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IMMUNIZATION
AGAINST
DENTAL CARIES

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A thesis submitted in partial
requirement for the
DIPLOMA IN PUBLIC HEALTH DENTISTRY

Department of Preventive Dentistry
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1982
ACKNOWLEDGEMENT

First and foremost I wish to thank Professor Peter D. Barnard, to whom I am greatly indebted for his valuable guidance, assistance and encouragement.

Thanks are also due to the Ministry of Defence, Malaysia for their sponsorship.

I also wish to thank my wife for her patience, encouragement and assistance during the preparation of this thesis.

Last but not least I am grateful to my children, Shireen and Kiran who have been a source of great inspiration during the course of my work.
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1 INTRODUCTION

Although dental caries is known to have occurred in ancient times it only reached epidemic levels when the human race adopted a soft diet which is rich in carbohydrates and sugars and low in fibers. Due to the fact that no organised dental surveys have been carried out before the 19th century (James 1975) on living subjects, we do not have much record of the prevalence and intensity of the disease. However the teeth being very durable organs remain in excellent condition for a considerable time after death for investigation into the epidemiology of dental caries to be carried out. During archaeological investigation, skulls discovered (Brothwell 1959; Moore & Corbett 1971, 1973) show that over the 2000 years between the beginning of the Iron Age and the end of the Middle Ages in Britain there were no major changes in the prevalence of caries. The prevalence of caries in the population sampled remained uniformly low compared with that found later.

In the 19th and 20th century the prevalence of caries has been on the increase. Epidemiological surveys carried out on Australian aborigines, New Zealand Maoris, Eskimos and other native populations show very low prevalence of caries when they were on their native diet. However with the introduction of European-type diet there has been an increase in caries prevalence. The problem of caries is not the same in all countries. It varies from place to place and between different social economic groups. Widely divergent figures have been reported from different parts of the world on caries. In a study of the caries prevalence and dental services in the Pacific Islands (Speake 1980), the DMF of 11 years old children was reported as low as 1.41 in Gilbert Islands and as high as 5.95 in the Cook Islands. In U.S.A. almost everyone suffers from dental caries to some extent before reaching adulthood (Scherp 1971 a).

This has been most frustrating for everyone concerned. With the advancement of civilization when standard of living and nutrition have improved, most other diseases have been brought under control, but dental caries has been on the increase. Hence dental caries seems to be a direct result of advancement of civilization.
1.1 METHODS OF CARIES PREVENTION

In the financial year 1975 - 1976 the total expenditure for dental health services was estimated at $254 000 000 in Australia. In the next financial year 1976 - 1977, approximately $285 000 000 was spent for dental health services. This was an increase of 12% over the previous year and only about 50% of the Australian population received dental services (Barnard 1982). If the total population was to receive dental health services the cost would be much higher. At the same time the cost of dental treatment is going up everyday and becoming a greater and greater financial burden on an ever increasing proportion of the population. Due to the enormous extent of the problem and tremendous cost involved, prevention seems to be the only way to solve all problems. If successfully carried out, it would reduce the prevalence of dental caries thus reducing pain and suffering and disability due to tooth loss. It would also cut down cost and manpower requirement. This is of special importance to under-developed and developing countries which cannot come up with the money and manpower for sophisticated restorative dental work.

Over the years various methods of caries prevention have been applied and improved to varying degrees of success. Some of the common ones are outlined below.
1.1.1 Oral Hygiene

In the maintenance of oral hygiene, toothbrushing is the most commonly advocated and socially accepted means of achieving good oral hygiene. However if viewed critically toothbrushing alone does not lead to much reduction in new carious lesions.

The basic concept in the maintenance of oral hygiene is the removal of plaque which is a major etiological factor in caries formation. Toothbrushing is more effective when used along with a disclosing solution or tablet. In this way the patient knows what he or she has to remove and will have a goal to achieve. The type of toothbrush and the frequency will not usually determine the effectiveness of toothbrushing as a preventive measure. As Silverstone (Silverstone 1978) puts it, it is better to clean the teeth once a day thoroughly than quickly and inefficiently after every meal. Also studies carried out on various designs of toothbrushes have not revealed any design to be superior than the others.

The problem of plaque removal from interproximal spaces is well known even when toothbrushing is carried out efficiently. Hence the use of dental floss is a must if all plaque is to be removed from interdental spaces.

1.1.2 Fluorides

The use of fluoride therapy is the single most effective method for a successful caries preventive program. Fluoride ions stimulate the formation of larger apatite crystals in the tooth structure. They also convert hydroxyapatite to fluor-apatite within the teeth and promote remineralization of hard tooth structure which is under caries attack. Fluoride ions also interfere with microbial metabolism by inhibiting membrane transport of substrate into plaque microorganisms on the teeth and by inhibiting formation of acid (WHO Report 1975). Fluoride reduces the intracellular storage of polysaccharides by bacteria and the proportion of plaque bacteria storing polysaccharides is also reduced thus resulting in less plaque formation.
(Jenkins 1975). All these actions tend to interrupt the carious process.

There are various types of fluorides in use today and various methods of application. These can all be basically divided into two groups. The use of systemic fluorides and fluorides used for topical application.

1.1.2.1 Systemic fluorides

If an optimal level of fluoride is ingested by the mother during pregnancy and the child through the years of tooth formation the reduction in caries will be greater than 50% in the permanent teeth (Backer-Dirks 1974) and the reduction slightly less than 50% in the primary teeth (Scherp 1971 b). The usual means of supplying fluoride to the population is through the domestic water supply. The fluorides are added to the optimal level which is usually around 1 ppm depending on the weather conditions and the amount of water consumed per individual.

In areas where the domestic water supply is not fluoridated, fluoride supplements are recommended from birth to the age of 14 years (Newburn 1978a). These supplements can be in the form of drops, tablets or lozenges. In his book "CARIOLOGY", Newburn (Newburn 1978b) recommends the following daily dosages:

- Infant under 2 years of age: - 0.2 to 0.3 mg. of fluoride.
- Children 2 to 3 years of age: - 0.5 mg. of fluoride.
- Children 3 years or older: - 1 mg. of fluoride.

Some countries where water is not fluoridated attempts have been made to fluoridate milk while others have tried fluoridating domestic salt. The means by which an individual obtains the fluoride is not important so long as an optimal level of intake is maintained for maximum protection.
1.1.2.2 Topical fluoride

It has been known for a long time now that fluoride used topically on the teeth have an additive effect when used along with systemic fluorides. Sodium fluoride, Stannous fluoride and Acidulated Phospho-fluoride are some of the common ones in use.

Before topical fluorides are applied it is recommended that the teeth are cleaned and polished well. However harsh abrasives should be avoided. Proximal areas should be cleaned with dental floss or a very fine abrasive strip. The teeth should then be isolated with cotton wool rolls, and dried well. Each quadrant is then treated at a time.

Sodium fluoride is usually applied as a 2 per cent solution in distilled water. It is sufficient to apply each quadrant for a period of 4 minutes. Stannous fluoride solution of 10 or 8 per cent in distilled water can be applied to the teeth for 2 minutes. Acidulated Phospho-fluoride comes in a solution or gel containing 1.23 per cent fluoride ions. The application time is 4 minutes.

Topical application of fluoride should be carried out 2 to 3 times a year and should be started with the deciduous teeth, preferably at the age of 2½ to 3 years.

1.1.3 Modify The Diet

The type of diet a person takes is directly related to the probability of him getting tooth decay. Sucrose is the biggest culprit in our modern diet which is taken up by microorganisms to produce acid leading to carious lesions. So too are other sugars and refined carbohydrates, though to a lesser extent.

It is however impossible to get the masses to avoid such foods completely. All the same attempts have been made to encourage patients to reduce the consumption of such cariogenic foods and increase the consumption of fruits and vegetables. Also snacks between meals are to be avoided as far as possible. Patients wishing to take any sweet foods are advised to take them at mealtime which should be followed by a thorough brushing.
1.2 HOW IMMUNIZATION CAME TO BE CONSIDERED

In the above discussion on methods of caries prevention, it is apparent that except for water fluoridation the rest of the means of caries prevention involves a great deal of motivation, cooperation and work on the part of the dentist and his patients.

Take the case of oral hygiene maintenance, most people do take an interest to some extent, in their oral hygiene. There are some who brush their teeth when they have the time, while others do it once a day and still others who make it a habit to brush after every meal but the effectiveness and efficiency with which it is carried out is questionable. To get all these people to even brush and floss after using a disclosing solution or tablet even once a day is no easy task.

In the same way it is difficult to get people to take the daily dose of fluoride if the water is not fluoridated or carry out home regimes of self applied fluorides at home. Even when topical application is done by the dentist or therapist not everyone turns up for all the necessary appointments. As Mosteller (Mosteller 1973) has pointed out that "If people will not lose weight, stop smoking or drinking, will not obey the speed limits, when they know all these things can kill them, how can we expect them to be too inconvenienced in order just to keep their teeth?"

Changing the dietary habits of the population is another major problem. In a survey carried out in Sweden (Koch & Mortinsson 1971), though most parents were aware of the cariogenicity of cookies, soft drinks, candies and chewing gum, 66% of the parents did not express any desire to change their children's consumption habits.

If however all preventive measures discussed are carried out, expect for modifying the diet, the cost can work up to quite a substantial sum for some under-developed and developing countries, and we will still not be assured of total protection against caries. At the same time if all preventive measures are to be applied to the entire population not many countries
at present have the required qualified manpower to carry it out nor would most of them be able to afford it in the near future. So the search for a cheaper, simpler and more effective means of protection against caries goes on. In the medical field a number of vaccines and anti-serums have been introduced for protection against specific diseases which are of microbial origin. Hence it is feasible to assume that a similar approach is very likely to produce results in the dental field. If successful such a method of protection against caries would be simple, cheap and hopefully very effective.

1.3 AIMS AND OBJECTIVES

To trace the history of attempts to immunize against caries.
To review some of the methods of immunization against caries.
To evaluate and compare various means of immunization against caries.
2 ETIOLOGY OF DENTAL CARIES

The earliest recorded theory of tooth decay dates back to 4000 B.C. (Levine 1977). This is the theory of the 'tooth worm'. Through the passage of time a number of theories have been postulated namely Humors theory, Vital theory, Chemical theory and the parasitic or septic theory. However the 'worm theory' was still widely accepted until just over a century ago.

In 1889 an American dentist W.D. Miller came up with the acidogenic or chemo-parasitic theory which was a combination of the chemical theory and parasitic theory. He supported his theory with a series of simple experiments and was widely accepted. According to Miller's theory acid was produced by metabolism of dietary carbohydrates by oral bacteria. The acid in turn caused demineralization of the mineral phase of the teeth followed by the breakdown of the organic matrix.

Later two more theories were postulated. In 1944 Gottlieb suggested that caries was initiated by proteolytic enzymes destroying the organic matrix which opened up pathways for bacteria to colonise. The bacteria then produce acid from sugars which remove the minerals. This is commonly known as the proteolytic theory. The second, the proteolytic-chelation theory was put forward by Schatz and Martin (Schatz & Martin 1962). According to this theory after the initial proteolytic attack on the enamel, the mineral phase was removed by chelation. The validity of these two theories are questionable due to the lack of experimental evidence and support of data.

On the contrary, Miller's basic concept has built up much support and a great deal of experimental evidence has been documented in its favour. Even the destruction of organic matrix which was not very well explained by Miller has been clarified by the discovery of proteolytic enzymes of bacterial origin in carious cavities.

Though it is now understood that caries is initiated by acid produced as a by-product of bacterial metabolism of dietary
carbohydrates within plaque, it is necessary to look into the various factors that take part in producing the varying patterns of caries attack in different individuals.

2.1 TEETH

It was at one time thought that caries susceptibility of a tooth depended upon the degree of mineralization. Except for cases of gross developmental disturbance there is little evidence to accept this as a contributing factor. There is however some evidence that a higher concentration of carbohydrates in the enamel content is related to lowered solubility to acid (Gron et al 1963). Of the trace elements in enamel, fluoride is undoubtedly the most important. An optimal level of fluoride renders it more resistant to acid attack.

Clinically it is observed that pits and fissures are highly susceptible to caries. This is due to the fact that food and bacteria can easily get impacted in them and are not easily removed. The extent and depth of these pits and fissures vary in different individuals and hence, the susceptibility to caries.

In a similar way crowding and overlapping of teeth renders them more susceptible to a carious attack. Microstructural defects on the enamel surface could also predispose the tooth to caries.
2.2 **SALIVA**

The susceptibility of an individual is very greatly influenced by the various characteristics of saliva.

2.2.1 **Rate of Flow**

A higher rate of flow aids in washing away food debris and possibly helps by diluting the acids formed. This is very clearly demonstrated in patients after radiation therapy. In such patients the salivary glands get irradiated in the process and the quantity of saliva is greatly reduced. This is usually followed by rampant caries.

2.2.2 **Viscosity**

Saliva of low viscosity would help cleanse the oral cavity and dilute the acids much better than saliva of high viscosity.

2.2.3 **Buffering Capacity**

The chief buffer systems are bicarbonate, carbonic acid and phosphate. These help to neutralise the acid and thus maintain a constant pH.

2.2.4 **Antibacterial Factor**

The last and possibly the most important is the antibacterial factor. Lysozyme, a hydrolytic enzyme in saliva, destroys bacteria by breaking down their cell wall while lactoperoxidase is active against microorganisms which accumulate peroxide. Saliva also contains immunoglobulins (Ig) which are specific antibody proteins. The different classes of immunoglobulins present in humans are IgG, IgA, IgM, IgD and IgE. The highest concentration of immunoglobulin present in serum is IgG. However the major immunoglobulin present in saliva is IgA. The IgA present in saliva differs from that present in serum in that it contains an additional glycopeptide referred to as the 'secretory component'. This IgA is synthesized by immunocytes in the salivary glands while the IgG and IgA in saliva are in part derived from their corresponding serum counterparts.
These antibodies may provide protection in three ways. Firstly these antibodies may lyse the bacterial cells. Secondly they may prevent adherence of the cells to the tooth surface and finally they may coat the bacterial cells and interfere with their metabolism.

2.3 **DENTAL PLAQUE**

Aristotle related tooth decay to the soft adherent food deposits on teeth in the fourth century B.C., but it was not until the seventeenth century with the introduction of the microscope that microorganisms were seen in dental plaque. In the years that followed various attempts were made to define this dental deposit and food debris was always associated with it. Of all the definitions, the definition that comes closest to describing dental plaque has been formulated by Loe in 1969. 'Plaque is the soft, non-mineralized, bacterial deposit which forms on teeth (and dental protheses) that are not adequately cleaned' (Loe 1969).

It is now understood that dental plaque is composed mainly of bacteria and their products. The first step in plaque formation is the deposition of an organic film, acquired pellicle, which takes place within two hours after the tooth has been cleaned and polished. The acquired pellicle is largely derived from salivary glycoprotein and though bacteria are not necessary for its formation, these early deposits already contain bacteria. As plaque progresses to develop, there is rapid bacterial growth. The early organisms that colonize the tooth surface get firmly attached to the pellicle and as they continue to multiply the layers of bacteria are held together by interbacterial adherence. The adherence of bacteria is further facilitated by the production of extracellular glucans by some oral streptococci, notably Strep. mutans and Strep. sanguis. The bulk of dental plaque finally consists of bacteria embedded in an organic matrix. The matrix is derived partly from salivary glycoproteins and partly from microbial extracellular polysaccharides. The
amount of plaque formed, varies on different teeth and on different areas of the same tooth and so does the microflora. Matured plaque undergoes constant remodelling as a bacterial mass is not a static entity. This plaque plays an essential role in the etiology of caries as it allows the diffusion of carbohydrates into it from the oral fluids. These carbohydrates are taken up by plaque organisms and acid is liberated resulting in a drop in pH. At the same time the complex structure of the plaque prevents the acid from being easily washed away from the enamel surface by oral fluid, thus allowing for prolonged action of the acid on the tooth surface to cause demineralization.

2.4 MICROORGANISMS

Dental caries cannot occur in the absence of microorganisms. This is clearly shown by the fact that germfree animals do not develop caries (Orland et al. 1954) and animals fed with antibiotics help in reducing the incidence and severity of caries (McClure & Helwitt 1946). Also unerupted teeth do not develop caries and oral bacteria can demineralize enamel and dentin in vitro to produce caries-like lesions (Von der Fehr et al. 1970). At the same time invading microorganisms have been demonstrated in carious enamel and dentin which can be isolated and cultivated.

Early plaque formation is mainly dominated by Streptococci with significant quantities of other cocci such as Neisseria and Veillonella and gram-positive rods. As the time of exposure is increased, the plaque undergoes a transition from a predominantly coccal flora to one which is increasingly filamentous particularly by the actinomyces species. At the same time there is a shift from mainly aerobic and facultative organisms to a more anaerobic flora.

