

A BIOCHEMICAL STUDY OF BACTERIAL METABOLISM IN ITS RELATION TO CHANGES IN THE DENSER TOOTH STRUCTURES IN THE MOUTH.

(Part 1, Fundamental Basis.)

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(Synopsis: 1. A study is reported of the underlying conditions in the destruction and formation of certain calcareous structures with especial reference to the relationship between bacterial metabolism and the changes in the denser tooth structures in the mouth. In the first (present) part the general fundamental basis, in the light of the accessible facts, is discussed, together with an attempt to indicate the stages and course in calcification and decalcification in the body as conditioned by underlying physico-chemical processes.

2. A fundamental similarity has been pointed out in the formation and general composition of calcareous structures in the body.

3. A regional difference frequently referred to, the effect of bacteria upon

crowns of teeth in the mouth, is regarded as a secondary matter.

4. The destruction of enamel of teeth, if an acid dissolution, must proceed at a rate conditioned by the local, NOT GENERAL OR AVERAGE, hydrogen ion concentration and be effected by other factors like aggregation state and surface active forces.

5. Bacteria of the mouth and alimentary tract as well as in artificial cultures are known to be related to the chemical character of the environment and probably to the physical; their metabolism is influenced by the physico-chemical conditions of their environment.

6. The evidence with regard to changes in bone and other calcareous structures, analogous or homologous to teeth, suggest that two possible destruc-

tive changes occur. One is a removal of salts of the alkalin earth metals, the other a removal of the nitrogenous portion. It is suggested that the course of dissolution or decalcification must follow the same laws as formation or calcification but probably in the reverse direction.

7. An attempt is made to aid the investigation by indicating tentatively, the course and stages in calcification and decalcification.

8. A further study, of the changes in hydrogen ion concentration effected by some characteristic oral bacteria and the results of the changes and the proteolytic activities of the same organisms upon the constituents of the teeth, is to follow.—Author.)

I. Introduction.

THE importance of the physical and chemical processes underlying tissue and bacterial metabolism has long been recognized. Until recently, the ideas of the particular transformations in tissues, and especially those induced directly or indirectly by micro-organisms, have come thru a consideration of analogous changes in physical and chemical systems. Advance has been made by the application of certain quantitative methods to the study of metabolic processes themselves, instances of which are the studies of agglutination and of immune bodies, of endogenous and exogenous metabolism, and bacterial nutrition. The development of methods to study the role of the hydrogen ion concentration in relation to carbohydrate and protein metabolism and bacterial growth, has added another chapter in this same direction. Information which we have regarding changes in the denser (calcareous) tissues is in contrast to this and it has seemed important to undertake at least an elementary study with regard to the principles involved, especially those associated with bacterial

metabolism in the mouth and the changes in crowns of teeth.

The external alterations in the calcareous tissues of the human teeth have been recognized by the dental profession as being of generally two orders: The disintegration of the enamel, one of acid dissolution, and that of the dentin, of a "liquefactive" nature. One difference has been presumed to arise from the chemical composition of the two tissues, the former having practically no organic matter, while the latter has as high as twenty-eight per cent. The studies of Magitot, Miller, Goadby, Black and others have tended to suggest the close relationship of bacteria to both changes; in the former tissue, a fermentation of carbohydrates of the mouth, etc., with the resulting acid formation and acid dissolution, while in the latter tissue, the more or less direct action of the enzymes of the bacteria upon the dentin (organic) substance. Since these ideas are held conjointly with one that the alteration of the coronal tooth substance is in the general direction of enamel to pulp the alteration of the enamel surface would proceed first, that of the dentin after. Opinions with regard to the matter are at variance and with the increase of empirical studies one can hardly draw definite conclusions. With a view to aiding in the better classification of the data which are scattered thruout the literature it has seemed wise to ascertain whether some of the organisms found in the human mouth can initiate in artificial cultures noticeable changes of hydrogen ion concentration, amino-acid-nitrogen and surface tension and whether these changes are of an order such as would pertain to the changes in the mouth and effect solution of the constituents of the teeth. It would appear further that if in artificial cultures certain organisms could or could not alter thru their own processes the permeability or resistance of the enamel layer, or of dentin, and if the same cultures could or

could not split any of the organic substance of bone or dentin, we would be in a position to prosecute further and more important investigations which would throw light upon the control of such conditions in calcareous tissues generally. Certain preliminary work has indicated that available facilities and methods were satisfactory for the study and upon this basis work is being conducted.

Our inquiry will be as follows: Are the fundamental factors underlying the changes in the crowns of teeth in the mouth a consequence of bacterial metabolism, and are they the same factors which condition the deposition and removal of calcium salts in other calcareous tissues or analogous structures? We shall not, for the present, attempt to explain caries of the bone, necrosis, nor the particular forms of various changed calcareous structures. Our concern is rather with regard to the physico-chemical processes associated with the principal changes and the order of events. It seems as tho this is the better course for the present. No attempt at completeness is made, and the frailty of the study is well recognized.

II. *General fundamental aspect of the problem.*

At the present time the underlying factors which govern the formation, preservation and destruction of bone and teeth are imperfectly known. It is not yet clear whether destruction and formation of these tissues stand in a reciprocal relationship. We are hardly able to define caries of the bone and teeth. The question is still open as to whether enamel of the teeth changes in structure or composition after initial formation. Generally speaking, however, the manner of the breaking down of such dense structures is known and roughly divided into two classes, viz. the destruction of the "matrix," on the one hand, e. g. necrosis, and the solution of the inorganic intercellular (or intertubular) substances on

the other, e. g. caries. Since, as has been referred to, enamel is presumed to have little or no organic matter, the latter process would with this tissue be only likely to proceed. But the subject cannot be left here. These dense tissues are fundamentally related in being formed by living cells under conditions which normally vary within quite narrow limits* tending to equilibration, in existing in different areas, but again, where the conditions are normally in a state of vital equilibrium, and are composed of substances which in chemical and physical behavior conform to known laws. One principal distinction is apparent and will bear first inspection. The crowns of teeth while generally existing in an environment with a tendency toward an equilibrium where the physical and chemical conditions are not fundamentally different from those in the environment of bones and the roots of teeth, they nevertheless are exposed to the action of bacteria, and are, in this respect, in contrast to the other calcareous tissues in the body. Resting here, lies the common assumption that bacteria are in some way related to caries of teeth, hence the offshoots—"A clean tooth never decays," "The micro-organisms of caries," etc. But the changes in the crowns of teeth have not been satisfactorily explained from this point of view. That some of the changes may be induced by bacterial metabolism is without a doubt, but further consideration of this point indicates that the fundamental processes underlying these changes are due to alterations in acid-base equilibrium, salt balance, surface active forces and ferment—antiferment balance acting independently or conjointly in the mouth and the body tissues and fluids. Since the bacteria are affected by alteration in the acid-base balance (expressed by the hydrogen ion concentration) and their metabolism leads to such a change, it is

*Temperature, pressure, hydrogen ion concentration.

not unlikely that they are related to this aspect of the problem. Since also bacterial action is largely thru enzymes they may likewise make impress upon the ferment—antiferment balance. Alterations in both equilibria would appear to have a pronounced effect upon the stability and functions of the teeth. The relation further of salt balance to all living tissues has been long known to be a close one and we have reason to expect that once the subject is carefully investigated, the stability of the calcareous tissues will in some measure be found to be dependent upon it. A consideration of the relationship between bacterial metabolism and the changes in some calcareous tissues with respect to the hydrogen ion concentration, salts, and the ferments would be materially helpful, not only to understand the true relationship, but to throw further light upon the factors which are related to similar processes.

