

# Human pulp reactions to resin restorations performed with different acid-etch restorative procedures

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Fifty-eight experimental resin restorations were performed in intact, human premolars, using different leakage-reducing restorative procedures. These were conventional acid-etching and acid-etching followed by cavity treatment with an intermediary layer of low-viscous resin or the dentin adhesive NPG-GMA/ethanol. The teeth were extracted after 4 months and examined for pulpal inflammation/necrosis (I), reduction of odontoblasts (OR), and formation of tertiary dentin (TD). By the general linear model procedure, 91%, 34%, and 56% of the variations in I, OR, and TD, respectively, could be explained by variations in the experimental conditions. The significant independent variables were jaw, stage of root formation, width of pulp, width of cavity, marginal leakage, bacteria in the cavity, bacteria in the exposed dentinal tubules, and the restorative procedure. With regard to the restorative procedure the analyses showed that application of low-viscous resin increased the pulpal reactions OR and I, whereas cavity treatment with NPG-GMA/ethanol had no adverse biologic effect. □ *Bacterial microleakage; composites; dental materials; dentin adhesives; histology*

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Research has clearly shown that several factors affect pulpal responses to dental restorations, including cavity preparation, toxicity of the materials placed, and subsequent leakage of bacteria and bacterial substances along cavity margins. It is no wonder, therefore, that recent studies on experimental resin restorations performed with modern techniques such as acid-etching and dentin adhesives have led to different conclusions as to their irritative effects (1–18). Reported reaction patterns range from 'none' to 'moderate' changes in the pulp tissue (2, 5, 9, 10, 13), but 'severe' reactions have also been observed (1, 3, 4, 6–8, 11, 12, 14–18). It is natural to interpret the extreme variation as being an indication of the complex interplay between the inherent toxic potential of the materials and the cavity treatments. In addition to their direct toxic potential the cavity treatments also promote a reduction in marginal leakage, known in

itself to be an important factor for pulpal reactions (2, 4, 7–9, 11, 17–20). In light of the growing use of resin restorative materials and the variety of cavity treatments, there is an obvious need for identification of relevant factors determining the pulpal reactions to resin restorations performed with leakage-reducing restorative procedures.

The first step in this direction is the availability of statistical procedures making it possible to treat both quantitative and qualitative data. It is well known that classical analyses of variance and regression, among other fundamental assumptions, require quantitative data measured in the least on an interval scale (21). However, many of the relevant variables involved in pulpal studies are qualitative ones, or they can only be measured on an ordinal scale, as, for instance, marginal leakage and different degrees of pulpal reactions. In a recent study it was documented, however, that general

linear model analysis (GLM analysis) (22) is capable of handling both qualitative and quantitative data for the identification of significant variables that can explain the pulpal reactions to restorative materials (23).

For this reason we have used GLM analysis in our present study, which deals with pulpal reactions to resin restorations performed with different leakage-reducing restorative procedures (24). These are conventional acid-etching and acid-etching followed by cavity treatment with an intermediary layer of low-viscous resin or the dentin adhesive NPG-GMA/ethanol.

## Materials and methods

Fifty-eight class V resin restorations were placed in intact premolars in children between 11 and 15 years of age. The teeth were in functional occlusion and scheduled for extraction for orthodontic reasons.

The following restorative procedures were studied: A) acid-etching and filling with composite resin (27 cavities); B) acid-etching, application of low-viscous resin, and filling with composite resin (6 cavities); C) acid-etching with cotton protection of the exposed dentin, application of low-viscous resin, and filling with composite resin (15 cavities); and D) acid-etching, application of the dentin adhesive NPG-GMA/ethanol, and filling with composite resin (10 cavities).