Not all organisms are cariogenic and those that are cariogenic, are not equally virulent. Furthermore, different organisms display some selectivity as to the tooth surface they will attack. Organisms capable of inducing carious lesions include Strep. mutans (several strains), a Strep. salivoruis strain,
a *Strep. millesi* strain, *Strep. sanguis* (several strains), a *Lactobacillus acidophilus* strain, a *Lactobacillus casei* strain, *Peptostreptococcus intermedius*, *Actinomyces viscosus* and *Actinomyces naeslundii*. Four types of *carious* processes have been described. They are pit and fissure caries, smooth surface caries, root caries and deep dentinal caries. The organisms and their degree of virulence in each of the carious processes have been taken from animal studies and tabulated in table 1.

Though the cariogenicity of all these microorganisms have not been directly demonstrated in humans, there is considerable indirect evidence and a great deal of emphasis has been given to the importance of *Strep. mutans* in the etiology of caries by most research workers.

### 2.5 Diet

There is enormous epidemiological and experimental evidence to show that carbohydrates are essential in the etiology of caries. Animal experiments (Konig et al 1969) have shown that the prevalence and incidence of dental caries is directly related to the frequency of carbohydrate ingestion. Investigations carried out in Sweden (Gustafsson et al 1954) showed that subjects who consume more sugar but at meal time only have fewer carious lesions compared with subjects who consume less sugar but with part of the sugar being taken in between meals. This indicates that the duration of sugar being in the mouth is of greater importance than the amount of sugar consumed. An interesting observation was made on institutionalized children at Hopewood House in Bowral, Australia (Harris 1963). Babies were either born at the home or taken into it in the first few weeks of life, gradually building up to a population of 60 children. From the beginning, sugar and other refined carbohydrates (e.g. white bread) were excluded from the children's diet. Carbohydrates were given in the form of whole-meal bread, soya beans, wheat germ, oats, rice, potatoes and some treacle and molasses. Dairy products, fruits, raw vegetables and nuts featured
Table 1. Types of dental caries in animal model system.

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<th>Type of Caries</th>
<th>Etiological Organism</th>
<th>Possible Significance in Human Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pit and fissure</td>
<td>Streptococcus mutans</td>
<td>Very significant</td>
</tr>
<tr>
<td></td>
<td>Streptococcus sanguis</td>
<td>Not very significant</td>
</tr>
<tr>
<td></td>
<td>Other streptococci</td>
<td>Not very significant</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus sp.</td>
<td>Very significant</td>
</tr>
<tr>
<td></td>
<td>Actinomyces sp.</td>
<td>May be significant</td>
</tr>
<tr>
<td>Smooth surface</td>
<td>Streptococcus mutans</td>
<td>Very significant</td>
</tr>
<tr>
<td>Root surface</td>
<td>Actinomyces viscosus</td>
<td>Very significant</td>
</tr>
<tr>
<td></td>
<td>Actinomyces naeslundii</td>
<td>Very significant</td>
</tr>
<tr>
<td></td>
<td>Other filamentous rods</td>
<td>Very significant</td>
</tr>
<tr>
<td></td>
<td>Streptococcus mutans</td>
<td>Significant</td>
</tr>
<tr>
<td></td>
<td>Streptococcus sanguis</td>
<td>May be significant</td>
</tr>
<tr>
<td>Deep dentinal caries</td>
<td>Lactobacillus sp.</td>
<td>Very significant</td>
</tr>
<tr>
<td></td>
<td>Actinomyces naeslundii</td>
<td>Very significant</td>
</tr>
<tr>
<td></td>
<td>Actinomyces viscosus</td>
<td>Significant</td>
</tr>
<tr>
<td></td>
<td>Other filamentous rods</td>
<td>Very significant</td>
</tr>
<tr>
<td></td>
<td>Streptococcus mutans</td>
<td>May be significant</td>
</tr>
</tbody>
</table>

(Source: Newbrun, E. 1978. p. 46)
prominently in the typical menu. Although this was a vegetarian
diet, it nevertheless provided an adequate amount of proteins,
fats, minerals and vitamins. Dental surveys carried out on these
children during the age of 5 to 13 revealed an average of def
and DMFT score of 1.1 or about 10% of the caries prevalence in
the general population of that age group. It is also interesting
to note that their water supply contained insufficient fluoride
(0.1 ppm) and their oral hygiene was poor. When the children
grew older and they left the home, their diet changed and a
steep increase of DMFT occured.

People who are fructose intolerant (Marthalar & Froesch 1967)
and have to restrict the intake of sucrose and sweet tasting
substances hardly ever have caries.

Experiments carried out (Kite, Shaw & Soguras 1950) where rats
were fed with cariogenic diet by gastric intubation, remained
caries free.

All carbohydrates are not equally cariogenic. It has been shown
in animals that monosaccharides and disaccharides are relatively
more cariogenic than starch (Shaw, Krumins & Gibbons 1967).
Within the sugars, sucrose is regarded as the most cariogenic,
though very little difference is indicated between glucose,
sucrose and fructose (Colwen, Bowen & Cole 1977).

Sugars taken in a liquid form tend to be less cariogenic than
sugars taken in a solid or sticky form such as toffees, candies
or cookies.

The consumption of sugar has an effect on the composition of
plaque microorganisms. There is considerable evidence to indic-
ate that the intake of sugars enhance the ability of Strep.
mutans to colonize tooth surface (Krasse 1965). Also micro-
organisms especially Strep. mutans and Strep. sanguis, synthe-
size glucan and fructan from sucrose which aid in their adhesion
to the tooth surface. At the same time these polysaccharides
act as a reserve source of carbohydrates and possibly protect
the plaque microorganisms from the harmful effects of the oral
enviroment.
The single most important factor that determines the cariogenicity of a carbohydrate is the ease with which it can be taken up and metabolised by plaque microorganisms to liberate acid. It is accepted now that enamel can demineralize below pH 5.5. A wide variety of acids are produced in plaque, the common one being lactic acid. It has also been shown that each time sucrose is introduced into the mouth the pH in plaque can fall as low as 4.1 (Hassell & Muhleman 1971). The greater the frequency of sugar intake by an individual and the longer the duration of the sugar being in his mouth, higher will be his risk of developing carious lesions.

2.6 SUMMARY

Dental caries is a localized, progressive decay of the teeth initiated by organic acids produced by bacteria which causes demineralization of the outer surface of the tooth and destruction of the tooth proteins by continued bacterial action. The acid is produced by bacteria through a fermentation process by taking up deposits of dietary carbohydrates. As demineralization by acids and proteolytic activity by bacteria continues it leads to the formation of a cavity.

Three principal factors (Newburn 1978c), the host (mainly the saliva and the teeth), the microflora and the substrate (i.e. the diet) are necessary for the initiation of caries. For caries to progress a fourth factor, time, has to be taken into consideration. The factors can be diagrammatically represented as shown in figure 1.
Figure 1. The circles diagrammatically represent the parameters involved in the caries process. All four factors must be acting concurrently (overlapping of the circles) for caries to occur.

(Source: Newbrun, E. 1978. p. 16)
3 IMMUNITY

The term immunity can be defined to include "all those physiologic mechanisms that endow the animal with the capacity to recognize materials as foreign to itself and to neutralize, eliminate, or metabolize them with or without injury to its own tissues" (Bellanti 1978).

Immunity against infection to parasitic organisms and against damage caused by their metabolic by-products results from a fine interaction of specific humoral and cell-mediated immunological mechanisms, with those mesenchymal cells which are present in all creatures and which have the power of ingesting and digesting foreign material.

Much of our immunity against infection is in fact innate or in-born, although immunity to certain microorganisms and their toxins develops following actual contact with the antigens in the microorganism or their toxic metabolic by-products. In certain cases immunity can be produced by injection of the dead organisms or their products. Such immunity can be transferred to a normal person through the transfer of serum of such an immune. Immunity of this kind can be transferred naturally from the mother to her young via the placenta or by the ingestion of colostrum in certain animals.

Immunity whether innate or secondary to an initial contact with the toxin or infective agent will in most cases be boosted by further contact with these antigens.

Immunity can be classified as follows:

I Non-specific immunity.

II Specific acquired immunity.
   a. active acquired immunity.
   b. passive acquired immunity.
3.1 NON-SPECIFIC IMMUNITY

The term "non-specific immunity" often equated with natural resistance and refers to the ability of an individual to resist infections through the normally present body functions of all members of his species. Non-specific immunity relies on the non-adaptive or normal activities of our phagocytic cells, blood and tissue proteins, certain smaller molecules of blood and tissue and the basic permanency and integrity of our cells and tissues.

3.1.1 External Defense System

The first resistance forces encountered by an invading microbe are actually outside the physiologic milieu of the body. The first body surfaces contacted by most pathogens are the skin mucous membrane. The keratinised outer layers of the skin which, when intact, constitute a highly effective structural barrier against invasion. Furthermore most bacteria fail to survive for long on the skin because of the direct inhibitory effects of lactic acid in sweat and the unsaturated fatty acids in secretions from the sebaceous glands. Both these acids are antibacterial in action.

The mucous membranes are structurally more susceptible to penetration than is the skin and, being moist, would theoretically permit a long-term survival of microbes on their surface. However, mucosal tissues are protected by several antimicrobial activities. The first of these is the mucous slime itself, a gelatinous-like bio-adhesive excreted by and coating the mucosal epithelium.

Microorganisms which enter the respiratory tract as a result of inhalation, are trapped by the mucosal flim which lines this tract. The cell layer beneath this mucous flim is ciliated and the cilia constantly sweep the mucous upwards. In this way the mucous with the trapped microorganisms reach the throat where they are swallowed except for instances of extensive mucous formation. Another important protective activity also occurs
in the mucous film — phagocytosis. A special kind of phagocytic cell known as the alveolar macrophage roams over the surface of the mucous membrane. The major function of this cell appears to be the phagocytic destruction of inhaled objects.

The process of phagocytosis includes first and foremost the ingestion of the microorganism by the phagocyte. The ingested microorganism is surrounded by the membrane of the phagocytic vacuole (phagosome) and by simultaneously ingested extracellular fluid. Lysosomal granules, which occur in large numbers in the cytoplasm of mature macrophages, contain a variety of hydrolytic enzymes with acid pH optima. These are the acid hydrolases which include acid phosphatase, lysozyme, acid ribonuclease and cathepsin. By fusion of the lysosomal membrane with that of the phagosome, its enzymes are transferred to the interior of the latter which becomes a phago-lysosome. The process of dissolution for most microorganisms is rapid and may be complete within 30 minutes.

Other mucosal surfaces have slightly different protective devices. In the male genitourinary system the periodic flushing with urine, which is normally somewhat acidic, provides protection to the mucous surfaces. The same may be stated for the female urinary tract. The reproductive tract of a mature woman has a thick underlying cell bed. These cells synthesize and store glycogen. As the cells die, the glycogen is degraded to lactic acid, creating a bacteriostatic enviroment in the vagina.

The mucosal surfaces of the eyes are partially protected by the tendency to weep when objects of any size enter the eye, and this is an effective cleaning action. Far more important, especially when microorganisms enter the eye without being borne on particulate matter and weeping is not induced, is the action of an enzyme known as lysozyme. Lysozyme has an isoelectric point near pH 11.0 and most bacteria have acidic isoelectric points; this ionic dissimilarity may promote the union of the enzyme with the bacteria. Lysozyme cleaves a β-1, 4 glycosidic bond that unites N-acetylglucosamine and muramic acid. This
exposes the underlying cell membrane to osmotic forces that rupture the cell.

Defense in the digestive tract is achieved in two ways. The first is through stomach acidity which may be as low as pH 1.0. This is not only an unfavorable environment for the growth of microorganisms but is actively lethal. Organisms that are markedly aciduric or protected inside poorly masticated pieces of food escape this acidity and are passed to the small and large intestines. These regions of the alimentary canal are heavily colonized with the normal flora, a heterogeneous collection of basically non-invasive bacteria. This flora which is quite stable, prevents the growth of others, including pathogenic organisms. Properties of the normal flora, responsible for this include certain acid and products and antibiotic-like substances.

3.1.2 Internal Defense System

Successful pathogens that escape the external defense system and penetrate into the true physiologic interior of the host are met with the internal armory of the host which is phagocytosis. Phagocytosis is promoted by several factors which include the naturally present opsonins (to prepare for eating). Many serum proteins serve as opsonins apparently by coating the particle or cell to be phagocytosed, altering its surface charge, making it approachable by phagocytic cells.

Another important stimulant of phagocytosis is leukotaxis or chemotaxis. Local tissue damage by the invading pathogen may liberate trypsin like tissue enzymes which cause the activation of the complement which is essential for immune hemolysis. Complement activation can also be initiated by the activation of plasminogen to plasmin or by the polysaccharide capsule or endotoxins from the invading organism.

Chemoattractants released by the neutrophils or by soluble factors excreted by the invading bacteria draw monocytes to the scene of the infection. In the tissues the monocytes transform into the highly phagocytic macrophages.
Macromolecules in blood may have antiviral or antibacterial properties. Glycoproteins, transferrin and ferritin are all antiviral. Beta lysin, leukin, plakin and other poorly described proteins are antibacterial. In tissues, polyamines and basic polypeptides are known to play a protective role against bacteria. Among these are the histones, protamines, spermine and spermidine.

3.2 SPECIFIC ACQUIRED IMMUNITY

The immunity an individual develops during his lifetime is called acquired immunity. Unlike natural or non-specific immunity which is a broad-spectrum type of resistance not directed against any particular pathogen, acquired immunity is expressed most typically against a specific pathogen and develops as a result of exposure to the specific pathogen.

Acquired immunity is based on the activities of three major cell types: the macrophages, the B lymphocytes and the T lymphocytes. Exposure of a host to antigens of a pathogenic or potentially pathogenic organism initiates a series of adaptive responses in these three cell types. Phagocytic cells, by virtue of their inherent cytophagic ability, ingest and degrade the antigen. Portions of the antigen are passed to B and T lymphocytes. This simulates growth and division in these cells so that immunoglobulin-forming plasma cells are formed from the B lymphocytes. T cells also undergo lymphocytic transformation and elaborate their lymphokines. Among these is a product that brings forth an activated macrophage which is more actively phagocytic and also more fully endowed with hydrolyses and other enzymatic functions, making it more lethal for its phagocytic victims. This vigorous new phagocytic activity is not specific against only the organism initiating the response but will encompass whatever foreign threat that may be present.

Other lymphokines associated with immunity are chemotactic factor, migration inhibition factor (MIF). Macrophage activating factor, lymphotoxin, interferon, and possibly blastogenic factor. Chemotactic factor draws monocytes into the arena where
the T cell has contacted the antigen, migration inhibition factor holds the macrophage at the scene of infection, and lymphotoxin causes a cytolitic destruction of at least certain types of foreign cells. Blastogenic factor activates surrounding T cells to join the secretion of these and other lymphokines.

Interferon was originally described as a virus-induced substance that was helpful in limiting or preventing a viral infection. Recent discoveries have shown that many nonviral agents—rickettsiae, mycoplasma and certain protozoa—are satisfactory interferon inducers. It is also recognised that interferon can suppress the replication of several intracellular parasites. It is believed that interferon excreted by stimulated lymphocytes or other cells, enters adjacent cells and derepresses them to permit their synthesis of a translation inhibitory protein. This protein restricts translation of foreign messenger RNA but has only a slight effect on host RNA, so the host cell retains its vitality while parasite replication is aborted. Interferon portrays a non-specific nature, that is, one species of virus administered as an interferon inducer would permit the host to resist disease caused by many different intracellular parasites. The action of interferon is host directed and not parasite directed.

3.2.1 Active Acquired Immunity

Actively acquired immunity is the result when the acquired is based on the acquisition of new defensive devices by the exposed host. Active acquired immunity is of two types. When a person contacts an overt clinical disease or an unrecognized subclinical illness, his body develops protective antibodies against that pathogen. A subsequent encounter with the same pathogen finds the host well prepared against reinfection. This type of immunity is classified as naturally acquired active immunity.

The second type is artificially acquired active immunity which is carried out through the use of vaccines or toxoids. This is safer and more economical means of acquiring protection against the ubiquitous pathogens. Vaccines used may be of the killed or
inactive variety which are vaccines composed of killed or inactive organisms. Attenuated vaccines, which are vaccines composed of living organisms of reduced virulence are in use against poliomyelitis, rubella, yellow fever, tuberculosis and rabies. In those instances in which the disease is more accurately classified as an intoxication, that is, due to specific toxins of the pathogen, toxoids are used as the immunizing principle. Artificially acquired active immunity is not achieved until 5 to 14 days after the immunization because of the time needed to reach protective levels of these immunoglobulins.

Since one's own cells participate in both forms of active immunity, the immune state may persist for months or years without reactivation by booster exposures to the antigen.

3.2.2 Passive Acquired Immunity

Passive immunity can also be acquired naturally or artificially. The former exists when maternal IgG transgresses the placental barrier from the mother to the unborn child and confers the mother's immunity related to that immunoglobulin class on the child. Some postnatal protection may be delivered to the nursing infant through the mother's milk and its secretory IgA. The heaviest flow of antibodies from the mammary glands is in the colostrum. A few days after birth the maternal milk decreases in antibody content and the developing digestive function of the child further restricts the role of these antibodies. Nevertheless, maternal milk borne antibodies may give low-level surface protection to the intestinal mucosa.