III. *Composition and structure of, and changes in, calcareous tissues.*

a. Formative conditions.

A brief description of the development of and changes in calcareous tissues in general and those particular ones with which we are concerned at the present time is necessary before we can proceed to study the processes underlying their destruction, since some of the reactions appear to depend upon form, structure and composition. Dense, hard tissues, largely of calcium salts are found in many phyla of the animal kingdom. These vary considerably, even in the same animal. Bone resembles other living tissues in that it grows and is subject to various alterations in the life of the animal. Shells of mollusks, teeth and placoid scales are not fundamentally different in this respect. Development is interstitial with certain modifications, e. g. basilar or peripheral. Calcification is the result of cellular action and it is reversible, differing presumably only quantitatively from ossification and ab-

sorption. Osteoblasts or their homologs are generated from the nuclei of tissue cells, have the power of rapid proliferation and are capable of dissemination. These peculiar cells have the power of surrounding themselves with a calcareous zone, or, in certain aggregates, of a calcareous layer on only one cell aspect, e. g. enamel, base of cell columns. At the time of calcareous deposition the cells are presumably mature and assume a fixed form, altho alterations occur thruout life. Shells, and the enamel layer of placoid scales, of tusks and teeth differ from bone in that the formed calcareous structure is finally separated from the ameloblasts.

Sudduth (1) defined calcification as that process by which (organic) tissues become hardened by deposition of salts of calcium in their intercellular substance, as exemplified in the formation of bones and teeth. In embryonal development calcification begins in two forms—ossification and amelification—the first arising from connective tissue cells and the second from epithelium. The essential difference between the two depends upon the matrix, and the manner in which the salts are deposited rather than upon the character of the cells. The enamel substance is deposited upon the already formed layer of the dentin, there being no basis-substance between the layer of ameloblasts and the formed dentin. What is significant here is that a crystallization takes place. This is not true of the interprismatic substance of teeth, of any age, which does not refract polarized light. Dentin, cementum and bone tend to be definitely crystalline and exhibit birefracton.* In bone the matrix is fibrous connective tissue (ossein?). The cement substance between the fibrils is largely composed of insoluble lime salts. The structure of the matrix is similar to protoplasm. The surrounding membranes act as molds and are therefore considered limiting mem-

*Unpublished reports.

branes. In calculi (renal calculi of Rainey) which can be duplicated outside of the body the salts are deposited in a gelatinous matrix (2). Presumably the lime salts in the body combine with the cell components; and probably similar to a combination with albumen or mucilage as in artificial foundations. Artificial structures are soluble in acids, except the matrix, which remains. Rainey, Hasting, Ord and Tomes refer to calco-globulin. Hasting observed that the treatment of calco spherites with acids changed the former albumen to a substance more resembling chitin "which became exceedingly resistant to the action of acids, alkalies and boiling water" and to the organic compound gave the name calco-globulin. Bone, Sudduth maintains, "is simply an aggregation of calcospherules" first thrown out as a thin covering of the osteoblasts. "The wall," he continues, "is thickened at the expense of the size of the osteoblasts." Since the osteoblasts are in contact with each other at the start it is not difficult to trace the formation of the fibres. The connection between the bone cells is, however, not understood. The subject of ossification is now divided into "interstitial," "intramembranous," "subperiosteal" and "intracartilaginous," depending upon the type of laying down of calcium salts. The cells which form these tissues are all osteoblasts.

Sudduth holds that fixed connective tissue cells are only modified white blood corpuscles; the osteoblasts, the ordinary embryonic connective tissue cells. These are in intimate contact with the vascular supply. The building up and breaking down appears to be due to functions which are common to the white blood corpuscles.

Cementification is a modified form of subperiosteal development of bone. The thickening of the cement is from the first layer deposited upon the dentin, enlarging the circumference. The pericemen-

tum is analogous and continues with the periosteum.*

The formation of dentin is referred to as tubular ossification or dentinification. The first-layer dentin is a permanent product. The odontoblasts are modified connective tissue cells, situated at the periphery of the pulp and send out rod-like processes to the inner side of the enamel organ in the crown. Dentin is formed by the mature cells around the dentinal fibrils. The thickening of the tissue is by the accretion of lime salts, lengthening the tubules and thus forcing a recession of the pulp. The dentin is deposited first at the apex of the dental papilla, gradually widening in area and thickening while the odontoblasts are proliferating rootward. Mature dentin is a solid mass of calcified tissue, the tubules gradually becoming cemented together as a solid. The occurrence of interglobular spaces in dentin are held to support the notion that "the dentin is secreted into a protoplasmic basis substance." The spaces are presumed to be masses of calculo-globulin not fully calcified and are pierced by the dentinal tubules. Manning (3) reports he has demonstrated semipermeability in dentin sections 0.25 mm. thick.

The enamel is deposited or secreted from lower, deeper ends of ameloblasts. The amount of organic matter in enamel is less than in analogous structures of mollusks. An interesting point yet to be investigated is the apparently different composition of the secretions from the lower side of ameloblasts and from the upper, respectively, as in enamel and shells. Sudduth and others are inclined to believe that the early enamel does contain some protoplasm in physical or chemical association with the lime salts. The interprismatic or fixed material of shells is known to be insoluble in acids and is rendered more insoluble by their action (generally spoken of as a hyalin

*Function of Periosteum, cf. Keith, *British Jour. Surgery*, 1918-19, and MacEwen—*Growth of Bone*.

formation). The interprismatic substance of enamel is dissolved by dilute chromic acid allowing the prisms to fall apart unchanged. This type of destruction is not known to occur in the mouth. The prisms are hexagonal—thru intra molecular pressure (?)—and match the interdigitation of the forming cells. Wave lines in the prisms according to Febiger are due to inequalities. Bödecker claimed that “the enamel is built up of columns of calcified substances between which spaces exist.” Eckermann and others have shown that the depth of staining of enamel increases with the age of the tooth; apparently indicating that the interprismatic substance undergoes alteration with age. The interprismatic substance often called “cement” is not uniform in thickness and has often been regarded as an active protoplasmic network. Bödecker believed it to be continuous with the dentinal fibrils. Klein believed that a homogeneous interstitial substance exists and that this was larger in amount in the immature than in the fully formed tooth. Sudduth points out that the first formed layer of enamel is deposited in more or less protoplasm and immature developing enamel may be decalcified, leaving a matrix. In the first formed layer the dentinal fibrils appear to interdigitate with the enamel prisms. Points or spaces sometimes occur in enamel which Sudduth believes are filled with calco-globulin. Where these occur upon the surface they lead to pitted and grooved points. This fixed material is dissolved out by soluble ferments and apparently insoluble in the ordinary acids of the mouth. Mrs. Whitman and Sudduth have noted that in the last stages of amelification the ameloblasts undergo an alteration. In growing teeth, near the last stage of enamel formation, the ameloblasts change from columnar type to flat horizontal cells on all the areas except near the neck, and a sort of cuticula appears, “a perfectly homogeneous membrane.” Apparently

