NATIONAL BUREAU OF STANDARDS REPORT

9820

Progress Report

on

THE REACTIVITY OF THE COMPONENTS OF TOOTH STRUCTURE

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THE REACTIVITY OF THE COMPONENTS OF TOOTH STRUCTURE

by
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The Reactivity of the Components of Tooth Structure

Eugene F. Huget and Gerhard M. Brauer

A restorative material capable of adhering to tooth structure is greatly needed. Bonding is complicated by the complex nature of tooth structure as well as the mechanical, toxicologic and bacteriologic conditions which occur in the oral cavity. Heats of immersion ($-\Delta H_w$) measurements for dentin, enamel, and anorganic whole tooth tissue of known specific surface have been used to study the reactivity and degree of surface modification produced by various ions and functional groups in an aqueous environment.

A decrease in the $-\Delta H_w$ value of dentin in water from 10.3 cal/g (4100 ergs/cm^2) to 4.02 cal/g (1590 ergs/cm^2) in 2% - 40% aqueous solutions of organic solvents suggests the strengthening of electrostatic bonds between polar groups of the collagenous matrix of dentin. In absolute ethanol and hexane, dentin absorbs heat indicating the weakening of hydrophobic bonds. $-\Delta H_w$ values for anorganic whole tooth in aqueous solutions of organic solvents are not appreciably different from those

obtained in water (109 ergs/cm^2). The presence of NO_3^-, NO_2^-, CNS^-, $SO_4^=$, and I^-, at pH 5 depressed the $-\Delta H_w$ of dentin to 4.81 cal/g (1910 ergs/cm^2). Other anions (F^- and Cl^-) had no effect. At pH 8, however, anions did not alter the $-\Delta H_w$ of dentin. The addition of aldehyde groups to the liquid adsorbate at pH 8, resulted in the lowering of the apparent $-\Delta H_w$ value of dentin. Significant changes in $-\Delta H_w$ values for anorganic whole tooth were not observed in the presence of anions or aldehyde groups. The surface of dentin may be modified by treatment with non-polar solvents. Ionic bonding to exposed polar groups can be achieved at the optimal pH.

1. INTRODUCTION

The most common cause of failure of a dental "filling" or restoration is the inadequate seal between the prepared surfaces of the hard tooth tissue and the restorative material. Faulty marginal seals facilitate the ingress and diffusion of salivary ions and bacteria which lead to further decay and often the eventual death of the vital pulp organ. The development and use of a restorative material capable of bonding to one or more of the components of tooth tissue would enhance the quality of the marginal seal and thus prolong the period of useful service of a finished restoration.

Bonding to tooth structure is complicated not only by the mechanical, toxicologic, and bacteriologic conditions which occur in the oral cavity, but also by the complex nature of tooth structure itself. Many investigators believe that bonding to tooth structure may best be accomplished by interaction of the restorative material with the mineral rather than the organic phase. Indeed, the only evidence obtained for the mechanism of a potentially successful dental adhesive suggests that interaction occurred mainly with the mineral component.[1]

Little information is available regarding the chemical and physical characteristics of the surfaces of the components of tooth structure. Such information would contribute significantly to the eventual solution of the many problems still to be resolved in obtaining a truly adhesive restorative material.

In previous studies, we have determined the apparent heats of wetting of well-characterized powders of the components of tooth structure,[2,3] as well as their heats of reaction in the presence of dilute acids, chelating agents and stannous fluoride. In this investigation, calorimetric measurements of the heats of immersion ($-\Delta H_w$) of human dentin, enamel, and anorganic whole tooth tissue of known specific surface have been used to determine quantitatively the nature and degree of surface modification produced by various ions and functional groups in an aqueous environment.

2. EXPERIMENTAL METHODS AND MATERIALS

2.1. Methods

A thermistor calorimeter similar to the instrument designed by Sunner and Wadsö[4] was used in this investigation. The techniques employed in calibration of the calorimeter, heats of immersion determinations, and specific surface measurements of finely ground human dentin, enamel, and anorganic whole tooth tissue have been described previously.[2,3] All heats of wetting measurements were made at least in duplicate.

