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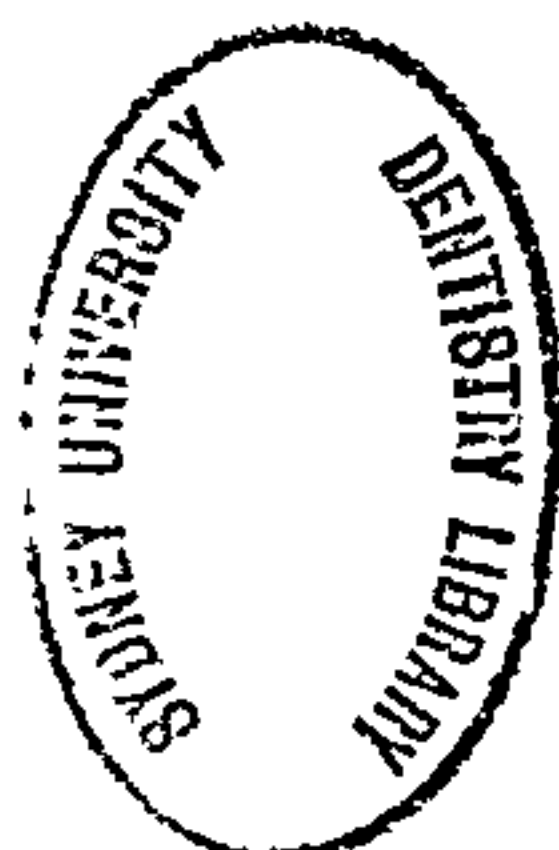
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THERAPEUTIC DENTIFRICES
IN
THE PREVENTION OF DENTAL CARIES

A thesis submitted in partial requirement for
the Diploma of Public Health Dentistry, in the
Faculty of Dentistry, University of Sydney.



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SECTION 1.

INTRODUCTION

The dental profession was first established to deal with pain; gradually developed to replacement of teeth, and then followed by the transition period from restorative to preventive era, which has largely been concentrated on dental caries. More useful tools become available for partial prevention of dental caries, and dentifrices have been utilized as a measure since the last two decades.

The apathy and neglect by the public in the matter of oral hygiene are unfortunately very real.¹ From a study in Great Britain, only one in seven persons owns a toothbrush, and less than one third of the population uses a dentifrice regularly, averaging two tubes per head a year. Despite the present trend, dentifrices seem to be an important adjunct to tooth-brushing, as shown by the participants in the study of Dudding, Muhler & Dahl. (1960).²

Exaggerated claims, from dentifrice manufacturers are prevailing. The money spent by the manufacturers on advertisements in 1963 amounted to £A322,000. Some authorities argue that more dentifrice that is sold to the public, the more dental health conscious will they become, regardless of what claims are made by the manufacturers, others consider these advertisements are misleading, such as : "Brush before breakfast and fight tooth decay - all day."³

That such advertising propaganda has had its insidious effect to the public, especially the children, many thousands of whom have genuinely believed that, so long as they use a certain toothpaste containing some magical agents, it was not necessary for them to clean their teeth regularly after meals, and that one brushing each day was sufficient to protect their teeth from decay. Those who rely upon the often bogus claims of proprietary companies tend to postpone professional treatment and endanger their dental health.

The influence of toothbrushing with a neutral dentifrice immediately after meals shown by Fosdick (1950)⁴ reduced dental caries by 50% in young adults. Similar experiments on hamster by Volker & Klapper (1954)⁵ showed the same trend in decreasing caries. However, only 9.3% reduction of caries in children was noted by Kerr & Kesel (1951).⁶

One of the major deterrents to the effective utilization of toothbrushing in the control of dental caries is that it demands a high degree of patient co-operation and the correct regime of oral hygiene. From Robinson's study⁷ on 405 persons, and the one by Eirman (1955)⁸, it is known that majority of the people do not practise toothbrushing in the effective manner. It is obvious that even with the discovery of a therapeutic dentifrice, such serious defects, as mentioned above, will well nullify the values of a promising agents. The question remains whether unsupervised

and inefficient toothbrushing with a therapeutic dentifrice can bring about caries reduction.

Despite the lack of base line information, attempts have been made to increase the effectiveness of dentifrices in limiting dental caries by incorporating within them various therapeutic agents. In theory at least by this means the whole population could benefit from the discovery of an effective agent, which serves as a double role in preventing dental caries by removing food debris with additional effect of protection against it.

One of the first widely used therapeutic dentifrices contained dibasic ammonium phosphate, and urea; then followed by agents such as chlorophyll, antibiotics, and in more recent years considerable interest was given to enzyme inhibitors, tetradecyclamine, and fluorides, especially the stannous fluoride.

The purpose of this thesis is to critically review the effectiveness of those agents, that have been incorporated into dentifrices with therapeutic claims in preventing dental caries.

HISTORY OF DENTIFRICES

The use of dentifrices for medicinal purposes has been practised in all ages and by all races.⁹ Dentifrices are mentioned in the work of Houang-ti, the Chinese Emperor and the founder of Chinese medicine, 2700 B.C. Salt, vinegar, and the ground bones of mice were recommended by him. In about the same era, Egyptians used the following prescription for "strengthening the teeth":¹⁰

Powder of flint stones	part 1
Green lead	part 1
Honey	part 1

To be rubbed on the teeth.

This might now be classified as antiseptic and abrasive tooth-paste.

Hippocrates mentions the use among the Greeks of powdered marble, wine and myrrh. While Cornelius, Celsus, Secibonius, Largus and Pliny of the time of Christ, referred to the use of such substances as rose leaves, nutgall, myrrh, wine, sea salt, nitre, pumice, powdered glass, sal ammoniac, ashes of burnt stag's horn, wolf, hare or mouse heads, hoof of the ox or goat, asses' teeth and egg shells as dentifrices. Urine was even once particularly popular for washing the teeth and gums, and at one period cisterns were provided where it might be readily obtained.

In the Epigrams of Martial (401-101 A.D.) reference is made to the use by the Romans of tooth picks of wood and

and metal, while Claudius Galen (131 B.C.) recommended salt, alum and vinegar as dentifrices.

A representative of Arabian science, Avicenna, (about A.D. 1000), pointed out that very hard powders should be avoided, as these were likely to damage the teeth, and he recommended the use of burnt hartshorn.

Guy de Chauliac, the greatest surgeon of the 14th Century, offered the following prescription for a dentifrice: cuttle bone, small white sea shells, pumice, burnt stag's horn, nitre, alum rock salt, burnt roots of iris, aristolochia, and reeds. All these substances must be reduced to a powder together, each one separately.

In the 15th Century, Giovanni of Arcoli, drew up ten Canons of Dental hygiene, one of which was that a dentifrice to be used last thing at night or early in the morning.

Anton Nuck, a celebrated 17th Century Dutch surgeon and anatomist, prescribed powdered cuttle fish, coral powder, cream of tartar, armenian, bole and powder of red roses.

In the next century many believed that the enamel could be regenerated by the use of dentifrice powder. Pierre Fauchard's book, "Le Chirurgien Dentiste" mentioned the use of horse hair for toothbrushes and recommended that teeth should be cleaned with a fine sponge and tepid water to which a $\frac{1}{4}$ pt. of aqua vitae was added. Hunter and Bell originated that

in order to keep teeth from tartar, it was desirable that coarse and gritty dentifrices should be used, and the resulting denudation of the enamel of the teeth did not matter at all, since it would be developed afresh (presumably like nails and hairs). In the middle of this century, Harris and Goddard stated that the use of chalk dentifrices would avoid dental caries. Goddard gave various formulae for antacid, detergent, and aromatic dentifrices.

In the early times, dentifrices were used as beautifying agents. Queen Shubad, Cleopatra, and the Queen of Sheba were habituated to the use of dentifrices.

We thus find from the very earliest time - from the dawning of a higher civilization - carbonate and phosphate of lime (as egg shells or burnt and powdered bones) were constantly prescribed as dentifrices; that alkalies in the form of ammonical salts were also frequently used and prescribed; that acids of all sorts were to be avoided; and that "aromatics" were usually combined with dentifrices.

COMPOSITION OF DENTIFRICES

A dentifrice is a substance used with a toothbrush for the purpose of cleaning the accessible surfaces of the teeth. Commercial dentifrices are available in the form of paste and powder.¹¹ The chief function of a dentifrice is to help cleansing the tissues of the mouth and to assist in maintaining these tissues in normal condition without producing injury.

Dentifrices, as at present compounded, consist chiefly of combination :¹²

1. Detergents.
2. Abrasives.
3. Thickeners and moisture-retaining agents.
4. Flavouring and sweeteners.
5. Colouring agents.
6. Therapeutic agents.

DETERGENTS

Detergents are, by definition, cleansing agents. They are used to lower surface tension with the purpose of breaking up adherent food residues and mucous plaques, penetrating areas inaccessible to the brush and assisting in the foaming properties.

Since the surfaces within the oral cavity are normally wet, unlike skin, the further use of a wetting agent is

unnecessary. Studies are lacking to show that surface-active agents in dentifrices have an inherent cleansing action. An abrasive is the essential ingredient for this purpose. The principal contribution which surface-active agents make to dentifrices may be the effect upon the consistency or other physical characteristics of the mixture.

Some chemicals with the surface-active properties, notably the quaternary ammonium compounds, also possess definite anti-bacterial properties. Their ability to reduce surface tension is not solely responsible for their anti-bacterial activity. Foaming agents employed in dentifrices have a limited effect in destroying or inhibiting the action of micro-organisms in the oral cavity.

Soaps produced from various animal fats and vegetable oils can be used singly or in combination as the detergent - for instance, white soap from tallow, castile soap from olive oil, sodium ricinoleate, a derivative of castor oil, and others.

Many synthetic chemicals which possess surface-active properties are commercially available. They are usually compatible with calcium and magnesium ions and for this reason there is a definite trend for them to replace soap, especially in hard water areas. In addition to their compatibility with divalent cations, these chemicals have an advantage over soap in that they may be used effectively

in neutral or slightly acid solutions. Relatively few of these chemicals have been shown to be suitable for incorporation in preparations to be used in the oral cavity. The ones that are incorporated in the dentifrices are: sodium lauryl sulfoacetate, sodium lauryl sulphate, sulfocolaurate, and sodium lauroyl sarcosinate. Under the conditions of their use, they are safe, but higher concentrations should be employed with cautions.

