

CARBOHYDRATE AND DENTAL CARIES

A critical review of the recent
current literature on carbohydrate
and dental caries submitted for the
Degree of Master of Dental Surgery.

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INTRODUCTION

W. D. Miller (1) in 1882 first advanced the theory that the source of the acids which decalcify the teeth in caries is in the activities of oral micro-organisms flourishing on carbohydrates. He said :

"A mixture of 68.0 grams saliva plus 1.0 bread plus 0.5 meat plus 0.5 sugar kept for forty-eight hours at the temperature of the human body, generated more than sufficient acid to decalcify the entire crown of a molar tooth."

Previous investigators - Robertson, Buehlmann, Erdl, Finicus, Leber and Rottenstein and Clark (1) - had recognised a relationship between oral micro-organisms and caries and that action of acids was suspected as the active demineralizing mechanism, but the source of the acids was not clearly demonstrated. Acids are known to be produced during the degradation of carbohydrates, and several observers have shown that in the complete absence of carbohydrates from diet, no caries occurs.

Recent histopathological studies on the solubility of enamel (2), composition of enamel (3-5), surface changes in enamel (6-7), and the formation of beginning caries of enamel (8-13), have attempted to prove or disprove the validity of acid attack as an initiating factor in dental decay.

The ingestion then of any carbohydrate that can be converted to acids by oral bacteria might favour the acceleration of caries. Different foodstuffs would hence act as caries accelerating factors to an extent varying with the carbohydrate content, with the nature of the carbohydrates, and with the tendency of the food-

stuffs to be retained in the oral cavity.

Carbohydrate and the main theories of dental caries are discussed, followed by a presentation of current research on animals and humans to illustrate the close relationship between the subjects.

The literature showing the direct actions of carbohydrate and their sequelae only is included; the various other factors, e.g. general diet, fluorine, and properties of saliva, although important in the aetiology of dental caries, are outside the scope of this thesis.

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CHAPTER 1.

CARBOHYDRATES, OR THE SACCHARIDES.

1. Occurrence:

More carbohydrate material is present in nature than all other organic substances combined, comprising most of the organic structure of the plants and, in part, animals.

The starches are abundant in grains, tubers and roots; cane sugar is present in the nectar of flowers, in fruits and in the juices of various plants, while large quantities of pentosans are found in seed husks, corn cobs, other fibrous structures, and in plant gums and mucilages.

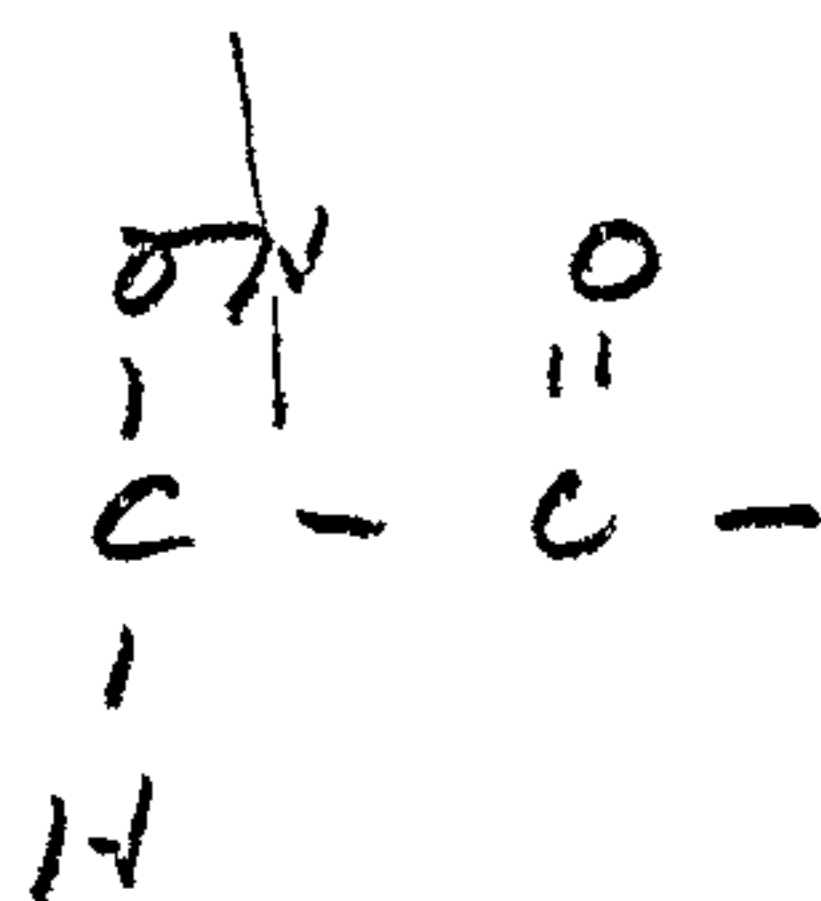
Carbohydrates and their derivatives are found in all animal tissues, tissue fluids, blood, and milk. Glucose is the sugar of the blood and other body fluids, blood normally containing from 60 to 90 mg. of glucose in each 100 cc.

2. Chemical characteristics:

The carbohydrates are all compounds of carbon, hydrogen and oxygen. All simple sugars contain a free "ose" or sugar group, $\begin{array}{c} \text{O} \quad \text{H} \quad \text{O} \\ | \quad | \quad | \\ -\text{C} - \text{C} - \text{C}- \\ | \quad | \quad | \\ \text{H} \quad \text{H} \quad \text{H} \end{array}$ - while all the compound sugars, which are made up of simple sugar molecules, contain the "ose" or sugar group in combined form, though a number also possess one free sugar group - an important factor in determining many of the chemical properties of carbohydrates.

3. Classification of carbohydrates:

The name Saccharide is derived from the Greek



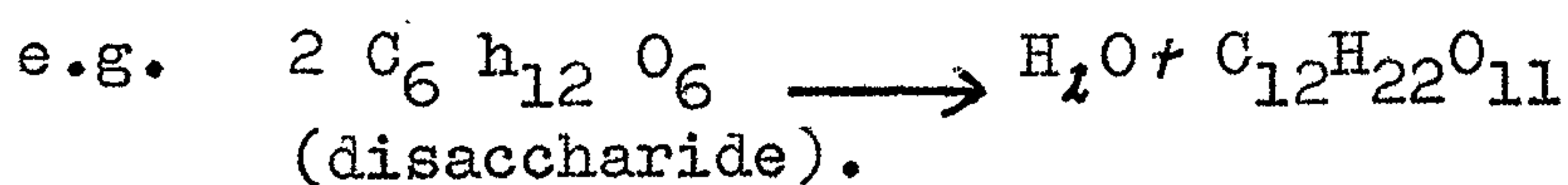
term Sakcharon, meaning sugar. Those carbohydrates such as glucose and fructose, which cannot be hydrolysed into simpler compounds, are referred to as simple sugars, or monosaccharides, or monosaccharoses, or simply monoses. Carbohydrates made up of two molecules of monosaccharide, e.g. sucrose, lactose, maltose, are named disaccharides, or disaccharoses, and similarly trisaccharides and tetrasaccharides are composed of three and four monosaccharide molecules. Polysaccharides are made up of many monosaccharide molecules.

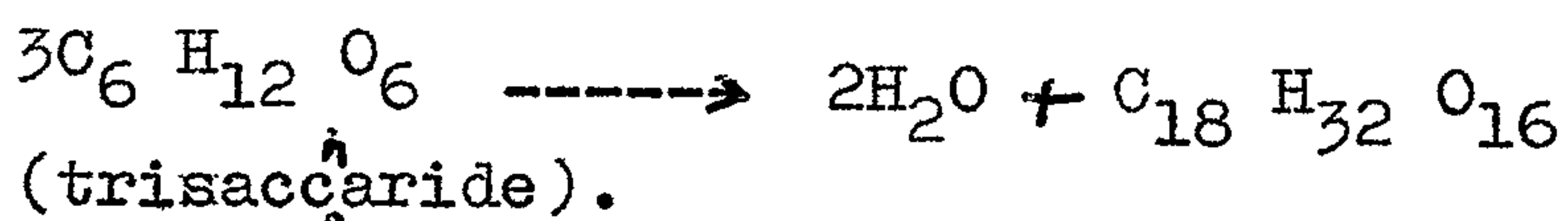
All these compound carbohydrates may be readily hydrolysed into their constituent monosaccharides by heating with dilute acids or by the action of specific enzymes.

Simple carbohydrates may not be hydrolysed into anything simpler, whereas compound carbohydrates are split by hydrolyses into their constituent simple carbohydrates :

(a). Simple sugars or monosaccharides
Those of this group are generally well crystallised solids, soluble in water; possess a sweet taste; reduce alkaline copper solutions; and give reactions characteristic of the free sugar group, such as with phenylhydrazine, hydrogen cyanide, and hydroxylamine. Included in this group are the aldoses and betoses.

(b). Compound carbohydrates or compound sugars
The compound carbohydrates are formed by condensation of two or more molecules of monosaccharide with the elimination of one molecule of H_2O for each linkage formed;





1. Oligosaccharides.

These substances are compound carbohydrates composed of only a few molecules of monosaccharides. The prefix "oligo" is from the Greek Oligos meaning "few". They are crystalline solids and soluble in water. Di-, tri- and tetrasaccharides are examples of this group.

2. Polysaccharides.

The molecules from these substances are very large, being composed of many (poly) molecules of monosaccharide. Generally they do not crystallise, are amorphous solids, are not sweet and, with few exceptions, do not reduce alkaline copper solutions or give other reactions of the free sugar group. Acids and enzymes hydrolyze polysaccharides and the general formulae of these substances may be represented by placing the formula of the constituent monosaccharide in parenthesis with "x" denoting the number of monosaccharide molecules, and subtracting hydrogen and oxygen equivalent to x-1 molecules of water; for example, if a polysaccharide is composed of a pentose, its formula may be written $(C_5H_{10}O_5) - (x-1)H_2O$

4. Metabolism

Carbohydrate metabolism in the animal body is essentially the metabolism of glucose and of substances related to glucose in their metabolic processes.

The digestion of food carbohydrates such as starch, sucrose and lactose produce the monosaccharides of glucose, fructose and galactose, the latter two being converted to glucose in the liver. The characteristic sugar of blood and tissue fluid is glucose.

More than half of the energy requirements of the body are derived from carbohydrate metabolism, particularly the brain in its entirety. The glycogen of liver, muscles, and other tissues is formed primarily from glucose, while much of the reserve fat in the body depots is formed from glucose in the liver. Glucose is the precursor of lactose formed by the mammary glands, as well as tissue glycolipids and glycoproteins. Again, the metabolism of many of the protein amino acids proceeds by the glucose pathway; thus it is apparent that glucose occupies the control position in carbohydrate metabolism.

The monosaccharides derived from the digestion of food are the primary materials for carbohydrate metabolism. The materials absorbed into the portal blood pass to the liver where fructose and galactose are converted to glucose and glycogen, and some of the amino acids are deaminized to form keto acids which are also converted to these carbohydrates. Lactic and pyruvic are always present in the blood as a result of metabolism in the muscles

and other tissues. These acids are continually being passed into the liver where they also are converted to glucose and glycogen. The numerous processes whereby the liver converts nonglucose substances into glucose constitute the gluconeogenetic mechanism of the liver.

Gluconeogenesis is increased on high protein diets when large amounts of aminoacids are absorbed into the blood, and decreased on high carbohydrate diets when there is an abundance of pre-formed glucose. It is increased during exercise when large amounts of lactic and pyruvic acids escape from the working muscles and there is the need to keep up the blood glucose and replenish the muscle glycogen supply during starvation. Gluconeogenesis from the amino acids of tissue protein which is the chief source of blood sugar and tissue glycogen. In diabetic states the rate of gluconeogenesis from both food and tissue protein may be greatly increased, contributing to body emaciation. The liver thus acts as a glucostatic mechanism to maintain the blood glucose within normal physiological limits.

Although both lactic and pyruvic acids are formed in the liver cells, none escapes into the blood, which is in direct contrast with muscle. This is due to the efficiency of re-conversion to glycogen in case the oxidation

of pyruvic acid cannot keep up with the rate of its formation in the glycolytic stage. Lactic acid is first converted to pyruvic acid in the liver, and the pyruvic acid is then converted to glycogen by reversal of the glycolytic reactions. These processes thus constitute the mechanism of gluconeogenesis from the lactic and pyruvic acids.

Muscle is limited essentially to blood glucose for its carbohydrate supply, and metabolism in muscle is highly specialised and designed primarily for the production of ATP as a source of energy for the contraction process.

The same phosphorylating reactions involved in the formation of liver glycogen are used, the glycogen yielding energy as A.T.P. through anaerobic glycolysis and oxidation of pyruvic acid in the tricarboxylic cycle.

In this case the reactions appear to proceed in an irreversible manner, particularly ^mmaximalian muscle, leading to an escape of the acids formed into the blood when the rate of their oxidation in the tricarboxylic cycle is less than the rate of formation in glycolytic breakdown. The rise in the blood level of these acids is particularly great under conditions of violent exercise, when the acids are formed much more rapidly than they can be oxidized. This conservation of the lactic and pyruvic acids from muscle is not entirely perfect since loss in urine becomes appreciable at high blood levels.

Pyruvic acid may be reversibly converted to lactic acid under the influence of the enzyme, lactic dehydrogenase.

In 1940 L. S. Fosdick reported that the process of degradation of carbohydrates in the mouth was the same or similar to that in muscle tissue during muscular activity and that, in the mouth, the series of enzymes probably were derived from the bacteria.

The Degradation of Starch:
 (From West and Todd (14) (page 982))

1. Starch (amylase) = maltose.
2. Maltose (maltase) = glucose.
3. Glucose plus adenosine triphosphate (i.e. ATP)(hexokinase) = glucose-6-phosphate and adenosine diphosphate (i.e. ADP).
4. Glucose-6-phosphate (phosphohexose isomerase) = fructose-6-phosphate.
5. Fructose-6-phosphate plus ATP (phosphohexokinase) = fructose-1-6-phosphate plus ADP.
6. Fructose-1-6-phosphate (aldolase) = dihydroxyacetone phosphate and 3-phosphoglyceric aldehyde (isomerase).
 Note: with isomerase, dihydroxyacetone phosphate and 3-phosphoglyceric aldehyde are in equilibrium.
7. 3-phosphoglyceric aldehyde plus H_3PO_4 = 1-3-diphosphoglyceric aldehyde.

8. 1-3-diphosphoglyceric aldehyde plus diphosphopyridine nucleotide (i.e. DPN) (dehydrogenase) = 1-3-diphosphoglyceric acid plus DPN . 2H.
9. 1-3-diphosphoglyceric acid plus ADP (phosphokinase) = 3-phosphoglyceric acid plus ATP.
10. 3-phosphoglyceric acid (phosphoglyceromutase) = 2-phosphoglyceric acid.
11. 2-phosphoglyceric acid - H_2O (enolase, Mg^{++}) = 2-phosphopyruvic acid (enol. form).
12. 2-phosphopyruvic acid-enol form plus ADP (phosphokinase, Mg^{++} K) = enol-pyruvic acid plus ATP.
13. Enolpyruvic acid (spontaneous) = ketopyruvic acid.
14. Ketopyruvic acid plus DPN.2H (dehydrogenase) = lactic acid plus DPN.

Note: 14 steps in all.

- * The parenthetic terms are the names of the enzymes and coenzymes which catalyze the reactions.

The Degradation of Fructose:

1. Fructose plus ATP (hexokinase) = fructose-6-phosphate.
2. Steps 5 through 14 of starch degradation follow, utilizing 12 steps in all.

The Degradation of Glycogen:

1. Glycogen (phosphorylase, adenylic acid, Mg^{++}) = glucose-1-phosphate.
2. Glucose-1-phosphate (phosphoglucomutase) = glucose-6-phosphate.
3. Steps 4 through 14 of starch degradation

follow, utilizing 13 steps in all.

