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CARIES INHIBITORY EFFECT OF FLUORIDE CO-CRYSTALLIZED SUCROSE - ESTABLISHING A FIELD TRIAL -

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SUMMARY

CARIES INHIBITORY EFFECT OF FLUORIDE CO-CRYSTALLIZED SUCROSE
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There is growing evidence that dental caries prevalence is increasing to epidemic proportions in many developing countries. Increasing economic growth, and the resultant ready access to information and products from developed countries, often results in a greater tendency for increased reliance in the diet on fast, refined foods, and reduction in the more natural traditional eating patterns. There is a great deal of evidence to show that this move to increased volume and frequency of refined carbohydrate consumption leads in most instances to a rapid increase in dental caries prevalence. Unfortunately, in developing countries, such changes are not followed by improvements in dental health services and education.

However, caries has been largely controlled in most developed countries, not by provision of treatment, but by the use of relatively simple public health preventive methods, in particular the widespread use of systemic and topical fluorides. Furthermore, this high level of reduction in caries prevalence has been achieved while the volume and frequency of refined carbohydrate consumption has remained relatively unchanged. Yet many of the methods that have been used to deliver fluoride ion to the population are not suitable for use in developing countries.

For example, in Indonesia, water fluoridation is not feasible because of the widely dispersed population, the wide use of well water,

and the very hot climate compared with most developed countries. Although tooth pastes are available in urban markets, they are not widely used by the population because of cost. Also dental health is not a high priority for many people.

Yet, the experience in developed countries leads to the conclusion that the widespread delivery of the fluoride ion in low dose to the population, would be the most cost and benefit effective method of controlling caries progression. Those methods which appear most suitable to achieve this in developing countries are the use of salt or sugar as fluoride vehicles. There is little information on the level of distribution and the utilization of salt in Indonesia, and this might be quite variable because of the wide diversity of cultural sub-groups who make up the population. Sugar would seem to be a most practical vehicle, its use being a major cause of caries development. However it has not been used on a population basis as yet, and there remains some concerns as to the economical, ethical and even political aspects of its use in such a way (Bratthall and Barmes. 1995). Yet, it has been proven very effective in the one human study so far conducted (Luoma et al, 1979), and in numerous animal studies (Mundorff, et. al., 1988, Bowen and Pearson, 1992; Luoma, et. al., 1972).

Considering the urgency of dealing with the rapid increase in caries prevalence in parts of Indonesia, the objective in this project was to design and implement a field trial, to test the effectiveness of a 10 ppm fluoride co-crystallized sugar in inhibiting caries. If successful, such a trial may assist in the conducting of more widespread trials in other developing countries, and provide support for the introduction of this method as a potentially inexpensive, though effective public health preventive method.

The primary requirement of such a trial is the safety aspect and a year was spent in :

1. conducting laboratory studies to determine that concentration of fluoride ion which would be most effective in caries inhibition, yet be clearly safe in terms of toxicity and in terms of providing minimal risk of fluorosis in young children.
2. determining the method of its combination with sugar to provide a homogenous product concentration and ready availability to the oral hard tissues.
3. selecting potential test populations, and determining their dietary patterns, background fluoride intake levels and ensuring that strict control could be maintained on sugar consumption.

The clinical trial was carried out in Medan, Indonesia in which subjects were children, 7-19 years of age, of mixed gender, living in two orphanages and a "pesantren" (a kind of boarding school) over a duration of 18 months period. The children were divided into a control group who had ordinary sugar and a test group who consumed a 10 ppm fluoride co-crystallized sucrose. Each child consumed approximately 60 gms sugar per day incorporating in the daily menu.

Data for DMFS scores were obtained, using a double blind clinical evaluation format, before and after 18 months utilization of fluoride co-crystallized sucrose. Analysis of Variance with significance level $p < 0.05$ were used to analyse the changes in the DMFS scores and their increments over the duration of the trial. Caries progression data calculated from the progress of the D, M and F components and the initiation/reversal of initial carious lesions, were also analyzed.

Analysis of Variance with significance level $p< 0.05$ revealed that significant increases in DMFS scores were found in the control and test groups, though the caries increment was significantly higher in the control group. Caries progression rates were significantly higher in the

control group. Development of initial carious lesions was found in the control group, while a slight reversal of such lesions was detected in the test group.

To assist in safety control, urine analyses of fluoride ion levels were carried out before the study and at six monthly intervals in sample individuals in both groups. A significant increase in urinary fluoride level was detected in the test group after a one-year period, while there was a tendency for a decrease in urinary fluoride concentration in the control group.

A further test carried out was to investigate whether 10 ppm co-crystallized sugar resulted in remineralization of previously artificially demineralized carious lesions in sterilized enamel slabs, bonded to the buccal surfaces of maxillary first molars of randomly selected subjects in both groups over a three week period. Previous *in vitro* tests , using an artificial remineralizing solution with 10 ppm fluoride in sugar had resulted in a substantial level of remineralization over 28 days. However the result of the *in vivo* experiment revealed an insignificant level of remineralization had resulted from the use of a 10 ppm fluoride co-crystallized sucrose.

Overall, the presence of 10 ppm fluoride co-crystallized with sucrose resulted in a significant difference in caries development between control and test groups. This result looks very promising, even though it needs to be viewed with caution, due largely to the short duration over which it was achieved. Futher field trials are needed, involving larger number of subjects over longer periods of time.

As the present study was carried out in a closely confined and controlled population of subjects, it would be preferable for studies involving local population groups, e.g. in a village, to be carried out. However, all such applications must consider very carefully all sources

of fluoride ion available and ensure safety in the overall levels of fluoride utilization.

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Signed : Date:

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CHAPTER I

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

I. THE POTENTIAL EPIDEMIC OF DENTAL CARIES IN DEVELOPING COUNTRIES

A. The Historical Spread of Dental Caries

The existence of dental caries was first recorded centuries ago, and it still continues to be a public health problem, even though much effort had been devoted to controlling it. For example, dental problems were reported by ancient populations which lived around 3500-3000 BC, such as the Mesopotamians, Phoenicians, Hebrews, and Egyptians. Various efforts and remedies were invented to alleviate the disease, usually without success (Ring, 1985). Ancient populations in Greece (Krikos, Cit. Nikiforuk, 1985) and England (Moore and Corbett, 1971,1973,1975) also suffered an increasing prevalence of dental caries.

Dental caries is a disease which is associated directly with the life style of an individual or a nation. Increasing affluence that leads to an increasing availability of refined food containing rich carbohydrate is believed to have caused the spread of the disease (Mandel, 1985). Caries until recently was regarded as a problem mainly in developed

countries, and in the 1960s it reached epidemic proportion in sections of those countries. Surveys in the USA in the 1980s showed that 20% of the children had nearly 60% of decay and in the UK, 60% of the DMFT was found in 25% of children (Bowen and Tabak, 1993).

As economies and technology of many previously underdeveloped countries now begin to develop rapidly, this leads to increased affluence. This subsequently leads to the availability of, and ready accessibility to, refined carbohydrate, and their people soon adapt to an easier way of life, purchasing highly processed foods, and bypassing traditional natural foods, and their preparation.

Data from many developing countries reflect a considerable increase of caries prevalence in recent years. In particular, caries prevalence in young children under 5-year of age is very high in some countries in Asia. In North East India the dmft score was 5.3/6.4 for urban and rural children respectively (Tewari, et. al., 1991). Thailand, Vietnam, China and Indonesia are countries with high dmft scores ranging from 6.3 in China to 8.4 in Thailand (Stephen, 1993). Vietnam showed a dmft score of 8.3 (Stephen, 1993) and a dmft score of 7.98 was recorded in West Java, Indonesia (Koloway and Kailis, 1990). This tendency to high caries prevalence has continued into the permanent dentition stage.

In Nepal, a country situated in the Himalayas, the mean population DMFT Score was 0.2 in 1977, and increased to 2.1 in 1985. This data shows a tendency toward increasing caries prevalence with age, for example the DMFT was 0.8 for 12 year-olds, 1.0 for 16-19 year old and 4.0 for 35-44 year olds (Tewari, et. al., 1991). Other example of high caries prevalence are DMFT scores of 3.0 to 6.4 in Thailand (Tewari, et. al., 1991), and 2.63 and 5.09 for 14 year olds and 35-44 year olds respectively in 1988 in Indonesia (Indonesian Department of Health

Report, 1992), 3.1 in Burma and a high DMFT score of 9.4 in 35-44 year olds in Sri Lanka in 1984 (Tewari, et. al., 1991).

It is important to note that the last 20 years has seen a decline in dental caries prevalence in developed countries. This has been achieved largely through the widespread use of systemic and topical fluorides (Newburn, 1989). Unfortunately in developing countries, the prevalence of dental disease is becoming greater and access to fluoride in any form as a major preventing substance is not possible to most people. In fact, there is of a limited knowledge of, and interest in the importance of avoiding dental disease. Its control remaining a low priority in most of those countries.

As the preceding data has shown, in many developing countries today, caries is a problem of all age groups. Depending on the oral environment, dental caries can be detected from early childhood to old age. Caries in preschool children is a huge problem in some populations, with rampant caries already present in children under two years (Koloway and Kailis, 1990; Matee, et. al., 1994). This will cause many long term oral and general health problems to the children involved. Caries in the deciduous teeth often will spread into the early permanent dentition (Johnsen, et. al., 1986). This condition continues into adulthood and the elderly where coronal caries gives way to root caries as the major problem, leading in most cases to a high level of tooth loss by mid life.

It is becoming obvious that dental caries may affect any individual from any population, given the right set of aetiological circumstances. Even in the era of declining dental caries in developed countries, still some groups of the population are at high risk of developing caries, and every attempt is made to diagnose the relevant cause, introduce preventive measure, and supply treatment.

At the present time, dental caries remains a significant problem in many developed countries, and is becoming a huge problem in many developing countries. It will reach epidemic proportions in those countries, unless a proper preventive program which is suitable to local conditions can be provided.

B. Evidence for Its Association with Increasing Levels of Refined Carbohydrate

Evidence of the relationship between sugar and dental caries has been obtained from laboratory experiments, animal and human studies as well as from epidemiological data.

From animal studies, it was found that sucrose showed the highest cariogenic potential (Mundorf, et. al., 1990), and its effect was shown to be more severe with the consumption of higher concentrations of sucrose (Luoma, et. al., 1982). Other experiments showed that sucrose was the most cariogenic substance compared to other sugars such as lactose, mannitol, sorbitol and melibiose (Koulourides, et. al., 1976). *In vivo* experiments also showed the effects of sucrose in plaque pH of either animals (Bowen and Pearson, 1967), or humans (Rosen and Weisenstein, 1965; Oliveby, et. al., 1990), in bacterial plaque colonization, and in increased bacterial counts (Mundorff-Shrestha, et. al., 1994).

Dental caries has reached epidemic proportions in many countries of the world over the last century. There is a great deal of epidemiological and clinical evidences to implicate the availability of refined carbohydrate, and its increasing availability in many countries, as the major contributing factor. For example, a three fold increase in sugar consumption was recorded in Britain from 1830 to 1880 and this rate continued to increase into the 1900s (Deerr, 1950). Corbett and

Moore (1976) recorded the increasing prevalence of fissure and smooth surface caries which coincided with this rapid increase in consumption of sugar.

Cause-effect evidence has come in particular from an introduction of refined food to many isolated communities, eg. Eskimoes, and the inhabitant of the remote island, Tristan da Cunha, where the populations had not experienced caries before the introduction of sugar, and which subsequently developed in epidemic proportions in these populations (Newburn, 1989).

Further epidemiological evidence for the association of increased sucrose consumption and caries experience came from Great Britain, where sugar consumption increased steadily in the last 100 years. This increase has been parallel with the increase of caries prevalence. The evidence of a positive relationship between sugar and dental caries was further strengthened by the decrease of caries prevalence following a reduction of sugar availability during World War II (Toverud, 1964: Takeuchi, 1961).

During the middle of this century, caries was clearly recognized as a side effect of increasing affluence, and the more developed countries had the highest prevalence rates, or DMF scores. In Australia, mean DMF Scores in the 60s reached amongst the highest level in the world. Sugar consumption at the same time reached approximately 50 kg/person/year, and this level of consumption has been maintained since then (Sivaneswaran and Barnard, 1993).

Newburn (1982b) summarized evidence to show that sugar consumption and dental caries are related, after reviewing studies correlating both factors. The dynamics of sucrose metabolism by cariogenic organism, investigations in experimental animals and clinical observations of the interrelationship of dietary sucrose intake and caries experience provide compelling evidence that the proportion of

sucrose in food, and the frequency of its consumption, are important determinants of its cariogenicity.

The Committee on Medical Aspect of Food Policy (COMA) in England was convinced that sugars are the most important dietary factor in the cause of dental caries (COMA Report, 1990), and that the amount and frequency of sugar consumption became risk factors for children susceptible to approximal caries (Burt, et. al.,1988).

Sheiham (1991) has proposed that the dose-response curve for sugar and caries is approximately sigmoidal (S-shaped). On the basis of his collected data, it was proposed that an acceptable level of sugar consumption is about 15 kg/person/year, without caries developing widely. If fluoride is readily available (Sheiham, 1991), an intake of approximately 25 kg/person/year could be tolerated without caries developing as a major problem in the community. This was placed at approximately 50 gms per day by Sreebny (1982).

Sundin and Granath (1992), also implicate sugar as the primary etiologic factor in dental caries development and its presence around the plaque covered tooth surface is essential for more than very limited caries development (Rugg-Gunn and Edgar, 1984). This is further confirmed in a study using dietary diaries which showed a significant relationship between DFS and sugar consumption (Akpata, et. al., 1992).

A common thread throughout these epidemiological studies has been the increasing availability of sugar and its increasing frequency of consumption with progressive affluence within a country (Naylor, 1986).

C. Fluoride as the Major Control Mechanism in Developed Countries

The incidence and prevalence of caries in developed countries in the mid 1900s was so great that there was a determined effort to introduce public health control measures. Those found most effective were the introduction of systemic and topical fluorides, and since their introduction, there have been dramatic reductions in caries incidence and prevalence recorded where ever they have been introduced (Naylor and Murray, 1989).

It was the discovery of fluoride in drinking water as the cause of endemic fluorosis (Dean and Elvolve, 1936), which lead to a separate finding that concentrations of 0.7 - 1.0 ppm of fluoride ion in drinking water could provide a high level of prevention of dental caries, in populations of children consuming that water from birth (Dean, 1938; Dean and Elvolve, 1942).

The initial trials of artificial water fluoridation at Grand Rapids - Michigan, Michigan USA, using 1.0 ppm F ion, generated about 36.4 - 59.8 per cent reduction in caries experience in 6-9 year-old children over a 5-year period in the experimental town in comparison to the control with 0.1 ppm F in natural drinking water (Dean, et. al., 1950). Over the period of 15 years of water fluoridation, total caries experience was reduced by 50 - 60 per cent in children aged 12-14 years (Arnold, et. al., 1962). This was followed by fluoridation of drinking water in other countries outside America, such as New Zealand and Australia, and to a lesser extent Europe.

However, with the subsequent widespread introduction of fluoride containing tooth paste in the 1960s in most developed countries, even greater levels of caries reduction have been recorded. A considerable reduction of caries prevalence associated with the used of fluoride tooth pastes was reported from European countries such as Denmark

(Fejerskov, et. al.; 1982), England (Anderson, et. al.; 1982), Ireland (O'Mullane, 1982), Netherlands (Kalsbeek, 1982), Norway (Von Der Fehr, 1982), Scotland (Downer, 1982) and Sweden (Koch, 1982). All reported a decline in caries prevalence from 1960's to 1980. Studies in the USA (Brunelle and Carlos, 1982) and New Zealand (Brown, 1982) found similar trends toward the decrease of caries prevalence in both countries.

Some of the most dramatic have been in Australia, where the percentage of children 12 years and under who developed no new caries each year over the period 1977-1986 increased from 48.6% in 1977 to 79.5% in 1986 (Carr, 1988). In South Australia, the number of new carious lesions developing in 12 year olds reduced from 7.86 per child on average in 1970, to 0.25 per child respectively in 1994, in fluoridated regions, and just slightly higher in non fluoridated regions (SA Dental Service Report, 1994).

Fejerskov, et. al., (1981) was the first to note the fact that caries prevalence was dropping almost as rapidly in regions where no water fluoridation was used, as in those areas where it had been introduced. It has now been recognized that the widespread, daily use of fluoridated tooth paste can result in even greater levels of reduction in caries experience through its topical (post-eruptive) action than can be achieved by systemic water fluoridation (pre-eruptive).

At the same time as there have been significant reductions in caries prevalence in developed countries despite small or almost non existence changes in sugar consumption in these countries, there have been marked increases in caries experience recorded in many developing countries. These changes have been generally summarized by Elderton (1990) in the following graph (Figure 1, over page). It is interesting to note that this graph predicts significant controls of caries before it reaches the epidemic proportions which were recorded in

developed countries. Whether this occurs or not will depend on the efficiency with which preventive methods, found to work in developed countries, can be adapted to work in these countries. The objective of this project is to investigate the effectiveness of one way in which this might be achieved.

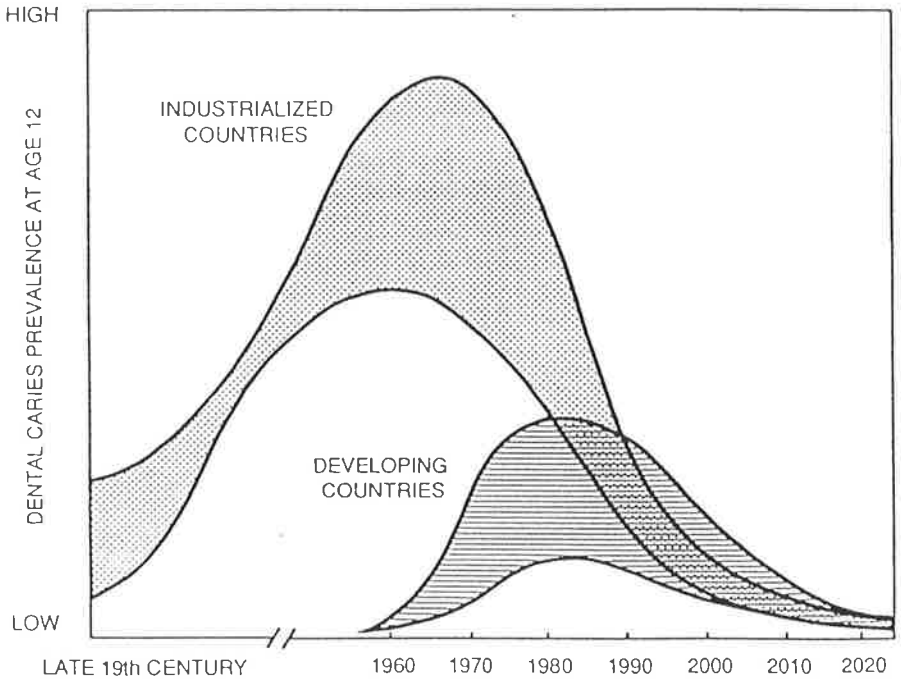


Figure 1: Past and projected dental caries experience at age 12, in developed and developing countries (Elderton, 1990).

D. Summary

Data has been presented to show that there is a great risk of dental caries increasing rapidly to epidemic proportions in many developing countries, at the same time as it is declining from such proportions in most developed countries. There is voluminous evidence to associate an increase of dental caries prevalence with increasing frequency of consumption of refined carbohydrate in most countries, though occasionally local increase can be associated with consumption of acidic or extremely starchy foods peculiar to a confined population. Yet, it is important to realize that caries incidence in most developed countries has declined significantly despite maintenance of the same high levels of consumption of refined carbohydrate which originally caused the epidemic.

In order to understand how this has been achieved, and to determine what might be the most appropriate methods of prevention to employ in developing countries, it is firstly necessary to review our current knowledge of caries, and mechanisms for successful prevention, both at the laboratory, and clinical and population level.

II. MODERN CONCEPTS OF THE NATURE AND AETIOLOGY OF DENTAL CARIES

A. Development of the Modern Concepts of Caries as a Dynamic Process

Theories of the etiology of dental caries developed as early as 5000 BC. The earliest theory known was "The Legend of Worm" as the cause of dental caries. Since that time, many different theories have emerged such as, the Humoral theory, Vital theory, Exogenous theory, Parasitic theory and others. One of the latest theories has been the Chemoparasitic theory developed by Miller (1890), which became the base of our modern concept of caries etiology.

In Miller's theory, dental decay was a two-stage process: decalcification of the tissue followed by the dissolution of the softened residue. This initial concept of dental caries as the resulting accumulation of a continuing process of demineralization, which once initiated could not be reversed, prevailed until the 1970s. It was during this time that an increased understanding of the nature of the incipient caries lesion in enamel from *in vitro* investigations, led to the realization that caries is a dynamic process involving changing levels of demineralization and remineralization (Silverstone, 1983; Featherstone, et. al., 1979) on a daily basis.

Critical to this understanding is the concept that the apatite demineralization process is reversible, resulting in the potential for reformation of enamel crystallites. Several factors in the mouth surrounding the tooth enamel control this equilibrium, and determine whether an initial caries lesion is developed, progresses to cavitation or is reversed.

Backer Dirks (1966) had previously presented clinical evidence suggesting that, it its early stages, enamel caries changed its activity

status quite often, though the significance of his study was not recognized until later. He found, for example, that of 72 white spot lesions noted in a group of children at age 9 years, 37 had reversed by age 15 without treatment, 26 had not changed in size and appearance, and only 9 had progressed to caries.

The current concept of caries then, is that each day as we eat, there is some fermentation of any carbohydrate present, resulting in acid production which causes some degree of surface enamel demineralization (Featherstone, 1987). However, where acids level produced are not excessive, salivary flow is normal, and its bufffering capacity and Calcium/Phosphate ion concentration normal, there is a rapid neutralization of produced acids and repair of any demineralized apatite structure in teeth. Caries progresses to an irreversible form (cavitation) in time if, over a prolonged period, the degree of demineralization is such that the natural and enhanced protection levels are insufficient to balance the degree of acid present at the tooth surface and its demineralizing effects.

On a population basis, the most common reason for caries to progress is that an increasing frequency of refined carbohydrate consumption causes an imbalance in the amount of fermented acid produced, which is beyond the capacity of the natural protective and repair processes to cope. At the individual level, however, there are many factors which can influence this demineralization/remineralization balance, such that a clinician may have difficulty in recognizing the multifactorial aetiology relating to this problem.

B. Intra Oral Factors Affecting the Demineralization-Remineralization Balance

1. Nature of tooth substance and anatomy

Enamel is the most highly mineralized tissue, consisting of 96% mineral and 0.4% - 0.8% organic material and 3.2% - 3.6% water by weight (tenCate, 1979). The inorganic content of enamel consists of a crystalline calcium phosphate salt known as hydroxyapatite (HA) or $Ca_{10}(PO_4)^6(OH)^2$. This salt is soluble in a certain pH range, depending on the nature of the environment at the tooth surface. The concentration of calcium is about 36% by weight, and of phosphorus about 18% by weight. Phosphorus occurs as a phosphate, $(PO_4)^{3-}$ and about 5% of the total inorganic phosphorus is in the form of $(HPO_4)^{2-}$ (ten Cate, 1979)

When teeth first erupt, the apatite contains many impurities, approximately 2.5% by weight of carbonate $(CO_3)^{2-}$; 0.6% Na; 0.3% Cl; 0.35% Mg and 0.03% K; as well as many other trace elements (Nikiforuk, 1985; Eanes, 1979). These impurities can be tolerated without changing the structure of the apatite crystall, for example sodium, magnesium and potassium may exchange for calcium, fluoride for hydroxyl ions and carbonate may replace hydroxyl and/or phosphate groups (ten Cate, 1979).

Some variation in concentration of minerals in enamel exists because of differences between persons, between teeth and even between parts of the same tooth. But in general, the components of mineral in enamel and cementum/dentine do not vary significantly between people, except where localized regions of hypoplasia are present (ten Cate, 1979).

As stated earlier, critical to the current concept of caries is the equilibrium nature of the acidic dissociation of apatite. Those factors

which most influence the reversal phase of the equilibrium reaction are reduction in pH levels, excess of calcium and phosphate ions and the presence of fluoride ions (Featherstone, et. al., 1979). This combination of factors is now regarded as the main factors controlling the rate and nature of the demineralization process (Featherstone, et. al., 1979; Thylstrup and Fejerskov, 1986).

It is important to recognize that the caries process is considered to be of a similar chemical nature in cementum and dentine (root and coronal) except that the end result differs considerably from that in enamel. The main difference is due to the differing mineral components of both tissue. (enamel 96% by weight; 86% by volume, cementum and dentine, 75 % by weight, 45 % by volume). Thus, when mineral is lost in cementum/dentine, the structural protein base (collagen) remains intact if hydrated, resulting in a softening of the surface to a "leathery" type of consistency (Newburn 1989), with much less marked surface hardening characteristics. On the other hand, remineralization can result in a reversal of this lesion, with a "rehardening" of the surface texture (Johansen, et. al., 1987).

A further factor relating to teeth is the anatomical structure and there is wide spread evidence showing pit and fissure lesions to be the first to develop when an increasing sugar frequency results in a cariogenic diet. (Moore and Corbett, 1971).

2. The oral environment of the teeth

This consist of a variety of factors which may be both potentially protective or destructive.

i. Saliva, is the most significant of the host protective factors

ii. Plaque

iii. Diet

i. Saliva: the major source of protective/repair factors

Saliva is fluid secreted by salivary glands. The major production of saliva comes from the parotid, sub-mandibular and sub-lingual glands. It covers the mouth as a thin film about 1/10 - 1/100 mm thickness. In adults the secretion of saliva is about 1 - 1.5 L per 24 hrs, though this varies according to the state of the body. During sleeping only around 0.25 ml/minute is secreted, while in chewing or conversation the secretion rate can reach to 7 ml/min. Organic, inorganic and cellular components in saliva vary between individuals. One ml saliva contains approximately 200 million organisms from 250 different species which build up a complex microbiota in harmonious equilibrium and balance.

The most convincing clinical evidence for the protective role of saliva is the serious and rapid damage to tooth structure which can result from its sudden loss, e.g. from drug or radiation induced loss of saliva (xerostomia). It is acknowledged that this result can be increased substantially by altered dietary patterns resulting from the dry mouth. There is now a substantial body of evidence to demonstrate that salivary factors in themselves play a major role in protecting the teeth against acidic challenge. The main protective constituents relating to this role are:

- The Ca or PO_4 ions, usually present in concentrations which are supersaturated with respect to enamel apatite at around neutral pH; and hence enhance the repair of partly dissolved apatite crystals. The PO_4 ion also provides a significant buffering capacity at resting pH and in the early stage of acidic challenge (Thylstrup and Fejerskov, 1986)
- Those proteins, forming a pellicle layer on the teeth which inhibit mineralization of apatite from the supersaturated levels of Ca and HPO_4 ions in saliva on the tooth surface (e.g. formation of calculus).

Pellicle also acts as a barrier to diffusion of acid ions into the tooth, or efflux of dissolution products of apatite out of the tooth. Evidence supports the concept of a well established pellicle providing a very high level of protection to the tooth surface against carious challenge (Thylstrup and Fejerskov, 1986).

- The bi-carbonate buffering system. This is the main buffering system in stimulated saliva, and contributes a high level of protection against both organic and the stronger erosive acids meeting the tooth surface.
- A further protective factors is salivary clearance of food, microorganism, and other debris from the mouth. Unfortunately a high salivary clearance rate will also remove topically applied fluoride from the mouth, and thus may increase the amount of topical fluoride required, to maintain optimal levels for tooth protection. However, overall, it provides a significant contribution to natural protection against caries.
- The fluoride content, though low (0.03 ppm on average) is considered on the basis of latest evidence to contribute to the overall protection and repair of tooth mineral (Featherstone, 1991).

The amount and quality of saliva secreted varies throughout the waking day and is depressed during sleep. Unstimulated flow rate saliva (on average 0.3 ml/min.) contains little bicarbonate buffer (1 mMol/l), and less Ca ion, though more PO_4 ion than plasma. Stimulation by chewing or eating, or through acidic food, e.g. citric acid, can cause up to 7 ml/min. to be produced, though on average within a population, it is more in the order of 1.5-2.5 ml/min.

Bicarbonate buffer concentrations can increase sixty times on stimulation, Ca ion levels increase, though PO_4 ions do not increase proportionately to flow rate (Kleinberg, et. al., 1982).

It is now generally accepted that saliva provides the major source of natural protection and repair to teeth, in the face of acidic challenge. The presence of saliva facilitates a relatively rapid process of regression of enamel white spot lesions (Ogaard and Ten Bosch, 1994). Depletion of salivary production to lower than 0.7 ml/min. is thought to increase caries risk, depending on other component aetiological factors, eg. diet. There is much evidence presented that a continually lowered salivary pH can correlate with increased caries risk (Stephan, 1944).

ii. Plaque

As stated in the introduction, this is generally recognized as a major factor enabling of production of acid ions at the tooth surface by plaque bacterial fermentation of carbohydrates in food and beverages. Plaque is particularly retentive in certain surfaces of teeth, where it contributes to higher risk of caries development, e.g. deep fissures and grooves, interproximal surfaces, cervical surfaces and near rough restorations (Thylstrup and Fejerskov, 1986).

It is important to recognize that plaque forms continuously in everyone's mouth, even when dietary carbohydrate frequency is low. It is the frequent ingestion of carbohydrate which substantially increases the thickness of plaque, and the level of carbohydrate fermentation to produce acids. The microorganism that form plaque comprise those which form the normal flora of the mouth. Hence it is difficult to inhibit plaque production by chemically controlling those microorganisms which form it without significatly affecting the natural oral ecology. This is why physical removal of plaque through oral hygiene is still the most effective method for its control (Thylstrup and Fejerskov, 1986).

Plaque must also be recognized for its ability to store PO_4, Ca and F ions in high concentrations, enabling it also to provide some

contribution to the protective factors in the mouth. It has been claimed to have a buffering potential ten times than that of resting phase saliva. Shellis, et. al., (1992) have claimed this buffering capacity to be largely due to the bacterial cell wall component of plaque. Once calculus precipitates in old plaque, its buffering capacity can reach 100 times than that of resting phase saliva (Thylstrup and Fejerskov, 1986). Thus, both are considered valuable in protecting teeth against erosive acids.

On the negative side, even a small sugary challenge to the plaque bacteria can result in a 2 to 4 point drop in pH at the tooth surface, depending on other factors contributing to caries risk (Stephan, 1944; Jensen and Wefel, 1989). This can take, at a minimum, 20 minutes for recovery to normal resting phase pH to be regained and several hours in people with a high susceptibility to caries. Of particular interest from the latter investigation is the reported significance of salivary flow on plaque pH (Dawes and Macpherson, 1992). This paper reported a rapid neutralization of plaque acid from stimulation of saliva production by chewing gum.

iii. Dietary Factors

The role of dietary carbohydrates in the aetiology of dental caries on a population basis has already been discussed. At the individual level the most significant external factor increasing caries risk is the frequency and time of consumption of refined carbohydrate intake. This may translate into an increased consumption overall, though there is good evidence that it is the frequency rather than the amount of refined carbohydrate, largely sucrose, that result in caries. This evidence was found in the Vipeholm Study (Gustaffson, et. al., 1954).

This comprehensive study which was designed to investigate the relationship between caries activity and carbohydrate intake was carried out in Vipeholm Hospital in Sweden for a period of 5 years. The

subjects in this study were mentally deficient patients who were treated in the hospital for a long period of time.

This continuous study comprised three periods of studies; Vitamin Study, Carbohydrate I and Carbohydrate II studies. For both carbohydrate studies, subjects were divided into four groups to provide a control group which had a basic diet, a sucrose group which had a basic diet with additional sugar solution at meals, a bread group which had a basic diet with additional 15% sugar in bread at meals and a sweets group which had basic diet and sugar in the form of sweets consumed between meals. The Vitamin study was regarded as preparation and supplementation to the subsequent carbohydrate trials. In the Vitamin study subjects consumed a regular diet with additional vitamins, calcium fluoride or bone meal. It was shown that caries activity was not affected by the addition of those substances. Marked increases in caries activity in the sweets group were detected throughout the Carbohydrate studies I and II over the period of 4 years. This study concluded that the frequent consumption of sugar increase caries activity, a great increase being found if the sugar was consumed in the form which tended to be retain on the tooth surface. The greatest increase was found if the sugar was consumed between meals. It was also found that elimination of sweets resulted in a lowering of caries activity to pre test levels.

As reported previously, Sheiham (1991) has estimated that once sucrose consumption exceeds 15 kilos/person/year, the rate of caries increases rapidly on a community wide basis. The sugar consumption in USA has declined about 47% of the total sugar consumption of 61 kg per person annually (Burt, 1993), while in Australia the consumption of sugar has remained at around 50 kilos/person/year. It appears that approximately 75% of consumed sugar is in manufactured goods, and

may not be recognised so readily as contributing to caries (Sivaneswaran and Barnard, 1993).

A further factor in diet often not recognized for its potential damage is the level of acidity of some foods and beverages. Cola drinks (pH 2.5), some fruit juices and most wine (pH 3.5), vinegar, pickles (pH 2.8), etc, can exert both direct demineralization (erosion) by overwhelming the buffering capacities of saliva and plaque, and thus directly dissolving apatite mineral from the tooth surface; as well as in most cases providing carbohydrates for fermentation (McIntyre, 1992). However, it is stressed that this is only when such foods or beverages are consumed very frequently in considerable quantities. Nevertheless, it is possible that acidic foods may hasten the demineralization process initiated as incipient caries, particularly where natural protective factors are depleted.

A further aspect of diet to consider is the potential for some foods., e.g. some milk products such as certain cheddar cheeses, and perhaps nuts to provide a protective role against dental caries (Jensen 1986, Rugg-Gunn, et. al., 1975).

Past investigations on reduction of cariogenicity of sucrose by the addition of phosphates (Lilienthal, et. al., 1966) or fluoride (Luoma, et. al.,.1979) have provided some success, though are not currently widely commercially used. Harris, et. al., (1968a, b) found approximately 25% reduction in caries increment using calcium sucrose phosphate as a food additive. Furthermore, Clark and Fanning (1971, 1973) in their studies showed that 1% concentration of calcium sucrose phosphate in a 10% sucrose solution altered plaque pH toward the alkaline direction.

The utilization of sugar substitutes has proven to be very effective in reducing the cariogenicity of sweetening agents, though they are expensive, and have some disadvantages (Newburn 1989). Numerous studies have been carried out on the effectiveness of the substitution of

sucrose with xylitol and other sugar alcohols in preventing dental caries. A very thourough study in the use of xylitol as sugar subsitute was carried out in Turku in which utilization of xylitol showed lowest caries incidence compared to fructose and sucrose (Scheinin and Makinen, 1975). Xylitols exert a capacity to affect the ecology of dental plaque (Bradshaw and Marsh, 1994) and its effectiveness as a caries preventive substance has been recorded (Imfeld, 1993; 1994). However sugar substitutes are quite expensive to purchase, and their utilization even in developed countries has been slow.