Of the various synthetic detergents that have been proposed sodium lauryl sulphate (Irium) and sodium N-lauroyl sarcosinate (Gardol) seem to have given most promise and so have received most attention. Sodium lauryl sulphate is an emulsifier and is used as a foaming agent in dentifrices. It has gram positive bacteriostatic action. Sodium N-lauroyl sarcosinate, a detergent and foaming agent, is also claimed to be an enzyme inhibitor which could be effective as an anti-caries agent. Although in some series of tests claims have been made for these reagents, the results have not been substantiated by subsequent check tests.

Hexachlorophene is sometimes used in conjunction with the soaps since, although not soluble in water, it is so in dilute alkalis, and it is a bacteriostatic and bactericidal agent against *Staph. aureus* and other gram positive bacteria, most active at acid pH. It is not precipitated by magnesium and calcium salt, and, therefore, can be used in hard water.

However, its action is easily nullified by agents likely to be present in the oral cavity and possibly by saliva itself. Although there is no indication that hexachlorophene may be harmful, there is yet very little evidence that it is beneficial in dentifrices. In 1% solution, it is used for disinfection of dental instruments.

Despite that these detergent agents have little cleaning action, they are, however, make up like half of the weight of the formulation of a dentifrice.

ABRASIVES

In 1948, Kitchen & Robinson¹³ reported that various people have different degrees of tooth staining and so need various degrees of abrasion to remove it. Some individuals may prevent stain formation by the use of only a brush and water, while others require a more vigorous agent. For those individuals where only a slight degree of abrasion is necessary, a mixture of baking powder, table salt or baking soda alone will suffice.

The abrasive agent used in a dentifrice must be carefully selected not only for its hardness but for its grading so that it will not cause more abrasion than is necessary to remove food debris and the mucous plaque. Any material that will scratch glass is unacceptable. One of the causes of abrasion is toothbrushing with an abrasive dentifrice. According to Manley¹⁴, the degree of tooth wear from tooth-

brushing depends upon the abrasive effect of dentifrice and the angle of brushing.

Various abrasive agents have been incorporated in dentifrices. These include: calcium carbonate, calcium phosphate, calcium sulphate, insoluble sodium metaphosphate, hydrated aluminium hydroxide, magnesium carbonate, magnesium phosphate, sodium bicarbonate and sodium chloride.

The information concerned with the abrasiveness of dentifrices is comparatively meagre. This is owing in part to the fact that other ingredients commonly found in the dentifrice may alter the behaviour of the abrasive itself. For example, the abrasiveness of a calcium carbonate slurry is reduced half when a certain amount of sodium lauryl sulphate is present. It is known that the particle size, shape, and the presence of impurities influence the behaviour of the abrasive. Comparison between relative abrasiveness of various compounds on tooth enamel is seen in Table 1.¹⁵

In addition to the primary purpose of a dentifrice to remove debris and stain, it should polish the tooth surfaces at the same time. There is a difference in the effect of various abrasives and commercial dentifrices upon the lustre of the tooth surface. Some such agents actually abrade enamel, leaving a dull surface, while others polish the enamel and produce a brighter appearing teeth. Abrasives such as extra heavy calcium carbonate will dull a polished

TABLE 1

Relative abrasiveness of various compounds
on tooth surface 15

<u>Abrasive</u>	<u>Abrasion Loss (mm.)</u>
Calcium carbonate, extra dense	0.012
Light chalk, U.S.P.	0.003
Extra light chalk, U.S.P.	0.003
Bentonite	0.006
Flour of pumice	0.300
Levigated alumina	0.300
Hydrated alumina	0.002
Dibasic calcium sulphate, dihydrate	0.001
Dibasic calcium sulphate, anhydrous	0.021
Tribasic calcium sulphate	0.001
Stannous oxide, C.P.	0.083
Zinc oxide	0.003
Calcium pyrophosphate	0.005
Magnesium trisilicate	0.009
Insoluble sodium metaphosphate & dibasic calcium sulphate dihydrate (1:1)	0.001

tooth, while a combination of insoluble sodium metaphosphate and tricalcium phosphate will rapidly polish a dull tooth. Still other abrasives and commercial dentifrices are apparently quite inert and have little effect on the enamel surface.¹⁶

There is another advantage of a highly polished tooth surface other than a cosmetic effect. The smoother the surface of a metallic restoration, the less will be the tendency for it to discolour. In addition, it has been demonstrated that bacteria will accumulate to a greater degree per unit of time, on a rough abraded enamel surface than upon a highly polished surface.

The exact polishing mechanism is still obscure. Electronic diffraction studies indicate that there may be a disorientation of the apatite crystals in the enamel, comparable to the Beilby layer formed during the polishing of the metal.

Types of abrasive have important bearing on the availability of the "therapeutic agents". These are dealt with in the respective categories.

Ware & Chong (1964)¹² have tested the dentifrices that are currently available on the Australian market according to the U.S. Federal Specification FFF-D-191b for Dentifrices with slight modifications. The gradings in abrasiveness are shown in Table 2. Table 3 lists the dentifrices that have passed all the tests.

Slightly Abrasive

Floran (Stannous Fluoride)

Ipana Chlorophyll

Kolynos New Super White

New Pepsodent

Nyal

Odol

Pepsodent

Sanos

Moderately Abrasive

Binaca

Ipana Fluoride

Kolynos Fluoride

Nyal Fluoride

Odol Mint Foam

Odol de Luxe

Excessively Abrasive

Colgate

Teelak

TABLE 2.Abrasiveness of Australian dentifrices. ¹²

Aseptor	Gibbs S.R.
Colgate Chlorophyll	Gibbs Stannous Fluoride
Colgate Fluoride	Ivorite Ammoniated
Colgate with Cardol	Jack and Jill
Dentax	Kolynos Chlorophyll
Dentax Chlorophyll	Pepinol
Durban's	Pepinol Chlorophyll
Euthymol	
Forhan's	

TABLE 3. The dentifrices that have passed all the tests
in the Bureau of Dental Standards, Australia.¹²

THICKENERS AND MOISTURE-RETAINING AGENTS

Glycerin and similar preparations such as propylene glycol and sorbitol are used to influence the viscosity, the consistency and other physical properties of dental products. The taste of glycerin and sorbitol is sweet, and they have the property of retaining moisture. Glycerin and the glycols may under favourable circumstances inhibit or destroy bacteria. Glycerin or some similar constituent appears to be necessary in the manufacture of paste type dentifrices, which would tend to dry out, cake or otherwise deteriorate physically. The dentifrice should flow from the tube without spilling and retain its form when placed on the toothbrush. Care should be taken in the selection of substitutes, especially in view of the known toxicity of some of the glycols.

FLAVOURING AND SWEETENING AGENTS

Suitable flavouring materials such as oils of peppermint, cinnamon, eucalyptus, wintergreen, and cloves, menthol, methyl salicylate, thymol, anise, musk and fruit flavours are among those used. The particular flavour is not of importance as long as it is such as to persuade the public to use it. With the variety in public taste, it may be said with a great deal of truth that the choice of a dental cream and its continued use could easily depend on this factor.

Cautions need to be exercised particularly with fruity flavours so that children are not tempted to eat the

dentifrice. With regard to both flavours and sweetening agents, fermentable sugars and alcohols should not be included. In most dentifrices, sorbitol and/or glycerine are used as dispersing agents and for retaining moisture; they are also sweet and add to the flavour. Saccharin, sodium cyclamate, and artificial sweetening agents are in common use.

COLOURING AGENTS

White dentifrice is popular in the market. It does not appear important what the colour may be as long as it is attractive and does not stain the teeth or other oral tissues. One toothpaste in the Australian market does appear to stain the soft tissues a redder colour making the teeth look whiter by comparison. Deeply staining mineral and vegetable artificial colouring matters have been gradually disappearing since 1911 (McGhee).⁹

THERAPEUTIC AGENTS

Many claims have been made for preparations which will render the teeth immune from attack and maintain the health of the teeth and periodontal tissues. In recent times, we have passed through the whole range of ammonia compounds, urea and urease for neutralizing acid attack, chlorophyll for deodorizing and inhibition of oral lactobacilli, followed by antibiotics, such as penicillin, and

tyrothricin to block or neutralize acid formation in saliva-sugar mixture. Then come the anti-enzymes including sodium N-lauroyl sarcosinate and sodium dehydroacetate, followed by other additive such as tetradecylamine, and more recently the stannous fluoride.

OTHER AGENTS

Manufacturers should ensure that a dentifrice does not contain harmful ingredients of sufficient proportions to cause poisoning or severe toxic effects if a tube of dentifrice should be eaten by a child. Specific tests should be conducted on animals and there should be an assurance that the dentifrice comply with any relevant food and drug regulations.

Having combined these various agents into a dentifrice, it is necessary that the total acidity or alkalinity be satisfactory, because of the detergents and abrasive agents usually employed, dentifrices range from neutral to alkaline, but can be slightly acid. There must be limits to the pH value because of the effect on the oral tissues and continued use of too acid a dentifrice may lead to decalcification of the teeth. Stannous fluoride dentifrice appear to require a slight acidity to be effective.

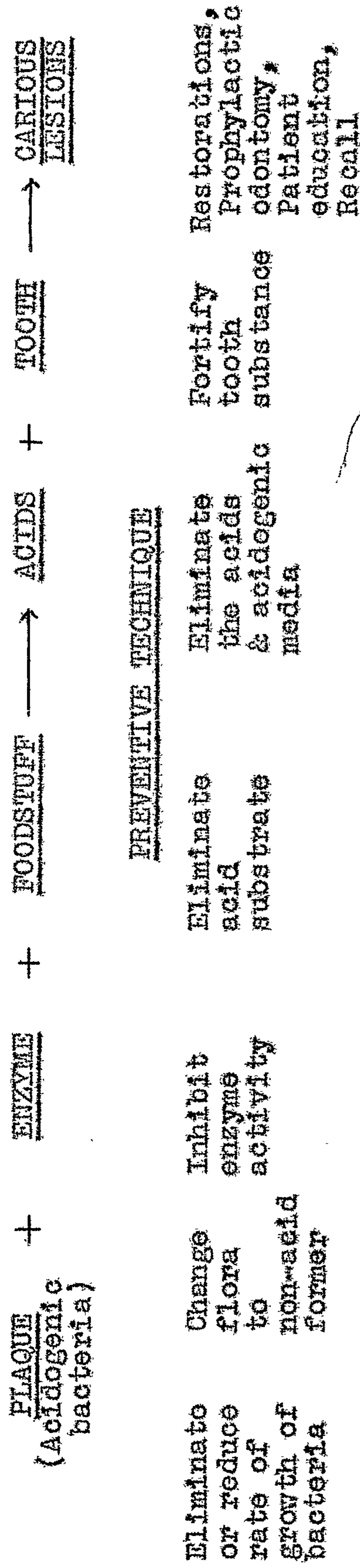
ROLE OF DENTIFRICES IN CARIES PREVENTION

Dental caries is a disease of the calcified tissues of the teeth, characterized by a demineralization of the inorganic portion and a destruction of the organic substance of the tooth. The effectiveness of this process can be reduced in either of the two ways:¹⁷

1. By destroying the attacking forces, or by so interfering with their metabolism process as to render them ineffective, or
2. By building up the defences of the tooth so that it is less vulnerable to the attack.