Summary of Enzymes utilized in
degradation of starch to lactic acid:

<u>Step:</u>	<u>Enzyme:</u>	<u>Step:</u>	<u>Enzyme:</u>
1.	Amylase	8.	Dehydrogenase
2.	Maltase	9.	Phosphokinase
3.	Hexokinase	10.	Phosphoglycer- omutase
4.	Phosphohexose isomerase	11.	Enolase, Mg
5.	Phosphohexokinase	12.	Phosphokinase, Mg, K
6.	Aldolase	14.	Dehydrogenase

CHAPTER 2.DENTAL CARIESDefinition:

Orland, Blayney and Harrison⁽¹⁵⁾ give an extended descriptive definition of dental caries in graded form without aetiological reference.

"

1. Dental caries is a destructive disease of the teeth.
2. It commences on an external surface and progresses in a selectively penetrating pattern by attacking and disintegrating the several tissues comprising a tooth.
3. The resultant lesion is characterised in the enamel by a loss of translucency, superimposed discoloration, and cavitation. There occurs a distinctive lateral extension of the lesion at the ⁱⁿdentoenamel junction, with subsequent softening and cavitation in the dentin assuming a conical pattern with the apex toward the pulp.
4. Carious lesions developing in pits and fissures of the molars of man, the golden hamster, the white rat, and the cotton rat have a number of characteristics in common. There is the impact-ion of food particles into the natural occlusal crypts. In contact with this environment there is penetration of the enamel, the lateral spreading of the lesion at the juncture of the enamel and dentin, and with progression in dentin there may be a perceptible retraction of this tissue from the enamel. The disintegration of dentin to an amorphous^u, soft yellow to brown

leathery mass seems to be preceded by changes in the intact dentinal tubules. ^hspheroidal and elongated bodies considered to be bacteria, as well as certain amorphous forms, are discernible within the tubules in decreasing numbers, leading from the advancing front of the dentinal lesion toward the pulp. With further disintegration undermining the *cusps* and other sound tissue, secondary fracturing occurs with subsequent enlargement of the carious cavity and with eventual pulp involvement."

Chemico-Parasitic Theory:

G. J. Cox⁽¹¹⁾ in 1952 presents in summary form W. D. Miller's chemico-parasitic theory which was formulated from 1883 to 1890 following a two year study of an estimated 8,000 teeth.

- "1. Amylaceous or saccharine food and saliva generate acid.
2. There is constant formation of acid in the mouth because of retention of food in cracks, pits and fissures of teeth and between teeth.
3. The degree of acidity depends on length of time of exposure.
4. A cavity of decay containing amylaceous or saccharine food always has an acid reaction.
5. The extent to which any tooth suffers from the action of the acid depends upon the density and structure, but more particularly upon the perfection of the enamel and the protection of the neck of the tooth by healthy gums. What we might call the perfect tooth would resist indefinitely the same acid to which a tooth of opposite character would succumb in a few weeks."

6. An occasional neutral or alkaline reaction in a cavity does not mean that acid did not produce the cavity.
7. A systemic condition which withdraws lime salts from a tooth or lowers its density or weakens the union of organic and in-organic matter renders it more liable to decay.
8. Strong acid and corroding substances brought but momentarily into the human mouth may give rise to lesions of the enamel at points where the ordinary agents alone could never have begun.
9. In Vitro^f, caries simulating natural caries in macro details can be produced with acid mixtures such as are found in the mouth.
10. Carious dentin is decalcified roughly in reverse proportion to depth.
11. Destruction of organic material follows, not precedes, decalcification.
12. Mouth fungi do not act directly in decalcification. Further study of their acid producing activities is needed.
13. Fungi produce great changes in decalcified dentin and finally reduce it to a mass of debris and fungi.
14. "The invasion of the micro-organisms is always preceded by the extraction of the lime salts."
15. Destruction of organic material is accomplished by fungi.
16. "Inflammation can hardly be looked upon as a very important factor in caries of the teeth."
17. Caries of the enamel is purely chemical, the decalcification resulting at once in the complete dissolution of the tissue.

18. Caries of cementum runs a course similar to
that in the dentin. »

"Miller, in 1889, published the results of his researches in German, with an English edition in 1890. It would be difficult to find anywhere in his work a statement of the "Miller theory", certainly not in the form understood today. He did not, for example, believe that any one organism could by acid formation decalcify dentin, but believed rather that any acid-former, if supplied with carbohydrate available to its metabolic mechanism, would de-mineralize dentin. It is not clear that he believed that bacterial acids initiated caries, or that he considered a distinction between initiation and promotion of caries, but it is overwhelmingly evident that he believed that such acids were the immediate cause of decalcification once initiation had been effected. »

Cox, in his conclusions on P.323 :

- "3. There is no evidence that acidogenic bacteria and carbohydrates can initiate dental caries.
4. The progress of dental caries, once initiated, is effected by bacteria acting during the acidogenic phase on a carbohydrate substrate. Destruction of the organic matter of teeth is probably effected by proteolytic action by bacteria."

Proteolytic Theories:

Recently several theories have been presented, based on degradation of the organic matrix as the mechanism of the initial attack of the carious process, although

decalcification of the inorganic structures is conceded.

The main theory of this school of thought was promulgated by Gottlieb⁽¹⁷⁾ in 1947, with minor additions since, and depends principally on histologic evidence. In brief, according to his theory, caries is apparently a proteolytic process, progressing along organic pathways - the lamellae prism sheaths - and destroying them. A brownish-yellow pigmentation is present from the beginning and characterizes the process, which distinguishes natural caries from that produced by acid in vitro. He admits that acid action is a component of the process and it is shown in the radiograph, but the yellow pigmentation is not. Different groups of micro-organisms are apparently responsible for both components of the process for the two factors occur in varying proportions and their location is also variable.

The yellow pigment producing groups proceed faster along the lamellae, and may reach the dentine long before the acid-producers, who proceed better along the prism sheaths even in thick enamel, if the yellow pigment producers are present. By shrinkage of the dentine, they produce the second kind of undermining dental caries evident in the x-ray picture.

If the opening to the enamel is small shrinkage of the dentine creates a vacuum and dental lymph is drawn in and advanced caries develops from this focus. Arrested enamel caries is denoted by an extended brownish pigmentation on an intact

enamel surface with the dentine still roentgenologically intact.

The necrotic tissue in a carious cavity indicates the final stage of the caries process; the initial caries lesion is shown by the brownish yellow pigmentation of the tooth tissue in which all normal histologic characters are present.

A discoloured, depressed point indicates an invasion of lamellae, even if the X-ray picture is negative, while the belt-like extending pigmentation along the gingival margin remains shallow, seldom reaching dentine in thicker enamel layers. Such caries develops mostly on a base of chalky enamel and is a product of acid action and is not real caries. It produces almost regularly a barrier of transparent enamel, which stops bacterial invasion.

By using extracted teeth, soft enamel cannot be produced by acid action and the removed calcium salts in caries are apparently replaced by the bodies of the invading micro-organisms, and thus the protein content of the enamel is enriched.

Gottlieb suggests that some element of saliva may continuously impregnate the tooth, blocking the invasion roads and producing natural immunity since areas where the flow of saliva is impaired show a greater disposition to caries.

"Obstruction of open roads" is also the principle of chemicals which act to overcome the sensitivity of tooth surfaces.

He concludes his theory by agreeing that dental caries may be produced in extracted teeth since it may be identified by the yellow pigmentation.

A somewhat similar theory had been advanced by Frisbie, Nuckolas and Saunders⁽¹⁸⁾ in 1944. Their theory also is presented from studies of histologic observations, using their special technique for the isolation and examination of the organic matrix and its resulting degradation. However, the initial carious lesion on the surface of the enamel is conjecture, though it is probably associated with the formation of the bacterial plaque and the breakdown of the enamel cuticle; thereby uncovering the ends of the enamel rods and inter-rod matrix.

The penetration of this calcified homogeneous cuticle could possibly be due to acidogenic bacteria removing the more readily soluble inorganic constituents from this layer.

Acid action may, therefore, account for the opening up of the rods and inter-rod matrix for invasion by spheroidal Gram-positive micro-organisms.

Once the primary cuticle is destroyed they believe that the primary pathologic lesion is proteolysis of the matrix; the extension of this proteolytic process and liquefaction of the matrix frees the less soluble basic tricalcium phosphate from its organic bond, thus favouring its solution by products of acidogenic bacteria which secondarily penetrate along widening pathways of ingress.

The earliest pathologic change observed in the matrix is an increasingly intense acidophilic reaction to stains immediately underlying the surface plaque. From then on there is a progressive loss of structural detail in the enamel elements, and when advanced in development the matrix becomes almost homogeneous, the affected area eventually undergoing solution. In the early stages these areas are devoid of organisms, the process being proteolytic and is probably produced by extra-cellular enzymes.

Continued proteolysis results in the exposure of individual rods or groups of rods which have not as yet undergone so complete a change, and project beyond the area of liquefaction. In this manner the rod core and inter-rod matrix are opened up - thus establishing portals of entry for invasion by other organisms. Gram-positive organisms, spheroidal in form after penetrating the lysed surface matrix, advance deeply into the matrix well beyond the general area of decomposition.

Depending on the mode of ingress, the organisms are found either within the core of the rod or the inter-rod matrix, and changes in the staining reaction may be observed in the immediately surrounding matrix.

Superficial carious areas, other than those of active penetration, appear to be remarkably resistant to further solution, expansion of the cavity being purely a surface phenomenon. They regularly observed that such portions of the cavity be always in relationship to areas where the thread-like and mixed organisms predominate.

Following the opening up of the rod core and inter-rod matrix, there is an invasion of Gram-positive spheroid-shaped organisms. The process is observed to extend through the full thickness of the enamel, leading to the establishment of foci at the dentino-enamel junction. The pathways of penetration are narrow, irregular tracts and may resemble the classically described lamellae.

Using very high magnification and bacteriological stains, these tracts are shown to consist of one or several isolated rods whose cores are packed with organisms; Gram-positive spheroidal forms.

Invasion along the so-called lamellar-tracts, having reached the dentino-enamel junction, establishes a secondary focus of the carious process, with proteolytic changes extending laterally along the junction and opening up the dentrinal extremities of the rod and inter-rod matrix.

In the earlier stages the foci are small and the organisms found are exclusively Gram-positive and spheroidal in form. With expansion of the tract, secondary organisms of diverse morphology make their appearance with resulting cavitation and lysis of the matrix elements.

From such dentino-enamel foci retrograde spread into the overlying enamel matrix proceeds. The frontal areas of attack present the same picture as that observed in superficial caries.

Extensive caries is characterized by penetration of the matrix through the development of multiple irregular channels of dissemination. Each channel has its inception similar to superficial caries as

as described above. This process produces islands of healthy matrix by fusion of the spreading channels until the entire matrix is broken up.

As the breakdown nears completion, the process leads to rapid and extensive cavitation. The newly invaded periphery of these areas shows relatively few organisms, all of the spheroidal type, and the margins of the lesion are acidophilic in reaction. Secondary invaders present a variety of organisms of heterogeneous morphology and in great profusion.

They conclude that in caries the underlying organic structure is fundamentally related to the pathological process and offer as a working hypothesis that with the establishment of the lesion caries of the enamel is primarily a proteolysis of the organic matrix resulting from the enzymatic action of micro-organisms, followed by the subsequent dissociation of the inorganic constituents.

"With the establishment of the lesion"? - these authors surmise that superficial or initial caries may be due to acidogenic bacteria removing the more readily soluble homogeneous cuticle and hence concede the essential presence of acid. Only after the surface is invaded can proteolysis occur.

A very recent theory supporting the proteolysis theme has been offered by Martin, Schatz and Karlson⁽¹⁹⁾ in 1955 which combines keratinolysis with removal of the mineral of enamel by chelation complexes. The authors state their hypothesis in the following quotation :

"Unfortunately, there had been few and unsuccessful attempts to demonstrate that oral microorganisms are capable of breaking down keratin, a protein which is the major organic constituent in tooth enamel. From this it was generally concluded that keratin-digesting microbes are not present in the mouth and that enamel keratin is resistant to enzymatic attack. It is thus understandable why most attention has been focused on the acidogenic theory, despite excellent histological evidence supporting the proteolytic concept. Since tooth enamel and consists exclusively of mineralized/organic components, one has no choice but to concentrate on the former if there is no mechanism for breakdown of the latter. Consequently, the role of acids in dissolving apatite has received major study.

However, the application of new microbiological and biochemical techniques⁽²⁰⁻²¹⁾ has recently proved that keratin-digesting bacteria and molds do indeed occur in the mouth, and that the enamel organic matrix can be rapidly destroyed by these cultures. These experimental observations constitute microbiological and biochemical aspects which complement the fundamental histological work of Dr. Charles F. Bodecker⁽²²⁾. Moreover, various proteolytic and other enzymes, proteins, and protein breakdown products such as polypeptides are capable of forming water-soluble compounds (known as chelate or chelation complexes) with calcium.

It thus appears that the dual proteolysis-chelation reaction may cause breakdown of the enamel organic matrix and, at the same time, dissolve enamel apatite. The proteolysis-chelation theory may therefore account for destruction of both the organic as well as the inorganic constituents of enamel, simultaneously or in sequence. But this raises a question as to the role of acidogenic bacteria and acids in dental caries. Lactobacilli and other acid-producers are widely distributed throughout nature, but are particularly abundant where carbohydrates are available and where the amount of oxygen is partially or wholly reduced. These microbes are therefore to be expected members of the oral population; it would be most surprising if they were not present in the mouth. What then is their relationship and the function of acid? Throughout nature, proteolytic bacteria are generally more active under alkaline conditions; and throughout nature acid-producing organisms tend to control and inhibit proteolytic activity⁽²³⁾. The same relationship between these two groups of micro-organisms applies in the mouth just as it holds true in many other places.

There is another aspect that is of interest with respect to the etiology of dental caries. Bacteria become established in the mouth of the human immediately after birth and long before

tooth eruption. Since the mouth and nasopharynx is both a portal of entry and major reservoir of numerous pathogenic bacteria and viruses, and tooth cavity is potentially one of the most dangerous kinds of infections, especially during the early years of life when resistance is at a minimum. If lactobacilli or other acid-producing bacteria are responsible for tooth decay, then nature has planted "seeds of destruction" in the mouth and before the teeth appear. However, if proteolytic bacteria are the cause of dental caries, it follows that nature has established acid-producing bacteria as a defence factor in the mouth to protect the forthcoming teeth. They not only tend to check tooth decay, but also serve to eliminate a major portal of entry for serious disease producing germs during a period when the individual has minimum resistance to infection.

In this light, lactobacilli and acid-forming bacteria in general are viewed not as the cause of dental caries, but as an effort by nature to prevent tooth decay."

H. Eggers Lura⁽²⁴⁾ has also proposed a caries-hypothesis supporting the premise that the organic matter of the enamel is first attacked by caries and not the inorganic. To him the caries lesion is not a simple acid decalcification and removal of the inorganic salts, but rather a pathologic enzymatic action caused by general disturbances of the phosphorus metabolism and by local abnormal accumulation

of phosphates in suspect areas. It is proposed that caries is a disturbance of phosphate metabolism, a phosphorus deficiency in the diet causing an unbalance in the organic PO_4 - inorganic PO_4 equilibrium which is restored in the oral cavity by the provision of organic PO_4 from the matrix of the enamel. However, this presumes the presence of organic PO_4 ^{in the matrix} - an unsubstantiated fact.

Lura⁽²⁵⁾ goes further, saying that there are three non-acid mechanisms which, either singly or in co-operation, are able to dissolve the tooth enamel at neutral pH. These mechanisms are :

1. The bacterial keratinolysis which attacks the organic compounds of the enamel,
2. the bacterial synthetizing phosphatase effect which attacks the phosphate of the enamel,
3. the chelation mechanism which attacks the calcium and other metals of the enamel.