Artificial means of receiving passive immunity rely on the injection of the gamma globulin fraction or whole hyperimmune serum into the person needing this protection. The benefits are instantaneous and persists according to the half-life of the immunoglobulin. For the intraspecies transfer of IgG this is about 30 days, so the immunity might extend over several weeks or a few months depending on the dose and the potency.
of the antiserum administered. The half-life of interspecies globulin transfers is only a few days and has the risk of causing serum sickness or, on reinjection, anaphylaxis.
4 EARLY DEVELOPMENTS IN IMMUNIZATION AGAINST CARIES

4.1 INTRODUCTION

A crude form of immunization was practiced in India and China in ancient times (Roitt 1971), where protection against smallpox was obtained by inoculating live organisms from disease pustules. This was dangerous and often fatal. Then in the eleventh century, Chinese physicians observed that the inhalation of smallpox crusts prevented the subsequent occurrence of the disease. Still later a similar form of immunization was practiced in the Middle East where powdered scabs were applied interdermally (Bellanti 1978).

This crude form of immunization reached England in the 18th century and Edward Jenner in 1798 discovered that inoculation with cowpox crusts protected man from smallpox. This was the result of his observation that milkmaids who had contacted cowpox were resistant to infection with smallpox. However it was Louis Pasteur who coined the term vaccine and his research led to development of the germs theory of disease. He also developed techniques for culture of microorganisms in vitro. These cultures provided material for developing vaccines with living, heat-killed or attenuated (living but with reduced virulence) organisms. Then in 1881, he developed a vaccine for anthrax using attenuated organisms.

In the years that followed many other vaccines were developed against various microorganisms and their products.

Dental caries is an infectious disease and the role of microorganisms in its etiology has been well documented. It is thus not surprising that the dental profession has been in search for a vaccine against caries.
4.2 EARLY ATTEMPTS TO IMMUNIZE AGAINST CARIES

The history for the search of a vaccine against caries goes back to the early part of the 20th century. The idea was first brought up by Von Beust in 1912, when he wrote that it is "possible, even probable that the immunity to caries .......... is the result of the formation of antibodies." (Von Beust 1912). Then in 1926 Fish (Fish 1926) considered it "worthwhile investigating the possible existence of an active immunity to caries in the blood stream".

However the first attempt to immunize against caries was carried out by Jay (Jay et al 1932) and his associates in 1932. They made a filtrate from a collection of cultures of Lactobacilli (forty oral strains believed to be the cariogenic organisms at that time) and used that filtrate to conduct skin test on individuals and determine the relationship between the skin reaction of the individual to his susceptibility to dental caries. They found that those persons who reacted to the filtrate, following intradermal injection, did not have Lactobacillus antibodies in their blood serum, while those whose skin test were negative did have Lactobacillus antibodies in their serum. The present or absence of antibodies in the serum was carried out by an agglutination test. They also noted that individuals with antibodies in their sera were immune to caries and Lactobacilli were absent in their saliva. However, individuals without antibodies had high caries scores and Lactobacilli could be cultured from their saliva.

In another investigation (Jay 1933) rats failed to develop antibodies to Lactobacilli Acidophilus of the rough type, the smooth type or the mixed vaccine (rough or smooth depends upon the type of colonies they form on culture). Subcutaneous injections were given, totaling 4.5 c.c. over a period of ten weeks. The animals that received the smooth vaccine had no abscesses while the rats that received the rough vaccine developed severe abscesses. The rats receiving the mixed cultures had moderate abscess formation. However when rabbits were given subcutaneous
injections of killed Lactobacilli Acidophilus of the rough type they developed antibodies. The investigation was carried further and cultures of Lactobacilli were prepared for a series of injections for persons whose sera did not contain antibodies. This was to determine if antibodies could be stimulated in them. The vaccines were prepared from both the rough and smooth strains of Lactobacillus. The vaccine was administered subcutaneously in 0.5 c.c doses (200 000 000 organisms per cubic centimeter), one week apart for four weeks. It was found that the two children that received the rough type of organisms developed abscesses and their antibody titer increased from 0 to 1 - 640 and from 1 - 30 to 1 - 640 respectively. The children who did not have sore arms showed no appreciable change in agglutinin titer. Saliva was cultured from these persons ten days after the last injection, but because of the short observation time it was impossible to draw any conclusion from these tests, as to the effect of this increased antibody titer on the Lactobacillus in the mouth.

Then in 1934, Clough (Clough 1934) reported that saliva had an inhibiting effect on the growth of certain bacteria. Saliva samples from ten people were tested on one or more different days. A zone of inhibition 4 mm. or wider invariably occurred around the wells containing fresh unfiltered saliva in plates inoculated with B. Megatherium and Sarcina Lutea. The degree of inhibition diminished in the following order, B. Subtilis, Proteus Vulgaris, Bact. dysenteriae, Bact. coli, M. lysodeiktics and S. albus. The same individual's saliva gave similar results when tested on different days. The following year Clough (Clough 1935) reported inhibitory action of saliva on Lactobacilli Acidophilus grown on Kulp's tomato-juice agar. Of the 133 specimens of saliva taken from 77 healthy adults, 131 inhibited growth of Lactobacillus Acidophilus. Similar findings were reported by Hine (Hine 1936) when he observed that in 91 percent of tests there was an inhibition of bacterial growth from a series of 1038 tests carried out. In yet another investigation, (Bibby et al 1938), of 169 bacteria investigated, 110 were inhibited by saliva. The following year Hill (Hill 1939)
attempted to draw a relationship between the inhibitory effects of saliva and dental caries. He wrote "There is present insaliva some factor which effects the growth in vitro of Lactobacillus Acidophilus. There is a variation in the intensity of this unknown factor and its presence or absence is consistent with the presence or absence of dental caries in the mouth."

A period of nine years had laped since the last attempted vaccination against caries. This time Canby and Bernier (Canby & Bernier 1942) who had been studing Lactobacilli for several years, became interested in developing a vaccine for Lactobacillus which could be injected without the development of abscesses as described previously. They selected certain strains of Lactobacilli by the process of cross-agglutination. The idea was to select a Lactobacillus which would not give rise to abscesses and, at the same time, stimulate antibody formation against a wide range of strains of Lactobacilli. The selected strains were heat-killed at 56°C., maintained for one hour and preserved with 0.5 per cent phenol. Each suspension was then standardized, so that 1 c.c. contained approximately 450 million organisms. This dilution of vaccine was designated, dilute antigen. Before human volunteers were vaccinated, tests were carried out on guinea-pigs and rabbits.

Twenty caries susceptible patients were included in the study. They were enlisted men, eating at the soldier's mess, except two (R.M.L. and J.M.N.). The number of Lactobacilli in the saliva of volunteers were determined and blood samples were taken. The Lactobacilli count was continued at weekly intervals. Each patient received the vaccine by intracutaneous administra- tion, in amounts of 0.1 c.c. at a single site of inoculation. The area of inoculation was observed as to the degree of skin reaction at 24 hours, 48 hours and subsequent times. If no local reaction was seen or the reaction was mild in character following several successive administration, the vaccine was changed to the concentrated antigen (twice the concentration of organisms than the dilute antigen). The volunteers were
vaccinated every 5 days for a start and later changed to three-
day intervals. The skin reaction in the majority of the cases
were essentially negative. One patient (W. J. T.) showed a local
skin reaction to the extent of abscess formation. In his case
the stock vaccine was withdrawn and an autogenous vaccine was
prepared from two Lactobacillus Acidophilus strains isolated
anaerobically from his saliva. One of these vaccines (D-T-A)
did not produce abscesses in this patient.

Lactobacilli count of saliva was carried out again and samples
were also taken. The results showed a drop in the number of
Lactobacillus Acidophilus in the mouth of all vaccinated persons
except one. The results also showed an increase in the agglutinin
titer in most of the vaccinated volunteers. The data on the
human vaccination experiments is given in table 2.

A follow up of the above investigation was carried out in 1944
(Williams 1944). Vaccines were prepared from Lactobacillus
Acidophilus of the 4s and 13c strains. A total of 33 volun-
teers took part, 20 in the experimental group and 13 in the
control. A number of prebreakfast salivary samples were taken
from all the volunteers prior to vaccination and a Lactobacilli
count done. Also blood samples were taken to determine the
agglutinin titers. Subcutaneous injections were given at weekly
intervals for four weeks, one arm receiving a living suspension
of a mixture of the two Lactobacillus strains and the other arm
receiving a heat-killed suspension of the same mixture. Salivary
counts of Lactobacilli were done and sera was obtained at regu-
lar intervals from both the experimental and control groups,
beginning two weeks after the last injection and continuing
through five months after completion of vaccination. Sera were
tested for agglutination reaction with the organisms that had
been used for injection purpose and the highest titers were
generally found about two weeks after the last injection. The
titers then decreased gradually until they were near the pre-
vaccination levels at five months. The titers from the control
group remained constant.
Table 2. Data on the human vaccination experiments.

<table>
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<tr>
<th>Patient</th>
<th>Vaccine</th>
<th>Period of Vaccination</th>
<th>Number of Intracutaneous Doses</th>
<th>Average Number of Lactobacilli per Ce. of Saliva</th>
<th>Titer of Serum</th>
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<td></td>
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<td>Dilute Antigen</td>
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<td>R.C.S.</td>
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<td>6/16/41</td>
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<td></td>
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<td></td>
<td></td>
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<td>7/21/41</td>
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<td>8/16/41</td>
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<td>7/15/41</td>
<td>9/5/41</td>
<td>21</td>
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<tr>
<td>H.G.H.</td>
<td>D-30-2</td>
<td>7/16/41</td>
<td>9/5/41</td>
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<tr>
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<td>D.T.A.</td>
<td>8/6/41</td>
<td>9/2/41</td>
<td>5</td>
<td>113,600</td>
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</table>

(Source: Canby, C.p. & Bernier, J.L. 1942)
The Lactobacilli count of the volunteers saliva was also carried out. Only 5 volunteers or 25% showed statistically significant reduction in the Lactobacilli count. The rest were not statistically significant.

Figure 2 shows the average agglutination titers of persons having reduction in average salivary counts which were greater than could be accounted for on the basis of chance. It is seen that at the start the average was between 100 and 200. The peak was reached about 2 weeks after the immunization period when antibodies to one strain 4s reached about 700 and to about 500 in the case of strain 13c. Table 3 shows the Lactobacilli count for the same group of volunteers.

Figure 3 shows the average agglutination titers of persons having changes in average salivary counts which were within limits of chance variation. In this group the peaks were also reached about 2 weeks after immunization although they were somewhat lower. Table 4 shows the Lactobacilli count for this group.

Figure 4 and table 5 show the agglutination titers and Lactobacilli count for the control group.
Figure 2. Average agglutination titers of persons having reductions in average salivary counts which were greater than could be accounted for on basis of chance.

(Source: Williams, N.B. 1944)
Figure 3. Average agglutination titers of persons having changes in average salivary counts which were within limits of chance variation.

(Source: Williams, N.B. 1944)
Figure 4. Average agglutination titers of unvaccinated controls.

(Source: Williams, N.B. 1944)
### Table 3. Lactobacilli counts in persons having reductions in salivary counts which were greater than could be accounted for on the basis of chance.

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### Table 4. Lactobacilli counts in persons with changes in counts within limits of chance variation.

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### Table 5. Lactobacilli counts in controls.

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*All figures given are in thousands.

(Source: Williams, N.B. 1944)
5. THE FALL AND RISE IN INTEREST IN IMMUNIZATION AGAINST CARIES

5.1 THE DECLINE IN INTEREST

After the attempt to immunize against caries in 1944 by Williams, there was a sudden decline of interest in immunization against caries. There are a number of reasons that could be attributed to this decline of interest. In the years that followed, it was becoming increasingly apparent that Lactobacilli did not occupy such a central role in caries as had been thought. As early as 1924 (Clarke 1924), Clarke observed that in cultures of material from carious lesions, in 72% of all plates a Streptococcus was present, which he thought to be of etiological importance. He gave a good description of the isolated organism and named it Streptococcus mutans. Then in 1938 (Turnnicliff & Hammond 1938), Turnnicliff and Hammond described the isolation of a greening Streptococcus from dental caries, which in its smooth phase grew as a diplococcus or in short chains. In the rough phase this Streptococcus showed bacillary and coiled form, or crescent, straight and undulating filamentous form. Then Hammond and Turnnicliff (Hammond & Turnnicliff 1940) isolated Streptococcus viridans from carious dentin and demonstrated their caries inducing capacity in vitro. In 1942 Bibby and associates (Bibby, Volker & Van Kesteren 1942) made it clear that the number of acidogenic organisms in saliva from mouths with active caries, indicate that Lactobacilli are a minor constituent of the oral flora. They also reported that the rate of acid formation by various organisms showed that Streptococcus and Actinomyces-like organisms formed acid more rapidly, and that Lactobacilli and other organisms were less active in this respect. In subsequent years more reports were made linking various Streptococcus and other organisms as causative organisms in the etiology of caries. Then in 1960 (Fitzgerald, Jordan & Stanley 1960) Fitzgerald and his associates, demonstrated the induction of caries in gnotobiotic rats with a single strain of Streptococcus, and Fitzgerald and Keyes
(Fitzgerald & Keyes 1960) induced dental caries in a strain of albino, "caries inactive" hamsters by oral inoculation of single or pooled cultures of five biochemically similar strains of Streptococcus isolated from a carious lesion in a hamster. After this most of the research work indicated that Strep. mutans played a greater role in the etiology of caries than had been thought before (Gibbons et al, 1966; Krasse 1966; Edwardsson 1968; Guggenheim 1968).

Another reason for the decline of interest could be due to the fact that the teeth seemed immunologically to be "outside" the body and unavailable to the antibodies in serum.

Yet another reason could be the diversion of interest towards fluorides as a preventive measure against caries. Fluorides were first deliberately added in control amounts to community drinking water in 1945 (McClure 1970). In the same year trial studies involving large groups of people were began in Michigan, New York and Ontario, Canada. In the years that followed a great deal of interest was channeled towards various aspects of fluoride research.
5.2 THE RISE IN INTEREST

With the passing of the years it became more and more apparent that fluorides alone could not fulfil the role of a total preventive programme against caries. At the same time Geller and Rowelstad (Geller & Rowelstad 1959) associated increased amounts of salivary gamma globulin with caries resistance in naval recruits. On similar lines Green (Green 1959) found that caries resistance in a group of dental students was associated with an increased gamma globulin fraction in saliva. It was also reported that antibodies could leak out of the serum through the oral tissues, (Brill 1962; Brandtzaeg 1965; Khurana 1969; Oppenheimer 1970) mainly gingival crevice, and is markedly increased when they are inflamed. Of greater importance was research indicating that the principle antibodies in saliva, as in other mucous secretions, are of the secretory IgA (S IgA) variety (Tomasi & Bienenstock 1965; Heremans 1968). It was further reported that secretory IgA antibodies were produced locally by plasma cells in the salivary glands and the submucous tissues (Claman, Merrill & Hartley 1967; Genco & Taubman 1969; Smith 1969), and that since this IgA antibody is locally produced in response to local antigenic stimulation, the amount in serum and saliva are unrelated. This made it clear that the level of serum antibodies, specific for Lactobacilli or Streptococci, alone are not related to the degree of dental caries experienced by an individual.
5.3 IMMUNIZATION ATTEMPTS AFTER 1960

After a lapse of 16 years Fitzgerald and Keyes (Fitzgerald & Keyes 1962) attempted to immunize albino hamsters against induced dental caries. In a series of experiments they vaccinated albino hamsters using phenolized cells of Streptococcus strain HS-6 (a strain of Streptococcus mutans) subcutaneously or intraperitoneally. The results showed no difference in caries experience in vaccinated or non-vaccinated animals nor were significant levels of antibodies reached. Their attempt to passively immunize hamsters by administering anti-HS-6 rabbit sera with agglutination titers of 1-320 to 1-640 also failed to render protection against dental caries.

Another attempt was made to passively immunize caries susceptible rats in 1966 (Sweeney, Shaw & Childs 1966). The gamma globulin preparation used was prepared from pooled serum obtained from 250 matured stock rats of the Harvard caries-resistant strain. The gamma globulin was prepared with distilled water for injection. Thirty-three rats of the Harvard caries susceptible strain were distributed by litter-mate between a control and an experimental group, at weaning and were maintained on a cariogenic diet.

The rats were given the equivalent of 2.5 ml. of whole rat serum in divided doses over three days subcutaneously. The half-life of gamma globulin being about 2 weeks, the procedure was repeated every 13 days. Similar procedures and schedules were carried out for the control group using buffer salts in distilled water, without the gamma globulin.

On the 80th day the animals were sacrificed. Saliva and serum samples were taken before sacrificing and caries scores taken after sacrificing.

The results did not show any significant difference in the caries scores between the control and experimental groups whether separated by sex or combined. At the same time no differ-
ence in concentration of any component was noted in either the serum or the saliva.

Then Wagner (Wagner 1967) successfully vaccinated gnotobiotic rats infected with Strep. faecalis. He vaccinated paranterally using homologous strains in adjuvant. He reported that the immunized animals had higher agglutinin titers and fewer bacteria in their saliva than the control animals. The immunized animals also had less carious lesions. In another series of experiments Wagner and Orland (Wagner & Orland 1967) reported that rats vaccinated with formalin killed Strep. mutans serotype C, Strep. sanguis or L. casei in Freunds complete adjuvant remained virtually free of dental caries, despite being infected with similar strains on the day immunization commenced. The vaccinated animals had increased salivary agglutinin titers to the strains used in the vaccines.