### *Clinical procedure*

Under local anesthesia a slightly undercut cavity with a depth and diameter of approximately 2 and 3 mm, respectively, was prepared on the buccal surface of each tooth with a carbide bur used at high speed with abundant water spray. All cavities and 1 mm of the surrounding enamel surface were etched for 60 sec with 37% phosphoric acid (Concise Etching Liquid, batch 61042, 3M Co., Minneapolis, Minn., USA), sprayed with water for 15 sec, and dried with air blowing for 5 sec. During acid-conditioning the exposed dentin in 15 cavities was protected with hydrophobic cotton (C). An intermediary layer of low-viscous resin (Con-

cise Enamel Bond, batch 8320 K1/L1, 3M Co.) was applied to the etched area in 21 cavities (B + C). Ten other cavities (D) received a double application of a 2% w/w ethanol solution of the dentin adhesive N-phenylglycine-glycidyl methacrylate (NPG-GMA/ethanol) (Cosmic Bond, De Trey Division, Dentsply Ltd., Weybridge, England) followed by brief air-blowing. After cavity treatment a chemically cured composite resin (Concise, batch 7117 FG 23, 3M Co.) was inserted into all cavities with excess and covered with a cervical matrix that was pressed against the tooth surface. The restorations were not finished, but great excess of polymerized resin was removed with an excavator, leaving the margin of the restorations undisturbed.

### *Histologic procedure*

After an observation period of 4 months the teeth were extracted under local anesthesia, fixated in 10% neutral formalin for 4 days, and demineralized in 10% neutral ethylenediaminetetraacetic acid (EDTA). The fillings were then loosened from the cavities and coated for scanning electron microscopy (SEM) examination, and the teeth were embedded in paraffin. Approximately 200 serial sections at 6–8 µm were cut through each cavity pulp area and stained sequentially with hematoxylin and eosin, Movat's tetrachrome staining, and a modification of Hucker's II Gram-staining for microorganisms (25). Examination of the stained sections was carried out without knowledge of the treatment procedure.

For each of the experimental restorations the maximum and the average degree of the pulpal reactions were recorded as infiltration with inflammatory cells or necrosis (I) (Fig. 1), reduction in number of odontoblasts (OR), and width and area of apposition of tertiary dentin (TD) (23). The average recordings were based on scorings and measurements performed on 10 sections selected equidistantly within each tooth from the cavity pulp area. The quantitation, range, mean, and standard error of the mean for each of the pulpal reactions are given in Table 1, together with similar information with