2.2. Materials

Freshly extracted human teeth were cleaned by brushing and rinsing with water. The crown portions were removed with carborundum disks and the necrotic tissue from the pulp chambers and canals removed with round steel burs. No. 4 and No. 8 steel burs were then used to grind out the dentin which was subsequently sieved for 16 hours on a mechanical shaker using No. 100, No. 200, and No. 325 mesh sieves. The powder passing through the No. 325 sieve was placed on a No. 400 mesh sieve and shaken for six hours. The powder which passed through the No. 400 sieve was examined petrographically for impurities. It was then stored in a deep freeze unit and later placed in a desiccator over anhydrous calcium sulfate for at least one week before use. Some samples were further dried under vacuum (10^{-1} to 10^{-3} mm Hg at room temperature). Other samples were saturated with water vapor prior to their use.

The enamel hulls which remained after the removal of the dentin were then ground to a fine powder with No. 558 carbide cross-cut fissure burs. The finely ground product was analyzed petrographically, sieved and stored in the manner described for the sieving and storage of powdered dentin. Finely ground whole tooth tissue (enamel, dentin, and cementum) was rendered anorganic by ethylene diamine extraction of the organic constituents.[5]

3. RESULTS

Since all determinations were made at least in duplicate, it was possible to obtain a measure of the reproducibility of the measuring process among replicates. The standard deviation of reproducibility did not appreciably depend on the magnitude of the measured value but was somewhat less for anorganic whole tooth than for dentin. The pooled estimates obtained from all individual sets of duplicates or triplicates are: for dentin: $\Delta = 0.095$; for anorganic whole tooth: $\Delta = 0.039$ where Δ is the estimated standard deviation for individual measurements.

All but a few of the values reported in Tables 1 through 4 are averages of duplicates. Their standard error is therefore: for dentin: 0.067; for anorganic whole tooth: 0.028. For convenience, all values are reported to the nearest 0.01 cal/g.

After conversion to ergs/cm^2, (1 cal = 4.1840 10^7 ergs), the rounding was made at the nearest 10 unit for dentin, and the nearest whole unit for anorganic whole tooth.

The apparent $-\Delta H_w$ values obtained for the three powdered constituents of human tooth structure are presented in Table 1.

The observed $-\Delta H_w$ values were highly dependent upon the residual water content of the dentin sample powders prior to immersion. The careful drying of dentin powders under vacuum resulted in a significant increase in the apparent $-\Delta H_w$ values. With the exception of anorganic whole tooth powder which had been equilibrated with water vapor prior to immersion, the method of sample powder storage and relative degree of dryness of anorganic whole tooth and enamel did not appreciably effect the apparent $-\Delta H_w$ values.

3.1. In Organic Solvents

In 2 to 40 percent (by volume) aqueous solutions of organic solvents (ethanol, acetone, butanol, ethylene glycol), a decrease in the apparent $-\Delta H_w$ value of dentin from that obtained in water (4100 ergs/cm^2) was observed (Table 2). When immersed in either absolute ethanol or hexane, dentin absorbed rather than evolved heat. On the other hand, $-\Delta H_w$

values for anorganic whole tooth in organic solvents (~ 84 ergs/cm^2) were not appreciably different from those obtained in distilled water (100-117 ergs/cm^2).

3.2. In Salt Solutions

The immersion of dentin powders in water neutralized with sodium hydroxide and made basic so that the resulting solution had a pH of approximately 8 yielded slightly lower $-\Delta H_w$ values than those observed upon the immersion of this substance in water (Table 3). No significant differences in $-\Delta H_w$ values accompanying the immersion of anorganic whole tooth tissue in dilute sodium hydroxide and water were found.

Using two percent solution of the respective salts, it can be seen from Table 3 that the presence of NO_3^-, NO_2^-, $SO_4^=$, CNS^- and I^- at pH 5 depressed the $-\Delta H_w$ value of dentin to as low as 4.81 cal/g, (1910 ergs/cm^2). Presence of fluoride or chloride, however, did not change the $-\Delta H_w$ values. Adjustment of the solution to pH 8 by addition of aqueous NaOH gave $-\Delta H_w$ values for dentin and anorganic whole tooth that differed little from those obtained in water adjusted to pH 8.

