Toxicity tests on dental filling materials

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Biological testing of dental amalgams and composite filling materials has been the subject of many publications. Recently, new developments have taken place. New high-copper amalgams are being manufactured as well as new composite materials which contain mainly organic fillers. On the other hand national and international institutions are working out programs for standardizing biological testing procedures. The objectives of the present investigation were both to compare the biologic reaction of two new filling materials with well-known ones and to look for a method which could become part of an international biological standardization protocol and which is already part of the Pharmacopeia of the United States (1970).

Materials and Methods

The materials under investigation were:

1. A fine-cut alloy amalgam—Amalcap (Vivadent, Schaan/Liechtenstein. Batch 120977 F 237321).

2. A spherical alloy amalgam—Spher-A-Cap (Kerr Sybron Corporation, Michigan, USA. Batch 0504763070).

3. A high copper two-component amalgam—Amalcap non-γ-2 (Vivadent, Schaan/Liechtenstein. Batch 090277 412).

4. A conventional composite—Compocap (Vivadent, Schaan/Liechtenstein. Batch 010277 425).

5. A new composite with mainly organic fillers—Isopast (Vivadent, Schaan/Liechtenstein. Batch Base 030577; Batch Catalyst 060577).

All materials except Isopast were delivered in capsules, Isopast as two pastes. The materials were mixed according to the manufacturer's instructions. The samples were tested at different ageing times (times between the beginning of the mixing and the beginning of the experiment): 5 min, 60 min, 1 day and 7 days.

The toxic potential of the materials was evaluated by the rabbit muscle implantation test as described by Lawrence et al. (1972). With this method the cylindrically shaped test materials were implanted into the paravertebral muscles of the rabbit by means of a hypodermic needle. Seven days after implantation the biologic response was recorded, both macroscopically and microscopically, according to the following scheme:

1. No or uncertain reaction, like the negative controls.

2. Moderate reaction, stronger than the negative control, but not as strong as the positive control.

3. Strong reaction like the positive control.

Glazed porcelain was used for the negative control and a known toxic polyvinylchloride plastic for the positive control. For macroscopic evaluation 10 specimens of each material at a given ageing time were obtained from two rabbits. The criteria for macroscopic evaluation were the zones of pyogenic reaction and abscess formation.

For histological evaluation the specimens were fixed in a 4 per cent buffered formalin, routinely processed and stained with haematoxylin and eosin. For each material and ageing time 40 sections were coming from 4 specimens. Histological criteria were: (*a*) size of total zone of reaction, (*b*) degree of necrosis and (*c*) degree of cellular infiltration.

Statistical analysis was carried out by using 2-times x or 3-times x contingency tables. The distributions were compared for significance by the χ^2 test at the 0·05 level. The influence of the ageing time upon the toxicity of the sample materials was statistically analysed by a test for trend (Everitt, 1977).

Results

Macroscopic evaluation showed a marked pyogenic reaction of the muscle tissue around the positive control and the lack of any reaction around the negative controls. *Fig.* 1 shows a strong reaction with non-γ-2 material at 1-h ageing time, graded 3. A reaction with Spher-A-Cap at 7 days, graded 1, is demonstrated in *Fig.* 2. The amalgams produce a significantly stronger reaction compared to the composite materials reaction (*Table I*). The new composite material Isopast evokes a more toxic reaction than the conventional composite Compocap at 5 min ageing time.

At 1-h ageing time a decrease in toxicity of Amalcap, Spher-A-Cap and Isopast can be observed. The non-γ-2 amalgam has kept its toxicity and even shows a tendency to increase (*Table II*). There is a further decrease in toxicity with all the amalgams at longer ageing times (*Tables III* and *IV*). No change in toxicity can be observed with the composite materials. Compared to the negative control, only the non-γ-2 material is statistically different at the 1-day ageing time period.