In 1969 (Bahn et al 1969) an attempt was made to immunize rats with enzymes which aid in the production of extracellular polysaccharides by cariogenic Streptococci. In this study an extract from the culture fluid of strain FA-1 was used to immunize the rats. Fifty three weaning rats were randomized into two groups; 24 immunized and 29 controls. The rats were immunized intraperitoneally with the enzyme extract in Freunds incomplete adjuvant. After 14 days of immunization all rats were challenged with swabbing and daily doses of $10^3$ cells of FA-1 in their drinking water and fed a cariogenic diet. The experiment was continued for 112 days, when the animals were sacrificed and the caries scores taken. The immunized rats had a mean caries score of 17.24 while the control rats had a mean of 42.45. The results were statistically significant ($p < 0.001$).

In the same year (Berkenbil, Matsutani & Bahn 1969) it was reported that significant titers of antibodies to the cell walls of caries inducing Streptococci have been observed in the sera of caries immune naval recruits, when compared to the sera of individuals with rampant caries.
Then for the first time Bowen (Bowen 1969) attempted to immunize monkeys. He used six young female monkeys, two aged 17 months and four aged 11 months. The younger animals had complete deciduous dentitions while the older ones were about to erupt their first permanent molars. A Streptococcus isolated from a carious lesion in a human was used for vaccination. Live organisms were washed in saline and resuspended in saline to give a reading of 100 in an Eel spectrophotometer at 420 m.

One in the older and two in the younger group were vaccinated. The others acted as the controls. Before vaccination, blood samples were taken. The three animals were then given 1 ml. of the vaccine intravenously 8 times over a 3 week period. After 12 weeks they were given a single booster dose. All the animals were infected by mouth with homologous Streptococcus. These microorganisms were rendered resistant to erythromycin and streptomycin to facilitate re-isolation.

The monkeys were maintained on a cariogenic diet rich in fermentable carbohydrates. They were examined monthly with mirror and probe and radiographed every alternate month. The carious lesions were classified as early or established.

After vaccination the antibody titer of serum of vaccinated animals had risen to 1:1280.

At the end of the seventh month there were a total of 9 well developed lesions and 17 carious lesions in the unvaccinated animals. The vaccinated animals had 1 well developed lesion and 6 early lesions.

In the older group of two monkeys the unvaccinated animal had 11 carious cavities and 3 early lesions, while the vaccinated, one had five cavities and two early lesions after 18 months.

The results of the younger group of 4 monkeys were taken at the 13th month. The controls had 27 cavities and 10 early lesions, while the two vaccinated animals had 2 early lesions and 1
caries cavity.

The sum total of the caries experience of the 6 animals after the 18 month and 13 month period was that the control had 38 cavities and 13 early lesions, while the vaccinated animals had 6 cavities and 4 early lesions. The results were significant at the 5% level (P<0.05).

The following year in 1970 (Gaffar et al 1970) Gaffar and his associates, reported a successful attempt to immunize hamsters with formalin killed Streptococcus (strain ss-2) in complete Freund's adjuvant. The immunized group showed a 68% reduction in caries which was statistically significant at the 1% level (P<0.01). However, Tanzer in the same year (Tanzer; Hageage & Larson 1970) reported a failure to protect rats against smooth surface caries by immunization.

Then in 1972 Hayashi (Hayashi, Shklair & Bahn 1972) tried immunizing rats with two different enzymes by different routes.

In the first experiment dextransucrase (glycosyltransferase) from Strep. mutans FA-1 in Freund's incomplete adjuvant was used. Fifty-six weaning rats (21 days old) were separated into three groups. The first group of 24 rats were given 0.5 ml. of the vaccine intraperitoneally. A month later, a second injection was given. The second group of 29 rats were given Freund's incomplete adjuvant in saline. The third group of three rats were not injected.

In the second experiment glycosidic hydrolases from Strep. mutans strain 20 (isolated by Irving L. Shklair) in Freund's incomplete adjuvant was used. Forty-four (12 days old) rats were separated into two groups. The first group of 26 were injected with 0.5 ml. of the vaccine into the submaxillary gland. The second group of 18 rats received 0.5 ml. of Freund's incomplete adjuvant in saline. The injections were repeated after one month.
All rats in both the experiments were given cariogenic diets except the three in the third group of the first experiment. The rats were challenged with homologous strains of Strep. mutans over the period of the experiments.

At the end of the experiments (112 days for the first experiment and 91 days for the second experiment) caries score were taken and the results are shown in table 6 and table 7.

Rats immunized with dextranase from strain FA-1 had a 59.4% lower caries rate, based on the means and the difference was statistically significant (P<0.001). However the protection or lack of protection was not uniform in all the rats. The serum antibodies of immunized rats was also reported to be higher than non-immunized rats.

Rats immunized with glycosidic hydrolases resulted in a reduction of caries of 28.3%. However the difference was not statistically significant.

In 1973 Tanzer et al (Tanzer, Hagege & Larson 1973) using NIH strains of specific pathogen-free Osborne Mendel rats (the strain remains essentially free of smooth surface caries unless infected with Strep. mutans while consuming a cariogenic diet, high in sucrose), attempted to determine (1) if high humoral antibody titers are elicited in rats in response to antigenic challenge by formalin killed plaque-forming Strep., (2) if salivary antibody response is so elicited and (3) if the immunization regimen confers immunological protection against smooth surface caries resulting from infection by plaque-forming Strep. mutans strain 6715.

The vaccine was prepared from formalin-killed Strep. mutans 6715 and suspended in formalinized saline. The animals were fed a cariogenic diet containing 56% sucrose.

Weaning rats (21 days old), were immunized by daily subcutaneous, 0.1 ml. antigen injections, distant from the salivary
Table 6. Score of carious lesions in the molars of rats immunized with dextranucrase extract intraperitoneally.

<table>
<thead>
<tr>
<th></th>
<th>Immunized</th>
<th>Nonimmunized</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>24.00</td>
<td>29.00</td>
<td>3</td>
</tr>
<tr>
<td>Mean caries score</td>
<td>17.42</td>
<td>42.25</td>
<td>0</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>15.05</td>
<td>18.29</td>
<td>0</td>
</tr>
<tr>
<td>Standard error</td>
<td>3.16</td>
<td>3.40</td>
<td>0</td>
</tr>
<tr>
<td>$P$ value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>


Table 7. Score of carious lesions in the molars of rats injected with glycosidic hydrolase extract into the salivary glands.

<table>
<thead>
<tr>
<th></th>
<th>Immunized</th>
<th>Nonimmunized</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>26.00</td>
<td>18.00</td>
</tr>
<tr>
<td>Mean caries score</td>
<td>29.00</td>
<td>54.39</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>16.00</td>
<td>16.74</td>
</tr>
<tr>
<td>Standard error</td>
<td>3.77</td>
<td>3.281</td>
</tr>
<tr>
<td>$P$ value</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

glands, for 5 days. This was followed by weekly injections of 0.2 ml. Non-immunized rats were injected with formalinized saline.

In each experiment animals were divided into three groups (A) immunized and infected; (B) non-immunized but infected; and (C) neither immunized nor infected. The first two groups were infected with Strep. mutans, 17 days after the initial immunization injections. The animals were sacrificed 42-66 days after the infectious challenge.

Attempts were made to recover Strep. mutans one week after infection and prior to the termination of the experiment. Also one week after the five daily injections and one week after the last weekly injection, blood samples were taken. Saliva samples at high rate of flow were taken before sacrificing the animals.

At the end of the experiment caries scores were taken. In all, five experiments were carried out, and the results are tabulated in table 8.

In all the experiments the serum antibody titer was high (640-2560), in all the immunized animals.

Estimates of the salivary antibody titer was not attempted in experiment 1. In experiment 2 and 3 it was not possible to evaluate salivary agglutinin titers due to the variable tendency of all saliva to agglutinate glucose-grown strain 6715. However in experiment 4 the salivary agglutinin titers were successfully evaluated and showed a rise, ranging from 0 to 48.

The caries scores in table 8 represent the average number of carious areas for the total smooth surface (buccal, lingual and approximal) and for the total sulcal surfaces (minor occlusal fissures and deep occlusal fissures). In experiment 1, the average number of carious enamel areas was not significantly different in the sulci of the three groups; on the smooth surfaces the scores in the infected groups were higher than in the unin-
Table 8. Summary of infectious challenges, animal weights, antibody titers, bacterial recoveries, and caries scores.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Infectious challenge (microorganism) (days)</th>
<th>Rat weight (g) (mean±SEM)</th>
<th>Terminal serum titer median (range)</th>
<th>Salivary titer median (range)</th>
<th>Recovery of Strep. mutans</th>
<th>Caries scores (mean±SEM)</th>
<th>Total smooth surface</th>
<th>Total smooth total nodule</th>
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<tbody>
<tr>
<td>Experiment 1</td>
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<td></td>
<td></td>
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<tr>
<td>A (n = 9) 6715-9</td>
<td>290 ± 10-6 1280(1280)</td>
<td>ND (mod-high)</td>
<td>42.8 ± 7.8 44.5 ± 2.6</td>
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<td></td>
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<tr>
<td>B (n = 6) 6715-9</td>
<td>202 ± 6-9 400(80-160)</td>
<td>ND (mod-high)</td>
<td>43.5 ± 0.26 46.5 ± 2.8</td>
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<td>C (n = 6) None</td>
<td>292 ± 8-9 100(0-10)</td>
<td>ND</td>
<td>67 ± 1.9 410 ± 2.3</td>
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<td>Experiment 2</td>
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<tr>
<td>A (n = 12) 6715-11</td>
<td>271 ± 7.5 1280(640-2560)</td>
<td>ND (low-high)</td>
<td>20.5 ± 4.1 9.4 ± 1.7</td>
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<tr>
<td>B (n = 11) 6715-11</td>
<td>278 ± 9.0 800(160)</td>
<td>ND (low-high)</td>
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<tr>
<td>C (n = 11) None</td>
<td>208 ± 7.2 800(160)</td>
<td>ND</td>
<td>6.0 ± 9.0 2.6 ± 0.8</td>
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<td>Experiment 3</td>
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<tr>
<td>A (n = 12) 6715-13</td>
<td>214 ± 4.9 1280(1280-2560)</td>
<td>ND (mod-high)</td>
<td>41.5 ± 8.1 24.9 ± 5.3</td>
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<tr>
<td>B (n = 11) 6715-13</td>
<td>254 ± 9.0 40(20-40)</td>
<td>ND (mod-high)</td>
<td>55.1 ± 3.8 31.3 ± 1.9</td>
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<tr>
<td>C (n = 9) None</td>
<td>227 ± 5.7 400(20-160)</td>
<td>ND</td>
<td>40 ± 1.1 21.9 ± 2.3</td>
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<tr>
<td>Experiment 4</td>
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<td></td>
</tr>
<tr>
<td>A (n = 11) 6715-15</td>
<td>213 ± 5.3 1280(1280-2560)</td>
<td>ND (mod-high)</td>
<td>37.9 ± 6.1 22.2 ± 2.4</td>
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<tr>
<td>B (n = 11) 6715-15</td>
<td>211 ± 5.0 20(0-40)</td>
<td>ND (mod-high)</td>
<td>51.1 ± 5.6 29.9 ± 2.6</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (n = 12) None</td>
<td>214 ± 3.2 40(20)</td>
<td>ND</td>
<td>1.9 ± 0.5 9.0 ± 1.4</td>
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<td></td>
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<tr>
<td>Experiment 5</td>
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<td></td>
</tr>
<tr>
<td>A1 (n = 9) 6715-17</td>
<td>209 ± 6.0 1280(640-1280)</td>
<td>ND</td>
<td>5.6 ± 1.213.5 ± 2.7</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B (n = 10) 6715-17</td>
<td>202 ± 4.1 1280(540-5120)</td>
<td>ND</td>
<td>5.4 ± 0.8 11.4 ± 3.0</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>C (n = 9) None</td>
<td>192 ± 1.7 40(0-160)</td>
<td>ND</td>
<td>17.3 ± 4.4 28.0 ± 2.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values significantly different (p < .05) from value of same parameter for group B.
† Monitored by indirect fluorescence technique.

fected but not different from one another.

In experiment 2, all the animals had unusually low caries scores when compared with those of other experiments in this series. It should be noted that the recoveries of strain 6715 ranged from low to high in this experiment.

Experiments 3 and 4 gave caries scores similar to each other. There was a slight but statistically significant reduction of smooth surface caries scores, 25 and 34 per cent respectively. There was also some reduction in sulcal caries in experiment 3.

Experiment 5 was carried out with two immunized and infected groups: group $A_1$ animals were immunized with glucose-grown and group $A_2$ animals with sucrose-grown 6715-13. This was done to further study the lack of protection with antigens from glucose-grown organisms in experiment 1 in contrast with the apparent protection with sucrose-grown organisms in experiments 3 and 4. The infected-immunized animals (groups $A_1$ and $A_2$) had 68 and 86 per cent reduction of caries scores on total smooth surface and 52 and 59 per cent reduction of caries scores on total sulcal surfaces, respectively, when compared to the infected-non-immunized animals (group B).
6 RECENT ATTEMPTS AT IMMUNIZATION AGAINST CARIES

So we have seen that by the 1970's a great deal of interest had been generated once again to develop a vaccine against caries. As Sims wrote (Sims 1972), "As before when Lactobacilli were thought to be the organisms responsible for caries, the idea of preventing or controlling the disease by immune responses is again in fashion".

6.1 ATTEMPT IN 1974

In 1974 Taubman and Smith (Taubman & Smith 1974) carried out a series of experiments using conventional and gnotobiotic rats in an effort to stimulate, by immunization the secretion of IgA antibodies directed to Strep. mutans into saliva. Strep. mutans of strain 6715, killed in formalin were used in the experiments. Five immunization experiments (P₁ to P₅) were performed in presumptively pathogen-free (did not harbour Strep. mutans indigenously) rats and two (G₁ and G₂) in germ-free rats. In the P experiments 30 to 40 pathogen free rats of both sexes were divided into four groups:–

(I) Non-immunized and non-infected.
(II) Non-immunized and infected.
(III) Sham immunized and infected.
(IV) Immunized with 0.1 ml. of vaccine (10⁹ Strep. mutans 6715) incorporated with 0.1 ml. of complete Freund's adjuvant and infected.

The animals in group III and IV were injected subcutaneously in the vicinity of each parotid and submandibular gland 4 to 5 times at approximately 10 days interval prior to infection. Rats in experiment P₄ were injected without adjuvant 20 times over a period of 7 weeks prior to infection. Subsequent injections were given at 20-day intervals after infection. All animals (except P₅) were maintained on a cariogenic diet after weaning.
(19 to 21 days) until the experiments were terminated. In experiment P₅, the animals were maintained on low carbohydrate diet from weaning and given a cariogenic diet one week prior to infection until the termination of the experiment. Experiments in the groups of germ-free rats (G₁ and G₂) corresponded to groups III and IV of the pathogen-free rats. Whole saliva and blood was collected at intervals and Strep. mutans 6715 agglutination activity was determined. Salivary and serum agglutination titers were also recorded. The rats in groups II, III and IV were orally infected with cultures of Strep. mutans 6715 (approximately 10⁸ colony forming units) 10 to 22 days after completion of the initial immunization. The time was chosen when salivary antibody could be demonstrated. At the termination of the experiments (61 to 120 days after infection) saliva was collected and the caries scores taken.

In the pathogen-free rats, it was seen that after three immunizations, the salivary agglutinin levels of the immunized group IV rats remained consistently higher throughout the experimental period and, in general, increased with continued immunization. Also the serum agglutinating activity in group IV rats was considerably elevated above that of the control groups. The antibodies in saliva directed to Strep. mutans 6715 were primarily of the IgA class. Similar results were seen in the germ-free rats.

The caries scores were taken and are shown in table 9. The immune group IV always had lower mean caries scores than either of the other infected groups. These difference were statistically significant in two of the seven experiments and were of borderline significance in two others (P₅, G₂, P<0.10).
Table 9. Results of caries scores (group means and standard errors) in the seven experiments carried out on rats, at the end of the experimental period.