3.3. In Dilute Aldehyde Solutions

The addition of aldehyde groups to the aqueous phase at pH 5, (glutaraldehyde or dialdehyde starch), had only a slight effect upon the observed $-\Delta H_w$ of dentin and anorganic whole tooth powders (Table 4). At pH 8, however, a decrease in the $-\Delta H_w$ of dentin to approximately 4.8 cal/g (1900 ergs/cm^2)

was found. The $-\Delta H_w$ values of anorganic whole tooth powders in aldehyde solutions (pH 8), however, were essentially the same as those obtained upon the immersion of these powders in water.

4. DISCUSSION

The cutting of tooth structure, whether in vivo or in vitro, will result in significant structural changes in the organic matter near the surface. It is hoped that the technique employing rather mild conditions used in the preparation of the odontic powders produced surface changes approximating those encountered under clinical conditions. Freshly cut dentin presents surfaces that are especially complex by virtue of the deposition of non-homogenous collagenous films. However, despite the difficulties in preparing reproducible surfaces, the surfaces of the dentin powders used in this study should be not unlike those occurring at the dentinal floors and walls of actual cavity preparations. The treatment of these-dentinal surfaces with the reagents used in this study should yield modifications similar to those obtained with powdered dentin. Comparison of the reactivities of the surfaces of dentin and anorganic whole tooth powders strongly suggests that the organic matrix of dentin presents reactive sites at which bonding may occur. Modification of the surface of dentin with the appropriate reagent may also result in the formation of easily accessible reactive groups that could act as potential sites for bonding.

The apparent $-\Delta H_w$ values obtained for human dentin upon immersion in water greatly exceeded the $-\Delta H_w$ values for inert solids (29 to 628 ergs/cm^2) previously reported by other investigators for other substances.[6] Therefore, the collagenous component of dentin binds water through hydrophilic groups and may further absorb, dissolve or react upon contact with water. The observed $-\Delta H_w$ values for anorganic whole tooth, however, were found to be within this range. Zettlemoyer[7] has suggested that an inert substance must present a reasonably large surface area (10-20 m^2/g) to yield a measurable evolution of heat upon immersion. The specific surfaces of the human enamel powders used in this study were apparently much too small to promote the evolution of sufficient amounts of heat to allow measurement by means of the calorimeter employed. The small, but reproducible, endothermic values obtained for enamel cannot be readily explained.

Analysis of human dentin by Hess[8] has revealed that this substance contains 18-19 percent proteinacious material and that the most abundant dentinal protein is collagen. Kanagy[9] has shown that bovine corium collagen has a greater capacity for the adsorption of water than other fibrous proteins, i.e., wool, nylon, and silk. Differences in capacity for water adsorption are related to both physical and chemical structure. A material capable of adsorbing vast quantities of water must not only contain water-attracting groups, but must also possess physical characteristics that permit water to come into contact with potentially available hydrophilic groups. The amine, guanidyl and carboxyl groups, as well as the hydroxyl groups of serine and hydroxyproline, are important in the adsorption of water by collagen. Spreading of the peptide chains and the accompanying access to polar groups is facilitated by the presence of large concentrations of proline and hydroxyproline and the long side chains of arginine and lysine that probably inhibit the formation of extensive crystalline areas. Although subtle variations in sequential arrangements may exist, the characterization of dentinal and corium collagens by Veis and Schlueter[10] did not reveal the presence of striking differences in regard to amino acid moieties. Heats of wetting values of +37 cal/g have been reported by Kanagy[11] for purified bovine corium collagen. Since the $-\Delta H_w$ values for anorganic whole tooth are low (-2.06 cal/g), it is likely that the unexpectedly large $-\Delta H_w$ values obtained for dentin were caused by the presence of collagenous material at the surface of the powder particles. Hence, the immersion of dentin powders resulted in the binding of water by the accessible polar groups of the collagenous matrix as well as the adsorption of water by the inorganic phase.

The $-\Delta H_W$ values obtained upon the immersion of powdered dentin in aqueous solutions of organic solvents also reflected the presence of a coating of collagenous material at the surfaces of the powder particles. The substitution of mixed solvents having lower dielectric constants for water as adsorbates yielded significant reductions in the magnitudes of the observed $-\Delta H_W$ values obtained for powdered dentin.

