Acta odont scand 1972 *30:* 39—47

Keywords:
Dental pulp
Dental pulp exposure
Blood circulation
Blood vessels
Radioisotope scanning
Hydrogen peroxide

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INFLUENCE OF CAVITY WASHING AGENTS ON PULPAL MICROCIRCULATION IN THE CAT

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The rate of disappearance of radioactive iodide from a deep dentinal cavity has been used as a means to measure the influence of various cavity washing agents on pulpal microcirculation. Hydrogen peroxide in concentrations of 2—4 % exerted a pronounced influence when it was allowed to act in deep dentinal cavities where the red colour of the pulp was barely visible through the dentin. Repetitive treatments of 3 % hydrogen peroxide of 40 sec duration caused a potentiated depression of the microcirculation. This was true when the dentinal wall between the cavity and the pulp was intact. In cases of pulp exposure the influence seemed to be much less. All other agents tested failed to influence the pulpal microcirculation as reflected by the present method.

In the treatment of deep dentinal cavities and pulp lesions a large variety of agents is available for cleansing the dentin. It is apparent that such a washing procedure should not exert any insult on the pulp (*Nyborg*, 1955; cf. *Brännström*, 1961).

Few investigations have dealt with this problem by means of studying the blood circulation of the pulp (*Kozam & Burnett*, 1959; *Pohto & Scheinin*, 1961).

The aim of this paper was to use the tracer disappearance method (*Kety*, 1949) as a means of studying the influence of such procedures on the microcirculation of the pulp. We have investigated the effect of several agents with cleansing and antimicrobial properties.

Received for publication, September 9, 1971.

METHODS AND MATERIALS

Operative procedure. Experiments were performed on 9 adult cats (2.5—3.6 kg and about 1—6 years old) anesthetized with sodium pentobarbital (30 mg/kg i.v. with supplement as necessary). The trachea was cannulated and provision made for recording femoral blood pressure. The rectal temperature was continously monitored and the body temperature was kept constant at about 38°C by heating lamps. X-ray pictures were taken of the canine teeth to determine the form of the pulp chamber. The head was immobilized by means of a steel rod inserted between the jaws and secured in place by dental acrylic. Two canine teeth remained exposed for insertion of a water circulated thermode in contact with one of them to ensure maintenance of a constant temperature of 37—39°C; this was monitored by a thermocouple.

Based on the information from the x-ray film, cavity positions were chosen, one over the pulp horn (coronal cavity) and one within the gingival half of the crown (test cavity). At these locations the enamel was removed using a diamond instrument operated at slow speed. Bodywarm Macrodex ® 6 % with sodiumchloride 0.9 % (Pharmacia) was used to prevent drying of the dentin. The cavities were deepened by means of a carbide tipped end cutting bur (Meisinger 207 L 1) rotated by a holder held between the fingers and observed through a binocular microscope. Both cavities were deepened until the pulp was barely visible through the dentin. In some test cavities the preparation was extended until the pulp was clearly visible through a very thin layer of dentin and a small exposure was produced with the tip of a No 2 pulp canal file (Kerr). After placement of Plastibase (Squibb) insulating gel on the enamel around each cavity they were filled with Macrodex solution and covered with a thin plastic film to prevent evaporation.

Tracers and washing agents. Radioactive tracers were obtained in isotonic carrier-free solutions from AB Atomenergi, Studsvik, Nyköping, Sweden; I^{125} and I^{131} (80 μCi/μl) as iodide dissolved in phosphate buffer (pH 7—8) containing sodium thiosulphate.

The washing agents used (Table I) were dissolved in distilled water. As controls Macrodex solution and distilled water were used.

Disappearance measurements. The Macrodex solution in the coronal cavity was replaced by a depot of I^{125} solution (0.1—0.2 μl) which was again covered with a thin plastic film. The disappearance of the tracer depot was monitored by an external scintillation detector. The detector output was fed into two recording channels, each containing a single channel pulse height analyzer and two scalers with digital print out. Radioactivity was counted for one

min periods. In agreement with observations previously reported (*Edwall & Kindlová* 1971) the disappearance rate from the dentinal cavity was mono-exponential for more than 1 hr. Two or three subsequent tracer placements were usually made in the same cavity. After correction for total final background the rate of disappearance of the tracer was determined using the relation

$$k = (\log C_1 - \log C_2) / 0.4343 (t_2 - t_1) \text{ (Kety, 1949).}$$

where C_1 and C_2 are the recorded net count rates of the depot at times t_1 and t_2. The calculations were made for each 1 min period. The k-values thus obtained represent the running average of the fractional elimination of the depot per min. For further technical details, see *Edwall* and *Kindlova* (1971), and *Edwall* and *Scott* (1971).

