BIOCHEMICAL STUDIES ON DECIDUOUS TOOTH SUBSTANCES

PART I. APPLICATION OF SILVER NITRATE

BY

Ikuo OHMORI*

INTRODUCTION

It is an obvious fact that the deciduous teeth has considerable difference from the permanent teeth in various aspects[1].

As one of these differences, one may point out the spreading rate of dental caries in the both dentitions. As a matter of fact, the extensive caries in deciduous dentition is noted very often in our clinical practice. The author, therefore, has naturally arrived at a question why the spreading rate of dental caries is so different between deciduous and permanent dentition.

On the other hand, S. Nagura[2] observed positive correlation between calcium content of the deciduous enamel and dental caries susceptibility, that is, low calcium content of the deciduous enamel was responsible for high caries susceptibility. Various values about calcium content of dental hard tissues have been reported by many investigators[3]. Some of them pointed out different values between the deciduous and permanent teeth, while Bird, M. J.[4] and others found no difference about them. In fact, the author did not find any considerable difference about calcium and phosphorus content between the deciduous and permanent teeth, as the values shown at Table I (Table I).

Table I.

materials		Ca (%)	P (%)
deciduous teeth	enamel	37.0	17.3
	dentin	25.4	10.8
permanent teeth	enamel	35.7	16.5
	dentin	26.6	11.8

Consequently, it was supposed that the calcium content of the teeth is not the critical factor for estimating the caries susceptibility.

And then, the author tried a dynamic study such as an investigation

* 大森郁朗: Dept. of Pedodontics (Chief. Prof. Hiroshi Yamashita).
Received for publication, December 30, 1960.

of the reactivity of these tooth substances to some chemical agents, e.g., silver nitrate, EDTA (ethylendiamintetraacetate), and stannous fluoride, with the intention of finding the chemical characteristics of deciduous tooth substances.

EXPERIMENTAL AND DISCUSSION

Materials

Human deciduous teeth, permanent teeth and bovine bone were used for the experimental materials. All of these stocked in acetone, were triturated by a jaw crasher and a coffee mill and the powders were limited in 100 to 200 meshes by screening with standard sieves. Iron chips mixed up during the trituration, were eliminated by magnet. And the triturated deciduous teeth and permanent teeth were separated into enamel and dentin by Manly and Hodge's flotation method[5]. The purity of enamel and dentin was more than 99% and more than 97% respectively.

Determination of calcium and phosphorus contents of the tooth substances

Each material was weighed by 50 mg with a torsion balance. And 10 ml of 1 N HNO_3 was applied for the decalcification of the sample. After the sample was perfectly decalcified, the solution was filtered by No. 2 filter-paper (Toyo Roshi K.K.) and diluted quantitively to 100 ml by adding distilled water. A part of this solution was used for the determinations.

1. Determination of calcium[6,7]

Reagents

M*/100 EDTA solution* (as the titrant): 4.0 grams of DiNa-EDTA were weighed and made up into 1 liter with distilled water. Small amount of 1 N NaOH was added simultaneously to this solution for adjusting the pH of this solution to a value of about 12. It is convenient for the titration.
Alkaline buffer (pH=12.7): The buffer solution was prepared by mixing 800 ml of 0.1 N NaOH and 200 ml of 0.1 M glycin solution; the solution is indefinitely stable at room temperature.
Indicator: A fluorescein-iminodiacetic acid complex named "calcein" was used as a metal indicator other than ammonium purpurate (murexide). This indicator was prepared by following procedure, i.e., 200 mg of calcein, 20 grams of KCl, and 120 mg of thymolphthalein were ground together into fine powder with an agate mortar.
M*/200 $CdCl_2$ solution*: 1.14 grams of $CdCl_2$ were dissolved in distilled water and made up into 1 liter.
N*/25 NaCN solution*: 1.96 grams of NaCN were dissolved in distilled

water and made up into 1 liter.

Technique

On the direct determination of calcium by using M/100 EDTA solution as the titrant, the pH was made up to about 12 by an addition of the alkaline buffer solution, and about 1 ml of N/25 NaCN solution was added as a masking agent to obviate the interfering ions. Just before the titration carried out with a 2 ml microburette, a knife point of indicator was added.

As the titration procedure is performed at high pH, some calcium is precipitated as the hydroxide at the beginning. Vigorous stirring, therefore, is necessary to dissolve the hydroxide as the titration progresses.

A definite end point was obtained with calcein.

Table II shows the results of the model test to examine for accuracy, specificity, and reproducibility of this method for calcium determination in the presence of magnesium ions (Table II).

Table II.

Sample was prepared only with 5 ml of 1 mg/ml calcium solution

Sample No.	titration value (ml)	value-Bl.	Ca (mg)	theoritical value
1	1.260	1.245	0.498	0.500
2	1.265	1.250	0.500	0.500

Sample was prepared with 5 ml of 1 mg/ml calcium solution and 1 ml of 1 mg/ml magnesium solution

Sample No.	titration value (ml)	value-Bl.	Ca (mg)	theoritical value
1	1.255	1.240	0.480	0.500
2	1.259	1.244	0.496	0.500
3	1.268	1.253	0.501	0.500

(Bl: Blank)

2. Determination of phosphorus

Fiske-SubbaRow's method[8] was used for the determination of phosphorus. A spectrophotometer (Hitachi, Type EPO-B) was used for colorimetric measurement.

