A STUDY OF SOLUBILITY OF THE DENTAL ENAMEL OF CARIES-RESISTANT AND CARIES-SUSCEPTIBLE ALBINO RATS

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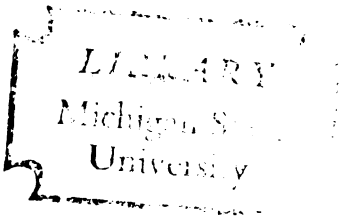

A STUDY OF SOLUBILITY OF THE BINARY SYSTEM OF CADMIUM IODIDE AND CADMIUM SUCCINATE AT ROOM TEMPERATURE

By

Gerda Mootee

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE

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During the decades which followed the suggestion of Miller, that dental caries is due to solution of the inorganic portion of enamel and dentin in acids much progress has been made in revealing the factors involved. To present a complete picture of the development of caries, the structure and composition of the tooth must be included.

Evidence of the varying resistance of teeth to dental caries has been presented by Hunt and Hoppert who bred a caries-resistant and caries-susceptible strain of albino rats. The availability of these strains made it possible to attempt to find out whether dental caries was correlated to the composition of dental enamel. Consequently a study of the solubility of powdered enamel from caries-resistant and caries-susceptible albino rats in buffered solutions of a pH 4-6 was undertaken. The determination of calcium and phosphorus is well adapted to the study of solubility of the tooth enamel since enamel comprises about 36 per cent Ca and 17 per cent P. Calcium was precipitated as the oxalate and titrated with $0.01N\ KMnO_4$. Phosphorus was determined colorimetrically by the Briggs modification of the Bell-Doisy method using a photoelectric colorimeter.

No difference was found in the solubility of the tooth enamel of the two strains. The enamel of the incisors of both strains showed somewhat lower solubility than the enamel of the molar teeth.

Unless it can be shown that the solubility of the dental enamel of the intact teeth of the rats used in this study differs from that of the enamel powder it must be concluded that other factors account for the different caries rates.

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INTRODUCTION

INTRODUCTION

In 1944 Hunt, Hoppert and Erwin (1) published data that with environmental factors held fairly constant, most of the difference between the caries-susceptible and caries-resistant lines of albino rats was hereditary.

These two strains of rats were developed using phenotypic selection, brother and sister inbreeding and progency testing of breeders.

A full understanding of the structure and behavior of the tooth can be obtained, however, only by applying all available methods of study, physical, chemical and anatomical, and by interpreting these in terms of the general biochemical and physiological principles which govern living organisms.

In this investigation a comparison of the enamel solubility of the resistant and susceptible strains of albino rats was made with regard to (a) the position of the tooth in the dental arch (b) the pH of the solvent.

Ever since the acid theory of caries was formulated by W. D. Miller, great interest has been shown in the solubility of teeth. Since the primary attack in caries is on enamel, almost all the solubility studies have been made on this tissue. Being composed of only around one per cent organic material, chiefly a modified protein, the enamel should be subject to total disintegration by exposure to acid.

Dallemagne and Melon (2) indicated that the enamel of the tooth is composed of about 60 per cent carbonate-apatite, 30 per cent -tricalcium phosphate, and 1 per cent calcium carbonate, the residue being minor minerals and organic matter. Since apatite is denser and less readily attacked by acids than is tricalcium phosphate, the chief constituent of tooth dentin and cementum, much of the strength of enamel may be due to its content of carbonate-apatite.

Miller's (5) main conclusion following his two year study of an estimated 8000 teeth was: " Caries of the enamel is purely chemical, the decalcification resulting at once in the complete dissolution of the tissue." But Miller did not associate any enamel structures with either freedom from or susceptibility to decay.

The present study was carried out to determine whether caries susceptibility might be associated with the relative solubility of the dental enamel.

HISTORICAL

HISTORICAL

Miller's theory has been until recently the foundation upon which almost all theoretical considerations of caries have been built. The solubility of tooth enamel has been studied by various investigators. The earlier work was inaccurate, because the effect of dissolved material on the acidity of the solvent was disregarded.

Rodriguez (4) and McIntosh et al (5) immersed whole teeth in acidic solutions and did not find any decalcification of the enamel. Hartzell et al (6) observed only slight opacity. Mummery (7) exposed the crowns of various teeth to 0.075 per cent lactic acid and tested the surfaces daily by scratching. He observed decalcification of cusps and incisal edges first. Bunting et al (8) and Friesell et al (9) exposed small areas of teeth to acidic solutions and examined the exposed area microscopically. Thurlow and Bunzell (10) investigated the activity of various organic acids in decalcification of enamel and noticed that the solubility appeared to depend upon factors other than pH alone. Whereas oxalic acid actually tends to prevent solution of enamel, citric acid dissolves enamel strongly. McClelland (11) used buffer solutions of various pH levels in which to suspend pieces of enamel and determined the rate of dissolution by weighing before and after immersion. Enright et al (12) maintained a constant hydrogen ion concentration by allowing a continuous stream of acid to flow over the surface of enamel, so that the dissolved products did not retard the progress of the reaction. They found that all the lactate and citrate buffer solutions from pH 4 to pH 8 decalcified the enamel. When these buffers had been saturated with tricalcium

phosphate, only those buffers with a pH more acid than 5.0 etched the enamel.

