

Analysis of pH variation of various calcium hydroxide compounds in vitro

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SUMMARY

Among the reasons for the use of calcium hydroxide products, there is their alkalinity. Variations in the alkalinity of six commonly used calcium hydroxide compounds were studied in vitro at different time intervals. All these compounds rendered the saline solution strongly alkaline. Dycal[®], Life[®], Nucap[®] and Reocap[®], had a weaker effect as compared with Contrasil[®] and to Pulpdent[®] paste. Such differences in the pH values were accompanied by differences in calcium loss, as revealed by scanning electron microscopy. Differences in the alkaline pH values and calcium losses among these calcium hydroxide compounds may account for their different clinical effectiveness in vivo.

KEY WORDS:

Calcium hydroxide, pH.

RÉSUMÉ

Une des raisons de l'utilisation des produits à base d'hydroxyde de calcium est leur alcalinité. Les variations alcalines des 6 matériaux à base d'hydroxyde de calcium couramment utilisés ont été testées in vitro à différents intervalles de temps. Dycal[®], Life[®], Nucap[®] and Reocap[®], avaient un effet plus faible que Contrasil[®] et Pulpdent[®] paste. Les différences de valeurs de pH étaient accompagnées de pertes de calcium. Les différentes valeurs de pH ainsi que les pertes de calcium pourraient expliquer leur diverse efficacité in vivo.

MOTS-CLÉS:

Hydroxide de calcium, pH.

INTRODUCTION

The effect of calcium hydroxide on human pulp has been widely investigated (Schroder, 1985). When calcium hydroxide is in contact with pulp tissue, a caustic effect is observed leading to a superficial zone of necrosis. It has been shown that the lower layer of the zone of necrosis, closed to the pulp, irritates the underlying tissue and stimulates the production of mineralized matrix. The exact mechanism is still not fully understood, but probably the calcium ions play a role (Schroder, 1985; Granath, 1982). Many histological studies have been performed to analyze

the pulpal response to different Ca(OH)₂ agents (Heys *et al.*, 1980; Pereira *et al.*, 1980; Baume and Holz, 1981; Franz and Holz, 1984, Stanley, 1989). Recent reports have demonstrated that an in vitro alternative method to the in vivo direct capping, can be applied successfully to compare the biocompatibility of dental materials using cultures of human pulpal cells explanted to form cell monolayers (Christen *et al.*, 1989; Regad *et al.*, 1989). Among the various important reasons in favour of the use of liners containing calcium hydroxide, there is their alkaline pH. Highly alkaline pH of calcium hydroxide

containing materials is considered responsible for its biological activity, keeping the immediate zone of the capping site in a state of alkalinity, a condition favorable for the hard tissue barrier formation. Previous studies suggest that the effects of calcium hydroxide is pH dependent (Schroder and Granath, 1971; Gordon et al., 1985). The aim of this investigation was to analyze and compare the pH behaviour of six of these pulp-capping agents at different time intervals. Knowledge of the pH variations among these different materials could be important in addressing their appropriate clinical use allowing the major effectiveness.

MATERIAL AND METHODS

The materials used in this study were bought in the open market. The following compounds were employed as test materials: Dycal® (L.D. Caulk Co., Milford, Del.); Pulpdent® paste (Pulpdent Corp., Brookline, Mass.); Life® (Kerr Sybrorn, Romulus, Mich.), Contrasil® (Septodont, Paris, France), Reocap® (Vivadent, Schaan, Liechstenstein), Nucap® (Coe Laboratories, Inc.). Each product was prepared according to the manufacturer's instructions. Five, 0.5 mm thick round specimens with a diameter of 3 mm, were allowed to set at room temperature ($22^{\circ} \pm 2$) within a polypropylene plastic mold. The resultant samples were placed into separate tubes, each containing 5 ml of normal saline solution, as described by Forsten and Soderling, 1984). Then, the pH of each solution was recorded, with a pH meter (Metrohm) previously calibrated with a known standard pH solution. The pH values were measured in 5 replicates after 1/2 hr., 1 day, 2 days, 3 days, 7 days, 10 days and 14 days. The significance of the difference between the various groups was analyzed by means of the student t-test. A value of $p < 0.05$ was considered as statistically significant.

To analyze the effects of the loss of Ca^{++} ions from the surface, one sample of the tested products was examined under a scanning electron microscope (Siemens) immediately after hardening; the other two were left in 5 ml saline and examined on day 3 and 7.