the oldest enamel-forming cells undergo a metamorphosis. After the individual prisms, which are each the work of a single ameloblast, are formed, the outer layer of enamel is deposited by the transformed membrane. This membrane, known as “Nasmyth’s membrane,” is hence a metamorphosed ameloblastic layer. The “cortical layer” of enamel is deposited by this membrane; a layer analogous to the “nacreous” layer is found in shells. Mature enamel varies in hardness from specie to specie and apparently from tooth to tooth. Pickerill (4) found that the surface resistance was variable. Such variations are seen in shells. Carpenter is quoted by Sudduth as follows: “This (increased) hardness appears to depend upon the mineral arrangement of carbonate of lime.” (p. 603). Interprismatic matrices have seldom been reported by histologists and the idea has been generally discounted. The enamel is regarded generally as a “coat of mail,” only protecting the dentin. Rickert and Bunting (5), Gies (6) and Eckermann (7) have shown that the enamel serves as a boundary membrane and permits the diffusion of solvents and some salts. The surface is generally polished but varies. Its thickness generally decreases to the neck of the tooth, and the developmental grooves of the molars and bicuspid. Some differences have been pointed out in chemical composition of the enamel layer between different races and the sexes. Sudduth, referring to Von Bibra’s analyses, speaks of a difference in organic material of the enamel in men and women. One infers from his statement that the resistance of the tooth to acids of the mouth varies in proportion to the organic constituent, but this should be more carefully studied. The enamel changes have in part been attributed to dessication, an increase in the hardness and decrease of interprismatic substance. Alterations in molecular structure of crystals thru loss of water of crystalliza-

tion is known and has been considered analogous to the changes in the enamel. Pigmented enamel is known and may be induced thru madder root ingested with the food or with trypan blue when the teeth are being developed. The pigmentation occurs in bands or striae. Mottled enamel has been studied for some time (8) and appears to be an alteration in the interprismatic substance. With the death and other changes in the pulp some pigmentation occurs. Differences in structure of the enamel layer have only partially been correlated to faulty calcification. One of the experimental attempts to show the influence of diet on teeth formation and also on eruption is that of Mrs. May Mellanby (9). Types of hypoplasia she writes "characterized by bands of defective enamel have been traced to specific fevers or to some acute illness as regards the more generalized types of gross hypoplasia. The parts of the teeth usually affected point to some cause acting during the first two years or so of life." Mrs. Mellanby found with reference to this malformation, as the results of experiments with puppies, that the abundance of articles with which the "fat-soluble A accessory-food-factor" is associated allows the development of sound teeth. Deficiency in these substances is associated with delayed loss of deciduous teeth, to the delayed eruption of permanent teeth, irregularity in position, partial absence of or defective enamel, and low calcium content. She further points out that "this is an instance of diet affecting teeth from the inside and is independent of bacterial sepsis and other oral conditions associated with food." She does not consider that the conditions can be attributed to malnutrition nor acute illness in her experiments since the improvement by addition of fat-soluble A "is as characteristic as the deleterious effect of deficient diet" and also that "the defective teeth are most pronounced in the rapidly growing puppies, and it

is difficult to associate rapid growth with illness or malnutrition as generally understood."

b. Post-formative changes and relation of bacteria to them.

It is undoubtedly significant that the formed enamel is exposed to less constant conditions than any other calcareous tissues. Post-formative changes in calcareous structures have been more especially studied by the dental profession but the underlying principles have been largely overlooked. That acids act destructively upon these structures out of the body has long been known. Certain difficulties in conception have, however, grown up which have led to lengthy discussions without great profit. The fact that usually the mouth is neutral and that the saliva, like body fluids and tissues, does not change from neutrality for long, was an apparent inconsistency. The character of dental caries with respect to location and duration has further had varying effects upon an acid dissolution theory. The demonstration by Miller and others some years ago, that bacteria from the mouth produced lactic acid among other substances and that the tooth crown is apparently soluble in this acid, was more favorable and has since been accepted with various qualifications. Many of the post-formative changes of enamel, e. g. caries, are generally periodic. The subject is at present in an unsatisfactory state. Certainly means have not been established for the control of the condition; and if bacteria are the cause, say some, why can we not overcome them? We are probably in difficulty because of a lack of definite ideas regarding the underlying physical and chemical processes and the limiting ratios.

c. Analogous changes: Tricalcium phosphate and bacterial metabolism in soil.

Since Hopkins and Whiting (10) (referred to by Hinkins (11) demon-

strated that tricalcium phosphate (one of the components of calcareous tissues) is altered thru bacterial action in the soil, the chemical changes involved have been made more clear. In the soil where conditions are somewhat similar to the mouth, e. g., a colloidal aggregation and a generally alkalin or neutral environment, they found that there may be acid production at local points. Their investigation indicates that tricalcium is changed to dicalcium or monocalcium phosphate. This may occur thru the relatively strong nitrous acid, the weaker acetic, butyric and lactic acids, or the still weaker carbonic acid. These acids are all produced thru bacterial action upon various proteins, sugars, starches, fibers and other compounds. It is significantly pointed out by Hopkins and Whiting that "in the first stage of decomposition it has been impossible to measure any soluble phosphorus without the growing plant as an indicator." A brief summary of their study may be of value, for in a number of instances it illuminates our problem. They point out that the decay of organic matter is not brought about by one kind of bacteria only, nor the result of but one process. Ammonia production and acid production occur at the same time as carbon dioxid production; in manure piles with raw rock phosphate present, and nitrate being produced, the general reaction may be alkalin. Further, the growth of bacteria depends upon specific compounds. In the presence of moisture and ferments, oxidations are carried out, the acids tending to be produced and requiring neutralization to the optimum bacterial needs. There tends also to be a utilization of calcium and phosphorus by the bacteria. The bacteria, after dissolving the phosphate, by subsequent action on the solution, make some of the phosphorus insoluble again, possibly by utilizing it for growth. Bacteria which utilize nitrites are principally involved

in the phosphate change, whereas nitrate bacteria are not. Carbon dioxid is produced in considerable quantities by bacteria and in carbon-dioxid-saturated soils some raw rock phosphate can be found in a soluble form. It is to be noted there that the general impression of dentists is that the acids and other substances produced by bacterial action on the saliva and food remnants are an important element in the decay and corrosion of teeth, or in the deposition of the tartar on the teeth. It is not impossible that, as indicated by Hopkins and Whiting's studies, the calcium phosphate of the teeth will neutralize the acids (a so-called "buffer action") produced by the bacteria and become more soluble. Whether this action favors the direction of caries toward the pulp, on the mesial and distal surfaces, and often on teeth where salivary calculi are being deposited remains for the present unanswered. It is beyond doubt that there tends to be in the mouth an acid-base equilibrium but what its relation is to true neutrality and whether the phosphates of the saliva and those of the teeth aid in its maintenance or only the latter, is open to experimental investigation. It is likely, however, that the reactions involving the formed tooth and bone structures must follow the general laws of solution, diffusion and precipitation and be effected in various stages.