He concludes that all of these caries mechanisms can be activated by the presence of refined sugars.

A further supporting proteolytic theory is by Paul Pincus⁽²⁶⁾, but he limits his theory to explain pit and fissure caries only. In summary form, Pincus considers dentine protein contains a polysaccharide combined with organically bound sulphate. Enamel contains a similar ~~micro~~^{muco}protein which again contains sulphate.

In the presence of bacteria which contain the enzyme sulphatase, this sulphate can be liberated as sulphuric acid, which will at once combine with any calcium present to form calcium sulphate. Sound teeth contain organically bound sulphate, while carious lesions contain calcium sulphate. Bacteria from a

carious lesion, growing on a mixed medium which contains no glucose, can produce a caries-like attack on teeth. It is thus suggested that the teeth themselves contain the necessary substances to produce acid under bacterial attack and that a continual supply of fresh glucose from the exterior is not necessary.

Thus, both the chemico-parasitic theory, dealing principally with degradation of the inorganic tooth structure, and the opposing proteolytic theories of organic tooth destruction are presented.

H.B.G. Robinson⁽²⁷⁾, in Haslick's textbook on dental caries, expresses this rather forceful opinion :

"There is an opposing, or different, concept of dental caries based on the idea that in the enamel we have organic material chiefly in the interprismatic substance that is between the enamel rods, and it also forms so-called "lamellae" the proponents of the proteolytic theory say that caries progresses primarily in the organic pathways rather than the way that we have seen through the years. There is very little evidence, in my concept, to support this theory.

The proponents of this theory occasionally find a section that shows caries at the dentino-enamel junction with a lamella coming into it and no evidence of caries above it (toward the surface). That is their chief evidence, but it is very poor evidence because if sections are cut through the edges of the conelike

or pyramidal areas of caries, the same picture can be reproduced as an artifact. In order to prove this concept of the carious process it would be necessary to have serial sections so that we would know that there wasn't caries above the dentino-enamel junction. I just can't believe that this concept fits and the burden of proof lies on the individuals who want to substitute it for the decalcification theory."

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CHAPTER 3.RESEARCH - ANIMALSSuitability:

Most research has been carried out on animals mainly because of the ease lesions may be sectioned and studied, and also the speed animals reproduce.

"At the present time Albino rats, Cotton rats and Hamsters are used almost exclusively. These animals have a relatively high degree of susceptibility to the disease under laboratory conditions and are easy and inexpensive to maintain. Guinea pigs and rabbits have been used but found unsatisfactory because of their continuously growing molars. Dogs are quite resistant to dental decay under experimental conditions and are no longer used. White-tail rats and mice have recently been tried, but not enough information is available to predict their usefulness. Various species of monkeys would for many reasons be the animals of choice, but they are expensive to maintain and to date have been found to be relatively resistant to dental caries under experimental conditions.

The basic characteristics of lesions found in experimental animals appear to be similar to those seen in man. Such differences as these are appear to be related to the morphological characteristics of the dentition and anatomical peculiarities of the mouth."⁽²⁸⁾

Stephen and Harris⁽²⁹⁾ justify the study of dental caries in animals :

- (a) On the basis that it permits much greater scientific control of many variable factors than may be achieved with humans.

- (b). On the basis that the development of caries can be studied more quickly in animals than in humans.
- (c). On the basis that the use of animals allows experimentation with new drugs and procedures of questionable safety. They continue :

"In the past a common objection to the study of caries in rats was that coarse particles of food were required to initiate caries in their teeth. This objection was removed by Sagnals who demonstrated occlusal fissure caries in rats fed a fine particle diet.

Another objection ... only caries beginning in occlusal fissures of molar teeth could be commonly produced in these animals. This is no longer valid because Stephen in 1951 and McClure in 1952 demonstrated the similarity of the areas of localization of carious lesions to humans. In the past it has been considered a rule that the incisor teeth of rats would not develop caries. These teeth are normally in continuous eruption, and through attrition the upper and lower incisors usually wear each other down at a rate which practically equals the rate of eruption. Although the exposed portion of the incisor remains relatively unchanged in appearance, the incisor tooth substance exposed to the oral environment is continually being replaced and normally does not remain in the mouth long enough to be attacked by caries to a detectable extent. However, the exception which proves the

rule seems to be in the rare occurrence of caries of the incisor teeth of rats when there is a failure of an incisor to erupt continuously owing to some defect or injury, or when there is a failure of the incisors to wear themselves down through displacement or a lack of attrition, then there appears the possibility of having the incisor tooth substance remain in the oral environment for a sufficient time for definite caries lesions to develop. Under these circumstances caries of the incisors has been found in some of the animals."

Keyes⁽³⁰⁾ described the macroscopic character and distribution of lesions in hamsters maintained on a semi-purified high carbohydrate diet consisting of :

Whole wheat flour	...	20%
Corn starch	...	25%
Confectioner's sugar	..	20%
Whole powdered milk	...	30%
Alfalfa	...	5%

and observed the most frequent sequence of events in cavity development appeared to be :

- (a). Plaque accumulation.
- (b). Superficial yellow-brown pigmentary change in the cuticle and enamel.
- (c). Enamel penetration with opaque changes developing in zones of pigmentation.
- (d). Enamel undermining and dental invasion.
- (e). Enamel fragmentation.
- (f). Rapid and extensive cavitation.

Caries production in laboratory animals is determined by five major influences and in chronological order from the period of conception to function are as follows⁽³¹⁾:

1. Genetic history.
2. Developmental-nutritional background.
3. Salivary-dental inter-relationship.
4. Oral micro-organisms.
5. Oral food debris.

Sagnæs further adds :

"Few, if any, can be studied singly without due regard to incidental, but nevertheless important, overlapping variables."

1. Genetic History:

Several research centres now breed rats of two strains; caries-resistant and caries susceptible. A suitable method is well described by Hunt, Hoppert and Rosen⁽³²⁾ :

"The development of dental caries in rats is definitely dependant upon a number of environmental factors."

They commenced the experiment with 119 rats from three sources using the well known diet of Hoppert, Webber and Canniff. The animals were placed on this cariogenic diet when 35 days old and examined approximately every two weeks for macroscopic caries. Before this age, the same diet was used, but with the rice ground finer. Animals which showed a relatively high degree of resistance in the first generation were mated to produce foundation stock for a caries resistant line; other

individuals which showed caries relatively early were bred with each other to build a caries susceptible line.

Breeders in subsequent generations of both these lines had been selected not only on the basis of their own behaviour with respect to the time required for developing cavities in the lower molars, but also on the basis of the behaviour of their full brothers and sisters. Such a procedure thus constitutes a progeny test of the breeding pair that produced this family.

Their study continued and by 1955 more than 9,900 albino rats had been bred extending over the previous 17 years. Twenty five generations of susceptibles and 17 generations of resistants had been bred.

In the words of the authors, Hunt, Hoppert and Rosen :

"It will be noted at once that the susceptible line immediately showed a low average caries time (a measure of caries resistance equals the number of days elapsing from the date on which the animal was placed on the cariogenic diet to the date on which the first caries cavity could definitely be established in a lower molar) and has maintained that low average consistently since the second generation. The curve for the resistant stock presents a striking contrast. The averages of sibship means have steadily, though somewhat irregularly, increased."⁽³²⁾

Keller et al⁽³³⁾ is quoted as confirming these results from their studies of the caries incidence of molar teeth in the different strains. Caries was much less frequent in the upper-molar teeth than in the lower and the resistant animals were almost immune to caries in the upper molars.

Stewart, Hunt and Hoppert⁽³⁴⁾ showed that the genetic difference between the two strains - susceptible and resistant - is not specific for the Hoppert, Webber, Canniff diet. In their experiments both strains were fed a ration which contained a very high percentage of granulated beet sugar. The average caries time for the susceptible animals on this diet was 92 days and for the ~~the~~resistants 368 days. Thus the difference between the two lines was demonstrated on an entirely different diet.

Different strains of rat were used in the experiments of Stephan and Harris⁽³⁵⁾; the Sprague-Dawley, Holtzman and Osborn-Mendel strains, and they further support that it is genetic variation responsible for much of the experimental variation observed in the development of smooth surface caries in the rat.

It is misleading, however, to compare results of experiments using different strains of animals, as it is ^{animals} obtained from a parent laboratory and try to duplicate previous results.

Hartles, Lawton and Slack⁽³⁶⁾ experimenting using albino rats, descendants of 4 pairs from the

Harvard (U.S.A.) School in the production of caries on a 67% sucrose, highly purified diet, make these observations :

1. Caries was produced in six months as against 3/4 months in Harvard using descendants of four pairs from Harvard, both on a high sugar diet.
2. There is no obvious explanation why their rats should be less susceptible to caries than the parent colony.
3. Their results emphasize, however, the dangers of comparing findings of different laboratories since small changes in the diet or a change in environment may influence the susceptibility to dental caries.

More recently, Keyes⁽³⁷⁾ raises doubts on the authenticity of breeding caries-susceptible and caries-resistant strains of rats. In his experiment :

"Over 500 rats were examined in the survey of 7 different stocks of rats: Osborn-Mendel, Fischer, M520, Hunt resistant, Sprague-Dawley, M520X (A x C), and N.I.H. Black.

His conclusions were :

1. Almost all strains of rats produced at the National Institutes of Health develop active caries on occlusal and circumferential surfaces of the molar teeth when they are fed high carbonate, low fat rations. The incidence of lesions and the pattern of caries varied considerably for each type of rat and diet used.
2. Lesions can be placed into the following classifications :

sulcal (fissure type), proximal buccal, lingual

morsal and cervical or root surface; Sulcal lesions have been by far the most prevalent. In general, it has not been possible to predict a correlation between the incidence of one type of lesion and another.

3. An accurate appraisal of the caries pattern induced in rats fed the diets used in this study requires grinding or sectioning of the teeth so that sulcal and proximal caries can be diagnosed with a degree of sensitivity equal to that for lesions on buccolingual surfaces.

Keyes⁽³⁸⁾ continued his work using Syrian hamsters in a somewhat similar study demonstrating the need for more definite proof that a strain of animals is indeed caries-resistant for all diets and not only for particular diets.

"In the past three years, over 1,000 golden and 850 albino hamsters have been raised and studied. In the process of screening hamsters a series of 30 albino hamsters were placed on the caries test diet including confectionery sugar 59%, skim milk powder 27%, whole wheat flour 6%. No caries was observed until the period reached approximately 100 days, at which time lesions had developed in third molars.

An assumption, which later proved to be incorrect, was made that these animals might be used to establish a "resistant line". Therefore, several of the most inactive males and females were selected for breeding. When the offspring from these animals were examined after 21 days on the test diet, caries activity was unexpectedly high. Up to seven generations of albino hamsters

have now been bred and none of these animals has shown any loss of activity unless specially treated. In some cases we have bred the same female several times to the same male findings to be published in a subsequent paper strongly suggest that the explanation of the sudden change in "susceptibility" between the first animals tested and the subsequent generations produced from these animals was due to the establishment of a "cariogenic" flora in the alimentary canal of the females. This probably happened during the long exposure to test diet."

2. Developmental-nutritional Background:

Sagnaes(39) studied developmental influence of diet upon the caries susceptibility of three species of rodents - rats, hamsters, and mice - and writing in "Advances in Experimental Caries Research" as late as 1955, said⁽⁴⁰⁾:

"When a natural ration, laboratory ^{chow}~~chaw~~ was fed throughout the period of pregnancy and lactation and then to the offspring after they were weaned, the incidence of tooth decay was very low, regardless of whether the number of carious molars, the number of carious lesions, or the caries score was used as the criterion. However, when the natural diet was used throughout pregnancy and lactation and the purified diet was used after weaning, post-developmentally there was an appreciable increase in the incidence of dental caries. Since both groups had the same diet during tooth development, this could be described as a clear-cut difference between

two diets on a post-developmental or oral environmental basis If the purified diet was fed to the mothers throughout the pregnancy and lactation periods, then to the babies thereafter, an appreciable higher incidence of caries and caries score was observed. Thus the teeth of the offspring of female hamsters fed the purified diet throughout pregnancy and lactation were 3 to 4 times as caries susceptible as the teeth of the offspring of hamsters which were fed a natural diet throughout pregnancy and lactation."

A similar study on hamsters was conducted by Mitchell and Shafer⁽⁴¹⁾ and the same general trend was observed.

Volker⁽⁴²⁾ showed that the susceptibility to caries could be greatly increased in hamsters by transferring the animals to high carbohydrate diets at birth rather than at weaning time, as had been the customary procedure.

Again, Segnnaes⁽⁴³⁾ suggests that the erupting teeth are subject to a certain aging process or maturation which might have an important bearing on future caries susceptibility. He goes on by saying :

"In this process the excessive intake of sugar and other refined carbohydrates may operate through more than one mechanism :

1. by a process of replacement - that is, by interfering with the normal intake of nutrients essential for normal maturation, fluorides and other trace elements

included, and,

2. by retention on the tooth surfaces by interfering with the normal salivary relationship with the emerging teeth. Little is known about the latter mechanism whatever the mechanism by which excessive consumption of sweets and other refined carbohydrates affect the teeth, there is no reason to suggest that this mechanism is identical prior to, during and after eruption of the teeth."

With regard to the fully formed functioning teeth, it would appear that the effect is to a large extent a direct external one, acting primarily as a substrate for the micro-organism within the environment of the mouth prior to tooth eruption; on the other hand, it would appear that the effect is an indirect one - a replacement effect, as suggested previously. As the teeth pierce the gum and emerge into the mouth a third intermediary mechanism may be active. This mechanism is as yet poorly defined, but it appears to be related to the caries resistance of the teeth themselves, rather than to the direct injury of the oral micro-organisms, their substrate or their products."

Shaw, Ensfield and Wollman⁽⁴⁴⁾ experimented on 669 rats of the Harvard caries-susceptible strain to test the post-eruptive and developmental influence

of the supplementation of a cariogenic regimen with either milk, chocolate drink, chocolate milk, a mixture of milk, vanilla ice-cream, and cheddar cheese, or a mixture of chocolate milk, vanilla ice-cream, and cheddar cheese. The levels of supplementation, varied with the age of the experimental subject, and were chosen to approximate levels of human consumption. All supplements of dairy products caused major reductions in the incidence of dental caries when fed on a post-eruptive basis only.

None of the supplements caused any detectable influence on the development of the teeth to alter their caries susceptibility. When the supplements were provided continuously through both the developmental and post-eruptive periods, there was no demonstratable supplementary effect beyond the major influence caused by post eruptive supplementation along.

Shaw and Sagnnaes⁽⁴⁵⁾ completed a study whereby a salt mixture from the stock diet was added to the ^{which} purified diet/was used during the period of tooth development. This inorganic ash contained, in addition to adequate amounts of required minerals, various amounts of trace elements. Teeth developed and calcified under this regimen proved to be significantly more resistant to decay when exposed to the same post-eruptive diet previously used. Conversely, a high caries susceptibility was observed in teeth which developed during subsistence on the purified diet, which contained 67% sugar as the

carbohydrate fraction, but otherwise was complete in all known nutritional essentials. In this case the degree of purification of the diet is believed to be more important than the type of carbohydrate in producing the greater susceptibility to decay.

These authors conclude that an indirect nutritional effect may occur when highly refined and sweetened products occupy a large portion of the caloric intake so that certain trace elements occurring in natural foods are being replaced and this factor, or factors, takes on major significance after prolonged ingestion by the mother and offspring during, as well as after, pregnancy.

However, Watson and Muhler⁽⁴⁶⁾, experimenting with rats, found that there was no dental caries in any of the animals born to parents receiving high sucrose diets, and whose pups remained on it throughout the study. Their data differs absolutely from those of Sagnnaës which, as we have seen above, suggested that the caries incidence in those progeny from parents receiving a high carbohydrate diet is significantly increased. They offer by way of explanation that the only apparent difference in experiment design between their study and Sagnnaës is in the length of time the female rats received the sucrose diet prior to pregnancy. In Sagnnaë's study the animals were given the carbohydrate diet not only during pregnancy but for "some time" before conception, whereas in their study the animals were

on the high carbohydrate diet just before breeding.