<table>
<thead>
<tr>
<th>Expt</th>
<th>Group</th>
<th>I Noninfected, nonimmunized</th>
<th>II Infected, nonimmunized</th>
<th>III Infected, sham-immunized</th>
<th>IV Infected, immunized</th>
</tr>
</thead>
<tbody>
<tr>
<td>P 1</td>
<td></td>
<td>30.5 ± 9.7</td>
<td>48.9 ± 7.2</td>
<td>49.2 ± 6.8</td>
<td>29.7 ± 7.6*</td>
</tr>
<tr>
<td>P 2</td>
<td></td>
<td>9.6 ± 1.5</td>
<td>28.8 ± 5.6</td>
<td>22.0 ± 3.9</td>
<td>18.6 ± 3.3</td>
</tr>
<tr>
<td>P 3</td>
<td></td>
<td>31.2 ± 12.0</td>
<td>48.6 ± 6.7</td>
<td>44.2 ± 10.6</td>
<td>36.6 ± 7.5</td>
</tr>
<tr>
<td>P 4</td>
<td></td>
<td>24.9 ± 7.7</td>
<td>32.8 ± 3.1</td>
<td>33.1 ± 16.3</td>
<td>21.3 ± 9.0</td>
</tr>
<tr>
<td>P 5</td>
<td></td>
<td>14.9 ± 2.6*</td>
<td>32.5 ± 6.9</td>
<td>41.9 ± 7.4</td>
<td>22.3 ± 5.6</td>
</tr>
<tr>
<td>G 1</td>
<td></td>
<td></td>
<td>19.8 ± 2.9</td>
<td>8.3 ± 1.1*</td>
<td></td>
</tr>
<tr>
<td>G 2</td>
<td></td>
<td></td>
<td>50.9 ± 8.6</td>
<td>47.8 ± 12.6</td>
<td></td>
</tr>
</tbody>
</table>

*Group means and standard errors; each group represents the scores of at least five animals.

Statistically significant. $P < 0.05$.

Statistically significant. $P < 0.005$.

(Source: Taubman, M.A. & Smith, D.J. 1974)
6.2 ATTEMPTS IN 1975

In 1975 Lehner, Challacombe and Jill (Lehner, Challacombe & Jill 1975) attempted immunizing monkeys. 23 rhesus monkeys were used; 13 females and 10 males. All the animals had fully erupted deciduous dentition but no permanent teeth. They were maintained on a human type of carbohydrate-rich diet.

A streptomycin-resistant Strep. mutans (Ingbritt) was used for the vaccine. The organisms were washed, heat-killed and resuspended in saline, corresponding to $10^9$ organisms per ml. For subcutaneous immunization, 1 ml. was mixed with an equal volume of Freund's incomplete adjuvant and for intraoral, submucous immunization about $2 \times 10^9$ organisms per ml. were added to 1 ml. of the adjuvant.

Six monkeys were immunized subcutaneously with 0.5 ml. of the vaccine into the contralateral limbs and six by the oral submucous route with 0.125 ml. of $2 \times 10^9$ organisms per ml. The remaining 11 control animals were divided into three groups; four had subcutaneous injections of the adjuvant only, four had submucous intraoral injections of Strep. CHT (a non-cariogenic organism) vaccine prepared in a similar way and three animals were given subcutaneous injections of saline alone.

The first injection of Strep. mutans/adjuvant vaccine was followed by four injections of Strep. mutans at 4, 8, 12 and 16 weeks and a booster injection of the organisms in adjuvant was administered at week 36.

Attempts were made to implant streptomycin resistant Strep. mutans (Ingbritt) in all the animals over a period of 28 weeks. Plaque samples were collected at intervals of 2 weeks up to 28 weeks and then monthly. These were cultured with and without streptomycin, and the number of colony forming units were determined. Blood and saliva was taken at intervals and examined for antibody titers. Clinical and x-ray examination for dental caries was also done.
The cultures of plaque failed to reveal any gross difference in the number of streptomycin-resistant Strep. mutans colony forming units. The attempt to implant streptomycin-resistant Strep. mutans was not successful.

In serum the complement fixing antibodies to HAGS (hydroxyapatite fraction of the culture supernatant of Strep. mutans) were found in log₂ titers of 0-3 before immunization. In the monkeys immunized by the subcutaneous and submucous routes, two animals from each group showed a brisk antibody response (table 10; figure 5 & 6); a titer of greater than log₂ 4 was reached within 4 weeks of an injection of Strep. mutans in Freund's incomplete adjuvant. In the remaining four monkeys in each group a slow antibody response was elicited; a titer greater than log₂ 4 was reached only after four to five booster injections of Strep. mutans, 20-48 weeks after starting the experiment. The antibody titers in the three control groups remained around log₂ 2.

Salivary antibodies to HAGS were found before immunization in low titers and an increase from log₂ 1 to 4 was found in the brisk responders of the subcutaneously immunized group within 8 weeks and fell to log₂ 2 by 36 weeks (table 10; figure 5). In contrast the slow responders failed to yield a mean titer greater than log₂ 2. The mean salivary titer in the immunized animals by submucous route increased to a titer, greater than log₂ 2 by 8-16 weeks (figure 6), but a differential antibody level was not found between brisk and slow responders. The antibody titer in the three control groups remained less than log₂ 2.

The two routes of immunization with Strep. mutans showed no significant difference in the caries score either between the brisk responders or between the slow responders. The onset of smooth surface caries was 17 weeks in the saline group (table 11) but 70 weeks in the brisk responders (P < 0.05). The difference was very significant if the brisk responders were compared with slow responders (P < 0.001) and to a lesser extent
Table 10. Results of serum and salivary antibody titers and caries scores of the four monkeys representing the brisk responders.

<table>
<thead>
<tr>
<th>Monkey no.</th>
<th>Index</th>
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</table>

1 and 2 had subcutaneous and 3 and 4 had submucous injections of Strep. mutans/adjuvant; antibody titres expressed as log₂ reciprocal.

(Source: Lehner, T., Challacombe, S.J. & Caldwell, J. 1975)
Figure 5. Sequential caries scores •——•, serum complement fixing O——O and salivary haemagglutinating A———A titers to HACS. The injections of Strep. mutans were administered subcutaneously.

Figure 6. Sequential caries score •——•, serum complement fixing O——O and salivary haemagglutinating A———A titers to HACS. The injections of Strep. mutans were administered submucosally.

(Source: Lehner, T., Challacombe, S.J. & Caldwell, J. 1975)
when compared with the Strep. CHT (P < 0.05) group of animals.

Significant difference in the smooth surface caries score were found between the combined brisk and slow responders from 9 to 24 months (P < 0.001). Differences were also observed between the brisk responders immunized submucously with Strep. mutans and the Strep. CHT group immunized submucously (P < 0.01). The combined brisk responders showed much less caries than the saline group and was statistically significant at 36 weeks (P < 0.05). After 2 years, the mean number of carious cavities per animal was 12 in the saline group and only 2.7 in the brisk responders.

Fissure caries developed later than smooth surface caries from about 36 weeks and the mean onset in the Strep. mutans immunized animals (71 weeks) was delayed as compared with the saline (56 weeks) injected animals by 11-19 weeks (P < 0.05; table 11). Fissure caries score was decreased in both of the Strep. mutans immunized groups, as compared with the three control groups of animals and at the 5 per cent level.

A correlation was found between serum complement fixing antibodies to HACS and the smooth surface caries index. Animals showing a brisk antibody response with a titer \( \log_2 4 \) had either no caries or developed substantially less caries at a late stage.

This was followed by an attempt by Emmings, Evans and Genco (Emmings, Evans & Genco 1975) to immunize monkeys (Macaca fascicularis). Eight female monkeys between 3 to 6 years of age, maintained on a high-sucrose, low-fiber diet were used for the study. Sucrose was also added to their drinking water at a concentration of 3% (wt./vol.). All monkeys had 28 teeth when the experiments were begun, though in a few deciduous molars were still present.

Formalin killed Strep. mutans 6715 suspended in 0.2% formalin in phosphate-buffer saline at a concentration of \( 10^9 \) organisms per ml. were used both for vaccination and for the immunofluo-
Table 11. Details of onset of caries in weeks.

<table>
<thead>
<tr>
<th>Type of caries</th>
<th>Weeks</th>
<th></th>
<th></th>
<th></th>
<th></th>
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<tr>
<td></td>
<td></td>
<td>Whole group</td>
<td>Responders</td>
<td>Slow</td>
<td>Whole group</td>
<td>Responders</td>
</tr>
<tr>
<td>Smooth surface</td>
<td>Mean (± S.E.)</td>
<td>37 (± 13.5)</td>
<td>70 (± 34)</td>
<td>21 (± 1)</td>
<td>40 (± 15.9)</td>
<td>88 (± 16)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>12-104</td>
<td>36-104</td>
<td>12-24</td>
<td>4-104</td>
<td>84-104</td>
</tr>
<tr>
<td>Fissure</td>
<td>Mean (± S.E.)</td>
<td>67 (± 11.9)</td>
<td>75 (± 14.2)</td>
<td>24-104</td>
<td>58 (± 11.8)</td>
<td>60 (± 22)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>40-104</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* SC = subcutaneous.
† SM = submucous.

(Source: Lehner, T., Challacombe, S.J. & Caldwell, J. 1975)
resistant antibody assay. Crude glucosyltransferase preparation (CFS) were derived from culture supernatant of Strep. mutans strains 6715, GS-5, and BHT.

The eight monkeys were divided into two comparable groups according to dental age. The schedule for immunization is shown in figure 7. Four monkeys (group I) were injected in the vicinity of the four major salivary glands 10 times during a 35-day period. The vaccine was prepared by combining the formalinized Strep. mutans 6715 with CFS in a buffer. Group II was similarly injected with the diluent buffer alone. Intraductal immunization was subsequently carried out on the parotid glands of the animals in group I using the same vaccine.

All monkeys were infected with Strep. mutans 6715 on days 241, 248 and 278, and with Strep. mutans GS-5 on days 432 and 446.

Saliva and blood was collected at regular intervals and serum was recovered from blood after allowing it to clot overnight. Two assays for antibody were used: (i) indirect immunofluorescent staining (IFS) and (ii) inhibition of glucosyltransferase activity.

After seven subcutaneous injections of the vaccine in the vicinity of the salivary glands during 15 days, the mean serum immunoglobulin M (IgM) antibody titer was elevated, and IgG and IgA antibody was also detected (figure 7). No antibody could be detected in 20x-concentrated parotid fluid (PF) from any of the monkeys. However PF collected 7 days after intraductal (PD) immunization showed IgA antibody to Strep. mutans 6715 in the PF's of three of the four animals in group I. No antibody was detectable in concentrated fluids from group II animals. Further PD immunization resulted in increase in the mean PF IgA antibody titer to peak at 13.5 (figure 7) and IgM antibody was infrequently found at low levels. Peaks of mean IgA and IgG antibody titers were found in the serum on day 200. Serum IgG antibody reached titers above 800, the highest dilution tested.
Figure 7. Titers of antibody to Strep. mutans 6715 as measured by indirect IFS. Titers are expressed as the reciprocal of the dilution. Each point represents the mean of samples taken from four animals. Vertical bars indicate subcutaneous (SC) immunizations. Arrows indicate immunization via the PD. The double vertical lines signify the beginning of PD immunization. The letter at each point denotes the immunoglobulin class of the antibody. All points on abscissa (<1) indicate no detectable antibody.

(Source: Emmings, F.C., Evans, R.T. & Genco, R.J. 1975)
After the first six immunization via the PD route the monkeys were rested for 5 weeks. Antibody levels in both fluids fell rather sharply during this interval. A single immunization on day 267 resulted in transient elevation of antibody titers in both PF and serum. During the next 9 weeks mean titers declined until antibodies were undetectable in the PF. Four subsequent PD immunization beginning on day 372 and evenly spaced over the next 7 weeks resulted in PF and serum responses with kinetics similar to those seen after the first course of PD immunization. Mean PF IgA antibody titer rose during immunization and afterwards fell sharply to undetectable levels after 9 weeks. Serum IgG, IgM and IgA antibody titers all increased during immunization and fell when immunization was curtailed.

One monkey, previously unexposed to the vaccine by any route, was immunized by instillation of the vaccine into the right PD while the left duct was instilled with diluent buffer. The response pattern (figure 8) was similar to that seen in the above groups of animals. IgA antibody was detected only in the secretions from the immunized gland, except in one sample.

Significant concentrations of antibodies inhibiting glucosyltransferase activity were not found in the serum until 4 months after initial immunization. Over the next 4 months they remained at essentially the same level during repeated immunization via the PD route. Significant inhibition was not detectable in the PF at any time.

A similar study was carried out by Evans, Emmings and Genco (Evans, Emmings & Genco 1975). Monkeys were immunized with formalin killed Strep. mutans 6715 cells and their products by injection in the vicinity of the major salivary glands and by instillation into the parotid glands via the ducts. Both the immunized and the control groups were infected orally with Strep. mutans 6715. Results showed that the immunized monkeys had fewer infected surfaces and fewer organisms on the infected surfaces than the control animals.
Figure 8. Titers of antibody to Strep. mutans 6715 as measured by indirect IF. Data were obtained from a single monkey instilled with Strep. mutans vaccine in the right PD and with a buffer solution in the left. Titers are expressed as the reciprocal of the dilution. Arrows indicate immunization via the PD. The letter at each point denotes the immunoglobulin class of the antibody.

(Source: Emmings, F. G., Evans, R. T. & Genco, R. J. 1975)
Another series of experiments were reported by Bowen, Cohen and Colman (Bowen, Cohen & Colman 1975). They attempted immunization of monkeys (Macaca fascicularis), with preparations of whole cells, broken cells and glucosyltransferase (GTF). The immunogens were derived from Strep. mutans, Bratthall serotype c, strain Ingbrit. Carious lesions were detected with the aid of a mirror and probe and bitewing radiographs were taken at every second clinical examination. The carious lesions are shown in graphs (figures 9 to 15), and the points plotted represents the number of lesions recorded at a particular examination. The number of carious lesions recorded in the deciduous teeth thus fell as these teeth were shed.

The first two groups of animals were immunized with suspensions of washed cells of Strep. mutans, serotype c, intravenously. In the first experiment (group A) there were three monkeys immunized and three acted as the controls. In the second experiment (group B) two were immunized and two acted as the controls. The animals in group A were immunized 8 times over a 3 week period and group B received 7 injections during 3 weeks. The animals were changed to a cariogenic diet and strain Ingbrit was implanted into their mouths before immunization was commenced. Group A was re-immunized on 4 occasions after an interval of just over 2½ years and a further single injection was given almost 3 years later. The animals in group B were re-immunized on 4 occasions after the original injections.

The caries scores of the animals in groups A and B are shown in figures 9 and 10 respectively. Experiment B was concluded after 48 months. The total caries scores at this time in the immunized animals was 20 and 43 for the controls, both in group A and B. The difference was not significant. Experiment B was continued and the caries scores of the immunized animals fell further below the scores of the controls. The difference was however largely due to one control animal developing rampant caries and one immunized animal remaining almost caries-free.
Figure 9. Carious lesions in Group A. Immunization with whole cells intravenously. 3 controls, 3 experimental.

Figure 10. Carious lesions in Group B. Immunization with whole cells intravenously. 2 controls, 2 experimental.

Figure 11. Carious lesions in Group C. Immunization with broken cells submucosally. 5 controls, 4 experimental.

(Source: Bowen, W.H., Cohen, B. & Colman, G. 1975)
In the third experiment (group C) broken cells were used. Four monkeys were immunized with cells of strain Ingbritt that had been broken in a Mickle tissue disintegrator with small glass beads. After centrifugation the material deposited was used as the immunogen. Five monkeys acted as controls. At the start of the experiment, the experimental animals were aged 11, 11, 14 and 21 months and the controls were 10, 11, 13, 16 and 24 months old. The broken cells were injected submucosally. A second injection was given 14 days later and the third, fourth and fifth given after 2, 4 and 5 months. At this point the animals were changed to a cariogenic diet and the strain Ingbritt was introduced into their mouths. They were again given injections at the 14, 26 and 36 months.

The caries scores are shown in figure 11. The immunized animals remained caries-free except for one animal which developed caries-like lesions at the edges of the 4 upper incisors; these were in consequence of trauma resulting from habitual biting of the metal struts of the cage. The control animals had developed a total of 64 lesions and these were distributed among the five animals as follows: 7, 30, 14, 11 and 2 cavities. The results were obviously very significant. However Strep. mutans counts were carried out and there was no significant difference between the immunized and the control groups. The sera had high agglutinin titers and inhibited GTP activity.

In the next set of four experiments (groups D, E, F and G) GTP was used to immunize the animals. Group D, was composed of 3 experimental and 4 control animals. The experimental animals were injected submucosally on 4 occasions with a partially purified enzyme preparation before challenge, and they received a total of 3 more injections of the same material in the fourth and fifth years of the experiment. In group E there were 2 in the experimental batch and 2 acted as the controls. All the animals in groups D and E were about 12 months old when the experiments were began. The immunogen was an invertase-free GTP preparation that was injected intra-mucosally. Four injections
were given before challenge and a total of 3 more were given in the third and fourth years of the experiment. In groups F and G there were 3 experimental and 3 control animals. The animals were about 4 months old. The immunogen used had some invertase activity. The animals in group F were immunized submucosally 13 times during the first year of the experiment and 5 times subsequently. The animals in group G were immunized 9 times in the first year and on 4 occasions subsequently.

The caries scores of the animals are shown graphically in figures 12, 13, 14 and 15. In brief, immunization with OTP did not protect these animals against dental decay. The number of Streptococci in plaque were determined and there were significantly more Strep. mutans in samples from the immunized animals. However, the sera of immunized animals contained antibodies to immunogens in OTP, and exhibited variable degrees of inhibition of enzyme activity.
Figure 12. Carious lesions in Group D. Immunization with partially purified GTP submucosally. 4 controls, 3 experimental.