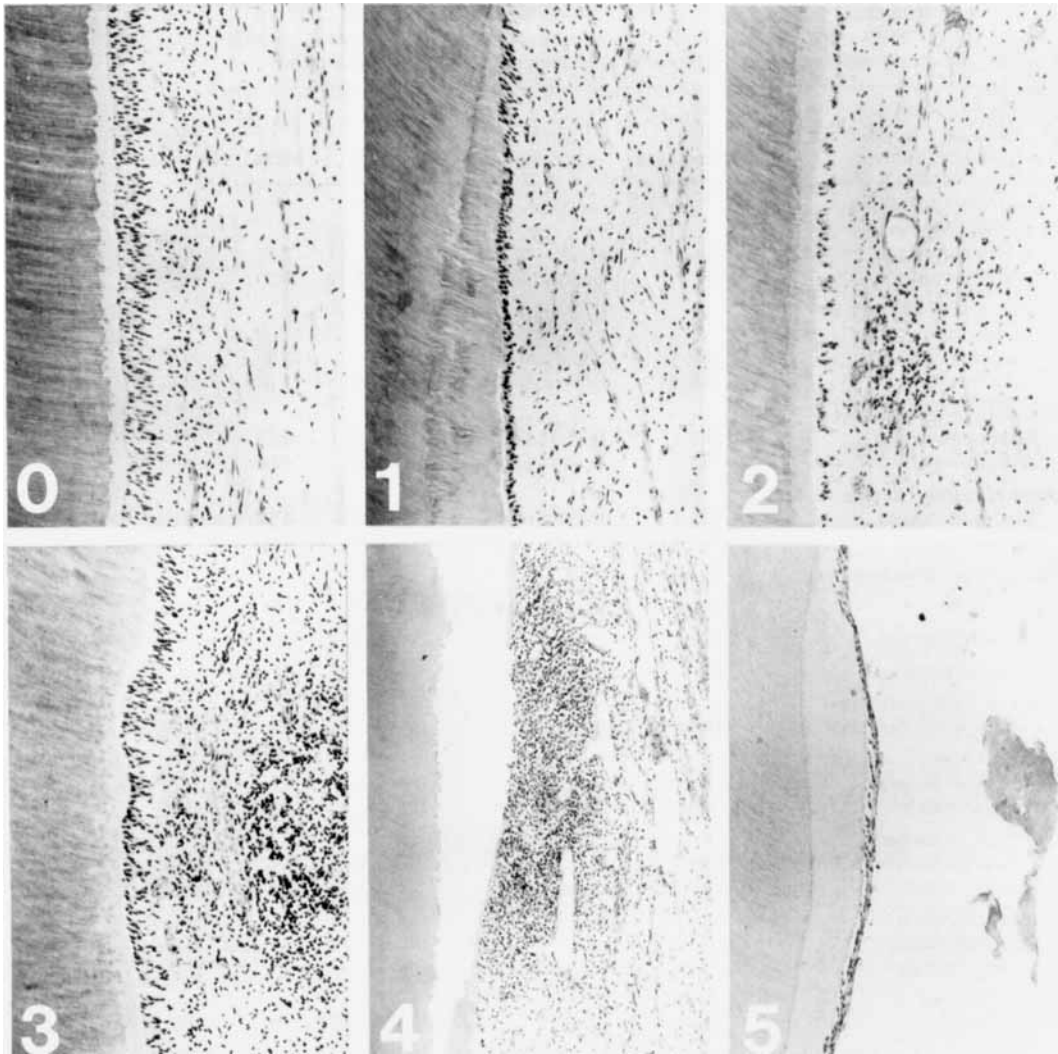


Fig. 1. Examples of scores, ranging from 0 to 5, of long-term inflammatory reactions to experimental restorations. 0 to 1 indicate 'none'; 2, 'mild'; 3, 'moderate'; and 4 to 5 'severe' reactions. Original magnifications; 0-3,  $\times 50$ ; 4-5,  $\times 33$ .

regard to the different independent variables that might have influenced the reactions. These are the restorative procedure (Group), the age of the child (Age), the observation period (Time), the location of the tooth (Jaw), the stage of root formation (Root), the width of the pulp (Pulp), the dentinal extension of the cavity (Cav), the amount of remaining dentin measured in the direction of the tubules (RD) (Fig. 2), the occurrence of 'burned lesions' in the dentin (Burn), and the occurrence of bacteria

beneath the restorations in the cavity and in the exposed dentinal tubules (Bac). On the basis of the results from a previous study bacteria in more than 10% of the Gram-stained sections were further used as a criterion for marginal leakage along the restorations (Leak) (26). An estimate of the resin penetration into the exposed dentinal tubules beyond the restorations was finally obtained by SEM examination of the inner cavity-faced surface of the fillings (Resin) (27).

Table 1. Abbreviation, scale of measurement, range, mean, and standard error of mean for the dependent variables—that is, the pulpal reactions—and for the independent variables—that is, variables that may influence the pulpal reactions to experimental resin restorations