A precise explanation for the decrease in the $-\Delta H_W$ values of dentin as the solvent medium became more organic in nature cannot be offered. This behavior may be attributed to the strengthening of electrostatic linkages of the dentinal collagen which in turn could result in (1) the reduction of the number of sites accessible to water or (2) a slight decrease in the surface area of the specimen through tightening of the structure of collagen. An endothermic interaction of the substrate with compounds such as butanol or ethylene glycol could also be a possible cause. The apparent absorption of heat which was observed when dentin was immersed in absolute ethanol and hexane could also be the result of the endothermicity of the transfer of the non-polar side chains of the collagenous matrix of dentin from the aqueous environment provided by its residual water content to an overwhelming hydrophobic environment.[12]

Because of the absence of water reacting groups at the surface, reductions of the dielectric constants of the adsorbate mixtures induced by the addition of organic solvents did not profoundly alter the $-\Delta H_w$ values observed for anorganic whole tooth tissue. The magnitude of the observed heat of immersion value of a polar solid is dependent not only upon available surface area, but also upon the ability of the polar solid to orient the dipoles of the adsorbate.[13] Even in a less polar environment, as long as small quantities of water are present, the heat of immersion of this polar solid is essentially the same as that in water itself. The striking influence of the presence of trace amounts of water has been shown by Boyd and Harkins[14] for the immersion of anatase in benzene.

The ability of a protein to interact with ions or functional groups to which it may be exposed is dependent, among other considerations, upon the pH of the ambient environment. The isoionic point of native collagen has been located in the pH range of 7 to 8. The exact location of this value is highly dependent upon the previous history of the tissue in question.[15]

It can be assumed that the isoionic point of the major protein constituent of human dentin, was approximately 7.

The depressed $-\Delta H_w$ values observed for powdered dentin upon immersion in dilute aqueous solutions containing certain anions (I^-, NO_3^-, NO_2^-, $SO_4^=$, CNS^-) at pH 5 suggest the direct endothermic interaction of certain anions with $R-NH_3^+$ groups. An alternative mechanism may involve the preferential binding of these anions to positively charged sites on the dentinal collagen at the expense of water. Chloride and fluoride ions apparently possess a low affinity for dentinal collagen. The affinity of the chloride ion for other proteins has also been shown to be less than that of other anions.[16]

Although a slight decrease in the apparent $-\Delta H_W$ values for dentin upon immersion in dilute base was observed, ionic bonding to charged groups of the dentinal collagen was not assumed to have taken place. It is more likely that the exposure of dentin to this medium resulted in the removal of protons from positively charged amine groups, thereby limiting the ability of these groups to bind water. The binding of anions to dentinal collagen did not occur at pH 8 as evidenced by $-\Delta H_W$ values approximately the same as those obtained for the immersion of dentin in a slightly basic medium (pH 8).

Although it has been shown by Gustavson[17] that aldehyde groups react with amino groups in the acid region, causing the discharge of hydrogen ions from the NH_3^+ groups which exist under acid conditions, the adsorption and binding of aldehydes by dentinal collagen did not appear to occur at pH 5. Interaction between amino groups and the aldehyde groups supplied by the adsorbate mixture did appear to occur at pH 8, however. This observation is in agreement with the older belief that aldehyde groups can react with amino groups only in the uncharged state.[18] However, since $-\Delta H_w$ determinations are concerned with nearly instantaneous measurements of changes in heat content, it is possible that the rate of interaction between charged amino groups and aldehyde groups proceeded at too slow a rate to allow detection by this means.

5. CONCLUSIONS

The magnitudes of the observed $-\Delta H_w$ values of human dentin in water indicate that factors other than the wetting of a crystalline inorganic solid are involved. The collagenous material present at the surface of the powder particles readily adsorbs water.

Under the proper environmental conditions, certain ions and functional groups interact preferentially with the collagenous matrix. The reactions which occur at the surface of dentin are similar to those exhibited by purified collagen.

The collagenous material at the cut dentinal surfaces of a tooth which has been mechanically prepared to receive a restoration offers potential sites to which bonding of a restorative material may be achieved.