IV. *Bacteria of the mouth and alimentary tract.*

It will now be important to refer briefly to the facts regarding bacteria in association with the tissues such as we are studying. With reference to bacteria Vignal found microorganisms in healthy human mouths as follows: Out of the nineteen, ten dissolved fibrin; nine, gluten; ten, casein; five, albumen; nine converted lactose into lactic acid; seven inverted cane sugar; seven caused fermentation of glucose; seven coagulated milk. Sanarelli showed that normal saliva destroyed the vitality of a limited

number of pathogenic bacteria—*B. typhi abdominalis* and *S. cholerae asiaticae*. Kligler (12) using as a basis in disease the clinical diagnosis of the single tooth with respect to its flora and in health the more general conditions, found the flora generally the same in character, streptococci and cocci predominating. The prevailing flora on "healthy teeth is approximately the same in all cases." He found an increase in the total number of bacteria during the night and during and immediately following a meal. With "diseased teeth" there was a decided difference in the numbers of bacteria present at the different stages of decay, with a complete change in the character of the flora and the relative prevalence of types from that of the healthy teeth, with a further difference between the types of bacteria in the early and later stages. It appeared to Kligler that "the early stages are due to one and the same process, characterized by a great decrease in the total number of bacteria accompanied by a drop in the relative number of cocci and a marked increase in the number of acidific bacilli and thread-forming organisms." He recognized the difference in decay of the pulp from that of the enamel and dentin and that there was an association with changes in flora. Studying children alone, Howe and his assistants confined their attention to the flora of carious teeth, especially the six and twelve year molars. They found principally members of the Moro-Tissier group. Members of this acidific group were always present, and it appeared to the investigators that the acid-forming properties limited the character of the flora in the carious tooth. Gillette and Howe (13) have shown that "as far as aerobic microorganisms are concerned the number of bacteria in the mouth are directly proportional to the amount of deposit present, regardless of the presence of caries." "The difference between the flora of immune and carious mouths appeared to be qualitative with a pre-

dominance of organisms capable of producing larger quantities of acid when caries are present than when there are no caries."

Meyer (14) reports that Brailowsky-Lounkervitch (1915) has demonstrated apparently that a notable change in distribution and frequency of flora occurs with dentition. The buccal surface and palate, gums and tongue have apparently marked differences. The observation was made that the bacterial flora of a clean mouth, on account of alkaline or neutral reaction, is aerobic or facultative anaerobic and poor in proteolytic organisms, in contrast to a dirty mouth.

The absolute statement of bacteria in the mouth, or even the ratio of microorganisms to unit of saliva, or debris, or tooth substance is, however, subject to later qualifications. We are really interested in the "working residue." No matter, e. g. carbohydrates in the mouth, can be changed without *relative* increase in one agent, say the bacteria, or a *relative* decrease in another, say the substances which tend to inhibit bacterial growth, and, since we are dealing with physico-chemical processes, the reaction must proceed thru a certain period of time. The buccal cavity is continually drained, its concentration of various agents is fluctuating, tending toward an equilibrium. The substances which give rise to bacterial growth, those which inhibit them, the bacteria and their metabolites are in an ebb and flow.

We have learned (15) that no less than five to eight grams of bacteria, dead for the most part, are excreted every day with the feces, and that the character and quantity of bacteria bear a direct relationship to the food digested. It now appears that lactose and dextrin with meat and rice cause a marked development of aciduric bacteria of the *b. acidophilus* type, predominating the fecal flora, tending to suppress the proteolytic types. Glucose, maltose and succrose cause either none or a minor alteration.

These results persist as long as the diet is maintained. Starchy foods tend to induce elimination of the obligate putrefactive bacteria, while proteins vary in their effect. Milk casein has a less tendency to give rise to putrefaction than meat protein. Fats and vegetable proteins are either without determining influence or stand in contrast to meat proteins in the encouragement of putrefaction. Milk led to a variation in the intestine from *B. coli* and streptococci in unboiled, to streptococci and *B. acidophilus* in boiled milk. Torrey correlates the types of bacteria vegetating in the intestinal tract to the chemical character of the food ingested. As secondary controlling factors he indicates the rate and degree of digestion and absorption of the food and the character of the end products. It excites considerable interest to read that in both the intestine and carious teeth, *B. bifidus* and *B. acidophilus* predominate (compare Howe, Kligler, Meyer), presumably under similar chemical conditions and in relation to the chemical character of the food.

V. *Bacterial metabolism.*

We are now interested in the question: What is the relation of bacteria to their environment, chemically? Bacteriologists have found that certain amino acids are utilized by different groups of bacteria and that some, more complex, products may be split. It has long been known that gelatin is liquified by certain organisms. Berman and Rettger, (16), Itano* and others have shown that *b. subtilis* is able to break down protein in the presence of fermentable sugar and in absence of a "buffer" (a substance like carbonates, phosphates, amino acids, which "soak up" excess of hydrogen or hydroxyl ions). The organism attacks glucose slowly and for this reason is able to produce a proteolytic enzyme before its activity is checked by the hydrogen

ion concentration. *B. coli* in a glucose or lactose medium with utilizable nitrogenous substances available maintains a suitable hydrogen ion concentration without the aid of a buffer. In this case the nitrogen metabolism neutralizes the acid formed from the sugar decomposition. Berman and Rettger suggest three types of bacterial metabolism—one where the organism attacks protein in the presence of fermentable sugar, because the sugar is fermented slowly and the nitrogen and carbohydrate metabolism are maintained in equilibrium. The second type is where the organism is able to regulate its own environment with a rapid sugar fermentation where little acid occurs among the end products. The third type is where sugar fermentation is rapid with the formation of large amounts of acid. With the last type, acid production tends to prevent further metabolism. Buffers like dipotassium phosphate tend to prevent such inhibition. These authors observed that a bacterial proteolytic enzyme is not as a rule produced within the first twenty-four hours. Previous to this some building up of bacterial substance (anabolism) must take place unless some enzyme is transferred with the organisms. With reference to their experiments they found that fermentable sugars in moderate amounts do not affect the nitrogen metabolism of bacteria in an otherwise fermentable environment. In confirming previous experiments they found that the bacteria require food substances of simple constitution and readily absorbed by the bacterial cell. Their conclusions were that bacteria are unable to decompose coagulated native protein in the absence of other sources of available nitrogen; that the purified proteose is resistant to direct action by bacteria; that gelatin and casein resemble proteose in their resistance to bacterial attack; and that the ability of an organism to liquify gelatin does not necessarily indicate that it possesses proteolytic properties. It is recognized that the enzymes

*Itano, Mass. Agric. Coll. Exp. Sta., 1916.

of *B. subtilis* and *proteus vulgaris* types are similar in their action to the pancreatic digestion of protein. Their bacterial enzymes appear to rapidly cleave some proteins, forming similar products to those in tryptic digestion. We also know that all organic matter is not food for bacteria but that it must be of simple composition before being utilized. It seems then that similar to other living cells, bacteria construct their own substances of these simple products. The body fluids or tissues apparently contain or provide the simple nitrogenous substances suitable to bacterial anabolism. Amines and amino acids are undoubtedly present.