Steinman and Haley⁽⁴⁷⁾, after administering carbohydrate solutions to rats commencing the day after birth and progressively later, observed that the earlier the drops of solutions were started the greater the number of teeth were totally destroyed. By varying the carbohydrate solutions, sucrose consistently affected the later caries experience of the teeth to a greater extent.

The conclusions of this experiment possibly involve the oral environment of the teeth as much as developmental factors in nutrition.

Steinman, Hardinge and Woods⁽⁴⁸⁾ fed diets representing the average American diet to their experimental rats composed of human foods as customarily prepared and eaten. The foods were thoroughly mixed to make a uniform moist mass of rations and fed to the animals ad libitum. They summarise their conclusions:

"The nutritional status and physical well-being of all animals showed an inverse correlation with incidence. It would appear that with these diets, good nutritional status is associated with a low caries incidence while nutritional deterioration is accompanied by an increase in caries."

The Syrian hamster was used in the experiments of Buxbaum, Kohn, Proutt and Oster⁽⁴⁹⁾ who found that animals maintained on a low carbohydrate diet

exhibited less caries than the animals maintained on a high carbohydrate diet, and on further analysis of the teeth showed that the former had a lower glycoprotein content in the enamel matrix. They maintain that their results indicate a positive relationship between the presence of glycoprotein in the teeth and the incidence of dental caries and, further, that this quantity varies with dietary factors prevailing during the time of tooth formation.

The effect of tooth maturity has recently been studied by Constant, Sievert, Phillips and Elvehjem⁽⁵⁰⁻⁵¹⁾ in their experiments with different minerals and fat in various diets on cotton rats. They conclude that withholding cariogenic diets until the teeth were more mature decreased the caries susceptibility of the teeth. Increasing the fat content even to 15% of a low calcium diet did not protect the teeth against high cariogenicity.

Shaw⁽⁵¹⁾ writing for the Journal of the American Medical Association states with emphasis :

"Every mature tooth has an inherent degree of proneness to decay that is dependent on its physical form, histologic structure, and chemical composition. These characteristics are determined during development as a result of the imposition of genetic and nutritional influences. Unquestionably, these characteristics result in individual variations in proneness to tooth decay ... that remain essentially unchanged through the the post developmental life of the tooth."

Bagnall⁽⁵²⁾ is sceptical of this concept of caries susceptibility influenced by a mechanism operating before tooth eruption and contests the previous experiments of Sagnals^e (45) on rodents, saying :

"It is hard to see how this new concept, if it is applied to man, fits into the well established observations that people on a primitive type of diet, who are relatively caries immune, rapidly lose this immunity when they come into contact with modern diets. If his hypothesis is correct, these people would still continue to be caries resistant, while their children would lose their immunity."

Post-Eruptive Effects Oral Environment

1. Carbohydrate Diet:

Orland⁽⁵⁴⁾ has produced two remarkable experiments which lead him to the following conclusions :

"It has become increasingly clear that development of carious lesions in the teeth must be a convergent biological phenomenon - one in which a number of variable factors must co-exist in the immediate environment of the tooth in order for caries to occur.

There are at least three such factors which can be stated in a rather definite manner :

- i. the resistance of the tooth must be below an optimal level,
- ii. a nutritive substrate for microbial growth must be available, and
- iii. the presence of certain micro-organisms is conjectured to be essential."

In arriving at his conclusions he fed white rats a caries-producing diet, but in such a way as

to completely bypass the oral cavity by means of a stomach tube made of polyethylene tubing. This idea had previously been used by Kite, Shaw and Segnnaes⁽⁵⁵⁾.

The animals used for these experiments had demonstrated a high degree of susceptibility to molar caries, being selected from those that had been maintained on a purified ration for the two previous generations. The stomach tubes were passed through oesophagus of recently weaned rats for rapid delivery of the aqueous suspension of the diet containing over 60% confectioner's sugar. Twenty to Fifty ml syringes connected via an adaptor to the anterior end of the tubing fed the animals, the suspension having a ratio of two parts diet to one part water. The rats received between 3 ml to 8 ml three times daily, the amounts varying as they reached maturity. However, in the final part of the experiment - lasting 16 to 25 weeks - one feeding of 15 ml and 12 ml was provided for male and female rats respectively.

Quoting Orland reviewing his findings :

"In the complete oral absence of food substrate, but not bacteria, no caries developed."

Shaw⁽⁵⁶⁾ also is definite in his thoughts on the importance of carbohydrate in the diet for the development of dental caries in the white rat. He declares :

"After intensive tests, carbohydrate-free and a low-carbohydrate ration were incapable

of producing tooth decay in a highly-carries susceptible strain of rats. Even in trials as long as one and two years after the rats were desalivated to increase the susceptibility to tooth decay no caries lesions developed."

Zepplin, Smith et al⁽⁵⁷⁾ studied dental caries in the cotton rat by feeding a natural diet comparable to a human diet. They found that a natural diet, containing foods used frequently in the human diet, and with only 17% sucrose (the proportion consumed on an average by the U.S. population), produced as many and as severe carious lesions in the cotton rat as did a basal cariogenic diet with 67% sucrose. Reducing the sucrose level of the diet to 0% or 2% reduced the occurrence of caries by 80% and 60% respectively.

Shaw⁽⁵⁸⁾ carried out a series of experiments comparing caries incidence in the white and cotton rat. He summarises his findings thus :

"Weanling caries susceptible albino rats were used as the experimental subjects to determine whether the initiation and development of carious lesions would be influenced by dietary procedures already demonstrated to alter the incidence of tooth decay in caries susceptible cotton rats.

The isocaloric substitution of fat and protein for part of the sucrose in the purified ration resulted in substantial reductions in the incidence of tooth decay. Administration of either mineralised fresh or

evaporated milk as the sole source of nutrients resulted in very low degrees of dental decay.

The addition of 10% sucrose by weight to either mineralised fresh or evaporated milk did not result in any appreciable increase in tooth decay above the average for the animals on the comparable milk diets alone.

Caloric restriction resulted in appreciably less decay than in the control rats which were allowed to eat ad libitum.

In each of the above results the influence of dietary variation on the initiation and development of carious lesions in the caries susceptible white rat is extremely comparable to the effect obtained with the same regimens in cotton rats.

In contrast, the type of caging arrangement employed throughout the experimental period was not found to influence decay in the white rat, unlike its strong influence in the cotton rat."

Phillips⁽⁵⁹⁾ in the Journal of the American Dietetic Association wrote :

"One of the factors contributing greatly to the development of dental caries is the carbohydrate portion of the diet. J.H. Shaw, in a private communication to the author, said :

"Compelling evidence has been compiled with experimental animals to show that

the carbohydrates in general and the mono- and the disaccharides in particular are responsible for the initiation and progression of the carious lesion. The degree of influence is closely dependent upon the form in which the carbohydrate is ingested and the rate at which it is cleared in the oral cavity."

Carbohydrate - Physical Characteristics

(a). Particle Size:

McClure(6) questions the identification of the aetiology of experimental rat caries produced by coarse particle diets or finely powdered diets usually containing a high % of sugar as the cariogenic agent.

"Both of these types of diets produce carious lesions which occur almost exclusively on occlusal surfaces and originate, apparently, with few exceptions, in the deep fissures. A most disconcerting feature of this type of induced experimental rat caries is the absence of carious lesions on buccal, lingual and proximal surfaces, whereas the same tooth surfaces in the human dentition are very susceptible to caries attack."

He experimented on rats using diets which did not depend on these two different physical properties for their cariogenicity and which would likewise produce carious lesions closely identified with human dental caries. This initial study pertained to a diet containing processed cereal foods together with a low content of refined sugar; then on to diets

containing dry skim milk powders and dry whey powders. The following results were obtained :

1.
"A diet containing four heat-processed cereal foods, i.e. corn grits, oat meal, white bread, and rye bread, and 18% cere-lose developed caries in white rats.
2. Diets containing roller and spray process skim milk powders also developed caries which severity and incidence seemed to parallel the severity of commercial heat processing of dry milk powders.
3. An additional heat treatment brought about by dry autoclaving the skim milk powders was responsible for an increase in cariogenicity of the milk powders.
4. Diets containing either a roller process or a spray process dry whey powder were responsible for the development of caries similar to that produced by the diets containing dry skim milk powder.
5. An additional heat treatment brought about by autoclaving was responsible for an additional cariogenicity of the roller whey powder, but not the spray process whey powder.
6. The caries developed by these diets included a large percentage of smooth surface lesions found mostly on lower teeth and on buccal surfaces. It bears a striking resemblance to human smooth surface caries.

7. Caries was developed by these diets without the presence of coarse particles or the presence of an excessive amount of sugar."

The question of particle size of the diet has by no means been solved, nor are results consistent.

Muhler⁽⁶¹⁾ studied the cariogenicity of various corn diets on rats and, although three diets with identical constituents, but containing different types of corn, were used they produced different caries pictures. The ones with the largest and smallest particles produced approximately the same degree of caries, the corn grits - intermediate in size between the two - were more cariogenic. Even increasing the amount of corn grits from 46% to 64%, at the expense of sucrose, did not increase caries.

Cox⁽⁶²⁾ in reviewing the literature up till then states emphatically :

"There can be no doubt that coarse cereal particles initiate lesions in rat molars that simulate human caries. This initiation is not dependent upon adequacy of the diet since by fine grinding of the maize or rice the initiating power of the ration is lost, with no change in the chemical composition."

Miller and Schlack⁽⁶³⁾, experimenting with two strains of rats, fed diets of two degrees of fineness and found that the number of

carious teeth, of carious areas, and a score representing the extent of the carious areas, were all significantly smaller after feeding a diet ground to pass a 48-mesh sieve than when the same diet was passed through a 28 mesh sieve. This seems to support Cox above, although Cox stresses the initiation of the lesion and so-called "fracture lesions" could account for the increase in surface lesions in the latter's work. Cox states that caries in the rat, once initiated, is accelerated in its development by fermentable carbohydrates; however, fermentable carbohydrates, either with or without added acidogenic bacteria, do not initiate caries in the rat.

Keyes⁽⁶⁴⁾ remains undecided after studying the distribution of lesions induced by high-carbohydrate low-fat diets in the molar teeth of rats. His results showed that the incidence of lesions and the pattern of caries varied considerably for each type of rat and diet used.

Finn, Klapper and Volker⁽⁶⁵⁾ consider that caries in hamsters more nearly approaches human caries, not only in the mode of production, but also as to the site of development of the lesions. These workers at the University of Alabama, U.S.A., after producing experimental caries in the hamster maintain that a suitable carbohydrate substrate is essential. In con-

trast to the experimental caries in the rat, fracturing of the cusps by coarse particles in the diet does not appear to be a potential variant that must be considered. They quote Arnold who, using a diet containing 66% corn starch, showed that powdered corn starch was more effective than larger corn particles in producing caries.

Much earlier in 1946 Keyes⁽⁶⁶⁾ had placed a group of hamsters on the Standard, Hoppert, Webber, Cannuff diet containing 60% corn meal which produced low caries scores. When one-third of this diet was replaced by sucrose, the caries score was over 5 times as great as produced on the standard diet alone, and over 50 times greater than that produced on whole yellow corn.

(b). Physical State:

The relative cariogenicity of sucrose when ingested in the solid form and in solution by the Albino rat was examined by Haldi, Wynn and Coworkers⁽⁶⁷⁾.

Using Albino rats of the Wistar strain, no carious lesions developed when they were fed entirely by stomach tube with a high sucrose diet which previously had been shown to be cariogenic if ingested orally. When all the components of this food mixture, except sugar, were fed by stomach tube and granulated sugar was ingested orally, the animals having been desalivated for the greater part of the experiment, there developed an appreciable number of carious areas.

However, when under the same experimental conditions, sugar was ingested in a 40% aqueous solution, the number of carious areas were markedly less than when the same amount of sugar was eaten in the solid form. Sugar in solution was therefore found in their experiments to be much less cariogenic than granulated sugar.

Constant, Phillips and Elvehjem⁽⁶⁸⁾ showed that when cotton rats were fed a diet of dry oatmeal, milk and sugar, 26 cavities were found per animal, but when the diet with the same composition and sugar content was fed in a liquid rather than a dry form only one cavity was found per animal.

Harris and Stephan⁽⁶⁹⁾ confirmed this observation in rats that sugar in a liquid form is not actively cariogenic when they found that the number of carious teeth per rat fell from 2.87 when the diet was dry to 1.16 with a small water addition, and to 0.37 with a large addition.

More recently, Davies and Bibby⁽⁷⁰⁾, using the Osborne-Mendel strain of rats, after placing them on a cariogenic diet until dental caries was detected in the occlusal surfaces of lower first or second molars, do not consider that there is any difference between the effect on the spread of caries of sucrose in aqueous solution as against using sucrose in solid form. However, when sucrose was fed in the form of a carbonated beverage and as a dilution of orange juice concentrate, dental caries

progressed at a significantly slower rate.

- (c). Natural or Processed Foodstuffs: (71) Constant, Phillips and Elevhjem, experimenting on cotton rats with a processed cereal diet of corn flakes and 30% sugar, produced more caries than 60% of sugar in an unprocessed corn diet, demonstrating that the manner of processing foods - and hence changing the physical characteristics - would influence caries incidence.

Further studies by these workers (72) on the stage of refinement of cereals with the cotton rat found that only fats gave partial protection against tooth decay when substituted for the "natural" fat of a cereal milk diet.

Bibby (73) quotes a previous work by J. K. Smith in 1948, that there is no greater caries production from refined sugar than natural sugars. Smith showed that natural syrup in a purified diet produced as much caries activity as sucrose when fed in a similar way. This laboratory result was confirmed by Constant et al (68) who summarised their finding thus :

"A significant difference between the cariogenicity of natural or refined sugars has not been demonstrated. The incorporation of natural sugar into various purified and neutral diets produced dental decay similar to that produced by refined sugars fed at equivalent levels."

- (d). Different Carbohydrates:
Shaw(74) and his co-workers, over a period of four years, showed that a high dental caries index resulted from feeding a purified diet containing a large proportion of simple sugars such as glucose, dextrose-maltose, fructose, maltose or lacto-sucrose, but a lower index from feeding starch or dextrin. They noted that the replacement of one half of the sucrose with fine dextrin did not reduce the severity of tooth decay as compared to animals which received the sucrose diets, while the replacement of $\frac{3}{4}$ of the sucrose with fine dextrin reduced the incidence and extent to a small degree. Partial caries protection was afforded cotton rats when 50 parts of dextrin and 17 parts of sucrose were fed in place of 67 parts of sucrose, and when dextrin was fed as the sole carbohydrate a very low incidence of carious lesions was noted.

Further studies on the effect of carbohydrates in diets containing added lard or increased amounts of protein indicated that starch and dextrin are the only carbohydrates tested that have a protective layer effect over the tooth, as opposed to sucrose, glucose, dextri-maltose, fructose, maltose or lactose which appeared to increase the severity of the lesions. When honey was fed to one group of animals as the source of carbohydrate, some small degree of protection was noted.

Hartles and Lawton(72) studied the incidence of caries in albino rats maintained on powdered

purified diets containing either 67% sucrose or 67% potato starch. They observed the effect of replacing the whole of the sucrose in the diet with potato starch and noted that there was a marked decrease in the incidence of dental caries - the number of carious teeth per animal reduced from 1.8 to 0.5.

Much earlier, Stephan⁽⁷⁶⁾ had pointed out that, of the carbohydrates which people commonly consume, the monosaccharides and disaccharides generally are fermentable by acidogenic oral micro-organisms, they rapidly lower the pH in plaques and carious lesions and they produce caries in rats and hamsters under suitable experimental conditions, whereas the polysaccharides, starch and dextrans, are not fermentable directly by acidogenic oral micro-organisms.