TABLE 1

Heats of Immersion of Odontic Powders in H_2O at 30°C

Powder	Specific Surface (BET) m^2/g	Powder Stored	Apparent Heats of Immersion $-\Delta H_R$	
			cal/g	ergs/cm^2
Dentin	10.5	Anhydrous $CaSO_4$	7.71	3070
Dentin	10.5	10^{-1} mm Hg	10.29	4100
Dentin	10.5	10^{-3} mm Hg	12.11	4810
Dentin	10.5	100% R.H.	0.31	121
Anorganic Whole Tooth	75	Anhydrous $CaSO_4$	1.98	109
Anorganic Whole Tooth	75	10^{-1} mm Hg	2.06	117
Anorganic Whole Tooth	75	10^{-3} mm Hg	1.85	100
Anorganic Whole Tooth	75	100% R.H.	0.66	38
Enamel	3.9	Anhydrous $CaSO_4$	- 0.50	----
Enamel	3.9	10^{-1} mm Hg	- 0.58	----
Enamel	3.9	10^{-3} mm Hg	- 0.64	----
Enamel	3.9	100% R.H.	- 0.07	----

TABLE 2

Heats of Immersion of Odontic Powders in Aqueous-Organic Mixtures at 30°C

Adsorbate Mixtures		Heats of Immersion, $-\Delta H_R$			
		Dentin		Anorganic Whole Tooth	
		cal/g.	ergs/cm^2	cal/g.	ergs/cm^2
Water		10.29	4100	2.06	117
Ethanol	2%	6.34	2510	1.85	100
Ethanol	40%	4.20	1670	1.69	92
Ethanol	Abs.	Heat Absorbed*	Heat Absorbed*	Heat Evolved*	Heat Evolved*
Acetone	2%	6.39	2550	1.67	92
Acetone	20%	4.02	1590	1.57	84
Butanol	2%	6.10	2430	1.61	88
Ethylene Glycol	2%	4.19	1670	1.51	84
Hexane	Abs.	Heat Absorbed*	Heat Absorbed*	Heat Evolved*	Heat Evolved*

Specific surface of dentin powders (BET) = 10.5 m^2/g
Specific surface of anorganic whole tooth powders (BET) = 75 m^2/g
Powders stored at 10^{-1} mm Hg prior to immersion
*Energy equivalent of the calorimeter was not determined for these adsorbates; quantitative ΔH_w values were not obtained

TABLE 3

Heats of Immersion of Odontic Powders in Dilute Aqueous Salt Solutions at 30°C

Solution		Heats of Immersion, $-\Delta H_R$							
		Dentin				Anorganic Whole Tooth			
		pH 5		pH 8		pH 5		pH 8	
		cal/g	ergs/cm^2	cal/g	ergs/cm^2	cal/g	ergs/cm^2	cal/g	ergs/cm^2
H_2O		10.29	4100	8.32	3300	2.06	117	1.90	104
NaCl	2%	10.34	4100	8.60	3430	1.72	92	1.79	100
NaF	2%	10.10	4020	8.79	3510	2.02	108	1.91	104
$NaNO_3$	2%	5.64	2230	8.13	3230	1.19	66	1.41	75
$NaNO_2$	2%	6.15	2430	8.48	3390	1.22	66	1.40	75
Na_2SO_4	2%	6.26	2510	8.04	3180	1.61	87	1.60	87
NaCNS	2%	5.21	2070	8.19	3260	1.80	100	1.81	100
NaI	2%	4.81	1910	8.39	3350	1.22	66	1.40	75

Specific surface of dentin powders (BET) & 10.5m^2/g.
Specific surface of anorganic whole tooth powders (BET) = 75 m^2/g.
Powders stored at 10^{-1} mm. Hg prior to immersion.

TABLE 4

Heats of Immersion of Odontic Powders
in 2% Aqueous Aldehyde Solutions at 30°C

Aldehyde	Apparent Heats of Wetting, $-\Delta H_R$							
	Dentin				Anorganic Whole Tooth			
	pH5		pH8		pH5		pH8	
	cal/g	ergs/cm^2	cal/g	ergs/cm^2	cal/g	ergs/cm^2	cal/g	ergs/cm^2
Glutaraldehyde	9.70	3860	5.04	1990	1.89	104	1.62	87
Dialdehyde Starch*	8.97	3590	4.83	1910	1.92	104	1.62	87

Specific surface of dentin powders (BET) = 10.5 m^2/g.
Specific surface of anorganic whole tooth powders (BET) = 75 m^2/g.
Powders stored at 10^{-1} mm. Hg prior to immersion.