Testing procedure of the washing agents. The experiments were started 7—15 min after the placement of tracer in the dentinal cavity when the control disappearance rate was constant. The Macrodex solution in the test cavity was absorbed with a strip of filter paper and replaced by a washing agent to be tested. The cavity was filled with the agent for periods of 5—7 min. In a series of experiments on hydrogen peroxide shorter times were also used (40 sec — 2 min). The time of action of the agent was measured from the moment it was present in the cavity until it was replaced with the control solution, which was allowed to stay in the test cavity for at least 6 min. The sequence in which the agents were tested was randomized. In order to check whether the change of liquid *per se* or whether the solvent *per se* influenced the disappearance rate, the Macrodex present in the test cavity during control periods was replaced by a new volume of Macrodex or distilled water. This procedure resulted in change in the disappearance rate in two cases, probably due to contamination of the test cavity by the tracer, and these were excluded from the material.

In order to check whether the dentinal wall between the test cavity and the pulp was permeable to small water soluble ions the test cavities without pulp lesion were labelled with a tracer depot of I^{131} in the end of the experiment. The disappearance of this depot was monitored simultaneously with the disappearance of I^{125} from the coronal cavity. This double tracer technique enabled the independent recording of the disappearance rate of iodide from the two sites in the tooth. For further details concerning the double tracer technique see *Edwall* and *Scott* (1971).

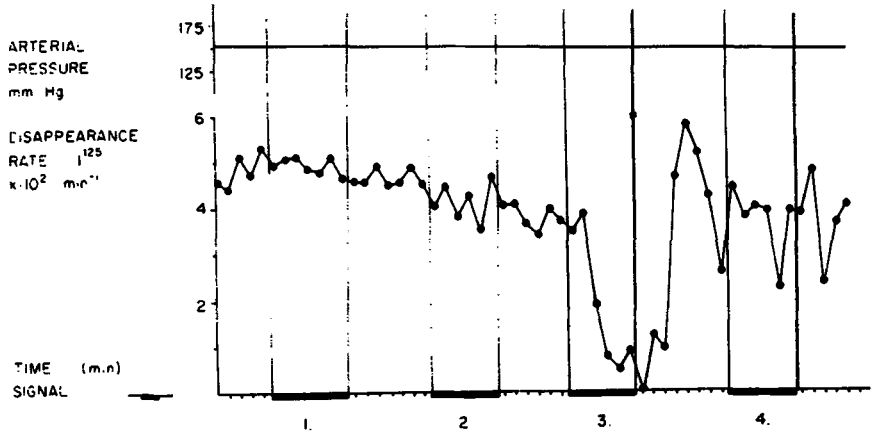

Fig. 1. Influence of washing agents on the disappearance rate of I^{125} from a dentinal cavity over the pulp horn. The following solutions were used:
1. Sodium hypochlorite 1 %
2. Biosept® 1 %
3. Hydrogen peroxide 3 %
4. Iodine-potassium iodide 5 %

During control periods the test cavity was filled with Macrodex. Temperature on the tooth was constant at 38.5°C. No exposure of the pulp.

RESULTS

Long term influence. In one series of experiments (8 teeth on 6 cats) the test cavity was filled with the washing agent for 5—7 min. A typical protocol from an experiment without pulp exposure is shown in Fig. 1. During the control periods the test cavity was filled with Macrodex solution. As can be seen from Fig. 1:1 and 1:2 the solutions of sodium hypochlorite and quarternary ammonium compound (Biosept®) did not cause any observable effect on the disappearance rate (k-value). In contrast 3 % solution of hydrogen peroxide (Fig. 1:3) caused a reduction of the k-value to near zero level after a delay of 2—3 min. Recovery was seen 2—3 min after the agent was replaced by Macrodex with a rapid rise to values above control during the first 3 min of recovery. Iodine-potassium iodide 5 % solution (Fig. 1: 4) did not induce any detectable change in k-value.

Summarizing the findings (Table I) shows that the following agents did not induce any detectable change in k-value irrespective whether there was a pulp exposure or not: ethyl alcohol 70 %, Biosept 1 %, saturated calcium hydroxide solution, chloramine 5 %, iodine-potassium iodide 5 %, Iodopax 0.4 % and sodium hypochlorite 1 %. In contrast, hydrogen peroxide in concentrations of 4 %, 3 % and 2 % consistently reduced the k-value in

Table I.