3. The concept of maturity of enamel

The concept of maturity of enamel, or its increasing resistance to carious demineralization with age, has been found to be a result of the progressive interaction of a number of trace elements and other ions with apatite over time. The effects of some of these elements is a decrease the solubility of apatite in those acids which may be present from time to time at the tooth surface. For example, strontium, vanadium, molybdenum and boron have been shown in some studies to increase resistance of enamel to acidic dissolution (Newburn, 1989). However, the element which has been shown consistently to have the most significant role in caries inhibition is fluoride.

The influence of fluoride ion in the caries process has been shown to occur in all persons, irrespective of their access to supplemented levels of systemic or topical fluoride. This is because of the widespread availability of fluoride ion in naturally occuring drinking water and food products. For example, many communities in South East Asia, China and India use well water, and frequently this water contains such high levels of fluoride ion as to result in endemic fluorosis in those communities. This condition is recorded in Sri Lanka (Warnakulasuriya,

et. al., 1992) and South India (Kodali, et. al.,1994), where endemic fluorosis is recorded present in approximately 51% to 78% of the population.

Fluoride ion is also widely available in many food product such as fish and other sea foods, and in beverages e.g. tea. As a result, teeth generally contain high concentrations of fluoride ion, accumulated from consumption of this ion in such products. Weatherell, et. al., (1977) have shown that concentrations of fluoride ion in enamel range from over 2000 ppm at the outer surface, to a few hundred ppm around the dentino-enamel junction.

Normally its concentration in saliva is quite low, around 0.02 ppm. At such concentrations it exerts a small effect on the development of enamel maturity, though this is mostly influenced by the fluoride present in foods and beverages. As stated previously, this "maturation" process results from the exchange of hydroxyl and fluoride ion for the carbonate and magnesium contaminants in apatite at times of acid interaction with the surface enamel. However, these levels of fluoride ion cannot assist in helping protect enamel against greatly increased levels of acid resulting from the frequent ingestion of refined carbohydrates, as is present in the "affluent" diet resulting fron increasing industrialization. In such cases, it is necessary for increased concentrations, or increased frequency of use, of fluoride ion to be constantly bathing the surface of the teeth. These may be achieved through pre-eruptive, or systemic uptake during tooth development, where the increased concentration present at the surface of the enamel (up to 4000 ppm) provide free fluoride ion on dissolution. Or they may be achieved through the daily or frequent utilization of a form of topical fluoride, as in fluoride containing dentifrices, or through the use of commercially available special products such as mouth rinses, gels, varnishes, or strong solutions.

These methods will be discussed in detail later. However Featherstone, et. al., (1981) have pointed out that there is a limitation to the effectiveness of fluoride ion, when acidic levels are too high and prolonged at the tooth surface. Even so, McIntyre and Blackmore (1993) have demonstrated that even the most extreme caries rate can be significantly curbed using a variety of preventive methods, and including the use of concentrated topical fluoride.

4. Mechanism of action of fluoride

Evidence currently available indicates that the main caries inhibiting actions of fluoride ion are achieved through the following effects:

a. Inhibition of the demineralization process,

b. enhancement of the remineralization process, and

c. inhibition of bacterial metabolism

It was previously thought to also exert an effect by pre-eruptive absorption into enamel during formation such as to alter the shape of crystallites, resulting in reduction in deep fissure formation. However, more recent evidence does not support this concept at the dosage levels involved in human consumption of fluoride ion.

Fluoride exerts a capacity to effect oral microorganism by inhibition of some aspect of bacterial metabolism. inhibition of bacterial growth and killing of bacteria. This ability requires different concentration of fluoride ion to be present in the bacterial millieu, and will inhibit production of acid by bacteria. Decrease in acid production will prevent the pH dropping below the so-called "Critical pH".

The concentrations required to achieve a detectable inhibition in bacterial metabolism has been as low as 19 ppm (Marsh and Bradshaw, 1990). One of the major effect of fluoride on oral bacteria is

reduction of acid tolerance which is depend on the weak acids property of fluoride and the ability of HF to act as a carrier of protons across the cell (Marquis, 1989). However, in general, much higher concentrations, maintained over a prolonged period of time, are considered necessary to show a detectable level of inhibition of caries clinically.

i. Nature of the basic acid interactions with apatite at the tooth surface

As stated previously, caries is a result of a number of interacting factors. At the tooth surface, the main factors are the acids produced by plaque bacterial fermentation of carbohydrates. Also present are salivary buffers and Ca & PO_4 ions at a concentration which is saturated in relation to that in the surface apatite.

Oral concentration of calcium and phosphate will determine the pH at which the oral fluid will reach saturation with respect to the enamel apatite. Saliva is just saturated with respect to enamel apatite at pH 5.2 to 5.5. At pH below 5.2 hydroxy apatite will dissolve starting the development of caries lesion. If fluoride ion is available at tooth development stage, the ion will subtitute the hydroxyl groups to form fluor apatite which has smaller crystall dimension. This substitution will improve the crystal's solubility and crystallinity and strengthen the resistance to acid attack (Nikiforuk, 1985).

Fluor apatite has a critical pH at 4.5. It means that fluor apatite will dissolve at pH below 4.5. At pH below 5.2, hydroxy apatite dissolves while saliva is still supersaturated in respect to fluor apatite. This condition may repeatedly occur when calcium and phosphate dissolve as a result of hydroxy apatite dissolution, at the same time, fluor apatite may be deposited onto the enamel. Once the remineralization occur, the enamel is more resistant to acid attack.

The presence of fluoride in saliva will inhibit demineralization as well as enhance remineralization of early caries lesion. Inhibition of demineralization was shown in numerous *in vitro* studies in which at concentration of 1 ppm F in solution the rate of demineralization was slower than of fluoride pre-treated enamel in a fluoride free buffer (Nikiforuk, 1985). Remineralization of caries lesions was found to occur by a deposition of crystalline hydroxyapatite. In *in vitro* experiments the initial remineralization of white spot lesion is increased by the addition of fluoride to the remineralizing solution, and results in an increase of the hardness of the surface of the demineralized region. The presence of fluoride in low concentration (1 ppm) continuosly in demineralizing solution results in a 2 to 3 fold increase in rate of precipitation (Koulourides, et. al.,1974; Gelhard and Arends, 1984).

The following diagram (Figure 2) from McIntyre (1992) illustrates the hypothetical nature of these interactions when a pulse of acid ions is produced at the tooth surface. This model is based on an interpretation of the concepts outlined by Larsen (1974, 1991), ten Cate (1990), Thylstrup and Fejerskov (1986), and Featherstone (1984).

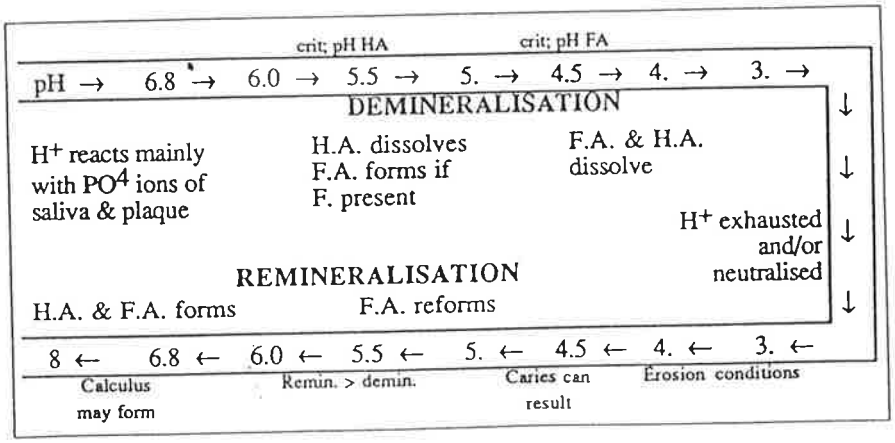


Figure 2: Nature of a hypothetical pH cycle at the tooth surface (McIntyre, 1992)

As the pH decreases, the acid ions react largely with the phosphates in saliva and plaque (or calculus) until the critical pH for dissociation of HA is reached (5.2-5.5). Further decrease in pH ceases progressive interaction of the acid ions with the phosphate groups of HA resulting in partial or full dissolution of the surface crystallites. Any stored fluoride released in this process reacts with Ca and HPO_4 ion breakdown products, forming FA, or fluoride enriched apatite. If the pH further decreases below 4.5, which is the critical pH for FA dissolution, then even FA dissolves, and progressively less remineralization is able to occur. If acid ions are neutralized, and the Ca and HPO_4 ions retained in this hypothetical model, then the reverse processes of remineralization are able to occur as described in the diagram.

ii.The potential outcomes of the acid ion/apatite interactions at the tooth surface

It is the enamel surface which most frequently interacts with acid ions at the tooth surface. In reality, there will be variable levels of acid ions produced, and varying degrees of neutralization of these different individual's mouths at different times, resulting in only partial expression of the above pH cycle operating at variable time intervals. Furthermore, the likelihood of loss by diffusion of dissolved Ca and HPO_4 ions to the tooth surface is high, particularly in the more advanced reactions, resulting in only partial or sectional remineralization occuring in the deeper sub-surface region. Nevertheless, the critical factor is that the factors favouring remineralization outweigh those favouring demineralization for much of each day. This will result in at least stasis or arrestment of the caries process, and hopefully high levels of replacement of lost mineral. As shown by Backer Dicks (1966), at best, this may result in dissapperance of incipient enamel lesions, though

frequently residual internal porosities will result in continuing altered translucency (i.e. the white spot lesion). Where a cavitated lesion is arrested, the dentine base will often become darkly stained with absorbed stains from foods, beverages, and medicines. Root lesions have been shown to be able to achieve high levels of remineralization, resultant volume percentages of apatite exceeding those originally present (Zuidgeest, et. al., 1993).

There are some studies which demonstrate the increased resistance of arrested, remineralized enamel carious lesion to further acidic chalenge (Kidd, 1989). Though with resumption of high levels and frequency of sugar intake, especially with accompaying levels of food acids (e.g. as in carbonated beverages); even these lesions are susceptible to regeneration of active caries. Hence the current concept of caries is of a dynamic process, the progress or control of which is determined by the overall continuing balance of demineralizing and remineralizing factors.

III. THE BASIS OF PREVENTION

A. The Multifactorial Approach to Prevention

As discussed in the previous sections, dental caries is the result of prolonged acidic dissolution of tooth mineral, mostly from organic acids produced as a result of fermentation of refined carbohydrate by plaque organism. It should be added that the level of demineralization must be consistently greater than the potential for remineralization or repair intraorally.

As such, there are perhaps four major components of this cariogenic interaction at the individual level: the plaque, the dietary carbohydrate frequency, the susceptibility of the tooth mineral to dissolution, and the protective and repair abilities of saliva. It is possible to view this interaction in a number of ways. Classically, the interaction has been presented as a series of three or four interactive circles, as in Figure 3, representing these factors.

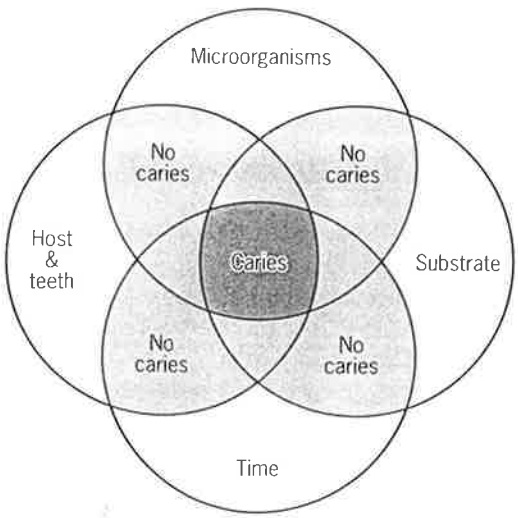


Figure 3: The four circles represent the factors involved in the carious process (Newburn, 1989).

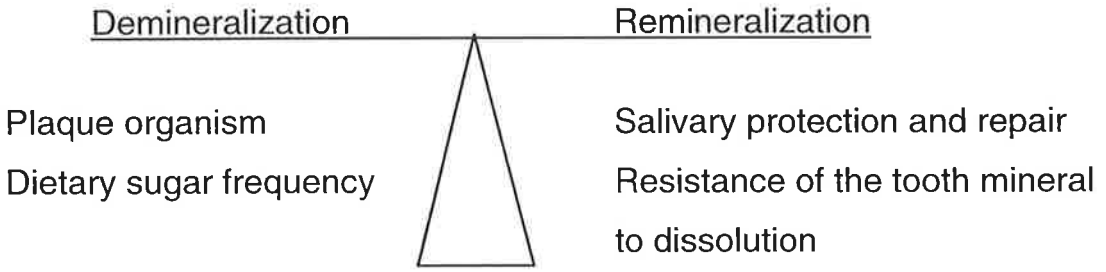
In this model, when all circles connect in the centre, caries results. In other words, this model is intended to represent the multifactorial nature of the aetiology of caries, resulting in caries when all factors interact, ie, when bacteria ferment dietary sugars, on susceptible tooth in a sufficient length of time. In this model, removing one circle breaks the resultant caries experience.

A further model is of these factors forming a chain or an equation, e.g.

Bacteria + Diet + Susceptible tooth → Caries

This concept is that if one link in the chain is removed, then caries can be controlled. Both models are too simplistic to cater for the modern concept of caries, and to provide a suitable model for prevention.

A further model is of a balance of those factors contributing to demineralization on one side, and those contributing to protection and repair on the other, as below:



Using this model, a representation of the delicate balance between demineralization and remineralization at the tooth surface as described by ten Cate (1979), Featherstone (1984), is provided.

Caries results when either, there is a prolonged excess of demineralization which cannot be adequately balanced by repair and

protective mechanisms, or when there is natural deficiency in the protective and repair capacity of saliva, given only moderate levels of acid being produced at the tooth surface.

Using this model, prevention is a matter not just of controlling one factor, e.g. bacterial plaque or diet, but also of enhancing the remineralization balance factors, e.g. salivary stimulation, and increasing resistance of the teeth to demineralization. In most cases, it is a matter of having some effect on all, ie. reducing the severity of the factors causing demineralization, while enhancing those causing remineralization. In other words, a multifactorial aetiology is best dealt with using a multifactorial approach to prevention.

This model applies particularly at the individual patient level in practice, though can have application on a population basis. Thus at the population level, a prevention program must have the following components:

a. Dental health education, involving education as to the importance of good dental health, the problem of neglect of oral health, the most significant aetiological factors, ie. dietary sugar frequency and plaque micro-organism, and the means of preventions, i.e. control of those factors through effective oral hygiene, and discipline control of dietary factors, and through enhancement of natural protection.

b. Provision of methods of enhancement of natural host protection, through clinical practice or on a public health basis, including:
    - Use of systemic or topical fluoride
    - Provision of physical protection of vulnerable tooth surfaces by use of sealants

- Use of other chemical protection systems as are available, e.g. antibacterial mouth rinses, in cases of extreme risk. These may be of particular value when gingival tissues are inflammed.

B. The Concepts of Prevention and Control

Traditionally, the concept of prevention of dental caries implies the ability to stop caries developing at all. However, taking into account the current concept of reversalibility of the early stage carious lesion, it is important to expand this concept to include control and reversal of those incipient lesions present. Hence, the preventive measures planned to stop caries becoming a problem must include those measures which will result in control of existing early stage carious lesions. In general, those methods necessary to achieve this result are similar to those necessary for primary prevention, though may be of a more intense nature, ie. more effective plaque and diet control, or more frequent use of topical fluorides.

C. The Utilization of Fluoride as a Mechanism for Caries Control and Prevention

Since the early discovery by Dean (1938) of an inverse relationship between natural water fluoride concentration, and the caries experience in four cities in Colorado and eight cities in Winconsin and its relation to dental caries, there has been a widespread introduction of this factor to influence the prevalence of caries worldwide. Initially it was thought that its systemic (pre-eruptive) use provided the largest reductions in caries prevalence. These appears now to be widespread acceptance that the topical (post-eruptive effect) have the potential for the largest reductions in caries

(ten Cate, 1979; Featherstone, 1991), although the combined effect is still regarded as providing recognisable advantages (Newburn, 1989).

As the use of systemic and topical fluorides is widely accepted as being the primary factor causing significant reductions in dental caries prevalence in developed countries, it is important to analyze closely why it has been so effective, and the relative effectiveness of the various methods of utilization.

D. Critical Requirements and Dose Regulation

It is now accepted that even low concentration of fluoride ion (e.g. 0.03 ppm), if present at the site of a demineralization reaction at the tooth surface, can inhibit the reaction while simultaneously enhancing the potential for remineralization to occur (Featherstone, 1991). Concentration of as low as 19 ppm have been shown to inhibit metabolism of *Streptococcus Mutans* and other bacteria (Marsh and Bradshaw, 1990). In general the requirement for effective inhibition of caries is the continuing presence of certain optimal concentrations of fluoride ion at the tooth surface, particularly during the presence of acid production in plaque and as pH returns to normal.

This may be achived by continual use of low concentration topical fluorides, as in twice daily use of fluoride containing dentifrices, and by the occasional use of higher concentration products, or by the use of mechanism to promote storage of fluoride ion in tooth structure, so that it is immediately available on acidic challenge. This may be achieved through:

a. Systemic use, enhancing concentration of fluoride ion present within the tooth structure
b. Use of highly concentrated topical fluoride products and those which are acidulated to enhance transport into tooth structure

Both systemic and topical fluoride may be used on a population or an individual person basis. The relative effectiveness of these methods will now be considered as used in developed countries.

1. Systemic Fluorides

i. Water fluoridation

This is the oldest form of fluoride ion delivery for systemic use, being first artificially used in 1945-50 as described previously. In North America and Australia, trials have frequently resulted in up to 60 % reduction in caries over a five year period (NHMRC Report, 1991). Based on Dean's original work, a figure of 1 ppm fluoride ion added to water is considered the optimal concentration to provide maximum protection with minimal fluoritic hypoplasia resulting (Dean, 1938) for children with developing permanent teeth. Above 2 ppm (2 mg fluoride ion /day), the risk of fluorotic hypoplasia in young children was found to increase significantly.

The WHO has recommended certain precautions in the use of artificial water fluoridation to control caries.* In hotter climates, eg, more water is consumed, and hence the concentration needed for children to consume is around 0.5-0.7 mg/day which provides optimal protection without complication. However, we should also take into account the cumulative effects of fluoride from tap water, processed foods, ingested toothpaste and also baby formula to reduce the risk of fluorosis in children (Van Winkle, et. al., 1995; Shulman, et. al., 1995). Overall, water fluoridation provides a very safe form of continual uptake throughout each day.

* NB: 1994 WHO Report, " Fluoride and Oral Health" recommends that Fluoride concentrations in water do not exceed 0.5-1.0 ppm..

ii. Tablets and drops

This form of systemic fluoride is intended for use where fluoridated water is not available, and provides 1 mg of fluoride ion at the most for daily consumption during tooth development. The fluoride supplementation begins at 6 months at 0.25 mg per day until 2 years of age in which the dosage changes to 0.5 mg per day. At the age of three, the dosage changes to 1 mg per day (Shulman, et. al., 1995).* The major disadvantages of these methods compared to water fluoridation are:

- The use of peak dose once a day, means less is adsorbed into the hard tissues overall, with higher temporary blood concentrations being present for periods each day.
- Risk of accidental overdose. Trials have reported up to 80 % reduction in caries over two year trial periods. However in many developed countries, where mild levels of fluorosis have been recorded, government health departments are now moving to severely reduced use of these supplemental systemic fluoride (NHMRC Report, 1991).

iii. Salt

The use of salt as a fluoride vehicle was first trialled in Switzerland (Wespi, 1961). Initially, 90 ppm of fluoride ion was added, but this was found to be too little. Currently 250 ppm fluoride ion are added, and the efficacy of this form of systemic fluoride in resulting in caries inhibition has been thoroughly tested (Toth, 1984). In the use of 250 ppm fluoridated salt over a 10-year period of clinical trial, there was a reduction in caries intensity, being 67.54% in age group 2-6 years, 60.35% in age group 7-11 years and 50.82% in age group 12-14 years (Toth, 1984).

* Also current recommendation of the National Health and Medical Research Council of Australia.

iv. Milk

Initially, milk was tried and dis arded as a fluoride vehicle, as it was argued that fluoride ion become bound to firmly to the calcium components. However, gastric acids (pH:1) should clearly free all fluoride bound to metallic ions, and milk is currently again being used as a major vehicle for fluoride ion ingestion in many Eastern European and South American Countries. Concentration used are 1.0 ppm, resulting in ingestion of approximately 0.8 mg fluoride ion per day, resulting in a 48% reduction in caries over a 5-year period (Stephen, et. al., 1984).

v. Sugar

The first to report fluoride ion addition to sugar for human use was Luoma, et. al., in 1979. The trial was conducted with ethical approval in an institution for the mentally handicapped. The mean age of the children was 10.7 years, indicating that the mechanism of action of the fluoride ion for many was almost entirely topical rather than systemic, though both action would have operated in the younger children.

In this trial, NaF was added to various food products, eg. marmalade, biscuits, jam and fruit juice, for the test group. These products accounted for approximately 60 gms. of sugar intake daily. Before the trial, the estimated sugar intake was 85-90 gms. daily.The fluoride was present at a concentration of 10 ppm. or 10 mg./ kgm. sugar. A Phosphate-Bicarbonate replacement was included in two of the sweets ingested per day. The estimated daily intake of Fluoride (F) from this source was 0.56 mg/ day.

The trial continued for three years, there being a linear increase in caries in the control group during this time. The test group demonstrated a total arrest of caries in the third year of the trial, averaging 42% reduction over three years.

Numerous researchers have added fluoride to sugar in animal caries experiments, though frequently only as a way of achieving ingestion of fluoride by the rats ; Mundorff, et. al., 1988; Bowen and Pearson, 1992). In all cases, reduction in cariogenicity were achieved, usually with the use of around 10 ppm fluoride ion equivalent.

2. Topical Fluoride

i. Dentifrices

The incorporation of fluoride into dentifrices has proven to provide one of the most effective of all fluoride vehicles for topical use. Initially, Sodium Fluoride was added to pastes using calcium phosphate abrasives, resulting in reduced availability of the fluoride ion due to its bonding with calcium. The subsequent utilization of monofluorophosphate proved beneficial, with numerous studies resulting in between 30-40% reductions in caries prevalence following two year trials (Nikiforuk 1985; Thylstrup and Fejerskov, 1986).

In retrospect, these trials grossly underestimated the long term effectiveness of fluoride dentifrices, as they did not take into account the fact that such a form of fluoride use has an accumulating effectiveness over many years, with the potential 100 % protection after many years of use, provided no severe deficiencies in natural host protection exist, and that the diet is not extreme in its frequency of carbohydrate consumption. It has been pointed out by Scheie (1992) that there is a compounding effect of protection, as originally proposed by Koulourides (1968).

Dentifrices are now moving steadily to NaF use again, using silicate abrasives which do not react with calcium. Most contain 1 mg

F/gm of dentifrice. A new junior toothpaste has been developed containing 0.4 mg F/gm of paste, to counteract the increasing tendency to mild fluorosis being seen in children (Winter, et. al., 1989; Horowitz, 1992).

ii. Other forms of topical fluoride

These are generally not applicable as community wide, public health measures, and are applicable at clinical practice level, involving professional application, or instructed self application, or under supervision of dental personnel at schools or institutions.
Those suitable for self application include:

a. Mouthrinses

Usually of NaF at neutral pH ranging in concentration from 0.02 to 0.2 % F ion. These have good retentivity, with elevated levels of fluoride in saliva for up to 5 hours after use. Some studies revealed that mouthrinses provide protection more effectively to smooth surface than pit and fissure caries (Rugg-Gunn, et .al., 1973; Brand, et. al., 1972).

b. Gels

Fluoride gel is easier to use and avoid the possibility of ingestion in young children. It is available as Acidulated Phosphate Fluoride gel containing 1.23% F ion (12.300 ppm) acidulated at pH 3.5, or NaF gel containing 2% F ion (10000 ppm) neutral, or SnF_2 at 0.4% concentration (Murray, 1989)

c. Concentrated Fluoride Solution

Concentrated Solutions of SnF_2 20% for local application to at risk surfaces in early lesions.

d. Fluoride Varnishes

Varnishes containing 25.000 ppm Fluoride ion (Duraphat) or 8.000 ppm F ion (Fluor Protector). These comprises natural resins or polyurethane lacquers dissolved in alcohol, which form a film on the tooth surface, permitting slow release into the tooth surface or oral environment (Naylor ann Murray, 1989).

3. Combined systemic and topical effect of fluoride

It is now recognized that many systemic fluoride systems result in some topical effect being exerted immediately, and vice versa. Findings by Featherstone, et. al., (1979) that even very low levels of fluoride ion, when present at the site of acid challenge to the tooth surface, can exert an inhibitory effect on caries development, have increased our understanding of the potential topical effects of even systemic fluoride. This may be exerted when the particular material enters a person's mouth, and be maintained by slightly elevated salivary levels of fluoride ion as it is absorbed into the plasma and eventually into saliva (Ekstrand, et. al., 1988).

Conversely, it is now recognized that ingestion of topical fluorides, in particular dentifrices, can cause increased levels of overall fluoride ingestion, such as to result in mild fluorosis being observed in many developed countries (Clark, et. al., 1994; Ismail, 1994; Levy, et. al., 1993).

In the case of sugar with fluoride added, where a range of age groups is ingesting the product, the effect would be largely topical, only those young children with developing teeth receiving any subsequent systemic benefit, after permanent teeth erupt.

A number of investigations (Marthaler, 1971; Horowitz, 1980) have pointed out the benefit of a combination of systemic and topical

fluorides as providing the highest level of protection, though this needs to be closely monitored for young children for the reason outlined above.

E. Safety Factors in the Use of Fluoride

1. Toxic aspects

Despite numerous claims by "Anti Fluoridationists", there is no evidence that both systemic or topical fluorides, if used as advised by manufacturers or scientific research personel, exerts any discernible toxic effect. Toxicity such as to cause death, called the "Acute Lethal Dose" is almost 34 mg/kg of body weight, or about 5 gms for an average size adult. Death has occurred in young children who swallow large amount of fluoride tablets or where excess quantity of topical fluoride have caused vomiting and inhalation. Systemic sources of fluoride such as water supply, salt or milk would be unable to result in poisoning.

A more useful concept is the "Probable Toxic Dose", or that dose which may make hospitalization and observation desirable. This has been set at 5 mg/kg of body weight by fluoride physiologists (Whitford, 1990). Again this may be exceeded only by excessive supplemental systemic fluoride tablet ingestion, but with normal caution, should not be exceeded by other systemic or topical use.

Ekstrand, et. al., (1981) claimed that blood plasma levels exceeded the 1 ppm mark, considered by physiologists to represent the point at which chronic toxicity may be experienced by tissues, if children were given topical fluoride treatmens using 5 mls of APF gel in trays. However, these trials did not include normal precautions, such as suction of excess saliva and spitting out of excess, the children

swallowing all excess. Subsequent trials have shown that when normal precautions are taken, this level of ingestion does not occur.

2. Fluorosis

The potential for young children to ingest excess fluoride ion, resulting in fluoritic hypoplasia of permanent teeth on later eruption, is a great concern. A number of investigators have recently reported a widespread evidence of mild fluorosis in developed countries (Clark, 1994; Szpunar and Burt, 1992). This has been considered to result largely from ingestion of tooth paste by children in their early years, when swallowing reflexes are not well developed(Levy, et. al., 1993). Barnhart, et. al., (1973) have found up to 30% of paste being ingested by 2-4 year-olds, with 13% of paste ingested even by 5-7 year-olds. Other sources of excessive fluoride ingestion have been excessive intake of fluoride supplements, and high levels in some baby formulae (McKnight-Hanes, et. al., 1988). Dean (1938) proposed that above 2 mg intake of fluoride perday, or 0.2 mg/kg of body weight, the risk of fluorosis increased greatly. * for a 10 kgm. one year old child

The incidence of endemic fluorosis in specific regional areas is high in South East Asia in particular, where well water is a major source of water supply. It is estimated that approximately 30 million Chinese people suffer from endemic fluorosis. In Sri Lanka (Warnakulasuriya et al, 1992), Antigua (Vignarajah, 1993), Kenya (Ng'ang'a and Valderhaug, 1993) and in Indonesia (Wibowo, 1978), endemic fluorosis has been detected in areas with high fluoride concentration in the natural water supply. Hence, widespread investigations into all sources of fluoride ion is essential before any systemic programme of fluoride ion supply is considered in such countries.

F. Relative Benefit of the Component Factors of Prevention in Developed Countries

1. Plaque Control

Plaque control was performed as early as the 3000 BC, using gold tooth picks, and various devices such as cotton balls, wooden sticks, and also finger nails. Tooth brush, dental floss and tape are a modern devices to keep oral cleanliness. These mechanical procedure are considered effective means for controlling plaque in individuals who are well motivated and properly instructed, and willing to spend time and effort to obtain good oral hygiene. (Newburn, 1989).

It is evident that plaque control does not have a large benefit in controlling dental caries, although it is essential for periodontal disease control. For example, there are low caries rates in Africa, not merely as the result of the use of traditional oral cleaning devices, but can largely be explained by dietary habits of low sugar consumption and its frequency. (Newburn, 1989). Sutcliffe (1989) pointed out the evidence from Tristan da Cunha population who originally showed very low caries rates, inspite of very poor oral hygiene. Caries rates soon became higher as sugar was introduced to this population, while oral cleanliness still remained low.

The apparent failure of tooth brushing to control caries may be due to the relative inaccessibility of interproximal and fissure plaque to the tooth brush filaments. There is a lack of evidence that mechanical cleaning can be carried out sufficiently to prevent caries in susceptible individuals (Addy, 1986).

2. Diet Control

As a refined carbohydrate diet is the cause of increased caries prevalence, the simplest method of reducing caries by dietary means would be to eliminate sucrose from the diet. However, this is very difficult to apply. Sucrose is a rich source of energy, cheap, available in huge amounts and its taste is enjoyable by most people (Naylor, 1986). At the individual level, this effort needs discipline and a well educated background. At a population level, market pressure through advertising and the basic liking of sweet foods by most people, make it inevitable that refined carbohydrate use will be adopted by most countries as their economies increase.

In developed countries, despite the enormous evidence of the link between sugar and caries and fourty years of dental health education, sugar consumption still stay the same. In Australia, sugar intake has been maintained as in the 60's (Sivaneswaran and Barnard, 1993) and slightly reduced in USA, although it is replaced by other refined foods such as protein (Burt, 1993).

3. Systemic Fluorides

Systemic fluorides provide good outcomes in caries prevention on a population basis. Water fluoridation is the most effective mean of caries preventiion in developed countries such as the USA, Canada, Australia and New Zealand, and has resulted in up to 60% of caries reduction. Water fluoridation is not widely accepted in Europe. Only a few cities in European countries have been fluoridated. Some cities such as Tiel in Holland, Tirgu-Mures in Rumania had the water supply fluoridated for some years before it had been ceased, due to various reasons (Naylor and Murray, 1989). Technical difficulties, argument

against mass medication and the right to choose become the basis of objection.

As fluoride is readily available to the people in developed countries from different sources such as dentifrices, and other topical products, and the fact that the same result in caries reduction can be achieved through the use of fluoride dentifrices, the concentration of fluoride in drinking water has been reduced to 0.6-0.7 ppm in many cities (e.g. Adelaide, Australia).

In Australia, fluoride supplementation is not recommended anymore, in the presence of water fluoridation, because of concern at mild fluorosis that can be developed following this procedure.

4. Topical Fluorides

Fluoride dentifrice is one of the most effective topical fluoride procedures on a population basis. Besides concern for ingestion of high fluoride concentration tooth pastes by young children (Beltran and Szpunar, 1988), this method is generally considered safe. Horowitz (1992) proposed the use of low fluoride concentration tooth pastes for young children, to reduce the risk of fluorosis.

Topical fluorides designed for specific needs are also available. For example, individual with low caries risk would use products with low fluoride concentration while high risk individual can use product such as fluoride varnishes, topical fluoride gel or strong fluoride solution.

Some home applied topical fluorides such as mouth rinses have the potential for a high level of protection though it arises safety concerns for unsupervised use in children.

5. Fissure Sealants

The use of fissure sealants to prevent dental caries seems to be effective, although the cost is very high. The procedure of applying

fissure sealants will not cause any difficulty in children on an individual basis in private practice. It is apparent that this procedure is less effective under condition of mass prophylaxis (Gordon, 1989).

IV. DEVELOPMENT OF THE MOST SUITABLE PREVENTION PROGRAMMES FOR INDONESIA

A. Dental Caries in Indonesia

Indonesia has a very large area consisting of thousands of islands, which are inhabitated by various ethnic groups with different ways of life and habits.

The organization of oral health care in Indonesia is managed by the Department of Health which established dental health programs for Community Health Centres and Hospitals. The Health Centres provide basic health care including dental health to the general community, school children, pregnant/nursing mothers and children under five years of age. The hospitals provide a broad range of dental health care in all aspects of dentistry.

Primary oral health care was incorporated into primary health care in 1979, emphasizing preventive measures through an educational approach with the primary target of high risk groups within low income communities. The management and implementation of the Primary Oral Health Care Programme in Indonesia still needs improvement and will be continuously upgraded to achieve the oral health goals in the year 2000, i.e. DMFT figure of not more than 3.0 and at least 20 functioning teeth in the age group of 35-44 years and up (Adyatmaka and Lahey, 1994).

The spread of dental caries is quite different from place to place, depending upon the geographic area, food and also certain habits.

Differences in caries experience between rural and urban populations were reported. It was found that the caries experience in urban area was much higher than that of rural area (Barmes, 1977).

Effendi, et. al. in 1981 (Cit. Tewari, et. al., 1991) stated that the DMFT score from the southern part of Sumatera was 8.7, greater than nearby Java. This condition was caused by a certain food which had a very sour sauce eaten by the people, and dental caries in this area was a continuation of erosion (Rahardjo, 1989). In other areas, surveys in 20 provinces in Indonesia on dental caries prevalence, revealed that in 51.31% of 8-years old children, dental caries was found with a DMFT 1.08 and 72.76 % of 14-year old children with DMFT 2.65. The prevalence increased for 35-44 year-olds with DMFT 4.61 (Wibowo, 1978). An increase in caries prevalence was recorded in which 10-12 years old school children in East Java showed DMFT scores of 3.34 for boys and 3.55 for girls (Kristanti, et. al., 1986). Even in a special group such as soldiers, DMFT scores between 3.0 - 4.9 (Gaare, et. al., 1989) were recorded.