Cox and co-workers⁽⁷⁷⁾ emphasize that initiation and extension of the carious lesion are two separate and distinct processes and that the fermentable carbohydrates, sucrose and glucose, permit more rapid extension of existing cavities than does uncooked corn starch.

Finn, Klapper and Volker⁽⁷⁸⁾ refer to a previous work of Shafer, who compared the cariogenicity of diets containing 61% of the following :

a pabysaccharide (starch), a monosaccharide (glucose) and a disaccharide (sucrose). He found that starch played little or no significant role in the initiation of gross caries in the hamster. Glucose produced intermediate caries scores. Sucrose produced over twice as great destruction as did glucose. Their caries-producing potential was roughly proportional to their respective solubilities.

Shafer, however, did not contend that the solubility factor was directly related to the caries producing potential.

Orland, Hemmens and Harrison⁽⁷⁹⁾, experimenting on Syrian hamsters produced considerable caries on a 60% sucrose diet. When dextrin was substituted for the sucrose, the caries index was reduced by about 50%.

In 1953 and 1954 Klapper and Volker⁽⁸⁰⁻⁸¹⁾ gave the results of their experiments on hamsters. Firstly, they placed three groups of hamsters from 45 to 70 days of age, where salivary glands were removed or rendered nonfunctional, on two modified Happert, Webber, Canniff diets containing in order 66% sucrose and 66% corn starch, and on a commercial laboratory pellet diet. Those hamsters on the sucrose diet averaged 10.5 carious teeth with 13 lesions and an average score of 39.4 after 79 days on the diet. Those receiving the corn starch had an average of 8.6 carious teeth, 12.2 lesions, with an average caries score of 23.4 after 110 days on the diet. Those ingesting

the stock laboratory diet had individually 6.4 carious teeth, 9.2 lesions and a caries score of 14.6. Those hamsters on the starch and stock diets, presumably disaccharide in nature, had minimal to moderate caries in contrast to the sucrose diet. The moderate caries produced by the starch diet may be the result of stagnation of the starch so that hydrolysis might occur, either by the saliva from the minor salivary glands, or by bacterial enzymes.

Secondly, they placed desalivated hamsters on a basal carcinogenic diet supplemented with a different form of carbohydrate. The first, in the form of an alcohol (sorbitol), the second as a ketone (fructose) and the third in the form of an aldehyde (sucrose). Preliminary findings indicated that sorbitol and fructose produced only about half the amount of caries that was produced by sucrose, although fructose produced slightly more than did sorbitol.

(e). Oral Clearance Time

1. Adhesiveness of food:

Much more work has been done in this field on human subjects; the work by Bibby, Goldberg and Chen⁽⁸²⁾ being particularly well done. However, included in this section could be the previous studies on particle size, "natural" or processed food-stuffs and the method by which the processing was done; the use of different carbohydrates in the diet - stickiness as against a fibrous structure, solid or a liquid diet.

Saliva:

2. Saliva:

"The action of the saliva appears to be, in the main, one of cleansing the tooth surfaces by carrying away the carbohydrate substrate and preventing stagnation of the carbohydrates on the self-cleaning surfaces". (78)

Baxter and Muhler⁽⁸³⁾ studied the relationship of food consumption and salivary flow to the incidence of caries in the rat, and found that the absence of saliva was associated with a significant increase in caries. Muhler and co-workers⁽⁸⁴⁾ further studied the salivary flow, viscosity and pH in groups of rats at varying ages and found that flow was found to increase with advancing age, while both viscosity and pH decreased from weaning to adulthood. The sublingual gland, being the most significant for saliva viscosity - a lower viscosity associated with an increase in caries activity⁽⁸⁵⁾.

Schwartz, Resnich and Shaw⁽⁸⁶⁾ as a result of their experiments state that the removal of either the parotid or submaxillary glands resulted in a highly significant increase in dental caries incidence, as compared to the control intact animals. The removal of the major sublingual or the extra-orbital lacrimal glands did not produce significant increases in caries incidence. They⁽⁸⁷⁾ had previously studied the effect of selective desalivation

on the dental caries incidence of albino rats which led them to conclude that the parotid glands were the most important of the salivary glands in relation to caries resistance in the white rat.

Quantitatively the parotid secretion is over 50% of saliva and so exerts a major influence on cleansing the teeth of carbohydrate substrate.

Sortino⁽⁸⁸⁾ produced caries in three generations of rats, studied the decrease in the rate of flow of the saliva associated with the diminished rate of degradation of thyroid hormone and noted a sharp increase in caries with the decreased flow.

Rosen et al⁽⁸⁹⁾ recently experimenting with salivariadenectomized rats in a study of oral clearance summarise their results :

"Since a salivariadenectomized rat spent more time in feeding than unoperated controls, an experiment was designed so that the feeding time, as well as the amount of food consumed, was approximately the same for the operated and normal rats.

Rats were fed so that the above two conditions were the same, with the results that caries time for salivariadenectomized rats was less than that of unoperated controls. Lactobacilli, but not other groups or micro-organisms, were recovered in higher numbers

consistently from salivariadenectomized than from unoperated control rats."

There is some evidence that saliva has some effect on tooth structure which adds to caries resistance. Fanning, Shaw and Sognnaes⁽⁹⁰⁾ found that the amount of caries occurring in rats increased proportionately to the length of time the animals had been deprived of their major salivary glands prior to being placed on a cariogenic diet.

"However, other salivary influences such as its buffering action, ammonium content, enzymatic activity, and anti-bacterial properties, should not be overlooked."⁽⁷⁸⁾

(f). The Oral Flora:

Williams (91), after studying the effect of sugars and other carbohydrates on the oral flora, concludes thus :

"The effect of carbohydrates on populations of bacteria in the oral cavity is not known completely. It is probable that carbohydrates in the concentration usually put into the mouth, do not directly affect the micro-organisms. Thus any possible effect is dependent on how the micro-organisms act on the carbohydrates.

Carbohydrates are extremely important in the metabolic activities of the oral flora, as well as all other micro-organisms :

1. They serve as the chief source of energy for cell functions, and

2. They are the principal, if not the only, source of carbon for synthesis of cell substances. Monosaccharides such as glucose, disaccharides such as sucrose and fructose, and polysaccharides such as starch are intermittently available in the oral cavity from remnants of food or liquids. Polysaccharides, other than starch, are present continuously in the oral cavity, but since they are attached to proteins in mucin, dead tissue and bacterial cells, enzymatic degradation of mucin (a mucopolysaccharide) and utilization of the by-products of the polysaccharide moiety which includes glucose, have been described by Knox⁽⁹²⁾."

Orland et al⁽⁹³⁾ made another major contribution to research in recent years using a germ free technique for their studies of dental caries. Their procedure used a total of 22 white rats (*Rattus norvegicus albinus*) which were reared in R.G.F. units - containers having a series of air filter tubes and a sterile transfer lock attached on the front side, a glass viewing port was at the top, and on the back were attached long rubber gloves which permitted manipulation within a germfree enclosure without contamination - and maintained in a germfree environment.

The animals ingested a laboratory prepared, steam sterilized diet, which in a normal

conventional rat would produce abundant tooth decay. The animals ranged in age from 163 to 256 days and were fed the caries-producing diet for 137 to 236 days respectively. A control group of 39 rats living under conventional conditions, which included the usual bacterial flora, ingested the same caries-producing diet for 137 to 240 days while aged 158 to 260 days respectively. The diet included casein polished rice, yeast extract, and starch, as well as essential dietary factors for the animals. In addition to the solid portion, a 5% sucrose solution in distilled water was fed ad libitum because sugar in a solid state would caramelize if steam sterilized.

Food and small equipment entered or left the internal environment by means of an attached sterile lock wherein all items were rendered sterile by steam under pressure. Air was filtered back both ways and a positive pressure was always maintained on the inside over that of atmospheric pressure on the outside of the germfree rearing unit.

Sterility tests were conducted on a variety of specimens taken from within the unit - from the animals, food, faeces, urine and equipment. In the words of these authors :

"No viable micro-organisms were ever found in the environment of the animals studied."

At the end of the time period almost every one

of the two groups of conventional control rats showed gross dental decay. Specimens were prepared from the germ-free animals and examined for signs of dental decay, using a thorough macroscopic and histologic techniques. The results these workers obtained :

"In marked contrast to these conventional rats with their usual microbial flora, the twenty-two rats reared and maintained entirely free of all discernible microbial life, but ingesting the same diet, showed the complete absence of any carious lesions as determined by the same microscopic methods."

From the two experiments of Orland and co-workers - using a germ-free environment and stomach tube feeding so as to bypass the oral cavity - one may deduce from this negative evidence that both oral food debris and bacteria must be essential for caries to occur in the rat.

Various bacteria have been associated with the aetiology of dental caries and lactobacilli strongly favoured.

Rosen, Hunt and Hoppert⁽⁹⁴⁾ studied the frequencies of oral lactobacilli in the Hunt-Hoppert caries-resistant and caries-susceptible rats and recovered lactobacilli more frequently and in greater numbers from the caries susceptible. The percentage of resistant animals from which lactobacilli were recovered were high when the rats were very young, but after the animals were 50

days old, lactobacilli were recovered from approximately 20% of the examinations.

Lilienthal⁽⁹⁵⁾ is convinced that oral lactobacilli are not aetiological agents in dental caries and among other reasons cites further experiments of Orland and co-workers⁽⁹⁶⁾ where caries can be produced in germ free rats by the introduction of an acidogenic organism, enterocci, and a high carbohydrate diet where, in this instance, caries is produced without lactobacilli being present in the oral cavity.

Ragosa et al⁽⁹⁷⁾, using hamsters, found no positive relationship between salivary Streptococci and dental caries in either male or female hamsters. No correlation was observed between salivary lactobacilli and dental caries in females. A positive relationship was found between lactobacilli and dental caries, but only after very extensive development of lesions. However, the final mean caries scores of both male and females in their study were not significantly different.

"For almost three-quarters of a century, since the human animal was first cognizant of the existence of micro-organisms as agents of disease in his own tissues and organs, bacteria of one type or another have been ascribed as the specific

or probable cause of tooth decay. As to which bacteria are capable of inducing dental caries is still too vast an area to consider in any definite sense."⁽⁹⁸⁾.

CHAPTER 4.RESEARCH - HUMANS1. Sweden:

"Dental caries is one of the greatest medico-social problems of today, as it affects such a high percentage of the population and shows a tendency to increase with the standard of living."(99)

Thus begins a very comprehensive study carried out by Gustafsson and co-workers in Sweden from 1945 to 1951 in an effort to "yield direct information about the effect of various factors on caries."

This study, the "Vipeholm Dental Caries Study", in its relation to carbohydrate will now be described.

The authors feel that long-term studies on human beings seem to be the only method capable of answering the question of causal relationship between carbohydrate intake and dental caries. The action or effect of the experimental variable on dental caries activity must therefore be assessed by continuous direct determination of the number of new carious lesions of the teeth, per unit of time. This is the only satisfactory method available for measuring caries activity in a scientific investigation until an indirect method has been devised and found satisfactory,

i.e. capable of reflecting clinical dental activity in detail. The high consumption of refined sugar in certain countries in which the frequency of dental caries is also high, suggests the possible importance of sugar as a causal factor.

The purpose of their examination was to find answers to the following questions :

- A. Whether and, if so, how caries activity, as studied under controlled conditions, is influenced :
- (a). by the ingestion, at meals, of refined sugar with only a slight tendency to be retained in the mouth (non-sticky form),
 - (b). by the ingestion, at meals, of sugar with a strong tendency to be retained in the mouth (sticky form, sugar-rich bread),
 - (c). by the ingestion, between meals, of sugar with a strong tendency to be retained in the mouth (sticky form, sweets, etc.).
- B. Whether and, if so, how dental caries activity is influenced by the omission of a variable proved to be capable of increasing caries activity.
- C. Will any new carious defects arise if the consumption of sugar is reduced as far as practically possible?

General considerations on the planning of the investigation:

An institution for the mentally deficient was chosen since the patients, due to their condition, might be expected to remain for a long time, considered so necessary.

After a preliminary registration of the patients' dental status, those considered suitable from a medical and an odontological viewpoint were chosen to be included in the investigation. The subjects were then examined dentally at regular intervals and at least once a year. On the basis of these examinations, numbered here as first to ninth, dental caries activity was assessed.

For a certain period all patients of the entire institute received the same basic diet which, judged by accepted standards, was adequate in all respects, except regarding calories. To the basic diet of every experimental group was added various differing supplements which constituted the experimental variables.

The first year of the investigation beginning in 1945 was a preliminary and adjustment period, the next 18 months various vitamins and mineral substances were studied for their effect on caries in different groups. The caries activity in this series was found to be low and without any significant differences between the groups.

The period from 1947 to 1949 referred to as Carbohydrate Study 1 was of investigation,

including an initial stage during which the experimental conditions should represent extremes as far as the carbohydrate consumption is concerned. The amount of refined sugar used in the preparation of the basic diet was kept to a minimum, but the diet was not "sugar free" due to the natural sugar content of the foodstuffs.

In some groups the subjects received nothing to eat between meals, in others they received a certain amount of sweets which they were allowed to eat when they liked. The sweet ration varied between groups.

In other groups the subjects received a supplement of refined sugar at meals which brought the total sugar ration up to about twice as much as the daily consumption of sugar per head of the Swedish population.

Some groups also received sugar-rich bread, while others received only the basic diet plus fat to bring up the caloric level.

Carbohydrate Study 11 was the title given to the experiments lasting from 1949 to 1951. The basic diet was made to resemble that of an ordinary Swedish household, the amount of sugar used per day in the preparation of the food corresponding roughly to the average daily household consumption per head of the Swedish population. Between meals eating was forbidden except in those groups where sweets were offered to the patients between meals in such amounts that the total consumption

of refined sugar did not exceed the average Swedish consumption. One group received sugar dissolved in the beverages they drank at meals, while another group received only the basic diet plus fat to make up for the calories. Finally, some patients received sugar-rich bread instead of the ordinary bread of the basic diet.

Thus, in both of the studies, four essentially different types of groups were represented, so that the relationship between carbohydrate intake and caries could be studied from the following viewpoints :

- (1). Basic diet without additional carbohydrates but with supplementary fat to bring up the caloric level. Observations made in this group elucidate the relationship between the basic diet and dental caries activity. (Control group).
- (2). Basic diet with additional sugar in solution (not sticky form at meals). The amount of sucrose is somewhat larger than in any of the other groups. Observations made in this group elucidate the relationship between the total sugar intake and dental caries activity. (Sucrose group).
- (3). Basic diet plus addition of sugar in bread (sticky form) consumed at meals. Observations made in this group elucidate the relationship between dental caries activity and the retention of sugar consumed in low

concentration at meals. (Bread group).

- (4). Basic diet plus addition of sugar in the form of sweets (sticky form) consumed between meals. Observations made in this group elucidate the relationship between caries activity and sugar retention, between meals, from preparations containing sugar in high concentration (chocolate, caramel, 8-toffee, 24-toffee groups).

Material:

The Vipeholm Hospital receives patients from all parts of Sweden and so the selection is geographically representative of the entire country. The inmates are not allowed outside except under supervision, and during the daytime patients were under continuous observation so that the dietary regime was adhered to by the different groups. The fluoride content of the water measured from 0.7 to 0.2 p.p.m. over 8 determinations from 1945. Only those patients in the same groups at the commencement and the end of the study (436, 355 males, 81 females) - the constant material - are included in this account. The subjects were willing, showed no signs of any disease requiring dietary measures, and had a minimum of 10 teeth. The males' average age was 31.9 years, females 30.5 years. The ability of the patients to sit at a table and eat without help varied widely and the personnel often had to help them. Of the entire series, 82 subjects brushed their teeth regularly, while the nurses brushed the patients' teeth in the female bread group, thus explaining their better oral hygiene.