These qualitative results can not safely be used for comparison. The size of surface of the whole teeth as well as of pieces of enamel varies and often one or only few teeth were used. This procedure is not satisfactory, since it is now known that individual intact teeth vary greatly in their resistance to acid decalcification.

The factor of individual variation in composition of the teeth can be overcome by using a statistically significant number of teeth. The variation of enamel surface exposed to the acids action was overcome by using a relatively homogeneous enamel powder. Procedures commonly employed for the preparation of enamel most of which utilized mechanical forces-(a) splitting off flakes of enamel from the dentin (b) grinding out the dentin from the pulpal side or (c) grinding off the enamel from outside-usually gave low yields and impure products.

An outstanding contribution was made by Brekhus and Armstrong (13) in 1935 by the introduction of a flotation method for separating enamel, dentin and cementum. The method takes advantage of the differences in the specific gravity of the various dental tissues.

The method was greatly improved by Manly and Hodge (14) who applied a centrifugal technique to the flotation method. By this method the degree of purity of the fractions obtained can be followed by determining the indices of refraction of the dentin and enamel particles.

Benedict and Kanthak (15) determined the rate of solution of powdered human enamel in various buffered solutions and found the rate to be proportional to the acidity of the solvent. The rate was rapid during the first 15 minutes and then reached an even, and slower rate during the next hour. Volker (16) explained the rate difference due to the greater rapidity of solution of smaller particles of enamel.

Benedict et al were the first to observe a difference in solubility related to enamel structure. Studying the decalcified enamel under the reflecting microscope, they noted that the rods were more easily decalcified than the interprismatic material.

Dallemagne et Melon (17) explained that the presence in and around the interprismatic material of organic matter slowed down the rate of solution, and so gave the effect of a greater solubility of the rods. When the organic matter was removed, the greater solubility of a -tricalcium phosphate over the carbonate apatite of the rods became apparent.

Karshan and Rosebury (18) determined the pH relationships at equilibrium to dissolved phosphorus and calcium. The results indicated that in lactate buffers, pH change at each time level was proportional to dissolved phosphorus at that level. The calcium values were about twice the phosphorus concentration.

Forbes (19) showed, that the solubility of sound enamel from carious and noncarious teeth was the same.

Volker (20) used a standard technique in his quantitative measurements on solubility of tooth tissues. He noted that if the powder was air dried, the solubility did not change, but if it was

dried in a vacuum, the solubility decreased as much as 25 per cent. He studied the effect of surface area on the amount of enamel dissolved. He also observed the greater solubility of deciduous enamel and explained it on the basis (a) of the greater content of Mg salts or (b) lower content of fluoride. Volker did not find a significant difference between the solubility of enamel from carious and non-carious teeth and concluded, that the resistance or susceptibility of a tooth to caries is not dependent on the relative decalcification of the enamel in acid, but that all teeth would be dissolved by acid solutions at approximately the same rate. He noticed a slightly lowered solubility of carious enamel. The first observation on dentin was recorded by Volker (20) who found that it was always more soluble than enamel.

A number of substances with respect to their power to reduce the solubility of powdered enamel was studied by Buonocore et al (21). In general, they used ions which could be expected to enter the apatite lattice and alter its solubility. Lead fluoride proved to decrease the solubility of powdered enamel most.

Several of the substances which can reduce enamel solubility in vitro have been tested for their action in vivo. Manly et al (22) attributed the difference between the clinical and the in vitro tests to the resistance of the protective layer on the intact enamel surface. Enough of the results were negative to invalidate a generalization that acid-solubility reduction invariably brings about caries reduction.

Although the destructive effects of acids were confirmed by many investigators, the question arose, as to whether any specific organic ion might not have been particularly active in destroying the tooth surfaces.

McClure et al (23) exposed the enamel of rats' molar teeth to the action of diluted solutions of Na-citrate at pH 5.5 - 7.2 such as prevail within the oral cavity. Although the solubilizing action of the citrate ion on the enamel bears no gross similarity to ordinary dental caries, it is suggestive of the erosion observed in human teeth. The action of the ion was explained as due to binding of the Ca of the enamel to form a soluble, slightly ionized Ca-citrate complex. The authors suggest that in considering the etiology of dental caries less emphasis should be placed on acid decalcification and that possibilities of a non-acid decalcification, brought about by bacterial metabolites, be given more attention in dental caries research.

One may question the importance of tooth resistance to decalcification and the applications of solutions of the teeth to decrease it when there normally exists a wide variation in resistance to decalcification among non-carious teeth from the same individual as well as among different individuals.