Furthermore, Koloway and Kailis (1992) found a very high caries experience for pre-school children in rural and urban area in West Java, with dmft scores of 7.98 and 7.92 in rural and urban area respectively.

Morgan, et. al. (1992) found a moderate caries experience in school children in Jakarta, Indonesia, in which the DMFS for 12 year-olds was 3.65 and 6.23 for 15 year-old children

The report of the Indonesian Department of Health (1992) stated that an increase was found in the prevalence of dental caries over a 4 years duration. The average DMFT scores for urban areas were 1.14, 2.63 and 5.09 for an 8 year-old, 14-year old and 35-44 year old range respectively in Pelita III (1984-1988). A marked increase was found in the 35-44 year old range in 1990 in which the DMFT score was 8.68.

* This may have included some Periodontally involved teeth contributing to the M component.

There is a tendency for dental caries to continue in adult life at a high rate. Water fluoridation is unavailable to the people as water supplies are not fluoridated and only provide reticulated water to 20 % of the population. The rest of the population use well water, bore water, rain water and water from the river. Dental health education for the community is not evenly provided as the population is scattered in quite large areas. Even though other fluoride vehicles such as fluoride tooth pastes and fluoride rinses can be found in the markets, these are not economically within reach of most people. Taking all factors into consideration an increase in caries prevalence in the younger age group is certain.

A 3-year trial in tooth brushing in school children once a day is being carried out in West Kalimantan (Brathall, personal communication), and other methods of delivering fluoride on a community wide basis should be explored to counter the rapid increase of dental caries.

B. Dietary Patterns and Measures of Prevention Used in Indonesia

The diet of the people in Sumatera is generally quite similar to that in other parts of Indonesia. This consists of rice and various side dishes. The side dishes are normally vegetables, soups, fish, meat and poultry. However, there are also exist in Sumatera, different tribes with different habits and quite different foods prepared in different ways. Staple foods such as rice, corn and sago serve as basic carbohydrate sources, usually steamed or boiled. A certain food eaten in South Sumatera is very sour and causes dental erosion (Rahardjo, 1989). This dietary pattern does not change much in the country side. Fruits will be served after dinner as desserts, while small cakes and nuts are served before dinner.

As the economy of Indonesia grows, the living conditions of the community become better. It is the intention of the goverment to provide basic food items such as rice, flour and sugar without the need to import these from other countries.

However, as communication and the economic system expands globally, Indonesia and its population are subjected more widely to information and influences from other countries. There is a tendency of a change in life style of the community, especially the wealthy; for example, diet changes to easy and fast prepared foods, as life becomes busier.

Snack and fast foods are now widely available anywhere, from a little shop to local supermarket. Sugary foods, sweets, chocolates are now sold in school canteens, replacing fruits and traditional cakes. Besides school canteens, in the front gate of any school can be found people selling all of the snacks such as ice creams, cold drinks, sweets, cakes etc. There is no restriction for students to go out and buy these foods in school breaks and after school. Even though the standard menues are still the same, the frequency of snacking between meals has become more pronounced.

Traditionally, preventive measures in Indonesia particularly in keeping good oral hygiene, are different for different tribes. Some of the tribes in North Sumatera chew betle nut and then wipe their teeth with tobacco. They believe this strengthen their teeth. In fact, the rigorous chewing and the roughness of the preparation cleans the teeth, but if this habit is retained for a along time, a heavy accumulation of calculus may results in periodontal failure. Other measures can be found in other areas in the country side, such as wiping the teeth with sand, terracota powder or a kind of grass found near the river.

As the level of affluence becomes greater, fluoridated or non-fluoridated tooth pastes, tooth brushes, dental floss, fluoride mouth

rinses and other oral hygiene devices become available widely, especially in the cities. The unfortunate situation is that, dental health information and education are not available nationwide. This is a reflection of the strategy of the development of the country. As a developing country, Indonesia relies on its 5-year development plans established by the government starting in 1966. The first five years were spent in combating economic ruin, in which stabilization and economic growth were in greatest focus. In health management, preventing the outbreak of epidemic disease, eliminating infectious disease, were most important. The attention to dental health started in the fourth five-year plan. Dental health education and dental care are gradually being provided even though these can not reach the remote areas. Compared to the wide availability of sugar and sugary foods, dental education is way behind, which leads to a higher caries rate in the country. Also the acquisition of oral hygiene devices is still unreachable to the majority of the community.

It is now clear that Indonesia may need a very comprehensive means of prevention, which can be provided nationwide with a very low cost to the people and to the government. Water fluoridation is not viable as Indonesia consists of nearly 3000 islands. Even in the areas where water supplies are available, only 20% of the people can be served. Other forms of systemic fluoride, e.g. tablets and milk are not feasible, though salt may be of value. Topical fluorides are in increasingly available to the wealthy, though not to the majority of the population.

C. Advantages and Disadvantages of Fluoride Use in Developing Countries

Eventhough the use of systemic and topical fluorides has resulted in marked reductions in caries prevalence in developed countries, it should not be assumed that the same approach can be considered for developing countries.

There are many reasons why this is so.

a. The cost of many of these systems is relatively high, and unless the caries rate is increasing rapidly, implementation may not be considered a high priority.
b. Water fluoridation is unsuitable for many developing countries due to:
    - a low level of reticulation of public water supply
    - many developing countries are in the tropics and the rate of water consumption and evaporation factors make it a less controllable source of systemic fluorides. Systemic fluoride is contraindicated where there is extreme poverty, as the fluoride ion then comprises an exaggerated proportion of ion intake, resulting in severe osteofluorosis, as occurs from excessive fluoride levels in natural well water in India (Gupta, et. al., 1994).
c. Food intake patterns vary considerately in many developing countries, e.g. milk is not a common food item in many tropical countries, and salt is used to variable degrees. There is also a major campaign to lower salt use in developed countries, even though this has not extended into developing countries as yet.
d. Those topical fluoride agents currently commercially available are very expensive to buy, and not widely available in regional areas in many developing countries.

e. Well water is widely used in developing countries and this may already contain high level of natural fluoride ion.

Overall, considering the situation in a country like Indonesia, with such a large population, the majority of whom live in rural areas and with many competing areas of economic priority, there is an urgent need for a population based, dental public health prevention programme for caries control. While every effort economically and geographically possible to provide effective dental health education, with emphasis on personal oral hygiene and dietary control programmes especially at school levels, should be persued, those are very labour intensive, and have limited effectiveness in the long term. This was evident from a study which monitored such systems in the Indonesian Dental Service. In this study, Matram (1986) proposed a model which involved cadres in villages and school teachers to obtain data of carious lesion, gingivitis and calculus and to provide oral hygiene instruction and calculus removal to the community including school children and villagers. Even though this model was likely to be feasible and valid as a routine activity in the Indonesian Dental Service, it was realized that limited financial resources and geographical conditions would impede the application of this mode nation wide.

Those public health measures which might have greatest effectiveness against caries in the short term are to provide some form of low concentration fluoride. The vehicles which might be most useful are salt or sugar.

The availability of salt amongst the whole population in Indonesia is not known. Consisting of so many culturally diverse groups, the population dietary pattern of salt use in cooking will vary widely. Hence, a great deal of research would be needed into the utilization pattern, and the background fluoride levels in foods and beverages currently

* ıs still essential to provide Dental Health Education as part of an overall ıtive Public Health Programme. The point being emphasised is that, on a c ıparative Cost Effectiveness basis, both systemic and topical Fluorides have been most effective in reducing caries incidence.

consumed, before the most appropriate concentration of salt-fluoride ion could be decided. The evidence from Central European studies would be useful to initiate such decisions, though eating patterns would vary markedly between these two locations.

While sugar has been used as a vehicle for fluoride only in the studies of Luoma, et. al., (1979), it would seem to provide many benefits in developing countries, though would obviously provide some concerns.

Firstly, as it is the frequency of refined sugar intake which is the main cause of caries in most populations, then the fluoride ion would be supplied with this product, and be present in the mouth at the same time when the sugar was being fermented to acids in plaque. Up to a certain limit, the amount of fluoride ion delivered would be proportional to the amount and frequency of sugar use. The limits would be where, in young children, there is a risk of levels sufficient to result in fluoritic hypoplasia of enamel. Even so, where such a pattern of sugar consumption was maintained, the inevitable consequence will be rapid caries development and possible eventual permanent tooth loss anyway. Hence mild fluorosis might be a preferred alternative to this outcome.

The major difficulties on a population basis, would be controlling its use to groups with alternative sources of fluoride ion intake, ie from reticulated or well water, and foods and beverages.

As the use of sugar as a fluoride vehicle has not been trialled as yet in a developing country, it is proposed in this project to do so, on a limited group trial basis. The factors which need to be considered in planning such a trial, so as to ensure maximum safety to the trial populations, and to obtain the most valid and reliable result, will now be considered. Such considerations will be made in reference to other

population based studies of substances considered likely to have a prevention outcome in relation to dental caries.

V. TRIAL OF EFFECTIVENESS OF A CARIES PREVENTIVE MEASURE

A. Type of Study Necessary

It is widely recognized in caries research, that it is necessary to conduct laboratory and clinical studies to test the effectiveness of a caries preventive measure, before it can be applied on a community wide basis. The studies carried out should consist of several *in vitro* and *in vivo* preliminary studies, to assess the suitability, safety and potential of the measure to be assessed in a clinical trial. As a fundamental method for population based research, in a clinical trial the intervention is systematically introduced by the investigator so that any observed treatment effects are free from bias.

The purpose of conducting a clinical trial is to provide a scientific basis for a preventive measure to be applied at the community level. It is essential that the result of a clinical trial can be interpreted by any one in the caries research field. This led to a considerable effort by the FDI, to produce guidelines for conducting a clinical trial (COCSTOC, 1972).

Luoma, et. al. (1979) had employed a clinical trial in the effectiveness of fluoride added sugar products in preventing dental caries in a mentally handicapped population. A further trial is needed to test the effectiveness of sugar as a fluoride vehicle in a general population. The most appropriate study to be carried out for this purpose would be a randomized clinical trial

The preferable design of such a trial is one in which a control and an experimental group are nominated such that the two groups are comparable and any variable beyond the research requirement could be strictly controlled. The employment of appropriate blindness to prevent bias in generating data is desirable in a clinical trial. Double

blind procedures should be maintained through out the study and the integrity of the blindness should be maintained to ensure that all the effort in carrying out the study is not wasted by an unsustainable result.

The ethical aspects of the study should be considered which include gaining subject participation, informed consent, adequacy of design, determining potential noxious effects of the agent being tested, and the possible withholding of beneficial treatment (Stamm, 1984).

B. Selection of the Population in the Study

1. Constancy of population

In conducting a clinical trial, it is very important that the subjects are randomly allocated to eliminate bias, and if the trial will extend over a considerable time period, continuous monitoring should be easily carried out over the period of the study. It should be predictable that the drop out of subjects from the study can be limited. A homogenous group can be selected to reduce sampling error for the study which is valuable to demonstrate the effectiveness of a preventive or treatment effect.

Groups of children who live in an institution such as orphanages are homogenous groups. As the groups are divided into the test and control groups it is required that both groups represent relatively equal mix of age and gender, similar food intake pattern and amount, and reasonably large caries increment.

2. High caries incidence

A group with high caries prevalence will provide good sample subjects in which the assessment of the preventive measure

subsequently can show the effect of the preventive agent employed over the period of the study. Significant differences between groups can be more readily detected in groups with high caries increments compared to groups with low caries increments.

3. Controllability of aetiological factor

A clinical trial, in which effectiveness of a preventive measure will be assessed, needs to be carried out in a population with a similar and regular dietary pattern. This is in conjungtion with the fact that caries, as a multifactorial disease, primarily depends on the diet of the population. Populations with similar diets will show a similar pattern of dental disease, and thus aetiological factors can be controlled through out the period of the study.

4. Controllability of preventive factor

If the preventive measure is fluoride, any other source of fluoride should be controlled or calculated. Any systemic or topical fluoride such as from drinking water, fluoride dentifrices or fluoride rinses should be avoided. A population living in an unfluoridated area and having drinking water with low natural fluoride concentration would be suitable for the study.

C. Selection of Preventive Measure

1. Cost

In a clinical trial, cost can be viewed as the cost of the product to be tested and the cost of trial to be conducted. In the developed countries, the usual outcome of a clinical trial is the comparison of effectiveness of two or more products, as it is difficult to test a new

product with a placebo, in finding a group of the population which is not using any particular preventive measure, either systemic or topical, such as water fluoridation or fluoridated dentifrices.The cost of a clinical trial of comparing the effectiveness of two or more preventive products will be very high, since the trial will be carried out at least for 3 years duration.

As stated by Luoma (1985) fluoridated sugary products showed some advantages as a fluoride vehicle for populations in developing countries. Low cost together with high effectiveness in caries prevention meet all the requirement of preventive measures in developing countries. A clinical trial in a population with high caries increment will keep the cost low as the trial will show differences between control and test group in a shorter duration.

Cost of a preventive product to be selected in a clinical trial in developing countries should be minimal with the intention that the product could be obtained easily by the people. The cost in the production of fluoridated sugar should not be very high as the addition of fluoride to sugar will not change the overall system in sugar production. Fluoridated sucrose which can be produced in a large scale in the sugar mill, should not increase the production cost except for the cost of Sodium Fluoride to be added and special equipment for spraying unit which is only an initial cost. The price of fluoridated sugar in the market should not differ much from ordinary sugar, and this certainly can be obtained by the community.

As sugar remains a basic food requirement in the community, it is realistic, with view to the cost of the trial and the product, that fluoridated sucrose could be appropriate as an effective caries preventive agent in developing countries.

2. Practicability

A preventive method should be easily applicable by the individual and distributed nationwide. The advantage will be more obvious if the method functions automatically, needing minimal or no effort from individuals. In clinical trials of such products, the production site needs to be near and a facility to test the safety of the product before being supplied to the subjects should be available.

3. Facility for close monitoring of implementation and outcomes

Location of the study groups in a close area will provide easy access for monitoring of implementation. The standard facilities for conducting examinations of outcomes such as physical equipments and diagnostic aids should be available. If radiographs are used, standardized method in exposure, development and reading radiographs should be followed.

4. Monitoring fluoride intake

Estimation of daily fluoride intake from food, beverages and drinking water should be carried out prior to the study to ensure that with the addition of fluoridated sugar, the children will not consume an excess of fluoride ion which may cause fluorosis or exert any toxic effect. Taves Method of fluoride ion separation and analysis (Taves, 1968) can be carried out to estimate fluoride content in those substances.

Monitoring fluoride intake can be carried out through the analysis of plasma fluoride or urinary fluoride. Urinary fluoride analysis is more convenient to the subjects and it is also easy to obtain a serial sample of urine for a prolonged time. Urine analysis is a reliable indicator to monitor the fluoride intake as nearly 50- 60% fluoride ingested will be excreted in urine in a 24 hour period (Singer et al, 1979).

D. Estimating Effectiveness

1. Use of DMFT, DMFS and changes in these scores

DMF index is a quantitative expression of a person's life time caries experience in the permanent teeth. It is important that the D (decayed) component represents a large proportion of the DMF score, otherwise disadvantages such as the treatment problem, i.e. most fillings are placed under unknown diagnostic criteria, may be raised in the study. If the changes in DMFS score are largely composed of F (filled) component, the reliability of the study may be questioned, as different dentists will have different criteria in placing a filling. Furthermore, a thorough analysis of caries progression rates in which the decayed component is divided into categories such as initial caries, enamel caries and dentin caries can be obtained from such populations.

2. Variations in traditional approaches, e.g. The Turku Studies

Turku Sugar Studies (Scheinin and Makinen; 1975) is a collaborative study carried out to investigate the effects of chronic consumption of various sugars on dental, oral and general health. Evaluation of caries incidence as influenced by sucrose, fructose and xylitol consumption was carried out, using the data from clinical examination and radiographic finding. Progression and reversal of caries lesions were analyzed through clinical and radiographic data.

Caries lesions were graded as primary or secondary caries without defect (C1 or CS1) and primary or secondary caries with defect (C2 or CS2). The radiographic lesions were also graded as primary or secondary caries with or without defect. The total clinical and radiographic caries were analyzed separately from clinical or radigraphic caries alone. The result showed lowest caries incidence in

the xylitol group and a significant difference was found between the xylitol group and the sucrose group. After two years the mean DMFS increment in the xylitol group was 0.0, while in the fructose group was 3.8 and in the sucrose group was 7.2.

3. Use of combined laboratory techniques to estimate effectiveness

It is clear that adequate laboratory and animal studies are essential to the selection of an anti caries agents. It is usual to assess their potential effectiveness before conducting a clinical trial. In assessing the potential effect of any substance for prevention of dental caries, we can initially attempt to examine its effect on the tooth or artificial caries lesions in a laboratory or in an animal studies.

Combined laboratory techniques with clinical trials provide usefull information in biological effects of the substance before a clinical result can be detected. This was showed by the Turku Sugar Studies in which several chemical and microbiological tests on oral fluids were conducted.

Laboratory tests can also be used to monitor the safety level of fluoride containing preventive products. Levels of fluoride ion in urine are directly related to the level of fluoride ingested. Approximately 50 % of a dose ingested by an adult will be excreted in urine (Ekstrand, et. al., 1988). Monitoring level of urinary fluoride will ensure the level of fluoride ingested, and if urinary fluoride levels increase substantially, the supply of the product can be reviewed immediately. Monitoring urinary creatinine levels is particularly useful when a series of daily specimen is being collected, to check the reliability of 24 hour urine collections.

4. Statistical methods

The purpose of statistical analysis is to ensure that a valid comparison is produced which truly reflects the effect of the experimental factor; to conduct a valid test of significance and confidence limits; and by detailed analysis to gather all possible explanations on the mechanism of action of the agent by revealing variations in effectiveness associated with such factors as age, tooth surface, etc., (Grainger, 1968).

In the usual clinical trial, a group of available children is divided at random into two (or more) groups, one to receive a presumably active treatment, the other a control treatment (placebo). Significance of the results obtained, can be tested by the use of the t-test and Analysis of Variance.

A comparison can be made before and after utilization of fluoridated sucrose to both groups. The appropriate analysis with measurements is the paired t-test, to test for significant difference between the two groups (Chilton, 1982).

VI. CONCLUSION

Caries experience is likely to be higher in developing countries in the near future. Economic growth and the accessability of communication and transportation make it possible for developing countries to gain more information and products from anywhere in the world. Within the developing country, there is tendency that the community will adapt to an easier way of life, including changing dietary pattern as they gradually leave the traditional life style. Dietary pattern changes in the community toward refined carbohydrate rich food and snacking between meal to staple foods. These changes lead to a steady increase in caries experience as such changes are not followed sufficiently by great improvement in dental health services and education.

In Indonesia and many developing countries, water fluoridation seems to be infeasible because of the widely dispersed population, the wide use of well water, and the climate, compared to most developed countries. Although other means of fluoride vehicles such as tooth paste are available in the market, it is not widely used in the community; some people cannot afford it, and others may not have dental health as a high priority.

Since this project was commenced, a report of a WHO conference on using sugar as a fluoride vehicle in developing countries has been published (Bratthall and Barmes, 1995). This study group investigated the issues relating to the ethical aspects of use of sugar as a vehicle, the concentrations which might be most effective, yet safe, and the problems associated with any proposed clinical trial. The major point to arise from this conference proceeding are (Bratthall and Barmes, 1995):

a. The objective of using sugar as a fluoride vehicle is to provide the lowest effective fluoride concentration, without hazardous effect, even in high sucrose consumption subjects.
b. Significant effects on caries from the use of fluoridated sugar is obtained from the use of over 10 ppm fluoride (dry weight), and total intake of fluoride depends upon the total sugar consumption.
c. It is difficult to find an area in developing countries to conduct a clinical trial which has a fairly high caries incidence and substantial sugar consumption. In addition, other preventive programs should not be available and consumption of sugar should be monitored closely.
d. Introduction of fluoridated sugar in the market also involves safety, ethical, political and economical issues.

The ability of fluoride to reduce the cariogenic effects of sugar while incorporated in the sugar was evident from theoretical and laboratory studies and from limited clinical studies (O'Mullane, 1995), especially if it is applied to the community in which it is impractical to use other fluoride vehicles.

Despite the fact that clinical studies in the utilization of fluoridated sugar are very limited because of the difficulty in finding suitable locations and subjects in developing countries (Bratthall and Barmes, 1995), some institutions such as orphanages would provide groups in which a clinical trial can be performed. The scope could be broadened to a set of villages, which have quite stable communities. Control groups can be selected randomly, as generally systemic or topical fluorides are not available to these communities.

There is a need to provide controlled yet optimal concentrations of fluoride to these communities, since this ion is still regarded as the

most effective caries preventive substance. Animal and human studies in implementing fluoridated sugar for caries prevention have been conducted (Luoma et al., 1972; Mundorff, et. al., 1988; Turtola and Luoma, 1972; Luoma, et. al., 1979), which provide promising results in using sugar as a fluoride vehicle.

The use of sugar as a fluoride vehicle has been seen from different perspectives. Not only should oral health issues and safety be taken into account, but also economical, ethical, and even political matters should be considered (Bratthall and Barmes, 1995). Despite all the related matters discussed, the fact that caries experience is increasing substantially in the developing countries, needs to be given urgent consideration. Clinical trials are needed to test the effectiveness of fluoridated sugar in inhibiting dental caries not only in Indonesia, but also in different developing countries, provided that laboratory experiments support its effectiveness and safety in regard of the dose to be implemented. The present study is being carried out to provide more understanding of the use of fluoridated sugar as a caries preventing substance.

VII. HYPOTHESIS

The hypothesis of this research project is:

> The utilization of a 10 ppm Fluoride co-crystallized sucrose over a period of 18 months provides a significant inhibition in the development of carious lesions.

CHAPTER II

CHAPTER II

MATERIALS AND METHODS

The overall objective of the project was to establish a field trial to determine whether sucrose could be effectively used as a vehicle for fluoride delivery, in regions where the more traditional systemic and topical fluoride vehicles eg. water, salt, milk and tooth pastes were not readily available. This type of project had not been attempted since Luoma's studies in 1979, and then within a confined groups of disabled children on a restricted control basis. As it was being held within a very different environment and as the project was likely to generate some level of controversy, the establishment of the field trial was proceeded by a number of experiments to provide more specific guidance, as to the most effective dose, oral clearance rates, and effectiveness *in vitro* in achieving some degree of remineralization. Precise observations were also carried out on prospective study populations in term of their suitability for the study.

For these reasons, the project is separated into three sets of investigations. However, a particular aspect of the proposed study needs to be first clarified. While sucrose is being tested as a vehicle resulting in systemic ingestion of fluoride ion, the effectiveness of the fluoride ion is expected to be almost entirely topical in this study. The use of such a method where the systemic effect is to be tested requires a prolonged period of evaluation, involving delivery at a very early age

and determining of inhibition of caries in permanent teeth once they erupt. This is the major mode of action of fluoride in artificially fluoridated water supplies. The present study is similar in some ways to the use of fluoride ion in salt, as used in Switzerland, except that a much higher dosage rate is involved (250 ppm) in salt because of low consumption rates.

In this project, it is expected that the study population will have had all permanent teeth erupt, and thus the design of the evaluation will monitor changes in caries experience over approximately 18 months following commencement of sugar intake.

The three sequential stages of the project are described below:

I. Preliminary Experiments (Including Results)

A. *In vitro* assessment of the most appropriate dose of fluoride to achieve caries control in enamel.

B. Assessment of oral retention rates of such a dose.

C. Initial investigation into the mode of delivery involving sucrose.

D. *In vitro* assessment of the effectiveness of fluoridated sugar in promoting remineralization of artificially generated enamel caries lesions.

II. Observations and Planning for a Field Trial in Medan, North Sumatera, Indonesia.

A. Selection of suitable groups of subjects for the proposed trial.

B. Precise observation and recording of the sucrose eating pattern of proposed subjects, over a prolonged period of time.

C. Assessment of the fluoride content of normal foods and beverages consumed throughout the year.

D. Selection of a sugar mill to assist in permitting co-crystallization of fluoride with sucrose samples, and determination of the precise method of co-crystallization to be followed.

E. Manufacture of fluoridated sugar, monitoring of fluoride levels in batch samples and storage of the sugar.

F. Choice of study format and of records to be used.

G. Summary

III. The Field Trial, Carried Out in Medan, North Sumatera, Indonesia.

A. The establishment phase:

1. Appointment of Co-ordinator,
2. Establishment of Double Blind Study condition
3. Pre - study clinical examination and record taking,
4. Distribution of sugar and control of intake

B. The continual monitoring of urinary fluoride levels of subjects.

C. The clinical evaluation of effectiveness of 10 ppm fluoride co-crystallized sucrose in inhibiting dental caries, using clinical and radiographic means of caries monitoring, following 18 months of intake

D. The evaluation of demineralization/remineralization balance resulting from use of 10 ppm fluoride co-crystallized sucrose.

NB: Ethical approval for all experiments involving human subjects was sought and given, by The Committee for the Ethics of Human Experimentation of the University of Adelaide. Consents were given by subjects and/or their guardian/principal to participate in the study (Appendix 1).

I. The Preliminary Experiments (Including results)

A. *In vitro* estimation of the effectiveness of various concentrations of fluoride ion in inhibiting the development of artificial caries lesion in tooth enamel

Sound premolar tooth crowns were cut into buccal and lingual halves and entirely coated with coloured nail varnish except for two windows measuring approximately 3 mm x 1 mm, exposing the enamel on the buccal or lingual surfaces (Figure 4).

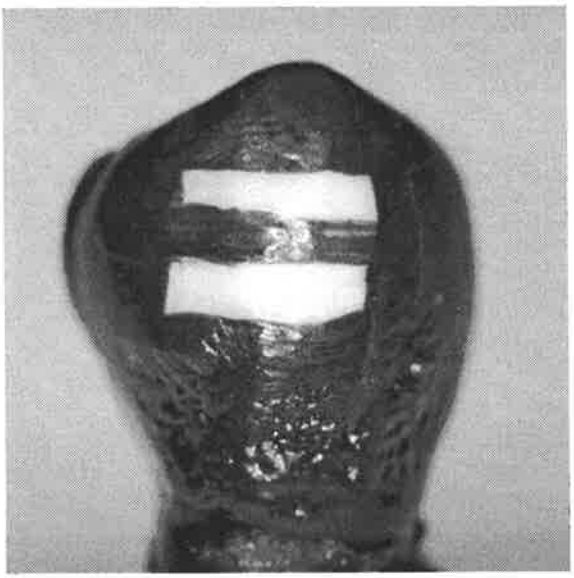


Figure 4: Buccal surface of a premolar showing two windows

An artificial caries generating system, commonly used in caries research (Featherstone, 1987) was prepared. This was an Acetate demineralizing solution at pH 4.3, containing 0.05M Acetic Acid and 2.2mM $CaHPO_4$. Fluoride ion resulting in a concentration of 0, 0.5, 1, 5,

10, 20 and 40 ppm respectively was added to batches of the solution to test the ability of fluoride ion present to inhibit the demineralization process. Each specimen was placed in 40 mls of the solutions respectively in which 5 specimens were used for each solution, in each category of time. The specimen jars were placed in an orbital mixer incubator (Ratek Instrument, Australia) without agitation at 37°C for 7, 14 or 28 days (Figure 5).

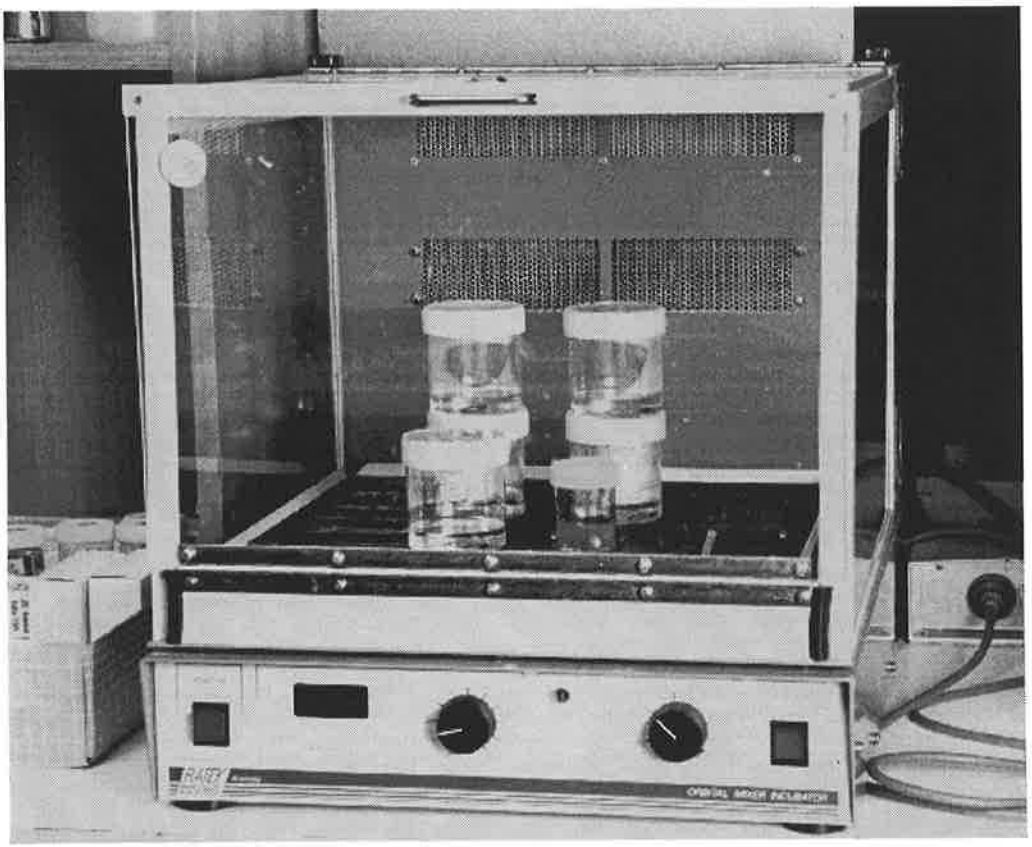


Figure 5: Orbital Mixer Incubator (Ratek Instrument, Australia)

After the set time elapsed, the crowns were imbedded in araldite as descibed below:

Araldite M and Hardener HY 5160 at 10:4 ratio were mixed using a rotator (Ratek Instrument) for 30 minutes. The container was then

placed in a vacum until the mixture was cleared of air bubbles. Each of the crowns was placed into a cubical mould (1,25 cm^3), and poured with araldite mixture and placed in an oven at 65° Celcius for 48 hours. The specimens were then sectioned using a bench sectioning machine (a modification of the Gillings-Hamco Hard Tissue Sectioning Machine, Figure 6) with a 0.13 mm thick circular diamond saw blade (Van Moppes, specification, D54T00-NP) and polished to a thickness of 100 micron using silicone carbide paper No. 800, 1200, and 4000. The lesions were then imbibed in quinoline and the depths of the lesions were measured using a polarized light microscope (Olympus BH-2, Figure 7).

The result of the experiment is illustrated in Table 1.

Table 1: Effect of various concentrations of F ion in demineralizing solutions on lesion depth over 7, 14 and 28 days (N=5)

time	Mean lesion depth (in µm)						
	F concentration in solution (in ppm)						
	0	0.5	1	5	10	20	40
7 days	186.6	96	96	32	14	8	0
14 days	362	210	118	74	27	15	6
28 days	517	395	230	106.6	80	67	33

It was found that a concentrations of 10 ppm F ion and above in the solution provided high level of inhibition of the development of caries. Thus, it was decided that a concentration of 10 ppm be chosen as that providing a high level of protection and having less chance of causing fluorosis by ingestion by infants and young children. This was

in confirmation with Luoma, et. al., (1994), who suggested that optimal protection was obtained from a 10 ppm F ion in the solution. Further tests however, examine the consequences of concentrations of fluoride ion ranging from 5 ppm to 20 ppm.

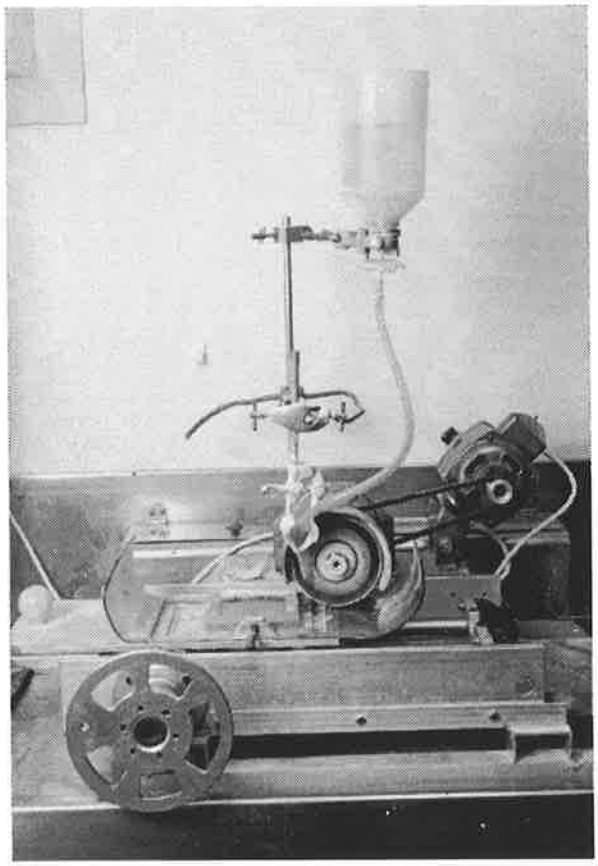


Figure 6: Diamond Saw Sectioning Machine

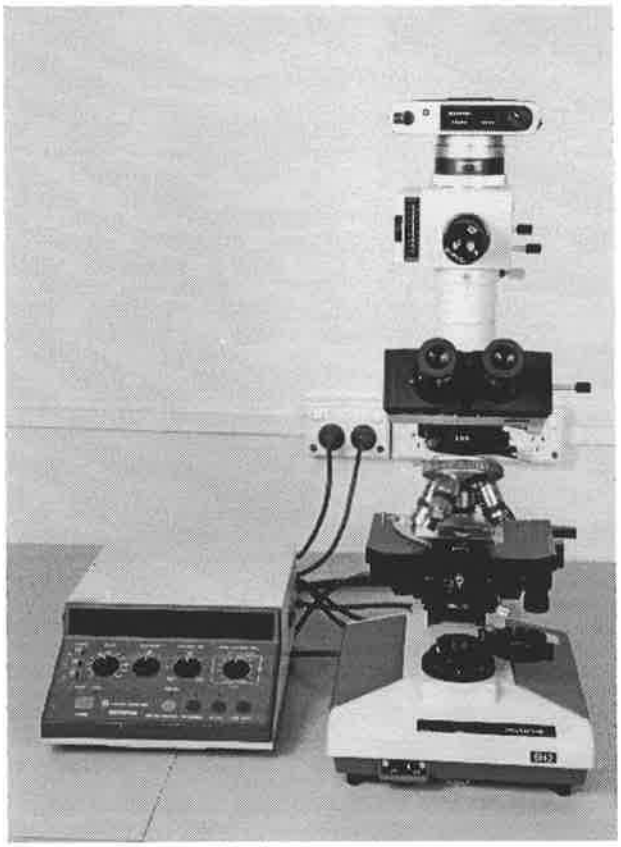


Figure 7: Polarized Light Microscope (Olympus BH-4)

B. Oral clearance/retention of fluoride from sugared solutions and local cakes containing 10 ppm and 20 ppm fluoride ion.