At present the Acidic theory is mostly accepted, therefore most efforts in counteracting caries have been directed towards reducing acid productions in the mouth. Figure 1 briefly represents the possible ways by which dental caries can be controlled or prevented.

Drastic reduction in the intake of carbohydrates has been shown to lead to a reduction in dental caries. In the majority of the world's population the consumption of carbohydrates is high. It is necessary to accept a high consumption of carbohydrate in the diet and to devise preventive measures which will mitigate the dentally harmful effects of such a regime. It is from this point of view that search is being made for chemical substances which will inhibit acid production in the mouth, and can be applied in either a mouthwash or toothpaste.



CONTROL METHOD

Drugs (Penicillin, F) Hygiene, Immunity	Ammonia, & Urea, dentifrice and gums, Protein, fat food	Fluorides, Sarcosinate, Dehydroacetic esters	Control acid-forming foods (sugar, flour)	Brush, floss, & rinse teeth after eating	Adequate diet (minerals, vitamins, & fluorides)	Professional oral health service (Dentist, Hygienist, Public health service)
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FIG. 1 Preventive and Control methods of dental caries. 19

Many different varieties of dentifrice have been used to prevent dental caries, and unfortunately, although several offered promises initially, in most cases the earlier claims for them proved to be optimistic.

There are four possible ways in which a dentifrice acting locally could prevent caries.¹⁸

1. By removing the fermentable carbohydrates.

The removal of fermentable carbohydrates is probably achieved by most dentifrices; it is not a specifically chemical reaction but rather mechanical. In so doing the microorganisms are deprived of the substrate upon which the acidic products are produced.

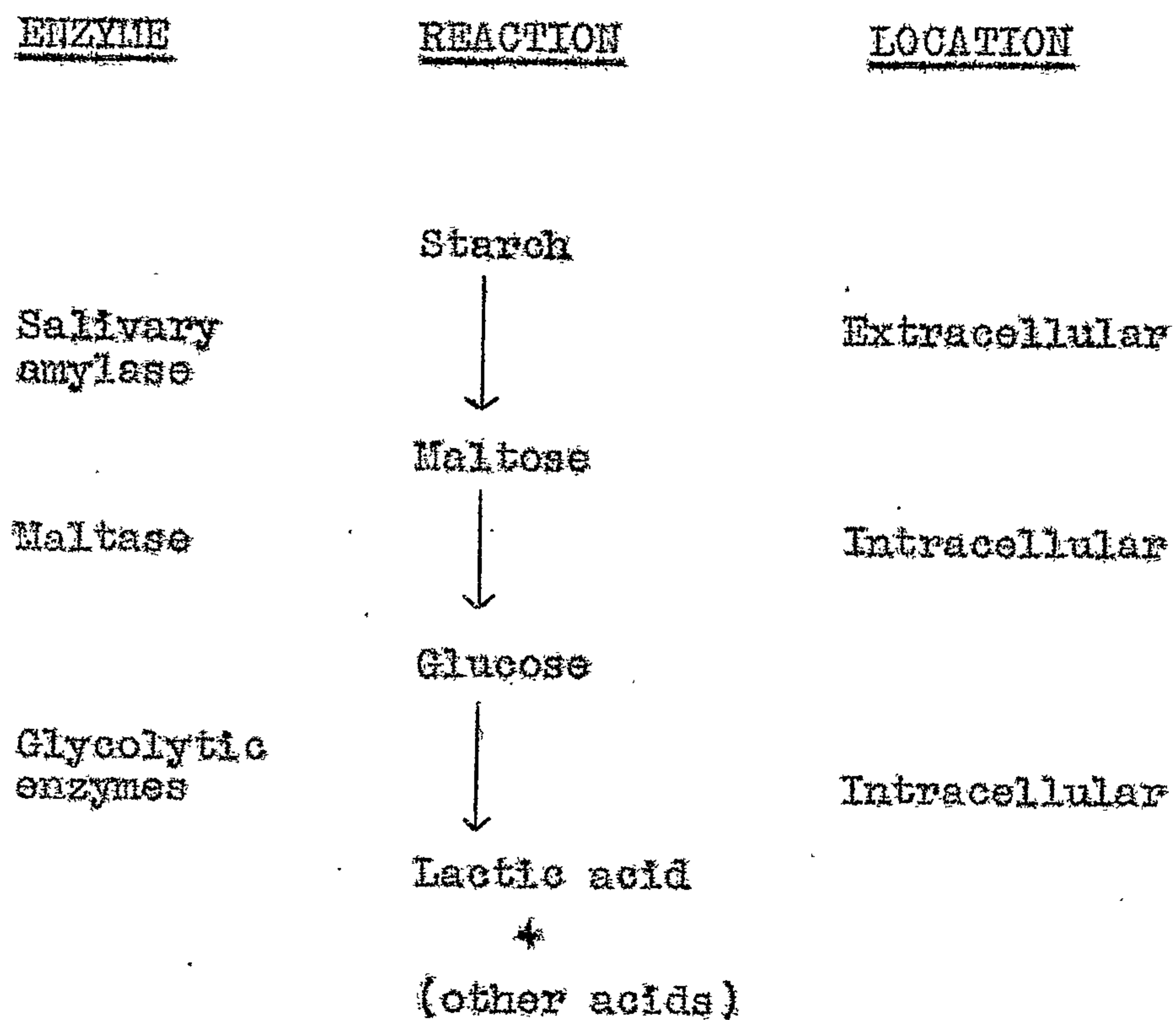
2. By interfering with bacterial activity on carbohydrates.

The two most important dietary carbohydrates are starch and sucrose. The only carbohydrate which has been shown without doubt to be a product of salivary glandular secretion is α -amylase (ptyalin). It splits starch to the disaccharide maltose and the trisaccharide maltotriose. No glucose is formed by the action of salivary amylase on starch. Neither parotid saliva nor centrifuged whole saliva is able to hydrolyse maltose or sucrose. Yet, whole saliva can metabolise sucrose and maltose just as readily as their component hexoses, glucose and fructose. The further metabolism of these low sugars will, therefore, depend on the ability of the oral flora to split them into

hexoses. The breakdown of starch to maltose is an extracellular process, whereas the hydrolysis of maltose and sucrose to glucose and fructose is an intracellular reaction. The further metabolism of glucose or fructose to acid products is also intracellular. This chain of reactions is summarized in Fig. 2.

The problem is to find some means of altering this breakdown process so that harmful acidic substances are not produced in a quantity sufficient to cause tooth demineralisation.

The ultimate end-products of the complete breakdown of carbohydrate yielding the maximum energy are carbon dioxide and water. To achieve this not only are the glycolytic enzymes necessary but also a further group of enzymes including a cytochrome system capable of making use of oxygen. Some oral microorganism (e.g. *Lactobacilli*) lack cytochromes and, therefore, cannot make use of oxygen, and their metabolism of carbohydrate ends at lactic acid or some other organic acid. Other organisms which have a cytochrome system will, in the presence of oxygen, oxidise glucose to carbon dioxide and water. If, however, the oxygen supply is withheld, these organisms will break glucose down only as far as lactic acid. Anaerobic conditions in the mouth will lead to an increased production of acid. This has been demonstrated using samples of whole saliva.

FIG. 2.

Degradation of starch in the mouth
to acidic products.

Theoretically, therefore, there are two ways in which acid production could be reduced:

- (1) By inhibiting the enzymes producing the acid, or
- (2) By encouraging those organisms possessing enzymes which will further metabolise lactic and other acids.

So far serious attention has been given only to the first method using inhibitors, and this includes agents, such as penicillin, sodium lauryl sarcosinate, and fluorides.

3. By protecting the enamel surface.

Tetradecyclamine has been used to act as a protective barrier on the tooth surface. Amine fluoride is also utilized partly in this respect.

4. By strengthening the tooth itself.

The mechanisms by which fluoride are thought to prevent caries are the anti-enzymatic action and enamel solubility reduction.

SECTION II.

THERAPEUTIC DENTIPRICES

AMMONIATED DENTIFRICES

Grove & Grove (1934)²⁰ indicated that rinsing the mouth with a very weak solution of ammonium hydroxide (0.006%) had a cleaning effect on the teeth, as well as a caries-reducing influence. Since then a great deal of interest has been stimulated in the use of ammonia as an aid in reducing the incidence of dental caries. These authors felt that the action of ammonium hydroxide was to loosen the bacterial plaques, thus making them yield to the action of toothbrush, for apparently it was felt that ammonia is a solvent for plaques and salivary mucin. They also found that in the saliva of caries-resistant persons there was a higher ammonia content in contrast to a lower ammonia concentration in caries susceptible patients. This, however, was not substantiated subsequently by Jenkins & Wright (1951),²¹ and Ludwick & Fosdick (1950).²²

Hill (1939)²³ reported that *L. acidophilus* placed in saliva collected from persons who were immune to dental caries was rendered non-viable in a few hours despite incubation at body temperature and the addition of glucose to the saliva to favour its growth. Attempts have been made to plant *Lactobacilli* in the mouths of caries-inactive persons by having them rinse their mouths with 48-hour broth cultures of these organisms. The mouths were cultured at hourly intervals after inoculation and it was found that the number of viable *Lactobacilli* was rapidly reduced, these micro-organisms completely disappearing in 9 to 12 hours.

Kesel and his co-workers (1946)²⁴ reported that cultures of human saliva collected from persons having no dental caries developed during an 8-day incubation period, the ability to inhibit the growth of oral Lactobacilli, as well as the conversion of glucose into acid because of its ammonium nitrogen production. They felt that the inhibition of oral Lactobacilli was not due to any alkalinity developed by the ammonia, for the same degree of alkalinity produced by sodium acetate did not prevent the inhibition that ammonium acetate did. Apparently, the source of the ammonium nitrogen was not entirely from urea, for other agents, such as amino acids in the saliva, contributed to its formation, but there is as yet no apparent correlation between the amount of these substances present in the saliva and caries activity.