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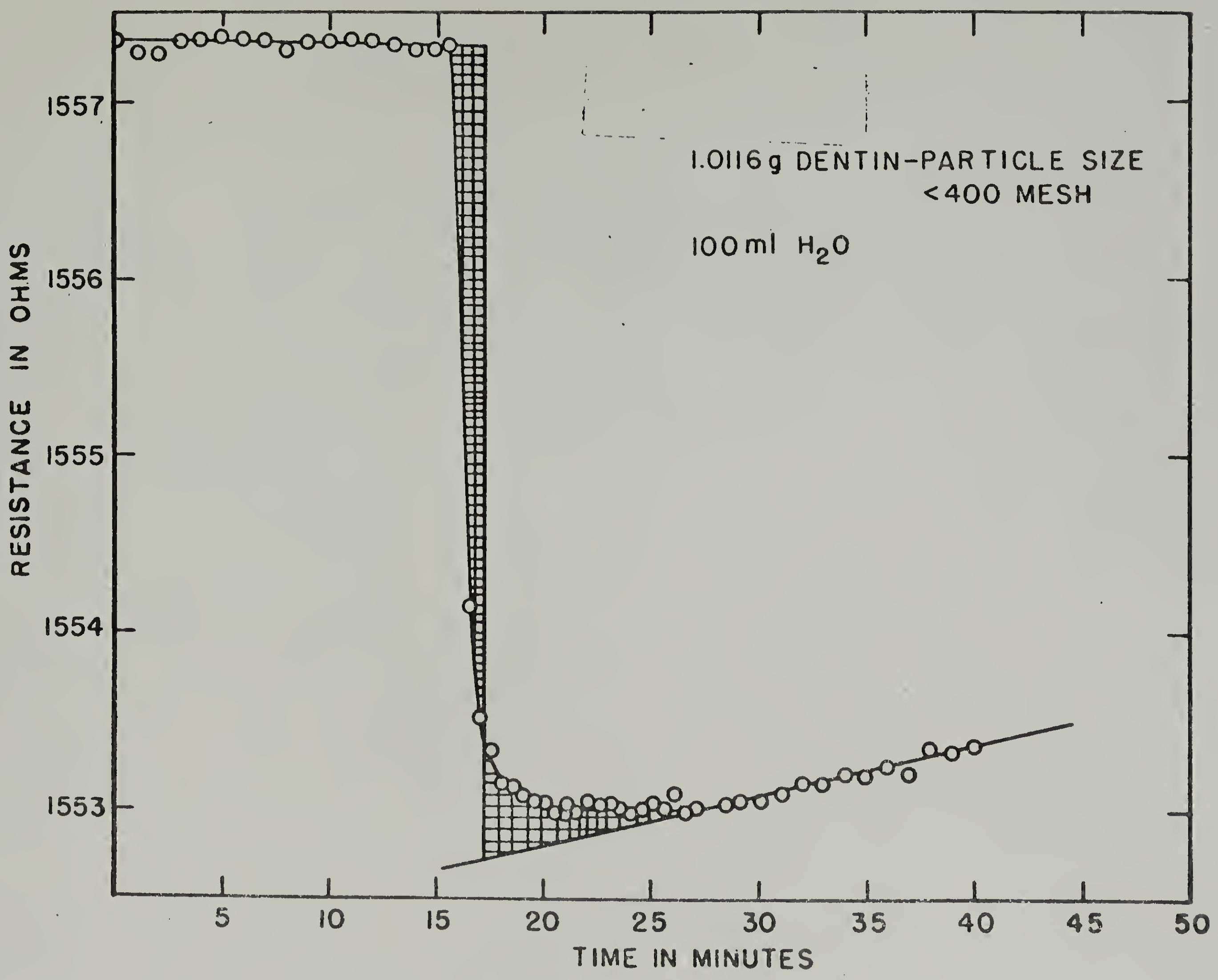

Fig. 1. Typical Heat of Immersion Curve Obtained for Dry Powdered Dentin.

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