Figure 13. Carious lesions in Group E. Immunization with invertase-free GTP. 2 controls, 2 experimental.

(Source: Bowen, W.H., Cohen, B. & Colman, G. 1975)
Figure 14. Carious lesions in Group F. Immunization with GTP containing invertase activity. 3 controls, 3 experimental.

Figure 15. Carious lesions in Group G. Immunization with GTP. 3 controls, 3 experimental.

(Source: Bowen, W.H., Cohen, E. & Colman, G. 1975)
6.3 ATTEMPTS IN 1976

This year Lehner, Challacombe and Jill (Lehner, Challacombe & Jill Chadwell 1976) reported another attempt to immunize rhesus monkeys. A total of 37 young monkeys with fully erupted deciduous dentition but without any permanent teeth were used for the study. The monkeys ranged from 11 to 21 months in age. They were supplied a human type of carbohydrate-rich diet that contained about 15% sucrose. The monkeys were distributed into seven groups (table 12). The immunization schedule is given in table 12; subcutaneous or submucous injections were adminis-tered at week 0,4,8,12,16 and 36 to monkeys in groups 1,2,3,4,5, 6 (four monkeys) and 7 (three monkeys), and subcutaneous injections were given at week 0,4,8,16 and 56 to monkeys in groups 3,6 (four monkeys) and 7 (three monkeys).

The vaccines were prepared as follows:

1. A human Strep. mutans (HSM) Ingbritt strain vaccine was prepared, as described in their 1975 study.
2. Passaged human Strep. mutans (PHSM) was prepared by first implanting the HSM into the mouth of a rhesus monkey, then reisolating the organism from which the vaccine was prepared in the same way as for HSM.
3. The HACS vaccine was prepared from Strep. mutans (Ingbritt) and 5 mg/ml. containing 10 glucosyltransferase units, was injected into each monkey with adjuvant (table 12).
4. Streptococcus GHf is a noncariogenic organism and the vaccine was prepared in the same way as HSM.

Repeated attempts were made to implant Strep. mutans in the monkeys but failed to do so. However no attempt was made in group 3, four in group 6 and four in group 7. At monthly intervals blood and saliva samples were taken. At the same time clinical and x-ray examination done and caries score taken.

The development of caries is presented during a period of 21 to 33 months (figures 16 and 17). The development and incidence of
Figure 16. Mean sequential smooth surface caries indexes in the seven groups of monkeys.

(Source: Lehner, T., Challacombe, S.J. & Caldwell, J. 1976)
Table 12. Schedule of injections in the seven groups of monkeys.

<table>
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<tr>
<th>Group</th>
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<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of monkeys</td>
<td>4</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Material injected</td>
<td>HSM*</td>
<td>HSM†</td>
<td>PHSMS</td>
<td>HACS§</td>
<td>CHT</td>
<td>FIA</td>
<td></td>
</tr>
<tr>
<td>Route of injection</td>
<td>2SC/2SM**</td>
<td>4SC/4SM</td>
<td>SC</td>
<td>SC</td>
<td>SM</td>
<td>SC</td>
<td>SC</td>
</tr>
</tbody>
</table>

*HSM, human 5 strains; brisk responders.
†Slow responders.
‡PHSM, passage human 3 mutants.
§HACS, hydroxyapatite fraction of culture supernatant of 3 mutants.
||FIA, Freund's incomplete adjuvant; all except group 7 had FIA in the first injection and groups 1, 2, 4, 5, and four in group 6 had FIA also administered at week 56.

Figure 17. Mean sequential fissure caries indexes in the seven groups of monkeys.

(Source: Lehner, T., Challacombe, S.J. & Caldwell, J. 1976)
smooth surface caries (figure 16) fall broadly into two divisions in the nonprotected monkeys (groups 2, 4, 5, 6 and 7), caries started from week 4 to 24. Once caries was initiated a rapid increase followed, reaching a high index plateau of between 10 and 16 cavities per monkey, between 48 and 120 weeks. However group 6 showed a delay in onset of caries and a lower plateau. The protected groups 1 and 3 could be clearly differentiated in that the onset of caries was delayed to week 36 and then was followed by a slow increase in caries, reaching a low plateau of 3.0 cavities per monkey in group 1 and 2.3 in group 3 by week 34 to 120. Significant differences at the 5 to 1% levels were found between the protected and the nonprotected groups.

Caries in the fissures developed later than smooth surface caries. Though the onset of caries in the protected animals (groups 1 and 3) was delayed and they had lower caries score, the difference from the nonprotected groups was significant only at the 5% level.

The serum antibody titers were also raised in the protected animals. The serum antibody titers in the nonprotected animals were variable and in general lower than the protected groups. The salivary antibody titer showed a modest increase to a titer \( \log_2 2 \), within four to eight weeks only in groups 1 and 3.

This year Emmings, Evans and Genco (Emmings, Evans & Genco 1976) continued their studies to stimulate salivary IgA in rhesus monkeys to Strep. mutans 6715. The studies were essentially the same as their 1975 studies using similar animals, organisms and routes of vaccine administration.

They reported that subcutaneous immunization induced only a serum response, whereas intraductal infusion stimulated both an IgA antibody response in the parotid fluid and a serum response. They also observed that the establishment of Strep. mutans 6715 was noticeably inhibited in immune monkeys. The IgA antibodies cross reacted with a few other strains of Strep. mutans. However inhibition of colonization on tooth surface in immune monkeys showed specificity for the immunizing strain
suggesting that inhibition was antibody mediated.

Another attempt was reported in the same year by Bahn, Cummings, and Hayashi (Bahn, Cummings & Hayashi 1976). They studied the inhibition of glucan and levan synthesis and neuraminidase activity of oral Streptococci by monkey antiseraums.

Twenty monkeys were divided into four groups. The injections were given monthly into the mucobuccal fold in the region of the first molar in all four corners of the mouth. Serum was collected about one month later. The first five monkeys were given a glucosyltransferase preparation from Strep. mutans strain FA-1. The second five were given a fructosyltransferase preparation purified from Strep. mutans strain JC2. The third group of five were injected with a mixture of glycosidic hydrolases prepared from Strep. sanguis strain 20. The remaining five monkeys were sham-immunized with sterile saline.

The results showed an inhibition of glycosyltransferase activity, partially inhibiting the production of the glucans. There was also a reduction of fructosyltransferase activity and a reduction in the amount of levan produced. There was an inhibition of neuraminidase activity as shown by antiseraums of the glycosidic hydrolases-immunized monkeys.
6.4 ATTEMPTS IN 1977

Lehner, Jill and Challacombe (Lehner, Jill Caldwell & Challacombe 1977) carried out another study, to study the effects of immunization on dental caries in the first permanent molars in rhesus monkeys. A group of 19 rhesus monkeys maintained on a human type of diet containing 15 per cent sucrose were used in the study. All animals had a fully erupted deciduous dentition, but the permanent first molars were erupted only in 4 monkeys. The animals were divided into five groups: group 1 and 2 consisted of nine monkeys which were sham-immunized with saline, group 3 consisted of 4 monkeys injected with Freund's incomplete adjuvant (FIA), group 4 and 5 consisted of 6 monkeys immunized with a whole cell vaccine of Strep. mutans (table 13).

The vaccine was prepared from Strep. mutans (Ingbritt; serotype c) in the same way as described in their 1975 study. The injections were given subcutaneously into each of the contralateral limbs; 0.5 ml. into each limb and when FIA was used it was mixed with an equal volume of the vaccine.

Serum IgG and IgM were assayed by the indirect fluorescent antibody (FA) test. Whole saliva was collected after subcutaneous injections of 0.5 mg. per kg. of pilocarpine and the hemagglutinating antibodies were determined. Plaque samples were collected from the fissures of the upper left permanent molar and the cervical and approximal sites of the adjacent deciduous molars of 3 monkeys each from groups 2 and 5. The samples were cultured and the colony-forming units (CFU) of Strep. mutans determined. Clinical and x-ray examinations were carried out at monthly intervals and caries were scored.

There was little difference between caries in groups 4 and 5, which had been immunized 5 and 2 times respectively with whole cells in FIA, these were combined for all statistical analyses (table 14). The mean number of fissure caries at 36 weeks was statistically significant at the 5% (P<0.05) level as compared with the saline groups or the combined control groups 1,2 and 3.
Table 13. Schedule of injections in the 5 groups of monkeys.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of monkeys</th>
<th>Injection</th>
<th>Site of injection</th>
<th>No. of injections</th>
<th>Times of injection (weeks)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>Saline</td>
<td>SC</td>
<td>5</td>
<td>0, 4, 8, 16, 56</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>Saline</td>
<td>SC</td>
<td>2</td>
<td>0, 30</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>FIA*</td>
<td>SC</td>
<td>5</td>
<td>0, 4, 8, 16, 56</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>Whole cells/FIA*</td>
<td>SC</td>
<td>5</td>
<td>0, 4, 8, 16, 56</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>Whole cells/FIA*</td>
<td>SC</td>
<td>2</td>
<td>0, 30</td>
</tr>
</tbody>
</table>

* Freund's incomplete adjuvant given at week 0, followed by saline, or *Strep, mutans* whole cells at the given times, respectively.

(Source: Lehner, T., Caldwell, J. & Challacombe, S.J. 1977)
Table 14. Analysis of caries in the monkeys.

<table>
<thead>
<tr>
<th>Group</th>
<th>Injection</th>
<th>No. of monkeys</th>
<th>Fissure caries (Week 36)</th>
<th>Fissure caries (Week 72)</th>
<th>Smooth surface caries (Week 72)</th>
<th>Fissure/SS caries (Week 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (1 and 2)</td>
<td>Saline</td>
<td>9</td>
<td>20 (2.2 ± 0.57)</td>
<td>20 (2.2 ± 0.57)</td>
<td>15 (1.7 ± 0.62)</td>
<td>35 (3.9 ± 1.0)</td>
</tr>
<tr>
<td>Controls (3)</td>
<td>FIA*</td>
<td>4</td>
<td>16 (4 ± 0)</td>
<td>16 (4 ± 0)</td>
<td>1 (0.25 ± 0.25)</td>
<td>17 (4.25 ± 0.25)</td>
</tr>
<tr>
<td>Controls (1, 2 and 3)</td>
<td>Saline/FIA</td>
<td>13</td>
<td>36 (2.8 ± 0.45)</td>
<td>36 (2.8 ± 0.45)</td>
<td>16 (1.2 ± 0.47)</td>
<td>52 (4.0 ± 0.7)</td>
</tr>
<tr>
<td>Immunized (4 and 5)</td>
<td>Strep. mutans/ FIA</td>
<td>6</td>
<td>8 (1.3 ± 0.6)</td>
<td>8 (1.3 ± 0.6)</td>
<td>0</td>
<td>8 (1.3 ± 0.6)</td>
</tr>
</tbody>
</table>

* Freund's incomplete adjuvant

(Source: Lehner, T., Caldwell, J. & Challacombe, S.J. 1977)
At week 72 there was a significant difference between the immunized groups as compared with the group having FIA alone (P<0.01) but the difference with the saline control failed to reach the 5 per cent level of significance. No smooth surface caries was observed at 72 weeks in the immunized group and there was significantly more caries in the saline-injected group (P<0.05). When the fissure and smooth surface caries were combined, the mean caries score in the immunized group (1.3) was significantly less than in both the FIA injected group (4.25; P<0.01) and the combined control groups 1, 2 and 3 (4.0; P<0.05).

The serum and salivary antibody titers are given in table 15. In the case of serum antibodies, the difference between the immunized and both control groups was very significant (P<0.0001), for both IgG and IgM at all the sampling times. The salivary antibodies in both the control groups showed a titer between log₂ 1–2 from week 8 to 36 and 72; the corresponding titers in the immunized groups varied between log₂ 2–3. These differences were not statistically significant.

There were consistently more colony-forming units of Strep. mutans in the saline controls than immunized animals. These were however not statistically significant.

Another investigation was carried out in the same year by Bahn, Shklair and Hayashi (Bahn, Shklair & Hayashi 1977), in an attempt to immunize monkeys with enzymes from oral Streptococci. Two experiments were carried out. In each, a group of 20 rhesus monkeys of approximately one year of age were used. All monkeys had fully erupted deciduous dentitions. A modified cariogenic diet with reduced fluoride than the standard monkey laboratory diet was used. The diet also included 10 two-gram sugar cubes and two caramel candies per day.

In the first experiment the monkeys were divided into two groups. Each group had 5 males and 5 females. The animals
### Table 15. Antibodies to Strep. mutans in the control and immunized monkeys.

<table>
<thead>
<tr>
<th>Group</th>
<th>Injection</th>
<th>No.</th>
<th>8</th>
<th>0</th>
<th>0.55</th>
<th>0.57</th>
<th>0</th>
<th>0.33</th>
<th>0</th>
<th>1.43</th>
<th>1.57</th>
<th>1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (1 and 2)</td>
<td>Saline</td>
<td>9</td>
<td>0.1</td>
<td>(±0.1)</td>
<td>(±0.37)</td>
<td>(±0.3)</td>
<td>0</td>
<td>(±0.23)</td>
<td>0</td>
<td>(±0.37)</td>
<td>(±0.2)</td>
<td>(±0.3)</td>
</tr>
<tr>
<td>Controls (3)</td>
<td>FIA</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0</td>
<td>1.5</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Controls (1, 2 and 3)</td>
<td>Saline/FIA</td>
<td>13</td>
<td>0.1</td>
<td>(±0.1)</td>
<td>(±0.27)</td>
<td>(±0.17)</td>
<td>(±0.1)</td>
<td>(±0.2)</td>
<td>(±0.2)</td>
<td>(±0.24)</td>
<td>(±0.76)</td>
<td>(±0.31)</td>
</tr>
<tr>
<td>Immunized (4 and 5)</td>
<td>Strep. mutans/FIA</td>
<td>6</td>
<td>6.5</td>
<td>6.5</td>
<td>6.2</td>
<td>3.2</td>
<td>3.2</td>
<td>2.7</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>FIA</td>
<td></td>
<td>(±0.9)</td>
<td>(±0.9)</td>
<td>(±0.5)</td>
<td>(±0.8)</td>
<td>(±0.6)</td>
<td>(±0.6)</td>
<td>(±0.6)</td>
<td>(±0.37)</td>
<td>(±0.3)</td>
<td></td>
</tr>
</tbody>
</table>

(Source: Lehner, T., Caldwell, J. & Challacombe, S.J. 1977)
were weighed and blood samples taken. The teeth of the animals were radiographed and examined for carious lesions. Plaque samples were also taken. In the second experiment 20 monkeys were divided into four groups of five monkeys each.

The vaccine for glucosyltransferase was prepared from Strep. mutans strain FA-1. For fructosyltransferase the vaccine was obtained from Strep. mutans strain JC-2 and glycosidic hydrolases from cultures of Strep. sanguis strain 20.

In the first experiment a mixture of the glycosidic hydrolases preparation and Freund's incomplete adjuvant was used. The injections were given intraorally - 0.25 ml. into the buccal area adjacent to the parotid gland and a second injection of 0.25 ml. into areas of the submaxillary and sublingual glands in the mucobuccal fold. Injections were given on each side so that a total of four injections of 0.25 ml. each were given at one time. Monthly injections were given and due to trismus the adjuvant was withdrawn. Sham immunization with Freund's incomplete adjuvant alone was given to control animals initially, then saline injection was used in all control animals.

In the second experiment, 0.25 ml. each of glucosyltransferase, fructosyltransferase and glycosidic hydrolases were injected intraorally without Freund's adjuvant into each of the four quadrants. Each enzyme preparation was injected in a separate group of 5 rhesus monkeys. The last 5 monkeys in the second experiment acted as the controls and were given saline injections only. Each animal was injected monthly for the duration of the experiment. In the first experiment, there were 12 injections and in the second experiment 18 injections.

Strep. mutans were implanted into the monkeys mouths. Of the 40 animals, 30 retained the implanted flora throughout the experiment, the remaining 10 were reimplanted until the Strep-tococci remained.

Blood was taken from the femoral vein at monthly intervals. Radiographic and clinical examinations for caries were per-
formed monthly. A plaque sample was obtained and the amount of plaque in the animals noted. At the completion of each experiment, the animals were sacrificed, blood samples taken and the salivary glands were dissected and ground; an extract of the salivary glands was obtained for determination of salivary antibodies.

The caries scores were classified into white spots, brown spots and gross lesions. The results of caries scores are presented in tables 16, 17, 18 and 19. There was a reduction of 68.6 per cent in the total carious lesions in the animals immunized intraorally with glucosyltransferase, 62.4 per cent reduction in those injected with fructosyltransferase, and 57.4 per cent reduction in total lesions in those immunized with glycosidic hydrolases after 19 months, as compared to the control group. There were no gross lesions apparent in the group immunized with glycosidic hydrolases. There was also an inhibition in the glucosyltransferase, fructosyltransferase and neuraminidase activity in the immunized animals.
Table 16. Caries scores of monkeys immunized intraorally with Streptococcal glycosidic hydrolases.