The concentration of amino acid present in saliva varies from one person to the other, but is usually below 2 mg/100 ml. Urea, creatinine, uric acid and ammonia represent in saliva, but their significance, if any, is known. At least some of the ammonia in saliva is formed from urea and amino acid by bacterial ureases and deaminases respectively.²⁵

Wach et al. (1942)²⁶ noted with the quinine-urea solution (pH 6.8), the salivary pH jumped immediately from 6.5 to over 8, and remained this high for about an hour and with this mouthwash, the total bacterial count was markedly reduced. This led Stephan (1943)²⁷ to study the effect of

urea in counteracting the influence of carbohydrates in lowering the pH of dental plaques for a period of up to 24 hours by application of urea in 40-50% concentration for a period of about 4 minutes. According to Stephan, this inhibitory effect on the pH change depended not only upon the concentration of the urea, but also upon the duration of its application.

The normal limits of urea in the blood are from 20 to 30 mg. per cent, while the average concentration of urea in resting saliva is about 20 mg. per cent and in stimulated saliva, about 13 mg. per cent. From the concentration of urea necessary to exert a neutralizing effect on intraoral acids, it would appear that the concentration of urea in saliva is insufficient to prevent a marked fall in the pH of plaques after a heavy ingestion of fermentable carbohydrates.

Subsequently, Stephan & Miller (1943)²⁸ used a 45% solution of urea in a clinical study, which involved 6 subjects suffering from severe dental caries. The study lasted approximately two years. They showed that the use of this urea solution resulted in a marked decrease in the incidence of new lesions, besides retarding the progress of the lesions already present. However, this study is a too small group to be conclusive. McClure has indicated that large doses of urea are effective in reducing the incidence of dental caries in rats. According to McClure, a pronounced effect

on reducing the incidence of dental caries in the rat, using urea, requires somewhat massive doses.²⁹

Kesel et al (1947)³⁰ noted that dibasic ammonium phosphate used orally in a mouth rinse and dentifrice caused a marked reduction in oral *Lactobacillus* counts, but did not reduce the number in some of the subjects to a level that would indicate an arrest of dental caries. In vitro experiments indicated that a combination of 5% dibasic ammonium phosphate and 3% urea is more effective as an anti-bacterial and anti-acid agent than was either of these substances alone.

There was no definite knowledge as to the mechanism by which alkaline phosphate reinforced the inhibitory action of urea. One logical reason given by Jenkin & Wright (1951)²¹ was that the phosphate may act as a buffer and prevent the slight fall of pH towards the optimum for acid protection which occurs with urea alone. Thus, the placing of an ammoniated mouthwash or dentifrice in the mouth after a meal might be too late to prevent the acid from being produced and the damage done. Stephan has shown that urea is effective in preventing a severe drop in pH if applied before a glucose rinse. This observation indicates that dentifrices containing urea should be used before a meal to be most effective.

It has been recognized that urea in high concentrations is a bacterial and enzyme inhibitor, and that its action is not

limited to specific or harmful bacteria. As early as 1906, Wilson³¹ reported that urea in culture medium inhibited the growth of such organisms as *Bacterium coli*, *Bacterium typhosum* and *Bacterium enteritidis*. If the medium contained 0.5, 1, 2 or 3% urea, *Bacterium coli* was as active as it was in medium containing no urea. At 4 and 5% concentrations a fair growth was obtained, at 7% there was little growth and at 8% none.

Stephan (1950)³² investigated, in vitro, the effect of various compounds on the growth of oral microorganisms, and also their ability to dissolve tooth salts. Only slight stimulating or inhibiting effects were observed with Na_2HPO_4 or $(\text{NH}_4)_2\text{HPO}_4$ for the 30 strains of *Lactobacillus* strains, and no significant difference was observed in the effects produced by the sodium or ammonium ions. However, Kirchheimer & Douglas (1950)³³ failed to find any inhibition in the growth of *Lactobacilli* even by ammonia. Kesel (1951)³⁴ pointed out that the failure was due to heavy inoculation of *Lactobacilli* and the reduced amount of ammonia nitrogen available.

The work of Ballantyne, et al (1952)³⁵ indicates that the activity of the ammonia-producing mechanism appears to be unrelated to caries activity as measured by the oral *Lactobacillus* count, and that there is no relationship between the urease content of saliva and the *Lactobacillus* count. These workers further point out that the relation between urease activity and ammonia production is not significant.

ANIMAL STUDIES

The effect of the daily brushing of hamster's teeth with non-ammoniated and ammoniated dentifrices, as well as the effect of ingestion was studied by Chernauek & Mitchell (1950).³⁶ Pertaining to the report of feeding the ammoniated compound to 13 female and 12 male hamsters, the results indicated an inhibition of caries as compared with a control group of 14 male and 14 female hamsters receiving a stock caries-producing diet without any urea or ammonia compounds added. The experimental group received the same stock diet plus 1% dibasic ammonium phosphate and 0.6% urea - the test compounds being in the ratio commonly found in ammoniated dentifrices.

They also carried out experiments on daily brushing of the molar teeth of hamsters with a control and an ammoniated dentifrice. There was a significant reduction of caries experience of approximately the same magnitude in both groups, whereas the ingestion of the compounds incorporated into the caries-producing diet resulted in a further reduction of the incidence of caries.

CLASSIFICATION OF AMMONIATED DENTIFRICES

Ammoniated dentifrices can be divided into two general types :

1. Low urea group; those in which the active agent is

5% dibasic ammonium sulphate, usually accompanied by 3% urea.

2. High urea group: those in which the active agent is 12-22.5% urea and may contain up to 5% dibasic ammonium sulphate as well.

LOW UREA GROUP

Summing up, the rationale given as the basis for the incorporation of 5% dibasic ammonium phosphate into a dentifrice is threefold :

1. Higher concentration of ammonia found in immune caries mouth - this is based on the work of Grove & Grove. This salivary ammonium-caries relationship has been studied by seven groups of investigators, none of whom have found a significant difference between caries-free and caries-susceptible persons.
2. Suppression of bacterial growth by ammonia - it has been suggested that the antibacterial property of the saliva is due to ammonia.
3. Dibasic ammonium sulphate inhibits growth of bacteria - in vitro experiment, such compound can inhibit the growth of *L.acidophilus* on tomato juice agar plates. It was also found that a combination of 5% dibasic ammonium phosphate and 3% urea was more effective as an anti-bacterial and anti-acid agent than was either of these substances alone.

The evaluation of 5% dibasic ammonium phosphate as a caries prophylactic agent should be based, however, on its clinical efficacy. Three studies have been reported to date.

The most effective results, in vitro, were obtained when a combination of 3% urea and 5% dibasic ammonium sulphate was used. This combination was then incorporated in a dentifrice and tested on a group of 33 subjects. The result of this preliminary trial (1948)³⁷ were promising so a large controlled study was then carried out.

The first clinical trial on ammoniated dentifrice was carried out by Kerr & Kesel (1951),³⁴ on 592 children, 10 to 11 years old. The children were divided into 3 groups: the first group brushed their teeth with dibasic ammonium phosphate and urea dentifrice under supervision; the second group, also supervised, brushed with a non-ammonium dentifrice and the third group brushed with a dentifrice of their choice without supervision. From this study, they concluded that "the results... showed a trend which indicates the usefulness of good oral hygiene and of ammoniated dentifrice." However, the proper control group for the experimental group is the group who used a non-ammoniated dentifrice under the same conditions as the experimental group, and when their reduction in caries was compared with the control group with no brushing in the school, there were reductions of 20.5% and 9.3% respectively. Davis & King point out that a statistical analysis of these results reveals that the difference between

these groups is not significant.

In a study of comparable size in New Zealand, Davies & King (1951)³⁸ used similar dentifrices to Kerr & Kesel. In this study, however, the toothpowders were not used under strictly controlled conditions in schools as the previous study. Instead an attempt was made to determine the effect of an ammoniated dentifrice under the usual conditions that apply at home. They found no difference between their experimental and control groups. The main criticism that can be levelled at this study is that it was conducted over 9 months, a too short period of time.

The latest report on this group of low urea dentifrice is by Dirks et al. (1953),³⁹ who studied the effect in 166 school boys, aged 10 to 14 years. The experimental group brushed their teeth with a dentifrice containing 5% urea and 3% dibasic ammonium phosphate under the supervision of the teaching personnel. After one and one-half years there was no significant difference in the caries or the Lactobacillus count between this group and the control.

HIGH UREA GROUP

As mentioned previously, the use of high concentrations of urea in a dentifrice is predicated primarily on the work of Stephan. Henschel had originally presented formulae for a liquid dentifrice containing 45% and a powder

containing as high as 66% of urea. After much experimentation, he found that 22.5% of urea to be the highest practicable concentration of urea, which could safely be incorporated into a dental powder for universal use for home oral hygiene, without irritation.

This group also give conflicting results, but on the whole the results are more promising than those obtained with low urea dentifrices, which can only be concluded as the active agent that have little or no value in the prophylaxis of caries.

The first clinical test of a dentifrice containing a 22.5% urea and 5% dibasic ammonium phosphate was investigated by Henschel & Lieber (1949).⁴⁰ A test group of 100 cases used cosmetic dentifrices an average of 7.8 years, with an average DMF per year of 2.39. After using the therapeutic dentifrice for a period of 34.3 months the average DMF per year was 1.55. Available data do not permit analysis of the yearly DMF increments in terms of increase or decrease with age. A control group of 50 used a cosmetic dentifrice throughout the observation period of 13 years with an average DMF of 2.48. A reduction in the incidence of 37.5% was claimed for the test group over the control group. This study was conducted by examination of records of private patients. Details of the method of examination of each patient were not sufficiently recorded. The importance of oral hygiene in influencing the results was noted by the authors in the statement "some proportion

of the apparent caries-incidence reduction may be attributed to enthusiastic oral hygiene heightened by the knowledge of the possible therapeutic benefit of the dentifrice."

Lefkowitz & Ventri (1951)⁴¹ carried out a 1½-year study of ammoniated dentifrice with a urea content of 22.5%. The study was conducted on 217 children ranging from 5 to 19 years of age. This number was reduced to 120 after one and one-half years. The difference in caries incidence between the two groups at the end of one year was not significant. In the third six-month period the caries incidence of previously non-carious permanent teeth was 60% less than in the control group. The weakness in this experiment lies in the obvious numerical disparity between the 31 patients in the control group and the 120 in the experimental group. The authors do not provide evidence, apart from age, to support the contention that the two groups were similar at the beginning of the experiment.

