EXPERIMENTAL STUDY ON THE PREVENTION OF PERIODONTAL DISEASE

Ⅲ. The Chemical Composition of Plaque in Heavy Calculus Former

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齒周疾患發生機轉 및 豫防에 關한 實驗的硏究

Ⅲ. 齒石多沈着者의 齒苔의 化學成分含量

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齒石多沈着者의 齒苔의 化學成分含量을 究明하기 위하여 齒石除去後 6個月에 齒石沈着度가 甚한 사람과 輕한 사람을 分類하여 全顎으로 부터 齒苔를 採取하였다.

그리고 採取된 齒苔를 細胞性分劃과 非細胞性 部分으로 分離하여 各 分劃에서 炭水化物, 蛋白質, 脂質, Ca, Mg, K 및 P의 含量을 化學的으로 分析測定한 結果는 다음과 같다.

1. 齒石多沈着者의 齒苔는 乾燥重量當 10%의 炭水化物, 43%의 蛋白質, 18%의 脂質, 4.3%의 Ca, 0.15%의 Mg, 0.29%의 K과 2.87%의 P으로 構成되어 있다.
2. 炭水化物, 脂質, K은 非細胞 性分劃에서 蛋白質, Mg, 細胞性分劃에서 많이 차지하였다.
3. Ca, Mg, P의 含量은 齒石多沈着者의 齒苔에서 K의 含量은 齒石輕沈着者의 齒苔에서 높았다.
4. Ca/P比는 非細胞性分劃보다 細胞性 分劃에서 훨씬 낮았다.

INTRODUCTION

Much of interest in dental plaque stems from the fact that it is generally considered to be a paramount significance in the etiology of dental caries and periodontal disease. Shortly after plaque from the tooth's surface, the process of mineralization resulting in the formation of calculus begin. Calculus matrix reflects the composition of dental plaque in general and not derived from any specific source as salivary glycoproteins(Little et al. 1966).

Theories regarding the mechanisms whereby plaque in mineralized to form calculus fit into two principal concepts (Mukherjee, 1968). According to the first

concept, mineral precipitation results from a local rise in the degree of saturation of calcium and phosphate ions (Bibby, 1935, Hodge and Leung, 1950, Naselund, 1926). According to the second concept, seeding agents induce small foci of calcification, which enlarge and coalesce to form a calcified mass (Neuman and Neuman, 1958). The tendency of the individual to form calculus would be influenced by the component of plaque. It is apparent that plaque varies from mouth to mouth, from tooth to tooth in the same mouth, and even from surface to surface on the same tooth. The components of plaque has receied a great deal of attention from many workers over many years (Krembel et al., 1969, Mandel, 1974, Kleinberg, 1970). Dental plaque consists of approximately 80% water, most of which is found within bacterial cells. The remainder is in the acellular portion of plaque either bound to other components such as proteins or free within the matrix.

The starting time and rate of calcification and accumulation of calculus vary person to person in different teeth, and at different time in the same person (Muhler and Ennever, 1962). Based on these difference, individual may be classified heavy, moderate, or slight calculus formers, or as nonformers

There is a great deal of variation in the rate of calculus formation from plaque in individuals and so the author have carried out to determine the chemical composition of dental plaque in heavy calculus former by fractionating the plaque into acellular and cellular portion in order to analyze its components.

MATERIALS AND METHOD

Subjects of plaque collection: The subjects of the plaque collection was selected patient who visited to dental clinic, ranging in age of 20—40 years. The six individuals, who indicated 0 as calculus criteria in periodontal indes (Ramfjord, 1962) at six month after professional cleansing, were regarded as the light calculus formers. And the other six individuals, who indicated 3 as calculus criteria in periodontal index at six month after professional cleansing, were regarded as the heavy calculus formers. Dental plaque was collected from every subjects who had no toothbrushing for three days after oral prophylaxis.