<table>
<thead>
<tr>
<th>Number of Monkeys Injected with:</th>
<th>White Spots</th>
<th>Brown Spots</th>
<th>Gross Lesions</th>
<th>Total Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycosidic Hydrolases</td>
<td>mean 16.2</td>
<td>3.5</td>
<td>0.2</td>
<td>19.9</td>
</tr>
<tr>
<td>10 (Immunized)</td>
<td>s.d. 8.5</td>
<td>6.0</td>
<td>0.6</td>
<td>12.8</td>
</tr>
<tr>
<td>Sterile Saline</td>
<td>mean 16.2</td>
<td>8.5</td>
<td>1.1</td>
<td>25.8</td>
</tr>
<tr>
<td>10 (Control)</td>
<td>s.d. 7.08</td>
<td>13.00</td>
<td>2.45</td>
<td>17.4</td>
</tr>
</tbody>
</table>

*Caries scores following 11 monthly injections during a 12-month period. s.d. = standard deviation

Table 17. Caries scores after 19 months of monkey immunized intraorally with Streptococcal glycosidic hydrolases.

<table>
<thead>
<tr>
<th>Number of Monkeys Injected with:</th>
<th>White Spots</th>
<th>Brown Spots</th>
<th>Gross Lesions</th>
<th>Total Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycosidic Hydrolases</td>
<td>mean 19.8</td>
<td>0.8**</td>
<td>0 **</td>
<td>20.6**</td>
</tr>
<tr>
<td>5 (Immunized)</td>
<td>s.d. 4.9</td>
<td>0</td>
<td>0</td>
<td>5.3</td>
</tr>
<tr>
<td>Sterile Saline</td>
<td>mean 25.0</td>
<td>5.6</td>
<td>17.8</td>
<td>48.4</td>
</tr>
<tr>
<td>5 (Control)</td>
<td>s.d. 7.8</td>
<td>4.7</td>
<td>9.1</td>
<td>13.3</td>
</tr>
</tbody>
</table>

(Source: Bahn, A.N., Shklair, I.L. & Hayashi, J.A. 1977)
Table 18. Caries scores of monkeys immunized intraorally with Streptococcal glucosyltransferase.

<table>
<thead>
<tr>
<th>Number of Monkeys Injected with:</th>
<th>White Spots</th>
<th>Brown Spots</th>
<th>Gross Lesion</th>
<th>Total Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucosyltransferase mean</td>
<td>5.0**</td>
<td>3.0</td>
<td>7.2**</td>
<td>15.3**</td>
</tr>
<tr>
<td>5 (Immunized) s.d.</td>
<td>4.3</td>
<td>0.0</td>
<td>5.1</td>
<td>6.1</td>
</tr>
<tr>
<td>Sterile Saline mean</td>
<td>25.0</td>
<td>5.6</td>
<td>17.8</td>
<td>48.4</td>
</tr>
<tr>
<td>5 (Control) s.d.</td>
<td>7.8</td>
<td>4.7</td>
<td>9.1</td>
<td>13.3</td>
</tr>
</tbody>
</table>

*Caries scores following 18 monthly injections during 19 months.
**Significant at the 1% level.
s.d. = standard deviation.

Table 19. Caries scores of monkeys immunized intraorally with Streptococcal fructosyltransferase.

<table>
<thead>
<tr>
<th>Number of Monkeys Injected with:</th>
<th>White Spots</th>
<th>Brown Spots</th>
<th>Gross Lesion</th>
<th>Total Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructosyltransferase mean</td>
<td>7.2**</td>
<td>2.8</td>
<td>8.2**</td>
<td>18.2**</td>
</tr>
<tr>
<td>5 (Immunized) s.d.</td>
<td>0</td>
<td>0</td>
<td>-4.6</td>
<td>10.7</td>
</tr>
<tr>
<td>Sterile Saline mean</td>
<td>25.0</td>
<td>5.6</td>
<td>17.8</td>
<td>48.4</td>
</tr>
<tr>
<td>5 (Control) s.d.</td>
<td>7.8</td>
<td>4.7</td>
<td>9.1</td>
<td>13.3</td>
</tr>
</tbody>
</table>

*Caries scores following 18 monthly injections during 19 months.
**Significant at the 1% level.
s.d. = standard deviation.

(Source: Bahn, A.N., Shklair, I.L. & Hayashi, J.A. 1977)
6.5 ATTEMPTS IN 1978

Lehner et al reported an attempt this year to passively immunize rhesus monkeys against dental caries with serum and immunoglobulins (Lehner et al 1978). Altogether 38 monkeys were used. Of these, 20 were donors, 14 recipient and 4 were sham-immunized. 14 donor monkeys were immunized with six subcutaneous injections of heat-killed cells of Strep. mutans (serotype c). The first and last of these injections were with Freund's incomplete adjuvant. The 6 non-immunized donor monkeys had six subcutaneous (s.c.) injections of saline at the same time. The mean log₂ serum antibody titers of the immunized donor monkeys were IgG 8.9, IgM 5.3 and IgA 4.8. The corresponding titers in the sham-immunized donor monkeys were IgG 0.9, IgM 1.1 and IgA 1.1. Blood was withdrawn every 3 weeks from these animals to obtain the serum and immunoglobulins for passive immunization of the recipient monkeys.

All the recipient monkeys had a fully erupted deciduous dentition, no permanent teeth and were free of Strep. mutans. They were maintained on a human type of diet, containing 15% sucrose, beginning on the day of immunization. 3 monkeys were given intravenous infusions of 15 ml. non-immune plasma and 4 received 15 ml. of immune plasma, at intervals of 17-22 days. IgG, IgM and IgA were administered to 7 monkeys (table 20) in the amounts calculated to be present in 15 ml. of monkey plasma. The other 4 monkeys were not immunized.

Sequential analysis of class-specific antibodies to Strep. mutans (serotype c) was done. Whole saliva was collected every month and the haemagglutinating antibodies were determined. Clinical and radiographic examinations were done every three weeks and caries scores taken. Plaque samples were also taken and cultured. The results were expressed as colony-forming units (C.F.U.) of Strep. mutans.

IgG, IgM and IgA classes of antibodies were found in the
Table 20. Table showing the grouping of the 18 recipient Rhesus monkeys, the caries indices and Strep. mutans colony forming units (C.F.U.).

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of monkeys</th>
<th>Passive transfer</th>
<th>Amount administered every 3 wk</th>
<th>No. of cavities in individual monkeys</th>
<th>C.F.U. Mean (±s.e.)%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>Nil</td>
<td>...</td>
<td>6,7,10,17</td>
<td>39.4 (±5.4)</td>
</tr>
<tr>
<td>2</td>
<td>3, 4</td>
<td>Non-immune plasma Immune plasma</td>
<td>15 ml, 15 ml</td>
<td>4,5,10, 6,6,10,16</td>
<td>27.3 (±6.8), 39.9 (±5.3)</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>IgG</td>
<td>177 (±2-5) mg</td>
<td>1,2,3</td>
<td>45.3 (±6.1)</td>
</tr>
<tr>
<td></td>
<td>2, 2</td>
<td>IgM, IgA</td>
<td>11.7 (±1-1) mg, 28.4 (±1-6) mg</td>
<td>5, 7, 10</td>
<td>44.8 (±7.5), 33.7 (±6-6)</td>
</tr>
</tbody>
</table>
monkeys given immune plasma but there was only an occasional titer of \( \log_2 1 \) in those given non-immune plasma or in the controls. When separated Ig classes of antibodies were injected the resulting circulating antibodies were predominantly in the same class, the titers of the other two Ig classes being usually below \( \log_2 1 \).

At 39 weeks the average numbers of lesions per monkey in the control non-immune plasma and the group with immune plasma were much the same. Clearly, passive transfer of immune serum failed to protect the animals against caries (table 20). The least number of carious lesions was found in the group receiving separated IgG (mean of 2.0), and this increased to 5.5 with IgM and 8.5 with IgA. Significant protection was induced with IgG (\( X^2 = 15.33, \ D.F. \ 2, \ P < 0.001 \)), but not with IgM or IgA, when the total number of carious lesions was compared with that found in the groups receiving immune serum, non-immune serum or saline injections. IgA class of antibodies in whole saliva failed to show a significant difference between any of the groups of animals. There was also no significant difference in the colony-forming units of Strep. mutans in any groups of the animals.
6.6 ATTEMPTS IN 1979

This year an attempt was made to immunize hamsters by oral administration of glucosyltransferase antigen (Smith, Taubman & Ebersole 1979). The glucosyltransferase antigen was prepared from Strep. mutans strain 6715.

Three immunization experiments were performed: H1 in NIH white hamsters, and H2 and H3 in LHC/1ak cream hamsters. In each experiment hamsters were randomized into three groups:

(I) Nonimmunized and uninfected.
(II) Sham-immunized with buffer and infected with Strep. mutans 6715.
(III) Immunized with 0.2 ml. of glucosyltransferase in buffer and infected with Strep. mutans 6715.

Immunization was accomplished by daily oral administration of antigen. Oral immunization was initiated in H1 when hamsters were 23 to 24 days old, in H2 when hamsters were 21 to 22 days old, and in H3 when hamsters were 23 to 24 days old. Antigen was fed for 21, 27 and 26 consecutive days in experiments H1, H2 and H3 respectively.

The hamsters in groups II and III were orally infected for three consecutive days starting on day 50, 51 or 53, with Strep. mutans 6715. Prior to infection salivary inhibition or glucosyltransferase-binding activity could be demonstrated in all the glucosyltransferase-fed animals. At the termination of the experiment (41 to 56 days after infection) saliva and sera were collected. The dental surfaces were swabbed and cultured. The jaws were defleshed, and all caries and lesions scored.

In general, saliva of glucosyltransferase-fed hamsters from experiments H1, H2 and H3 had an inhibiting effect on glucosyltransferase activity. The buffer-fed group II showed little or no inhibition. A comparison of the colony-forming
units of Strep. mutans (table 21) recovered from the buffer-
fed and antigen-fed groups of the three experiments indicated
that significantly fewer Strep. mutans organisms colonized
the tooth surfaces of the antigen-fed animals. These differ-
ences were observed early (4 to 7 days) and late (41 and 58
days) in the course of infection and were statistically signi-
ficant on six of the nine swabbing occasions.

The caries and lesions scores are given in table 22. The
nonimmunized uninfected animals in group I had negligible
disease at the end of the experiment. Of the groups which
were infected, the orally immunized group III always had
lower mean caries scores than the respective sham-immunized
group II. These differences were statistically significant
(P<0.001) in two of the three experiments (H1 and H2). The
scores and lesions of the buffer-fed groups were compared
with those of the corresponding immune animals and the reduc-
tion in the immunized animals is expressed in percentage,
and given in table 23.

In this year Cohen, Colman and Russell (Cohen, Colman &
Russell 1979) reported an extension of the experiments carr-
ried out in their laboratory and reported in 1975 (Bowen,
Cohen & Colman 1975). Of the experiments reported in 1975,
none showed as great a degree of protection as group C.
Over a period of five years one of the immunized animals had
shown 4 caries-like lesions and the remaining 3 were caries-
free; the 5 control animals had, at that time, developed a
total of 64 lesions.

By 1979 the experiment had been in progress for nine years.
There were still 5 surviving animals, 2 of the immunized
group and 3 control animals. The 3 surviving control animals
had rampant caries, having 56, 69 and 93 decayed surfaces
respectively. Both immunized survivors continue to exhibit
a remarkable degree of protection. One small lesion had
developed in a lower premolar in one of them and the other
remained completely caries-free. No booster doses had been
Table 21. Bacterial recoveries from dental surfaces of hamsters during infection and at experiment termination.

<table>
<thead>
<tr>
<th>Expt</th>
<th>Group and treatment</th>
<th>Infection strain</th>
<th>No. of S. mutans cells*</th>
<th>Total colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st swabbing</td>
<td>2nd swabbing</td>
<td>Expt termination</td>
</tr>
<tr>
<td>H1</td>
<td>I. Nonimmunized</td>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6715</td>
<td>1,900</td>
<td>1,850 x 10^6</td>
</tr>
<tr>
<td></td>
<td>II. Sham-fed</td>
<td>6715</td>
<td>700</td>
<td>378 x 10^6</td>
</tr>
<tr>
<td></td>
<td>III. Glucosyltransferase-fed</td>
<td>6715</td>
<td>17 x 10^3</td>
<td>17 x 10^3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>170 x 10^5</td>
</tr>
<tr>
<td>H2</td>
<td>I. Nonimmunized</td>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6715</td>
<td>75,500</td>
<td>676 x 10^6</td>
</tr>
<tr>
<td></td>
<td>II. Sham-fed</td>
<td>6715</td>
<td>479 x 10^6</td>
<td>204 x 10^6</td>
</tr>
<tr>
<td></td>
<td>III. Glucosyltransferase-fed</td>
<td>6715</td>
<td>52 x 10^6</td>
<td>52 x 10^6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>154 x 10^6</td>
</tr>
<tr>
<td>H3</td>
<td>I. Nonimmunized</td>
<td>None</td>
<td>ND*</td>
<td>ND*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6715</td>
<td>78,700</td>
<td>879 x 10^6</td>
</tr>
<tr>
<td></td>
<td>II. Sham-fed</td>
<td>6715</td>
<td>811 x 10^6</td>
<td>451 x 10^6</td>
</tr>
<tr>
<td></td>
<td>III. Glucosyltransferase-fed</td>
<td>6715</td>
<td>53 x 10^6</td>
<td>53 x 10^6</td>
</tr>
</tbody>
</table>

* The first and second swabblings were performed 7 and 23 days (H1), 4 and 22 days (H2), and 5 and 20 days (H3) postinfection. Experiments were terminated 58 days (H1), 41 days (H2), and 56 days (H3) postinfection.

Statistically significant, P < 0.05.

Statistically significant, P < 0.001.

Statistically significant, P < 0.005.

ND, Not determined.

(Source: Smith, D.J., Tauben, M.A. & Ebersole, J.L. 1979)
Table 22. Effect of oral immunization with glucosyltransferase (GTF) on pathogenesis of Strep. mutans 6715.

<table>
<thead>
<tr>
<th>Expt</th>
<th>Group and treatment</th>
<th>No. of hamsters</th>
<th>Mean caries score ± SE*</th>
<th>Mean lesions ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>I. Nonimmunized, noninfected</td>
<td>9</td>
<td>1.8 ± 2.6</td>
<td>6.1 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>II. Sham-fed, infected</td>
<td>14</td>
<td>16.1 ± 2.6*</td>
<td>34.5 ± 3.4*</td>
</tr>
<tr>
<td></td>
<td>III. GTF-fed, infected</td>
<td>14</td>
<td>4.6 ± 1.1*</td>
<td>15.5 ± 3.2*</td>
</tr>
<tr>
<td>H2</td>
<td>I. Nonimmunized, noninfected</td>
<td>6</td>
<td>1.2 ± 0.4</td>
<td>4.7 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>II. Sham-fed, infected</td>
<td>13</td>
<td>12.7 ± 2.0*</td>
<td>32.8 ± 2.9*</td>
</tr>
<tr>
<td></td>
<td>III. GTF-fed, infected</td>
<td>11</td>
<td>3.3 ± 0.5*</td>
<td>13.0 ± 1.6*</td>
</tr>
<tr>
<td>H3</td>
<td>I. Nonimmunized, noninfected</td>
<td>5</td>
<td>2.3 ± 0.6</td>
<td>8.2 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>II. Sham-fed, infected</td>
<td>11</td>
<td>15.0 ± 3.7</td>
<td>33.4 ± 4.1*</td>
</tr>
<tr>
<td></td>
<td>III. GTF-fed, infected</td>
<td>11</td>
<td>8.0 ± 2.8</td>
<td>13.9 ± 2.9*</td>
</tr>
</tbody>
</table>

*SE, Standard error.

*Statistically significant, P < 0.001.

*Statistical significance, P < 0.051. Excludes one animal in group III in which the caries score was greater than the two standard deviations from the mean.

Table 23. Reduction of caries scores or lesions on occlusal or smooth surfaces of glucosyltransferase-fed compared with buffer-fed (sham-immunized) hamster groups.

<table>
<thead>
<tr>
<th>Expt</th>
<th>Hamster strain</th>
<th>Percentage of reduction*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Caries</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Occlusal surfaces</td>
</tr>
<tr>
<td>H1</td>
<td>NIH White</td>
<td>71</td>
</tr>
<tr>
<td>H2</td>
<td>LHC/Lac Cream</td>
<td>64</td>
</tr>
<tr>
<td>H3</td>
<td>LHC/Lac Cream</td>
<td>41</td>
</tr>
</tbody>
</table>

*Percentage of reduction = 100 – (mean caries score or mean number of lesions of immune group/mean caries score or mean number of lesions of identically challenged sham group) × 100.

(Source: Smith, D.J., Taubman, M.A. & Ebersole, J.L. 1979)
given since 1974. The caries scores are given in table 24.

In the same report they reported three more experiments, experiments 11, 14 and 19. Experiment 11 was designed to compare (a) subcutaneous with submucosal administration; and (b) the effectiveness of a whole cell preparation with one in which both whole cells and their extracellular products were included. Three groups of eight monkeys each, were used in this experiment. The first group was given washed whole bacterial cells (Strep. mutans strain Ingbritt). The second group was immunized with a similar preparation to which had been added protein-rich extracellular material precipitated, by the addition of ammonium sulphate, from the broth in which the cells were grown. The third group acted as control. Aluminium hydroxide was used as an adjuvant in the experimental groups. Half of the animals in each group were immunized subcutaneously, and half submucosally in the mouth.