Brudevold (24) has developed a method for quantitatively testing the surface solubility of enamel in acid solutions. The tooth is covered with wax except for a standard-sized window, and after exposure to acid the amount of dissolved P is analysed. In a study of forty permanent teeth, four consecutive exposures of an area with a

diameter of 4 mm to 5 cc of acetate buffer of pH 4 for 10 minute periods showed wide variations. The first exposure showed lower P values in 85 per cent of teeth, indicating that the surface is more acid resistant than deeper layers. In contrast ground surfaces were more soluble than intact enamel surfaces. The first exposure of a ground surface showed a higher average solubility than the second. Solubility of different areas on one tooth may vary as much as 50 per cent. In thirty areas on twelve erupted teeth average deviation was 19.5 per cent. In six areas on three teeth the average deviation in solubility of each tooth was 17 per cent. Brudevold showed also that brown pigmentation of enamel was frequently associated with low solubility. Repeated acid extracts from intact and ground enamel surfaces frequently showed variations in the Ca/P ratio. He suggested that there may be differences in the inorganic composition of the enamel from the same tooth.

Yardeni (25) exposed whole teeth and powdered enamel (for comparison) to buffers at varying pH levels and measured the weight loss. The rate of dissolution of teeth was essentially a linear function of hydrogen ion concentration. The loss of weight did not appear to increase linearly with the time of exposure, i.e., the decomposition did not proceed at a constant rate. Powdered enamel showed a linear course probably because of its greater homogeneity. The attack on intact teeth was at first slow, a lag period being present. He explained it as a threshold necessary to overcome some kind of a biological barrier against acid diffusion, probably the continuous organic matrix evenly distributed throughout the enamel.

The slight dissolution near the neutral region he interpreted as due to the carbonate fraction of enamel. With decreasing pH the dissolution steadily increased up to a certain critical point. Further loss was very slight either because of a decrease of rate of attack with the loss of minerals by the tooth structure or because of a change of electrical charge at the isoelectric point of the tooth at a certain low pH value.

The diminishing rate of dissolution is in agreement with the clinical observation that carious enamel is more resistant to acids. He postulated that in vivo the decalcification depends on the quality of the structure of the tooth, the size of the apatite crystallites, and the width of the organic channels open for diffusion.

In 1952 Yardeni (26) published data on solubilities of powdered enamel and dentin. He criticized the postulation that the more mineralized enamel is more susceptible to decalcification than dentin. According to Armstrong (27) the apatite crystallites are smaller in dentin than those in enamel, and hence present a greater surface for absorption. Yardeni says:

> "The fact is, that enamel contains larger apatite crystallites, is more compact, and therefore, less permeable to the acid solution. The quality of the mineral is the same in enamel as in dentin. Possibly, it is the anions which determine the different rate of solubility; first the carbonates readily go into solution, then the phosphates, and finally the more resistant fluorides. But there may be also a reaction of the enamel protein with the components of the buffer solution."

Swartz and Phillips (28) determined the hardness of the tooth enamel and tried to correlate it with solubility. Enamel varied widely in hardness and solubility from tooth to tooth as well as from area to area on the same tooth. The maximum area of approximately uniform hardness was found to be 2 mm in diameter. No correlation between hardness and solubility was detected. The decalcified areas of intact teeth were examined microscopically. When the depth of penetration was measured, the floor of the decalcified area was uneven, indicating that the acid had penetrated to greater depths in some areas than others. The Ca/P ratio of the enamel dissolved during the solubility tests was slightly higher than the 2:1 known to exist in enamel.

EXPERIMENTAL

EXPERIMENTAL

ANIMALS

Twenty-fifth generation albino rats from Hunt and Hoppert's caries-susceptible and twentieth generation caries-resistant strains were used for the dental tissue studies. The animals were killed with ether.

TREATMENT OF TEETH

The teeth of sacrificed animals were dissected out, stripped of adhering tissues and separated into collective groups of upper and lower incisors and upper and lower molars of caries-susceptible and caries-resistant strains. The incisors were split into fragments to facilitate the removal of dental pulp. Subsequently the teeth were extracted with absolute ethanol for twelve hours followed by a similar extraction with ethyl ether. Extraction appeared to decrease the tendency of particles to adhere during subsequent pulverization. Samples were dried at 60° and pulverized in an agate mortar with repeated sifting until the whole quantity passed a sixty mesh screen. The powder was again extracted with an ethanol-ether mixture and dried.