List of variables included in the statistical analysis	Abbreviation	Scale of measurement	Range	Mean	Error of mean
Inflammatory reaction from none to necrosis					
Maximum values	I 1	Scores: 0-5	0-5	1.7	0.21
Average values	I 2	Scores: 0-5	0.0-5.0	1.0	0.18
Reduction of odontoblasts from none to necrosis					
Maximum values	OR 1	Scores: 0-5	1-5	3.4	0.12
Average values	OR 2	Scores: 0-5	0.2-5.0	2.3	0.13
Width of tertiary dentin					
Maximum values	TD 1	In 0.01 mm	0.00-0.45	0.13	0.01
Average values	TD 2	In 0.01 mm	0.00-0.22	0.06	0.01
Area of tertiary dentin					
Maximum values	TD 3	In 0.01 mm <sup>2</sup>	0.00-0.99	0.19	0.03
Average values	TD 4	In 0.01 mm <sup>2</sup>	0.00-0.48	0.09	0.01
Restorative procedure	Group	A/B/C/D	—	—	—
Age of child	Age	In 0.1 years	10.8-14.9	12.9	0.18
Observation period	Time	In days	116-149	127	1.0
Position of tooth	Jaw	Upper/lower	—	—	—
Stage of root formation					
Unfinished to finished	Root	Scores: 1-3	2-3	2.6	0.06
Width of pulp					
Maximum values	Pulp 1	In 0.1 mm	0.6-5.0	2.5	0.12
Average values	Pulp 2	In 0.1 mm	0.5-3.2	1.7	0.07
Extension of cavity					
Maximum values	Cav 1	In 0.1 mm	1.4-3.8	2.4	0.05
Average values	Cav 2	In 0.1 mm	1.3-3.3	2.2	0.05
Remaining dentin					
Minimum values	RD 1	In 0.1 mm	0.3-2.7	1.7	0.06
Average values	RD 2	In 0.1 mm	0.6-3.1	2.0	0.06
'Burned lesions' on cavity walls	Burn 1	-/+	—	—	—
	Burn 2	% of sections	0-100	48	5.0
Bacteria on cavity walls	Bac 1	% of sections	0-100	40	6.2
Average thickness of layer of bacteria	Bac 2	In 3.2 µm	0-10.7	2.5	0.41
Bacteria in dentinal tubules	Bac 3	% of sections	0-100	37	6.2
Marginal leakage					
Bac 1 > 10%	Leak	-/+	—	—	—
Resin penetration into dentinal tubules	Resin	Scores: 0-12	0-11.5	2.3	0.34

### Statistical analysis

The general linear model (GLM) from the SAS (statistical analysis system) package

(22) was used to analyze the significance of each of the independent variables Group, Age, and so forth on the dependent vari-

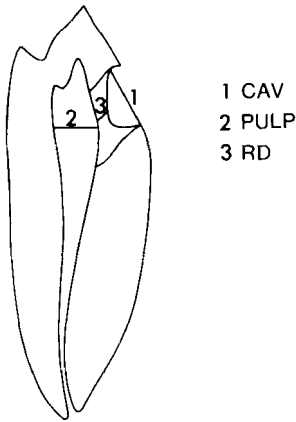


Fig. 2. Schematic illustration of recordings of dentinal extension of cavity (Cav), width of pulp tissue (Pulp), and remaining dentin (RD) for an experimental restoration performed in a lower premolar.

ables, the pulpal reactions I, OR, and TD. In the GLM procedure, as in the analysis of variance or regression, the total variation in the dependent variable consists of an explained part due to the variables included in the model and an unexplained part. The unexplained part represents individual and biologic differences in the pulpal responses to the experimental restorations, the effects of unidentified variables and interactions, and errors of measurement.

At each step in the analysis the independent variable explaining the smallest part of the variation in the pulpal reaction was

identified and removed from the model. The resulting increment in the unexplained part of the variation was tested for significance by means of an F test. This procedure was continued until the model only contained variables significant at a 5% level. Interactions between these variables were then included and tested for significance by the same procedure. For the 'final' models an estimate of the effect on the pulpal reactions of each of the significant independent variables and interactions was calculated.

### Results

The pulpal reactions to the restorations are summarized in Tables 1 and 2, in accordance with the different histologic criteria. The percentage distribution of none, slight, moderate, and severe inflammatory reactions (Fig. 1) to each of the restorative procedures is given in Table 3, together with information on the amount of remaining dentin beneath the cavities, the resin penetration into the exposed dentinal tubules, and the marginal adaptation of the restorations.

Cavity treatment with low-viscous resin (groups B and C) and the dentin adhesive NPG-GMA/ethanol (group D) both reduced the leakage occurrence in comparison with conventional acid-etching (group A) (Table 3). This may explain the

Table 2. Mean, range, and explainable part of pulpal reactions to resin restorations performed with different acid-etch restorative procedures. For explanation of the abbreviations, see Tables 1 and 3