Approval for this experiment was obtained from The Ethical Committee of Human Experimentation of The University of Adelaide, and written consents from the subjects were obtained prior to the beginning of the experiment (Appendix 2).

As a concentration of fluoride ion between 10 and 20 ppm in food and beverage was likely to provide caries protection, the next experiment aimed to provide samples of food or solution, similar to those which would be used in Indonesia, which contained these

concentrations of fluoride ion. The objective was to determine the oral retention rate, or conversely, the clearance rate of fluoride ion, when these types of ingestion were used. This would assist in knowing the concentrations retained at the tooth surface, and the time of retention. This information in turn would provide some indication of the elevations in salivary fluoride which might be experienced several times a day, and the likely degree of remineralization.

Two different sugar solutions (25 % sugar solution with 10 ppm fluoride ion and 25 % sugar solution with 20 ppm fluoride ion) and two different cakes (cakes made with sugar containing 10 ppm F ion and with 20 ppm F ion) were prepared for the experiment. These cakes were of the variety commonly consumed in Indonesia which consisted of flour, coconut and sugar.

Four healthy consenting subjects were asked to hold the solution in their mouths or to chew the cake for one minute before swallowing it. The subjects were asked to collect 3 mls of unstimulated saliva into measuring vials through a small funnel, before, immediately after and 10 minutes after swallowing the solutions or the cakes.The collected saliva samples were directly subjected to fluoride separation by rapid diffusion using Hexamethyldisiloxane (Taves, 1968). The separated fluoride ion content was determined using a fluoride specific electrode (Orion, model 9609-00) connected to an Expandable Ion Analyzer EA 940 (Orion).

The Taves rapid diffusion, closed acid hydrolysis system for separating fluoride ion from mixed substances (Taves, 1968) relies on hydrolysis of products with 6M Hydrochloric Acid (HCl), resulting in the formation of Hydrogen Fluoride (HF). The HF reacts with the Hexamethyldisiloxane (HMDS) present in saturated levels in the acid,

forming a volatile fluorosiloxane. This volatile component is caught in a fluoride sink containing 0.5 ml of 1.65M Sodium Hydroxyde (NaOH) and neutralized. The siloxane component is released to continue to take part in the reaction, while all fluoride ion is eventually transferred into the sink. Following several hours, the sink is removed, water evaporated and the fluoride ion is reconstituted with 1 ml 1.34M Acetic Acid.

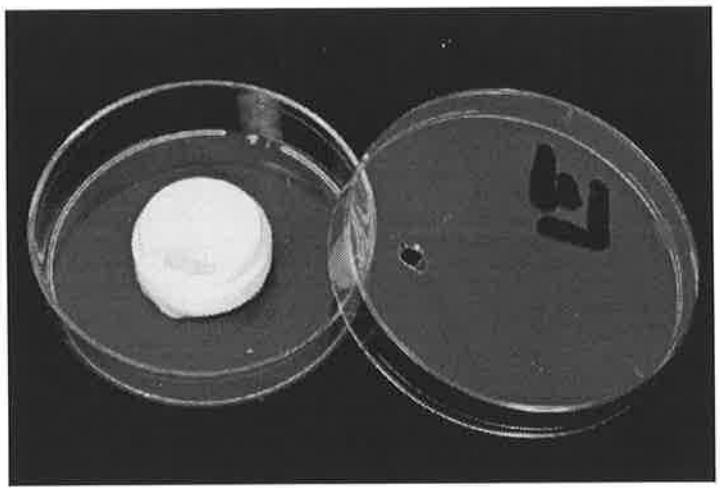


Figure 8: The petri dish used in the Taves method

The procedure was carried out as followed:

1. A 2-mm hole was made on the lid of a 65-mm plastic petri dish, and the edge of the lid of a No. 4 plastic vial was trimmed about 1 mm to permit entry of volatile agents once the petri dish cover was in place. This vial lid was then stuck onto the base of the plastic dish by petroleum jelly. This would acting as a fluoride sink (Figure 8).

2. A volume of 0.5 ml of 1.65 M NaOH was placed in the fluoride sink and one gram of solid material to be tested was crushed and placed in the outer area and 3 mls of DDW were added (3 mls of material were needed where liquid material was being analyzed). Fluoride standard solutions of 0.01 ppm, 0.1 ppm, 1 ppm 10 ppm and 100 ppm were included in the test.
3. The petri dish was then covered with the lid after sealing it with petroleum jelly. One ml HCL saturated with HMDS was injected through the hole and covered immediately with petroleum jelly and a piece of parafilm. The petri dishes were then shaken for at least 8 hours on an Orbital Shaker SS70 (Chiltern Scientific) at a minimum rate (Figure 9).
4. The fluoride sink was then removed and placed in a drying oven at 70^{O} C until all moisture dissappeared.
5. The fluoride was reconstitued with 1 ml of 1.34M Acetic Acid, before placing the base on the caps and the whole vial shaken on a vortex mixer for 10 seconds. Care was taken to hold the lid firmly to the vial, as gases were produced during this shaking.
6. The millivolt (mV) reading of sample was carried out using a Fluoride specific electrode (Orion, model 9609-00) in conjunction with an Expandable Ion Analyzer EA 940 (Orion; Figure 10).

A curve was constructed on semilog graph paper from the fluoride standard readings and the fluoride concentrations of the samples were determined from the resulting curve (Figure 11).

The result of the experiment is illustrated in Table 2:

Table 2: Oral clearance/retention of fluoride from sugared solutions and cakes containing 10 ppm and 20 ppm fluoride ion (N=4)

Time	Fluoride concentration in saliva (ppm)			
	10 ppm F solution	20 ppm F solution	10 ppm F cake	20 ppm F cake
Before	0.013	0.032	0.00	0.00
Immediately following	0.088	0.178	0.021	0.105
After 10 minutes	0.012	0.032	0.006	0.005

The result revealed that the fluoride concentration of saliva was elevated immediately after drinking the solutions or eating the cakes, and reached the baseline concentration in 10 minutes. It was also clear that the elevation of fluoride concentration was more pronounced from the solutions than the cakes as the solutions would spread more evenly and readily in the mouth than the cakes, which were swallowed more in bulk.

While these concentrations were low, it was recognized that the consumption of foods and beverages in a daily basis would result in multiple periods of such elevations, and this may be sufficient to result in the ability to inhibit demineralization and enhance remineralization. Furthermore, a certain proportion of the retained fluoride would have been absorbed into plaque, and stored at the tooth surface. The retention from the 20 ppm products were significantly higher than that from 10 ppm products. However, again, the balance needed between dose and effect indicated that a 10 ppm level of fluoride ion be initially chosen for testing.

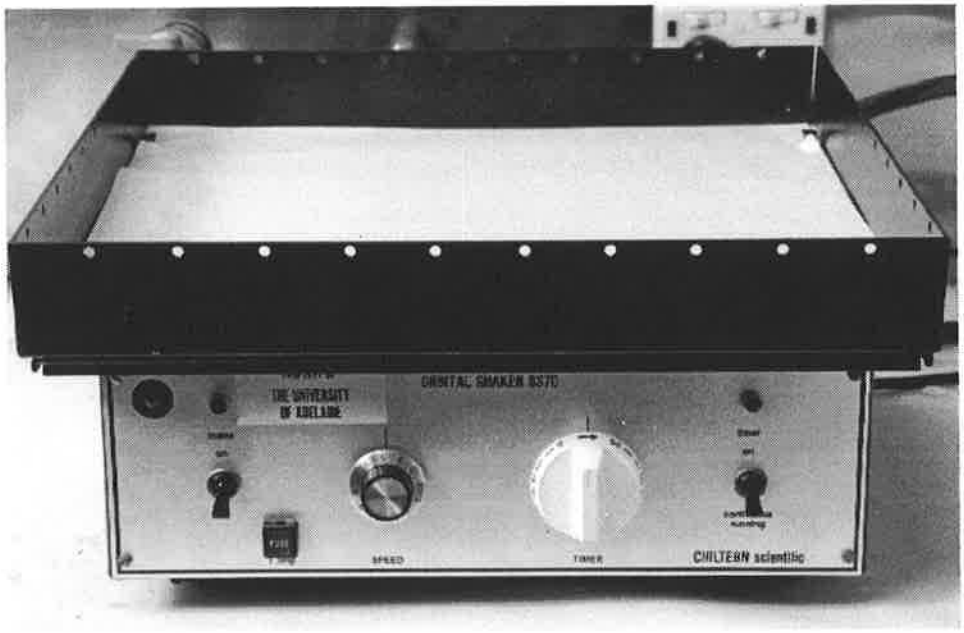


Figure 9: Orbital Shaker (Chiltern Scientific)

Figure 10: Fluoride Ion Electrode and Expandable Ion Analyzer (Orion)

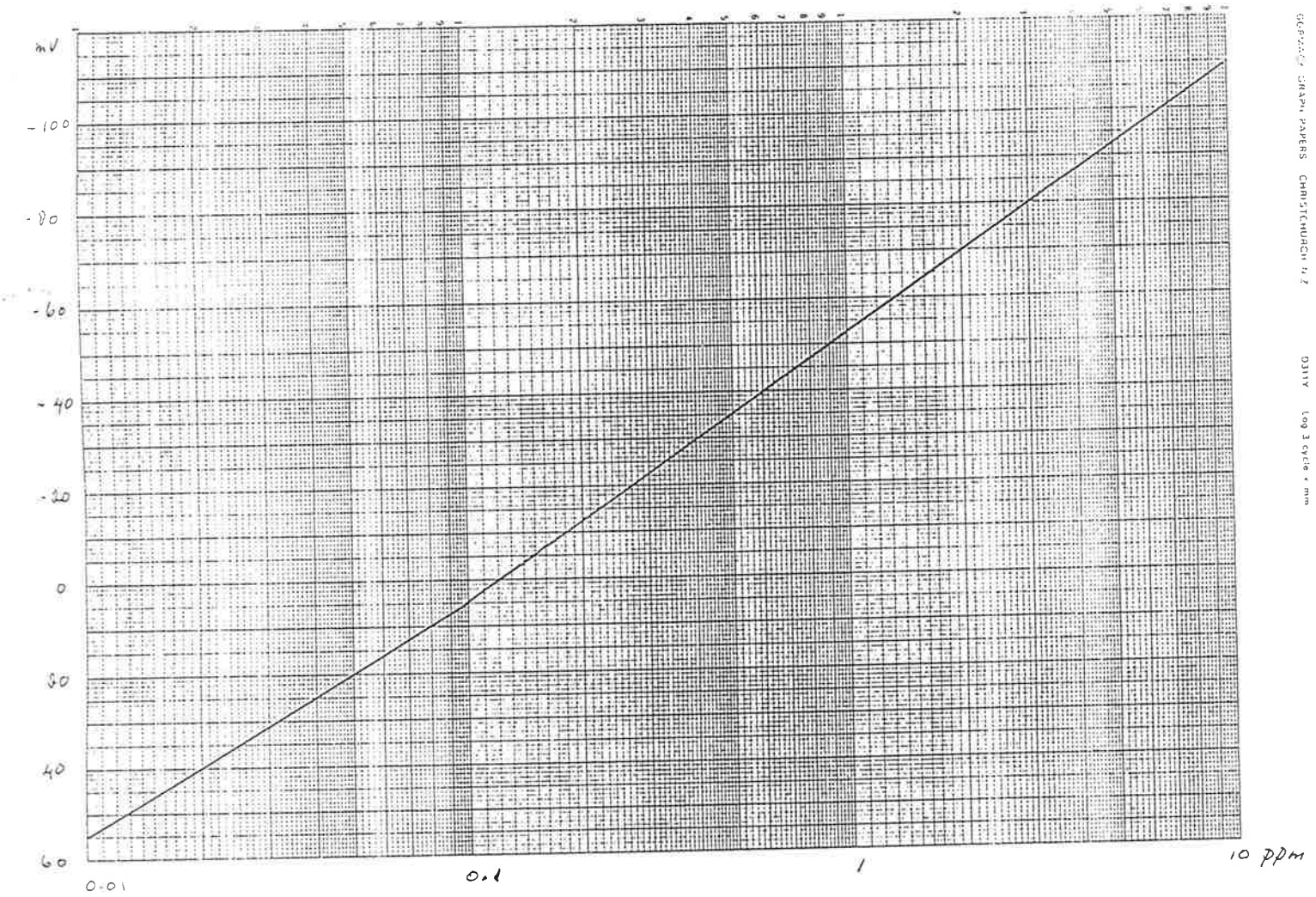


Figure 11: Curve of fluoride standard readings on semilograph paper

C. Laboratory preparation of a 10 ppm fluoride co-crystallized sucrose

As the aim of the project was to evaluate sucrose as a fluoride vehicle to prevent development of dental caries, it was necessary to develop a method where by fluoride could be attached to the sugar crystal. A review of the literature which discussed the used of chemically bonded fluoride to sucrose molecules (1'-Fluorosucrose), as used extensively in plant growth research, made it evident that it was difficult to break this bond under the conditions present in the oral cavity. Furthermore, replacement of one hydroxyl group by fluoride ion to form 1'-fluorosucrose only occurs in a very complicated chemical reaction under strict laboratory conditions (Hitz, et. al., 1985; Lemoin, et. al., 1988). Hence, it was realized that the fluoride needed to be loosely bound to the sucrose crystal, so that it could be readily released on placement in the mouth. The straight addition of NaF powder to sucrose was not suitable, as it fell to the bottom of the packet and was thus not evenly spread through the sugar bulk.

It was decided to use the method of Mundorff, et. al., (1988) and Bowen and Pearson (1992) in co-crystallizing the fluoride with the sucrose crystal. This was attempted by a variety of methods, in which concentrations of 250 ppm, 500 ppm, 750 ppm and 1000 ppm F solution were added to sugar using different ways of mixing the solution and the sugar. These included direct addition of fluoride solution to 250 gms of sugar, followed by thorough mixing, and redrying; and the use of a fine spray to apply the fluoride solution over a thinly spread layer of sugar.

It was realized that the method of spraying the solution onto the sugar would be that most likely suitable in the sugar mill. This was confirmed later, following discussion with the administrator of the sugar mill. He proposed that it would be feasible to spray the solution on sugar at the end of the conveyor belt before it flowed into the drying tube. If the mixing was to be carried out at another stage in sugar processing such as at the mother liquor stage, significant amounts of the fluoride would be washed away when the sugar went through further processes, and it would be very difficult to calculate the exact amount of NaF needed.

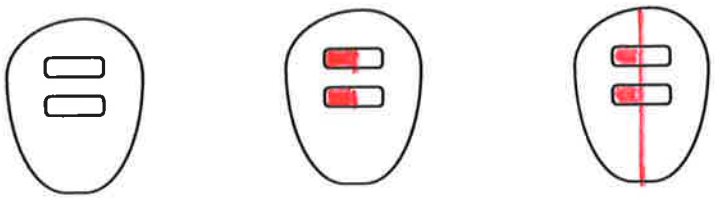
As this was difficult to simulate in the laboratory, the method used to determine the concentration of NaF needed, involved spraying the fluoride solution onto 250 gms of sugar. This was then placed in a rotator (ordinary kitchen) mixer at a slow speed, for 10 minutes until thoroughly mixed. The sugar was stirred and dried on a hot plate under an infra-red light for 15 minutes at low heat. The concentration of fluoride in the sugar was determined using a fluoride specific electrode (Orion, model 9609-00) after subjecting the sugar to fluoride separation by rapid diffusion using hexamethyldisiloxane (Taves, 1968). After some trials at different concentrations of fluoride solution, it was found that a fluoride concentration of 10 ppm co-crystallized in sugar was obtained from 10 mls of a 650 ppm fluoride solution mixed with 250 gms sucrose.

D. *In vitro* assessment of effectiveness of 10 ppm fluoride co-crystallized sucrose in promoting remineralization of artificial caries lesions

It was realized that with such low concentrations of fluoride, it may take some years before significant clinical results could be obtained. Thus, there was a need to explore a system where by a more rapid determination of whether some degree of inhibition of demineralization, or enhancement of remineralization might be achieved. It was decided to investigate a test using artificially generated caries in enamel slabs for use initially *in vitro*, placed in a remineralizing solution simulating those condition present in the oral cavity. If this provided a significant result, then this method could be applied in an *in vitro-in vivo* study.

Sound human premolar crowns were cut into buccal and lingual halves, dried and entirely coated with coloured nail varnish except for a window measuring approximately 3mm x 1 mm, exposing the enamel on the lingual or buccal surfaces (Figure 12a). Specimens were immersed in demineralization solution described in experiment IA (page 68) for 8 days to create artificial caries approximately 300 microns in depth. Half of each lesion was then coated with coloured nail varnish to protect it from remineralization while the uncoated lesion would be remineralized (Figure 12b) when the crowns were immersed in remineralization solution. The remineralization solution used was developed by ten Cate (1979) to provide levels of remineralization similar to saliva, to which was added fluoridated sugar. The solution contained 1.5 mM Calcium Chloride ($CaCl_2$), 0.9 mM Potassium Hydrogen Orthophosphate (KH_2PO_4), and 150 mM Potassium Chloride (KCl), and was adjusted to pH 7. This was mixed with 5 ppm, 10 ppm

or 20 ppm fluoride co-crystallized sugar respectively at 1:1 ratio (1 gram sugar/1 ml remineralization solution). The final concentration of fluoride ion in the solution would be 2.5 ppm, 5 ppm and 10 ppm respectively.



a b c

Figure 12 : Coating and sectioning of demineralized and remineralized crown
a. Premolar crown with two windows
b. Coating of half of the lesions after demineralization
c. Cutting line after remineralization

Groups of 5 crowns were immersed in remineralization solution mixed with fluoride co-crystallized sucrose with a final concentration of 2.5 ppm, 5 ppm and 10 ppm fluoride ion in the solutions respectively and were put in the incubator at 37^{o} C for 28 days. Following remineralization the crowns were imbedded in araldite as described in A1 (page 68), and cut through the middle of the lesions at the border of the nail varnish coating (Figure 12c), using Van Moppes circular diamond saw blade (0.13 mm thick) attached to a bench sectioning machine. Both pieces of the crown were placed in a circular mould so as to expose the cut surface to the surface of the mould and were subjected to a microhardness test as described below:

1. Araldite Imbedding

Araldite was mixed as described previously in experiment IA (page 69). The inside wall of a 2.5 cm high circular mould cut from a PVC water pipe was coated with vaseline and placed on the surface of a piece of glass which was also coated with vaseline. Two pieces of the crowns bearing demineralized and remineralized parts of the lesions were then placed in the mould with the cutting surface of the lesions facing the surface of the glass and then poured with araldite mixture and placed in the oven for 48 hours at 65^{o} C. The imbedded lesions were then polished using an Abramin Polisher (Figure 13) with Silicone Carbide paper No. P.1000 and P. 1200 to get two parallel surfaces followed by polishing with a cloth with 2.5 micron and 0.25 micron diamond paste incorporated.

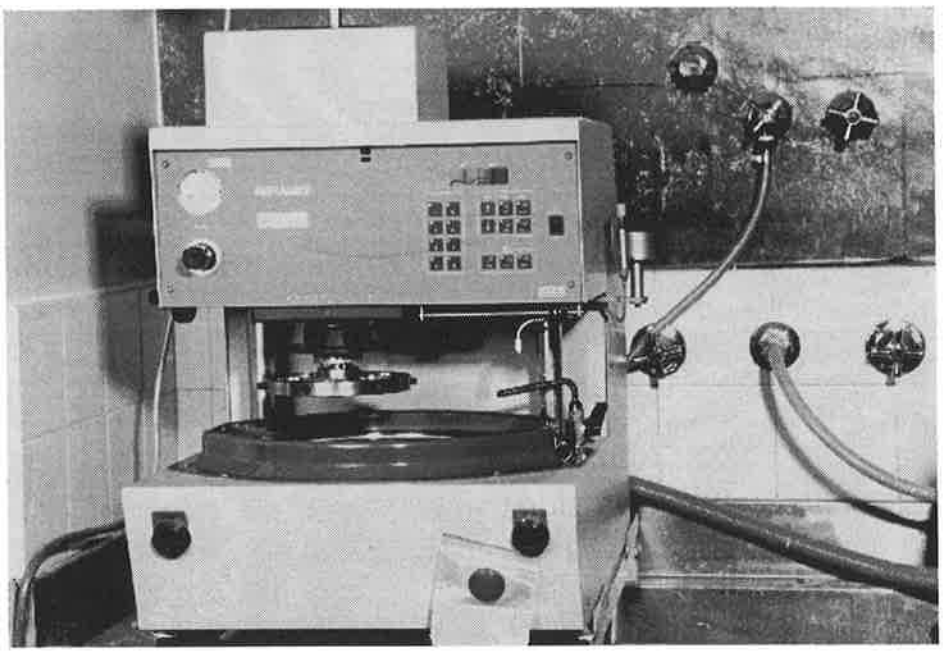


Figure 13: Abramin polisher

2. Microhardness test

Microhardness tests were carried out on the lesions using a Leitz Miniload Hardness Tester (Figure 14). The hardness profiles across lesions were produced by a Knoop Diamond Indentor at 25 micron intervals from the surface using 50-gm or 25-gm weight loaded for 10 seconds.

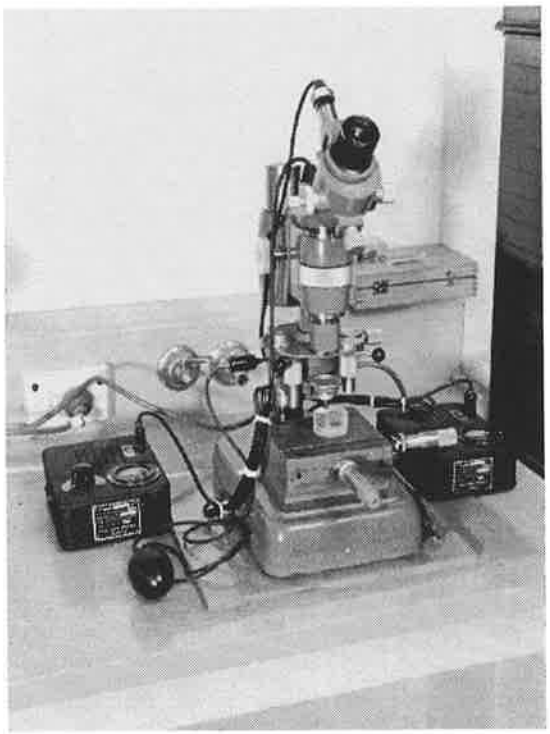


Figure 14: Miniload Hardness Tester (Ernst Leitz)

The Knoop Hardness Number was calculated using the formula:

$$KHN = \frac{14130\ K}{L^2}$$

Where : KHN = Knoop Hardness Number
K = Force (in gram)
L = Observed Indentation Length (in micron)

The Volume % mineral was calculated using the formula: (White and Featherstone, 1987):

$$\textbf{Vol. \% mineral} = 4.3\sqrt{\textbf{KHN}} + 11.3$$

Following the conversion to mineral content values, serial data of each lesion was normalized by assuming that the sound enamel mineral content within each specimen measured 85% volume mineral (White and Featherstone, 1987).

3. Determination of surface profile loss or gain of mineral across the lesion

Lesion progression or reversal can be evaluated by comparing the mineral depth profiles that are constructed using longitudinal microhardness measurement at 25 microns intervals within the specimen. The quantitative evaluation of lesion progression/reversal is restricted to each of these intervals.

White and Featherstone (1987) found that it was advantageous if the comparison was made on the area of lesion progression/reversal which represents a useful format to compare treatment effects. This evaluation was facilitated by using Simpson's Rule of approximating a definite integral for the area under a curve (Thomas and Finney, 1979). The formula of Simpson's Rule, illustrated in Figure 15, can be expressed as:

$$\textbf{Ap} = h/3\,(Y0 + 4Y1 + 2Y2 + 4Y3 + 2\,Y4 + \ldots + 2Yn\text{-}2 + 4\,Yn\text{-}1 + Yn)$$

Where : Ap = Area under the parabola (curve)
h = interval
Y = ordinates of the curve as a function of x

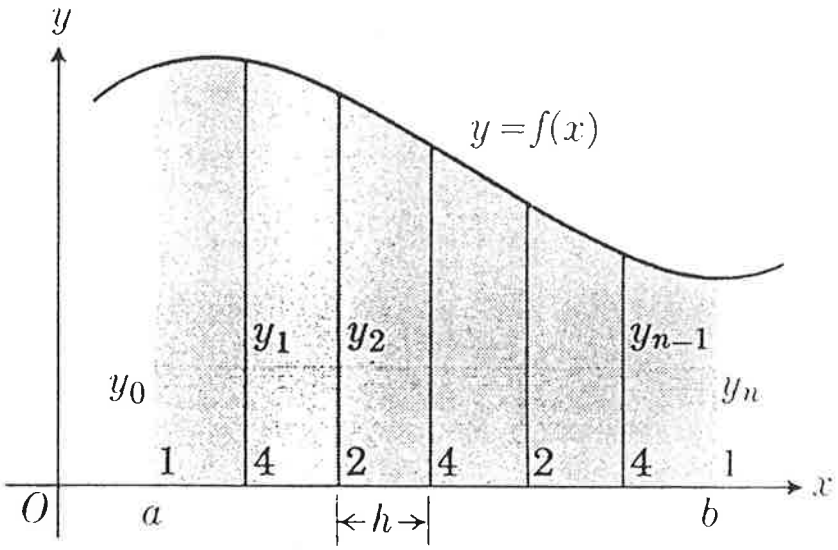


Fig.15: The Area under a curve with even subdivisions can be calculated using Simpson's Rule (Thomas and Finney, 1979).

A computer program applying Simpson's Rule (Microsoft Excell ver.5) has been used to curve-fit the discrete interval measurements and integrate the area under the microhardness traced curve.

Differences between area (ΔZ) of demineralized and remineralized lesions of each groups (remineralization in 2.5 ppm F, 5 ppm F and 10 ppm F ion respectively) were calculated and these differences were analyzed using One-way Analysis of Variance with a multiple range test (Scheffe test) with significant level $p< 0.05$.

4. Replicability Study

A replicability study was performed in which double determinations were carried out on separate occasions for 40 indentations of 4 lesions. Values of these two determinations were subjected to an Analysis of Variance, Intraclass Correlation Coefficient of Reliability (R) was calculated using the formula (Fleiss, 1987):

$$R = \frac{N(IMS - EMS)}{N.IMS + (k-1)\,MMS + (N-1)(k-1)EMS}$$

Where : N : Number of measurements (40)
IMS : Mean Square of Indentation length
MMS : Mean Square of Times of measurement
EMS : Mean Square of Error
k :Time of measurements (2)

The result of the reliability study as presented in Table 3.

Table 3: Reliability of measurements of indentation length of microhardness test (N: 40)

Source of variation	df	SS	MS	F ratio	R
Indentation length	39	309856.05	7945.03		
Measurement	1	216.15	216.15	3.42	0.98
Error	39	2466.72	63.25		

The result of the reliability study indicating that reliability of the measurement is very high (R=0.98).

5. Result of experiment

The data relating to the *in vitro* remineralization experiment are presented in Appendix 3. The result of this experiment is illustrated in Table 4, and in Figure 16 a, b and c.

Table 4: Differences of demineralized and remineralized lesions (ΔZ) in 2.5 ppm, 5 ppm and 10 ppm F ion in remineralizing solution (N = 5)

Solution	area		differences (ΔZ)
	demin	remin	
2.5 ppm F in remin solution	13071.20	14310.65	1239.45
5 ppm F in remin solution	12475.18	14575.55	2100.37
10 ppm F in remin solution	6646.56	9113.34	2466.77

The results of the experiment showed that subsequent remineralization can be obtained from the use of 10 ppm and 20 ppm fluoride co-crystallized sucrose in the remineralization solutions resulting in 5 ppm and 10 ppm fluoride ion in the solutions respectively. Analysis of Variance with Scheffe Test with significant level at $p < 0.05$ revealed that there was no significant difference between the three groups, even though there was a tendency toward greater remineralization using higher fluoride concentration in remineralizing solution. However, remineralization obtained from 10 ppm F in solution was not much greater compared with remineralization obtained from 5 ppm F in solution (10 ppm F ion added)

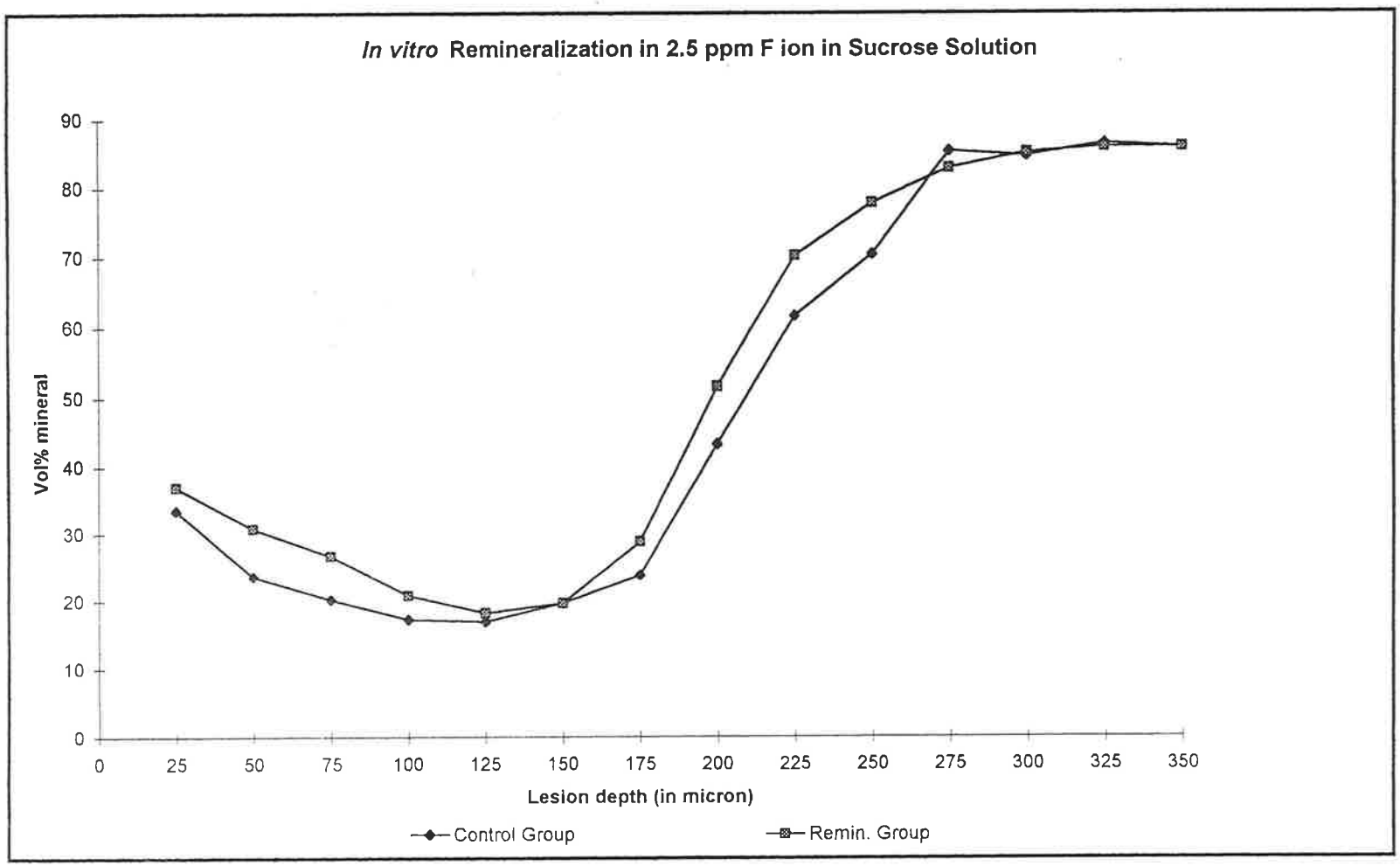


Figure 16 a: Profile of demineralized and remineralized lesions in 2.5 ppm fluoride ion in sucrose solution

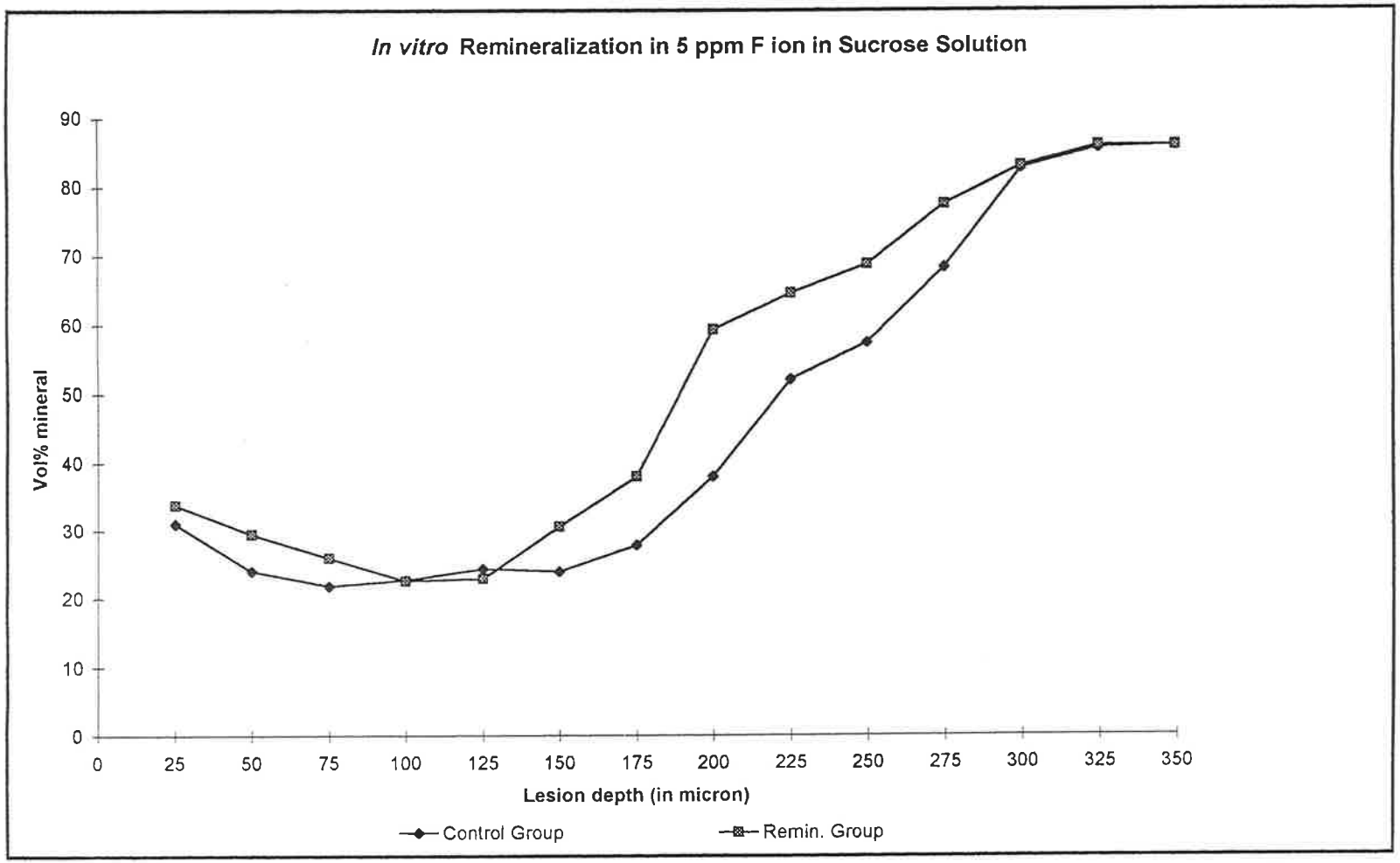


Figure 16 b: Profile of demineralized and remineralized lesions in 5 ppm fluoride ion in sucrose solution