The powdered teeth were fractionated into enamel and dentin and cementum components by the flotation method of Manly et Hodge (14) who used a bromoform-acetone mixture of sp. gr. 2.70 for separating human enamel. It was found that the corresponding rat tissues appeared to be slightly less dense and in a liquid of sp. gr. 2.70

practically no enamel separated. Consequently a mixture of bromoform and acetone of sp. gr. 2.65 was used and centrifugation carried out at 1500 RPM in a small clinical centrifuge. The percentage composition of the fraction of enamel was determined by direct count of one hundred particles under the microscope, using the Becke line based on the different refractive indices of dentin and enamel as a means of differentiating. (14) The enamel fraction was considerably contaminated, the less dense material carried along into the outer tube. Purity rose as teeth were ground finer to pass the two hundred mesh sieve. To insure uniform particle size for solubility studies only that portion that passed the two hundred mesh sieve and failed to pass a 325 mesh screen was used. The separation of the two fractions appeared to be complete after the fourth refractionation. The enamel samples were air dried, stored in a vacuum desiccator over anhydrous $CaCl_2$ to constant weight and weighed.

ANALYTICAL PROCEDURE

The phthalate buffers were prepared from pH 4 - pH 6 and checked by means of the pH meter. Ten milligram samples of the powdered enamel were added to 10 ml of the buffered solutions, shaken for fifteen minutes and filtered.(29) The analytical figures refer to the acid soluble Ca and P.

Ca DETERMINATION

Suitable samples of the filtered enamel solutions were analyzed by precipitation of the Ca as oxalate in 15 ml centrifuge tubes. Further operations of centrifuging, washing and titration with 0.01N $KMnO_4$ were all carried out in the centrifuge tube by the Clark-Collip modification of the Kramer-Tisdall method. (30)

P DETERMINATION

Suitably diluted portions of the filtered enamel solutions were analyzed for P by the method of Fiske-Subbarow modified by Briggs using a photoelectric colorimeter. (31) Both methods gave reproducible results.

RESULTS

TABLE I

THE ENAMEL SOLUBILITY AT pH 6

(expressed as mgs. Ca/100cc)

TOOTH	I	II	III	Average
Upper resistant molars	2.62	2.59	2.6	2.6
Lower resistant molars	2.55	2.57	2.6	2.57
Upper susceptible molars	2.59	2.6	2.57	2.59
Upper resistant incisors	2.43	2.4	2.42	2.42
Lower resistant incisors	2.4	2.42	2.42	2.41
Upper susceptible incisors	2.4	2.42	2.42	2.41
Lower susceptible incisors	2.38	2.4	2.4	2.4

(expressed as mgs. P/100 cc)

TOOTH	I	II	III	Average
Upper resistant molars	1.22	1.24	1.25	1.24
Lower resistant molars	1.2	1.24	1.23	1.22
Upper susceptible molars	1.23	1.2	1.23	1.22
Upper resistant incisors	1.14	1.12	1.15	1.14
Lower resistant incisors	1.12	1.13	1.15	1.13
Upper susceptible incisors	1.15	1.13	1.08	1.12
Lower susceptible incisors	1.15	1.14	1.13	1.14

TABLE II

THE ENAMEL SOLUBILITY AT pH 5.5

(expressed as mgs. Ca/100 cc)

TOOTH	I	II	III	Average
Upper resistant molars	4.6	4.58	4.59	4.59
Lower resistant molars	4.62	4.6	4.63	4.62
Upper susceptible molars	4.61	4.63	4.58	4.61
Upper resistant incisors	4.18	4.2	4.17	4.18
Lower resistant incisors	4.19	4.21	4.2	4.2
Upper susceptible incisors	4.2	4.19	4.18	4.19
Lower susceptible incisors	4.2	4.23	4.2	4.21

(expressed as mgs. P/100 cc)

TOOTH	I	II	III	Average
Upper resistant molars	2.2	2.19	2.2	2.2
Lower resistant molars	2.18	2.19	2.2	2.19
Upper susceptible molars	2.3	2.2	2.2	2.2
Upper resistant incisors	1.97	1.96	1.92	1.95
Lower resistant incisors	1.92	1.99	1.95	1.95
Upper susceptible incisors	1.97	1.99	1.95	1.97
Lower susceptible incisors	1.94	1.97	1.98	1.96

TABLE III

THE ENAMEL SOLUBILITY AT pH 5

(expressed as mgs. Ca/100cc)

TOOTH	I	II	III	Average
Upper resistant molars	8.4	8.54	8.53	8.49
Lower resistant molars	8.57	8.6	8.55	8.57
Upper susceptible molars	8.6	8.58	8.57	8.58
Upper resistant incisors	6.7	6.9	6.78	6.79
Lower resistant incisors	6.9	6.8	6.82	6.84
Upper susceptible incisors	6.84	6.9	6.86	6.87
Lower susceptible incisors	6.85	7.0	6.83	6.89

(expressed as mgs. P/100cc)

TOOTH	I	II	III	Average
Upper resistant molars	4.1	4.07	4.08	4.08
Lower resistant molars	4.13	4.09	4.12	4.11
Upper susceptible molars	4.1	4.12	4.09	4.1
Upper resistant incisors	3.27	3.29	3.27	3.28
Lower resistant incisors	3.27	3.29	3.29	3.29
Upper susceptible incisors	3.3	3.28	3.27	3.28
Lower susceptible incisors	3.28	3.3	3.29	3.29