Methods:

Records were made of the number of decayed teeth, the surfaces involved, the number of teeth extracted, not erupted or retained and only primary caries was included, i.e. :

1. Macroscopic defects of the enamel, dentine cementum as did not show characteristics of hyperplasia^{s a} or erosion.
2. Caries without defect (precaries) visible decalcified spots of enamel that had lost their normal translucence and assumed a chalk-like consistence.
3. Pit and fissure caries, when the probe "caught".
4. Roentgen caries, well defined decalcified areas of the proximal surfaces in the X-ray film which could not be verified as cavities on inspection with mirror and probe.

The lesions thus observed were classed according to the Scandinavian mosaic^Mlage system, devised in accordance with suggestions made by Western (1940), Dahlberg (1940) and Lindstrom (1940). According to this method, a cavity is compared with respect to position, depth and extent with models of teeth with cavities, each of which has been made on the basis of a large number of observations of all types of caries lesions. Every type of caries is represented by a series of models containing cavities of varying size and depth. Every lesion has its own size and type number. With this system then it is possible to describe :

- i. The position, type and size of any cavity at a given time,
- ii. The number of cavities occurring between two consecutive examinations,
- iii. Any increase in the size of the cavities recorded earlier.

This method is superior to simply recording whether or not a tooth or dental surface is decayed.

Carbohydrate Study 1:

The caries activity was high in groups which received sugar in sticky form between meals. In the control, sucrose and bread groups the activity was low.

The authors remark on the development in the control group and the later 8-toffee group, i.e. the groups that received the basic diet plus 150 grams of margarine. During this period the caries activity was low in the group with the older patient material, with a mean value of 0.12 and 0.07 new carious surfaces per person. The younger group had 0.80 new carious surfaces per person. Thus the activity did not drop to nil, despite the fact that the patients had received a diet as sugar-free as was practically possible, and a large additional fat ration.

Although the patients in the sucrose group received 300 grams of sugar per day, caries activity did not distinctly increase in this group, or in the later chocolate group, which during Carbohydrate Study 1, had lived under

the same conditions.

In the 24-toffee group, 8-toffee group, and caramel group an increase was observed in the mean caries activity. This increase started immediately after introduction of the experimental conditions. When initial stages are not recorded as caries, there will be a retardation in the increase in dental caries activity. It will be much higher during the second year than during the first.

Carbohydrate Study 11:

During this period, caries activity in the control and sucrose groups was on the whole on the same level as during carbohydrate study 1. In the bread groups there was a certain increase during the second year of the period.

In the 24-toffee group, the activity returned to the same low level as during the Vitamin Study in connection with the withdrawal of the addition (and the replacement with fat). This also applies to the caramel group, for which the change occurred during Carbohydrate study 11.

The 8-toffee group persisted at roughly the same activity level as during Carbohydrate Study 1. In the chocolate group there was a moderate increase in caries activity on the introduction of chocolate.

The frequency of caries, based on the D.N.F. system also revealed a higher caries activity when the sugar was given between meals in a form with a strong tendency to be retained. Curves plotted on this data recovered their usual slope as soon as the consumption of sweets

between meals was stopped, as was the case in the 24-toffee group and the caramel group.

In order to give a more correct statistical analysis of the experimental results, complex statistical methods were employed showing intra-group and inter-group comparisons.

A. Intra-group comparisons

(1). Control Group:

This group of 60 males, average age 34.9 years, after elimination of practically all refined sugar from the diet, maximum restriction of natural sugar in foodstuffs, and reduced carbohydrate ration to about half the ordinary Swedish standard, rendered the diet caloric poor, and so the patients received an additional ration of 150 g. margarine per day to bring up the caloric level. The reduced caries activity thus may be explained by reduction of carbohydrate diet or an increased fat ration, which raises doubts as to the use of this group as a control. Caries activity was depressed to practically nil.

After two years this diet was replaced by an ordinary diet with a ration, at meals, of refined sugar corresponding to what may be regarded as the average Swedish household consumption in 1948. This change produced a small increase or decrease in caries activity, depend-

ing on the withdrawal or addition of fat to supplement the diet. Nothing was given to the subjects between meals.

(2). The Sucrose Group:

A group of 57 male patients, average age 34.7 years, who had for 1½ years been on an ordinary Swedish diet and who had received 1 mg. fluorine daily in the form of a tablet, were placed on a diet in which the major part of the carbohydrate ration was consumed in the form of refined sugar, mainly in solution at meals, the amount of sugar representing about twice the average total Swedish consumption. After a further two years the distribution, but not the quantity, of the carbohydrates was again changed so that the amount of refined sugar consumed at meals was slightly more than the average Swedish consumption. During these study periods all consumption of food between meals was forbidden. These changes in the distribution of the carbohydrates produced no statistically significant changes in caries activity.

(3). Bread Groups:

Forty-one males and forty-two females, average age 30.4 and 28.0 years respectively, received sweet bread at one meal

every day for two years; the consumption of this bread did not produce a demonstratable increase in caries activity. During the following two years the same sort of sweet bread was served at all meals (breakfast, dinner, afternoon coffee and supper). During the second year an increase was observed in the mean caries activity. The increase recorded was statistically significant for the males, but not for the females. The female group differed from the male group in that their oral hygiene was better and, secondly, they did not eat so much of their ration. Consumption of food between meals was forbidden.

- (4). The Chocolate Group:
This group of 47 male patients with an average age of 29.1 years, received a diet containing an ordinary amount of carbohydrates, but an amount of sugar corresponding to twice the average Swedish consumption. Caries activity, which was known to be fairly low before the commencement of the study, remained low. The following three years the patients were placed on an ordinary Swedish diet and received a daily ration of 65 g. milk chocolate, which they ate between meals.

This change in the experimental conditions was accompanied by a statistically significant increase in caries activity. However, another change in the diet was made when the chocolate consumption started. An extended analysis was made, therefore, of all patients in the chocolate group. This analysis comprised also these patients not included in the main material. The correlation between the consumption of 65 g. chocolate per person per day between the meals was studied in 87 patients for 3 years. At the same time 95 patients were studied in the control group. It was shown in the under 30 year old patients that the consumption of chocolate was accompanied by a dental caries activity three times greater than before. This difference was found between the periods with and without chocolate consumption in the same individuals as well as between the chocolate and control group. In older individuals (over 30 years old) the increase was insignificant.

In a parallel investigation on a group of the personnel (mean age 34 years) at the hospital, no difference could be observed between a group who ate 54 g. chocolate daily and a control group who ate no chocolate. Experimental control of these last groups, however, was unsatisfactory.

- (5). The Caramel Group:
Sixty-two males, average 35.6 years, were given 155 g. (= 70 g. sugar) of sweets between meals and this consumption was accompanied by a statistically significant increase in caries activity. On withdrawal of the caramel ration without any reduction in carbohydrates, caries activity diminished to its previous level. The decrease occurred while the patients were on a dietary regime corresponding to ordinary Swedish standard diet with a consumption of refined sugar at meals corresponding to the household consumption.
- (6). The 8-toffee Group:
Forty males, average age 26.3 years, at the commencement of the study were given a low-carbohydrate, high-fat diet for one year. Practically no refined sugar was used in the preparation of the food. This diet had no demonstrable effect on caries activity, which was low from the beginning. With the patients on the same basic diet, but with 8 toffees served between meals for three years, the caries activity increased. This increase was noted during the first year. When calculated on the basis of definite cavities, the caries activity showed a maximum during the third year. During the last two years of the study period the patients were on an ordinary

Swedish diet, including an amount of sugar corresponding to the average Swedish consumption.

- (7). The 24-Toffee Groups:
Forty-eight males and thirty-nine females, average age 31.0 and 31.1 years respectively, at the beginning of the study received the amount of sugar present in the ordinary Swedish diet. During the following two years the total carbohydrate consumption remained unchanged, but the proportion between the different carbohydrates was varied, so that they received refined sugar in a quantity equal to twice the average consumption of the daily ration of carbohydrates 160 g. was given as toffees, which was consumed mainly between meals. During the two subsequent years the total carbohydrate consumption remained unchanged, but then the patients received refined sugar at meals and in a quantity corresponding to the average household consumption in 1948. During these last two years all consumption of any food between meals was forbidden. These changes in the carbohydrate distribution were accompanied first by a marked increase in caries activity, i.e. during the time the patients were offered toffees, and by a marked fall to the original level on withdrawal of the toffee ration.

The loss of surfaces because of enlargement of existent cavities did not significantly increase in association with the increase of caries activity.

Discussion of Material Suitability:

The high average age, the fairly low caries frequency observed at the beginning of the trial, the poor oral hygiene, and the habit of some of the patients to swallow the sweets without chewing them, as well as the mental deficiency of the series, make it difficult to judge the caries susceptibility of the patients.

Age as such, in the authors' opinion, is the decisive factor and the other factors only have a modifying influence. In view of the high average age of the patients it is, therefore, highly probable that their resistance to caries was greater than in young non-institutionalised people.

This infers that any variable producing an increase in caries activity in these patients will more than probably do so in a normal younger population.

Also, any of the factors that produced only a slight or doubtful increase in caries activity in the patients, or an increase that could not be demonstrated with certainty owing to the sources of error of the method and to the smallness of the groups, might possibly cause a more distinct tendency or even statistically significant increase in caries activity in ordinary young people.

Summary of the Vipeholm Investigation:

The evaluation of the effect of the sugar intake on dental caries is not only :

- (a). the quantity of sugar consumed, but also
- (b). the form in which it is served, and
- (d). whether it is consumed at meals or between meals.

That this is so was apparent in the Chocolate Group in which a reduction in the total sugar consumption was followed by an increase in caries activity when the subjects received a small portion of the reduced amount of sugar between meals.

The authors, at the end of their experiments, suggest the following conclusions :

1. The consumption of sugar can increase caries activity.
2. The risk of sugar increasing caries activity is great if the sugar is consumed in a form with a strong tendency to be retained on the surfaces of the teeth.
3. The risk of sugar increasing caries activity is greatest if the sugar is consumed between meals and in a form in which the tendency to be retained on the surfaces of the teeth is pronounced with a transiently high concentration of sugar on these surfaces.
4. The increase in caries activity under uniform experimental conditions varies widely from one person to another.
5. Increase in caries activity due to the intake of sugar rich foodstuffs consumed in a manner favouring caries disappears on withdrawal of such foodstuffs from the diet.

6. Carious lesions may continue to appear despite the avoidance of refined sugar, maximum restriction of natural sugars and total dietary carbohydrates.

2. England
King, Mellanby and co-workers⁽¹⁰⁰⁾ carried out three experiments in the London, Liverpool and Sheffield areas of England to test the effect on the initiation and spread of caries of different amounts of sugar taken as ordinarily consumed in the diet. Their studies were limited to children of various institutions and included both deciduous and permanent teeth, although deciduous teeth predominated. The children's age ranged from 2 - 14 years and were receiving basal diets of good nutritional quality.

In each study there were control and test groups, the latter receiving additional sugar as part of the diet. In London, both white (refined) and brown (unrefined and semi-refined) sugars were tested, whereas in Liverpool and Sheffield only the white variety was used. It was found that many of the London children could not tolerate over a long period a total intake from all sources of more than approximately 22% of sugar per week, a figure which was slightly higher than the estimated pre-war consumption for this young group. It was, therefore, arranged that the control group should have 11 oz., which was rather less than their war-time and early post-war amount, and that the high sugar groups should have the

22 oz. which they could manage without difficulty. In Liverpool and Sheffield, where the children were older, the test was so planned that the control groups should have the total amount then current in the homes, while the test groups had an additional 22 oz., bringing their total to 37 oz. in Liverpool and $41\frac{1}{2}$ to $45\frac{1}{2}$ oz. in Sheffield - up to or above the estimated appropriate pre-war level.

In the London study, infants 2 - 4 years old, all deciduous teeth present at the first inspection together with the few molars which had previously been extracted because of caries, were included in the caries estimations.

In the other two studies, children 4 years to 14 years, only the deciduous first and second molars of the children aged 4 - 10 years, and the permanent first molars of those aged 7 - 14 years were used for analysis.

Their results on page 42 of this publication, and printed in heavy type, were :

"The results of the investigation are, in the main, clear cut and therefore can be stated quite briefly. They demonstrate that relatively great differences in the total sugar content of the diets of children in institutions had no significant effect on the initiation or spread of dental caries in periods of 1 to 2 years."

They further state that regarding the deciduous teeth, as a whole, there was no evidence that

additional sugar, white or brown, as they administered it - diet addition - resulted in any increase in caries. Generally, there was actually in each centre an apparent tendency for a reduction in caries of the deciduous teeth in the groups taking additional sugar.

Concerning the permanent first molars, while the sugar groups tended to show a slightly greater increase in and spread of caries than the control groups, on balance the total figures available for these teeth did not disclose any consistently significant differences.

"As it turned out high dietary sugar per se had no caries promoting effect ... it is clear .. that the high refined sugar content of our "civilized" diets, provided they are otherwise nutritionally good, especially as regards the calcifying properties of the diet during the period of development, is not necessarily harmful to the teeth."

The main line of thought of these investigators, that carbohydrate does not play an important role in dental caries, is given in their conclusion thus :

"If, as there is reason to think, correct nutrition is important in tooth structure and in the resistance of erupted teeth to caries, then, in addition to the known factors mentioned above, there must be others, still to be identified, which can be, and often are, deficient in our modern diet."

3. America
 In a study of pre-school children in Tennessee, U.S.A., Frithart and Weiss⁽¹⁰¹⁾ concluded that there appeared to be a direct and consistent relation between the number of d.e.f. teeth and the number of times confections were eaten between meals. On almost consistent progression of one additional d.e.f. tooth for each additional between-meal confection was also noted.

4. Australia
 An excellent scientific and thorough study of 82 "institutional" children has been made by the staff of the Australian Institute of Dental Research at Hopewood House, Bowral, N.S.W.

Sullivan and Goldsowrthy⁽¹⁰²⁾ in their review of studies conducted over the preceding twelve years make the following relevant points :

(1). Diet
 Certain limitations in the types of food offered to the children have been observed. Meat and refined sugar are not eaten at all, and white flour is used only occasionally as bread - oven-baked. Vegetables, principally raw, constitute the major part of the meals; fruit, milk and cream are produced, while eggs, butter wholemeal bread and, to a lesser extent, white bread are purchased locally. The meal plans are designed at rigid restriction of refined carbohydrate, and moderate amounts of milk and fruit only are provided for between-meal eating.

(2). Observations at half-yearly intervals revealed that the prevalence of clinical caries was negligible, the average annual increment in the number of lesions was also very small. The amount of caries of the permanent teeth in children of similar age was only 1/10th the amount seen in the general population, while 25 of the 82 children remained caries-free during the whole of the five-year period under review.

5. Cuba
Dreizen and Spies⁽¹⁰³⁾ carried out an investigation of 147 persons in West Central Cuba and found that only one subject was completely free from dental caries. They observed that the high caries incidence and extensive amount of tooth destruction was not consistent with the hypothesis that raw sugar cane and unrefined carbohydrates contain a protective factor in amounts sufficient to prevent tooth decay.

One must take into account the fact that any institution should be regarded as a special environment and results obtained may not be transferred to the general population. A major consideration, as well as the actual diet, should be the rigid adherence to eating only at regular meal times and, hence, the limited period of caries attack.

6. Wartime Diet
Shaw⁽¹⁰⁴⁾ reviewed the observations made by Toverud in 1945 and Sagnnaes in 1947 and 1948 on the relationship of wartime conditions on

children in Norway resulting from a decrease in the consumption of sugar.

In Norway the sugar consumption dropped to about half of the pre-war level and reached a minimum in 1944. The consumption then gradually rose, and in 1949 the amount of sugar supplied to the manufacturers of sweets and various kinds of foodstuffs had surpassed the pre-war figures. The frequency of caries in children decreased to 20% of the pre-war frequency.