At the 7-day period the biologic reaction of all materials is not different from the negative controls (*Table IV*). However, there is a trend indicating that even at the 7-day period the non-γ-2 material is slightly more toxic than the two other amalgams, just as Isopast is slightly more toxic than Compocap.

Microscopic evaluation showed necrotic muscle tissue and the dead inflammatory cells inside the zone of reaction of the positive control (*Fig.* 3). At the border line between the dead inflammatory cells and the sound muscle tissue, leucocytes and many lymphocytes are present.

The negative control (*Fig.* 4) shows only a thin zone of fibrous tissue and capsule formation beginning around the implant. *Fig.* 5 shows a strong reaction, graded 3, with Spher-A-Cap at 5-min ageing time, *Fig.* 6 a moderate reaction graded 2 with Isopast at 1-day ageing time and *Fig.* 7 no reaction with Amalcap at 7-days ageing time. The results of the microscopic evaluation for the short ageing times are shown in *Tables V* and *VI*. As expected from macroscopic observations, all the amalgams show a strong reaction. At the borderline between sound and necrotic tissue in the cases of Amalcap and Spher-A-Cap a marked infiltration of eosinophilic granulocytes, some leucocytes and many lymphocytes are observed.

The non-γ-2 material shows less eosinophilic cell infiltration, even less than the negative controls. At the 1-h ageing time a decrease in toxicity with the amalgams and the Isopast can be observed. The difference

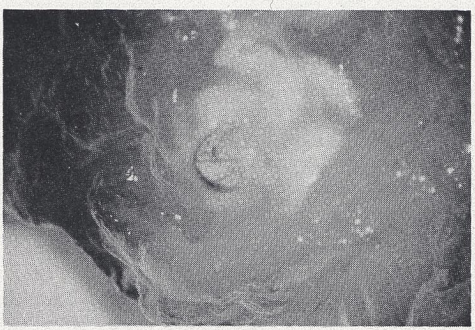

Fig. 1. Macroscopic reaction: Amalcap-non-γ-2: 1-h ageing time: graded 3.

Réaction macroscopique: Amalcap-non-γ-2: vieillissement 1-h: niveau 3.

Makroskopische Reaktion: Amalcap-non-γ-2: 1 Stunde Alterungszeit: Reaktionsgrad 3.

Reacción macroscópica: Amalgama-non-γ-2: 1 hora de antiguedad, grado 3.

Fig. 2. Macroscopic tissue reaction: Spher-A-Cap: 7-day ageing time: graded 1.

Réaction tissulaire macroscopique: Sphere-A-cap: vieillissement 7 jours: niveau I.

Makroskopische Gewebereaktion: Spher-A-cap: 7 Tage Alterungszeit: Reaktionsgrad 1.

Reacción tisular Macroscópia: Spher-A-cap: 7 días de antiguedad, grado 1.

Table I
Frequency distribution of results—macroscopic evaluation—ageing time: 5 min

Materials	No. of samples	Macroscopic reaction No	Mild	Strong
Amalcap	10	0	3	7
Spher-A-Cap	10	0	1	9
Non-γ-2	10	0	8	2
Compocap	10	10	0	0
Isopast	10	5	5	0
Pos. control	10	0	0	10
Neg. control	10	10	0	0

Table II
Frequency distribution of results—macroscopic evaluation—ageing time: 60 min

Materials	No. of samples	Macroscopic reaction No	Mild	Strong
Amalcap	10	4	5	1
Spher-A-Cap	10	2	1	7
Non-γ-2	10	0	0	10
Compocap	10	10	0	0
Isopast	10	8	2	0
Pos. control	10	0	0	10
Neg. control	10	10	0	0

Table III
Frequency distribution of results—macroscopic evaluation—ageing time: 1 day

Materials	No. of samples	Macroscopic reaction No	Mild	Strong
Amalcap	10	4	5	1
Spher-A-Cap	10	8	1	1
Non-γ-2	10	2	7	1
Compocap	10	9	1	0
Isopast	10	7	3	0
Pos. control	10	0	0	10
Neg. control	10	10	0	0

between the two conventional amalgams on the one hand and the non-γ-2 material on the other is statistically significant as is the difference between the positive controls and the conventional amalgams. This means that at the 1-h period the conventional amalgams show a moderate tissue response, whereas the high copper amalgam produces a strong reaction. The difference between the two composites is statistically significant also. The reactions at the longer ageing times (*Tables VII* and *VIII*) show a further decrease in toxicity for amalgams.