TABLE IV

THE ENAMEL SOLUBILITY AT pH 4

(expressed as mgs. Ca/100 cc)

TOOTH	I	II	III	Average
Upper resistant molars	22.98	23.03	23.05	23.02
Lower resistant molars	22.99	23.05	23.03	23.02
Upper susceptible molars	23.1	23.2	23.05	23.12
Lower resistant incisors	18.27	18.3	18.32	18.29
Lower susceptible incisors	18.34	18.4	18.3	18.35

(expressed as mgs. P/100 cc)

TOOTH	I	II	III	Average
Upper resistant molars	11.04	11.1	11.0	11.05
Lower resistant molars	11.02	11.06	11.0	11.03
Upper susceptible molars	11.18	11.04	11.18	11.13
Lower resistant incisors	8.59	8.73	8.69	8.64
Lower susceptible incisors	8.68	8.6	8.7	8.66

DISCUSSION

DISCUSSION

The popularly accepted concept of dental caries, namely, that acid formation on the surface of the tooth is the complete cause of its destruction, has been followed for more than half a century. It has not led to a complete comprehension of the dental caries mechanism as evidenced by clinical observation.

The experimental biological approach to the problem of dental caries is comparatively recent. The hypothesis of this thought has been set up that the factors causing dental caries are twofold:

1. The presence of exciting (exogenous) factors, (food retention, bacteria) has been demonstrated.

2. The predisposing (endogenous) factors, which could explain the immunity to dental caries of certain individuals, still are somewhat obscure.

It has been proven that susceptibility and resistance to caries in rats are in part due to heredity. (1) Genes produce their effects by initiating events which lead to the development of structures, and to chemical processes. Both the susceptibles and the resistants have consumed the same kind of food, drunk water from the same source, lived in the same kind of cage, and been handled by the same caretaker in the same building.

The process of caries is very complex, involving the interplay and interaction of a great many different factors, no one of which alone can be considered the cause of caries, yet any one of which may

be decisive in the progress or prevention of the condition. Variable resistance of teeth to attack appears to be linked more with endogenous factors. These factors may relate to abnormal structural variations, such as permeable enamel lamellae, varying chemical composition, chemical differences in tissue fluid within the tooth, which in turn is dependent on blood conditions.

The present experiment considers the possibility that the genes for caries susceptibility act through producing an effect on the composition of the enamel.

In solubility studies we have to consider the chemical composition as well as the anatomical structure of the teeth. That they both influence the solubility has been confirmed by various investigators. Dallemagne et al (17) showed the difference in solubility to be due to the difference of the organic matter in enamel. Enamel protein has caused much controversy which may be due in part to the rather wide variation in amount. Rosebury (32) decalcified the enamel with acetic acid and obtained an insoluble protein, very resistant to hydrolysis with hydrochloric acid or potassium hydroxide. According to Volker (16) the difference in moisture content of the enamel preparation may affect the solubility as much as 25 per cent. Yardeni (26) postulates the solubility to be dependent on the structure of the tooth as well as on the chemical composition.

There is abundant evidence that intact teeth are highly variable in their resistance to acid decalcification in vitro and there is indication that the resistance is associated with the nature of the surface.

The only plausible explanation of failure of some teeth or areas of teeth to be attacked in an in vitro experiment is a difference in surface structure between different teeth and different parts of the same tooth. However, if there are indeed real structures of dental enamel surfaces that confer resistance to caries, the origin is in vivo (33). By inspection of enamel with the Roetgenspectroscope, differences in crystalline structure between outer and inner layers can be demonstrated.

Our data compares only the mean inorganic phase of the tooth enamel of these two strains of rats. On the basis of these results one may conclude that there are no hereditary differences as to the inorganic composition of the enamel in the two strains. In our experiments all the samples were treated similarily and conditions were kept constant. (In order to avoid the differences in the moisture content or of the organic matter.) By refractionation the samples got very "hard" treatment. The enamel tended to carry less dense material along. In order to avoid such contamination, the powder was added stepwise in four portions with a short centrifugation immediately after each addition. Although the bromoform was redistilled freshly under high vacuum etc, the centrifuge used did not permit control of the temperature.

Gilda (34) studied the Manly-Hodge (14) separation method as applied to dental tissues of rodents and explained the difficulties in separation due to a higher percentage of junction particles (a smaller volume of enamel per unit area). According to Gilda (35) the enamel of albino rats closely resembles hydroxyapatite in X-Ray

diffraction pattern. The lower density (2.720 - 2.920 Gm per cc) compared to human tissue he attributes to a relatively greater amount of organic matter in rat enamel. That would account for greater differences in solubility of a rat's intact tooth and of enamel powder prepared from rats' teeth. In the literature there is much confusion resulting from attempts to analyze and assign formulas to definite compounds which were thought to have been isolated from the tooth.