RESULTS

The average pH of the specimens of each compound with time dependent variations are shown in Figure 1. All the pulp capping agents used in the present investigation made the saline solution strongly alkaline. Contrasil® and Pulpdent® paste had a

significantly higher pH than the other tested materials ($p < .001$, for any day of testing). Their pH values ranged from 12.53 when measured after 30 min., to 12.40 after 14 days for Contrasil® or started from 12.22 to reach until 12.40 in the case of Pulpdent® paste. Both pH values of these two products, however, were quite stable over the time. The Nucap® pH average after 1/2 hr. of setting was 10.75 and remained stable on these values until the 14th day of determination (10.76). The initial Reocap® and Life® pH values were 10.48 and 10.41 respectively, increased slightly after 1 day, and dropped to 10.31 and 9.83 on day 7 reaching their minimal pH value on day 14: 9.38 for Reocap® and 9.71 for Life®. Finally the Dycal® pH average after 30 min. of setting was 9.75. It increased to touch the maximum pH value on day 2 and then started to drop until his minimal pH value (9.72) measured on the 14th day of analysis.

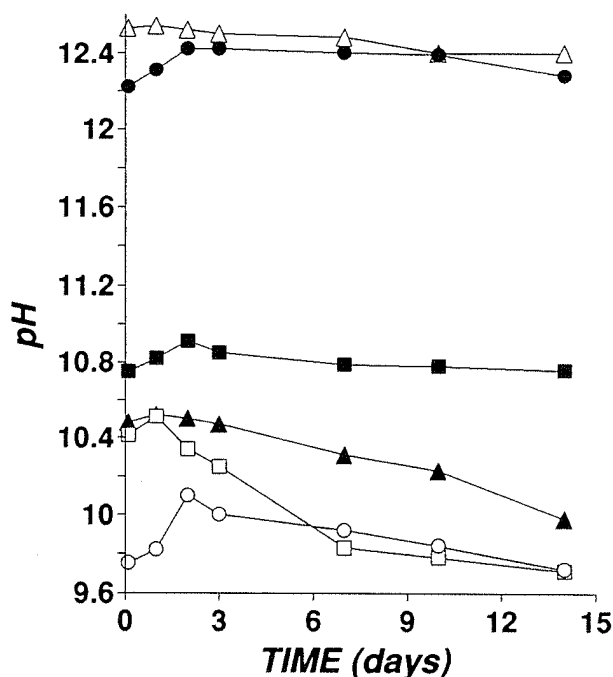
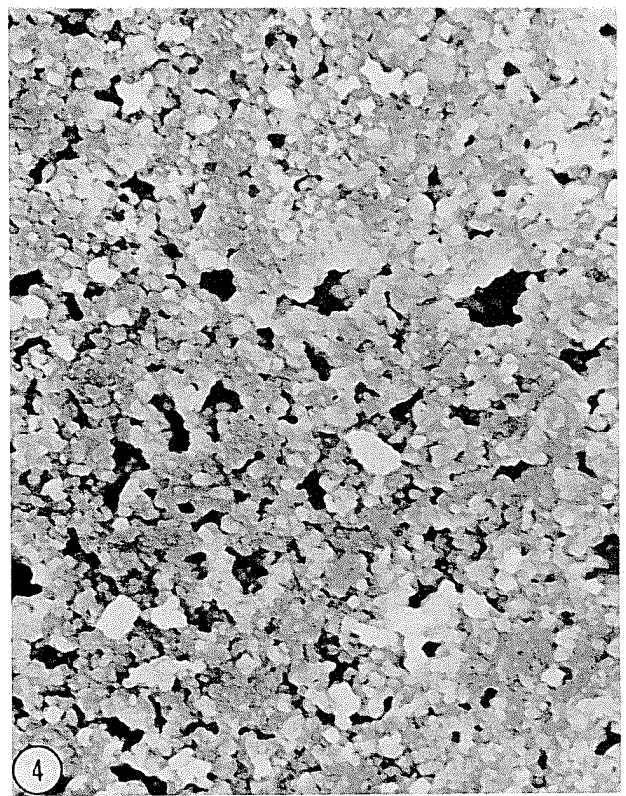
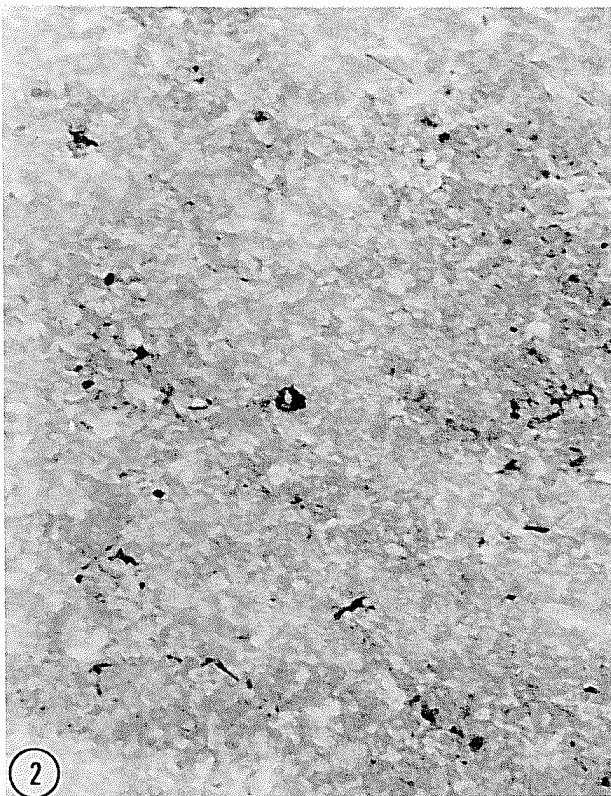
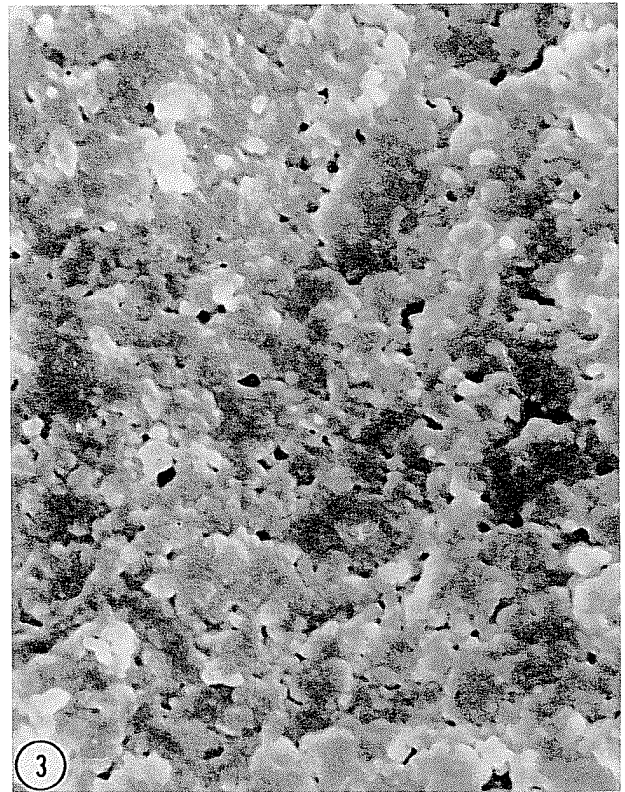