Gale (1951)⁴² in Scotland tested a commercial ammoniated dentifrice on a small group of pre-school children, under 3 years of age, for two years. The teeth were brushed under supervision after arrival in school and after lunch. Thirty children from six schools formed the experimental group, with another group of thirty children forming the control. At the end of the study, Gale concluded that there was a remarkable retardation of caries in the test group.

Unfortunately, the author does not give sufficient data to make a statistical analysis possible but on the evidence presented, the significance of his conclusion is open to questions for the following two reasons :

1. At the beginning of the experiment the test school children showed almost twice as many cavities as the control school children, 60 and 35 respectively. The groups were thus not similar at the beginning of the experiment.
2. At the end of the experiment, the number of cavities in the test group (89) was the same as in the control group (83), although it is true that the test group showed a lower increase (71% increase in the number of carious teeth) than the control group (161% increase). However, in view of the fact that the test group had a larger number of cavities at the beginning of the experiment than the control group the result could be interpreted as meaning that the test group had relatively fewer "susceptible areas" capable of being attacked. Thus, even if the ammoniated dentifrice under test was completely ineffective one would expect the control group to show a greater increase.

The first significant result was reported by Henschel & Lieber (1952)⁴³. In this experiment the control group of 75 persons used a cosmetic dentifrice and the test

group of 110 persons used a higher urea-ammoniated dentifrice. At the end of the 4-year test period the results were compared with those obtained from the control group. The mean annual increase of carious lesions in the experiment group in the test period of 48.8 months was also compared with the mean incidence during the pre-test period of 101 months when the same patients used a non-ammoniated dentifrice. In both cases a significant reduction in caries was obtained. When compared with its own pre-test experiment, the experimental group showed a reduction of 44.1%, and when compared with the control group the reduction was 43.6%. Of the 110 individuals in the experimental groups 87% experienced a reduction in caries incidence during the test period, 13% showed an increase incidence.

The authors concluded that the use of a high-urea dentifrice is more effective in reducing the incidence of dental caries than a non-ammoniated dentifrice, and that if an ammoniated dentifrice is used before meals it should be more effective than when used after meals, since this will enable the urea to diffuse into plaques before carbohydrates enter the urea.

The results of this study are well documented and carefully analyzed. The results indicate a significant reduction in the sample population studied, but it must be remembered that the sample is a selected one and that this naturally limits the width of inference that can be drawn.

The fact that a successful result has been obtained does not ensure that a similar result would be obtained in the population as a whole.

A dentifrice containing 12% urea and 1% urease was evaluated in a study by Hawes & Bibby (1953).⁴⁴ The urease was added to provide the necessary enzyme for the release of ammonium ions through the decomposition of urea to ammonium carbonate. The clinical trial of the dentifrice under supervised brushing was carried over a period of one year among 372 school children between the ages of 7 and 13 years. This study was supplemented with an eight-week bacteriological study in young women. Brushing twice daily with the urea dentifrice failed to produce any reduction in the occurrence of new caries in 196 children as compared with that in 177 children using a control dentifrice. The difference in the *Lactobacillus* counts between the two adult groups was not significant.

The latest experiment was carried out by Cohen & Sonzanti (1954)⁴⁵ resulting in a 25.2% reduction in new carious lesions in a 2-year period when a group of 169 children used a dentifrice containing 13% urea and 3% dibasic ammonium phosphate. The control group consisted of 137 children who used a neutral dentifrice. The average number of teeth/person which developed new carious lesions during the course of treatment was 1.70 in the experiment group and 2.27 in the control group.

CONCLUSION

1. A low urea ammoniated dentifrice is no more effective than a non-ammoniated dentifrice, and, therefore, has no therapeutic value in the prevention of dental caries.
2. High urea dentifrices appear to be more promising than low urea dentifrices. Final proof is not yet available, due to the inadequate experimental design of some of these studies, and the preliminary nature of the more recent reports on the clinical effectiveness of dentifrices containing high concentrations of urea.

<u>Date</u>	<u>Authors</u>	<u>Test</u>	<u>Subject</u> <u>Control</u>	<u>Age</u> <u>Groups</u>	<u>Super-</u> <u>visions</u>	<u>Period</u>	<u>Reduction</u>	<u>Concentration</u>
1951	Korr & Kesel	483	301	10-11	Yes/No	2 yrs.	9%	3% urea and 5% dibasic $(\text{NH}_4)_2\text{SO}_4$
1951	Davies & King	171	168	2-18	No	9 mon.	nil	As above
1951	Gale	23	23	2-3	Yes	1 1/2 yr.	Significant	
1951	Leikowitz & Ventil	120	31	5-19	Yes	1 1/2 yr.	60% - 6 mon. Nil - 1 yr.	22.5% Urea 5% dibasic $(\text{NH}_4)_2\text{SO}_4$
1952	Henschel & Lieber	110	75	Average 32.5	No	4 yr.	43.6%	3% urea & 5% dibasic $(\text{NH}_4)_2\text{SO}_4$
1953	Haves & Bibby	196	177	7-13	Yes	1 yr.	Nil	12% urea & 1% urease
1953	Dirks et al.	83	83	10-14	Yes	1 1/2 yr.	Nil	3% urea & 5% dibasic $(\text{NH}_4)_2\text{SO}_4$
1954	Cohen & Donzanti	169	137	9-13	Yes	2 yr.	25%	13% urea & 3% dibasic $(\text{NH}_4)_2\text{SO}_4$

TABLE 4. Ammonium salts and urea dentifrices - Clinical trials.

CHLOROPHYLL IDENTIFICATION

Chlorophyll is the major chromogenic material of green plants and is the agent responsible in catalyzing the photosynthesis process. The chloroplasts are the pigment-bearing structures of plants. The solids are composed approximately of protein 80%, lipid 10-20%, and pigments 5%. Considerable amounts of copper and iron are present in the chloroplast. The pigments consist of chlorophyll, the xanthophylls, and the carotene. The role played by the chlorophyll is not properly known. Chemically, it is classified as a porphyrin, and contains magnesium and has a long chain alcohol group attached to a peripheral carbon atom. This is commonly referred to as a phytol group and is linked to the porphyrin nucleus by means of an ester bond.⁴⁶

Numerous reports have been published describing the successful treatment of chronic infected wounds with water-soluble chlorophyll preparations in the medical literatures.⁴⁷ In many cases healing has been indirect where there had been no response to conventional treatment. Whereas chlorophyll derivatives in wound healing are generally regarded as promoters of healthy granulations, and some clinical reports suggested that they have anti-bacterial properties.

Smith (1944)⁴⁷ observed, in vitro, bacteriostasis of Staphylococci, Streptococci, and anaerobic spore-bearers with chlorophyll compounds.

Shafer & Hoin (1950)⁴⁶ also investigated the effect of chlorophyllin on acid production in saliva-carbohydrate mixtures. The experiments showed that chlorophyll was effective *in vitro*, in preventing the falling of pH in saliva-carbohydrate mixtures and effective in reducing the incidence of caries in the hamster. No reduction in oral *Lactobacillus* counts in the animals was observed after the administration of a chlorophyll solution that was effective in reducing the incidence of caries in these animals.

Griffiths & Rapp (1950)⁴⁸ showed that water-soluble chlorophyll is able to lower the *Lactobacillus* counts of the mouth. Preliminary work by these authors also had indicated that other organisms of the oral cavity were adversely influenced by this material.

Nevin & Bibby (1951)⁴⁹ showed that water-soluble chlorophyll exerted a bacteriostatic effect on various oral organisms. The evidence indicated that *Lactobacilli*, *Streptococci*, *Micrococci* were inhibited by various concentrations of water-soluble chlorophyll. They indicated further that the inhibition of respiration is roughly proportional to the concentration of chlorophyll used.

McBride (1952)⁵⁰ demonstrated that various other porphyrins also had inhibitory activity of acid production. It is clear that the inhibition of acid production by the

chlorophyll is not a specific activity, but rather a general property of several porphyrin compounds for certain types of organism, whereas other types of acidogenic organisms are stimulated by these compounds, and that porphyrins directly stimulate or interfere with the synthesis of some enzyme, or is actually included as a co-enzyme or an anti-vitamin.

ANIMAL STUDIES

Shafer & Hein (1950)⁵¹ first showed the effectiveness of chlorophyll on dental caries in animal experiments. The hamsters were given a 1:500 solution of chlorophyll in the drinking water. The results showed 93% reduction of caries in female hamsters, given a 1:500 solution of chlorophyll in the drinking water and a 67% reduction in female hamsters given a 1:1000 solution. No significant difference was observed in males. According to the authors this suggested some usefulness of chlorophyll. No changes of *Lactobacillus* counts in the hamster when administered with such solution were noted. In the same study, chlorophyll was also tested for a systemic influence on caries in 25 animals by administering the drug during the formation of teeth. A 0.2% chlorophyll drinking solution was given to the mothers of these animals from mating to 13 days after birth. The same caries-producing diet was given to the mothers of 26 control animals. At sacrifice, no difference was observed in the mean score of the control and experimental groups.

Shaw (1950)⁵² found that 0.2 or 0.5% sodium copper chlorophyll did not alter the initiation or progress of carious lesions in the white and cotton rats. The only effect was intense green colouration of food debris in molar fissures.

Glass (1952)⁵³ found that brushing the teeth of hamsters with several different copper chlorophyll dentifrices gave significant reductions in comparison with those obtained when control pastes were used.

Hein (1953)⁵⁴ reported that a magnesium chlorophyll appeared to increase dental caries in male hamsters. The latter finding indicates that the metallic portion of chlorophyll apparently plays an important role in the activity at least in so far as hamsters' caries is concerned. Experiments with low levels of copper as copper sulphate have shown it to be highly effective against dental caries in the hamster. It may well be that the small amounts of free copper ions released from certain copper chlorophyll samples, mentioned earlier, accounts for their caries-preventive action.

CLINICAL TRIAL

Werner & Hafer (1952)⁵⁵ studied the effect of chlorophyll dentifrice on Lactobacillus count. 19 patients, 17-43 years old, were examined clinically, bacteriologically and chemically for oral conditions. 11 were re-examined 3 months later after using chlorophyll toothpaste twice daily after eating. The Lactobacillus counts were reduced in some

cases where the original count was high. Caries developed in the high Lactobacillus group in spite of using the chlorophyll dentifrice. However, the author believed that trial in larger groups would show a beneficial effect of chlorophyll on caries.

There has been no clinical study reported testing the effect of water-soluble chlorophyll on dental caries experience.