The stable entity in the tooth is the apatite lattice itself, the arrangement of atoms in space being relatively constant. The outstanding peculiarity of this lattice is that it can tolerate a large number of substituents for the usual Ca, P, O_2 and H_2 atoms which it contains. If the substituting elements are about the same atomic radius as the elements which they replace, the unit cell of the apatite lattice is not greatly altered, and the diffraction pattern which it gives remains essentially the same. That the composition of any apatite will depend upon the conditions which prevail at the time it is formed has been shown by Thewlis et al (36). In the case of teeth this will obviously reflect the composition of the blood serum from which it originated.

Sobel et Hanok (37) confirm that there is a relationship between the inorganic composition of the upper incisor of the albino rat and blood serum, which in turn is regulated by the diet. They do not express an exact quantitative relationship because the activities of the tooth forming ions at the site of deposition and exact relationship between composition of the tooth and the activities of the ions that form the tooth are not known.

In view of the findings, that the PO_4:CO_3 ratios in rat enamel are in all cases lower than in dentin (38) (In human teeth Armstrong and Brekhus (39) reported higher PO_4:CO_3 ratios in the enamel than in the dentin) they pointed out that the difference in composition between enamel and dentin of human and rats may be due to the particular diet given rather than to species differentiation (40).

The investigations by Deakins (41) indicate the inorganic composition of enamel to be constant. In developing pig enamel the Ca, P and CO_2 contents were found to increase linearly and in constant ratio to each other throughout the range of calcification. It was concluded that these elements are deposited as a complex compound having a fixed composition. The calcification gradients in the same tooth they attributed to the start of infiltration of the mineral phase into the substance of a preformed organic matrix at the cusp tips and to the gradual spread over the whole crown of the tooth.

We do not know whether the limitations of the analytical methods for determining the fine structure of tooth substance led to varying results and conclusions.

It is possible to sum up the foregoing discussion by the statement that the nature of the apatite lattice possesses great significance from the standpoint of the structure and behavior of the tooth. It explains the constancy of the chief properties of the tooth in spite of variations in composition. It explains also the extreme sensitivity of the developing tooth to any metabolic changes as well as some of the changes which the fully formed tooth can undergo.

There is, however, evidence that teeth are tissues in equilibrium with body fluids. Greenberg (42) Armstrong et al (43) fed Ca^{45} and P^{32} to albino rats and measured the amounts found in the teeth. They found that the turnover of the isotopes in the incisor enamel was about one-third of that in the femur. In the case of molar enamel the turnover rate was only from 1.5 to 3 per cent of that of the femur.

As permanent teeth become more completely calcified, equilibrium with body fluids slows up to such an extent that only minor changes can be observed. According to Gilda (35) comparatively small changes in the composition of the tooth may affect tooth solubility.

From this we can conclude that the caries-resistant and caries-susceptible strains of albino rats have fairly constant inorganic composition of tooth enamel with no correlation with susceptibility or resistance to decay of the teeth. This is in agreement with the results of Armstrong et al (39). The same Ca:P ratio indicates a homogeneous inorganic phase.

The lower solubility, of the incisor enamel of albino rats is hard to explain. It may merely be incidental to the need for structural material that is best suited for the special function of the incisors. Perhaps pigmentation may be of some significance for it is well known that the incisors of rats receiving an excessive amount of fluoride show depigmentation and are easily broken. If solution in acid is a prime factor in the initiation of caries, it will be easy to see the basis of the statement of Hunt and Hoppert (44):

"In more than 6,400 rats we have never observed a carious lesion in the incisors."

The enamel powder of upper and lower teeth seems to have the same composition. This is not in agreement with the work of Matsuda (45) who investigated the composition of whole incisors of rats and found a difference in composition of upper and lower teeth. The fact that enamel and dentin were not separated makes those results less significant.

The increased solubility with decreasing pH may be explained on the basis of a different rate of solubility of different anions. Yardeni (26). The fact, that rat enamel and dentin show about the same solubility as the corresponding human tissue preparations of Volker (46) gives more significance to the present work. Thus the solubility of enamel powder was relatively slight above pH 6.0 but increased with decreasing pH.

We should also consider the possible role of genes influencing the tooth form, which could be correlated to the susceptibility to caries.

It has long been recognized that tooth form and the shape of contacts between teeth may influence the onset of dental caries. In 1952 Nakfoor, Hunt, Hoppert (47) measuring the resistance of lower molar teeth to mechanical fracturing, observed greater mechanical weakness in the susceptible rats. They also observed (both with the unaided eye and with a binocular microscope) that the crevices of the molars of the susceptible strain were wider than those of the resistant rats. Wide crevices would permit impaction of food more readily which in turn would allow formation by bacteria, and finally caries. As a matter of fact, 60.4 per cent of the caries in susceptibles has occurred at the major crevices of the first and second lower molars, whereas in the resistants only 15.7 per cent of the cavities appeared at those locations.