There is a significant difference between the Amalcap and the non-γ-2 material, but not between Spher-A-Cap and the high copper material at the 1-day period (*Table*

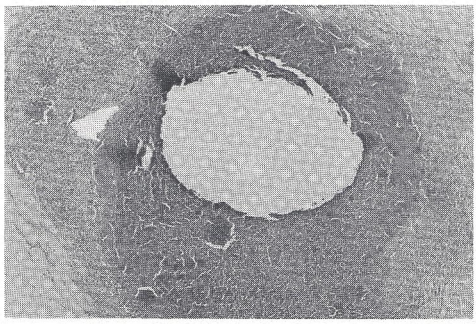

Fig. 3. Microscopic tissue reaction: positive control: graded 3 (× 18)

Réaction tissulaire microscopique: contrôle positif: niveau 3 (× 18).

Mikroskopische Gewebereaktion: positive Kontrolle: Reaktionsgrad 3 (× 18).

Reacción tisular microscópica: control positivo: grado (× 18).

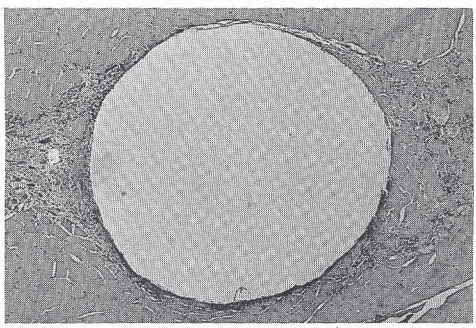

Fig. 4. Microscopic tissue reaction: negative control: graded 1 (× 18).

Réaction tissulaire microscopique: contrôle négatif: niveau 1 (× 18).

Mikroskopische Gewebereaktion: negative Kontrolle: Reaktionsgrad 1 (×18).

Reacción tisular microscópica: control negativo: grado 1 (× 18).

Table IV
Frequency distribution of results—macroscopic evaluation—ageing time: 7 days

Materials	No. of samples	Macroscopic reaction No	Mild	Strong
Amalcap	10	8	2	0
Spher-A-Cap	10	9	1	0
Non-γ-2	10	6	4	0
Compocap	10	8	2	0
Isopast	10	6	4	0
Pos. control	10	0	0	10
Neg. control	10	10	0	0

Table V
Frequency distribution of results—microscopic evaluation—ageing time: 5 min

Materials	No. of samples	Microscopic reaction No	Mild	Strong
Amalcap	40	0	0	40
Spher-A-Cap	40	0	0	40
Non-γ-2	40	0	0	40
Compocap	40	40	0	0
Isopast	80	0	80	0
Pos. control	40	0	0	40
Neg. control	40	40	0	0

Table VI
Frequency distribution of results—microscopic evaluation—ageing time: 60 min

Materials	No. of samples	Microscopic reaction No	Mild	Strong
Amalcap	40	0	25	15
Spher-A-Cap	40	0	20	20
Non-γ-2	40	0	0	40
Compocap	40	40	0	0
Isopast	80	24	56	0
Pos. control	40	0	0	40
Neg. control	40	40	0	0

VII). At the 7-day period (*Table VIII*) the high copper amalgam exhibits greater toxicity than the other amalgams and the negative control. There is also a marked difference between the two composites.