A number of investigators have reported significant variations in the growth of bacteria with respect to temperature and other factors. These are referred to respectively as stationary lag, logarithmic growth, negative growth acceleration, stationary growth, accelerated death phase, and logarithmic negative growth (17 and 18). Rettger (19) points out that the so-called bacterial lag is likely a temporary absence of the substances which are necessary for bacterial growth in the new environment to which the organisms are transferred. Satisfactory culture media which practically eliminate bacterial lag would have the necessary simple intermediate bodies and possibly growth accessory factors. Salter found that bile salts, peptone up to 5%, beef extract, dipotassium phosphate up to 1%, and the age of the culture influence the rate of growth, with *b. communis*.

VI. *Special considerations regarding relation of bacterial metabolism to changes in calcareous tissues in the mouth.*

a. Aggregation states.

This general survey now leads us to a discussion of another grouping of the evidence with respect to the formation or destruction of calcareous tissues and bacteria generally. We have seen that the

osteoblasts are similar in function to the white blood corpuscles; that bone formation, cementification and dentinification are alike in principle and differ from amelification only by reason of the enamel being finally separated from the secreting tissue; that bone, dentin, cement and enamel are subject to many (but similar fundamental) influences in formation. The enamel during development may be related to accessory food factors; and appears to increase in density later; and probably calcareous tissues, generally, tend to be affected likewise. We have seen that bacteria are related to an acid-base balance, are generally affected by the changes in hydrogen ion concentration, produce acids thru their metabolism, and in the soil at least, change tricalcium phosphate to monocalcium phosphate. The bacteria of the mouth appear to fluctuate, with a tendency in health and disease, respectively, to a constant flora; that the bacterial groups change in accordance with definite conditions, notably a fluctuation with changes in dentition, and decrease in total numbers at the early stages of decay with a rise of acidific bacilli and thread-forming organisms; and that there appears to be a characteristic flora of carious teeth. The flora of the alimentary tract appears to change in character and quantity in relation to the chemical character of the food as indicated by conditions in the intestine and the mouth, with a distinct tendency to the survival of certain groups. We have further learned that bacteria utilize amines and amino acid nitrogen; that their metabolism is related to temperature, carbohydrates and salts in solution; that, with certain exceptions, they do not cleave gelatin, casein and proteoses; that liquefaction of gelatin by them is probably not a splitting but a gel to sol transformation; that their enzymes may not completely decompose substances like glycoproteins but may cleave glucose from the molecule. It also appears significant

that bacterial growth is periodic; that bacteria require definite time, as well as other factors, for changes in structure and composition. In sum, we realize that the stability of the calcareous tissues and the bacteria is dependent upon (1) environment, (2) specific cell composition and structure, and (3) cell or tissue functional activities. Growth, repair and destruction of most living processes seem to be fundamentally related to the environment with respect to the acids, bases, salts or their ions and probably physical forces which we shall later examine in certain quantitative relations.

Among the physical and chemical aspects of our problem, the state of aggregation is important. The growth of the calcareous tissues appears to depend upon the state of solution of the calcium salts; the stability of the bone and teeth, upon the peculiar conditions of the solid state in contact with other solids, liquids and gases. The condition of and some of the changes in the teeth may be explained on the basis of dilute solid solutions. The matter is open to investigation but at present is little understood. The observation that solids, such as we are dealing with, conduct electrolytically suggests solid diffusion; and diffusion thru plates of calcium salts may be explained thru transport of ions since the two stand in intimate relations. Some of the phenomena, however, appear to be typical of alterations in colloidal solutions. The condition of most of the fluids in the body is analogous to suspensoid sols. This is true of the blood and lymph and it appears to be true of the saliva. Chemical reactions in the body are characteristic of those in colloidal suspensions. In fact it is upon the properties of colloids that the cell functions depend. The precipitation of mucin and agglutination of bacteria, and likely solidification of calcium salts in the body, for examples, may be explained by the play of surface active forces, ions and various salts in colloidal solutions. The true explanation of the

transformations of the calcareous tissues will not be forthcoming without consideration of the aggregation states. This must be borne in mind as we consider the various physico-chemical processes which underlie vital phenomena.

b. Hydrogen Ion Concentration.

The work of L. J. Henderson in making more clear the mechanism of moderating the hydrogen ion concentration in protoplasmic processes has had a pronounced effect upon studies of changes in the living cells or those related to their growth. The various processes taking place in the living organism are known to be very sensitive to changes in the acidity and alkalinity, and some processes result in the production of acids or bases, as referred to in the case of bacterial growth. The numerical values of the concentration of hydrogen and hydroxyl ions serves to express the true condition. The increase of hydrogen ion concentration is known to be taken care of partly by removal of CO_2 in respiratory ventilation, and lactic acid by oxidation in the blood tissues, but other means are provided, e. g. bicarbonates and carbon dioxide; the acid or alkaline phosphates; proteins, as amphoteric substances; and adsorption (altho probably a subordinate factor in the equilibrium process). It is important to mention here the properties of the hydrogen and hydroxyl ions. One may well recall the fact that some acids will turn out others from combinations and some solutions of them invert solutions of cane sugar more rapidly than others, in the same relative concentration. The common character to acids is the hydrogen ion, and to bases, the hydroxyl ion. The alteration of litmus and phenolphthalein depends upon the relative concentration of these oppositely electrically charged ions in solution. The great activity of hydrogen and hydroxyl ions in physiological processes is undoubtedly in many cases connected with the relatively great rate of migration, which is affected by

the state of aggregation and viscosity of the solution. Very small concentrations of H^+ and OH^- have pronounced effects on colloidal solutions. Faraday's original work (1858), with that of Schultze (1882), Hardy and others, relate the electrical charge of the ions to observed effects upon colloidal states, e. g., coagulation, precipitation, etc. The activity of enzymes (cf. Bayliss and others) appears to be due to the colloidal state and changes in the hydrogen ion concentration (expressed frequently as pH). To similar changes we know the heart is effected, and the respiratory center lies in a still more sensitive class. (cf. J. S. Haldane and Y. Henderson.) Bacterial metabolism, as we have seen is affected by changes in pH. Working with *b. subtilis*, Itano, for example, found its activity to be greatest at $pH=7.5$ to 8.5 , to stop below $pH=4.18$ and above $pH=9.43$. The optimum concentration for proteolysis in the cases of *Streptococcus erysipalatis* and *S. lacticus* was $pH=7.62$ and 7.02 respectively.*

c. Salt content.