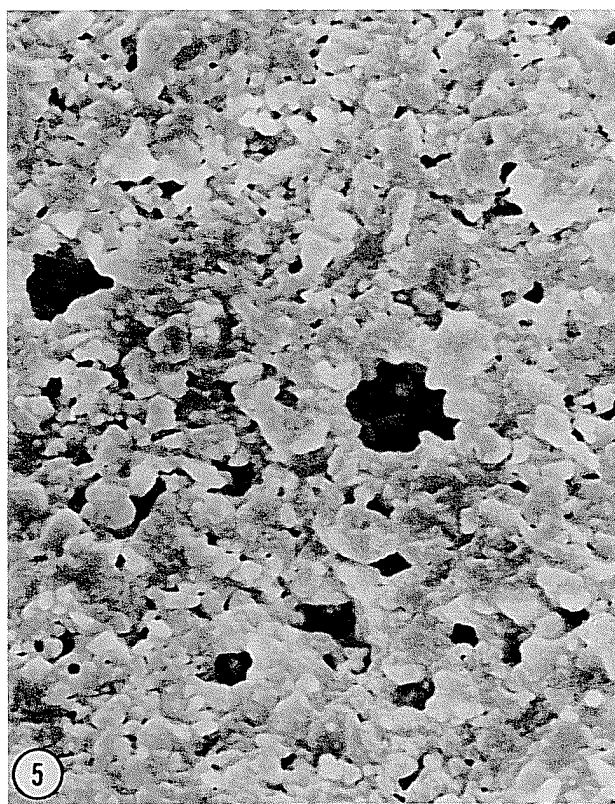
Figure 1
Mean pH levels at the different times of determination for the six solutions. Each point represents the average of five individual determinations. pH values at any day of evaluation of Contrasil® (Δ) and Pulpdent® paste (●) were significantly higher ($p < 0.001$) than values obtained with Nucap® (■), Reocap® (▲), Dycal® (○) and Life® (□).