Preparation of samples: All collected materials from each individuals was pooled in two groups; heavy calculus former and light calculus former, rehydrated with 2 ml of distilled water. And each samples was stirred in aqueous suspension at room temperature by using glass rod. And then 8ml of distilled water was added to make 10 ml of final volume, and homogenized. This homogenized samples were divided into three part as following; 1) 1 ml for determination of dry weight of plaque, 2) 3 ml of sample for whole fraction, 3) 6 ml for acellular fraction and cellular fraction. The remained 6 ml of samples was centrifuged at $10,000 \times G$ for 30 min. The supernatant from centrifugation was regarded as cellular fraction. And 6 ml of distilled water was added to the resulting precipitate to make aqueous suspension, which is regarded as cellular fraction (Silverman and Kleinb-

erg, 1967).

Determination of dry weight of dental plaque: The first part of homogenized plaque material was dehydrated in crucible jar at 60~70°C. The dried deposit of the crucible jar was weighed(W_1) and the jar was re-weighed(W_2) after washing by distilled water and dried. The deposits weight was obtained from the value between W_1 and W_2.

Chemical analysis: The total carbohydrate content from each fraction was determined by Anthrone method(Dische, 1955). And the protein content from each fraction was determined by the method of Lowry et al. (1951). Free lipid content from each fraction was determined by the method of Connetry et al. (1961). Calcium, magnesium and potassium components of each fraction was determined by Flame photometer. The inorganic phosphate components of each fraction was determined by Fiske-Subbarow method(1929)

RESULTS AND DISCUSSION

Table 1. Organic components of dental plaque in heavy calculus former and light calculus former (per cent of dry weight)*

Fractions	Carbohydrate		Protein		Lipid	
	Heavy	Light	Heavy	Light	Heavy	Light
Whole fraction	10	15	43	45	18	12
Acellular fraction	31	36	11	7	31	28
Cellular fraction	8	11	51	55	4	3

*Each data represents the mean value from triple determination.

Table 1 lists the levels of the organic major components of plaque. The possible extreme variability in the organic compostiion of different fraction of plaque is apparent in these analyses. Organic components of dental plaque in heavy calculus former is composed of 10% carbohydrate, 43% protein, and 18% lipid in % of dry weight. Carbohydrate and free lipid predominate in the acellular fraction or matrix fraction, whereas proteins predominate in the cellular or bacterial portion.

The carbohydrate content of plaque is extremely variable because many factors can affect it. For example, plaque taken within minutes after eating has much higher carbohydrate content than that collected following a period of fasting, because the polysaccharide used as energy storage compounds, especially amylopectins and levans, are consumed during the fasting period. An individual's diet can also determine the carbohydrate composition of plaque. Plaque taken from subjects on a high sucrose diet has been shown to have a carbohydrate/nitrogen ratio five times higher than plaque of subjects on a high glucose diet. The protein found in the matrix of plaque is primarily of salivary origin, since the amino acid composition of water extractable plaque protein is similar to that of salivary glycoprotei-

ns. Thus, the protein of the extracellular plaque matrix and that of acquired pellicle are derived from the same source and probably deposited by some of the same mechanism.

Although there is a high lipid content in plaque, little work has been done in plaque lipid research. Krembel et al. (1969) reported that the free lipids of the acellular fraction of plaque comprised abouth 20—26% of dry weight of plaque.

The levels of the major inorganic component of plaque in heavy and light calculus former was listed in Table 2.

Table 2. Inorganic components of dental plaque in heavy calculus former and light calculus former(per cent of dry weight of plaque)*

Fractions	**	Ca**	Mg		K		P		Ca/P	
	H	L	H	L	H	L	H	L	H	L
Whole fraction	4.3	0.76	0.15	0.08	0.29	0.30	2.87	0.53	1.5	1.4
Acellular fraction	2.1	0.43	0.02	0.01	0.31	0.93	0.87	0.16	2.4	2.7
Cellular fraction	2.6	0.46	0.23	0.11	0.06	0.19	2.85	0.52	0.9	0.9

* Each data represents the mean value from triple determination
** H; Heavy, L; Light

Inorganic material in dental plaque of heavy calculus former is composed of 4.3% Ca, 0.15% Mg, 0.29% K and 2.87% P in % of dry weight of plaque. As shown in Table 2, this data indicate that the heavy calculus former had higher levels of Ca, Mg, and P in their plaque, where as light calculus former had higher level of K in their plaque. Magnesium predominates in the cellular fraction or matrix fraction, whereas potassium predominate in acellular or bacterial portion.