Long term (5—7 min) influence of washing agents on pulpal microcirculation. (—) and (0) indicates reduction and no reduction of the k-values respectively

Washing agent	No exposure		Exposure	
	0	—	0	—
Ethyl alcohol 70 %	3		3	
Biosept® (Recip) 1 %	3		3	
Calcium hydroxide solution (saturated, pH 12)	3		3	
Chloramine 5 %	3		3	
Iodine-potassium iodide 5 %	3		3	
Iodopax® (Ferrosan) 0.4 % (of stock solution)	3		3	
Sodium hypochlorite 1 %	3		3	
Hydrogen peroxide 1 %	4			
Hydrogen peroxide 2 %		4		
Hydrogen peroxide 3 %		8	7*	
Hydrogen peroxide 4 %		1		
Macrodex® 6 % with sodium chloride 0.9 % (Pharmacia) (control)	63		34	
Distilled water (control)	3		3	

* a transient increase of the k-value was observed in some cases.

cases without pulp exposure. The reduction in k-value amounted to 90—100 % with a delay of 1—3 min before the effect on the k-value was observable. After replacement with Macrodex in the test cavity the depression of the k-value persisted for 2—12 min before recovery. This was shown in 13 procedures. A concentration of 1 % hydrogen peroxide consistently failed to reduce the k-value.

In cases when a pulp exposure was present (3 teeth and 7 procedures) the above mentioned response upon hydrogen peroxide (3 %) was different; no reduction of the k-value was observed. Instead a transient increase in k-value was sometimes noted.

Short term influence of hydrogen peroxide. Hydrogen peroxide in 3 % solution was used during shorter time periods (40—45 sec) in cavities without pulp exposure (7 procedures). A typical experiment is shown in Fig. 2, where 3 short periods of influence of hydrogen peroxide, separated by control periods of Macrodex, were instituted during 20 min (Fig. 2:2). As can be seen, the first period resulted in a barely detectable effect on the k-value, while the following period showed a clearcut reduction of the k-value. The third period showed a potentiated reduction of the k-value as compared with the previous period.

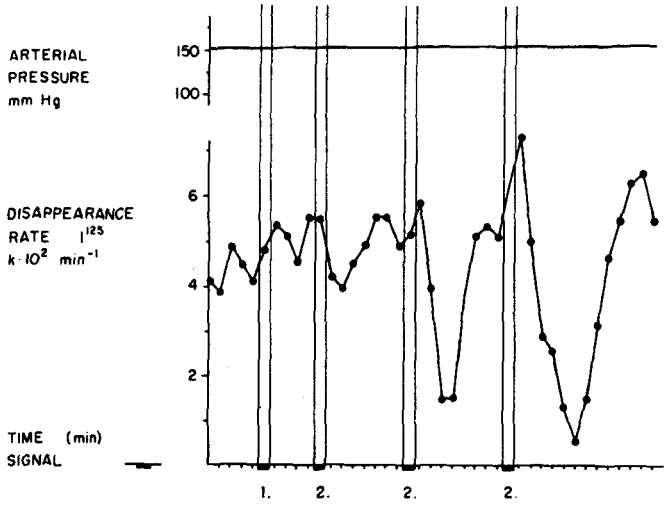

Fig. 2. Influence of repetitive 40 sec periods of hydrogen peroxide 3 %.
1. Control: change of Macrodex solution
2. Hydrogen peroxide 3 %
Temperature on the tooth was constant at 37.8°C. No exposure of the pulp.

The potentiated effect of hydrogen peroxide when used repetitively is demonstrated in Fig. 3 which also illustrates that an initial short application (Fig. 3: 1) sometimes resulted in a transient increase in k-value. The second application (Fig. 3: 2) reversed this response and produced a nearly total reduction of the k-value which was maintained during 6 min. This was followed by a rapid recovery with an overshoot. The third application which was identical with the first (Fig. 3: 1) showed a potentiated response of the k-value, amounting to a 90 % reduction which lasted for 6 min.

DISCUSSION

The present results clearly demonstrate that hydrogen peroxide affected the tracer disappearance rate but this was not the case with the other agents investigated despite they were tested for periods of 5—7 min.