The analysis of trend (*Tables IX* and *X*) shows, with both microscopic and macroscopic evaluation, that with the amalgams most of the overall variation in the contingency table can be explained by linear regression demonstrating the important influence of the ageing time upon toxicity. However, with the composites (*Tables XI* and *XII*) the contrary can be demonstrated: most of the overall variation in the respective contingency table can be explained by the deviation from linear regression indicating no

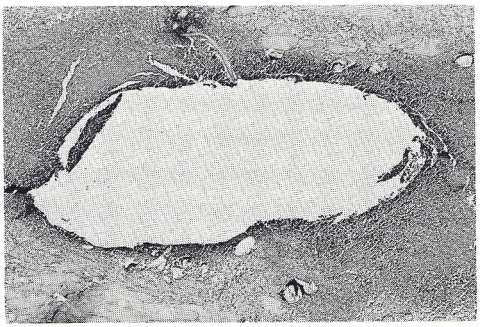

Fig. 5. Microscopic tissue reaction: Spher-A-Cap: 1-day ageing time: graded 3 (× 18).

Réaction tissulaire microscopique: Spher-A-Cap: vieillissement 1 jour: niveau 3 (× 18).

Mikroskopische Gewebereaktion: Spher-A-Cap: 1 Tag Alterungszeit: Reaktionsgrad 3 (× 18).

Reacción tisular Microscópica: Spher-A-cap: 1 día de edad, grado 3 (× 18).

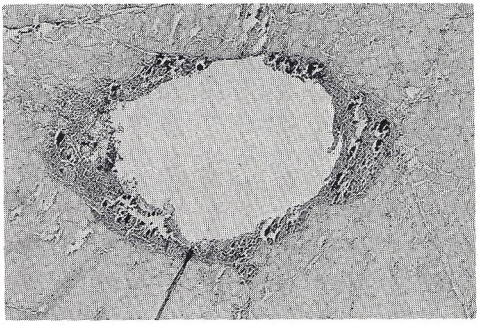

Fig. 6. Microscopic tissue reaction: Isopast: 1-day ageing time: graded 2 (× 18).

Réaction tissulaire microscopique: Isopast: Vieillissement I jour: Niveau 3 (× 18).

Mikroskopische Gewebereaktion: Isopast: 1 Tag Alterungszeit: Reaktionsgrad 2 (× 18).

Reacción tisular microscopica: Isopast: 1 día de edad, grado 2 (× 18).

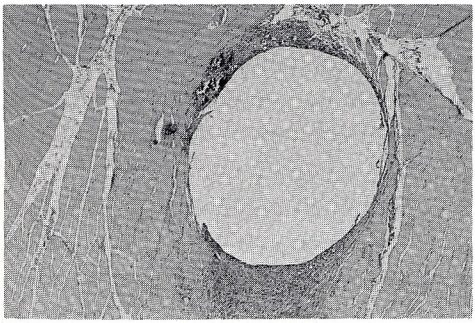

Fig. 7. Microscopic tissue reaction: Amalcap: 7-day ageing time: graded 1 (× 18).

Réaction tissulaire microscopique: Amalcap: vieillissement 7 jours: niveau 1 (× 18).

Mikroskopische Gewebereaktion: Amalcap: 7 Tage Alterungszeit: Reaktionsgrad 1 (× 18).

Reacción tisular microscópica: cápsula de amalgama: 7 días de edad: grado 1 (× 18).