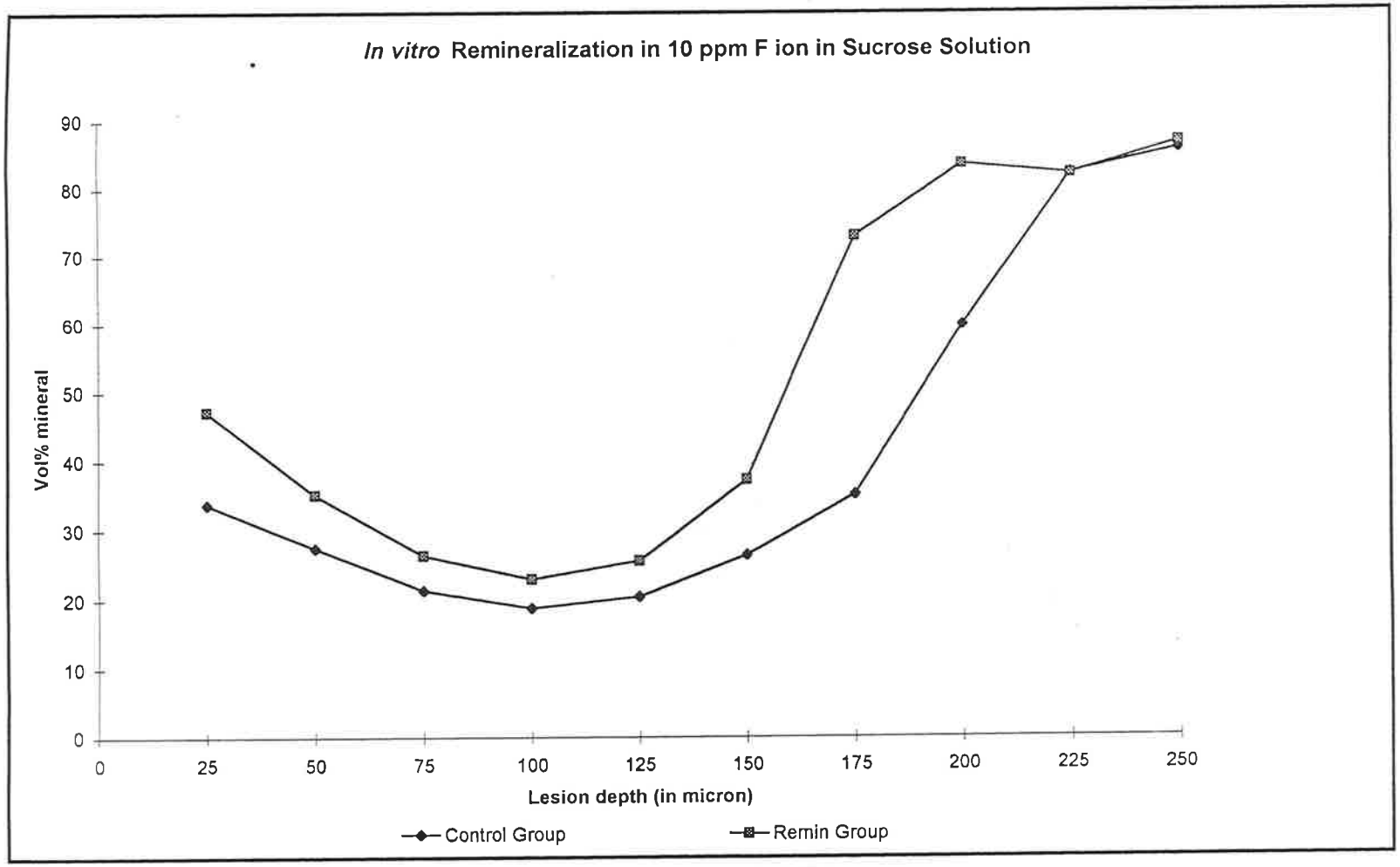


Figure 16 c: Profile of demineralized and remineralized lesions in 10 ppm fluoride ion in sucrose solution

E. Summary of Preliminary Investigations

The concentrations of fluoride ion in sugar found by Luoma, et. al. (1979) to result in high level of protection but not risk intake at levels likely to result in fluorosis in young subjects with developing teeth, was 10 ppm. The animal experiments of Mundorff, et. al. (1988) and Bowen and Pearson (1992) found lower concentration of fluoride ion to result in good protection. However, it is difficult to equate these findings with the human situation.

Clearly, a 20 ppm fluoride ion concentration provides a higher level of protection, retention in saliva, and remineralization than 10 ppm. However, further investigation into the amount of sugar eaten daily by subjects may show that a concentration of 20 ppm results in a daily intake in excess of that considered to increase the risk of fluorosis in young children with developing teeth.

Dean (1938) found that, above 1 mg fluoride ion per day, the risk of fluorosis increased substantially. Hence, eventhough 20 ppm provided some advantages, it was expected that this might prove eventually to be too high. Hence it was decided to plan to use the concentration described by Luoma (1985) of 10 ppm for the time being.

B. Planning for the Field Trial in Medan, Indonesia

A. Selection of suitable groups of subjects in Medan for the proposed trial

As the study aims to test the effect of 10 ppm fluoride co-crystallized sucrose on inhibition of caries, it was decided that, in selecting potential subjects, the following requirement/condition should be fullfilled.

- Relatively equal mix of age in which, as the fluoridated sugar would act topically, children with at least 10 permanent teeth erupted would be included in the study (7-18 years).
- Relatively equal mix of gender
- Relatively similar food intake pattern and amount,
- Reasonably equivalent, high DMFS Scores present,
- Similar background sources of drinking water with minimal fluoride ion present,
- Routine use of nonfluoridated tooth pastes
- Can be strictly controlled to prevent outside sources of sucrose being used by the children.

Two "Panti Asuhan" (a kind of orphanages, which is not only caring for orphans but also taking care of children of poor parents) and one "pesantren" (a kind of boarding school for children, which was not well funded) in Medan (Figure 17) were found to fulfil these criteria, and agreed to participate in the study. The children in the orphanages and the boarding school fitted to the above requirements as they are all

live in a kind of dormitory in which similar kinds of food were provided by the institutions.

a

b.

Figure 17: a. The building of the boarding school
b. The building of the orphanage

The children living in the orphanages, who were not all orphans came from different parts of North Sumatera, and were aged from 7 - 17 years. Various activities for the children were set by the board of the orphanages, besides school activities. Food was provided by the orphanages and occasionaly there would be some food as a charity from people living around the orphanages. Usually the food were main courses consisting of rice, meat and vegetables. Raw material such as rice, flour, eggs, sugar were sometimes received as charitable goods. In this regard where sugar could be obtained from different source, a condition of participation in the project, which provided sugar free of charge was that the orphanages would only use sugar provided by the project.

The children in the boarding school also came from different parts of North Sumatera, aged from 11 -19 years. They came from ordinary Muslim families and they paid only a small fee to the school. Overall, the boarding school was considered to be not as well funded as the orphanages. They live in the dormitory and the activities start from early morning untill about 10.00 pm. The children were permitted to go home in the school holiday at the end of the year. In this regard, it was agreed to provide sugar from the project to every child, to be consumed at home during the period of the holiday.

The three institutions obtained water from the state water supply which was not fluoridated and fluoride from other foods and beverages were found to be very limited (to be described later). In oral hygiene procedure it was found that the children in these institution brushed their teeth using unfluoridated tooth paste (Delident Brand; Red Box; Figure 18) which was chosen because it was the cheapest in Medan.

Figure 18: Unfluoridated toothpaste used in the clinical trial

Written consent (Appendix 1) was given by the guardians of the children in the orphanages and the principal of the boarding school. Consents from guardians/principal on behalf of the children were sufficient as, in Indonesia, it is customary for the children to accept any decision that had been made by their superiors that would be beneficial for them. As required by the Ethics Committee of Human Experimentation of The University Adelaide, a meeting was conducted in the boarding school in which all aspects of the project were explained to all students and staff of the school and questions about the project were answered.

At this observation stage, dental examinations were carried out in the orphanages to assess the approximate caries prevalence of the children. About 30% of the children had a DMFS Score of about 6. The result of this examination is described in Table 5:

Table 5: Caries prevalence in sample children (DMFS Score)

Group	N	Age	Sex	D	M	F	DMFS
Group I	47	8-17	F	3.21	1.70	0.06	4.97
	72	7-16	M	1.60	0.62	0.10	2.32
Group II	30	11-19	F	2.93	1.0	0.27	4.20
	27	12-19	M	1.40	1.67	0.15	3.22

A clinical examination was carried out on children in the orphanages to determine the DMFS scores. The result approved to be similar to that for the children in the boarding school (See Results Section for formal data). Only children with a good general health, were included for evaluation in the study.

B. Observations and recording of sucrose eating pattern of the proposed subject.

Daily sugar consumption for the children in the orphanages and the boarding school was determined at 60 grams/day/child, incorporated in their tea, breakfast porridge and afternoon tea by the guardians/principal of the institutions. This was calculated from monthly consumption of sugar in the orphanages and the boarding school. Sugar was also obtained from snacks which were prepared in their kitchens. There were no individual differences in daily sugar consumption rate as the children did not serve themselves. This daily consumption of sugar would be slightly increased in the fasting month of Ramadhan. In this month the children would not eat anything in the

day time, and would break the fast when the sun set with various sweet cakes or porridge and tea before they have dinner.

Exposure to other sources of sugar was limited as the children in the orphanages did not have enough money to buy sweets or cakes and the children in the boarding school had limited access to go out from the school/dormitory. This intake of sugar per day, with 10 ppm fluoride ion added, would result in ingestion of approximately 0.6 mg fluoride ion per day.

C. The fluoride content of normal foods and beverages consumed throughout the year

The diet consisted of mainly rice and various side dishes such as fish, meat, chicken and vegetables. The raw ingredients of the food were subjected to the Taves Method to test their fluoride contents (Table 6).

Table 6: List of some ingredients of food consumed in the institutions and their fluoride content (in ppm)

Ingredients	F content/ppm	Ingredients	F content/ppm
Rice	0.18	Green bean	0.23
Casava Leaf	0.11	Sumatera tea	2.12
Salted fish	4.08	Rose tea	2.98
Salted Bait	3.73	Coconut milk	0.88
Tempe	0.26	Tahu	0.30

Usually salted bait, salted shrimp were used in small quantity as spices in various soups or curry and fried salted fish will be eaten in small pieces.

The drinking water used in the institutions was also tested and the fluoride content was found to be in 0.00-0.20 ppm range. This is in accordance with Soeparto, et. al. (1974) who found the fluoride content in Medan was about 0.00-0.45 ppm in the rainy season and 0.00-0.55 in the dry season. The level of fluoride consumption from water subsequently could be neglected, while fluoride from food/beverages could be counted only from fish and tea. The fluoride concentration consumed per day from a sample food obtained from the kitchen was calculated as follow :

A. Breakfast

1. 250 gms rice = 250 x 0.18 µgm = 45 µgm

   50 gms tempe = 50 x 0.26 µgm = 13 µgm

or 2. 250 gms green bean porridge = 250 x 0.23 µgm = 57.50 µgm

3. 100 mls Sumatera Tea infusion = 100 x 2.12 µgm =212 µgm

Total F intake: 327.5 µgm

B. Lunch

1. 300 gms rice = 300 x 0.18 µgm = 54 µgm

100 gms veg. = 100 x 0.23 µgm = 23 µgm

10 gms salted fish = 10 x 4.08 µgm = 40.8 µgm

2. water

Total F intake: 117.8 µgm

C. Dinner

1. 250 gms rice = 250 x 0.18 µgm = 45 µgm

   100 gms veg. = 100 x 0.23 µgm = 23 µgm

   50 gms tahu = 50 x 0.30 µgm = 15 µgm

2. 125 mls Sumatera Tea infusion = 125 x 2.12 µgm =265 µgm

Total F Intake : 348 µgm

Total F intake per day = 327.5 µgm + 117.8 µgm +348 µgm

= 793.3 µgm

A total of 793.3 µgm (0.793 mg) was the highest amount of fluoride consumed per meal per child, as salted or fresh fishes was served not so often.

Together with that to be ingested by the experimental group (0.6 mg/day) total daily intake should be approximately 1.4 mg/day. In the age groups participating in this experiment, the concentration is not of great significance, as fluorosis is not a problem. However, any eventual application of this technique to infants would require no greater ingestion rate per day than was being used here.

D. Selection of sugar mill to assist in permitting co-crystallization of fluoride with sucrose samples and determination of the precise method of co-crystallization to be followed

A plantation company in Medan (P.T. Perkebunan -IX) which had two sugar mills was approached, and approval from the company was received for the addition of fluoride into batches of sugar in one of the mills.

The addition of fluoride into sugar was carried out in Kuala Madu sugar mill, located about 30 kms from Medan. The processing method used in this sugar mill is known as the "double sulphitation" process in which SO_2 is used in the clarifying stage and the sugar produced is called SHS sugar (Standard High Sugar). Figure 19, illustrates the working scheme of the production of sugar using double sulphitation process (Mathur, 1975).

In this diagram the sugar cane was carried to the milling plant by cane carrier and the juice which is extracted was heated in the boiler before flowing to the the juice clarification station. In this station lime milk was added to adjust the juice to pH 9.5-9.7, and after that SO_2 fume was sprayed to achieve further clarification. A further process is evaporation of water from the juice in an evaporator station to obtain a good result in the crystallization process. Some steps such as vacuuming and crystal seeding are carried out in the crystallization station and the sugar crystal is then chanelled to the centrifugal machine. From the centrifugal station the sugar is carried by a conveyor to the dryer and cooler tube, before being dropped to the sugar bin and put in 50-kgs bags.

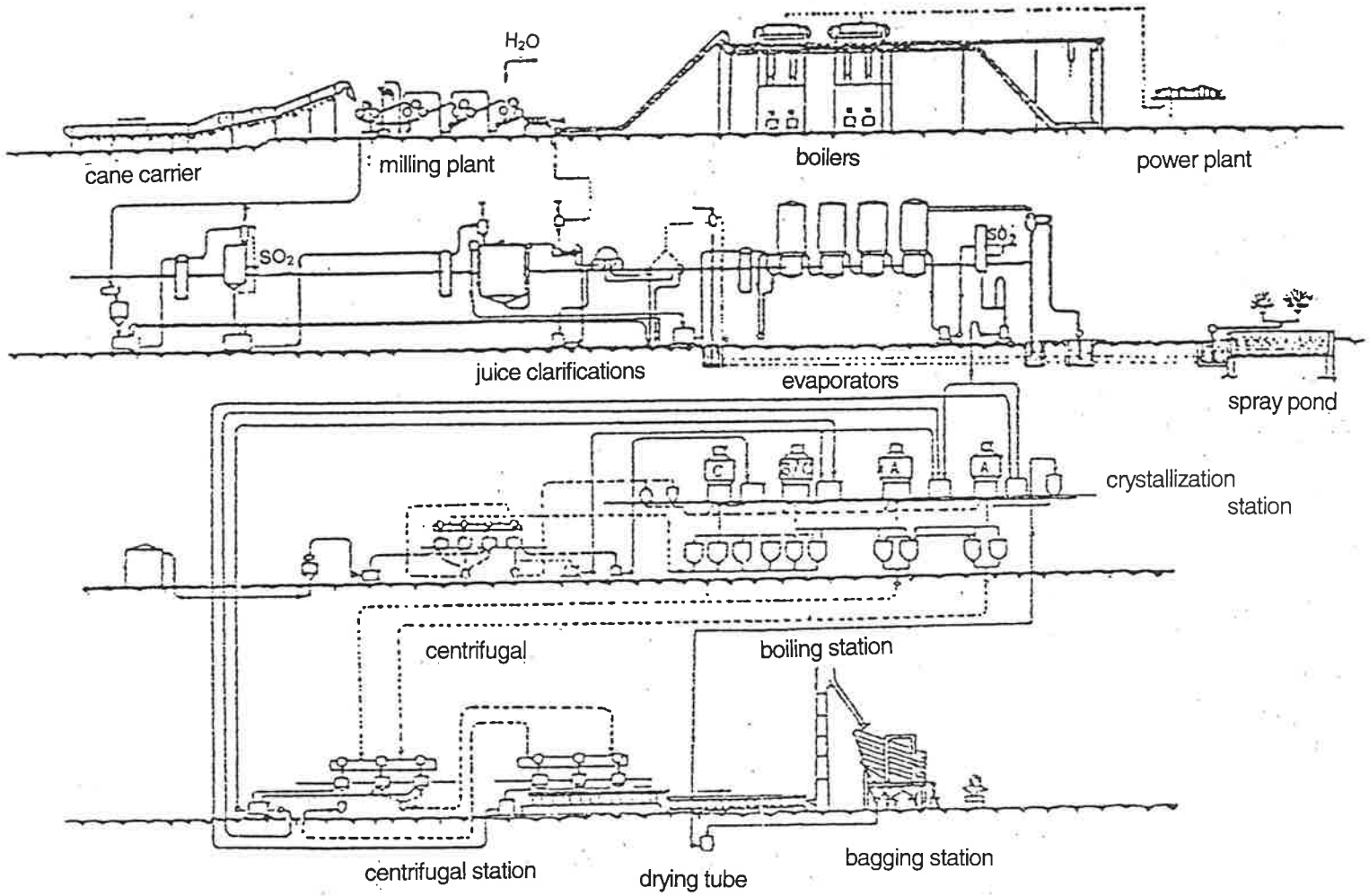


Figure 19 :Schematic diagram of sugar processing by double sulphitation process
Mathur (1975)

Taking into account the complicated sugar processing, it was decided to spray the sugar at the end of the conveyor belt while the sugar flowed down into the dryer which is illustrated in the diagram below (Figure 20).

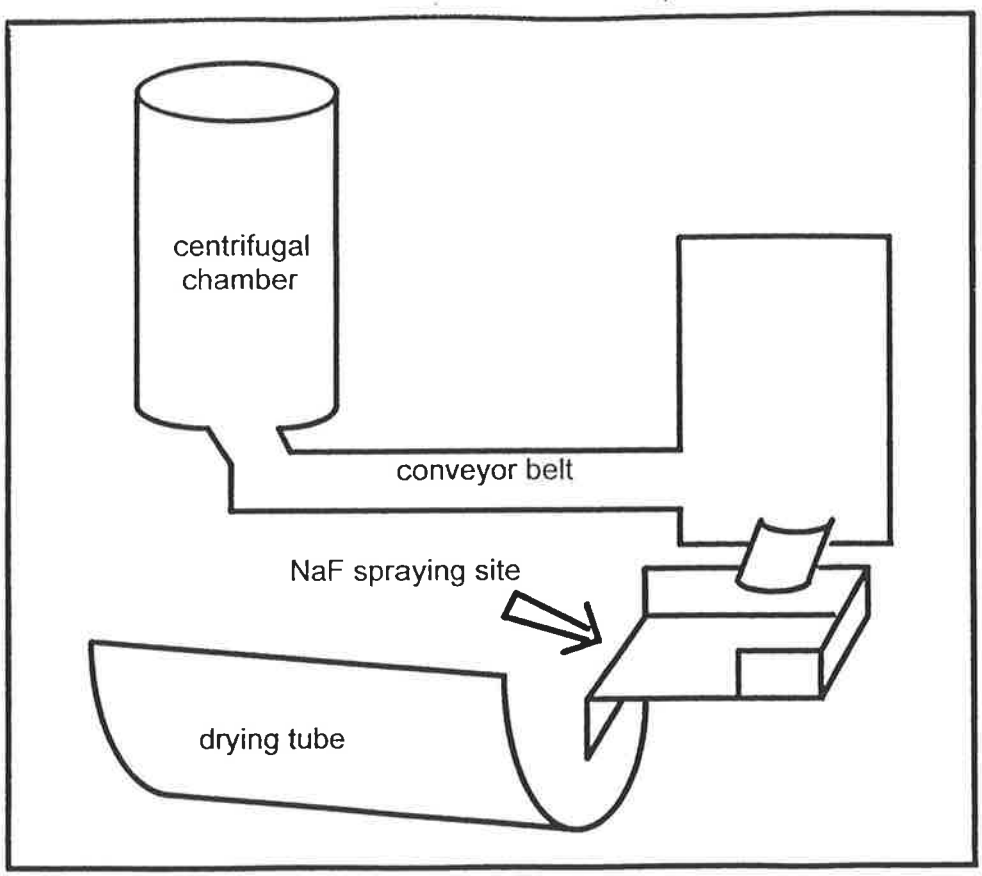


Figure 20: A diagram of the spraying site of the NaF solution.

A special spraying unit incorporating an electrically driven pump was constructed (Figure 21) in which the output flow rate could be adjusted at the end of a spray nozzle. The pump suction was connected to a plastic reservoir which held the fluoride solution.

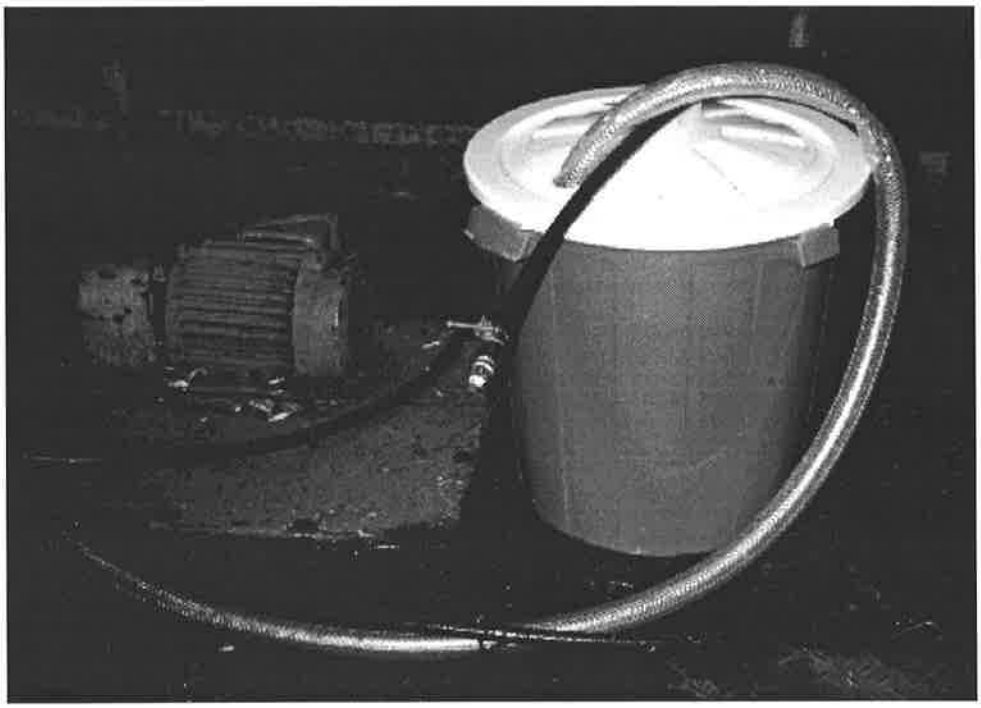


Figure 21: Spraying pump

A test spraying of the fluoride solution onto the sugar in the sugar mill was carried out to find out the exact amount of solution needed and its fluoride concentration. It was decided to adjust the nozzle to spray 25 ml/second, so as to prevent the sugar becoming too moist.

It was calculated that a basket of sugar weighing 300 kgs passed the end of the conveyor about every 120 seconds. As the sprayer was set up for 25 mls/second, the amount of the solution sprayed for 120 seconds was 3000 mls. According to the experiment in the laboratorium, in which 10 mls of 650 ppm fluoride solution should be added into 250 gms sugar to obtain sugar with 10 ppm fluoride, the amount and the concentration of the fluoride solution was calculated as followed:

300 kgs sugar	: 300 x 1000 gms = 300 000 gms.
The solution needed	: 300 000/250 x 10 mls of 650 ppm F solution
	= 12 000 mls of 650 ppm F solution

As the amount of solution that could be sprayed for 2 minutes was only 3000 mls, it was needed to concentrate the solution 4 times , so the concentration of the fluoride solution was 2600 ppm.

The resulting fluoride concentration in the sugar was determined from batch analyses. Batch samples of sugar were subjected to fluoride separation by rapid diffusion using hexamethyldisiloxane (Taves, 1968) and the fluoride content was determined using a fluoride specific electrode (Orion, model 9609-00). Initially it was found that the concentration of fluoride in the sugar was just around 5 ppm. Then it was decided to double the concentration of fluoride solution to 5200 ppm without any adjustment in the flow rate of the solution and the sugar. The decision to increase the fluoride concentration in the solution was made when it was realized that when the solution was sprayed manually, nearly half of the ~~sugar~~ fluoride solution dissappeared as mist or was sprayed onto the side of the platform. Increasing the amount of solution was not possible, as it was necessary to keep the dryness of the sugar. The result of spraying a 5200 ppm fluoride solution onto the sugar was approximately 10 ppm co-crystallized sucrose.

E. Manufacture of fluoridated sugar and monitoring fluoride level in batch and bag samples and storage

The addition of fluoride to sugar was carried out twice over the period of the study, and batch analysis carried out to confirm that it contained Fluoride ion at 10 ppm concentration. Samples for batch analysis were obtained when the sugar from the sugar bin was packed

into the bags before the bags were sealed and then numbered. The stock was kept in the sugar mill in a special vermin and moisture proof room. The sugar was packed in 50 kgs bags and each bag was tested for its fluoride content. Only bags which contained 9 ppm -10 ppm fluoride ion were taken to the laboratory, and subjected to further tests before being supplied to the children. This was carried out to double check fluoride concentration and to ensure that fluoride was evenly distributed in the bag. Five samples from every bag were tested for their fluoride content. This was carried out by taking approximately 10 gram of sugar from five different sites of the bag, two sites in the front and three sites in the back of the bag, as illustrated below (Figure 22). The result of the test of bag samples is presented in Table 7.

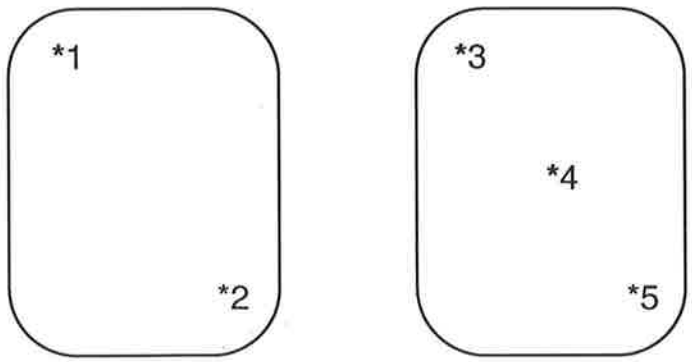


Figure 22: The sites of sugar samples taking from the bag

Table 7: Fluoride concentrations in some of bag samples (in ppm)

Bag No.	F Conc (in ppm)
1	10.5
2	9.62
3	9.77
4	9.0
5	9.92
6	9.48
7	9.50
8	10.4
9	9.0
10	9.10

F. Choice of study format and of records to be used

As the selection of subjects, and preparation of sugar had now been achieved, it was necessary to determine the format of the trial. A randomized clinical trial was set up in which a control group would have normal sucrose incorporated in their daily menu and the experimental group would have 10 ppm fluoride co-crystallized sucrose included. This design is usually applied in clinical trials of the effectiveness of a substance as a caries preventing agent.

Clinical examinations would be carried out in the beginning and at the end of the study period to obtain the DMFS score as the baseline and final data. The D component of the DMF was divided into initial caries lesions (white spot lesions) and cavitated lesions. Radiographic examinations would also be carried out to provide data of proximal caries of posterior teeth through the use of bitewing film. The

clinical and radiographic data would be recorded in separate sheets (Appendix 4 and Appendix 5) , to be statistically analyzed through the use of an SPSSX Program.

As reported previously, a proposal was submitted to the Ethics Committee for Human Experimentation of The University of Adelaide to seek ethical approval for the trial, and was granted by the committee. The project was also reported to The Department of Health in Medan in the beginning of the trial, and their approval obtained. Discussion of the safety aspects of the project was also conducted with the physician who was responsible for the health of the children in the orphanages.

F. Summary

A large study of this nature is difficult to plan, and also has constraints of time available and budget. Considering these limitations, the study population chosen appeared to demonstrate those characteristic required. They might be grouped as follows: the two orphanages as the first group, and the boarding school as the second group. They would provide the following data base:

Variable	Children in orphanages	Children in boarding School
age	7-18	11-19
gender	male and female	male and female
living condition	dormitory	dormitory
food	rice and side dishes	rice and side dishes
drinking water	nonfluoridated	nonfluoridated
tooth paste	nonfluoridated	nonfluoridated

As far as could be determined, the eating characteristics of both groups were very similar, with the exception that boarding school children went home in school holiday. To control the sugar consumption from other source, the children were given sugar to take home enough for the duration of the holiday. The children in the orphanages stayed in the dormitory during the school holiday, and were unlikely to indulge themselves to extra sugar as they did not have enough money to buy cakes or sweets. The preparation of fluoride co-crystallized sugar appeared to provide fairly constant batch analysis level of fluoride ion. The decision of study format, and determination of records to be used, now enabled the project to commence.

III. The Field Trial , Carried Out in Medan, North Sumatera, Indonesia.

A. The Establishment Phase

It was realized that the logistics of organising the supply of sugar to the subjects, the clinical examinations and recording of data, would be a very considerable task. Also it was decided that the PhD candidate should be the main examiner with the supervisor being the second examiner using the double blind study conditions. Hence it was considered necessary to appoint a project co-ordinator for the duration of the actual trial, who would, with a team of co-workers carry out these duties according to a strict timetable.

The time taken for the preliminary studies and the "on site" planning had taken more time than anticipated. Hence it was considered that a period of from 18 months to 2 years only of fluoridated sugar utilization would be the maximum possible, considering all factors. This period is referred to as "Actual Trial Time (ATT)".

The general time frame for the project to now be completed was :

1. ATT-1 month (1 month before ATT):
   a. Initial clinical and radiographic examination of all children.
   b. Initial urine analyses of sample children.

2. ATT
   a. 6 monthly monitoring of urinary fluoride levels.
   b. extra-investigation into remineralization activity using an *in vitro-in vivo* system of randomly selected control and test groups children for a period of 3 weeks.

3. End
   a. Final clinical and radiographic examination of all children
   b. Calculation of result

The first stage was to appoint a co-ordinator to administer the project according to this time table.

1. Appointment of co-ordinator.

A team of co-workers was established which was responsible for arranging the clinical examinations, the radiographic sessions and the laboratory tests and supplying the sugar to the children. The clinical examinations and radiographic sessions were to be carried out in the dental clinic, Faculty of Dentistry, The University of North Sumatera, and the laboratory tests were to be carried out in the laboratory of Faculty of Agriculture, The University of North Sumatera.

To overcome the many logistic problems in keeping the project running smoothly, it was decided to appoint a Project Co-ordinator. The duties of the co-ordinator were:

- Determine the control and the experimental groups and keep their identity confidential from everyone, and keep the records until the project is finished.

- Arrange laboratory tests of fluoride content of the sugar before it is supplied to the children.
- Maintain the continuity of sugar supply for each group
- Organize the clinical examinations and radiographic sessions.
- Organize the urine collection and the test of fluoride content in urine.

It was necessary that the co-ordinator determine the control and the experimental groups and keep all records until the project finished, to fulfill the double blind principle of the research. This format of experimental design was applied in which neither the other members and examiners, nor the guardians and the principal of the children, knew who were experimental or control groups until the project was completed.

2. Establishment of double blind study conditions, appointment of examiner and reliability study.

As the sugar manufacturing was finished, the handling of sugar was passed to the co-ordinator who arranged the transportation of sugar to the laboratory for fluoride testing and transport to the children. She decided which group served as the control or experimental groups and supplied the sugar according to this decision. The examiner only knew the group as Group I and Group II, in the examination sessions.

The examiner (MD) examined the children who were organized to come to the clinic by the co-ordinator. Second examinations on some randomly selected numbers of children of the three institutions were performed to provide analyses in intra-examiner reliability. Another series of examinations were also carried out by the supervisor of the

project, and a further test of inter-examiner reliability carried out. These were carried out at the baseline and final examinations.

The data were subjected to Analysis of Variance and the R (reliability) value was caclulated using the formula:

$$R = \frac{N(PMS - EMS)}{N.PMS + (k-1)\,MMS + (N-1)(k-1)EMS}$$

Where : N : Number of Patients
PMS : Mean Square of Patient Score (DMFS)
MMS : Mean Square of Examiners
EMS : Mean Square of Error
k : No. of Examiners

The results are presented in Table 8 to Table 11.

