetration of the radioactive monomer to some degree. This result might be related to the immediate polymerization of the methyl-methacrylate in the presence of the catalyst which reduces the amount of free monomer.

Hardened phosphate cement did not give any effective protection against the penetration of the monomer (cavity no. 4), which is in agreement with the findings reported by *Spreter von*

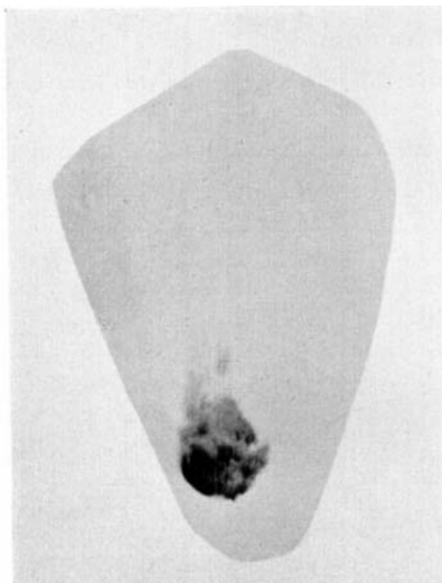

Fig. 3. Autoradiograph of cavity no. 2. Overexposure in lower portion corresponds to thinnest layer of dentine. Decreasing intensity in occlusal direction.

*Kreudenstein* and *Stüben*. A thin layer of silver amalgam was also tested as a cavity liner, but in our experiment the layer of silver amalgam was no longer plastic and was not protective (cavity no. 7).

In the unlined cavities (nos. 1, 2, and 8) the penetration of the monomer was clearly seen. The thickness of the dentinal layer seemed to exert some influence on the amount of monomer penetrating the dentine, as shown in Fig. 3. Here the overexposed autoradiograph (which is *not* to be used as a basis for comparisons) showed a much darker zone that corresponded to the very thin layer of dentine between the floor of the cavity and the cross-section. In the upper part of this cross-section the blackening is a little less pronounced and decreases gradually with the thickening layer of dentine between the cavity and the cross-section autoradiographed. Transparent dentine derived from caries does not offer any protection (cavity no. 8).

## DISCUSSION

The object of this pilot experiment was to find a way of determining the diffuse penetration of the monomer methyl-methacrylate into the dentine without using complicated apparatus or adding substances such as dyes or stains, since the results obtained by these dye-monomer complexes can be questioned. The problem was attacked by using $C^{14}$-labelled methyl-methacrylate monomer. The advantage of the $C^{14}$-monomer was its slow decay which is practically infinite ($T\frac{1}{2}$ is about 5570 years). In Handbook no. 48 of the National Bureau of Standards $C^{14}$ has been described as "moderately dangerous". Thus, the volatility of the monomer made it necessary to administer the radioactive methyl-methacrylate in a closed case which limits the types of cavity liners that can be investigated. It was impossible to evaluate the effectiveness of phosphate cement which had not set. Nor could thin layers of plastic silver amalgam be assessed.

Penetration of the radioactive monomer into the dentine was clearly demonstrated. Due to the limited material at their disposal, however, the present writes hesitate to draw concludings regarding the actual effectiveness of the liners tested as barriers to the penetration of methyl-methacrylate into the dentine.

## SUMMARY

Using a $C^{14}$-labelled monomer of methyl-methacrylate, the writers were able to demonstrate the diffuse penetration of the monomer into the dentine by autoradiography. As it is obvious from these pilot experiments that the method is feasible and convenient, it is hoped that further experiments may be conducted along these lines.

RESUME

DIFFUSION DANS LA DENTINE D'UN MÉTACRYLATE DE MÉTHYLE MARQUÉ AU $C^{14}$

Utilisant un monomère de métacrylate de méthyle marqué au $C^{14}$, les auteurs ont pu, à l'aide d'autoradiographies, mettre en évidence la diffusion dans la dentine d'un monomère radioactif. Grâce à cette méthode, ils ont en outre pu étudier l'efficacité de différents procédés d'isolement de la cavité destinés à arrêter l'infiltration de ce monomère radioactif dans la dentine, mais, en raison de la quantité limitée de matières radioactives dont ils disposaient, ils n'ont pu en ce qui concerne cette partie des recherches aboutir à des conclusions définitives.

Il apparaît cependant nettement que cette méthode est parfaitement appropriée et les auteurs ont l'espoir que des recherches complémentaires pourront être effectuées suivant les principes indiqués ici.

ZUSAMMENFASSUNG

DAS EINDRINGEN VON $C^{14}$-MARKIERTEM METHYL-METAKRYLAT IN DENTIN

Mit Hilfe von Autoradiographie ist es den Verfassern gelungen, das Eindiffundieren von Monomer in Dentin zu zeigen, indem sie $C^{14}$-markiertes Monomer des Methyl-metakrylats verwendeten.

Weiterhin wurde mit gleicher Methode die Wirkung verschiedener Kavitätsisolierungsmittel untersucht, um das Eindringen dieses radioaktiven Monomers in Dentin zu verhindern. Aber auf Grund der recht begrenzten Menge des zur Verfügung stehenden radioaktiven Materiales können aus diesem Teil der Untersuchung keine endgültigen Schlüsse gezogen werden.

Es hat sich jedoch deutlich gezeigt, dass es eine ausserordentlich geeignete Methode ist. Man hofft daher, recht bald weitere Untersuchungen nach den hier angegeben Prinzipien durchführen zu können.

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