Application of silver nitrate solution to the materials.

1. Preliminary experiment

2.5 grams of the triturated bovine bone (100~200 meshes) which had been estimated chemically similar to dentin in the previous study[9], were applied with 50 ml of silver nitrate solution of which concentration being varied M/50 to 1 M. This suspension was constantly stirred at 38°C by using a thermostat attached to a magnetic stirrer (Mitamura, Type IIA).

And the liberated amount of calcium from the material were determined by chelate titration method just described.

Prior to the titration, following procedures were carried out; the solution applied to bovine bone for a definite period of which time interval was varied from 1 minute to 120 minutes, was taken, filtrated with 3G4 glass filter using a water-jet pump, and diluted so adequately enough to titrate with M/100 EDTA solution. Then, a definite amount of 1 N NaCl solution was added to the aliquot being centrifuged for 5 minutes at 3,000 rpm, and the supernatant was used for the determination of calcium.

Table III shows the liberated amount of calcium in the silver nitrate solution (Table III).

Table III.

concentration of the applied $AgNO_3$ solution	1 M		M/10		M/50	
liberated amount of Ca period of application (min.)	mg	%	mg	%	mg	%
1	85.5	13.4	39.2	6.1	10.9	1.7
5	137.2	21.5	54.1	8.5	11.5	1.8
10	172.1	26.9	62.7	9.8	12.0	1.9
30	227.4	35.5	70.7	11.0	12.1	1.9
60	281.8	44.0	75.5	11.8	11.8	1.9
120	396.8	62.0	78.8	12.3	12.1	1.9

Total calcium content of 2.5 grams bovine bone is 640 mg.

By applying the silver nitrate solution to the bovine bone powder, considerable amount of calcium was dissolved out from the hard tissue, which was paralleled with the concentration of the applied silver nitrate solution and the applying period.

2. Application to the triturated human dentin

100 mg of the triturated human deciduous dentin and permanent dentin were applied with 20 ml of 20% (≒1.2 M) $AgNO_3$ solution for 1 minute.

These suspensions were constantly stirred at 38°C by using a thermostat attached a magnetic stirrer. And the liberated amounts of calcium and phosphorus from these materials were determined by means of the same method taken on the preliminary experiment.

Table IV shows the obtained values (Table IV).

A significant difference on the liberated amounts of calcium and phosphorus, especially on calcium, was found between the deciduous

Table IV.

Liberated amounts of calcium and phosphorus by application of 20% $AgNO_3$ solution for 1 minute

materials	Ca		P	
	mg	%	mg	%
deciduous teeth	8.4	33.1	1.5	13.9
permanent teeth	2.4	9.0	0.7	5.9

sample : 100 mg

dentin and the permanent dentin, i.e., on the permanent dentin, the liberated amount of calcium was much less than on the deciduous dentin, from which 33.1% of the calcium content were liberated in the 20% $AgNO_3$ solution and 13.9% of the phosphorus content were liberated simultaneously.

It is concluded, therefore, that the deciduous dentin would be more susceptible to silver nitrate than the permanent dentin. And this difference may suggest the difference in size of the crystalin of inorganic substance between the deciduous and permanent dentin.

But, R. F. Sognnaes et al.[10] reported in 1952 that the enamel and dentin of young teeth had higher radioactivity values than the enamel and dentin of teeth which had been in the oral cavity for extended periods of time, and they pointed out that this difference between erupting and fully erupted teeth might suggest a period of maturation of the teeth after their eruption into the oral cavity.

Accordingly, it should be investigated in further study whether the values obtained in this report indicate the essential difference between the deciduous and permanent dentin.

3. Application to the teeth of which the position, age, and sex were known

Samples are listed on the Table V (Table V). The "age" means the chronological age of the individual when the teeth have been extracted. As seen in the Table V, the deciduous teeth have been in the oral cavity for the almost full period of their functional role, while the permanent teeth have been still young.

Each of these samples was triturated and separated into enamel and dentin, and the dentin was used for the investigation which was performed by means of the same method as the previous experiment.

Calcium and phosphorus contents of each sample are shown at Table VI and VII (Table VI, VII).

About calcium and phosphorus contents, any considerable differences were not found between the deciduous and permanent dentin.

The amounts of calcium and phosphorus which were liberated from these samples by application of 20% silver nitrate solution to each sample

Table V.

Sample No.		position	age (y. · m.)	sex
deciduous teeth	1	III	7 · 2	♀
	2	III	7 · 4	♂
	3	III	10 · 5	♂
	4	IV	10 · 0	♀
	5	IV	10 · 0	♀
	6	V	8 · 0	♂
	7	V	10 . 5	♀
permanent teeth	1	1	11 · 4	♂
	2	1	12 · 6	♀
	3	2	12 · 6	♀
	4	4	10 · 5	♀
	5	4	10 · 6	♀
	6	4	11 · 11	♂

Table VI.

sample No.		calcium		phosphorus		
		mg	%	mg	%	Ca/P
deciduous teeth	1	13.49	27.0	6.26	12.5	2.15
	2	12.75	25.5	5.94	11.9	2.15
	3	13.32	26.6	6.06	12.1	2.20
	4	13.28	26.6	6.06	12.1	2.19
	5	13.10	26.2	5.72	11.5	2.29
	6	13.33	26.7	6.34	12.7	2.11
	7	13.41	26.8	5.88	11.8	2.28

sample : 50 mg.