Figure 1
Moyenne des pH à différents temps d'évaluation pour les six solutions. Chaque point représente la moyenne de cinq mesures individuelles. La valeur du pH à chaque jour de mesure pour Contrasil® (Δ) et Pulpdent® paste (●) était statistiquement plus significative ($p < 0.001$) que les valeurs obtenues avec Nucap® (■), Reocap®, (▲), Dycal® (○) et Life® (□).

Throughout the period of the experiment, the inter-sample coefficient of variation was consistently $<3\%$, except for the Dycal[®] tested on day 3, where the coefficient of variation was 10%. Control tube containing only the normal saline gave stable pH values, which ranged from 7.37 at the beginning to 7.45 at the end of the experiment.

The scanning electron microscopic examination of calcium hydroxide products in normal saline solution demonstrated a release of material as suggested by the presence of voids on the surface of the sample. The surface gaps were already present on day 1 and were increased on day 7 (Figs. 2-5). The different products showed, when compared, void differences at various extent. In fact, Pulpdent[®] and Contrasil[®] showed less voids when compared to Life[®] and Dycal[®], which possess voids greater in number and diameter. On day 7 the Life[®] disc was almost entirely crumbled and only part of material was still present.





Figures 2-5:
Pulpdent® immediately examined after setting (Fig. 2), 1 day (Fig. 3), 3 days (Fig. 4) and 7 days (Fig. 5) after the immersion in the saline solution. The voids, were few and small at day 1 of experiment, slightly increased at day 3, and further increased in number and size at day 7 (X 850).

Figures 2-5:
Pulpdent® examiné immédiatement après sa prise (Fig. 2), après 1 jour (Fig. 3), 3 jours (Fig. 4) et 7 jours (Fig. 5). Les espaces vides étaient peu nombreux et de petite taille au premier jour de l'expérience, augmentaient légèrement le 3ème jour, pour augmenter ultérieurement en nombre et taille au 7ème jour (X 850).

DISCUSSION

Calcium hydroxide was introduced as pulp capping agent in 1930 (Hermann, 1930). Since then, many investigations have reported on its effect on the dental pulp and its other biological activity (Heys *et al.*, 1980; Stanley, 1989; Phaneuf *et al.*, 1968; Stanley and Lund, 1972; Tronstad, 1974; Weiss and Bjorvatn, 1970). Histological studies have shown that $\text{Ca}(\text{OH})_2$ containing materials stimulate the pulp response leading to a dentin bridge formation (Heys *et al.*, 1980; Pereira *et al.*, 1980; Baume and Holz, 1981; Franz and Holz, 1984; Stanley, 1989; Schroder

and Granath, 1971; Glass and Zander, 1949). The ultrastructure during the dentinal bridge formation has also been studied by scanning electron microscopy (Schroder and Granath, 1972; Franz *et al.*, 1984); the mineralization process of the calcium-induced bridge has also been assessed by qualitative and quantitative microradiography (Franz *et al.*, 1985). Despite a large number of investigations, the precise action mechanism of calcium hydroxide is still not fully elucidated. Two properties of calcium hydroxide, calcium ions concentrations and alkaline pH, assure its success. Although earlier studies suggested that calcium in dentine bridge formation derives from calcium hydroxide material, it has been demonstrated that the calcium ions forming the dentin barrier derive from the blood stream and not from the calcium hydroxide (Sciaky and Pisanti, 1960). It has also been suggested that a high pH is necessary for dentin formation (Seltzer and Bender, 1975) and that the OH^- group provide the alkaline environment which pennits repair and calcification. A high pH not only buffers lactic acid but could also activate the alkaline phosphatase which is thought to play a role in dentin and bone formation (Seltzer and Bender, 1975; Stock 1985) and in the differentiation of precursor cells into odontoblasts with subsequent elaboration of dentin matrix (Attalla and Noujaim, 1969). Some investigations have also indicated that calcium ions may activate the adenosine triphosphatase activity, which accelerate the mineralization of hard tissue as in the case of bone or dentine (Abyko, 1977; Guo and Messer, 1976).