CHLOROPHYLL DERIVATIVES AS GINGIVAL DISEASE PREVENTIVE AGENTS

Studies of this kind were difficult as there was no satisfactory classification of the periodontal condition, and the soft tissues are constantly changing under the influence of both local and systemic factors.

Four reports of extensive clinical trials are available to date.

The first report was by Rapp (1940)⁵⁶ who showed that the use of a chlorophyll dentifrice was highly effective against periodontal disease if preceded by a prophylaxis. There was no control group in this study.

Costich & Hein (1952)⁵⁷ found that the use of a 0.1% of copper chlorophyll toothpaste by 19 to 22 year old women for 5 months failed to improve the gingival conditions.

McDonnell & Domalakes (1952)⁵⁸ reported that there was reduction in gingivitis in a group of adolescents after using chlorophyll dentifrice for 2 months of regular and routine brushing twice each day, but that continued use over a 9-month period resulted in no advantages over the non-chlorophyll dentifrice group.

The latest report by Kutscher & Chilton (1953)⁵⁹ failed to confirm the effectiveness of such chlorophyll dentifrice on gingiva.

CHLOROPHYLL DERIVATIVES AS ORAL DEODORISING AGENTS

A number of reports of in vitro and in vivo experiments has indicated that certain samples of the chlorophyll possess limited deodorising power. The amount of deodorising effect obtained with any given sample of chlorophyll depends on the concentration of both the chlorophyll and the odour agent. The sulphur-containing odoriferous agents are the most effectively combated by the chlorophyll samples.

In addition, since salivary flow rapidly decreases the concentration of agents in the concentration of agents in the oral cavity. According to Hein the proper concentration of effective samples of chlorophyll derivatives will have a demonstrable deodorising effect against many odoriferous agents present in the oral cavity at the time the chlorophyll sample is placed in the mouth.

VARIABILITY OF CHLOROPHYLL DERIVATIVES

The production of industrial quantities of chlorophyll derivatives is a difficult process. In the methods employed at present, the resulting product is probably a mixture of degradation compounds.⁵³

Much of the research on chlorophyll with samples different from each other in chemical, physical and biological properties. In Hein's work (1954)⁵³ on copper chlorophyll samples, the metallic derivatives most frequently employed, collected from 1947-1952 showed that they differed widely in copper content and, surprisingly enough, that in the majority of cases the copper present was only about 50% of the theoretical amount to be expected. It was further found that the characteristics of the absorption spectra denutralizing power of the derivatives also differed in an irregular manner. Also, no uniform relationship was found to exist between the so-called purity rating of the compounds and their ability to inhibit salivary acid production in the test tube. In addition, further work showed that when certain of the samples were treated with acid they released varying amounts of copper ions. Lastly, it was found that samples varied in their ability to deodorize, some having no capacity in this respect. In view of these findings, it is not surprising that conflicting research reports have appeared in the dental literature.

CONCLUSION

From the above evidence, chlorophyll products for the prevention of dental caries, and gingival disease, or as deodorizing agents, cannot be recommended, despite the presence of chlorophyll dentifrice on sale in the market.

ANTIBIOTICS

Antibiotics is the term to designate the metabolic products of one micro-organism that is detrimental or inimical to the life activities of other micro-organisms, usually even when present in extremely low concentrations. According to this commonly employed definition, anti-microbial substances derived from higher plants or from animals other than protozoa would be excluded from the subject matter of antibiotics.⁶⁰

Antibiosis, therefore, refers to an antagonistic effect of one organism upon another in its vicinity. Antibiosis was observed many years ago by bacteriologists who found that some micro-organisms did not grow well, or perhaps failed to grow at all, in the presence of other micro-organisms. This kind of observation led to the discovery that certain moulds produced substances which inhibited or destroyed bacteria.¹¹

A number of these agents has been used for the treatment of oral diseases and for prophylaxis before surgical procedures. Two types of agents have been incorporated into dentifrices as therapeutic agents. They are penicillin and tyrothricin. The purpose of incorporating antibiotics in dentifrice is twofold: (1) its deleterious effect in bacteria and (2) its deleterious effect in enzymes in the plaques.

PENCILLIN

Fleming (1929)⁶⁰ recognised an appropriate strain of

penicillin grown on a simple medium could produce specific substance, which can be extracted from the medium and endowed with selection of anti-bacterial activities. It was not until 1941 that penicillin came into clinical practice. Since then, it has found usefulness in every branch of the healing arts.

Penicillin is most active on Gram positive bacteria, but some Gram negative forms, such as the Gonococcus and Meningococcus are susceptible to it, as well as most spirochetes and the larger viruses of the psittacosis-lymphogranuloma group. It appears to act primarily as a bacteriostatic agent, though high concentrations may be bactericidal.⁶¹

Bacteria exposed to penicillin become swollen and fail to divide. In treatment of bacteria with penicillin, the antibiotic is irreversibly bound to the bacterial cell. The formation of enzymes concerned with the assimilation of glutamic acid is interfered with, non-amino nitrogenous material appear in the suspending medium and the ratio of ribonucleic acid phosphorus to deoxyribonucleic acid phosphorus decreases. The cell is apparently stripped of ribonucleic acid, and the synthesis of protein is disturbed with the accumulation of polypeptide that may or may not be a normal intermediate in that synthesis.

Penicillin is inactivated by an enzyme, penicillinase, which is produced by a wide variety of micro-organisms especially *Bacillus* species.

With use of penicillin, it builds up resistant strains, and upsets normal flora of the oral cavity. Prolonged administration often leads to monilial infection, in particular when the resistance of the patient is low.

ANIMAL STUDIES

In 1942, Hobby et al.⁶³ showed that in vitro *L. acidophilus* is susceptible to penicillin. Following this, many reports in the literature have shown that administration of penicillin inhibits the growth of a fraction of the normal oral flora, particularly the predominant Gram positive species, and permits a concurrent overgrowth of Gram negative organisms.

In 1946, McClure & Hewitt⁶⁴ demonstrated that dental caries could be controlled by penicillin. They fed 75 units of sodium penicillin per gram of diet, and the same amount per ml of drinking water to rats. Control rats received the same diet and drinking water without the addition of penicillin. After 125 days the experimental rats had no carious teeth, while 50% of the control rats showed caries. Administration of penicillin also resulted in marked reduction of *Lactobacillus* counts. Subsequently some investigators have found reductions.

A similar animal study by Webman and co-workers (1949)⁶⁵ on rats was not able to confirm complete inhibition of caries, although there was a trend towards less caries. The rats were fed on a coarse corn diet, and were given 5 units of penicillin per ml of water, and none in the food.

The effectiveness of penicillin dentifrice was demonstrated by Zander & Bibby (1947)⁶⁵ on hamsters. There was a significant inhibition of dental caries in these animals. It was noticed also that the reduction in both carious teeth and carious areas was consistent when the hamster studies were repeated. Their data also indicated that minute amounts (50 units) of penicillin inhibit acid formation in saliva-carbohydrate mixtures. The capacity of saliva to ferment carbohydrates is eliminated for several hours following the use of a penicillin mouthwash. These authors came to the conclusion that penicillin might have value in combating human dental caries.

CLINICAL TRIALS

A pilot study was conducted by Hill (1948),⁶⁶ who used 10 well selected students to test the effect of a dentifrice containing 1,000 units of penicillin in the inhibition of Lactobacilli. At the beginning of the experiment the students had an average Lactobacillus count of approximately 72,000. At the end of one week the count had dropped to 18,000 and it kept on dropping for 5 consecutive weeks, until it became approximately 300. The students used the dentifrice for an additional week, after which time they were allowed to use any other commercial non-penicillin dentifrice. Following this regime, the count remained at an average of 300 for 3 months and then during the fourth month jumped back approximately to its original value. When the students were put back on the

penicillin dentifrice, their counts dropped in the same degree, only somewhat more slowly.

Fitzgerald et al. (1950)⁶⁷ found that salivary *Lactobacillus* counts of children using a penicillin dentifrice for a year and a half, and a control group using the same dentifrice without penicillin showed no significant difference. The incidence of penicillin-fast *Lactobacilli* was not significantly greater in the group using the penicillin dentifrice.

Ludwick, Fosdick & Schantz (1951)⁶⁸ found that out of 13 potentially therapeutic dentifrices, only the powder containing 0.07% penicillin was the most effective. The oral *Lactobacillus* count was adopted as the criterion for measuring the efficacy of the agents.

By the use of the method of Fosdick and co-workers (1951)⁶⁹ for determining the absorption or attachment of substances to the dental plaque, it was found that penicillin, chloromycetin, oxytetracycline and subtilin were retained on the plaque for several hours. A tooth powder containing penicillin was tested on 60 individuals to see if it would prevent the tooth surface from becoming more acid than pH 5.5. The maximum acidity obtained after sucrose rinse was pH 6- well above the isoelectric point of enamel. Penicillin was retained on the dental plaque for from 12 to 24 hours. (Table 5)

<u>Inhibitor</u>	<u>Inhibitor Concentration mg/100 c.c.</u>	<u>Retention</u>
Tyrothricin	1.0	-
Gramicidin	80.0	-
Streptomycin	0.5	-
Bacitracin	1.0	-
Penicillin	1.0	-
Glyceric aldehyde	10.0	-
Menadione	1.0	-
Citrinin	10.0	-
Copper chlorophyllin	50.0	-
Ammonia	50.0	-

TABLE 5. Substances tested for retention by
dental plaque material.⁶⁹

All these studies give powerful evidence that antibiotics, which inhibit the Gram positive flora and which are retained by the dental plaque have a caries inhibiting effect. Also there is substantial agreement in laboratory and animal experiments that acidogenic flora, acid production and experimental caries can be reduced by a penicillin dentifrice. However, no such unanimity results have been reported in the five clinical trials of penicillin dentifrices on humans.

Hill & Kniesner in 1949,⁷⁰ using a toothpowder containing 1,000 units of penicillin per gram, noted no difference in decay at the end of a year between the control group of 68 and an experimental group of 108 institutionalized boys. Brushing was not under supervision. There was a greater reduction noted in Lactobacillus counts in the experimental group despite the absence of reduced caries incidence.

Zander in 1950⁷¹ reported a reduction of 54% in new decay with a tooth powder containing 500 units of potassium penicillin per gram. The study was conducted with an experimental group of 200 children and a control group of 150. Both groups brushed their teeth once a day under school supervision. Despite the 54% reduction there appeared to be no difference in the Lactobacillus counts in the two groups.