The Ca/P weight ratio in cellular fractions were marked lower than that in acellular fraction.

Concentrations of calcium and phosphorus in plaque are influenced by the age of the subject, age of plaque, pH of the plaque, location of plaque on the dentition, and the tendency of the individual to form calculus. The concentration of inorganic phosphate varies with the metabolic state in plaque, since organic phosphate formed during glycolysis. Kleinberg et al. (1969) measured calcium and phosphorus level in plaque 1, 2 and 4 days after cleaning the tooth's surface. Relative concentrations of these two minerals declined after the first day and remained relatively constant through day four. The investigators attributed the presence of the calcium phosphate carbohydrate-protein complex on tooth's surface. Initial deposition of these complex results in a high calcium and phosphorus to dry weight ratio. After initial deposition, the ratio changes as the proteins to which the calcium is bound are metabolized by plaque bacteria as the mechanisms of plaque deposition change.

SUMMARY

This present study was designed to determine the chemical composition of dental plaque and its variation in the rate of calculus formatiion in different individual. So the author divided the subject into two groups; heavy calculus former (rapid) and light(non) calculus formers. And dental plaque of both group was separated into its cellular and acellular component. The interpreted results from chemical analysis of dental plaque in each fractions were summarized as follow.

1. Dental plaque in heavy calculus former is composed of 10% carbohydrate, 43% protein and 18% lipid. Inorganic material in heavy calculs former is composed of 4.3% Ca, 0.15% Mg, 0.29% K and 2.87% P.
2. Carbohydrate, free lipids and potassium are predominate in the acellular fraction, whereas proteins and magnesium predominate in the cellular fraction of dental plaque.
3. Heavy calculus former had higher levels of Ca, Mg, P than light calculus former in their plaque, whereas light calculus former had higher level of potassium than heavy calculus former in their plaque.
4. The Ca/P ratio in celullar fractions were marked lower than that of cellular fraction.

REFERENCES

1) Little, M.F., Bowman, L., Cassiani, C.A., and Rowley, J.: The composition of dental calculus. Arch. Oral Biol., 11 : 385, 1966.
2) Mukerjee, S.: Formation and prevention of supragingival calculus. J. Periodont. Res., Suppl. 2, 1968.
3) Bibby, B.G.: The formation of salivary calculus. Dent. Cosmos, 77 : 668, 1935.
4) Hodge, H.C., and Leung, S.W.: Calculus formation. J. Periodotol., 21 : 211, 1950.
5) Naselund, C.: A comparative study of the formation of concretions in the oral cavity and in the salivary glands and ducts. Dent. Cosmos, 68 : 1137, 1926.
6) Neuman, W.F., Neuman, M.W.: The chemical dynamics of bone mineral. Chicago, University of Chicago Press, 1958, p. 209.
7) Krembel, J., Frank, R.M., and Deluzarche, A.: Fractionation of human dental plaques. Arch. Oral Biol. 4 : 563, 1969.
8) Mandel, I.D.: Relation of saliva and plaque to caries. J. Dent. Res. (Suppl.), 53 : 1974.
9) Kleinberg, I.: Biochemistry of the dental plaque. Adv. Oral Biol., 4 : 44, 1970.
10) Muhler, J.C. and Ennever, J.: Occurrence of calculus through several successive periods in a selected group of subjects. J. Periodontol., 33 : 22, 1962.
11) Ramfjord, S.P.: The periodontal indes(PDI). J. Perodontol., 38 : 602, 1967.
12) Kleinberg, I. and Silverman, G.: Fractionation of human dental plaque and the characterization of its cellular and acellular components. Archs Oral Biol., 12 : 1387, 1967.
13) Dische, Z.: New color reactions for determination of sugars in polysaccharides, In Glick, D.(ed.): Methods of biochemical analysis. Vol. 2. New York, Interscience Pub. Inc. 1965, p. 313.
14) Lowry, O.H., Rosenbough, N.I., Farr, A.L., and Randall, R.I.: Protein measurement with the Folin phenol reagent., J. Biol. Chem., 193 : 265, 1951.
15) Connetry, H.V., Briggs, A. R. and Eaten, E. H. Jr.: Simplified determination of the lipid compounds of blood serum. Clinical Chem. 7 : 37, 1961.