Beside the hydrogen ion concentration in importance, stands the salt content of the medium in which cells are living. The blood, lymph and saliva contain, at least, salts of sodium, potassium and calcium in quite definite ratios. In fact few, if any, living processes go on in the absence of these salts. Generally speaking the salts of the univalent ions are antagonized by traces of a salt with a bivalent cation. (Osterhout, Loeb and others.) The work of W. J. V. Osterhout, J. Loeb, and R. S. Lillie especially has indicated the relationship of these ions to the alterations in the semipermeability of membranes of living cells; but Clowes' work has shown the significant structural analogy in the alteration of emulsions of oil and water. Loeb (20)

it was who expressed the "theory of physiologically balanced salt solutions, by which we mean that in the ocean (as in the blood or lymph) the salts exist in such ratio that they mutually antagonize the injurious action which one or several of them would have if they were alone in solution." We have previously referred to their role in the maintenance of neutrality of organisms. We shall later point out the relation to solution and precipitation of solids.

d. Surface tension.

In transformations, such as must occur with reference to the dissolution of calcareous tissues, the growth and activity of bacteria and the functional activity of secreting cells, surface tension phenomena are to be considered and as well, in the former case, solution tension and sublimation tension. In the last few years since the work of Macallum has been brought to our attention and, later, the work of Madsen, Jorgensen and others in the field of agglutination, the role of surface tension in its relation to vital phenomena has served to acquaint us with some of the mechanisms involved in cellular function. Before the work of Macallum, surface phenomena had attracted the attention of physicists and chemists. The surface contact between a liquid and another phase (solid, liquid or gas) had come to be recognized as differing in many characteristics from the properties of the main body of either phase. Laplace showed that the surface tension has its origin in the forces of attraction between the molecules of the liquid. The surface energy was found to vary with the chemical nature of the liquid, some solutes acting to lower the surface tension, while some increased it. It later came to be recognized that substances which lower the surface tension tend to move to the surface, i. e., "Gibbs-Thompson law." The so-called adsorption phenomenon is really surface condensation and may be mechanical, elec-

*Explanation: The concentration of hydrogen ions in neutral (pure) water where $H_2O=H^+ + OH^-$ is 1/10,000,000 molecule in grams, per litre of solution; that is, 0.0000001 "normal" or 1×10^{-7} (H^+) which is expressed as $pH=7$.

trical or chemical. Rather rigid films are formed according to Ramsden with saponins, proteins, etc., and in these may be dissolved substances in a different concentration from those in the interior or main body of the phase. Chemical reactions which lower chemical potential are favored at a surface and the rate of these is controlled by the extent of the active surface. The phenomena characteristic of colloidal solutions are presumably the results of acting surface forces, that is, forces at play at boundaries. No direct application of the surface tension formula has been applied to the formation of bacterial films on the surfaces of teeth nor to the changes which appear to take place at the tooth-saliva boundary. It is undoubted, however, that the crown surface is always wetted by saliva, hence the forces which act at the tooth-saliva boundary are different from those which act in the saliva or in the body of the tooth. Since the tooth is electrically charged and the saliva is a suspensoid colloid (likewise charged), it is further probable that there is a tendency to surface adsorption. Pickerill appears to doubt this, altho he admits that the staining of teeth may be an adsorptive phenomenon. If it is later definitely established that the enamel is permeable or semi-permeable, which has been suggested by experiments of Gies (6), Eckermann (7) and Bunting (5), it may be that ionic phenomena there are analogous to phenomena observed by Bartell and Hocker (21) in clay partitions. The electrical charge of the saliva-half of the double layer may be due in part at least to hydrogen ions and the anode side (saliva) becomes alkaline; the cathod side (enamel), acid. It is also to be recognized that the hydrogen ion exerts an influence; on the positive sol opposed to precipitation, acting opposite to that of the hydroxyl ion; vice versa, of course, in the case of negative sols. Any solution or precipitation of solid particles would be expected in our case

to be in accord with these phenomena in colloidal solutions. Precipitation is due, if we may draw upon the work of Bodländer and others, to a balance between the two opposing influences, that is, the action of ions of opposite sign (isoelectric point). Whether or not reversible precipitation occurs at the tooth-saliva boundary is not yet known. The formation of salivary calculi and caries on the same tooth tends to suggest that in local regions, particularly, the opposite is true. The work of Clowes and Bancroft (22) may be mentioned here since it tends to offer an explanation of the distribution of calcium in surface films. They found that the solubility of calcium salts in the oil phase and of sodium salts in the water phase of oil-water mixtures affected the surface tension of the oil-water boundary. It is only significant here, perhaps, that the ratio of calcium to sodium as far as the ions are concerned is important in determining whether oil is suspended in water, or the reverse, or neither; and the dispersion of Ca soaps in oil more than in water, and the tendency of these ions to move toward the surface. If this be true, and it may be experimentally investigated, we may better understand the solution and precipitation of calcium salts under such conditions as exist in the body.

e. Sublimation and solution tension.

We are more likely dealing with the solution tension and sublimation tension in the dissolution of the enamel and the solid calcium phase of dentin, cementum and bone. The composition of the structures suggests an equilibrium existing between two or three solid phases and a different pressure of their vapors, the pressure of which is called "sublimation tension". This tension, which would appear to exist in the case of the enamel and calcareous structures must be altered before chemical reactions would proceed, since the complex salts must dissociate into their less complex relatives and the chemical reactions follow in the vapor

phase surrounding the solids. This cannot be conceived to take place except thru an alteration of the phase boundary conditions and decrease of the electrostatic force which holds the oppositely charged ions together in the solid phase. Now a solid in contact with a solution, e. g. teeth bathed by saliva, tends to send off its molecules so as to form a solution. This tendency, exerting a so-called solution pressure, is affected by the concentration of the ions in the solution. As the ions of the same kind in the solution are increased, their osmotic pressure opposes the passage of similar ions from the solid, and as the ions leave the solid, or are deposited upon it, it will become charged respectively, negative or positive.

VII. *Physical chemistry of calcification and decalcification.*

Unfortunately we have, in detail, no accurate picture of the composition of the calcareous structures, nor of the mechanism of the deposition and solidification in bone, teeth and shells. We have, from histological and embryological sources, mainly, only a rather vague idea of the structure of enamel at various stages of formation. It is suggested that this can be improved upon thru X-Ray studies and the application of optical petrographic methods as applied in crystallography. As regards calcification and decalcification we are far from having a clear understanding of the stages and the course or courses of the general process. To aid in this study a statement of the author's conception is given. The author believes that calcification, fundamentally, is the inverse of decalcification, in the living body, occurring under conditions not unlike those outside of the body, where solids arise from colloidal solutions as the result of (1) alterations in surface energy at phase boundaries, (2) the alterations in the ion or solubility product, thru infiltration, hydrolysis and synthesis, and (3) alterations in the hydrogen ion concentration.

The outline which follows is of course tentative and will be considered later in connection with experimental results. It is but a preliminary step and is unsatisfactory. A view of calcification as related to CO_2 content is presented by Wells, and will be referred to later; but this does not appear to provide an explanation of some of the changes in the formation of bone and tooth structures which are frequently observed.