Walsh & Smart in 1951⁷² found that a penicillin tooth powder containing 100 units per gram used under supervision produced no reduction in decay. It was stated that the

concentration of penicillin in this study was too low.

In 1953, Hill and co-workers⁷³ reported a questionably significant reduction in decay (about 15%) with a procaine penicillin toothpaste (1,000 units per gram) used under supervision. There were 492 children involved in the study. No difference in Lactobacillus counts was noted. The procaine salt has a lesser solubility and also is a larger molecule. It would not seem probable that the relatively lesser solubility of the procaine salt would adequately explain the difference. It is possible that the larger molecule of the procaine salt might have a lesser penetrability of the dental plaque. This has not been investigated.

The latest report by Lunin & Mandel in 1955⁷⁴ showed no reduction in decay with a potassium penicillin toothpowder containing 500 units per gram used without supervision. There was no difference between the control and experimental groups in the Synder test for acid production.

Of the 5 studies reported to date, only one (Zander 1950)⁷¹ has a substantial reduction in dental caries incidence been noted. The clinical effectiveness of penicillin dentifrice seems to be questionable. (Table 6.)

SENSITIVITY

It is well known that prolonged topical application of penicillin in the oral cavity will cause formation of

<u>Date</u>	<u>Authors</u>	<u>Subject</u>		<u>Age</u>	<u>Super-</u>	<u>Period</u>	<u>Reduction</u>	<u>Concentration</u>
		<u>Test</u>	<u>Control</u>	<u>Groups</u>	<u>visions</u>			
1949	Hill & Kriesner	108	68	8-15	No	1 yr.	Nil	1,000 units per gram powder
1950	Zander	202	150	6-14	Part	2 yr.	54%	500 units K penicillin G per gram
1951	Valley & Smart	30	25	5-15	Yes	2½ yr.	Nil	100 units per gram
1953	Hill, Sams & Newman	347	145	7-12	Yes	1 yr.	15%	1,250 units procaine salt/gram
1955	Luna & Mandel	77	34	6-12	No	1½ yr.	Nil	600 units K penicillin per gram powder

TABLE 6. Penicillin dentifrices - Clinical trials.

resistant strains of sensitive bacteria, and upset the balance of normal flora resulting monilial infections.

Zander⁷⁵ and Hill⁷⁶ have shown that sensitivity reactions are not frequent and are not serious enough to cause concern. The fact that penicillin dentifrices have been on the market for 3 years without causing serious difficulties points to the same conclusion.

Figure 3, sums up the three studies ⁷⁵⁻⁷⁷ on resistance by micro-organisms.

It can be seen that the curves representing sensitivity of Streptococci in penicillin dentifrice users are further to the right than those in non-users. In other words, in all three studies the distribution of penicillin sensitivity of Streptococci shows that the groups using penicillin dentifrices had more organisms resistant to higher concentrations of penicillin. Yet in all three studies there were organisms in the control groups that were also resistant in the higher unit concentration.

In Hill's study,⁷⁶ a greater percentage of Streptococci was found to be resistant to more than one unit of penicillin in the control group than were similarly compared organisms in the penicillin group in Welch's study and the one by Lind & Zander.

In interpreting these data, we can state that the

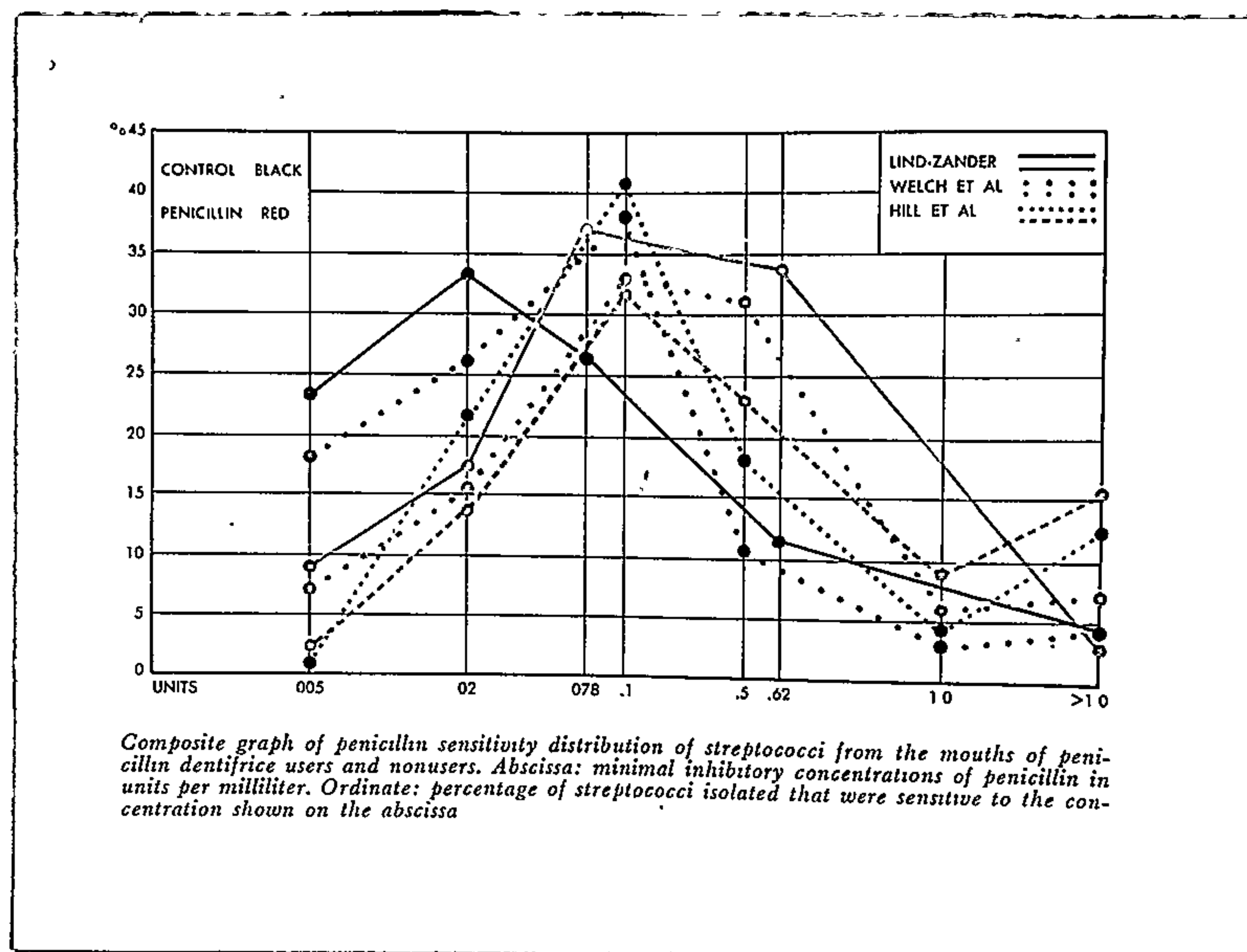


FIG. 3.

Ref. 183.

use of the penicillin dentifrice resulted in an increase in the number of penicillin-resistant Streptococci in the mouths of people using the penicillin dentifrice over those in the control group.

However, there was also a considerable percentage of Streptococci in the control group that were just as penicillin-resistant as the streptococci in the experimental group. The degree of resistance does not appear to be one in which treatment with penicillin would be impossible should these organisms become pathogenic. The chances for these mouth organisms to become pathogenic actually may be very slight. Under no circumstances can these data be interpreted that the mouth organisms build up a tolerance to penicillin as was stated recently by Fosdick.⁸³

Kalb⁷⁸ reported one case of urticaria medicamentosa followed the characteristic course seen following penicillin used in the customary manners. Urticaria appeared between 48 and 60 hours after initial brushing of the teeth containing powder 500 units per gram of crystalline potassium penicillin G. No other significant toxic reactions have been reported subsequently.

CONCLUSION

No substantial evidence available that penicillin dentifrice is effective in preventing dental caries, and although

the practical significance of these hazards has not been fully evaluated, there is a natural tendency to prefer dentifrices containing other therapeutic agents.

TYROTHRIN DENTIFRICE

Penicillin dentifrice trials stimulated interest in test of other antibiotics. Firstly, those antibiotics commonly used systemically were eliminated. In theory, if such systemic antibiotics were used as dentifrice, the body would develop micro-organisms resistant to them and create a general health hazard. Also eliminated were antibiotics known to sensitize the user. Among the remaining antibiotics is tyrothricin.

Tyrothricin was first isolated by Dubos in 1939 from *Bacillus brevis*,⁶⁰ and found to be an alcohol-soluble, water-insoluble polypeptide. It has two components, gramicidin and tyrocidin, both of which contain "unnatural" amino acids of the d series, although they have somewhat different properties.

The mode of action of tyrothricin is similar to that of some anionic detergents, being considered as a surface acting protoplasmic poison or as a specific inhibitor blocking one or more metabolic reactions. Tyrothricin is effective only when placed in direct contact with infecting bacteria.

This antibiotic agent is active in vitro against many gram-positive bacteria, including Pneumococci, Streptococci and some strains of Staphylococci; its activity against gram-negative bacteria is negligible.

The most important toxic effect of tyrothricin is its hemolytic action. This limits its use to topical applications. Agents which appear to control the hemolytic effect also interfere with anti-bacterial activity. Interference with clot retention has been reported following its use in post-extraction socket.¹¹

It has been used in the treatment of infected ulcers, wounds, infections of tonsils, rhinopharynx and other infections. The effectiveness of tyrothricin in Dentistry has not been evaluated. It has been shown to be effective against organisms isolated from infected pulp canals.

ANIMAL AND CLINICAL TRIALS

An in vitro screening programme was applied to a large group of compounds by Shiere (1957).⁷⁹ The results seemed to favour tyrothricin as an inhibitory agent. In contrast to this finding is the published literature on tyrothricin, which is nearly all unfavourable. Negative results were obtained as to its absorption on dental plaque by Fosdick (1951),⁶⁹ its effectiveness in dentifrice for reducing Lactobacillus counts in human saliva by Ludwick (1951),⁶⁸ and its influence in toothbrushing experimental caries in hamsters by Zander (1951).⁸⁰

It seemed possible that these negative results in dentifrice studies could be due to the absorption or the precipitation of the tyrothricin by the polishing agent used in the dentifrice, resulting in a loss of effectiveness of the tyrothricin. It was suggested that the activity of tyrothricin is retained when a component of the polishing agent in the toothpaste is insoluble sodium metaphosphate, and that the degree of retention of activity might depend upon the composition of the dentifrice.