Group	I1, x̄ score and range	I2, x̄ score and range	OR1, x̄ score and range	OR2, x̄ score and range	TD1, x̄ mm and range	TD2, x̄ mm and range	TD3, x̄ mm <sup>2</sup> and range	TD4, x̄ mm <sup>2</sup> and range
A	2.0 0-5	1.1 0.0-5.0	3.6 2-5	2.6 1.2-4.0	0.11 0.00-0.28	0.05 0.00-0.12	0.14 0.00-0.44	0.07 0.00-0.28
B	3.2 0-5	2.8 0.0-5.0	4.2 3-5	3.2 1.6-5.0	0.19 0.02-0.45	0.10 0.01-0.22	0.31 0.01-0.99	0.17 0.01-0.48
C	0.7 0-3	0.2 0.0-1.4	3.2 1-4	1.8 0.2-3.1	0.13 0.00-0.32	0.05 0.00-0.15	0.20 0.00-0.58	0.07 0.00-0.25
D	1.4 0-3	0.5 0.0-1.4	3.0 1-4	1.8 0.3-3.1	0.13 0.03-0.28	0.06 0.01-0.14	0.23 0.00-0.60	0.11 0.00-0.31
Explained part of the variation	63%	91%	34%	37%	29%	56%	32%	36%

Table 3. Remaining dentin, resin penetration, marginal leakage, and inflammatory reactions to resin restorations performed with different acid-etch restorative procedures

Restorative procedure	Group: no. of cavities	Remaining dentin, RD1, mm, $\bar{x}$ , range	Resin penetration into dentin, Resin scores, $\bar{x}$ , range	Marginal leakage, Leak	Inflammatory reaction II				
					0-1 None	2 Slight	3 Moderate	4-5 Severe	
Etching Composite resin	A: 27	1.8 0.8-2.7	1.1 0.0-4.0	52%	11	6	7	3	
Etching Low-viscous resin Composite resin	B: 6	1.1 0.3-1.9	3.9 0.5-11.5	50%	2	—	—	4	
'Cotton', etching Low-viscous resin Composite resin	C: 15	1.8 0.9-2.3	1.8 0.0-7.5	33%	13	1	1	—	
Etching NPG-GMA/ethanol Composite resin	D: 10	1.5 0.9-1.9	5.5 4.0-7.5	30%	6	2	2	—	

observed reduced inflammatory reaction after use of NPG-GMA/ethanol. However, application of low-viscous resin resulted in the most frequent occurrence of abscess formation and necrosis and the greatest reduction of odontoblasts and apposition of tertiary dentin beneath the cavities unless the exposed dentin was protected with hydrophobic cotton during acid-conditioning (Tables 2 and 3). The effectiveness of cotton protection during etching was also reflected in the reduction in resin penetration into the dentinal tubules beneath the restorations in group C versus group B (Table 3).

The GLM analyses disclosed that it was possible to explain from 29% to 91% of the variation in the degree of the eight pulpal reactions listed in Table 1 by means of the significant independent variables and interactions included in the 'final' models (Table 2). It can be seen in Table 2 that the explainable part of the variation varied with the type of reaction and the registration method. The greatest part of the variations could thus be explained when the analyses were performed on average recordings and when the width of tertiary dentin was used. The most informative histologic criteria, therefore, seemed to be I 2, OR 2, and TD 2. Corresponding to these reactions, 91%, 37%, and 56% of the variation could be explained. The unexplained part of the variations, the residuals, was distributed approximately as independent, normal deviates with a mean value at zero. Because of the extreme reactions observed for cavities treated with low-viscous resin the GLM procedure was duplicated excluding the six group-B restorations. These analyses confirmed the significance of the independent variables and interactions in the final models concerning I 2, OR 2, and TD 2, and it was also found that the explained part of the variations remained at approximately the same level as found for the total material.

Table 4 gives the level of significance for each of the independent variables in the final models for the three pulpal reactions. Apparently, the restorative procedure (Group) did not directly influence the formation of tertiary dentin (TD 2: B  $\cong$  D  $\cong$  A  $\cong$  C), even though it had a significant

Table 4. Level of significance for each of the independent variables in the final significant models for the three types of pulpal reactions. For explanation of the abbreviations, see Table 1