Sagnnaes pointed out that the caries curve reached a minimum several years after the deepest drop in the consumption of sugar. As an example, he quoted the consumption of sugar was lowest in Germany in 1919, while the lowest frequency of caries of permanent teeth of 7 year old children was not recorded until 1924.

The inference is that high sugar consumption affects the metabolism of the tooth during its development, as opposed to the local oral environment theory in its entirety.

The validity of conclusions of this type has, however, been questioned on the ground that many factors, other than sugar consumption, undergo changes in times of war. A large part of the carbohydrate reduction (candy, refined flour and soft drinks) was replaced by more nutritious, although in some cases, less palatable foods.

King⁽¹⁰⁵⁾ writing in the Journal of the American Dietetic Association quotes Sagnnae's^e study, also suggesting that the structure of the teeth is very important in determining their subsequent caries experience.

Toverud⁽¹⁰⁶⁾ still stresses, in 1957, that the reduced consumption of easily fermentable carbohydrates was the main factor in the great improvement in the dental condition of Norwegian pre-school and school children. The recently increased consumption of these cariogenic substances is responsible directly or indirectly for the increase now observed.

Segnnaes⁽³¹⁾ remains undecided on the main causes of the wartime reduction of caries, expressing his thoughts thus :

"The conflicting interpretations of the wartime caries reduction again indicate that the observed changes in caries prevalence have not been satisfactorily explained on the basis of contemporary changes in the intake of refined carbohydrates on the one hand, and in the oral environment on the other."

As a conclusion to this chapter, we may quote from Fosdick⁽¹⁰⁷⁾ :

"All of the methods which have been successful in the control of dental caries can be explained on the basis that :

- (a). the fermentable sugars are eliminated from the mouth.
- (b). they are prevented from being degraded to acids.
- (c). the acids are destroyed before they can attack the tooth or are prevented from reaching the tooth surface.
- (d). the tooth itself is made more resistant to acids."

CHAPTER 5.A. ORAL CARBOHYDRATE BIOCHEMISTRY

Fosdick and Burrill(108) noted in 1943 from chemical considerations that the only substrates from which acids can be formed are the carbohydrates in general, and that, in all probability, the fermentable carbohydrates in particular are the ones most likely to be converted to various acids under oral conditions.

Volker⁽¹⁰⁹⁾ reports on the oral biochemistry of carbohydrates, commencing with the statement that is in general agreement that the minimal oral conditions that must be fulfilled before the initial carious lesion may be anticipated are twofold; namely, the presence of micro-organisms and fermentable carbohydrates. The only way to explain the presence of sugars in and about the teeth is either their adherence to the teeth during digestion, or their being secreted as a constituent of normal saliva.

1. Glucose Levels in Saliva:

Volker⁽¹⁰⁹⁾, reviewing the current literature of investigations in this field, showed that virtually all present active research agrees that the quantity of sugar secreted in the saliva is negligible. He studied the normal salivary glucose level of 12 subjects over a period of six days. When samples were taken two hours after eating, values which ranged from 14 to 22 mg. per cent were found. Concurring with other workers that most, if not all, of this value is attributable to reducing substances other than glucose.

Pigman and Hawkins⁽¹¹⁰⁾ support this finding from their experiments as they were unable to produce any evidence of monosaccharides, oligo-

saccharides or hexas^amines.

The main point is the time when saliva samples are taken. High sugar values are present immediately after the ingestion of meals, whereas several hours later very low values, or none, are found. By suitable physical forms, stickiness, or a very hard candy lasting an appreciable time, it is possible to maintain high salivary glucose levels for an hour and a half.

Lanke⁽¹¹¹⁾ carried out an important investigation on the difference in the sugar elimination rate of different persons. The question was whether this was an individual property not varying from one type of food to another and so help explain the wide variation in the caries activity of different people on one and the same diet.

A study of the following factors by standardised chemical methods was completed.

1. The influence of different foodstuffs on the sugar content of the saliva when the foodstuffs were given in quantities containing equal amounts of carbohydrate,
2. Factors influencing the effect of different sorts of bread on the salivary sugar,
3. The sugar elimination of different subjects after consumption of the same foodstuff,
4. Factors possibly influencing the sugar elimination rate in different subjects.

The salivary sugar concentration was determined after ingestion of different sorts of starchy

foodstuffs and of sweets. Sugar was determined by means of copper-iodometric reagents after hydrolysis of the disaccharides.

Lanke made the following observations.

- "(1). When the influence of different foodstuffs was compared by conventional methods, i.e. by comparison between the mean salivary sugar level of a number of subjects significant differences were found only between few foodstuffs. The reason was the large standard errors of the means, which in turn were due to the differences between the subjects.
- (2). On comparison of the foodstuffs by differences calculated for each person, differences were demonstrable for a larger number of substances.
- (3). It was found that after the ingestion of foodstuffs the salivary sugar concentration was an exponential function of the time, i.e. the logarithms of the salivary sugar values as a function of the time was a straight line most comparisons were made using the time the salivary sugar was above a certain level, since this factor is probably more relevant in the caries process than the magnitude of the sugar concentration at certain intervals.

- (4). Chewing gum gave an enhanced salivary sugar level during a longer time than the other sweets ingested.
- (5). Potatoes gave an enhanced salivary sugar level during a shorter time than the other starchy products.
- (6). Macaroni gave an enhanced salivary sugar level during a shorter time than the various types of bread.
- (7). White rye bread gave an enhanced salivary sugar level during a longer time than the other types of bread investigated.
- (8). Brown rye bread and hard bread (both of whole-meal) gave an enhanced salivary sugar level during a shorter time than the other types of bread studied.
- (9). New wheat bread gave an enhanced salivary sugar level during a shorter time than stale bread of the same sort.
- (10). Unsweetened bread gave an enhanced salivary sugar level during a time equally long as a sweetened bread baked according to the same recipe but with sugar added. Both regarding sugar content and other properties, the sweetened bread was equivalent to the type of bread mainly consumed in Sweden.
- (11). Bread baked with proteins added to the flour gave an enhanced salivary sugar level during a shorter time than bread of the same flour without proteins added.
- (12). The differences in salivary sugar levels

between different individuals after the ingestion of the same foodstuff were greater than those in the same person after ingestion of various sorts of bread and sweets (except chewing gum).

- (13.) The effect of slow elimination in a subject appeared, especially during "chain-eating" of lozenges. Even when the amount of carbohydrates ingested at a time was small, the salivary sugar level remained elevated for a long time.
- (14). It was found that a factor with a marked influence on the course of elimination was the movements of lips and tongue after the substance had been swallowed.
- (15). It was observed that other factors which tended to accelerate sugar elimination from saliva were high frequency of chewing movements before the food was swallowed, rapid salivary flow and low viscosity of saliva and also a high activity of amylase in the saliva. It was further observed that the bacteria-containing saliva sediment from persons with a slow elimination of sugar, consumed sugar faster than did the saliva sediment from persons with a rapid elimination of sugar."

Another study of sugar clearance from the mouth or persistence of sugar in the saliva is that of Lund⁹qvist⁽¹¹²⁾, who compared 66 foods and beverages

and used his observations to calculate a "caries potentiality index" for the various foods. The lowest values were given by low carbohydrate foods such as beer, fish and meat. The lowest value for what was recognized as sugar-containing foods was given by lemonade, an index number of 2, then by fruit juice 3, and certain fruits such as oranges and strawberries 3. The highest values were given by toffee and caramel in 27.

Bibby⁽⁷³⁾, commenting on his previous work with Goldberg and Chen⁽⁸²⁾ on the "decalcification potential" of a food as a result of the determining of the amount of food which adhered to the teeth after eating and then by multiplying this value by the amount of the acid formed when the food was incubated in saliva, thinks that certain reservations should be made on the true accuracy of the results obtained. He feels that the procedure depended heavily on the suitability of the test subjects, which gave irregular results. Further, depending on the method used, only narrow differences were found among the amounts of acid formed from the different foods, the broad variations which appeared in retention unduly influenced the decalcification values.

2. Conversion of Sucrose to Glucose:
Experiments, both in vitro and in vivo of Volker⁽¹⁰⁰⁾ showed that appreciable quantities of dietary sucrose are converted to reducing sugars almost immediately on contact with certain enzymes normally present in the saliva or salivary micro-organisms.

3. Conversion of Sugars to Acid:

Volker⁽¹⁰⁹⁾ also studied the possible influence of the concentration of salivary enzymes on sugar breakdown by comparing acid production of the supernatant and sedimentary fractions of centrifuged, pooled saliva from caries active patients. His results support the belief that the concentrations of sugars and oral enzymes, likely to be found on the tooth surfaces, will accommodate appreciable acid production.

Neuwirth and Baerger⁽¹¹³⁾ reported on the production of acids from glucose by oral micro-organisms and by using paper chromatography showed that the following acids of the Krebs cycle were produced by the action of oral micro-organisms on glucose :

Malic, oxalacetic, alpha-ketoglutaric, succinic, fumaric, pyruvic, as well as lactic acid.

He found the concentration of these acids increases with time, except for lactic and citric acids.

B. THE DENTAL PLAQUE

Bruckner⁽¹¹⁴⁾ studied plaques which he obtained by suspending sheets of cellophane in a saliva-glucose mixture for three weeks. He noted the matrix of threadlike organisms and gave the following definition of a plaque :

"A thin, adherent film, white and cloudy in appearance, present on improperly cleansed tooth surfaces, and consisting of micro-organisms and debris, that is primarily of

organic nature. It cannot be removed from the tooth surface by a stream of water or by gentle swabbing, but can be removed by vigorous brushing, especially with the aid of a mild abrasive."

Cox⁽¹⁶⁾ states that plaques vary widely in their characteristics and especially in their constituent flora. They may or may not be associated with carious lesions, and some types may be protective against caries. A causal role for dental caries cannot be assigned to any micro-organisms present in plaques, or to the plaques themselves.

(a). Plaque as a Selective Membrane:

Huh, Blackwell and Fosdick⁽¹¹⁵⁾ made an artificial plaque from salivary sediment - a synthetic microbial plaque - and tested its permeability with glucose solutions. The microbial mats/mm thick were used and it was found that altho' the mat resisted the rapid penetration of sugar, the rate of penetration was roughly proportional to the concentration.

Fifty-two chemicals, selected because of initial promise as glycolysis inhibitors, were tested for their inhibitory action, ~~mainly~~ by Manly⁽¹¹⁶⁾ on three different thicknesses of thin films of salivary sediment. Film thicknesses of 0.2, 0.5, and 0.9 mm. were prepared and the layers 0.2 mm. thick were considered to represent the types of plaques that are formed within a period of 24 hours over the gingival third of the teeth.

The layers 0.9 mm thick would represent the amount of heterogeneous material that germicides or inhibitors would have to penetrate in order to be effective in interproximal spaces or occlusal pits and fissures. Manly's results showed that the most inhibitory group of chemicals were affected to the greatest extent by change in thickness. The groups of chemicals showing more than 40% recovery of the original pH on 0.2 mm films possessed little or no inhibitory action when tested on 0.9 films.

Forscher and Hess⁽¹¹⁷⁾ had previously questioned the validity of plaque pH measurements as a method of evaluating therapeutic agents as plaque samples from different regions of the mouth differed in pH. When the pH of a plaque is determined by a procedure that requires the sample to be removed from the mouth, no conclusions can be drawn as to the behaviour of that sample during the succeeding time interval.

(b). Acidity of the Dental Plaque:

According to Stephan⁽¹¹⁸⁾ microbial plaques remain as a possible source of acid production in the initiation of caries. They produce a rapid drop in pH when glucose is ingested in the mouths of caries-free individuals as well as in those with active caries, but the plaques in the mouths of caries-free individuals did not drop below pH 5.0, whereas in the mouths of caries-active individuals the pH sometimes reached 4.0. This observation suggests that the microbial

flora growing in the plaques of caries-active individuals differs from that of caries-free individuals. This apparent difference is not so much in the rate at which acid is produced at pH levels above 5.0, but rather in the relative ability of plaques in caries-active individuals to continue production of acid below 5.0 and the relative inability to remove the acid produced, as compared with plaques of caries-free individuals.

His work⁽¹¹⁹⁾ was considered so important by the Councils on Dental Health and Dental Therapeutics of the American Dental Association that part of his thesis of 1944 is included in their joint report of as late as 1953.

"The bacteria growing on tooth surfaces exist in concentrated masses or "plaques" which possess a maximum concentration of enzymes and coenzymes in a minimum space with minimum buffering capacity. The acid production by such bacterial masses is very rapid when carbohydrate is available and, since little initial buffering capacity must be overcome, the drop in pH is likewise very rapid The subjects (65 in number) were seated in a semi-reclining position in a chair equipped with a headrest ... direct pH determinations were made on the tooth surfaces with a polished stick antimony electrode using a vacuum tube potentiometer ... the mouth was rinsed with 25 cc. of a 10%

aqueous solution of glucose for two minutes following initial pH determinations. The pH determinations were repeated two minutes after the glucosal rinse and at 10-minute intervals thereafter until the pH had returned to approximately its original value. Before the application of glucose, the pH values of most areas were around neutrality, except in the cases of extreme caries activity in which pH values were generally considerably lower. In all cases there was a sharp drop in the pH of bacterial material in teeth following rinsing the mouth with the glucose solution. The drop in pH was greatest and lasted the longest time in the caries active cases ... only in the caries-active cases were pH values below 5.0 consistently produced by the glucose."

This joint report also refers to the work of Haldé and Wynn⁽¹²⁰⁾ which was being prepared for publication in 1953. On the basis of their study, findings were presented which indicated that drinking (5 unscreened subjects) or rinsing the mouth (8 subjects with rampant caries) with a 10% solution of sucrose did not produce a sufficient drop in pH to initiate decalcification of the surface of a tooth. This finding was directly opposite to Stephan's work above on 65 carefully chosen subjects. In Haldé and Wynn's report no significant differences in the pH developed from drinking or from rinsing out the mouth with a 10% solution of sucrose.

Tullar⁽¹²¹⁾ carried out some experiments on rapid acid formation resulting from the action of plaque or salivary sediment on sugars. His results showed that plaque or salivary sediment freshly removed from the mouths of healthy young adults is capable of producing enamel-demineralizing concentrations of acid(s) of pH 4.3 or under in from 8 to 281 seconds at room temperatures.

Ludwig and Bibby⁽¹²²⁾ studied acid production from different carbohydrate foods in plaque and saliva and concluded that :

"What goes on in the saliva is probably of less importance in the causation of decay than what happens to foods in the bacterial accumulations or plaque on the tooth surface."

Further, that sugar-containing beverages as a cause of caries only produced low and transient acidities in plaques being essentially the same as those produced by apples or fruit juice. This suggests a conclusion contrary to the common belief which possibly has originated from the assumption that the effects produced in the plaque by sugar-containing beverages will be similar to those produced by a glucose rinse.

Sullivan⁽¹²³⁾ used an acidulated beverage "lemonade" in his experiments and noted a drop in pH of all areas in the mouth after rinsing. This was particularly noticeable in carious lesions where the hydrogen ion concentration was well below the pH usually considered as critical for decalcification. The patients were all caries-

susceptible and had open carious lesions, sufficiently large to permit the insertion of the 1 mm. glass electrode and the hygiene of this group was unsatisfactory.

Nolte and Arnim⁽¹²⁴⁾ describe a novel technique of measuring in vitro changes in pH of dental plaque material and surrounding saliva by using two glass electrodes. They also conclude that the wide variations in the readings seemed to be related to the volume of saliva and the sample source.

(c). Plaque Microflora:

Harrison⁽¹²⁵⁾ made a longitudinal study of over 600 plaques taken from 87 proximal areas of the teeth of 44 children, and made a number of observations :

1. Acid-producing and acid-tolerating streptococci both were found associated with dental decay.
2. Aciduric streptococci were associated more persistently with advanced caries of the dentine, while lactobacilli were found more likely to be associated with the initiation of the lesion in the enamel.
3. Streptococci of the viridous type began to disappear in plaques as early as 42 weeks before the first appearance of cavitation, and disappeared rapidly with the formation of carious lesions.
4. Hemolytic streptococci, found in-frequently, showed a slight increase as lesions developed.