Table VII.

sample No.		calcium		phosphorus		
		mg	%	mg	%	Ca/P
permanent teeth	1	13.41	26.8	6.36	12.7	2.11
	2	12.74	25.5	5.85	11.7	2.18
	3	12.30	24.6	6.00	12.0	2.05
	4	12.40	24.8	5.75	11.5	2.16
	5	13.32	26.6	6.14	12.3	2.17
	6	13.50	27.0	6.42	12.8	2.10

sample : 50 mg

for 1 minute, are shown at Table VIII (Table VIII).

Concerning the liberated amounts of calcium and phosphorus, they were apparently much in the cases of the permanent dentin. Comparing the percentile values of Table IV with that of Table VIII, it is considered apparent that aging of the permanent teeth would account for this difference.

A significant difference, however, was found in the liberated amount of calcium between the deciduous and permanent dentin.

Consequently, it was concluded that the chemical reactivity of the deciduous dentin was significantly higher than that of the permanent dentin, according to the investigation by means of silver nitrate application.

Table VIII.

Liberated amounts of calcium and phosphorus by application of 20% $AgNO_3$ solution for 1 min.

	sample No.	calcium		phosphorus	
		mg	%	mg	%
	1	4.53	33.6	1.01	16.1
	2	3.98	31.2	0.89	15.0
	3	4.30	32.2	0.73	12.0
deciduous teeth	4	3.80	28.6	0.51	8.4
	5	4.55	34.7	0.72	12.6
	6	4.16	31.2	1.04	16.4
	7	4.39	25.2	0.75	12.7

sample : 50 mg

	sample No.	calcium		phosphorus	
		mg	%	mg	%
	1	4.26	24.3	1.08	17.0
	2	2.24	17.6	0.53	9.1
permanent teeth	3	2.65	21.5	0.86	14.3
	4	2.80	22.6	0.61	10.6
	5	3.49	26.2	0.86	14.0
	6	3.37	24.9	1.14	17.8

sample : 50 mg

Summary

For the purpose of finding the chemical characteristics of the human deciduous tooth substances, the chemical reactivity was investigated on the human deciduous and permanent teeth, and bovine bone.

1. Any considerable difference about calcium and phosphorus contents was not found between the deciduous and permanent teeth.

2. By applying the silver nitrate solution to the bovine bone powder, considerable amount of calcium was dissolved out from the materials, which was paralleled with the concentration of the applied silver nitrate solution and the applying period.

3. A significant difference on the liberated amounts of calcium and

phosphorus, especially on calcium, was found between the deciduous dentin and the permanent one. This phenomenon was also observed when the old deciduous dentin and the young permanent dentin were used for the investigation, although the liberated amounts of calcium and phosphorus were considerably high in the case of the young permanent dentin.

4. According to the investigation by means of silver nitrate application, it was concluded that the chemical reactivity of the deciduous dentin was significantly higher than that of the permanent dentin.

Acknowledgement

The author wishes to express their deepest appliciation to Professor Hiroshi Yamashita for suggesting this investigation and to Professor Shimpei Araya, Department of Biochemistry, for his kind advice and constant encouragement.

References

1) Brauer, J. C. et al.: Dentistry for Children, New York, Toronto, London, 1958, McGraw-Hill Co., p. 76.
2) Nagura, S.: Relationship between the histological structure of the deciduous teeth, their dental caries susceptibility, and child care, Kōkūbyō-Gakkai-Zasshi, **26**: 164–179, 1959.
3) Leicester, H. M.: Biochemistry of the Teeth, St. Louis, 1949, Mosby Co., p. 20.
4) Araya, S.: Oral Biochemistry, Kyoto and Tokyo, 1954, Nagasue Co., p. 90.
5) Manly, R. S. and Hodge, H. G.: Density and refractive index studies of dental hard tissues I., J. Dent. Res. **18**: 133–141, 1939.
6) Ueno, K.: Chelate Titration Method, Tokyo, 1596, Nankodo Co., p. 130.
7) Appleton, H. D. et al.: The rapid determination of calcium in biologic material, Clin. Chem. **5**: 36–44, 1959.
8) Hawk, P. B. et al.: Practical Physiological Chemistry, New York, 1951, Blakiston Co., p. 889.
9) Ohmori, I.: Decalcifying process of human teeth by EDTA (calcium and phosphorus), Kōkūbyō-Gakkai-Zasshi, **24**: 135–143, 1957.
10) Sognnaes, R. F. et al.: Salivary and pulpal contributions to the radiophosphorus uptake in enamel and dentin, J.A.D.A., **44**: 489–505, 1952.