Table VII
Frequency distribution of results—microscopic evaluation—ageing time: 1 day

Materials	No. of samples	Microscopic reaction No	Mild	Strong
Amalcap	40	40	0	0
Spher-A-Cap	40	11	29	0
Non-γ-2	40	21	19	0
Compocap	40	8	32	0
Isopast	80	24	56	0
Pos. control	40	0	0	40
Neg. control	40	40	0	0

Table VIII
Frequency distribution of results—microscopic evaluation—ageing time: 7 days

Materials	No. of samples	Microscopic reaction No	Mild	Strong
Amalcap	40	40	0	0
Spher-A-Cap	40	40	0	0
Non-γ-2	40	25	15	0
Compocap	40	0	40	0
Isopast	80	40	40	0
Pos. control	40	0	0	40
Neg. control	40	40	0	0

Table IX
Analysis for trend with amalgams—macroscopic evaluation

Source of variation	Degree of freedom	χ^2	Level of significance
Linear regression	1	52·97	$< 1 \times 10^{-9}$
Deviation from linear regression	5	7·10	0·21
Overall variation	6	60·07	$< 1 \times 10^{-9}$

Table X
Analysis for trend with amalgams—microscopic evaluation

Source of variation	Degree of freedom	χ^2	Level of significance
Linear regression	1	363·93	$\ll 1 \times 10^{-9}$
Deviation from linear regression	5	101·81	$< 1 \times 10^{-9}$
Overall variation	6	465·74	$\ll 1 \times 10^{-9}$

Table XI
Analysis for trend with composites—macroscopic evaluation

Source of variation	Degree of freedom	χ^2	Level of significance
Linear regression	1	0·37	0·54
Deviation from linear regression	5	2·24	0·82
Overall variation	6	2·61	0·86

Table XII
Analysis for trend with composites—microscopic evaluation

Source of variation	Degree of freedom	χ^2	Level of significance
Linear regression	1	1·84	0·17
Deviation from linear regression	5	18·83	0·002
Overall variation	6	20·67	0·002

influence of the ageing times used in these experiments.

Discussion

The demonstrated influence of the ageing time of amalgams upon toxicity is consistent with previous *in vitro* findings by Nunez et al. (1976a) and by Kawahara et al. (1975). This phenomenon may be due to the setting reaction or to the build-up of a non-toxic surface layer on the material. On human teeth Granath and Moeller (1971) and Moeller and Granath (1973) found initial pulp damage immediately after insertion of an amalgam filling. This was attributed mainly to the packing pressure (Granath and Moeller, 1971; Moeller and Granath, 1973). The more prolonged *in vitro* toxic reaction of a high copper two component amalgam is also consistent with findings of Nunez et al. (1976b). The *in vitro* toxic potential of copper ions has been established by Leirskar (1974). High copper amalgams form a copper–tin phase which partially comes to the surface of the materials as shown by Marek and Okabe (1977). Espevic (1977) found a high copper release of those materials into the surrounding fluid medium compared with other amalgams. At the 7-day period, we found a definite decrease in toxicity of the high copper material; however, it is more toxic than the conventional amalgams which is consistent with *in vivo* findings by Mjör (1978).

The comparatively low but still evident toxicity of the composite materials is in accordance with *in vivo* findings by Klötzer et al. (1977) with the Compocap and by Riethe et al. (1978) with the Isocap. In both investigations with monkey teeth, the test material, when directly applied onto the dentin, produced no reaction or a very mild reaction of the odontoblast layer. However, in both publications a liner was recommended as a base. Similar results were reported after clinical evaluation of the tissue compatibility of composite filling materials (Schmalz, 1981). The increase in toxicity with the composite materials and the difference between the two composites is not easily explained. However, former investigations (Schmalz, 1981) showed that materials delivered in capsules and to be mixed mechanically are generally lower in toxicity than the same materials which have to be mixed by hand.

As the results obtained are in general accordance with *in vivo* findings and clinical experience (Schmalz, 1981), the rabbit muscle implantation tests as used in this investigation is regarded as suitable as a standard test procedure for screening the chemically mediated acute toxicity of solid materials.

SUMMARY

The rabbit muscle implantation test as described by Lawrence et al. (1972) was applied to evaluate the acute non-specific toxicity of 3 amalgams and 2 composite materials, in order to test both the materials' toxicity and the suitability of the method used.

The materials were implanted after different ageing times: 5 min, 1 h, 24 h and 7 days. Macroscopical as well as microscopical evaluation was performed.