5. Yeasts were found in comparatively few cases and did not change with carious progress.

Green, Dodd and Inverso⁽¹²⁶⁾ compared the microflora of developing dental plaques in caries-immune and susceptible individuals, with special notice taken of the rate of increase of lactobacilli. They noted a slower rate of increase of several types of organisms, including lactobacilli.

Lanke⁽¹¹¹⁾, in studying the sugar consuming capacity of bacteria found in salivary sediment and that of bacteria present in dental detritus, found that the vast majority of the bacteria were in the salivary sediment. Although all material was carefully scraped off from the teeth, the amount of bacteria it contained was so small that on addition to centrifuged saliva it had no demonstrable effect. The amount of bacteria retained in the paraffin lump from experiments had negligible significance. However, no investigation was made of the tongue surface or mucosal surface of the oral cavity.

Stralfors⁽¹²⁷⁾ experimented to estimate quantitatively the aerobic respiration of oral bacteria as opposed to the anaerobic breakdown of carbohydrates to organic acids in the metabolism of oral bacteria. He used the Warburg constant volume respirometer to determine the aerobic and anaerobic breakdown

of carbohydrates, and made chemical determinations of the amounts of lactic acid produced and glucose remaining. The bacteria studied were : streptococci, lactobacilli, staphylococci, gaffkyacocci, sarcinacocci, and actinomyces.

Strålfors summarizes his results on page 182 :

- "(1). Endogenous respiration accounted for only a relatively small fraction of the respiration of the bacteria in glucose.
- (2). Streptococci and lactobacilli showed decreasing respiratory activity, in terms of oxygen taken up, as the experiment proceeded. The other bacterial groups had a more uniform activity curve.
- (3). The absorption of oxygen was on a lower level for the streptococci and lactobacilli than for the other bacteria.
- (4). It was possible to make an approximate calculation of the depth in the dental plaque, under which strictly anaerobic conditions would prevail (0.32 mm).
- (5). The respiratory quotient was especially high with lactobacilli, the mean value being 1.45. For streptococci it was significantly lower than (1), with a mean value of 0.89 ± 0.04 . For the other bacterial species, the quotient was very near to 1. Various possible explanations of the different values

are discussed. (pH, formation of Hydrogen Peroxide).

- (6). The relationship between the amount of glucose which was changed to lactic acid in the presence of air, and the amount which was, at the same time, aerobically oxidated, was variable, mean values for this relationship were: for lactobacilli 61, for streptococci 20, for yellow staphylococci 2.5, for white staphylococci 0.42, for gaffkya 0.20, for sarcina 0.10 and for actinomyces 0.07. Thus it appears that lactobacilli and streptococci predominantly break down carbohydrates anaerobically to acid even when they have good access to air.
- (7). The sum of the amount of glucose remaining after the experiment, the glucose changed to lactic acid and the glucose metabolized by aerobic oxidation was constantly lower than the total amount originally supplied. Staphylococci showed the lowest value. Various other possible ways of glucose consumption are discussed (other fermentation products than lactic acid part of the glucose may be assimilated).
- (8). The acid production, measured by the gasometric method under strictly anaerobic conditions, was of approximately

the same magnitude as was reported earlier by the present author using a method with pH determination (Stålfors, 1950). The gasometric method of determining acid production does not seem to be particularly dangerous."

C. SALIVA.

(a). Buffering Action:

Lilienthal⁽¹²⁸⁾ completed an extensive analysis of saliva and concluded :

- (1). Activated saliva owes its buffer capacity largely to the presence of bicarbonate. Phosphate is probably the only other buffer of significance. Although the micro-organisms act as buffers, they represent an insignificant part of the buffer mechanism. Mucoid plays no detectable role as a buffer.
- (2). In resting saliva, bicarbonate and phosphate also represent the buffer system. Mucoid was shown to have no influence on buffer capacity.
- (3). The buffering mechanisms of salivary sediment and plaque were investigated; the former had absorbed a variable amount of bicarbonate, but the plaque relies only on the organisms for its buffering action.

Turner, Scribner and Bell⁽¹²⁹⁾ state that reserve alkalinity is more closely and consistently related to caries experience than is either titratable acidity or pH. It would appear that the saliva of persons free from dental decay has a high potentially basic

conjugate or an enzyme system capable of yielding this effect. Muracciolo⁽¹³⁰⁾ supports their conclusion.

Englander, Mau, Hoerman, Chauncey⁽¹³¹⁾, after experimenting on 30 caries-free males and 52 with rampant caries, found no differences in pH, titratable alkalinity and rate of flow of the parotid salivas between the two groups.

Lanke's⁽¹¹¹⁾ thorough study of salivary sugar clearance included the following points :

- (1). The consumption of sucrose in centrifuged saliva and in sediment were diametrically opposite. The consumption of sugar in saliva being so low as to be hardly demonstrable, while the sediment from the saliva under the same conditions showed a high sugar consumption. The consumption of sugar by the bacteria is consequently much greater than that by the enzymes in the saliva.
- (2). No significant relationship could be shown between the salivary production and the salivary sugar content at a certain interval.
- (3). The saliva produced does not only influence the course of sugar elimination only by diluting the sugar-containing saliva in the oral cavity, as many other factors are involved. The frequency, for example, with which the saliva is swallowed must be of importance. Also the effectiveness with which a given amount of saliva removes

sugar from the oral cavity may vary with the energy of the movements of the tongue and lips.

(4). The viscosity of the saliva samples was not found to have any substantial influence on the shape of the sugar elimination curve.

(b). Microflora of Saliva:

Lilienthal⁽⁹⁵⁾ ~~et al~~ presented evidence to show that oral lactobacilli in the concentration found in saliva play an insignificant role in the formation of acid from glucose or sucrose. Even additions to saliva of 10 million lactobacilli per ml failed to increase the rate and amount of acid produced by saliva. They are convinced that other acidogenic organisms must play important roles in acid formation in saliva, streptococcus salivarius, an acidogenic organism present in saliva in large numbers, was shown to account for as much as 30% of the acid formed. After considering previous works, together with their own results, they are certain that oral lactobacilli are not aetiological agents in dental caries. Further work by Lilienthal⁽¹³¹⁾ in 1957, including neisseria in salivary acid formation, found on a numerical basis, strep. salivarius was four times as important as neisseria and three times as important as lactobacillus in forming acid from glucose and sucrose. However, Lilienthal agrees that lactobacillus count is a measure of dental caries activity⁽¹³²⁾ which is also supported by the results of Green and Dodd⁽¹³³⁾

in 1956 who found that :

"Quantitative comparison of gram-negative diplococci, gram-negative rods, yeasts, caseolytic organisms, streptococci, micrococci and lactobacilli in caries-immune and caries-susceptible salivas showed that only lactobacilli occurred in significantly different numbers, being much lower in immune salivas."

Rae and Clegg⁽¹³⁴⁾ say that in the cases they studied there is no apparent relation between lactobacillus count and lactic acid production since zero-count (Lactobacillus index) salivas produce considerable amounts of lactic acid. Quite low concentrations of glucose (0.1%) are sufficient for lactic acid production, the optimum pH being 6.5.

Gore⁽¹³⁵⁾ believes that it is the potential acidity of the spontaneously precipitated material of the unstimulated nocturnal stagnant saliva which determines susceptibility or immunity to caries. A single high carbonate meal can temporarily increase the carbohydrate concentration in the whole saliva. A prolonged high carbohydrate diet can produce prolonged high carbohydrate concentration in the whole saliva. Both the preparation of the mouth for collecting the saliva and the method of collecting influence the chemical and physical properties of the saliva.

Recently Green⁽¹³⁶⁾, after studying the saliva of caries-immune and caries-susceptible subjects, found a factor which inhibits growth of lactobacilli and streptococci in the saliva of the

caries-immune. This factor has biologic activities in vitro which may account for the lower number of salivary lactobacilli thus found. His work indicated that this peculiar antibacterial factor is a chemical entity, probably globulin.

SUMMARY AND CONCLUSION

More carbohydrate material is present in nature than all other organic substances combined, being present in all animal tissues and comprising most of the organic structure of plants. Hence it is a major constituent of food.

The breakdown of carbohydrate in all its various forms in the human and animal oral cavity is accomplished by enzymes, which may be furnished by the host or bacteria present. The end results of this degradation are acid in nature, the common ones being lactic and pyruvic which, in selective media, are capable of decalcifying tooth structure.

However, mere decalcification is not dental caries as proved by experiments conducted in vitro.

The proponents of W.D. Miller's (1) chemico-parasitic reasoning stress that acid derived from "amylaceous and saccharine food" by acid producing bacteria demineralizes the teeth which precedes, not follows, destruction of the organic constituents. However, it is not clear whether any differentiation is made between initiation and promotion of caries. Once commenced, however, the overwhelming importance of acids as the immediate cause of decalcification is maintained.

The five opposing exponents to this treatise believe that the dental caries process is primarily concerned with disintegration of the organic composition of the teeth. They say that proteolytic enzymes possibly "lyse" or dissolve the protein or organic portions of the dental tissues. This would help explain how

the organic cuticular structures of the enamel surface, the glycoprotein of the inter-rod substance, and the organic matrix of the dentine can be destroyed by the caries process. It is possible also that protein hydrolysis may liberate acids strong enough to affect the inorganic tooth structures.

Gottlieb⁽¹⁷⁾ considers that the organic pathways (lamellae prism sheaths) are invaded by proteolytic organisms (*staphylococcus aureus*) with the formation of a yellow pigment, a feature he claims distinguishes natural caries from that produced by acid (in vitro). Acid-action, a component of the process, causes decalcification which shows on the radiograph but the yellow pigmentation does not. By shrinkage of the dentine the second kind of undermining caries occurs.

Frisbie, Nuckolls and Saunders⁽¹⁸⁾ maintain that the initial caries lesion is associated with the formation of the bacterial plaque and the breakdown of the enamel cuticle, thereby uncovering the ends of the enamel rods and inter-rod substance. Penetration of this calcified homogeneous cuticle may be due to acid action by acidogenic bacteria; proteolysis of the matrix then follows, the process extending and freeing the less soluble basic tricalcium phosphate from its organic bond, thus favouring its solution by products of acidogenic bacteria. These secondarily penetrate along widening pathways of ingress and the organisms found occupying the tracts are invariably gram-positive spheroidal forms.

Apparently Iura⁽²⁴⁾ thinks the process is a pathologic enzymatic action caused by general disturbances of the phosphorus metabolism and by local abnormal accumulation of phosphatase in suspect areas.

The theory of Pincus⁽²⁶⁾ explains only pit and fissure caries, suggesting that the organic component (secondary cuticle) provides the acid whereby the tooth is decalcified.

Finally, Martin Schatz and Karlson⁽¹⁹⁾ maintain that keratinolysis is the proteolytic process present with removal of the mineral of enamel by chelation complexes. They advance an interesting point that acid-producing bacteria may be a defensive factor in the mouth to protect the teeth if proteolytic bacteria are the cause of caries.

Research on animals by Orland⁽¹⁵⁾ and others has shown the necessity of both carbohydrate and bacteria for caries to develop. Previously it was accepted that certain strains of animals could be bred with a pre-disposing characteristic either towards or against caries development. However, recent research has shown that this premise is incorrect since animals may be caries resistant for one special diet but develop caries when placed on different diets.

Keyes⁽⁶⁴⁾ was prominent in the denunciation of this previously accepted "fact".

That the nutritional background of animals influenced caries susceptibility in the developmental period has

demonstrated by Sagnaës⁽³⁹⁾, Mitchell and Shafer⁽⁴¹⁾ and Volker⁽⁴²⁾. After prolonged ingestion of carbohydrate, an indirect nutritional effect may occur where certain trace elements are replaced in the diet. Opposed to this are the writings, for example, of Watson and Muhler⁽⁴⁶⁾, Steinman and Haley⁽⁴⁷⁾, but the concensus of opinion favours the importance of developmental nutrition for caries resistance. Bagnall⁽⁵³⁾ remained unconvinced in his review of caries research extending up till the early 1950's.

Carbohydrate possesses different degrees of caries potential depending upon its concentration in the diet, the length of time it remains in contact with the teeth, and the frequency of ingestion. Such physical properties of the various carbohydrates, particle size, liquid or solid, natural or refined, adhesiveness, all influence the rate of oral clearance and hence the length of time carbohydrate is in intimate contact with the teeth. Finely powdered sugar is more highly cariogenic in the diet than starch which contains much larger molecules, and also needs more time for its degradation. Carbohydrate in liquid form produces less carious lesions than when ingested in the solid form and, similarly, for the natural state. The addition of fat to an otherwise cariogenic diet reduces caries incidence, possibly through a protective layer and/or quantity replacement.

The quantity, rate of flow and possibly the viscosity of saliva affect the oral clearance time of carbohydrate food and hence the period of susceptibility

to dental decay. Both dilution of any acids formed and mechanic washing are important here.

Research on human subjects has not been as exhaustive as with animals, but the Vipeholm Dental Caries Study in Sweden ranks very high in recent programmes of caries research. Light was shed on the important questions of :

- (a). The quantity of carbohydrate consumed,
- (b). The form in which it was served,
- (c). Whether it was consumed at or between meals, in their relation to caries experience.

A similarity to animal research was found regarding quantity ingested, length of time of oral clearance, and, most important, the risk of sugar increasing caries activity was greater if the sugar was consumed between meals and in a form in which the tendency to be retained on the surfaces of the teeth was pronounced.

Other institutional studies, particularly in England, suggest that carbohydrate either has little cariogenic effect, or indeed has a caries resistant effect. A balanced regular diet of good nutritional status may account for this apparent discrepancy.

The decrease in caries in the immediate post-war years in Western Europe has not been satisfactorily explained beyond all doubt. The immediate and then the more pronounced delayed reduction in caries in

the child age groups raise questions of quantity reduction of sugar intake and more nutritious diets giving a long-term immunity.

Results obtained from studies of patients or inmates of institutions have doubtful value as they cannot be transposed to the ordinary population.

The oral biochemistry of carbohydrates is complex; the various types of foodstuffs consumed, e.g. potatoes, white bread, rye bread, maccaroni, give widely different times for oral clearance movements of the lips and tongue after the food had been swallowed is a factor with a marked influence on elimination, as also is rapid salivary flow, and a high activity of amylase in the saliva.

The dental plaque is considered highly relevant in the initiation of dental caries, possibly providing the habitat for either acid producing or proteolytic bacteria. The ease with which plaques function as a semi-permeable membrane, the protection afforded bacteria and their acidic products has been demonstrated by many studies.

The Hydrogen ion concentration of plaques decreases rapidly after the ingestion of carbohydrate providing an acid environment, while the micro-flora of dental plaques contains widely differing bacterial strains ranging from streptococci, lactobacilli, staphylococci to actinomyces and many others, on which plaques rely for their buffering action.

Saliva has a strong buffer action against acid, activated and resting saliva owe their buffer capacity mainly to the presence of bicarbonate and phosphate.

Recent work by Lilienthal⁽¹²⁸⁾ shows evidence that oral lactobacilli in the concentration found in saliva, play a very small role in the formation of acid from glucose or sucrose and doubt is cast on any aetiological significance of lactobacilli in dental caries.

Streptococcus salivarius^u, an acidogenic organism, was shown to produce as much as 30% of acid formed. Lactobacilli counts are still useful in demonstrating caries activity however, and the suggestion has been made that lactobacilli act as a protective mechanism against caries.

Thus the important relationship between carbohydrate and the various theories of dental caries has been presented. The chemico-parasitic theory is considered the most suited and has also stood the test of time, being supported by active research. The proteolytic theories, on the other hand, rely heavily on histologic evidence, although mention is made of the presence of carbohydrate and acid.

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