Table 8 : Inter examiner reliability at baseline examination (DMFS Score; N = 41)

Source of variation	df	SS	MS	F ratio	R
Patient	40	1667.12	41.68		
Examiner	1	0.012	0.012	0.022	0.97
Error	40	22.49	0.56		

Table 9: Inter examiner reliability at final examination (DMFS Score; N = 31)

Source of variation	df	SS	MS	F ratio	R
Patient	30	969.74	32.33		
Examiner	1	2.73	2.73	3.30	0.91
Error	30	24.77	0.83		

Table 10: Intra examiner reliability at baseline examination (DMFS Score; N = 30)

Source of variation	df	SS	MS	F ratio	R
Patient	29	1053.00	36.31		
Examiner	1	1.68	1.68	6.59	0.92
Error	29	7.33	0.25		

Table 11 : Intra examiner reliability at final examination (DMFS Score; N = 33)

Source of variation	df	SS	MS	F ratio	R
Patient	32	1230.68	38.46		
Examiner	1	2.56	2.56	5.14	0.97
Error	32	15.94	0.498		

The results show that inter-examiner and intra-examiner reliability are very high (R values ranging from 0.91 to 0.97)

3. Clinical and radiographic examinations

i. Clinical examination

The children were grouped into two groups; the children from the orphanages were included in group I, while the children from the boarding school were included in group II. At every session each day only ten children were brought to the clinic and bitewing radiographs were taken after the clinical examinations.

The children were seated on the dental chair with a proper light source, and had their teeth polished before the examination. A mouth mirror and a sickle explorer were used and every surface of the tooth was dried to enable the examiner to see any white spot lesions. The examination was started from maxillary right second molar, to maxillary left second molar, and continued to mandibular left second molar to mandibular right second molar. Examination of each tooth was carried out from buccal/labial, occlusal, lingual, distal and mesial surfaces. The approximate location of the white spot lesions were drawn on the chart (Appendix 4).

White spot caries lesion need to be distinguished from enamel hypoplasia or fluorosis at their early stage. White spot caries lesion can be detected as a chalky-white or discoloured area at the cervical margin of enamel, just above the gingival margin at the buccal or lingual surfaces of the tooth; or along the fissures at the occlusal surface without any cavitation. Slight fluorosis can be detected as white opaque lines running across the tooth on all parts of the enamel and it is symetrically distributed. Hypoplastic enamel can be seen as an area of imperfection of enamel, in which enamel often is obviously grooved or defective.

Active enamel and dentine caries can be detected with visual and tactile examination. Cavitation should be detected with a soft enamel or dentine base that can be judged using an explorer.

Pulp involvement such as hyperaemia and pulpitis can be judged in very deep caries lesion by asking the subject whether he/she has experienced any hypersensitivity or pain on the suspected tooth.

Coding and scoring system

The grading and criteria of the carious lesions were recorded as follows:

Code	Category	Criteria
0	Sound enamel	There is no sign of enamel defect
1	initial caries lesion	On smooth surface; white spot as evidence of subsurface demineralization On pit and fissure; loss of normal translucency of the enamel adjacent; shadowing at the base of the fissure
2	Enamel Caries (Cavitated)	Cavitation in enamel only
3	Arrested Caries	cavitation into enamel or dentine with hard base, with or without staining
4	Active Dentine Caries	cavitation into dentine with soft dentine base
5	Pulpal Involvement	any signs or symptoms of pulpal involvement eg. pulpitis
Md	Missing due to caries	any missing tooth that had been caused by extraction due to caries, or root fragment without any crown
Mo/Mt	Missing due to orthodontic treatment /trauma	any missing tooth due to orthodontic treatment or trauma
P	Persistent tooth	Persistence of primary tooth
U	Unerupted tooth	Tooth unerupted at the due time
O	Restoration	Any restoration
H	Hypoplastic enamel	Enamel defect caused by hypoplasia

ii. Radiograph examination

Bitewing radiographs were taken by a trained radiographer using a radiograph machine (Pampas, Japan, 1985;) with 70 kV/10mA for 0.6 seconds. The child was seated upright and a film holder was used to hold the film.The use of film holders was necessary to facilitate a standardization in reproducing the relationship and distances between tooth, film and X-ray source as changes in angulation can cause changes in radiolucency. Kodak ultra-speed dental x-ray films size 1 to size 2 (Eastman Kodak Company) were used and routine processing procedures were followed.

The viewing of the radiograph was carried out using a viewing box which was covered with black paper except for a window measured as big as the bitewing film, in a room with a subdued light intensity (Figure 23). Only 10 radiographs were viewed in one time by using a magnifying glass with a fixed distance to the box.

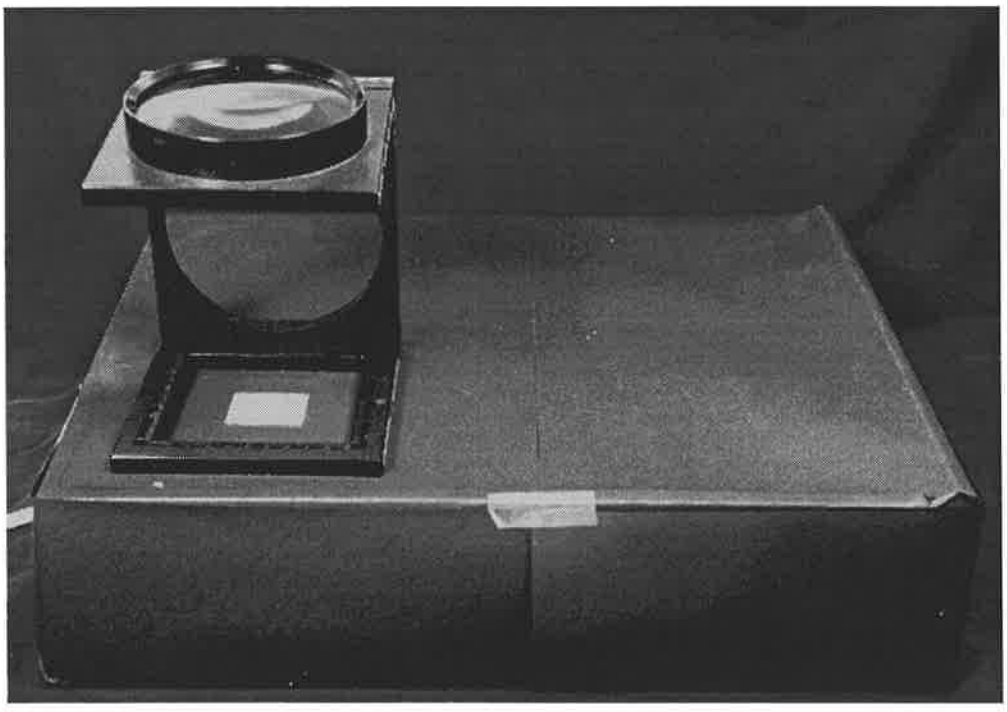


Figure 23: Radiograph viewing box

The caries lesions in the radiograph were scored using the scoring system proposed by Pitts (1985), as follows:

Code	Category	Criteria
R0	Sound	No radiolucency or restoration visible
R1	Outer half enamel lesion	Zone of increase radiolucency confined to outer half of enamel (no minimum limit)
R2	Inner half enamel lesion	Zone of increased radiolucency involving both inner and outer halves of the enamel, including lesions extending up to but not beyond ADJ (Amelodentinal Junction)
R3-0	Outer half dentine lesion	Zone of increased radiolucency penetrating enamel and ADJ but confined to the outer half of the dentine (suffix 0 where the surface has enamel overlapped provided radiolucency in dent ine is distinct
R4-0	Inner half dentine lesion	Zone of increased radiolucency penetrating into the inner half of dentine with or without apparent pulpal involvement (suffix 0 where surface has enamel overlapped provided radiolucency is distinct)
R5	Enamel overlap (no lesion in dentine)	Overlapped surface, Overlap of more than half of the thickness of the enamel but not beyond the ADJ. No zone of increased radiolucency in dentine
R6-0	Secondary caries	Zone of increased radiolucency associated with a filled surface (suffix 0 where obviously overlap)
R7-0	Filled surface	Radiographic appearance consistent with a restoration (suffix 0 where obviously overlapped)
R8-0	Excluded surface	Unerupted, extracted or missing from film (suffix 0 if unreadable overlap extending into dentine
R9	Partial overlap carious	: Overlap of less than half the enamel, zone of increased radiolucency in inner half of enamel including lesions extending up but not beyond ADJ
R10	Partial overlap sound	: Partial overlap of less than half of enamel thickness, no zone of increased radiolucency

In the radiograph reading each proximal surface was coded using the above criteria. The readings at the baseline and final examination were then compared, and any changes or transition of the reading was

scored according to the following scoring system. For example score 1 will be applied if a lesion changes from R0 at the baseline to R1 at the final examination.

The changes/transition in the radiograph reading of the caries lesion will be scored as follows:

Score	Transition
0	R0-0, 0-5, 0-8, 0-10 1-1, 1-5, 1-8, 1-10, 2-2, 2-5, 2-8 2-9, 3-3, 3-6, 3-7, 3-8, 4-4, 4-6 4-7, 4-8, 5-0, 5-1, 5-2, 5-5, 5-8 5-9, 5-10, 6-6, 6-7, 6-8,7-7, 7-8 8-0,1,2,3,4,5,6,7,8,9,10 9-2, 9-5, 9-8, 9-9, 10-0, 10-1, 10-5. 10-8, 10-10
1	R0-1, 1-2, 1-9, 2-3, 2-6, 2-7, 3-4, 5-3, 5-6 5-7, 7-6, 9-3, 9-6, 9-7, 10-2, 10-9
2	R0-2, 0-9, 1-3, 1-6, 1-7, 2-4, 5-4, 9-4, 10-3, 10-6, 10-7
3	R0-3, 0-6, 0-7, 1-4, 10-4
4	R0-4
-1	R1-0, 2-1, 2-10, 3-2, 3-5, 3-9, 4-3, 9-1, 9-10
-2	R2-0, 3-1, 3-10, 4-2, 4-5, 4-9, 9-10
-3	R3-0
Illegal Transition	R4-0, 4-1, 4-10, 6-0,1,2,3,4,5,9,10 7-0,1,2,3,4,5,9,10

Note: Illegal transition means that the changes/transition is imposible to happen and should be excluded from the analysis.

Reliability studies

Radiographic readings were carried out twice for bitewing radiographs of 30 children within a week interval. The mean differences between the two readings were then subjected to Analysis of Variance and the R (Reliability) value was calculated using the formula:

$$R = \frac{N(RMS - EMS)}{N.IMS + (k-1)\,MMS + (N-1)(k-1)EMS}$$

Where : N : Number of readings (30)
IMS : Mean Square of Reading
MMS : Mean Square of Times of reading
EMS : Mean Square of Error
k : Time of readings (2)

The results of the reliability study at baseline and final examinations are presented in Table 12 and 13.

Table 12: Reliability study of radiographic readings at baseline examination (N: 30)

Source of variation	df	SS	MS	F ratio	R
Reading	29	138.48	4.78		
Time of reading	1	0.15	0.15	1.00	0.94
Error	29	4.35	0.15		

Table 13: Reliability study of radiographic readings at final examination (N:30)

Source of variation	df	SS	MS	F ratio	R
Reading	29	371.93	13.10		
Time of reading	1	0.60	0.60	0.85	0.90
Error	29	20.40	0.70		

Reliability of radiographic readings were high with R values of 0.94 and 0.90 at baseline and final examination respectively.

4. Distribution of sugar and control of intake.

- Every child in the boarding school had 60 grams sugar/day incorporated overall in their drink at breakfast, afternoon tea time, and after they finished class at night. For the children in the orphanages, they had the sugar at breakfast, afternoon tea time and after they finished dinner.
- If in the menu the children had some sweet porridge or cake that had been made in the institution, for afternoon tea, they would not have a sweet drink at tea time and at night.
- The children had been advised to brush their teeth as usual using a non-fluoridated tooth paste (Delident Brand). As described previously this tooth paste is normally supplied either in the orphanages or boarding school as the price is much cheaper than the other brands. If the children in the boarding school went home for school holiday, enough sugar was given to them for consumption at home during the holiday time. The children in the orphanages were also allowed to go

home, but only for a very short period of time, usually about 3 to 7 days.

B. Monitoring Urinary Fluoride

Urinary fluoride was monitored regularly in six-month intervals starting before the utilization of the sugar until the end of the trial. Urine samples of all subjects were collected approximately 3 hours after breakfast in plastic containers and were directly brought to the laboratorium. The children were asked to collect the urine samples for three days in a row.

The pH of the urine samples was recorded using a pH electrode before and after the addition of TISAB II at ratio 1:1 to the urine (Mirth, et al, 1982; Whitford et al. 1991). TISAB II (Total Ionic Strength Adjustment Buffer) was added to urine to provide a constant background ionic strength, decomplex fluoride and adjust solution pH. The fluoride contents were recorded directly in urine samples after the addition of TISAB II using a Fluoride specific electrode (ORION, Model 90960).

The TISAB II was prepared from laboratory supplies as follows:

> About 500 ml distilled water was placed in a 1-litre beaker. To this was added 57 ml Glacial Acetic Acid, 58 gm NaCl and 4 gm CDTA (Cyclohexylene dinitrilo tetraacetic acid). This was stirred to dissolve. The beaker was placed in a water bath for cooling. A calibrated pH electrode was placed into the solution, and approximately 5 M NaOH was slowly added until the pH was between 5.0 and 5.5. The solution was cooled to

room temperature. The solution was poured into a 1-litre flask and diluted to the appropriate mark with distilled water (Orion F electrode handbook).

Monitoring urinary creatinine levels is particularly useful when a series of daily specimen is being collected, to check the reliability of 24 hour urine collections (Varley, 1967). In the present study, urinary creatinine levels were not carried out as the urine samples were collected once in the certain days as described above

C. The Clinical Evaluation of Effectiveness of 10 ppm Fluoride Co-crystallized Sucrose in Inhibiting Dental Caries, using Clinical and Radiographic Means of Caries Monitoring

Final clinical and radiographic examinations were carried out after a period of one and a half years. The same procedures were used as in the first examination, in the dental clinic of the Faculty of Dentistry, The University of North Sumatera. The results from the first and final examinations were then tabulated so that the caries increment and caries progression could be easily read.

1. Caries Increment

The data collected from both groups, in which DMFS scores would be compared were treated as followed:

Decayed component was divided into three categories :

- Cavitated lesions: consisting of enamel and dentine lesions

- Initial lesions : consisting of white spot lesions on smooth surfaces and initial lesions on occlusal surfaces (fissures)

- Radiographic lesions : posterior proximal lesions detected by bitewing radiographs

The DMFS scores were calculated from Cavitated lesions as the Decayed component, Missing component and Filled component.

Descriptive analyses were carried out on base line and final DMFS values for both groups. Caries increment can be analyzed by comparing mean DMFS scores between the base line and the final data of each group. Differences between groups were analyzed using Analysis of Variance with significant level at $p < 0.05$.

2. Caries progression/reversal

Caries progression/reversal was analyzed in two parts; assessment of initiation/reversal of initial caries lesions and assessment of caries progression. These changes from both groups were compared statistically using Analysis of Variance with significant level at $p < 0.05$

i. Assessment of initiation/reversal of initial caries lesions

Assessment of initial caries lesions was carried out to determine clinically the effect of 10 ppm F co-crystallized sucrose in inhibiting demineralization and enhancing remineralization in enamel.

The changes were scored as follows:

Score (+1) : transition from code : 0 - 1

Score (-1) : transition from code : 1 - 0

Note: If in the baseline, a surface was recorded at stage 1, and in the follow up it progressed to stage 2, this surface would not be assessed in caries initiation but in caries progression.

ii. Assessment of caries progression

Caries progression was assessed based on changes in Decayed, Missing and Filled components. The changes were scored as follows:

Scoring of Decayed component:

Score : + 1	transition from code : 1 - 2; 2 - 4; 4 - 5; 3 - 2; 3 - 4
Score : +2	transition from code : 0 - 2; 1 - 4, 2 - 5
Score : +3	transition from code : 0 - 4; 1 - 5
Score : -1	transition from code : 2 - 3; 4 - 3
Illegal transition	transition from code : 5 - 4; 4-2; 2-1; 2-0

Scoring of Missing component

Score : +1	if the status of a surface in the base line is decayed (code: 4), and in the follow up was indicated for extraction or was extracted

Scoring of Filled component

In this study, a filling would have usually been placed if the caries has already reached the dentin. In this case the caries is in stage 4. The scoring was carried out as followed:

Score : 0

- If the filling is still in place, with no secondary caries
- If the filling has already been lost, but the cavity has a hard dentin base and no further caries development

Score : +1

- If there is secondary caries around the filling
- If the filling has already been lost and there is caries with a soft dentine base with or without any symptoms of pulp involvement

D. The Interim Assessment of Demineralization-Remineralization Balance Resulting from Use of 10 ppm Fluoride Co-crystallized Sucrose of Randomly Selected Subjects

This experiment was carried out in an attempt to assess the possibility of remineralization of artificial initial carious lesions facilitated by the use of 10 ppm fluoride co-crystallized sucrose *in vivo*, over a short time duration. As the effect of a 10 ppm fluoride co-crystallized sucrose incorporated in daily diet may not be visible clinically in one and a half years duration, the demineralization/remineralization balance over a short period of time might be assessed by this *in vitro-in vivo* experiment.

Buccal surfaces of sound maxillary premolars were cut into enamel slabs measuring approximately 2 mm x 0.5 mm with some dentine included. The slabs were covered with varnish except for the enamel surfaces, and put in demineralizing solution, for 8 days. The slabs were then sectioned and polished to 300 micron thickness and the depth of the lesions were recorded. The surfaces of the enamel slabs were painted with varnish except for the surfaces of the lesions, and the slabs were mounted into a small amount of light-cured composite resin (Silux Plus - 3M) leaving the surface of the lesions exposed. The composite at the opposite surface of the lesion was left flat to enable it to be attached to the buccal surface of the maxillary first molar. The specimens were then sterilized with ethylene oxide in the Hospital Sterilization and Decontamination Unit, Royal Adelaide Hospital, Adelaide.

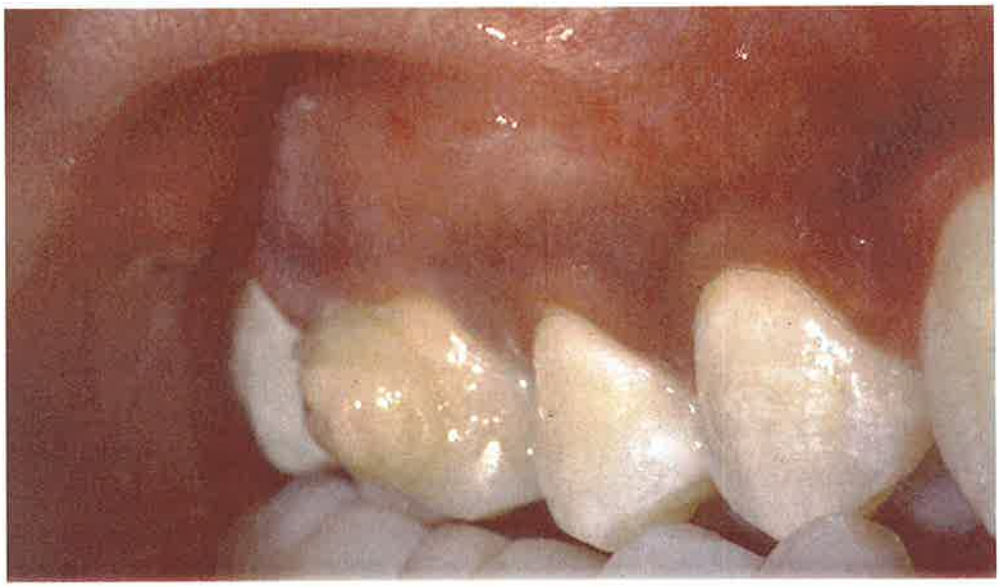


Figure 24: Carious lesion in enamel slab attached onto buccal surface of maxillary first molar using composite resin

Groups of 10 children from control and experimental groups who had DMFS Scores greater than 10 were randomly selected as subjects for the experiment. The buccal surfaces of the maxillary first molars were polished with non fluoridated pumice, and etched for one minute. After a thorough wash of the etching material, the flat surface of the specimen was attached to the tooth surfaces using composite resin, and light cured (Figure 24).

After 3 weeks in the mouth the specimens were taken out and imbedded in araldite and polished as described at experiment ID (page 83) and subjected to the microhardness test using the Leitz Miniload Hardness Tester (Leitz, Germany) using a 25 gm-load for 10 seconds.

The area of lesion progression/reversal was calculated using Simpson's Rule and the mean differences in lesions area between groups was tested using One way Analysis of Variance with multiple range test (Scheffe test) with significant level at $p < 0.05$.

1. Reliability study

A reliability study was carried out in which 48 indentation lengths from 4 lesions were measured twice and compared statistically using Analysis of Variance and the R value was calculated using the formula:

$$R = \frac{N(IMS - EMS)}{N.IMS + (k-1)\,MMS + (N-1)(k-1)EMS}$$

Where : N : Number of measurements (48)
IMS : Mean Square of Indentation length
MMS : Mean Square of Times of measurement
EMS : Mean Square of Error
k : Time of measurements (2)

The result of reliability study is presented in Table 14.

Table 14: Reliability study of microhardness test of *in vitro-in vivo* remineralization experiment.

Source of variation	df	SS	MS	F ratio	R
Indentation length	47	356508.31	7585.28		
Measurement	1	12.34	12.34	2.68	0.99
Error	47	217.48	4.63		

The result of the replicability study is very high with R value of 0.99.

CHAPTER III

CHAPTER III

RESULTS

In the present study Group I (control group) subjects consumed the ordinary sugar while the 10 ppm co-crystallized sucrose was provided to Group II (test group) over the period of 18 months. The raw data relating to DMFS scores, radiographic readings and initial lesions are presented in Appendix 6. Data relating to the *in vitro - in vivo* remineralization experiment and urine analysis are also presented in Appendix 7 and 8 respectively.

Some children in both study groups dropped out from the study for different reasons. Children in the orphanages went home to parents and children in the boarding school discontinued their study. Number of children who had been examined in the baseline and final examinations is illustrated in Table 15.

Table 15: Number of children included in the study

Group	Sex	Age	Baseline exam	Final exam	Drop out
Group I	F	8-17	61	47	14
	M	7-16	41	29	12
Total			102	76	26
Group II	F	11-19	73	62	11
	M	12-19	27	18	9
Total			100	80	20

In the beginning of the trial, 102 and 100 children from Group I and Group II respectively were examined, and with drop out of 25.5% in Group I and 20% in Group II, the number of children who continuously participated in the study was reduced to 76 and 80 in Group I and Group II respectively. The results of the present study were obtained from the analysis of the data of the children who were continuous participants in the study.

Children in age group 11-17 years were identified from both groups to present a sub-group which matched in that age range and which also included children who were not in the mixed dentition stage of development. The sex distribution of children in this age group is presented in Table 16.

Table 16: Distribution of subjects in age group 11-17 years

Sex	Group I	Group II	Total
Female	29	60	89
Male	22	17	39
Total	51	77	128

As described previously in Chapter II, the Decayed (D) component was divided into three categories: cavitated carious lesions, initial carious lesions and posterior proximal lesions detected in bitewing radiographs. Only cavitated lesions were included in the evaluation of DMFS scores as recommended by WHO (1987). The results from the study are presented under the following categories:

I. CLINICAL TRIAL

A. Evaluation of Age Group 7 - 19 Years

1. Evaluation of DMFS scores and D, M and F components and their increments.
2. Evaluation of initial lesions and proximal lesions detected in bitewing radiographs.
3. Assessment of caries progression.
4. Assessment of initiation/reversal of initial caries lesion.

B. Evaluation of Age Group 11-17 Years

1. Evaluation of DMFS scores and D, M and F components and their increments.
2. Evaluation of initial lesions and proximal lesions detected in bitewing radiographs.
3. Assessment of caries progression.
4. Assessment of initiation/reversal of initial caries lesion.

II. URINARY FLUORIDE LEVELS

Evaluation of urinary fluoride levels over the 18-month period of consumption of 10 ppm fluoride co-crystallized sucrose.

III. *IN VITRO-IN VIVO* REMINERALIZATION EXPERIMENT

Evaluation of demineralization/remineralization balance resulting from the consumption of 10 ppm fluoride co-crystallized sucrose.

I. CLINICAL TRIAL

A. Evaluation of Age Group 7-19 Years

1. Evaluation of DMFS scores and D, M and F components and their increments

i. DMFS scores of Group I and Group II (Age group 7 - 19 years) at baseline and final examinations and DMFS increments of both groups are presented in Table 17.

Table 17: DMFS scores of Group I and Group II (Age group 7-19 years) at baseline (BL) and final (F) examinations and their increments

Variable	Group I (N: 76)			Group II (N: 80)		
	Mean	SD	SE	Mean	SD	SE
DMFS (BL)	4.57*	4.01	0.46	5.61°	4.46	0.49
DMFS (F)	5.97*	4.53	0.52	5.90°	4.59	0.51
DMFS increment	1.40+	1.59	0.18	0.29+	0.62	0.07

*, o, + : Significant difference at $p < 0.05$ (Anova)

The DMFS scores in Group I and Group II were 4.57 and 5.61 at baseline examination respectively. Analysis of Variance with significance level $p < 0.05$ revealed that there was no significant difference between these scores, even though the DMFS score in Group II was higher than that of Group I. DMFS scores of 5.97 and 5.90 at final examination were recorded in Group I and Group II respectively, again without any significant difference between groups.

Significant increases in DMFS scores in both groups were detected over 18 months period with the DMFS increment in Group I (1.40) significantly greater than that in Group II (0.29).

ii. D component scores of Group I and Group II (Age group 7 - 19 years) at baseline and final examinations and D increments of both groups are presented in Table 18.

Table 18: D component scores of Group I and Group II (Age group 7 - 19 years) at baseline (BL) and final (F) examinations and their increments

Variable	Group I (N: 76)			Group II (N: 80)		
	Mean	SD	SE	Mean	SD	SE
D comp(BL)	3.71*	3.08	0.35	3.45@	2.52	0.28
D comp (F)	4.89* +	3.64	0.41	3.65@ +	2.50	0.28
D increment	1.18°	1.65	0.19	0.20°	0.85	0.09

*, o, +, @ : Significant different at $p < 0.05$ (Anova)

D component scores of 3.71 and 3.45 at baseline examination of Group I and Group II respectively were not significantly different at $p < 0.05$, while at final examination significant difference was found between the D component scores of 4.89 in Group I and of 3.65 in Group II. There were significant increases in D component scores in both groups over the period of 18 months. The D component increment of 1.18 in Group I was significantly greater than the D component increment of 0.20 in Group II.

iii. M component scores of Group I and Group II (Age group 7-19 years) at baseline and final examinations and M increments of both groups are presented in Table 19.

Table 19: M component scores of Group I and Group II (Age group 7-19 years) at baseline (BL) and final (F) examinations and their increments

Variable	Group I (N: 76)			Group II (N: 80)		
	Mean	SD	SE	Mean	SD	SE
M comp (BL)	0.79*@	2.27	0.26	1.84o @	3.08	0.34
M comp (F)	1.04*+	2.46	0.28	2.03o+	3.09	0.35
M increment	0.25	0.73	0.08	0.19	0.64	0.07

*, o, @., + : Significant difference at $p < 0.05$ (Anova)

Missing components of 1.84 in Group II at the beginning of the study was significantly higher than that of Group I (0.79) and it was also significantly higher at final examination (2.03) compared to Group I (1.04). There were significant increases of M component scores over the period of 18 months in both groups, even though M component increment of 0.25 in Group I was not significantly greater than that of 0.19 in Group II.

iv. F component scores of Group I and Group II (Age group 7-19 years) at baseline and final examinations and F Increments of both groups are presented in Table 20.

Table 20: F component scores of Group I and Group II (Age group 7-19 years) at baseline (BL) and final (F) examinations and their increments

Variable	Group I (N: 76)			Group II (N: 80)		
	Mean	SD	SE	Mean	SD	SE
F comp (BL)	0.05^{+}	0.28	0.03	0.33^{*+}	0.59	0.07
F comp (F)	0.04$^{@}$	0.20	0.02	0.23$^{*@}$	0.53	0.06
F increment	-0.01^{o}	0.11	0.01	-0.10^{o}	0.34	0.04

*, o, +, @ : Significant different at $p< 0.05$ (Anova)

Children in Group I had significantly less fillings (0.05) at baseline than Group II (0.33), and their mean number of fillings reduced at the final examination, being 0.04. The mean number of fillings also reduced over the study period for children in Group II (0.23). This reduction in mean number of fillings was only significant in Group II and children in Group II had significantly more fillings than those in Group I at the final examination. There was a significant difference in negative F increment between Group I (-0.01) and Group II (-0.10). The negative increment reflected no replacement of lost filling in both groups and no access to dental care over the study period.

2. Evaluation of initial lesions and proximal lesions detected in bitewing radiographs

i. As described previously initial carious lesions and posterior proximal lesions detected in bitewing radiographs of Group I and Group II were analysed separately from cavitated lesions which were included in the assessment of DMFS scores. The results for initial carious lesions and posterior proximal lesions are presented in Table 21 and 22.

Table 21: Initial caries lesions at baseline and final examinations of Group I and Group II (Age group 7-19 years)

Exam	Group I (N : 76)			Group II (N: 80)		
	Mean	SD	SE	Mean	SD	SE
Baseline	4.72°	4.10	0.47	3.81	3.51	0.39
Final	7.66*°	4.25	0.49	3.53*	3.39	0.38

*: Significant difference at $p < 0.05$ (Anova)

Analysis of Variance showed significant increase in initial carious lesions in Group I from 4.72 to 7.66, but in Group II, a decrease of initial carious lesion was detected, from 3.81 at baseline examination to 3.53 in final examination. This decrease was not significant . Between the two groups, the difference was only significant at final examination with Group I children having more than twice the mean number of initial carious lesions than Group II children.

Table 22: Posterior proximal lesions detected on bitewing radiographs at baseline and final examinations of Group I and group II (Age group 7-19 years)

Exam	Group I (N : 76)			Group II (N: 70)		
	Mean	SD	SE	Mean	SD	SE
Baseline	0.20	0.65	0.08	0.49	1.06	0.13
Final	0.32	0.70	0.08	0.84	1.64	0.20

Only 70 from 80 children in Group II had the radiographic data at baseline and final examinations due to the difficulty in the radiographic equipment on one of the examination day at baseline examination. Analysis of Variance revealed that there was no significant difference at the baseline examination between Group I (0.20) and Group II (0.49) and also in final examination, in which Group I and Group II showed insignificant increases to 0.32 and 0.84 respectively.

ii. Total D Component

As described previously, Total D component consisted of cavitated lesions, initial lesions and proximal lesions in Group I and Group II. As the radiograph data only covered 70 children in Group II, Total D component was analyzed based on the data of only 70 children in Group II. The Total D components in Group I and Group II are presented in Table 23.

Table 23: Total Decayed component at baseline and final examinations of Group I and Group II (Age group 7-19 years)

Exam	Group I (N: 76)			Group II (N: 70)		
	Mean	SD	SE	Mean	SD	SE
baseline	8.63°	5.30	0.60	8.45	3.99	0.48
Final	12.87*°	5.57	0.64	8.57*	4.34	0.52

*: Significant difference at p< 0.05 (Anova)

Total Decayed component in Group I (8.63) was not significantly different from Group II (8.45). Analysis of showed significant increase in Group I (12.87), while in the Group II increase in Total D component to 8.57 was insignificant. There was a significant difference in Total D component between Group I and Group II in final examination.

3. Assessment of caries progression

As described previously in Chapter II, caries progression was evaluated from the changes of progression of lesions from initial carious lesions to enamel lesions, dentine lesions, and pulpal involvement in which each change was given score 1. It also included changes in F component. Caries progression is presented in Table 24.

Table 24 : Caries progression in Group I and Group II (Age group 7-19 years)

Group	N	Mean	SD	SE
Group I	76	0.040*	0.039	0.004
Group II	80	0.012*	0.017	0.002

*: Significant difference at p < 0.05 (Anova)

Analysis of Variance revealed that caries progression in Group I (0.040) was significantly higher than that of Group II (0.012)

4. Assessment of initiation/reversal of initial caries lesion

In assessing initiation or reversal of initial carious lesions only development or dissappearance of new initial carious lesion were included. Progression of initial lesions to cavitated lesions was included in the assessment of caries progression, thus was not analyzed in the assessment of initiation/reversal of initial caries lesions. Initiation/reversal of initial caries lesions is presented in Table 25.

Table 25: Initiation/reversal of initial caries lesions in Group I and Group II (Age group 7-19 years)

Group	N	Mean	SD	SE
Group I	76	0.033*	0.045	0.005
Group II	80	- 0.003*	0.031	0.003

*: Significant difference at $p < 0.05$ (Anova)

A significant difference in initiation/reversal of initial caries lesion was found between Group I (0.033) and Group II (-0.003). Initiation of new initial caries lesions was detected in Group I, while in Group II a slight decrease of initial caries lesions was detected.

B. Evaluation of Age Group 11-17 Years

1. Evaluation of DMFS scores and D, M and F components and their increments

i. DMFS scores of Group I and Group II (Age group 11-17 years) at baseline and final examinations and DMFS increments of both groups are presented in Table 26

Table 26: DMFS scores of Group I and Group II (Age group 11-17 years) at baseline (BL) and final (F) examinations and their increments

Variable	Group I (N: 51)			Group II (N: 77)		
	Mean	SD	SE	Mean	SD	SE
DMFS (BL)	4.96*	3.92	0.55	5.74	4.23	0.59
DMFS (F)	6.43*	4.23	0.59	6.04	4.62	0.53
DMFS increment	1.47+	1.69	0.24	0.30+	0.63	0.07

*, + : Significant difference at $p < 0.05$ (Anova)

A significant increase in DMFS scores analyzed using Analysis of Variance was detected in Group I from baseline (4.96) to final (6.43) examinations. Slight increase was found in Group II, with DMFS scores being 5.74 and 6.04 at baseline and final examination respectively. However, there was no significant difference between DMFS scores of the two groups at baseline and final examination. DMFS increment in Group I (1.47) was significantly higher than that in Group II (0.30).

ii. D component scores of Group I and Group II (Age group 11-17 years) at baseline and final examinations and D increments of both groups are presented in Table 27.

Table 27: D component scores of Group I and Group II (Age group 11-17 years) at baseline (BL) and final (F) examinations and their increments

Variable	Group I (N: 51)			Group II (N: 77)		
	Mean	SD	SE	Mean	SD	SE
D comp (BL)	3.86*	3.04	0.42	3.50	2.56	0.43
D comp (F)	5.00*	3.50	0.47	3.70	2.53	0.29
D comp increment	1.14°	1.79	0.25	0.21°	0.86	0.10

*, o, : Significant difference at $p < 0.05$ (Anova)

D component scores of 3.86 in Group I and of 3.50 in Group II at baseline examination, and of 5.00 in Group I and of 3.70 in Group II at final examination were not significantly different tested by Analysis of Variance. A significant increase of D component score was found only in Group I, while D component increment in Group I (1.14) was significantly different from Group II (0.21).

iii. M component scores of Group I and Group II (Age group 11-17 years) at baseline and final examinations and M Increments of both groups are presented in Table 28.