Biological reactions of the amalgams proved to be dependent upon the ageing time. All amalgams were strongly toxic at the 5-min and 1-h periods. By the 7-day period the high-copper two component amalgam showed a moderate reaction, the other two amalgams no or uncertain reactions. The composite materials did not show a dependency of the toxicity upon the ageing time as did the amalgams. They evoked a slight to moderate tissue reaction.

The results obtained in this study are in accordance with clinical experience. We regard the rabbit muscle implantation test as a suitable method for screening the chemically initiated toxicity of solid materials.

TESTS DE TOXICITÉ DES MATÉRIAUX POUR OBTURATIONS DENTAIRES
RÉSUMÉ

Le test d'implantation dans un muscle de lapin tel que décrit par Lawrence et autres (1972) a

été utilisé pour évaluer la toxicité aiguë non spécifique de trois amalgames et de deux matériaux composites afin d'expérimenter à la fois la toxicité des matériaux et l'adéquation de la méthode utilisée.

Les matériaux étaient implantés après différents temps de vieillissement: 5 minutes, I heure, 24 heures et 7 jours. On procédait à une évaluation macroscopique aussi bien que microscopique.

Les réactions biologiques des amalgames ont montré une corrélation aves le temps de vieillissement. Tous les amalgames étaient fortement toxiques dans le délai de 5 minutes et d'une heure. Au bout de la période de 7 jours, l'amalgame comportant une forte teneur en cuivre présentait une réaction modérée, les deux autres amalgames pas de réaction ou une réaction incertaine. Les matériaux composites ne faisaient pas apparaitre la même corrélation entre réaction de toxicité et temps de vieillissement que les amalgames. Ils évoquaient une réaction tissulaire allant de légère à modérée.

Les résultats obtenus dans cette étude corroborent l'expérience clinique. L'auteur en déduit que le test d'implantation dans un muscle de lapin constitue une méthode qui convient pour l'étude de la toxicité d'origine chimique des matériaux solides.

TOXIZITÄTSTEST BEI ZAHNÄRZTLICHEN FÜLLUNGSMATERIALIEN
ZUSAMMENFASSUNG

Der Kaninchenmuskel-Implantationstest, wie er von Lawrence et al. (1972) beschrieben wurde, ist angewandt worden, um die akute, unspezifische Toxizität von 3 Amalgamen und 2 Komposit-Materialien zu untersuchen. Dabei wurde sowohl die Toxizität der Materialien wie die Eignung der angewendeten Methoden getestet.

Die Materialien wurden nach verschiedenen Alterungszeiten untersucht: 5 Minuten, 1 Stunde, 24 Stunden und 7 Tage. Es wurde eine makroskopische und mikroskopische Untersuchung durchgeführt.

Die biologischen Reaktionen auf die Amalgame zeigen eine Abhängigkeit von der Alterungszeit. Alle Amalgame waren stark toxisch nach 5 Minuten und 1 Stunde. Nach 7 Tage Alterung zeigte das stark kupferhaltige Zweikomponenten-Amalgam eine mäßige Reaktion, die beiden anderen Amalgame keine oder nur eine ungewisse Reaktion. Die Komposit-Materialien wiesen keine Toxizitätsabhängigkeit von der Alterungszeit auf wie die Amalgame. Sie riefen eine leichte bis mäßige Gewebereaktion hervor.

Die bei dieser Studie erzielten Ergebnisse stimmten mit klinischen Erfahrungen überein. Wir halten den Kaninchenmuskel-Implantationstest für eine geeignete Methode, die chemisch ausgelöste Toxizität fester Materialien zu prüfen.

TESTS DE TOXICIDAD EN LOS MATERIALES DE RELLENO DENTALES
RESUMEN

Los test de implantación en músculo de conejo se describen por Lawrence y colaboradores en 1.972 y fueron aplicados para evaluar la toxicidad aguda no específica de tres amalgamas y dos composites, para demostrar la toxicidad de ambos materiales y los resultados de los métodos utilizados.