A preliminary study was carried out on experimental animals with insoluble metaphosphate as a component of the polishing agent with reduction of caries in the hamster in comparison with a control toothpaste.

A clinical study was then carried out on children with dentifrice containing 0.05% tyrothricin and the similar polishing agent. The incidence of new decayed and filled permanent tooth surfaces was reduced by 35% after one year and by 26% after a total of 2 years' brushing with the tyrothricin toothpaste. The reduction in caries is highly significant.⁸¹

There has been no other report on tyrothricin dentifrice study appearing in the literature. No verdict can be given on this preliminary work.

ENZYME INHIBITOR DENTIFRICES

The cause of decalcification of enamel is the acid formed by the enzymic degradation of sugars by the oral bacteria, then in order to prevent dental caries, one of the ways is to have a substance on the tooth surface or in the immediate vicinity of the tooth which would block the formation of acid. These substances in general may be termed enzyme inhibitors.

The formation of acids may be blocked by the inhibition of any of the primary enzymes concerned in the initial stages of the anaerobic phase of carbohydrate degradation; or they also may be blocked by the inhibition of enzymes concerned in the metabolic process of the micro-organism associated with the degradation of carbohydrate to lactic acid. If the former is blocked, it would not be expected that the organisms would develop a tolerance, while in the latter case a tolerance is probable.

However, since carbohydrate is an essential nutrient, enzyme inhibitors used in the control of dental caries must have a local effect in the mouth only and must not interfere with the general metabolism of carbohydrate in the body; apart from this enzyme inhibitor must be non-toxic.

Such compounds as vitamin K, urea, ammonia and a host of others are known to interrupt chain of reaction which

is necessary for the production of acid. It is obvious that, in order for the glycolytic reduction reactions to be inhibited the enzyme inhibitors must be present at the time the fermentable carbohydrate is present. This means that the inhibitor may be incorporated into the sugar that is ingested, it could be placed in the mouth immediately before, after, or during the consumption of sugar, or it may be fastened to the tooth surface in some manner so that it will be present when sugars are ingested. A compound suitable for incorporation in the sugar at the refinery would be highly desirable. The introduction of enzyme inhibitors shortly before or after the ingestion of carbohydrates presents the great problem of oral hygiene and diet control and, therefore, necessitates interest and co-operation of the people.

It has been noted by many investigators that the carious lesion always occurs under dental plaque. The dental plaque is consistently present on the unclean surfaces of the teeth. It is difficult to remove and by the usual oral hygiene procedures can be cleaned only from those surfaces accessible to the toothbrush. It is a problem to keep the tooth free from plaque or even its formation. It acts as a sponge to hold the sugars and acids in contact with the tooth surface. In 1949, Fosdick ⁸² suggested that if the dental plaque could not be eliminated or modified so as to be harmless it might serve as agent for the retention of enzyme inhibitors on the tooth surface. If compounds could be found that would absorb, adsorb,

or otherwise attach themselves to the plaque, and at the same time be powerful enzyme inhibitors either in situ or by being gradually released from the plaque, these compounds could be incorporated into an effective therapeutic mouthwash or dentifrice.

A considerable amount of preliminary work has been done in an attempt to find a suitable enzyme inhibitor. Fosdick et al⁸³ studied 381 compounds and found that ten were absorbed by plaques and remained active for several hours. Of these, penicillin, sodium lauroyl sarcosinate, and sodium dehydroacetate were the most efficient in reducing acid production from glucose by bacterial plaques. Volker et al (1954)⁸⁴ and Tonzetich et al (1956)⁸⁵ found that sodium N-palmitoyl sarcosinate, a substance similar to sodium lauroyl sarcosinate (SLS), reduces the solubility of enamel in acid. Volker (1958)⁸⁶ suggested that selected sarcosinates alone or in combination, may combine with both plaque and enamel. In which case they might inhibit acid production by microorganisms resident in plaques and also reduce the solubility of the enamel in acid.

CHEMISTRY & BACTERIOSTATIC ACTIVITY

Sodium lauroyl sarcosinate and N-alkyl sarcosinates are true detergents and wetting agents, having excellent surface tension activity, high cleansing and foaming characteristics and emulsifying properties.⁸⁷

Detergents and wetting agents, in addition to their effects on surface tension, are known to demonstrate various degrees of anti-microbial activity. The most active of these are the cationic agents, usually based on a quaternary ammonia structure. These materials are true germicides but the mechanism of their action has not been clearly elucidated. Many theories have been advanced to explain their mode of action including enzyme-inhibiting effects, interferences with organic electrical charges, penetration of the cell membrane, interference with membrane gaseous exchange or other mechanisms. The theory that surface activity alone is responsible no longer seems tenable.

Both the nonionic and anionic agents usually show greater surface activity than the cationics but not as great a bactericidal effect. Epstein and co-workers were unable to find any marked relationship between quaternary detergents and bactericidal action.

It has been shown that SLS exert bacteriostatic effect against *L. acidophilus* in concentration as low as 0.5%. The sarcosinate has also been shown to have at least slight bacteriostatic effects against other commonly oral microorganisms including *Stap. aureus*, *Stap. pyogenes*, *Bacterium ammoniagenes*, and *Candida albicans*.

SLS or urea is absorbed on (or diffuse into) the salivary sediment and exerts an anti-glycolytic effect despite

the intermediate water wash. The combined activity exert a more pronounced effect than either material alone. This is due to urease activity, which raises the pH.

The only enzyme occurring naturally in physiological significant amount in the mouth is ptyalin from saliva. SLS has been found to inhibit ptyalin activity but to no greater extent than other commonly used dentifrice detergents.

Brudevold and Little (1954)⁸⁸ measured the fall in pH of the plaque material from proximal surfaces following a sugar rinse in subjects who were instructed to brush their teeth and rinse with a solution of SLS in the morning upon rising and before going to bed at night for seven days. Similar tests were performed after the subjects had been using toothpaste containing SLS and dehydroacetate for one week. Under the above test conditions neither of these procedures prevented a drop in the pH of the dental plaques. In another experiment the inhibitory effect of single mouth rinses of lauroyl sarcoside was studied. Measurement of pH of plaque material by an intra-oral technique showed that this compound exerted an inhibitory effect on the fall in pH after one-half hour.

CLINICAL STUDIES

A preliminary clinical study on the effectiveness of SLS involving 2,265 19 to 35-year old individuals was

reported by Foadick (1953)⁸⁹. A group that brushed their teeth according to their personal choice and a group that brushed their teeth under supervision using a placebo dentifrice comprised the controls. The experimental group was divided into one section that brushed their teeth twice a day and those that brushed their teeth after meal times, both groups using the test dentifrice. Thus, the two groups were not comparable in respect to toothbrushing procedures. At the end of one year a comparison between the test and control groups (combined) indicated that the increment of new carious lesions were 0.29 and 0.67 respectively, or a 57% reduction compared to the controls.

In 1956, Foadick reported a 2 year series of toothbrushing trials using SLS morning and night or after meals, which gave a 48% reduction in dental caries.⁹⁰

In 1948, Frasher & Hein⁹¹ reported a 27-month study on 365 Mexican children. The control group of 184 children, averaging 8.35 years of age at the onset, was supplied a dentifrice containing a detergent 2% sodium salt of sulphated glyceride or cocoanut fatty acids. The 181 children in the experimental group, averaging 8.23 years of age at the onset received the same dentifrice except substitution of 2% SLS as the detergent. A 48% reduction of caries in DMF, and 43% reduction in DMFS were noted.

Another study was conducted by Hayden (1959).⁹² The control group, averaging 11.6 years of age, used a dentifrice containing 2% sulphated, hydrogenated cocoanut oil monoglyceride as the detergent. The experimental group averaging 11.8 years of age, used the same base dentifrice, but with SLS as the detergent. At the end of a year, 332 subjects were available for re-examination. Only interproximal caries were evaluated, by posterior bite-wings and anterior vertical films. The experimental group showed a mean of increment of 0.67 DF interproximal carious surfaces compared to 1.09 in the control group. The difference was not statistically significant.

Backer-Dirks (1959)⁹³ compared the effectiveness of 2% SLS and sodium lauroyl sulphate in 174 boys between 10 and 13 years old in a Belgian boarding school. Proximal carious cavities were evaluated from radiographic examination, whereas occlusal cavities were diagnosed by direct observations. After 20 months, the incidence of proximal carious lesions in both groups was approximately equal in number as well as in distribution. The number of new carious lesion in the occlusal surfaces was slightly less in the experimental group than in the control group. The difference was not statistically significant.

CONCLUSION

At the present time dentifrice with SLS seems

unlikely to prevent or reduce dental caries in children or young adults, as shown by the four studies mentioned.

(Table 7.)

SODIUM DEHYDROACETATE & OXALATE

Sodium dehydroacetate has also been suggested as an anti-caries agent because like sodium lauroyl sarcosinate, when incorporated in a dental plaque it prevents the breakdown of fermentable sugar. Fosdick (1953)⁸³ in his preliminary experiments indicated that sodium dehydroacetate would attach itself to microbial plaque on the tooth surface and to the epithelial tissue of the mouth and persist in situ. The presence of sodium dehydroacetate could be detected for 48 hours and the effectiveness in preventing acid formation on the tooth surface persisted for from 12 to 24 hours. However, Brudevold⁹⁴ and Hein⁹⁵ partially confirmed this, and Foscher & Hess failed to do so.⁹⁶

Sulser (1958)⁹⁷ reported a clinical trial of a dentifrice containing 0.75% sodium dehydroacetate and 0.5% sodium oxalate were made on 22 to 24 year old subjects as follows :

1. 483 used the test dentifrice morning and evening.
2. 369 used the test dentifrice within 10 minutes after each meal.
3. 307 used a placebo dentifrice morning and evening.

4. 135 followed their usual oral hygiene routine.

Clinical and X-ray examinations were made before and after one and two years testing. Caries was reduced significantly by about 50% by the test dentifrice. Brushing after meals was slightly better than morning and evening.

No conclusion can be drawn from this single study on the effectiveness of sodium dehydroacetate and oxalate dentifrice.

<u>Year</u>	<u>Author</u>	<u>Subject</u>	<u>Age in yrs.</u>	<u>Period in yrs.</u>	<u>DMS % Reduction</u>
1956	Fosdick	1159	19-35	2	48
1948	Frasher	565	8.3 (aver.)	$2\frac{1}{2}$	43
1959	Hayden	532	11.6 (aver.)	2	-
1959	Backer-Dirks	174	10-13	$1\frac{3}{4}$	-

TABLE 7. Clinical trials of SIS dentifrices.