List of variables	Inflammatory reaction, I 2	Odontoblast reduction, OR 2	Tertiary dentin, TD 2
Group	0.0001	0.0010	NS
Age	NS	NS	NS
Time	NS	NS	NS
Jaw	0.0001	NS	NS
Root	0.0001	NS	NS
Pulp 1	NS	NS	NS
Pulp 2	NS	NS	0.0004
Cav 1	NS	0.0113	NS
Cav 2	NS	NS	0.0010
RD 1	NS	NS	NS
RD 2	NS	NS	NS
Burn 1	NS	NS	NS
Burn 2	NS	NS	NS
Bac 1	0.0001	NS	NS
Bac 2	NS	NS	0.0011
Bac 3	0.0010	NS	NS
Leak	0.0007	NS	NS
Resin	NS	NS	NS

Bac 3), and the marginal adaptation of the restorations (Leak: + > -). The reduction of odontoblasts increased with the number of exposed dentinal tubules measured as the extension of the cavity (Cav), whereas the formation of tertiary dentin was negatively correlated to this variable (Cav) and to the width of the pulp tissue (Pulp) but increased with the thickness of the layer of bacteria in the cavities (Bac 2), as an indirect effect of the restorative procedure.

The charts in Fig. 3 illustrate the relative importance of the significant independent variables for each of the pulpal reactions I 2, OR 2, and TD 2 on the basis of their respective mean square sums of variation. Thus, 16% and 26% of the explained part of the variation in the inflammatory reactions could be related to the position of the tooth (Jaw) and the stage of root formation (Root), respectively. The major part was, however, related to the treatment. Thirty-four per cent was caused by their leakage-preventing effect in terms of marginal leakage (Leak, 10%), bacteria in the cavity (Bac 1, 12%), and bacteria in the dentin (Bac 3, 12%). The remaining 24% was caused by the direct toxic effect of the different restorative procedures (Group) and thus reflects the significant increase in inflammatory reactions after use of an intermediary low-viscous resin. With regard to the degree of reduction in odontoblast cells and the formation of tertiary dentin, most of the variation was

effect on the degree of inflammation and the reduction of odontoblasts beneath the restorations (I2: B > A ≥ D ≥ C; and OR2: B ≥ A > C ≥ D). The inflammatory reaction was also influenced by the position of the tooth (Jaw: upper > lower), the stage of the root formation (Root: finished > unfinished), the occurrence of bacteria in the cavity and in the dentinal tubules (Bac 1;

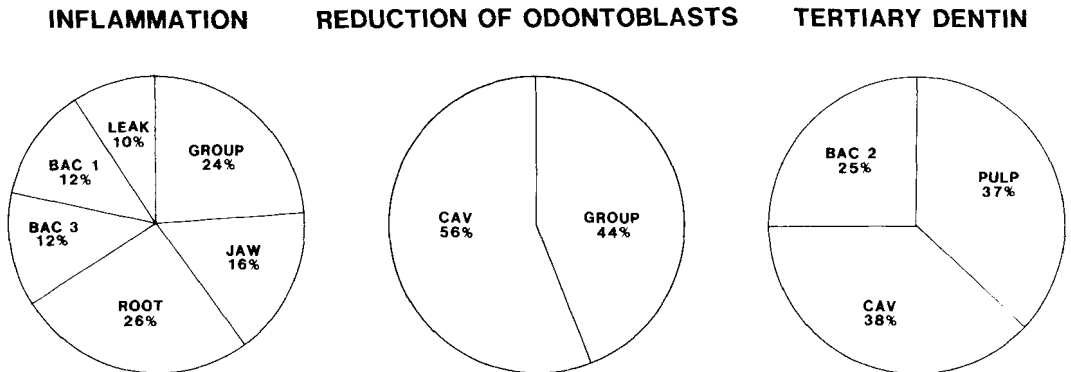


Fig. 3. The relative importance of factors determining the long-term pulpal reactions to experimental resin restorations performed with different acid-etch restorative procedures. For explanation of the abbreviations, see Table 1.

related to the amount of exposed dentinal tubules in the cavities (Cav).