Table 28: M component scores of Group I and Group II (Age group 11-17 years) at baseline (BL) and final (F) examinations and their increments

Variable	Group I (N: 51)			Group II (N: 77)		
	Mean	SD	SE	Mean	SD	SE
M comp (BL)	1.06	2.60	0.37	1.91	3.11	0.36
M comp (F)	1.39	3.12	0.36	2.10	3.12	0.36
M comp increment	0.33	0.86	0.12	0.20	0.65	0.07

M component scores in Group I and Group II were 1.06 and 1.91 at baseline examination, and 1.39 and 2.10 at final examination respectively. Analysis of Variance revealed that there were no significant differences among all scores, within and between groups. M component increment in Group I (0.33) was not significantly higher that that of Group II (0.12)

iv. F component scores of Group I and Group II (Age group 11-17 years) at baseline and final examinations and F Increments of both groups are presented in Table 29.

Table 29: F component scores of Group I and Group II (Age group 11-17 years) at baseline (BL) and final (F) examinations and their increments

Variable	Group I (N: 51)			Group II (N: 77)		
	Mean	SD	SE	Mean	SD	SE
F comp (BL)	0.06	0.31	0.04	0.34	0.60	0.07
F comp (F)	0.04	0.20	0.03	0.24	0.54	0.06
F comp increment	-0.02	0.14	0.02	-0.10	0.35	0.04

F component scores of 0.06 and 0.04 at baseline and final examinations respectively in Group I were not significantly lower than F component scores in Group II, being 0.34 and 0.24 at baseline and final examination respectively. Decreases of F component in both groups were detected, but were not significant, while F component increment in Group I (-0.02) and Group II (-0.10) were also not significantly different.

2. Evaluations of initial lesions and proximal lesions detected in bitewing radiographs

Evaluation of initial lesions and proximal lesions detected in bitewing radiographs and Total D component were carried out using the same procedure as those carried out in Age group 7-19 years. The results are presented in Table 30, 31 and 32.

Table 30: Initial carious lesions at baseline and final examinations of Group I and Group II (Age group 11-17 years)

Exam	Group I (N : 51)			Group II (N: 77)		
	Mean	SD	SE	Mean	SD	SE
Baseline	4.80^{o}	4.04	0.57	3.61	3.36	0.38
Final	7.76*^{o}	4.29	0.60	3.39*	3.24	0.37

*, ^{o} : Significant difference at $p<0.05$ (Anova)

The mean number of initial carious lesions in Group I (4.80) was not significantly higher than that of Group II (3.61) at the baseline examination. A significant increase was found in Group I with a mean number of initial carious lesion of 7.76 at final examination, while slight decrease was detected in Group II, being 3.39 at final examination. A significant difference was detected between those scores in Group I and Group II at final examination.

Table 31: Posterior proximal lesions detected on bitewing radiographs at baseline and final examinations of Group I and Group II (Age group 11-17 years)

Exam	Group I (N : 51)			Group II (N: 67)		
	Mean	SD	SE	Mean	SD	SE
Baseline	0.25	0.77	0.11	0.51	1.01	0.13
Final	0.39	0.80	0.11	0.88	1.67	0.20

Proximal lesions detected on bitewing radiographs were 0.25 and 0.39 at baseline and final examinations respectively in Group I, while in Group II, these scores were 0.51 and 0.88 respectively. There was no significant difference between and within groups, even though initial carious lesions were higher in Group II at baseline and final examinations.

Table 32: Total D (decayed) component at baseline and final examinations of Group I and Group II (Age group 11-17 years)

Exam	Group I (N : 51)			Group II (N: 67)		
	Mean	SD	SE	Mean	SD	SE
Baseline	8.92^{o}	4.88	0.68	8.32	4.00	0.49
Final	13.16*^{o}	5.51	0.77	8.54*	3.35	0.54

*, ^{o}: Significant difference at $p < 0.05$ (Anova)

Total D component at baseline examination in Group I (8.92) was not significantly different from Group II (8.32). A significant increase of Total D component in Group I to 13.16 at final examination was found, while the increase was not significant in Group II (8.54). A significant difference was found between Group I and Group II at final examination.

3. Assessment of Caries Progression

The same procedure in assessing caries progression as in Age Group 7-19 years was carried out. Progression of cavitated lesions are presented in Table 33.

Table 33 : Caries progression in Group I and Group II (Age Group 11-17 years)

Group	N	Mean	SD	SE
Group I	51	0.041*	0.04	0.006
Group II	77	0.012*	0.017	0.002

*: Significant difference at $p < 0.05$ (Anova)

A significantly higher caries progression was found in Group I (0.041) compared with Group II (0.012).

4. Assessment of initiation/reversal of initial caries lesions

Again, the procedure carried out in Age group 7-19 years was carried out in assessing the initiation or reversal of initial carious lesions. Initiation/reversal of initial caries lesions are presented in Table 34.

Table 34: Initiation/reversal of initial caries lesions in Group I and Group II (Age Group 11-17 years)

Group	N	Mean	SD	SE
Group I	51	0.032*	0.038	0.005
Group II	77	- 0.002*	0.030	0.003

*: Significant Difference at $p < 0.05$ (Anova)

A significant difference in initiation/reversal of initial caries lesions was found between Group I (0.032) and Group II (-0.002). Initiation of new incipient caries lesions was found in Group I, while in Group II a slight reversal of initial caries lesions was detected.

II. ANALYSIS OF URINARY FLUORIDE LEVELS

Analysis of urinary fluoride levels were carried out on randomly selected subjects before and during the utilization of fluoridated sugar in six monthly intervals. This analysis was carried out to ensure the safety aspect of the utilization of a 10 ppm co-crystallized sugar to the children, The results are presented in Table 35 and in Figure 25.

Table 35: Urinary fluoride levels before, and during the utilization of fluoridated sugar in six monthly intervals (in ppm)

Test	Group I (N:30)			Group II (N: 30)		
	Mean	SD	SE	Mean	SD	SE
Baseline	0.21	0.10	0.02	0.13	0.05	0.01
Follow up 1	0.19	0.13	0.02	0.17	0.07	0.01
Follow up 2	0.13*	0.04	0.01	0.34*	0.14	0.03
Follow up 3	0.17°	0.07	0.01	0.29°	0.14	0.03

*, °: Significant difference at $p < 0.05$ (Anova)

The results show that there were increases of urinary fluoride levels in Group II while in Group I there was a tendency for a decrease in urinary fluoride level over the 18 month period of the trial. Analysis of Variance revealed that there was no significant difference in urinary fluoride levels at baseline and the first follow up test, being 0.21 ppm and 0.19 ppm in Group I, and 0.13 ppm and 0.17 ppm in Group II respectively.

After a one year period of the utilization of fluoridated sugar, a significant difference was found in the second and third follow up tests

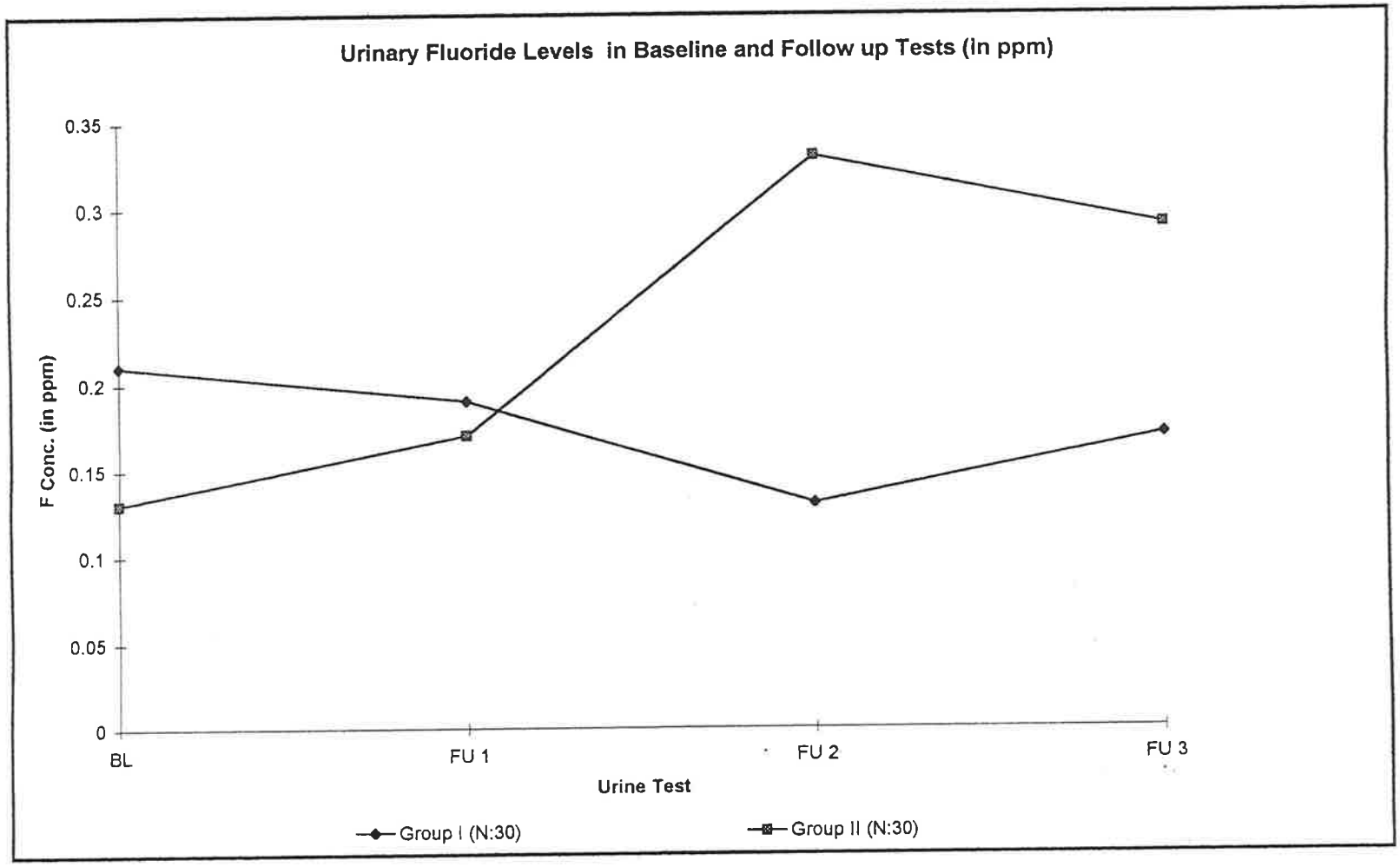


Figure 25: Urinary fluoride levels before and during the utilization of fluoridated sugar at baseline and follow up examinations

III. *IN VITRO - IN VIVO* REMINERALIZATION EXPERIMENT

Artificially generated carious lesions on enamel slabs were attached for three weeks duration on buccal surfaces of maxillary molars of randomly selected subjects in Group I and Group II. After the time elapsed, further laboratory analysis was carried out using a microhardness test to quantitatively determine the mineral content across the lesions as described previously in Chapter II. Comparison of remineralization of carious lesions in Group I and Group II and the original lesions as control was carried out using Analysis of Variance with the Scheffe Test with significance level at $p < 0.05$. The result of this *in vivo* remineralization experiment are presented in Table 36 and Figure 26.

Table 36: Lesion area in Group I and Group II after the *in vitro-in vivo* remineralization in Group I and Group II, and of the original lesions.

Group	Mean	SD	SE
Original (N:3)	13757.89	542.25	313.07
Group I (N:3)	13703.87	1796.59	1037.26
Group II (N:3)	12238.21	2040.27	1177.95

The lesion area was smaller in Group II than that in Group I and in original lesion. Analysis of Variance with the Scheffe test with significance level at $p<0.05$ revealed that there were no significant differences between the three groups.

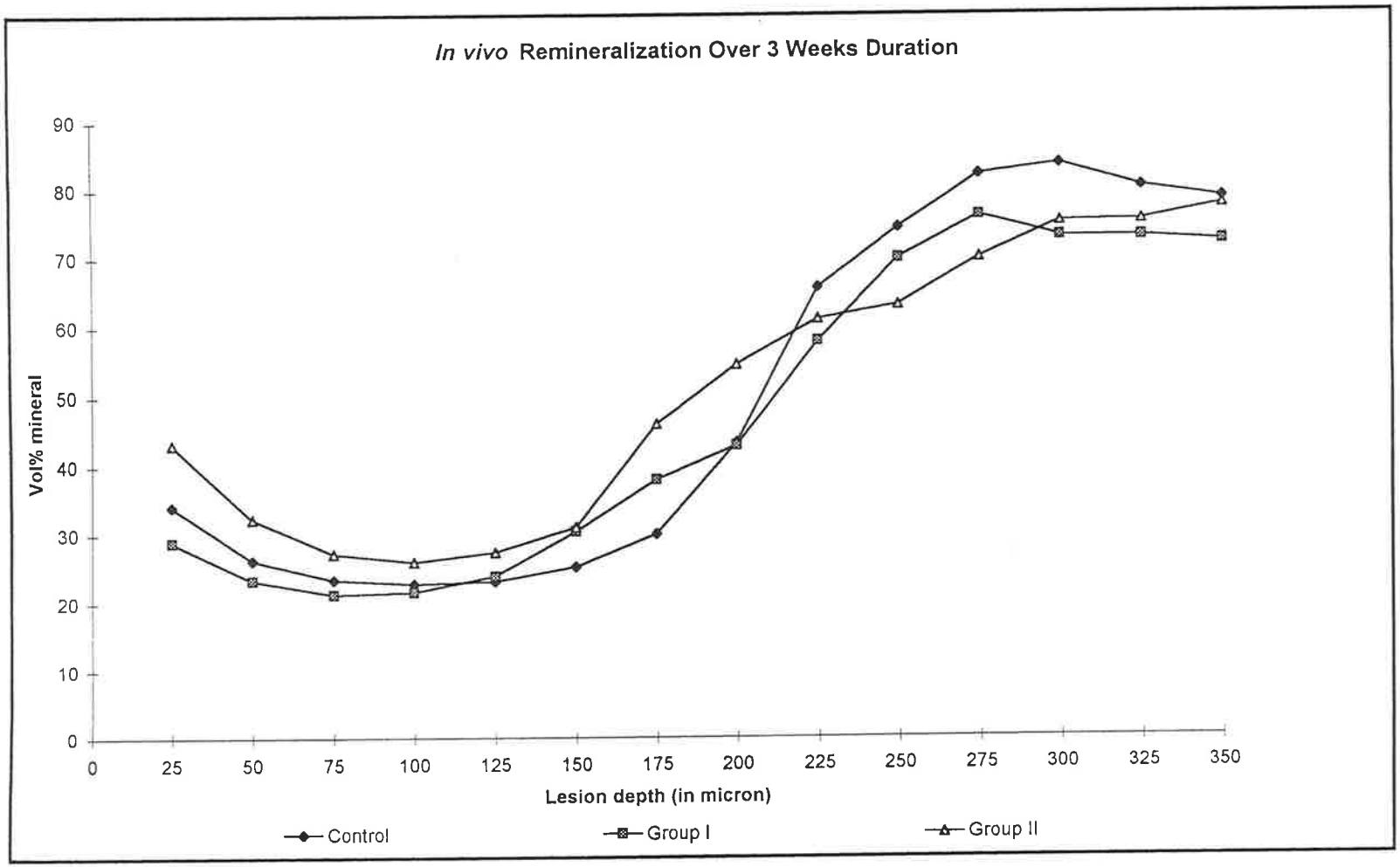


Figure 26 : Profile of original lesions, and in vivo remineralized lesions in Group I and Group II over a three weeks period

CHAPTER IV

CHAPTER IV

DISCUSSION AND CONCLUSION

NATURE OF THE PROJECT

The concept of using sugar as a vehicle for fluoride ion delivery would seem to be logically practical and useful as a means of caries inhibiton. It is now generally recognized that fermentation of sucrose provides the greatest sources of caries promoting acids, with fluoride as the most effective chemical inhibitor. Also with the bulk of the developing worlds' population, sugar is perhaps that factor which become initially the most widely accessible with increasing affluence. Yet it is evident from the recently published WHO report titled "Adding Fluoride to Sugar" that there is some concern that this process may increase the acceptibility of sugar as a dietary component, leading to its increased consumption to a level beyond that which fluoride can protect. It was also considered to have other political and ethical implications (Bratthall and Barmes, 1995).

Such an argument may apply to a small percentage of the more educated middle class sectors of developing countries, though would hardly apply to the overall mass of urban and rural populations who are the target of marketing propaganda to promote consumption of refined foods, with resultant detriment to oral health. It must also be recognized that despite 40 years of active education by dental professionals in Australia as to the cariogenic effects of sucrose, that countries' sugar

consumption has remained unaltered over this period of time (Sivaneswaran and Barnard, 1993). The harsh reality is that the marketing forces promoting sugar consumption are more heavily funded and acceptable to these populations than is health education, which is frequently seen as attempting to promote a futuristic concepts of prevention of an as yet unrecognized problems by such populations.

While such arguments against fluoridated sugar would seem to hold little weight, the matter of risk of excessive dose of fluoride ion during development is of great importance. It is for this reason, that a variety of field trials are essentials to determine not only the most effective dose/response effect across a population, but also to closely monitor long term outcomes in young children using the lowest doses possible.

In this study, the latter trial situation was avoided due to the time and cost constraints necessary for such a test. Instead, the potential effectiveness through predominantly topical action, of a 10 ppm co-crystallized sucrose in an older population of children was tested. Ideally it would have been preferable to have the study run over a much longer time period. However, cost and time available constrained the project to only 18 months of continuous utilization of the fluoridated sugar in the test population.

It was originally planned to continue the project beyond the 18-month data collection period, using this data only as an interim result. However, despite approaches to numerous bodies it was not possible to obtain the funding to permit this to happen.

The over-riding factor to consider in such a project is the safety of the subjects. For this reason, preliminary tests were carried out *in vitro* to help in the decision as to the safest, yet most likely effective dose of fluoride to be tested. Again, because the population were beyond the age where fluorosis might result from excessive intake, the safety factor

involved ensuring that unforseen problems in the manufacture of the fluoridated sugar might result in an unacceptably high dose for individual subjects. The frequent bag sample monitoring of fluoride levels in co-crystallized sugar, together with analysis of urinary fluoride concentrations, should have permitted such an error to be discovered before prolonged ingestion occured. The prior in depth analysis of background fluoride ingestion levels was also essential to ensuring no unforseen excessive intake occured.

The positive results recorded in the clinical evaluation of caries status after 18 months were very encouraging, though need to be viewed with some caution.

Firstly, these were mainly achieved within the incipient lesion data, and recording of such lesions is open to a more subjective interpretation than cavitated lesions. Even so, the evaluation was carried out under strict "double blind" conditions, and with careful monitoring of reliability, which was high. Both examiners had considerable experience in detecting caries in a "high caries risk" clinic at the Adelaide Dental Hospital, which would have contributed to the high reliability levels.

A further reason for caution is the short time period over which the results were achieved. While this may have a very positive interpretation, it would be preferable to have maintained this positive trend to a greater numerical outcome over at least three years.

Overall, the significance of the project is that a second attempt has been made, following that of Luoma, et. al. (1979), under very strict conditions of monitoring and evaluation, to determine the effectiveness of fluoridated sugar in inhibiting caries development. Hopefully, the outcomes of both the Luoma, et. al. (1979) study and this project, will stimulate further field trials of a more comprehensive nature, particularly in developing countries where sugar may well be the most practical and

useful form of delivery of systemic and topical fluorides until other more conventional methods, e.g. fluoride dentifrices, are more affordable and accessible.

EFFECTIVENESS OF THE PROJECT PLANNING

A. Preliminary *in vitro* tests

These tests, and the resultant data obtained, were considered sufficient to permit a decision to be made on the concentration of fluoride to be added to sugar, taking into account background fluoride intake data and sugar ingestion rate data.

The tests involving salivary retention of fluoride from a sweet drink and a cake may have provided little extra information, and may in fact have been a little misleading in retrospect. This is because a prolonged intake of these materials may have likely resulted in an increased salivary fluoride level through elevated plasma concentration with time. However, this may have taken a long time to show up in increased plasma fluoride.

The *in vitro* remineralization data, achieved using fluoridated sucrose, was encouraging in that a substantial degree of remineralization was achieved with both 10 and 20 ppm fluoride ion (diluting to 5 and 10 ppm respectively), even though no significant diifference in outcomes was noted between 5, 10 and 20 ppm concentrations of fluoride ion.

Therefore, it was subsequently disappointing that a similar result could not be obtained *in vivo*, though saliva itself may have not been as effective as its simulation remineralization solution used in this *in vitro* study. Also, a more acidic situation may have prevailed *in vivo* for longer periods of time, inhibiting a positive remineralization process *in*

vivo, i.e. the lesion remained static or arrested. The *in vivo* remineralization result will be considered later.

B. Subject selection, and monitoring of eating habits and background fluoride intake levels.

This component of planning appears to have provided one of the strongest bases for achievement of what might be considered a meaningful result. The essential requirement was for groups of sufficient size with matched age and gender, for whom a detailed knowledge of routine eating patterns and source of food were known, and where it was possible to ensure no extraneous sources of sucrose supply existed. As it turned out, these requirements were closely met, with an added experimental advantage that no dental treatment was available to the subjects during the course of the project. It should be pointed out that a sum of money was made available to provide basic treatment to both groups of subjects following the completion of the tests, which otherwise would not have been available.

From the ethical view point, the intake of sugar for one year prior to the study in both groups was closely recorded, to ensure that the supply of a "free" source of sugar during the project, did not result in an increased consumption. The managers of all institutions involved were very co-operative in this matter, and in ensuring that other conditions imposed were adhered to.

From the very strict epidemiological point of view, the "boarding school" group may provide a concern, in that, in western terms, a boarding school is usually considered a place for children of "well to do" parents, and secondly, that the children went to their home for a one-month period each year. However, it must be understood that the

"boarding school" in this study was less wealthy than the orphanages, and was an entirely different concept from that in western countries.

To overcome the latter obstacle, a supply of fluoridated sugar was sent home with the children, and parents co-operation sought to include it in their sweet drinks and cooking. It is highly likely they had access during this period to unfluoridated sugar. However, some children from the orphanages also went "home" for short periods, again indicating that they were different types of institutions from those in Australia.

A further point of concern may be the number of subjects. While larger groups were originally planned, such groups which satisfied the stringent requirements were not readily available. Also cost had to be considered. It was decided finally to choose two groups which it was felt closely satisfied the requirements of the study, where variables could be most closely controlled. It was considered that such groups should provide an increased potential for a meaningful result, even though numbers were below those originally planned. This decision appears to have been partly indicated in the results.

Background levels of fluoride ingestion were moderately low for both groups, making it easy to detect elevation in urinary fluoride levels in the test group with time. This factor was of particular interest, as orphanages for young children specifically, who had similar background fluoride ingestion levels and similar sugar ingestion levels, could have 10 ppm fluoridated sugar supplied with very low risk of endemic fluorosis resulting. (i.e. 0.793 mg from foods and beverages sources + 0.6 mg from the fluoridated sugar). Such a study would however require monitoring of infants until 8 - 10 years of age, when anterior teeth erupted, and this be very difficult to carry out within an orphanage over such a prolonged time period.

Overall, the choice of a 10 ppm co-crystallization level, appears to have been a good one, considering the background ingestion levels

and other data presented, e.g. urinary fluoride levels. Indeed, 20 ppm would clearly generate concerns in young children and in less controlled populations. However, as stated above, it may be that 10 ppm is suitable for older children and adults, while a slight lowering of concentration might be appropriate where young children are involved.

EFFECTIVENESS OF ORGANIZATION OF THE PROJECT

A. General Organization

Organization of a field trial even of this limited size proved to be very difficult, particularly with the organization spanning two countries. Appointment of a co-ordinator overcame many of the local organization difficulties in Indonesia, once the initial planning and manufacture of the fluoridated sugar had been achieved.

There was some difficulty initially in persuading management of a sugar mill to permit batches of the fluoridated sugar to be prepared, and then stored until required. A considerable debt of gratitude is owed to the company (P.T. Perkebunan IX - Kuala Madu Sugar Mill) which finally permitted this to be done.

This process proved not to be too difficult, and batch estimations of fluoride content of fluoridated sugar were relatively consistent. However, delay in obtaining permission, and in achieving the initial test batches, resulted in a six months delay in comencement of the field trial. This time, if saved, would have permitted a two-year test period to be achieved.

The co-ordinator was able to maintain secrecy on the identity of the test and control groups, though some indication from urinary fluoride data may have given some indication to one of the evaluating

dentists, before the final clinical evaluation. Even so, individual identities of subjects were not revealed to either examiner.

B. Urinary Fluoride Evaluation

The objective in using this method was to ensure excessive amounts of fluoride ion were not ingested by sample representatives of both groups. As it was carried out on a general comparative basis, as an adjunct to the project, creatinine levels were not also recorded in urine samples. Urinary fluoride in the test group did not reach levels of concern throughout the duration of the project, based on criteria generally used (Whitford, 1990)

One monitoring test considered in addition to urine analysis was that of salivary fluoride estimation at regular intervals during the test. However, this proved to be so low in the preliminary analysis, it was decided to concentrate only on urine analysis. Further more, previous studies had revealed that salivary fluoride was not necessarily indicative of fluoride ion ingestion rates (Dawes and Weatherell, 1990).

C. Effectiveness of Recording Taking, and the Clinical Assessment Process.

An attempt was made to collect a wide range of clinical data in order to detect even slight differences during the duration of the project. The Turku Study (Scheinin and Makinen, 1975) on the effectiveness of xylitol in reducing caries was used partly as a model for data collection. This widespread collection of data, while very time consuming, provided sufficient material for a fairly exhaustive descriptive analysis of the results. It enabled fairly small pre and post test differences to be supported from different set of data.

As stated previously, intra and inter examiner reliability was high, and secrecy as to the identity of subjects maintained while clinical results were called out by the dentist examiners to a recording assistant. It was considered that this component of the project went very well.

STUDY RESULTS

The high level of drop out was not expected (20% to 25.5%). Even so, the numbers remaining permitted statistical analysis to be employed with sufficiently high levels of significance. It will be noted that separate subgroups of subjects were chosen for comparison, where they provided a greater level of matching in terms of age and gender between groups.

Over all, the presence of 10 ppm fluoride co-crystallized with sucrose resulted on a significant difference in caries development between test and control groups.

In evaluation of DMFS Scores of the groups as a whole (age range 7-19 years), caries increment and caries progression are significantly lower in the group having 10 ppm Fluoride co-crystallized sucrose (Group II), being 0.29 and 0.012 compared with the sugar group (Group I) with caries increment of 1.40 and caries progression of 0.040. A similar tendency is also found in the evaluation of those age group 11-17 year olds. The Decayed component is significantly higher in Group I at final examination and resulting in higher increment in Group I compared with Group II. As both groups have very limited access to dental care, the Filling components in both groups were reduced in the final examination, resulting from no replacement of lost fillings.

Evaluation of the initiation or reversal of initial caries lesions also shows that fluoridated sucrose is primarily effective in the early stage of

the development of dental caries. Inhibition of the progression of the caries lesions and reversal of initial carious lesions are evident, even if a 10 ppm co-crystallized sucrose (dry weight) was incorporated in foods and beverages resulting in an even lower concentration of fluoride in the final product. As the ability of fluoride to affect dental caries depend on its ambient level in the oral cavity, it must be remembered that very low levels can lead to remineralization of early carious lesions (Marsh and Bradshaw, 1990).

In accordance with Morgan et al. (1990) the present study shows that the prevalence of proximal caries detected on bitewing radiographs is very low in both groups, with Group II showing a higher prevalence.

As stated previously, the *in vitro-in vivo* remineralization study in sampled children, resulted in a virtual stasis of caries progression, rather than a positive replacement of of lost mineral as occured on the *in vitro* study. This is consistent with the over all clinical result, which pointed to a stasis of early caries progression in the test group.

OVERALL SIGNIFICANCE OF THE RESULTS

As stated previously, the results need to be viewed with caution, though look promising. It is hoped they will stimulate and assist further field trials to take place, involving larger numbers of subjects over longer time periods. Hopefully these will include some involving infants, where the systemic effect can be followed over a 6 or 7 year period. Whether a lower concentration of fluoride might be considered in such cases, e.g. 5, 7 or 8 ppm, will need to be closely examined.

While this study was carried out in closely confined and controlled populations of subjects, it would be preferable for more wideranging studies to be conducted, e.g. among village in remote areas, where caries has become a problem due to the penetration of sugar. Such a

project was reported as being planned by Lahti, et. al. (1995) in Mauritius. Whilst the variables in such cases might be greater, and control of extraneous forms of sugar more difficult, this environment will provide a more realistic setting to model what might be the eventual use of fluoridated sugar in such communities.

Because of the wide ranging intake levels of sugar in most populations, where diets are not controlled so much as in orphanages, the question is whether it should be freely available to all who wish to use it, or only by prescription for specific groups or families by a dentist or dental health worker for limited time periods, accompanied by close monitoring of outcomes.It may be that slightly reduced concentrations of fluoride are also effective, and are such as to virtually eliminate the risk of fluorosis. Perhaps a more realistic judgement needs to be made by health professionals as to whether the risk of mild fluorosis is worth taking, in comparison to the gross disfiguration of rampant caries, as was reported so frequently in many developed countries twenty years ago.

The results of this project should require a closer evaluation by World Dental Health Authorities and those Dental Administrators in Developing Countries where there are insufficient dental resources to deal with the impending epidemic of caries, of the potential usefulness of fluoridated sugar as a relatively inexpensive means of controlling this problem, at least until other conventional means, e.g. fluoride tooth paste become more readily accessible.

As the present study was carried out only for a short time, it is intended to further request financial support for the same study to be carried out in the same groups of children, in reverse. This means that Group I will have fluoridated sugar in the future study. It would be also interesting before such project took placed, to monitor the rate of

development of dental caries after cessation of the consumption of fluoridated sugar.

CONCLUSION

The objective of the project was to design and carry out a field trial to test the effectiveness of a fluoride co-crystallized sucrose as an inhibition of caries development. The trial was succesfully carried out, and while it would have been preferable to have a longer term duration, the results of the 18 months period appear to give a great deal of optimism that sugar may be a very effective and safe vehicle for fluoride transfer.

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APPENDICES

Appendix 1

Ethical Approval and Written Consents for the Clinical Trial

THE UNIVERSITY OF ADELAIDE
THE REGISTRY
SECRETARIAT

Ref: HE/44/93
FILE 2603/75
Contact: Mrs R Hofmeyer
Committee on the Ethics of Human Experimentation
Tel: (08) 228 5871 Fax: (08) 232 4574

10 March 1993

Dr M Dalidjan
Department of Dentistry

Dear Dr Dalidjan,

H/03/93 EFFECTIVENESS OF A 10ppm FLUORIDE CO-CRYSTALIZED SUCROSE IN PREVENING DENTAL CARIES IN A GROUP OF INDONESIAN CHILDREN.

I am pleased to inform you that the Committee on the Ethics of Human Experimentation has considered and approved this project.

In view of the age range of 7-18 years of the children in the study, the Committee suggests that the older children (say 16-18 years) have the study fully explained to them and be given the opportunity of giving Informed Consent themselves.

Please note that any change to the project which may affect its ethical aspects will invalidate the project's approval. In such cases an amended protocol must be submitted to the Committee for further approval.

Subjects taking part in the study should be given a copy of the Information Sheet and the signed Consent Form to retain.

Project approvals are current for one year and the expiry date for your project will be 31 March 1994.

Applications for renewal must be accompanied by a brief report on the project's progress and any ethical issues which may have arisen. If the project is completed before that time, a brief report on its outcome and the ethical considerations must be made to the Committee.

I take this opportunity to wish you well in your research.

Yours sincerely,

FJ O'NEILL
Registrar

THE UNIVERSITY OF ADELALIDE

STANDARD CONSENT FORM FOR RESEARCH TO BE UNDERTAKEN ON A CHILD, THE MENTALLY ILL, AND THOSE IN DEPENDANT RELATIONSHIPS OR COMPARABLE SITUATIONS

FORM TO BE COMPLETED BY PARENT OR GUARDIAN

(See also Information Sheet attached)

1. I, Mrs. Rumindang Hutagalung (please print) hereby consent to allow children in P.A. Aisyiyah, Medan (please print) to take part in the research project entitled:

   Effectiveness of A 10 ppm Fluoride Co-crystalized sucrose in Preventing Dental Caries in A Group of Indonesian Children

2. I acknowledge that I have read the Information Sheet entitled:
   Efektifitas Penambahan 10 ppm Fluoride pada Gula Dalam Pencegahan Karies Gigi pada Grup Anak-Anak Indonesia

   and have had the project, as far as it affects the children fully explained to me by the research worker. My consent is given freely.

   IN ADDITION, I ACKNOWLEDGE ON BEHALF OF the children THE FOLLOWING:

3. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained to me that there may not be any benefit to the children by ~~his/her~~ their involvement.

4. I have been given the opportunity to have a member of his/her family or friend present while the project was explained to me.

5. I have been informed that the information he/she provides will be kept confidential.

6. I understand that he/she is free to withdraw from the project at any time and that this will not affect medical advice in the management of his/her health, now or in the future.

7. I am aware that I should retain a copy of this Consent Form, when completed, and the relevant Information Sheet.

SIGNED _____ DATE 28 -11- 1992
(Relationship to patient: ~~PARENT~~/ GUARDIAN)

NAME OF WITNESS DATE 28-11-1992

I, DR. Mulyani Dalidjan (Please print) have described to Mrs. Rumindang Hutagalung the nature of the procedures to be carried out. In my opinion ~~he~~/she understood the explanation.

_____ DATE 28-11-1992

STATUS IN PROJECT Post-Graduate Student

THE UNIVERSITY OF ADELALIDE

STANDARD CONSENT FORM FOR RESEARCH TO BE UNDERTAKEN ON A CHILD, THE MENTALLY ILL, AND THOSE IN DEPENDANT RELATIONSHIPS OR COMPARABLE SITUATIONS

FORM TO BE COMPLETED BY PARENT OR GUARDIAN

(See also Information Sheet attached)

1. I Drs. Supryatno (please print) hereby consent to allow children in PKU, Medan (please print) to take part in the research project entitled:

Effectiveness of A 10 PPM Fluoride co* crystalized Sucrose in Preventing Dental Caries in A Group of Indonesian Children

2. I acknowledge that I have read the Information Sheet entitled:
Efektifitas Penambahan 10 PPM Fluoride pada Gula dalam pencegahan Karies Gigi pada Grup Anak-Anak Indonesia
and have had the project, as far as it affects the children fully explained to me by the research worker. My consent is given freely.