Nevin and Walsh (48) investigated the possible effects of certain physico-chemical factors in relationship to the cause of caries. They obtained results which can explain the differences in interproximal surfaces of the same tooth and between interproximal surfaces in different parts of the mouth. The indications are that variations in degree of separation of teeth and the shape and width of contacts are more important than the buffering capacity of saliva.

Cox (49) showed that rat molars exhibited variations in (a) subdivision of major cusps, (b) fusion of minor to major cusps, (c) absence of minor cusps, (d) presence of extra cusps, and (e) bifurcation of minor cusps. Some of these variations are related, in frequency, to the diet of the mother during pregnancy and lactation; others may be related to familial, and hereditary factors.

From the present study it becomes obvious that further work will be necessary to gain a full understanding of the relationship of tooth structure to susceptibility to caries.

The exact path of every individual lesion may be different and will depend on two major factors. One, the external, is determined by the types of bacteria, acidogenic and proteolytic, together with the supply of suitable substrates upon which they can act, and the conditions of plaque, pit or fissure which bring them into contact with the enamel surface.

The second major factor is the morphological and histological state of the tooth itself, the condition of the surface and the amount and distribution of organic and inorganic matter available for attack at the surface and within the enamel itself.

In the background of the production of acid is the still greater problem of clinical caries with systemic factors not yet determined and bacterial antagonisms and synergisms undoubtedly playing a major role in the complex picture. Yardeni (25)

Although a genetic factor related to caries resistance has been clearly established in laboratory animals, only familial relations have been demonstrated for man.

SUMMARY

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1. A study of the solubility of powdered tooth enamel from the twenty-fifth generation of caries-susceptible and the twentieth generation of caries-resistant strains of albino rats in buffered solutions of a pH of 4, 5, 5.5 and 6 did not show significant differences between the two strains.

2. No difference in solubility was found between the enamel of the upper and lower molars of the resistant strain.

3. The incisor enamel powder was markedly less soluble than the powdered enamel of molars.

4. The soluble Ca:P ratio was in all cases essentially the same.

5. The solubility increased with decreasing pH. The increase was not proportional to the H ion concentration, but was much higher at the lower pH level.

BIBLIOGRAPHY

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1. Hunt, H.R., Hoppert, C.A., and Erwin, W.G., Inheritance of susceptibility to caries in albino rats., J. Dental Research 23:385 (1944)

2. Dallemagne, M.J., and Melon, J., Density and refractive index of bone and of tooth enamel, dentine and cement. First results on the determination of the chemical constituents of the mineral portion of the teeth. Bull. Soc. Chim. Biol. 27:85; 597 (1945) State of combination of the carbonate of tooth enamel, dentine and cement. Compt. rend. Soc. biol. 139:804 (1945)

3. Miller, W.D., Caries of the human teeth. Independent Practioner 4:629 (1883)

4. Rodriguez, F.E., Studies in the specific bacteriology of dental caries. Military Dental J. 5:199 (1922)

5. McIntosh, J., and Lazarus-Barlow, an investigation into the etiology of dental caries. I The nature of the destructive agent and the production of artificial caries. Brit. J. Exp. Path. 3:138 (1922)

6. Hartzell, T.B. and Larson, W.P., Preliminary report of some experiments on the destruction of human enamel. J. Am. Dental Assoc. 11:649 (1924)

7. Mummery, S.P., Some experiments on the relative susceptibility of different teeth to dental caries. Proc. Roy. Soc. Med (Sec. Odont.) 3:71 (1910)

8. Bunting, R.W., Palmerlee, F., The role of the Bacillus acidophilus in dental caries. J. Am. Dental Assoc. 12:381 (1925)

9. Friesell, H.E., and Vogt, C.C., A report concerning the etiology of dental caries. J. Am. Dental Assoc. 13:748 (1926)

10. Thurlow S., and Bunzell, H.H., Tooth decay studies. I On the effect of acids on tooth tissue. Dental Cosmos 69:29 (1927)

11. McClelland, J.R., Decalcification of human tooth enamel. Dental Cosmos 68:127 (1926)

12. Enright, J.J., Friesell, H.E., and Treschor, M.O., Studies of the cause and nature of dental caries.
J. Dental Research 12:759 (1932)

13. Brekhus, P.J., and Armstrong, W.D., A method for the separation of enamel, dentin and cementum.
J. Dental Research 15:23 (1935)

14. Manly, R.S., and Hodge, H.C., Density and refractive index studies of dental hard tissues.
J. Dental Research 18:133 (1939)

15. Benedict H.D., and Kanthak, F.F., The solubility of dental enamel in various buffered solutions.
J. Dental Research 12:277 (1932)