On the basis of the estimated parameters for the variables and interactions included in the final models the expectable mean reactions for the different types of restorations with and without marginal leakage were finally calculated. The figures given in Table 5 are based on 'average' restorations placed in upper premolars with completed root formation (Table 1). They reflect the effect of marginal leakage, including bacterial invasion, on inflammation and apposition of tertiary dentin and the effect of the restorative procedure on inflammation and odontoblast reduction. The calculations indicate that resin restorations performed with acid-etch procedures usually result in reduction of odontoblasts and formation of tertiary dentin beneath the cavities, whereas a prolonged infiltration by inflammatory cells seems to be closely connected with marginal leakage. However, the previously mentioned toxic effect of cavity treatment with an intermediary layer of low-viscous resin (group B) is clearly demonstrated, as this procedure enhances the inflammatory responses markedly, irrespective of marginal adaptation/leakage. However, the calculations also show that the toxic effect of this procedure can be prevented when the dentin is protected during the acid-etching (group C).

## Discussion

The most striking result of this study was the great variation in the pulpal response to the experimental restorations, ranging from barely visible reduction in the number of cells in the odontoblast layer, to limited apposition of tertiary dentin, to a localized necrosis or abscess formation in the pulp tissue. The variation reflects previous observations (1-18) and emphasizes the need for a better understanding of relevant factors determining the long-term pulpal reactions to resin restorations performed with acid-etch restorative procedures.

Traditional statistical testing of the effect of the restorative procedure and the

Table 5. Estimates of the average long-term pulpal reactions in human teeth to resin restorations in relation to restorative procedure and marginal leakage -/+ of restorations. For explanation of the abbreviations and ranging of the reactions, see Table 1 and Fig. 1

Group	Pulpal reaction—marginal leakage -/+				
	I2		OR2	TD2	
	-	+	-/+	-	+
A	0.6	1.8	2.5	0.04	0.06
B	1.5	4.1	2.8	0.04	0.06
C	0.0	0.8	1.9	0.04	0.06
D	0.4	1.3	1.6	0.04	0.06

numerous other factors that possibly influence the degree of the pulpal reactions may, however, lead to invalid conclusions because of the large number of tests necessary and because of possible mutual correlations between the different variables of significance for the reactions. Analysis of covariance, as used in the GLM analysis, seems to be a valuable tool to overcome these problems. In principle, one common test is performed for each of the pulpal reactions. The analyses thus disclosed that the significant independent variables and interactions could explain 91% of the variation in the degree of inflammation/necrosis (I), 37% of the odontoblast reduction (OR), and 56% of the formation of tertiary dentin (TD). Some authors have proposed the use of the overall, extreme pulpal response to evaluate the effect of different restorative procedures and materials (28-30). This easy and simple method may, however, lead to less reliable conclusions, as our results indicated that the pulpal reactions I, OR, and TD were influenced by different independent variables and that the explained part of the variations was increased when the analyses were performed using average recordings instead of extreme recordings of the reactions.

The most important pulpal reaction is the degree of inflammation/necrosis owing to its possible consequence for pulpal survival. Moreover, inflammation/necrosis was the most informative reaction with regard to the effect of different independent variables on

the long-term pulpal reactions to resin restorations performed with leakage-reducing restorative procedures. This result is noteworthy, as a previous GLM analysis of the 6-month reactions to tooth-colored restorations inserted in unetched cavities showed that only 16% of the variation in the inflammatory response could be explained, against 91% in the present study (23). The difference may in part be related to improvements in the experimental design such as the shorter observation period, the use of average registrations instead of extreme registrations, and the increased number of independent variables and interactions included in the present analyses. In addition, we found that the acid-etching restorative procedures add to a prolonged inflammatory reaction beneath resin restorations with marginal leakage. For this reason it is likely that the much more irritative restorative procedures used in this study also have strengthened the discriminatory value of the inflammatory reaction.

In spite of the high level of explanation only six variables seemed to be of significance for the degree of inflammation/necrosis beneath the restorations. Furthermore, it is tempting to assume that the effect of the stage of the root formation and of the tooth position may not be interpreted as a simple cause-and-effect relationship but as a strong indication of the significant role of vascularization for pulpal responses to restorative treatments. Previous findings with regard to the healing potential of the pulp tissue following traumatic injuries to teeth further contribute to this conclusion (31, 32). The significance of the vascularization in terms of Root and Jaw (and Pulp for OR and TD) should, therefore, be taken into account in the planning of future studies and in the interpretation of pulpal reactions.