As shown both in vivo (Torneck and Wagner, 1980) and in vitro (Torneck *et al.*, 1983), calcium-hydroxide containing materials possess a mitogenic activity on the pulp cells. However, factors other than stimulation of mitosis have been implicated to explain the effectiveness of the calcium hydroxide agents. Among these factors there is the bactericidal activity (Barkhordar and Mark, 1984; Fisher and McCabe, 1978; Tronstad and Birkeland, 1971; Forsten and Soderling, 1984; Craig, 1985; Lado *et al.*, 1986; McComb and Ericson, 1987). This activity is believed to permit pulp healing and to enhance dentinal bridge formation (Attalla and Noujaim, 1969).

Calcium containing compounds find a broad spectrum of application. The clinical situations where they are largely applied are endodontic, indirect pulp capping, direct pulp capping, root resorption and peri-endo lesions (Stock, 1985; Andreasen and Hjorting-Hansen, 1966; Frank, 1974; Heithersay, 1970; Jacobson and Kerekes, 1980; Girard and Holz,

1985). A different clinical effectiveness among calcium hydroxide materials have been reported. For example, Pulpdent® has been shown to be more successful than Dycal (Phaneuf *et al.*, 1968), while Heys and colleagues (1980), found the contrary to be true. Other investigators of testing eight products in vivo have reported best results in dentin bridge formation when Pulpdent® or Contrasil® were used; in the same report Reocap® showed toxic effect on the pulp and the absence of bridge repair (Liard-Dumtschin *et al.*, 1984). Concerning Reocap, this result is in agreement with a previous extensive study testing this agent for indirect capping (Holz and Baume, 1973). In fact, this report showed an insufficient protection as demonstrated by the formation of vacuoles in the odontoblast layer. A recent report (Kirk *et al.*, 1989) showed that the dentinal bridge formation after direct pulp capping of exposed rat incisors occurred more consistently with less inflammation with the use of Pulpdent® than with Dycal.

In our study, all tested materials consistently made the normal saline solution strongly alkaline. We have analyzed and compared the pH behavior of the six pulp-capping agents during two weeks. Moreover, it has been clearly shown that mineral deposition starts at week 2 (Franz *et al.*, 1985); therefore the analysis of the pH variations at this time is important in order to understand the relationship existing between pH behavior and mineralization process. Calcium hydroxide products used showed two main behaviors. Two calcium hydroxide materials such as Contrasil® and Pulpdent® paste exhibited an initial high pH values (pH > 12) which remained very stable over the time, when analyzed at different intervals. On the other hand, the remaining calcium hydroxide compounds expressed initial pH values less than 12 which dropped over the time at the different days of examination to reach pH values of about 10 as in the case of Dycal®, Life® and Reocap®. The Nucap® pH average tendency was peculiar, since this product expressed a pH average of 10.75 which remained stable during the period examined. It is well known that a strong alkaline pH buffers the acidosis of pulpal infection, possesses antibacterial activity, and favours differentiation and replication of odontoblasts (Stanley, 1989). Therefore, our findings support the clinical evidence showing that for direct pulp capping highly alkaline products, such as Pulpdent® paste or Contrasil® are fully successful if employed respecting indications and technics; moreover neutral calcium hydroxide based materials have shown failure in pulp healing.

Moreover, the clinical results obtained with Pulpdent® and Contrasil® are supported by in vitro, biological test using culture of human pulpal cells showing a better biocompatibility response of these calcium agents (Christen *et al.*, 1989; Regad *et al.*, 1989). In addition, the scanning electron microscopic examination of the different calcium hydroxide products, stored in normal saline solution, at different days revealed the presence of gaps on the surface. Such voids, indicating the calcium loss, were more numerous for Life® and Dycal®, when compared to Contrasil® or Pulpdent®. These results are in agreement with previous work (Straffon *et al.*, 1988), and with the pH variations observed with these products and reported in the present paper. In conclusion, we have demonstrated important time-dependent pH variations of six commercially available calcium hydroxide containing materials. These pH value differences and the different calcium ions release from pulp capping agents (Shubich *et al.*, 1978; Straffon *et al.*, 1988) could reflect the different behavior and therefore the effectiveness of calcium hydroxide compound in vivo. It is clear that a better knowledge of calcium hydroxide action mechanism will allow a more appropriate clinical use.

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