Structurally, bone, dentin and cementum may be placed in one group, enamel and shells in another. The difference between the two groups chemically lies in the relative amounts of nitrogen and carbon to calcium and magnesium. Bone, teeth and cementum differ from each other slightly as regards chemical composition and more as to the method of formation or growth and the histological form or structure. As solids (physically), the masses in mature organisms differ little from each other—tend to approach an ossified mass, more or less crystalline, with little protoplasm in the formed structure. This is least true of bone. In the second group, enamel differs from mollusk shells in being secreted from opposite faces of the ameloblasts, with less nitrogen and carbon than the latter, and a thinner, less permanent, "nacreous" layer. The enamel is not uniformly crystalline at least insofar as separate prisms can be viewed in plane polarized light along their short axis, and the interprismatic substance is apparently amorphous.* The crystallographic structure should be carefully studied to illuminate this and other structural questions and also to throw light upon phenomena, which appear, under various stresses, e. g. sudden alteration of temperature, friction and pressure, as electric currents. The much belabored problem of "sensitivity" of the dental structures may be cleared up thru a physico-chemical crystallographic investigation. Pyro-electric currents in

*Unpublished reports.

crystalline structures are well known. Of the minute structure, then, our knowledge is not satisfactory and especially with respect to the structural alterations in relation to changes in the body tissues and fluids.

Observations upon forming bone, teeth and shells indicate that the solid structures arise out of a gelatinous mass, but the composition of the mass is uncertain and its subsequent modifications are not clear. The following outline is drafted from the histological evidence and reactions of a chemical and physical nature in colloidal systems. Two formative courses suggest themselves which could give rise to calcified structures. We may consider in the first that the stages are as follows:

I. Secretion by cells of a substance which is, or gives rise to, a gelatinous (colloidal) mass.* (1) The alkalin earth salts** may be present in solution as intimate radicals of a protein or lipin (discontinuous phase); or (2) in solution, distributed between the discontinuous and continuous phases of the mass. Calcium is at least more soluble in lipins and proteins than in water.

II. The formation of phosphates and carbonates thru hydrolysis and molecular action in solution; and separation of the solid phase.

III. Solidification and crystallization.

A second view (which in part has been suggested by Williams (23) and perhaps others) is similar to the first except that

I. Secretion of gelatinous substance is without the alkalin earth radicals and dissolved or adsorbed ions which are

II. Diffused later as soluble salts with subsequent rise of phosphates and carbonates and then, as before,

III. Solidification and crystallization.

The secretion of the mass from which arises the solid structures does not appear to be different with respect to each special structure, i. e. bone, dentin, etc. Aggregations of secreting cells in layers are more or less intimately connected with a limiting tissue such as the periosteum, altho as Macewen and others have shown, in diaphyseal bone, that epiphyseal plates will give rise to bone free from periosteum. The secreted mass assumes a definite form in consequence of the proliferation and extent of the limiting tissues. It is not unlikely that the ameloblastic layer in enamel formation and the forming enamel serve, coronally, as a form governor, as the pericementum does with respect to the root. The growth of the structures is similar. In bone, cementum and mollusk shells the structure appears to be deposited periodically, that is, laid down in waves; but the true significance of laminations in bone and cementum, in apparent contrast to dentin and enamel, remains to be studied carefully.

The order of secretion, salt formation, saturation and solidification is indicated by events observed in the body and in physico-chemical systems. The peculiar form of the structures is undoubtedly related directly to the cells and their aggregates, but the mechanism of the secretion, and subsequent changes in the intercellular mass does not appear to depart from processes occurring outside of the body. Since we have presumed that the mass arises as a consequence of cellular functions the properties of cells must be considered. It is not unlikely that the gelatinous mass which has been observed in early calcification is but transformed protoplasm. A number of secreted body juices have been considered in this light. Saliva appears to arise as the result of cellular synthesis. Raw material brought to the cells thru flow of blood to the glands is made into crude

*Called crudely "calcoglobulin," but further than this, undefined.

**CaCl₂, CaF, CaCO₃, Ca₃(PO₄)₂, MgCO₃, X Mg PO₄ have been cited in such combination.

protoplasm; later, thru unknown processes, giving rise to mucin and other constituents which accumulate in the cells and then is secreted from their inner ends, as saliva. It is not to be overlooked that many of the salts with which we are dealing in calcification form, on precipitation outside of the body, gelatinous-like masses. In the light of cell reactions generally, solidification of the mass would occur outside of the cells and in areas away from the cell boundary. It is not known whether the phenomenon is like gelatin or petrification. It is not unlike some crystal and rock formations. Crystallization often occurs but amorphous masses and nitrogenous constituents are also present. This may occur independent of the cells but it is nevertheless intimately bound up with and is thus practically a part of the cellular functions. Evidence which we have indicates that during the life of the cells the structures are affected only thru them. Calcification thus usually proceeds or recedes thru the metabolic activity of the forming cells and any processes which affect cell metabolism would leave their impress for the time being, at least, upon the secreted mass. We have, however, come to recognize that cellular metabolism is affected by alterations of a physico-chemical nature and in the secretion of the bone, enamel, etc., we are fundamentally dealing with physico-chemical phenomena, resembling reactions in colloidal systems. The building stones brought to the cells are transformed by hydrolysis and synthesis, oxidation and reduction. The intake and outgo in cells resemble diffusion. Calcification may resemble precipitation and solidification; and decalcification, resolution. If such be the case, we thus would expect to observe the influence of surface active forces at phase boundaries, the effect of antagonistic ions, e. g. $\text{Ca}:\text{Na}$, the effect of the hydrogen ion concentration, and in our particular case the ion or solubi-

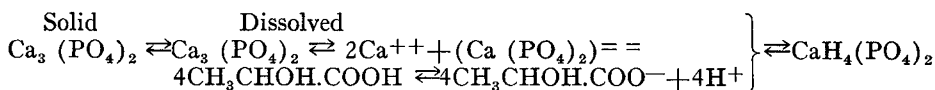
lity product. It is not improbable then that the conditions under which a substance, once formed in the body, may be dissolved or precipitated* and may move toward a liquid-solid boundary or away from it, are similar within and without the body. Hence while we regard the formation of bone, etc., as a cell phenomenon, we must not neglect the physical and chemical conditions determining the process.