The authors have used a modified tracer disappearance method (*Meyer*, 1966; *Edwall & Kindlová*, 1971; *Edwall*, 1971) as a means to measure change in pulpal microcirculation. Recent studies indicate that change in the recorded rate of tracer disappearance from deep dentinal cavities is closely related to change in pulpal blood flow but is also influenced by local

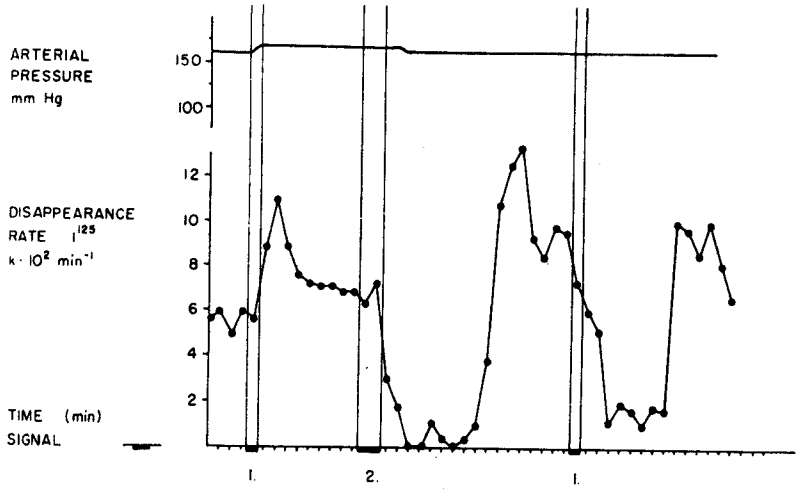

Fig. 3. Influence of repetitive periods of hydrogen peroxide 3 %.
1. Hydrogen peroxide during 1 min
2. Hydrogen peroxide during 2 min
Temperature on the tooth was constant at 38.0°C. No exposure of the pulp. During control periods the test cavity was filled with Macrodex.

factors, presumably in the capillary section affecting the exchange function (*Bolme & Edwall*, 1971; *Edwall & Kindlová*, 1971).

The localisation of the I^{125} depot over the pulp horn implies, however, that the observed changes in k-value in the present study refer to changes in the microcirculation in the pulp horn. Since the test cavity was localized in the gingival half of the crown the question arises whether the response on microcirculation in the pulp horn was accompanied with a similar response in the vicinity of the test cavity or if they differed. In order to examine this problem the double tracer experiments were performed, where the disappearance rates from both cavities were recorded simultaneously. The rates of disappearance from the two locations were practically identical during resting conditions after testing the washing agents. Moreover, when the test cavity was labelled with I^{131} during a depression of the microcirculation in the pulp horn caused by a previous action of hydrogen peroxide in the test cavity, both k-values remained depressed for the same period of time and recovered simultaneously. This observation indicates that the recorded changes in k-value refer to changes in microcirculation in the whole coronal pulp and not only in the vicinity of the coronal cavity. A complete standstill of tracer disappearance, as was observed following hydrogen peroxide, thus suggests a serious alteration of the blood circulation in the pulp.

The present observation that hydrogen peroxide in cavities without exposure influences pulpal microcirculation supports previous studies by *Pohto* and *Scheinin* (1958, 1961) where the formation of gas embolies under intact dentin occluding the vascular bed was described. However, on the exposed pulp we were not able to confirm the finding of these authors that hydrogen peroxide caused reduction in the pulpal blood circulation. It seems reasonable, however, that when a small pulp lesion is present and the vascular bed remains undamaged, the gas embolies will escape from the pulp chamber thus causing only superficial change and little effect on the vascular bed under the lesion. The vital microscopic study of *Pohto* and *Scheinin* revealed intravascular gas embolies in the exposed pulps where at least the terminal vascular bed was traumatized in connection with the relatively large area of exposure. The discrepancy between the two studies may thus be due to several causes, for example differences in preparation technique and size of the lesion.

The present observation that an initial short action of hydrogen peroxide sometimes increased the disappearance rate, while repetitive identical treatments induced potentiated reduction, shows that the initial treatment by the agent had an influence on the pulp although not enough to reduce the microcirculation in the coronal part of the pulp. However, from the clinical point of view this observation deserves consideration since the potentiated influence was seen when control periods of 8 min separated the short applications of hydrogen peroxide. This effect may be due to a residual of hydrogen peroxide in the dentin and small gas embolies in the pulp which add their influence to a new dose of the agent. Moreover, the potentiated influence was pronounced; long lasting nearly total reduction of pulpal blood flow was seen.

In all procedures when hydrogen peroxide reduced pulpal blood flow the effect was transient and blood flow recovered. It should be stressed, however, that the present investigation was carried out on healthy pulps which had not suffered from the influence of dentinal decay nor from insults related to local anesthesia or thermal trauma during cavity preparation. In the clinical situation, when such insults are present, hydrogen peroxide used in concentration of 3 % for short periods may cause irreversible disturbance in pulpal blood flow. The probable noxious effect of this agent when used in deep dentinal cavities has been pointed out earlier (cf. *Brännström*, 1961) based on the observations by *Pohto* and *Scheinin* (1958).

Acknowledgements: This investigation was supported by grants from the Swedish Medical Research Council (B72-24X-817-07) and Karolinska Institutet, Stockholm.

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