Los materiales fueron implantados después de diferentes tiempos: 5 minutos, 1 hora, 24 horas y 7 días. Se realizó una evaluación tanto macroscópica como microscópica.

Reacciones biológicas de amalgamas han demostrado na correlacíon con la antiguedad. Todas las amalgamas fueron muy tóxicas a los 5 minutos y a la hora. A los 7 días las amalgamas con alto componente en cobre demostraron una reacción moderada, las otras dos amalgamas no tuvieron reacción demostrable. Los materiales de composite no demostraron una dependencia de la toxicidad en relación con la antiguedad a diferencia de las amalgamas Ellas evocaron una reacción tisular moderada.

Los resultados obtenidos en este estudio están de acuerdo con las experiencias clínicas. Nosotros miramos al implantación muscular en el conejo como un método para investigar la toxicidad inicial química de los materiales sólidos.

REFERENCES

ESPEVIC S. (1977) The effect of Cu additions on the corrosion of amalgams. *J. Dent. Res.* **56,** A102 (Abstr. no 237).

EVERITT B. S. (1977) *The Analysis of Contingency Tables.* London, Chapman and Hall.

GRANATH L. E. and MOELLER B. (1971) Reaction of the human dental pulp to silver amalgam restorations. The effect of amalgam of high plasticity in shallow cavities. *Acta Odontol. Scand.* **29,** 165–172.

KLÖTZER W. T., ROSENDAHL R. and RIETHE P. (1977) Kompositfüllungsmaterialien im Tierversuch. *Dtsch. Zahnärztl. Z.* **32,** 367–372.

LAWRENCE W. H., DILLINGHAM E. O., TURNER J. E. et al. (1972) Toxicity profile of chloracetataldehyde. *J. Pharmacol. Sci.* **61,** 19–25.

LEIRSKAR J. (1974) On the mechanism of cytotoxicity of silver and copper amalgams in a cell culture. *Scand. J. Dent. Res.* **82,** 74–81.

KAWAHARA H., NAKUMURA M., YAMAGAMI A. et al. (1975) Cellular response to dental amalgam *in vitro. J. Dent. Res.* **54,** 394–401.

MAREK M. and OKABE T. (1977) Corrosion behaviour of structural phases in high copper dental amalgam. *J. Dent. Res.* **56,** A102 (Abstr. no 239)

MJÖR J. A. (1978) Biologic assessment of restorative dental materials. *Operat. Dent.* **3,** 9–13.

MOELLER B. and GRANATH L. E. (1973) Reaction of the human dental pulp to silver amalgam restorations. The effect of insertion of amalgam of high plasticity in deep cavities. *Acta Odontol. Scand.* **31,** 187–192.

NUNEZ L. J., SCHMALZ G. and HEMBREE J. H. (1976a) Influence of amalgam, alloy and mercury on the *in vitro* growth of streptococcus mutans. I. Biological test system. *J. Dent. Res.* **56,** 257–261.

NUNEZ L. J., SCHMALZ G. and HEMBREE J. H. (1976b) Influence of amalgam, alloy and mercury on the *in vitro* growth of streptococcus mutans. III. Effects of specimen age and composition. *J. Dent. Res.* **55,** 1001–1003.

RIETHE P., ROTGANS J. und SCHMALZ G. (1978) Tierexperimentelle Prüfungen mit einem neuen Füllungsmaterial (Isocap). *Dtsch. Zahnärztl. Z.* **33,** 609–615.

SCHMALZ G. (1981) *Die Gewebeverträglichkeit zahnärztlicher Materialien.* Stuttgart, Georg Thieme.

THE PHARMACOPEIA OF THE UNITED STATES OF AMERICA XVIII (1970) *Biological Tests: Plastic Containers.* Eaton P A, Mack Publ. Co.