There is some evidence that decalcification is effected thru cell metabolism. Presumably in this event there is an unstable equilibrium between the cell content and the nutrient plasm. As the concentration of constituents of the nutrient plasm rises and falls, the concentration of the cell would be expected to follow. Since the cytoplasm tends to be in equilibrium with the interstitial medium, the secreted calcareous mass would be noticeably affected if the circulation to the cell were interfered with beyond a certain limit. In cases of caries of the bone, rachitis and osteomalacia, the reduction in calcium content is often temporary; and circulatory disturbances have been considered. If our suggestion is correct the bone cells would be altered temporarily because of a change in equilibrium conditions. Many changes in calcareous structures, however, also appear to be effected thru cell metabolism. In necrosis the nitrogenous matrix of the bone is involved, whether thru autolysis or not, is not known; the cell circulation is usually cut off and the nitrogen content (not the calcium) is affected. From our point of view an equilibrium tends to be maintained. Hence when the circulation is interrupted the cells draw upon the secreted material which, next the cell is probably still in a gelatinous stage. Resorption of tooth roots and absorption of bone, two characteristic processes, involve an irreversible removal both of

*In other words, a liquid or a solid, a dissolved or a suspended particle.

nitrogenous and alkalin earth compounds. Histologists appear to hold that these processes are carried on by osteoclasts; but it is not yet clear whether they only are involved. Somewhat different from these phenomena are the changes

dissolved. Tricalcium phosphate passes into solution when in contact with acids—nitrous, sulphuric, lactic, carbonic—giving soluble monocalcium phosphate and a calcium compound—nitrite, sulphate, lactate and bicarbonate, e. g.



in dentin and enamel. In tooth caries the dentin is frequently decalcified, apart from cells, in the denser enamel region, and often following decalcification of the enamel. In all these cases we do not know what alterations of structure have preceded the lesions recorded, nor the effect upon the cells which give rise to the structure.

We have referred to decalcification as a physico-chemical process, not necessarily associated with cell phenomena, whence it would follow the same laws as the solution of similar chemical substances. The enamel of teeth is free from the cells which secreted it and is irreversibly decalcified. As different as the case might appear to be from decalcification of bone, there are, fundamentally, similarities and where the same laws would apply. Principally, both solids are in contact with a liquid phase of a colloidal nature, and comprising various salts in solution. The enamel is always wetted by saliva. Reactions occurring in this medium, like reactions in the gelatinous mass secreted by the bone cell, would affect the solid-liquid relationship. Hence we incline to the suggestion that alterations of the solubility product, the surface energy at phase boundaries, and the constituent in which the calcium and magnesium are soluble, would affect the stability of the solid phase. In the first case, for example, if the product of the concentration of the ions in, say the liquid (ions which can enter into combination with ions in an adjoining solid) is less than the ion product in a solid, formed by their union, the solid will be

The general statement in true solutions is that an insoluble salt of a given acid will interact and dissolve when treated with a solution containing another acid if certain conditions prevail, e. g. if the latter acid is much more highly ionized than the former, or the acting acid is more active than the acid formed, or the salt is of an acid which is not present, or if the union of the former anion to the cation in the solid state is less secure than in the case of the cation of the acting acid.

Altho somewhat apart from calcification, another problem is of interest here. The formation of salivary calculi seems not wholly to be explained on the basis of precipitation of calcium salts from true solutions. For one thing, mucinous plaques, salivary and serumal calculi are yet undefined and much confusion exists about them. Assuming—as analyses suggest—that salivary calculi and “tartar” are similar, an alteration of the salt concentration and acid-base balance would facilitate a precipitation of calcium salts upon the enamel. The presence of the calcium layer in contact with a solution laden with calcium salts would hasten this and tend to explain the precipitation at the tooth-saliva boundary rather than the gum-saliva boundary. The relation of mucin films to salivary calculi appears to be a false one, the two being distinct. The former is probably a precipitation of mucin from saliva thru an acid-base alteration or a bacterial development as the work of Gies (24) and Rettger (25) indicates; whereas the latter are, according to

Roberg (26) and others, calcareous concretions with organic matter of variable proportion.

In discussing the chemistry of pathological calcification, Wells (27) considers that the calcium salts may come from the blood, precipitated from protein suspensions of carbonates and phosphates or as calcium-ion-protein compounds, and susceptible to CO_2 influences; that calcification may accompany hyalin degeneration and necrosis; or may follow feeble circulation and low oxidation. In such cases the deposition of calcium salts depends upon one or more of the following conditions:

- (1) Increased alkalinity or decreased CO_2 .
- (2) Utilization of the protein of the calcium borne fluids by starved tissues, lessening the calcium suspension power.
- (3) Formation within degenerated area of a substance or substances with affinity for calcium.
- (4) Production of a physical condition favoring the local absorption of salts, the least soluble salts accumulating in excess.

Wells points out that the opposite of the first is more true in normal ossification. The second also probably cannot apply to normal ossification. The third explanation could be considered if the presence of phosphoric acid can be admitted (as has been assumed, arising from decomposed nucleoproteins and lecithin) along with fatty acids and some proteins like deuterioalbumose (present in autolyzing areas). But the calcification, whether normal or pathological, borders on proportional alterations of a similar nature and it seems as tho one explanation should care for both. The processes of ossification and pathological calcification Wells viewed as essentially

the same, and suggests, as explanatory of both, the alterations of CO_2 content in solutions, as suggested by Hofmeister.

VIII. *Conclusion.*

We have thus far considered the growth and destruction of calcareous tissues, as they may be attributed to certain fundamental physico-chemical processes. The relation of the changes in the calcareous tissues to the acid-base balance, salt content and phase boundary conditions appear to be important and the changes in the tissues may be explained by a careful study of the quantitative alterations of these factors. The changes in the oral flora appear to be dependent upon some of the same fundamental processes also. In just what relation the changes in the denser tooth tissues in the mouth stand to the oral bacteria or to other oral conditions independent of them it will be of value to investigate. The quantitative estimation of the alterations induced by the bacteria is but an aspect of the real problem. We must sooner or later know the structural and functional alterations of the calcareous tissues incident to age, disease and environment. In part only has this been suggested. The teeth, for example, appear to change with age and independent of bacteria in the mouth. Just what the degree of the change or changes is we can only guess at present. These alterations cannot be overlooked, in the control of affections of the denser (calcareous) tooth tissues at least, since they affect the course of changes induced by foreign agents like bacteria. Some will of course say that, with the teeth, bacteria are always present and hence this variable factor must always be considered. But this is only tenable while we are unaware of the relationship between the bacteria themselves and the fundamental factors to which we have referred. Then, too,

the processes in the mouth appear in certain aspects to be similar to some occurring elsewhere, e. g. rickets, caries of bone, where it is inconsistent, with our present ideas of health, to believe bacteria have any influence. The bacteria in the mouth can still be viewed as symbionts, parasitism resulting only from changes in their environment, perchance thru accidental alteration of the bacterial content, but more likely because of alteration attributable to body functional changes which, when made apparent, will likely be found to be within control. The development of a sound basis upon which changes in calcareous tissues can only result will tend to aid in clearing up the matter. Our lack now is the knowledge of the basic factors, criteria to be followed in estimating their role, and ready means of detecting the intensity and order of the changes.

Where we have, in what has gone before, suggested the importance of certain physico-chemical processes underlying the growth, repair, stability and destruction of calcareous tissues, processes which have appeared as significant fundamentally in most all living phenomena, we shall in the next paper examine two of these processes as they are related to the changes in the calcareous tissues of the mouth associated with bacterial metabolism. Experimental work has been undertaken with a view to investigating the changes in hydrogen ion concentration effected by some characteristic oral bacteria and the result of these changes and the proteolytic activities of the same organisms upon teeth and similar structures.

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