IN ADDITION, I ACKNOWLEDGE ON BEHALF OF the children THE FOLLOWING:

3. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained to me that there may not be any benefit to the children by ~~his/her~~ their involvement.

4. I have been given the opportunity to have a member of his/her family or friend present while the project was explained to me.

5. I have been informed that the information he/she provides will be kept confidential.

6. I understand that he/she is free to withdraw from the project at any time and that this will not affect medical advice in the management of his/her health, now or in the future.

7. I am aware that I should retain a copy of this Consent Form, when completed, and the relevant Information Sheet.

SIGNED _____ DATE 5 - 12 - 1992

(Relationship to patie

NAME OF WITNESS _____ DATE 5-12-1992

I, Dr. Mulyani Dalidjan (Please print) have described to Drs. Supryatno the nature of the procedures to be carried out. In my opinion he/~~she~~ understood the explanation.

SIGNED ____ DATE 5 -12 - 1992

STATUS IN PROJECT Post -/Graduate Student

070:HE:Protocol Info Form

THE UNIVERSITY OF ADELAIDE

STANDARD CONSENT FORM FOR RESEARCH TO BE UNDERTAKEN ON A CHILD, THE MENTALLY ILL, AND THOSE IN DEPENDANT RELATIONSHIPS OR COMPARABLE SITUATIONS

FORM TO BE COMPLETED BY PARENT OR GUARDIAN

(See also Information Sheet attached)

1. I, Mhd. Saleh Saifuddin (please print) hereby consent to allow children in TPI Darul Hikmah, Medan (please print) to take part in the research project entitled:

   Effectiveness of a 10 ppm Fluoride co-crystalized Sucrose in Preventing Dental Caries in a Group of Indonesian Children

2. I acknowledge that I have read the Information Sheet entitled:
   Efektifitas Penambahan 10 ppm Fluorida pada gula dalam Pencegahan Karies Gigi pada Grup Anak anak Indonesia

   and have had the project, as far as it affects the children fully explained to me by the research worker. My consent is given freely.

   IN ADDITION, I ACKNOWLEDGE ON BEHALF OF the children THE FOLLOWING:

3. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained to me that there may not be any benefit to the children by ~~his/her~~ their involvement.

4. I have been given the opportunity to have a member of his/her family or friend present while the project was explained to me.

5. I have been informed that the information he/she provides will be kept confidential.

6. I understand that he/she is free to withdraw from the project at any time and that this will not affect medical advice in the management of his/her health, now or in the future.

7. I am aware that I should retain a copy of this Consent Form, when completed, and the relevant Information Sheet.

SIGNED _____ DATE 24 ~~Agustus~~ 1993

(Relationship to patient: ~~PARENT / GUARDIAN~~)

NAME OF WITNESS Burhanuddin Nst) DATE 24 Agustus 1993

I, Dr. Mulyani Dalidjan (Please print) have described to Mr. Mhd. Saleh Saifuddin (Please print) the nature of the procedures to be carried out. In my opinion he/she understood the explanation.

_____ DATE 24 Agustus 1993

STATUS IN PROJECT Post-graduate Student

070:HE:Protocol Info Form

Appendix 2

Ethical Approval and Written Consents for Salivary Fluoride Experiment

THE UNIVERSITY OF ADELAIDE
THE REGISTRY
SECRETARIAT

Ref: HE/199/92
D.2603/75
Enquiries: Mrs R Hofmeyer
Committee on the Ethics of Human Experimentation
Tel: (08) 228 5871 Fax: (08) 232 4574

Tuesday, 21 July 1992

Dr JM McIntyre
Department of Dentistry

Dear Dr McIntyre,

H/40/92 CONCENTRATION OF FLUORIDE IN SALIVA AND PLAQUE AFTER DRINKING AND EATING SUCROSE PRODUCTS WITH FLUORIDE ADDED (Dr M Dalidjan)

I am pleased to inform you that at its meeting of 7 July 1992 the Committee on the Ethics of Human Experimentation considered and approved this project.

The Committee has suggested some editorial amendments to the Information Sheet for your consideration (copy attached).

Please note that any change to the project which may affect its ethical aspects will invalidate the project's approval. In such cases an amended protocol must be submitted to the Committee for further approval.

Subjects taking part in the study should be given a copy of the Information Sheet and the signed Consent Form to retain.

Project approvals are current for one year. The expiry date for your project will be 31 July 1993.

Applications for renewal must be accompanied by a brief report on the project's progress and any ethical issues which may have arisen. If the project is completed before that time, a brief report on its outcome and the ethical considerations must be made to the Committee.

I take this opportunity to wish you well in your research.

Yours sincerely,

FJ O'NEILL
Registrar

THE UNIVERSITY OF ADELAIDE

CONSENT FORM

See also Information Sheet attached.

1. I GEORGINA CRAIG (please print) hereby consent to take part in the research project entitled:

   Concentration of fluoride in saliva and plaque after drinking and eating sucrose product with fluoride added

2. I acknowledge that I have read the Information Sheet entitled:

   Concentration of fluoride in saliva and plaque after drinking and eating sucrose product with fluoride added

3. I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker. My consent is given freely.

4. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to me.

5. I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.

6. I have been informed that, while information gained during the study may be published, I will not be identified and my personal results will not be divulged.

7. I understand that I am free to withdraw from the project at any time and that this will not affect medical advice in the management of my health, now or in the future.

8. I am aware that I should retain a copy of this Consent Form, when completed, and the relevant Information Sheet.

SIGNED ..... DATE 14/8-92

NAME OF WITNESS ..... SIGNED .....
(Please print)

DATE .....

I, Mulyani Dalidjan ..... have described to
(Please print)

the nature of the procedures to be carried out. In my opinion she/he understood the explanation.

SIGNED .... DATE 14/8-92

STATUS IN PROJECT .....

THE UNIVERSITY OF ADELAIDE

CONSENT FORM

See also Information Sheet attached.

1. I DYAH ASTARINI (please print) hereby consent to take part in the research project entitled:

   Concentration of fluoride in saliva and plaque after drinking and eating sucrose product with fluoride added

2. I acknowledge that I have read the Information Sheet entitled:

   Concentration of fluoride in saliva and plaque after drinking and eating sucrose product with fluoride added

3. I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker. My consent is given freely.

4. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to me.

5. I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.

6. I have been informed that, while information gained during the study may be published, I will not be identified and my personal results will not be divulged.

7. I understand that I am free to withdraw from the project at any time and that this will not affect medical advice in the management of my health, now or in the future.

8. I am aware that I should retain a copy of this Consent Form, when completed, and the relevant Information Sheet.

SIGNED ..... **DATE** 14/8-92

NAME OF WITNESS ..... **SIGNED** .....
(Please print)

DATE .....

I, Mulyani Dalidjan (Please print) have described to the nature of the procedures to be carried out. In my opinion she/he understood the explanation.

SIGNED ..... **DATE** 14/8-92

STATUS IN PROJECT .....

THE UNIVERSITY OF ADELAIDE

CONSENT FORM

See also Information Sheet attached.

1. I _____ (please print) hereby consent to take part in the research project entitled:

   Concentration of fluoride in saliva and plaque after drinking and eating sucrose product with fluoride added

2. I acknowledge that I have read the Information Sheet entitled:

   Concentration of fluoride in saliva and plaque after drinking and eating sucrose product with fluoride added

3. I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker. My consent is given freely.

4. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to me.

5. I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.

6. I have been informed that, while information gained during the study may be published, I will not be identified and my personal results will not be divulged.

7. I understand that I am free to withdraw from the project at any time and that this will not affect medical advice in the management of my health, now or in the future.

8. I am aware that I should retain a copy of this Consent Form, when completed, and the relevant Information Sheet.

SIGNED ..... DATE 14/8-92

NAME OF WITNESS ..... SIGNED .....
(Please print)

DATE .....

I, Mulyani Dalidjan ..... have described to .....
(Please print)

the nature of the procedures to be carried out. In my opinion she/he understood the explanation.

SIGNED ..... DATE 14/8-92

STATUS IN PROJECT .....

THE UNIVERSITY OF ADELAIDE

CONSENT FORM

See also Information Sheet attached.

1. I [illegible] (please print) hereby consent to take part in the research project entitled:

   Concentration of fluoride in saliva and plaque after drinking and eating sucrose product with fluoride added

2. I acknowledge that I have read the Information Sheet entitled:

   Concentration of fluoride in saliva and plaque after drinking and eating sucrose product with fluoride added

3. I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker. My consent is given freely.

4. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to me.

5. I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.

6. I have been informed that, while information gained during the study may be published, I will not be identified and my personal results will not be divulged.

7. I understand that I am free to withdraw from the project at any time and that this will not affect medical advice in the management of my health, now or in the future.

8. I am aware that I should retain a copy of this Consent Form, when completed, and the relevant Information Sheet.

SIGNED ..... DATE 14/8-92

NAME OF WITNESS ..... SIGNED .....
(Please print)

DATE .....

I, Mulyani Dalidjan .....have described to [illegible]
(Please print)

the nature of the procedures to be carried out. In my opinion she/he understood the explanation.

SIGNED .... DATE 14/8-92

STATUS IN PROJECT .....

070:HE:Protocol Info Form
Wednesday, 28 November 1990

THE UNIVERSITY OF ADELAIDE

CONSENT FORM

See also Information Sheet attached.

1. I Dejanie De Jon (please print) hereby consent to take part in the research project entitled:

   Concentration of fluoride in saliva and plaque after drinking and eating sucrose product with fluoride added

2. I acknowledge that I have read the Information Sheet entitled:

   Concentration of fluoride in saliva and plaque after drinking and eating sucrose product with fluoride added

3. I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker. My consent is given freely.

4. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to me.

5. I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.

6. I have been informed that, while information gained during the study may be published, I will not be identified and my personal results will not be divulged.

7. I understand that I am free to withdraw from the project at any time and that this will not affect medical advice in the management of my health, now or in the future.

8. I am aware that I should retain a copy of this Consent Form, when completed, and the relevant Information Sheet.

SIGNED ..... **DATE** 14/02/92

NAME OF WITNESS ..... **SIGNED**
(Please print)

DATE .....

I, Mulyani Dalidjan (Please print) have described to the nature of the procedures to be carried out. In my opinion she/he understood the explanation.

SIGNED ..... **DATE** 14/2-92

STATUS IN PROJECT .....

Appendix 3

Data of the Microhardness Test in *in vitro* Remineralization Experiment

obs : number of sample
demin : area of demineralized lesion
remin : area of remineralized lesion
conc : fluoride concentration in solution
1 - 2.5 ppm F in sucrose solution
2 - 5 ppm F in sucrose solution
3 - 10 ppm F in sucrose solution

obs	demin	remin	conc
1	13073.25	14737.17	1
2	12783.21	14078.06	1
3	12594.19	14994.30	1
4	13856.94	16029.29	2
5	14432.77	16524.84	2
6	10903.48	12084.05	2
7	7847.12	8703.64	3
8	6202.10	9689.70	3
9	7620.32	8756.47	3

Appendix 4

Clinical Examination Chart

No :.....

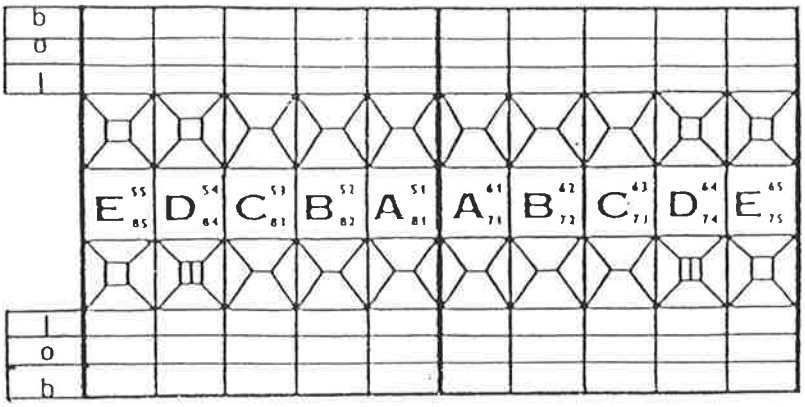
Name :.....

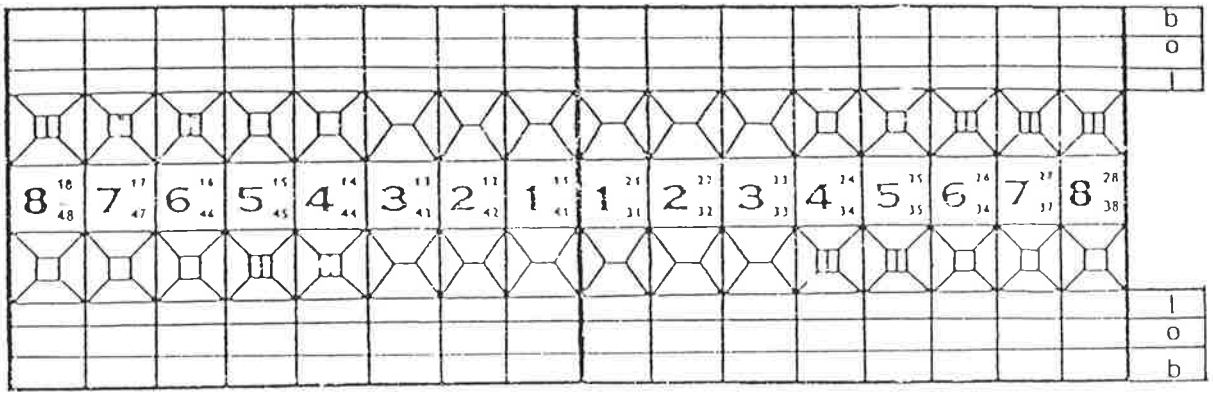
D.O.B :.....

Sex : F / M

Orph. :

Date:.....,.....1993





Code.

1 : White Spot Lesion

2 : Enamel Caries (Cavitated)

3 : Arrested Dentine Caries

4 : Active Dentine Caries

Md : Missing (Caries)

Mo : Missing (Orth.)

P : Persistent

U : Unerupted

O : Filling

DMFS Score

D	M	F	Total

Appendix 5

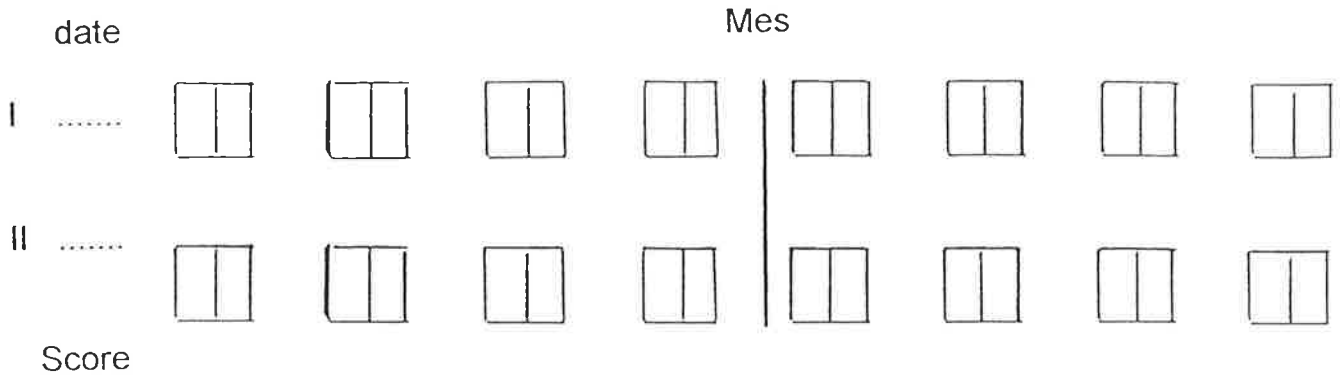
Radiographic Reading Chart

RADIOGRAPHIC READING CHART

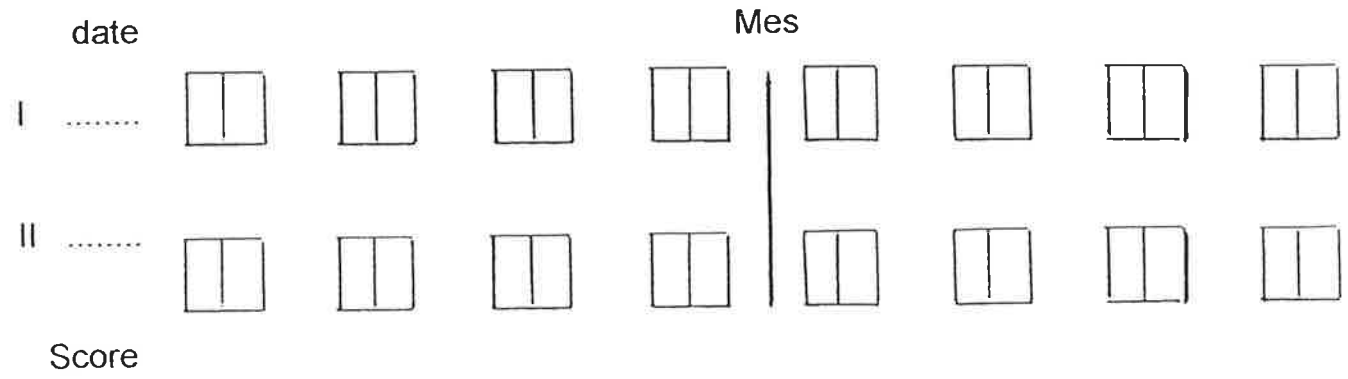
Name : .....
No. : .....

Group: .....
D.O.R. I : .....
D.O.R. II: .....

Maxilla



Mandible



Grading:

Code	Category
Ro	Sound
R1	Outer half enamel lesion
R2	Inner half enamel lesion
R3	Outer half dentine lesion
R4	Inner half dentine lesion
R5	Enamel overlap (no lesion in dentine)
R6	Secondary caries
R7	Filled surface
R8	Excluded surface
R9	Partial overlap: carious
R10	Partial overlap : sound

Appendix 6

Baseline anf Final Data of Clinical and Radiographic Examinations

group : group 1 - sugar group
group 2 - fluoridated sugar group
no : children number
age : age of the child
sex : sex of the child
DMFS1 : DMFS score at baseline examination
DMFS2 : DMFS score at final examination
D1 : Decayed component at baseline examination
M1 : Missing component at baseline examination
F1 : Filled component at baseline examination
D2 : Decayed component at final examination
M2 : Missing component at final examination
F2 : Filled component at final examination
Progress : Progress of cavitated lesions
Num : Number of surfaces
DI1 : Initial lesion at baseline examination
DI2 : Initial lesion at final examination
Init : Initiation of initial lesions
DR1 : Radiographic lesion at baseline examination
DR2 : Radiographic lesion at final examination
Progress : Progress of radiographic lesions
NDR : Number of surfaces examined by radiograph

group	no	age	sex	DMFS1	DMFS2	D1	M1	F1	D2	M2	F2	Progress		Num	DI1	DI2	Init		DR1	DR2	Progress		NDR
												+	-				+	-			+	-	
1	4	17	1	2	2	2	0	0	2	0	0	1	0	92	5	15	11	1	0	0	0	0	32
1	6	15	1	4	6	4	0	0	6	0	0	5	0	96	3	9	9	3	0	0	0	0	32
1	8	13	1	2	2	2	0	0	2	0	0	0	0	96	0	7	7	0	0	0	0	0	32
1	9	14	1	16	16	3	12	1	3	12	1	1	0	79	1	5	4	0	0	0	0	0	18
1	10	13	1	0	0	0	0	0	0	0	0	0	0	96	3	5	2	0	0	0	0	0	32
1	15	13	1	6	7	3	3	0	2	5	0	2	0	96	6	8	5	3	0	0	0	0	28
1	16	11	1	11	13	11	0	0	13	0	0	7	0	96	3	9	6	0	0	0	0	0	30
1	17	16	1	5	5	5	0	0	5	0	0	0	0	96	0	5	5	0	0	0	0	0	32
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1	24	14	1	1	2	1	0	0	2	0	0	2	0	96	2	5	4	1	0	0	0	0	32
1	27	13	1	0	0	0	0	0	0	0	0	0	0	96	0	4	4	0	0	0	0	0	32
1	29	13	1	2	4	2	0	0	4	0	0	2	0	96	2	3	3	2	1	1	2	0	32
1	31	12	1	7	9	1	6	0	3	6	0	5	0	92	6	8	6	4	3	3	2	0	28
1	32	8	1	6	10	6	0	0	10	0	0	13	0	96	3	7	7	3	0	0	0	0	32
1	33	10	1	2	2	0	0	0	2	0	0	0	0	93	7	7	7	7	0	0	0	0	30
1	34	8	1	5	5	5	0	0	5	0	0	1	0	67	2	4	3	1	0	0	0	0	18
1	35	9	1	6	10	6	0	0	10	0	0	8	0	96	11	2	1	9	1	1	0	0	32
1	36	9	1	0	0	0	0	0	0	0	0	0	0	61	2	5	4	1	0	0	0	0	12
1	37	9	1	0	0	0	0	0	0	0	0	0	0	70	4	2	2	4	0	0	0	0	18
1	39	8	1	1	1	1	0	0	1	0	0	0	0	51	1	7	6	0	0	1	1	0	10
1	40	8	1	3	5	2	0	1	4	0	1	4	0	88	0	8	8	0	0	0	0	0	32
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1	43	12	1	6	9	6	0	0	9	0	0	12	0	96	2	1	1	2	0	0	0	0	32
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1	48	13	1	3	4	3	0	0	3	1	0	4	0	96	6	8	5	3	0	0	0	0	30
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1	140	16	2	6	6	6	0	0	6	0	0	3	0	96	10	9	5	6	0	0	0	0	32

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2	4	13	1	3	3	3	0	0	3	0	0	0	0	96	8	5	2	5	0	0	0	0	32
2	5	12	1	6	6	3	3	0	3	3	0	1	0	96	5	1	0	4	0	0	0	0	30
2	6	12	1	3	3	3	0	0	3	0	0	0	0	96	7	0	0	7	0	1	1	0	32
2	7	12	1	7	9	4	3	0	6	3	0	5	0	88	5	8	6	3	1	2	3	0	30
2	8	12	1	8	9	8	0	0	7	2	0	2	0	93	5	0	0	5	0	0	0	0	28
2	10	11	1	1	1	1	0	0	1	0	0	0	0	96	10	9	3	4	0	0	0	0	32
2	13	13	1	7	8	6	0	1	8	0	0	3	0	92	2	3	3	2	0	0	0	0	32
2	14	14	1	6	6	5	0	1	6	0	0	3	0	96	1	4	4	1	0	2	2	0	32
2	15	15	1	6	6	5	0	1	4	2	0	2	0	96	0	3	3	0	0	2	3	0	28
2	16	13	1	0	0	0	0	0	0	0	0	0	0	93	2	3	3	2	2	2	0	0	32
2	17	13	1	6	7	4	0	2	5	0	2	4	0	96	0	5	5	0	2	5	3	0	32
2	18	13	1	5	5	4	0	1	4	0	1	2	0	93	5	4	3	4	0	0	0	0	30
2	19	13	1	5	5	4	0	1	4	0	1	1	0	96	15	13	2	4	0	1	1	0	32
2	20	13	1	11	13	2	9	0	4	9	0	4	0	96	3	0	0	3	0	0	0	0	26
2	21	13	1	7	8	4	0	3	5	0	3	6	0	96	2	1	1	2	0	0	0	0	30
2	22	13	1	1	1	0	0	1	0	0	1	0	0	96	7	0	0	7	0	0	0	0	32
2	23	13	1	5	5	4	0	1	1	3	1	0	0	96	3	2	1	2	1	2	3	0	30
2	24	13	1	6	6	5	0	1	5	0	1	0	0	96	0	5	5	0	0	0	0	0	32
2	25	13	1	3	3	2	0	1	3	0	0	1	0	96	1	1	1	1	0	0	0	0	32
2	26	13	1	9	10	9	0	0	10	0	0	1	0	96	1	1	1	1	0	0	0	0	32
2	28	13	1	15	16	12	3	0	10	6	0	5	0	92	2	9	8	1	0	0	0	0	26
2	29	13	1	2	4	1	0	1	3	0	1	3	0	96	4	5	4	3	0	0	0	0	32
2	31	14	1	5	5	2	3	0	2	3	0	1	0	96	10	10	6	6	0	0	0	0	30
2	33	14	1	4	5	4	0	0	5	0	0	2	0	96	8	3	0	5	0	0	0	0	32
2	34	14	1	0	0	0	0	0	0	0	0	0	0	96	2	2	2	2					
2	36	15	1	14	14	8	6	0	8	6	0	1	0	88	3	0	0	3	3	5	4	0	28
2	37	16	1	11	11	5	6	0	5	6	0	0	0	96	10	7	0	3	2	2	0	0	28

2	38	15	1	11	13	2	9	0	4	9	0	6	0	96	5	6	1	0	0	0	0	0	26
2	39	14	1	10	10	4	6	0	4	6	0	0	0	96	1	1	1	1	2	2	0	0	28
2	40	15	1	2	2	2	0	0	2	0	0	3	0	96	4	7	4	1	0	0	0	0	32
2	43	14	1	3	4	2	0	1	4	0	0	4	0	96	4	1	0	3	0	0	0	0	32
2	45	15	1	0	0	0	0	0	0	0	0	0	0	96	1	1	0	0					
2	47	14	1	2	2	2	0	0	2	0	0	0	0	96	4	5	1	0	0	0	0	0	32
2	48	15	1	1	1	1	0	0	1	0	0	0	0	96	5	8	8	5	5	7	4	0	32
2	49	14	1	9	11	6	3	0	6	5	0	5	0	96	9	6	3	6	2	7	10	0	28
2	50	17	1	0	0	0	0	0	0	0	0	0	0	96	0	0	0	0	0	0	0	0	32
2	51	14	1	1	3	1	0	0	3	0	0	2	0	96	6	5	3	4	0	0	0	0	32
2	52	15	1	5	5	5	0	0	5	0	0	0	0	96	9	13	9	5					
2	53	15	1	6	6	6	0	0	4	2	0	0	0	96	6	8	3	1	0	0	0	0	32
2	54	16	1	6	6	6	0	0	6	0	0	1	0	96	0	0	0	0	1	4	3	0	32
2	55	14	1	2	2	2	0	0	2	0	0	1	0	96	1	1	1	1	0	0	0	0	32
2	56	16	1	0	0	0	0	0	0	0	0	0	0	96	0	2	2	0					
2	57	15	1	3	3	3	0	0	3	0	0	0	0	96	4	8	4	0	0	0	0	0	30
2	58	15	1	0	0	0	0	0	0	0	0	0	0	96	0	1	1	0					
2	59	15	1	1	1	1	0	0	1	0	0	0	0	96	2	2	2	2					
2	60	15	1	0	0	0	0	0	0	0	0	0	0	96	0	1	1	0					
2	62	16	1	8	8	5	3	0	5	3	0	1	0	96	7	10	8	5	0	0	0	0	30
2	63	15	1	7	7	2	3	2	3	3	1	0	0	96	1	1	0	0	0	1	1	0	30
2	65	14	1	12	12	6	6	0	6	6	0	0	0	96	3	0	0	3	1	1	0	0	28
2	67	18	1	3	3	3	0	0	3	0	0	0	0	96	5	7	3	1	0	0	0	0	32
2	68	19	1	3	3	3	0	0	3	0	0	0	0	96	9	1	0	8	0	0	0	0	32

2	69	16	1	12	12	2	9	1	2	9	1	1	0	96	2	2	2	2	0	0	0	0	26
2	70	16	1	7	7	6	0	1	7	0	0	1	0	96	3	1	1	3	0	0	0	0	32
2	71	16	1	5	5	2	3	0	2	3	0	0	0	96	5	2	0	3	1	2	1	0	30
2	72	17	1	6	6	3	3	0	3	3	0	1	0	96	5	0	0	5	0	0	0	0	30
2	73	16	1	14	14	5	9	0	5	9	0	1	0	96	4	4	1	1	0	0	0	0	26
2	74	17	1	3	3	3	0	0	3	0	0	0	0	96	4	6	3	1	0	0	0	0	32
2	75	17	1	7	7	4	3	0	4	3	0	0	0	96	1	4	3	0	0	0	0	0	32
2	76	17	1	3	3	2	0	1	2	0	1	0	0	96	0	1	1	0	0	0	0	0	32
2	77	17	1	13	13	7	6	0	7	6	0	2	0	96	3	2	1	2	2	1	2	1	28
2	78	17	1	2	2	2	0	0	2	0	0	0	0	96	0	0	0	0	1	2	1	0	32
2	83	12	2	1	1	1	0	0	1	0	0	0	0	93	1	2	2	1					
2	84	12	2	6	6	6	0	0	6	0	0	1	0	96	3	3	2	2	0	0	0	0	32
2	85	12	2	7	7	7	0	0	7	0	0	0	0	96	0	4	4	0	0	0	0	0	32
2	87	12	2	2	2	1	0	1	1	0	1	0	0	96	5	5	3	3	0	0	0	0	32
2	88	15	2	9	9	3	6	0	3	6	0	1	0	92	0	3	3	0	0	0	0	0	26
2	89	14	2	19	19	10	9	0	10	9	0	0	0	96	0	2	2	0	0	0	0	0	26
2	90	14	2	0	0	0	0	0	0	0	0	0	0	96	0	1	1	0					
2	91	14	2	1	1	0	0	1	0	0	1	0	0	96	7	2	1	6	0	0	0	0	32
2	93	15	2	1	1	1	0	0	1	0	0	0	0	96	0	0	0	0					
2	97	17	2	9	9	3	6	0	3	6	0	1	0	96	0	2	2	0	0	0	0	0	28
2	98	15	2	7	7	3	3	1	4	3	0	2	0	96	2	1	1	2	0	0	0	0	30
2	99	16	2	4	4	3	0	1	3	0	1	0	0	96	4	0	0	4	5	5	0	0	32
2	100	15	2	7	7	7	0	0	7	0	0	0	0	96	0	1	1	0	0	0	0	0	32
2	102	16	2	4	6	4	0	0	5	1	0	3	0	96	12	9	2	5	1	1	0	0	30
2	103	19	2	1	1	1	0	0	1	0	0	0	0	96	13	13	7	7	0	0	0	0	32
2	105	17	2	18	18	5	12	1	6	12	0	2	0	89	5	4	2	3	2	2	1	0	22
2	107	17	2	14	14	5	9	0	5	9	0	1	0	96	4	0	0	4	0	0	0	0	26
2	109	17	2	5	5	5	0	0	4	0	1	1	0	96	4	2	2	4	0	0	0	0	32

Appendix 7

Data of the Microhardness Test in *in vivo* Remineralization Experiment

No : Number of sample
Area : lesion area
Group : Group of samples
1: original lesion
2: Lesion from Group I (sugar group)
3: Lesion from Group II (fluoridated sugar group)

No	area	group
1	13436.86	1
2	13452.85	1
3	14383.96	1
4	11649.31	2
5	14979.81	2
6	14482.48	2
7	10953.36	3
8	11170.50	3
9	14590.78	3

Appendix 8

Data of Urinary Fluoride Levels in Baseline and Follow Up Examinations

No. : Number of sample
Group : 1 - Group I (sugar group)
2 - Group II (Fluoridated sugar group)
Sex : 1 - female
2 - male
BL : Baseline
FU1 : Follow up I
FU2 : Follow up 2
FU3 : Follow up 3

No	Gr	Sex	BL	FU1	FU2	FU3
1	1	1	0.28	0.13	0.10	0.10
2	1	1	0.11	0.18	0.10	0.23
3	1	1	0.26	0.26	0.21	0.11
4	1	1	0.26	0.15	0.13	0.17
5	1	1	0.27	0.51	0.10	0.17
6	1	1	0.20	0.13	0.14	0.11
7	1	1	0.23	0.20	0.24	0.13
8	1	1	0.24	0.23	0.07	0.25
9	1	1	0.21	0.45	0.08	0.17
10	1	1	0.18	0.28	0.09	0.25
11	1	1	0.14	0.33	0.08	0.24
12	1	1	0.21	0.02	0.11	0.24
13	1	1	0.20	0.28	0.13	0.25
14	1	1	0.18	0.51	0.10	0.24
15	1	1	0.11	0.18	0.19	0.37
16	1	2	0.28	0.15	0.12	0.16
17	1	2	0.10	0.15	0.10	0.15
18	1	2	0.19	0.16	0.12	0.16
19	1	2	0.16	0.15	0.12	0.15
20	1	2	0.05	0.14	0.12	0.12

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No	Gr	Sex	BL	FU1	FU2	FU3
21	1	2	0.12	0.04	0.13	0.13
22	1	2	0.05	0.11	0.14	0.17
23	1	2	0.17	0.17	0.15	0.21
24	1	2	0.53	0.11	0.16	0.08
25	1	2	0.42	0.25	0.14	0.19
26	1	2	0.38	0.05	0.13	0.05
27	1	2	0.32	0.10	0.19	0.10
28	1	2	0.22	0.15	0.15	0.15
29	1	2	0.10	0.10	0.19	0.10
30	1	2	0.23	0.05	0.15	0.05
1	2	1	0.08	0.10	0.52	0.21
2	2	1	0.12	0.12	0.50	0.27
3	2	1	0.04	0.11	0.18	0.21
4	2	1	0.23	0.18	0.56	0.35
5	2	1	0.15	0.12	0.19	0.48
6	2	1	0.16	0.21	0.28	0.24
7	2	1	0.05	0.27	0.33	0.27
8	2	1	0.05	0.10	0.28	0.35
9	2	1	0.17	0.15	0.11	0.64
10	2	1	0.11	0.10	0.12	0.27
11	2	1	0.25	0.09	0.21	0.60
12	2	1	0.05	0.10	0.33	0.27
13	2	1	0.19	0.18	0.35	0.31
14	2	1	0.17	0.33	0.33	0.28
15	2	1	0.09	0.13	0.38	0.15
16	2	2	0.15	0.18	0.52	0.27
17	2	2	0.12	0.12	0.38	0.29
18	2	2	0.09	0.20	0.50	0.23
19	2	2	0.06	0.23	0.19	0.14
20	2	2	0.09	0.23	0.28	0.09
21	2	2	0.16	0.30	0.31	0.23
22	2	2	0.15	0.18	0.40	0.23
23	2	2	0.18	0.20	0.19	0.17
24	2	2	0.13	0.14	0.15	0.08
25	2	2	0.17	0.23	0.18	0.44
26	2	2	0.13	0.13	0.56	0.22
27	2	2	0.10	0.10	0.50	0.20
28	2	2	0.15	0.14	0.35	0.24
29	2	2	0.08	0.20	0.38	0.35
30	2	2	0.13	0.30	0.50	0.64