16. Volker, J.F., Studies on the acid solubility of human enamel.
J. Dental Research 19:35 (1940)

17. Dallemagne, M.J., and Melon, J.
Arch. biol. Paris 57:79 (1946)

18. Karshan, M., and Rosebury, T., Relationship of pH, Calcium and Phosphorus to solubility of enamel.
J. Dental Research 14:220 (1934)

19. Forbes, J.C., Solubility of enamel.
J. Dental Research 13:349 (1933)

20. Volker, J.F., Effect of fluoride on solubility of enamel and dentin.
Proc. Soc. Exp. Biol. Med 42:725 (1939)

21. Buonocore, M.G., and Bibby, B.G., The effects of various ions on enamel solubility.
J. Dental Research 24:105 (1945)

22. Manly, R.S., and Bibby, B.G., Substances capable of decreasing the acid solubility of tooth enamel.
J. Dental Research 28:160 (1949)

23. McClure, F.J., and Ruzicka, S.J., Destructive effect of citrate versus lactate ions on rat molar tooth surfaces, in vivo.
J. Dental Research 25:1 (1946)

24. Brudevold, F., A study of the phosphate solubility of the human enamel surface.
J. Dental Research 27:320 (1948)

25. Yardeni, J., The weight losses of intact teeth in different buffers at varying pH values.
J. Dental Research 29:278 (1950)

26. Yardeni, J., Further experiments with intact teeth placed in various buffer solutions.
J. Dental Research 31:595 (1952)

27. Armstrong, W.D., Proc. Soc. Exp. Biol. Med 44:28 (1940)

28. Swartz, M.L. and Phillips, R.W., Solubility of enamel on areas of known hardness.
J. Dental Research 31:295 (1952)

29. Suess, P.H., and Fosdick, L.S., The solubility of normal and fluoridized enamel.
J. Dental Research 30:177 (1951)

30. Clark, E.P., and Collip, J.B., A study of the Tisdall method for the determination of blood serum Ca with a suggested modification.
J. Biol. Chem. 63:461 (1952)

31. Briggs, A.P., A modification of the Bell-Doisy phosphate method.
J. Biol. Chem. 53:13 (1922)

32. Rosebury, T., Biochemical study of the protein in dental enamel.
J. Dental Research 10:187 (1930)

33. Committee on Dental Health. A survey of the literature of dental caries. (1952)

34. Gilda, J.E., Studies on physical properties of radiant enamel, I The Manly-Hodge separation method as applied to the teeth of the rat and hamster.
J. Dental Research 30:445 (1951)

35. Gilda, J.E., Studies on physical properties of rodent enamel, II Density distribution and X-Ray diffraction pattern.
J. Dental Research 30:828 (1951)

36. Thewlis, J., Glock, G.E., and Murray, M.M., Trans Faraday Soc. 35:358 (1939)

37. Sobel, A.E., and Hanok, A., Calcification of teeth. I Composition in relation to blood and diet.
J. Biol. Chem 176:1105 (1948)

38. Sobel, A.E., Calcification of teeth.
J. Dental Research 28:61 (1949)

39. Armstrong, W.D., Brekhus, P.J., Chemical constitution of enamel and dentin. I Principal components.
J. Biol. Chem. 120:677 (1937)

40. Kornhner, H., Sobel, A.E., Hanok, A., I Division of Biological Chemistry, American Chemical Society, 114th meeting, Washington, Aug. 30 - Sept. 3 (1948)

41. Deakins, M. and Burt, R., The deposition of Ca, P and carbon dioxide in calcifying dental enamel.
J. Biol. Chem. 156:77 (1944)

42. Greenberg, D.M., Studies in mineral metabolism with the aid of artificial radioactive isotopes. VIII Tracer experiments with radioactive Ca and Sr on the mechanism of vitamin D action in rachitic rats.
J. Biol. Chem. 157:99 (1945)

43. Armstrong, W.D., and Barnum, C.P., Concurrent use of radioactive isotopes of Ca and P in the study of the metabolism of calcified tissues.
J. Biol. Chem. 172:199 (1948)

44. Hunt, H.R., Hoppert, C.A., The distribution of carious cavities in the lower molars of caries-susceptible and caries-resistant albino rats.
J. Dental Research 29:158 (1950)

45. Matsuda, Y.A., Biochemical study of tooth growth.
J. Biol. Chem. 71:437 (1927(

46. Volker, J.F., Proc. Soc. Exp. Biol. and Med. 43:643 (1940)

47. Nakfoor, E.C., Hunt, H.R., Hoppert, C.A., Fracturing of the molar teeth in caries-susceptible and caries-resistant albino rats.
J. Dental Research 31:143 (1952)

48. Nevin, R.B., Walsh, J.P., Some physico-chemical factors in relation to the causation of interproximal caries.
J. Dental research 30:235 (1935)

49. Cox, G.J., Nathans, J., Wirth, L.J., A method of study of molar form in the rat.
J. Dental Research 31:466 (1952

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