In agreement with many other studies our analyses disclosed that marginal leakage and hence the occurrence of bacteria in the cavities and in the exposed dentinal tubules is a major causal factor in pulp tissue inflammation (2, 4, 7-9, 11, 17-20). Dental plaque or a mixture of dental plaque bacteria also contains components with the capacity to induce inflammatory reactions (33, 34). The

significant effect of marginal leakage (Leak), as observed by, us may therefore be due to pulpal penetration of toxins produced in the plaque on the oral surface of the restorations.

The importance of marginal adaptation of resin restorations emphasizes the need to develop leakage-preventing restorative techniques. Current attempts to reduce marginal leakage seem, however, to involve the use of materials with a toxic potential (35). To eliminate the possible effect of oral bacteria when testing the toxicity of new materials *in vivo*, an additional sealing procedure has been suggested by some authors (11, 17, 36, 37). Even though this procedure may have its advantages, it should also be acknowledged that none of the materials and techniques available today are able to prevent marginal leakage totally. For this reason it might be more appropriate to examine pulpal reactions resulting from the complex interplay of all factors involved in restorations, using methods of analysis which enable discrimination between the clinically relevant factors.

Considering the significant effect of the cavity treatments, a more even distribution of observations among the experimental groups would have been desirable. In the original design of the experiment the number of observations in group A was set to 30 and in groups B, C, and D to 15. Owing to an unforeseen decrease in suitable experimental teeth during the 2-year sampling period the numbers were reduced. However, it was still possible to demonstrate that cavity treatment with the dentin adhesive NPG-GMA/ethanol had no adverse biologic effect, whereas an intermediary layer of low-viscous resin significantly increased the pulpal reactions to resin restorations in acid-etched cavities, even though the leakage-reducing effect of both procedures was confirmed and thus could be taken into account in the analyses (24). In keeping with previous reports on the pulpal effect of various components in resin materials we also found that the increased resin penetration beneath cavities treated with low-viscous resin and NPG-GMA/ethanol had no significant influence on the

pulpal reactions (2, 38). However, cotton protection of the dentin during acid-conditioning minimized the irritative effect of the low-viscous resin, as well as the dentinal penetration of resin. In addition, this procedure may have reduced the pulpal penetration of ingredients with a more damaging effect than the BISGMA (2,2-bis[p-( $\gamma$ -methacryloxy -  $\beta$ -hydroxypropoxy)phenyl]propane) monomer, the main component of the composite filling material. In this context the toxic effect of TEGDMA (triethylene glycol dimethacrylate) used to dilute the resin and of the initiator system, which contains benzoyl-peroxide and a tertiary amine, should be considered. The higher concentrations of these agents in the low-viscous resin versus the composite filling material (39–41) probably explains the severe pulpal reactions observed beneath restorations performed with an intermediary layer of low-viscous resin.

In addition to the chemically induced histologic changes it seems reasonable to assume that parts of the reactions may be caused by physical injuries produced during the cavity preparation, such as heat generation or desiccation of the exposed dentin. However, the occurrence of burned lesions in the dentin (Burn) and the depth of the cavities (RD) did not influence the pulpal reactions, and neither did the dentin adhesive NPG-GMA/ethanol even though it may have a marked dehydrating effect as it contains 98% w/w ethanol. The major part of the long-term pulpal reactions observed in this study may therefore be of chemical-toxic origin.

Considering the irritative potential of acid-etching of cavities, which has been discussed in many papers (2, 4, 9–12, 15–20, 42–44) it is interesting that our estimates for long-term pulpal reactions to resin restorations performed with optimal marginal adaptation generally were confined to slight tissue changes (Table 5). Our results thus support the assumption that the phosphoric acid in itself only has an acute and minimal effect on the dentin pulp organ (2, 4, 9–11, 42). Nevertheless, the estimations also confirmed that the increased permeability of the dentin, which is the inevitable result of acid-etching

procedures (45), increases the toxic effect of materials and that of bacteria and bacterial toxins in connection with marginal leakage along resin restorations (12, 16–19, 43).

Because a perfect marginal adaptation cannot be totally ensured by any currently available restorative techniques, including acid-etching and the use of dentin adhesives, insulation of the exposed dentin in cavities to be restored with resin materials is still indicated.

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