After nearly 5 years the difference in the degree of protection present in the 2 groups was negligible. Statistical analysis of the caries scores of the various experimental groups showed that there was no significant difference between the groups immunized with cells or whole culture vaccine. This was true for both the subcutaneous route of immunization (P > 0.1) and the submucosal route (P > 0.1). The results obtained with the 2 vaccine preparations were then pooled to compare the effects of the different routes of immunization with the control group. The mean caries scores are illustrated in figure 18. The subcutaneously immunized group showed a 55 percent reduction in caries at 52 months, which is highly significant (P < 0.02), while the protection of the submucosally immunized groups was not significant (P > 0.1).

Experiment 14 was carried out to ascertain whether it was essential to use an adjuvant with the immunogen tested. However it was found that an adjuvant was not essential. Then experiment 19 was carried out to investigate if the rate of growth of cells influenced the protective effect of cellular
Table 24. Caries in permanent dentition of surviving animals in group C.

<table>
<thead>
<tr>
<th></th>
<th>Controls (3 animals)</th>
<th>Immunized (2 animals)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1975</td>
<td>1979</td>
</tr>
<tr>
<td>Number of carious surfaces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of carious teeth</td>
<td>48</td>
<td>218</td>
</tr>
<tr>
<td>Number of carious teeth</td>
<td>28</td>
<td>46</td>
</tr>
</tbody>
</table>

Table 25. Caries experience in experiment 19 after 1 year.

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Number of carious surfaces</th>
<th>Number of caries-free animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole cells (D = 0·5h⁻¹)* (4 monkeys)</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Broken cells (D = 0·5h⁻¹) (4 monkeys)</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Whole cells (D = 0·05h⁻¹) (3 monkeys)</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Broken cells (D = 0·05h⁻¹) (3 monkeys)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Controls (5 monkeys)</td>
<td>35</td>
<td>0</td>
</tr>
</tbody>
</table>

* Dilution rate in chemostat.

Figure 18. Experiment 11: Progression of caries in controls and monkeys immunized with 'whole cell' and 'whole culture' vaccines by submucosal (sm) and subcutaneous (sc) routes. Results are expressed as mean number of lesions per animal at successive examinations. Arrows indicate times of immunization.

(Source: Cohen, B., Colman, G. & Russell, R.R.B. 1979)
immunogens. Cultivation of bacteria in a chemostat permits the harvesting of cells from a culture growing in a steady state and also facilitates control of many different factors affecting the properties of cells. Cells were grown in a chemostat at both a low dilution rate (low rate of growth - $D = 0.05h^{-1}$) and at a high dilution rate (high rate of growth - $D = 0.5h^{-1}$). Some of the cells from each batch were washed, killed with formaldehyde and used as whole cell vaccine. Cell walls were also prepared from both batches by breaking the cells with glass beads in a Mickle Tissue Disintegrator. The preparations were administered submucosally without adjuvant.

The details of experiment 19 are shown in table 25. It was seen after one year that there was no clear indication of differences in the protective capacity of immunogens derived from cells grown at the different rates of dilution.
6.7 ATTEMPTS IN 1980

This time Lehner with Russell and Caldwell (Lehner, Russell & Caldwell 1980), reported an attempt to immunize rhesus monkeys with a purified protein antigen. The protein was isolated from Strep. mutans, having a molecular weight of about 185,000 daltons and designated Streptococcal antigen I/II.

Ten young rhesus monkeys, all having fully erupted deciduous teeth but no permanent teeth were examined and maintained on a human type of diet containing 15% sucrose. They were divided randomly into 3 groups; 3 monkeys were given 1 mg. of antigen I/II in adjuvant subcutaneously, in equally divided doses, into an upper and lower limb. The adjuvant used in 2 monkeys was Freund's incomplete adjuvant (FIA), and in the third monkey 'Alhydrogel' was used. The next three monkeys were injected with 5x10^8 formalin-killed cells of Strep. mutans (serotype c) in FIA, and 4 control monkeys were sham-immunized with saline. The whole-cell immunized monkeys were also given the vaccine without adjuvant at week 30. The monkeys were examined every 1-2 months when blood, saliva and plaque were collected. Clinical and radiographic examinations were carried out to record the caries scores.

The caries scores are shown at 80 weeks in table 26. The sham-immunized group of monkeys showed a progressive increase in dental caries score from week 12 to 80 reaching a mean of 13.5. In the antigen I/II group caries appeared at week 20 and the score increased only to a mean of 4.0. This was significantly less than that in the control group (P<0.025). The whole-cell immunized group showed a similar reduction in caries when compared with controls; mean score of 3.7 (P<0.02). Both immunized groups had similar reductions in numbers of smooth-surface caries (72%) and fissure caries (75% and 68%), when compared with those in the control groups.

The mean IgG antibody titer varied between 1:320 and 1:640 in the antigen I/II immunized group and between 1:160 and 1:640 in
Table 26. Total, smooth-surface and fissure caries-score in 3 groups of monkeys at the end of the experiment.

<table>
<thead>
<tr>
<th>Type of immunisation</th>
<th>Mean (± SE) of caries-score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Shun</td>
<td>13.5 (± 1.8)</td>
</tr>
<tr>
<td>Cells of Strep. mutans</td>
<td>3.7 (± 1.8)</td>
</tr>
<tr>
<td>Antigen I/II</td>
<td>4.0 (± 2.8)</td>
</tr>
</tbody>
</table>


Table 27. Schedule of immunization.

<table>
<thead>
<tr>
<th>Section</th>
<th>Group</th>
<th>Route</th>
<th>No. of monkeys</th>
<th>Vaccines</th>
<th>Adjuvant</th>
<th>Number of immunizations</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>A</td>
<td>SC</td>
<td>6</td>
<td>$5 \times 10^8$ Strep. mutans</td>
<td>FIA</td>
<td>6 over 36 weeks</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>SM</td>
<td>6</td>
<td>$5 \times 10^8$ Strep. mutans</td>
<td>FIA</td>
<td>6 over 36 weeks</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>Controls</td>
<td>7</td>
<td>Saline</td>
<td>None</td>
<td>6 over 36 weeks</td>
</tr>
<tr>
<td>II</td>
<td>D</td>
<td>Oral</td>
<td>4</td>
<td>$10^{11}$ killed Strep. mutans in capsules</td>
<td>None</td>
<td>Daily for 13 days, and then for 11 days</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>Oral</td>
<td>4</td>
<td>$0.5-2.5 \times 10^9$ live Strep. mutans in drinking water</td>
<td>None</td>
<td>Daily for 18 weeks</td>
</tr>
<tr>
<td>III</td>
<td>F</td>
<td>Controls</td>
<td>3</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>SC</td>
<td>3</td>
<td>$10^9$ killed Strep. mutans</td>
<td>FIA</td>
<td>2 at 0 and 30 weeks</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>SC</td>
<td>5</td>
<td>Pronased cell walls</td>
<td>FIA</td>
<td>3 over 30 weeks</td>
</tr>
<tr>
<td></td>
<td>J</td>
<td>Controls</td>
<td>5</td>
<td>Saline</td>
<td>None</td>
<td>3 over 30 weeks</td>
</tr>
</tbody>
</table>

SC: subcutaneous; SM: submucosal; FIA: Freund’s incomplete adjuvant.

(Source: Challacombe, S.J. & Lehner, T. 1980)
the whole-cell immunized group. IgA antibodies were detected in both immunized groups, but the titer was higher in the whole-cell (1:40 to 1:320) than the antigen I/II (1:10 to 1:40) immunized group. Only the whole-cell immunized monkeys showed IgM class of antibodies (1:10 to 1:40). No antibodies were detectable in the control monkeys. Salivary antibodies failed to show a significant increase.

Another investigation was carried out by Challacombe and Lehner (Challacombe & Lehner 1980), to determine the salivary antibody responses in rhesus monkeys immunized with Strep. mutans by the oral, submucosal and subcutaneous routes. Forty-three young monkeys maintained on a human type of cariogenic diet were used in the experiments. All monkeys had fully erupted deciduous dentition and in some the first permanent molars were erupting or erupted. The experiments were divided into three sections and the sections, groups, routes, number of monkeys in each experiment, the vaccines and adjuvants used and the number of immunization in each experiment are given in table 27.

Section I was for comparison of subcutaneous (SC) and submucosal (SM) immunization on the induction of salivary antibodies. Section II was for comparison of two types of oral immunization on the induction of salivary antibodies and section III was for comparison of different antigen preparations given subcutaneously on the induction of salivary antibodies.

Samples of blood and stimulated mixed saliva were taken at 3-5 weekly intervals, except for group D where samples were taken at 3-4 day intervals. In addition, stimulated parotid saliva was collected in group D, E and F.

In section I experiments, the haemagglutinating antibodies to c polysaccharide of up to log₂ 2 were found in pre-immune samples of saliva. After immunization, the antibody titers of up to log₂ 5 were detected. Titers were highest in the SM group B and at weeks 24 and 48 they were significantly greater than in the SC immunized group (P<0.05) and the controls (P<0.02).
The titers in group A (SG) were not significantly greater than the control group. Agglutination titers of between \(\log_2 1\) and 4 were present in the preimmune samples of saliva. In both immunized groups, a rise in the mean titer was found at week 24, but this was significantly greater than the controls only in group B (SM; \(P<0.05\)). Following the booster dose at 36 weeks, a further rise in the agglutinating titer was found in both immunized groups and at 48 weeks the mean titer was significantly greater than the controls in both groups (\(P<0.02\)).

The predominant serum antibody response was in the IgG class, but no significant differences in the mean serum antibody titers of any class were apparent between the two immunized groups. The IgG antibody titers were greater than \(\log_2 6\) by week 12 and did not change significantly in either group over the remainder of the experiment. The mean IgA titers varied between \(\log_2 1\) and 2.2, and IgM antibody titer was around \(\log_2 2.5\).

Immunization by oral route, with bacteria in gelatin capsules, resulted in increases in salivary agglutinating activity lasting for 25–30 days. Secondary immunization resulted in a more rapid response, but again of about 25 days. Administration of Strep. mutans cells in the drinking water led to a salivary antibody response in both whole and parotid saliva within 28 days. Antibody titers fell to pre-immune levels within 42 days of cessation of antigen administration.

Subcutaneous immunization with pronased cell-wall preparations of Strep. mutans led to greater increases in agglutinating activity in saliva than immunization with whole cells.

Analysis revealed that the increased agglutinating activity in saliva following immunization was mainly due to antibodies of the IgA class.
6.8 ATTEMPTS IN 1981

Russel and Colman reported an attempt to immunize monkeys this year using purified glucosyltransferase from Strep. mutans (Russel & Colman 1981). The purified glucosyltransferase was isolated from Strep. mutans serotype c. The control and test groups were composed of four animals each and were matched for age, sex, weight and number of erupted teeth.

The immunogen was injected at two sites on the lower limbs. The first four injections were given at monthly intervals, while the 5th, 6th, 7th and 8th were given on the 8th, 14th, 23rd and 29th month respectively. Two weeks after the second injection, the diet of the animals was changed to one containing about 20 per cent sucrose.

Every second month examinations were carried out with mirror and probe, and at every second examination bite-wing x-rays were taken. Blood samples were collected from the femoral vein, and the serum separated. Plaque samples were taken and the presence of Strep. mutans determined.

The antibody titers to glucosyltransferase in different animals in the non-immunized group is given in figure 19. The titers seem to increase with age, presumably as a response to antigenic stimulation by Strep. mutans in the plaque. The antibody titers of immunized animals are given in figure 20. All four monkeys immunized with glucosyltransferase displayed a good rise in antibody levels, which was maintained between booster injections. An absorbance of 0.4 of a 1:500 dilution of serum corresponds to a titer of approximately 1:10,000, so the antibody levels in immunized animals were well in excess of those found in control animals. The inhibition of glucosyltransferase activity are given in figure 21 for both the immunized groups.

Both the immunized and non-immunized groups developed caries. No differences were observed between the control and immunized
Figure 19. Level of IgG antibodies to GTF in four non-immunized monkeys. Each symbol represents a different animal.

Figure 20. Levels of IgG antibodies to GTF in 4 monkeys (continuous lines) immunized at the times indicated by arrows. All sera were assayed at 1:500 and control sera (dotted line) did not give significant reading at this dilution. All samples were assayed on the same day.

Figure 21. Effect of sera from immunized and non-immunized monkeys on incorporation of radioactivity from \textsuperscript{14}C-sucrose into glucose-containing polymers. All sera were tested at a final dilution of 1:40 and were from blood samples taken 29 months after the start of the experiment.

monkeys with regard to distribution or time of onset of lesions. In the deciduous teeth, the control animals had a total of 21 lesions, while the immunized group had 22 lesions. In the permanent teeth the control group had 3, 3, 5 and 7 carious lesions (mean 4.5) while the immunized monkeys had 1, 4, 5 and 7 lesions (mean 4.25) after three years.
7 DISCUSSION

We have seen that beginning from the 1930's a great deal of research has been carried out to develop techniques for immunization against caries. To determine the feasibility of immunization against dental caries certain criteria should be fulfilled if these immunization techniques are to be taken from the experimental stages to mass immunization of human beings as a preventative measure against dental caries. Firstly the vaccines developed should render protection against caries effectively. The results should be predictable and constant. An antibody response to the immunogen should be demonstrable and in turn, these antibodies should inhibit microbial activity, indicated by a reduction of microbial aggregation, adhesion of dental plaque to tooth surfaces, or plaque formation. The third and most important criteria is that the vaccine should be safe and within accepted guidelines for vaccination procedures in human beings.

The aim of this discussion is to compare and evaluate the studies carried out, and to come to a conclusion as to the best method of immunization against caries. Most of the researchers have used Strep. mutans for their vaccine preparation. Some of the methods of vaccine preparation have been formalin killed cells, heat-killed cells, whole live cells, broken cells or cell walls and enzyme preparations.

Preparation of formalin-killed cells have shown variable results. In the study carried out by Tanzer, Hageage and Larson (Tanzer, Hageage & Larson 1973), the serum antibody titers were high in all experiments, while the salivary antibody titers were either not determined or they were very low. There was not a significant reduction in the Strep. mutans recovered from the immunized rats. At the same time the reduction in caries was not significant in all the experiments. In the seven experiments carried out by Taubman and Smith (Taubman & Smith 1974), on gnotobiotic rats, only two of the experiments showed a significant reduction in caries. This results
were obtained inspite of significant increases in serum and salivary antibody titers. Emmings, Evans and Genco (Emmings, Evans & Genco 1975) reported greatly increased titers in serum and salivary antibodies, but no caries scores were given. Hence, it is difficult to come to a conclusion as to the success of the immunization process. In a similar study (Evans, Emmings & Genco 1975), significant increases in the salivary IgA titers and a decrease in the infected surfaces was reported. However no caries scores were given and the vaccine consisted of formalin-killed cells and their products. In another study (Emmings, Evans & Genco 1976) increases in serum and salivary antibody titers were demonstrated but no caries scores given.

Lehner, Challacombe and Jill (Lehner, Challacombe & Jill 1975) conducted studies with heat-killed organisms. One third of the monkeys immunized showed very significant reductions in caries and increases in serum and salivary antibody titers, while two third showed less significant results. The immunized animals also showed delayed onsets of caries. Similar results were reported in their 1976 study (Lehner, Challacombe & Jill 1976). In 1977 (Lehner, Jill & Challacombe 1977) they showed that there was no difference in caries scores in monkeys immunized with heat-killed organisms or formalin-killed organisms. They reported significant increases in the serum antibody titers but no significant difference in the salivary antibody titers. In yet another study (Challacombe & Lehner 1980), significant increases in serum and salivary antibody titers were reported but no caries scores were given.

The results obtained by Bowen, Cohen and Colman (Bowen, Cohen, & Colman 1975) when they injected whole live cells intravenously in monkeys were not significantly different from the control groups. When Challacombe and Lehner (Challacombe & Lehner 1980) attempted to immunize monkeys with live organisms in drinking water, they demonstrated significant increases in the serum and salivary antibody titers but no records on caries scores were given.
In 1975 Bowen, Cohen and Colman (Bowen, Cohen & Colman 1975) reported that of the four animals immunized with broken cells only one monkey developed caries-like lesions along the edges of the four upper incisors at the end of five years. This was due to habitual biting of the metal struts of the cage. On the other hand the five control animals had a total of 64 lesions. They also reported significant increases in the serum and salivary antibody titers. A follow up of this experiment was reported in 1979 (Cohen, Colman & Russell 1979). After nine years there were two surviving immunized monkeys and two control monkeys. By this time the control monkeys had 56 and 69 decayed surfaces. On the other hand only one of the immunized monkeys had one small lesion in the lower premolar and the other immunized monkey was completely caries free. It is important to note that no booster doses had been given since 1974. In the study by Challacombe and Lehner (Challacombe & Lehner 1980) the results showed that the salivary antibody titers of the monkeys immunized with pronase-treated cell walls was significantly higher than the control group. However it was not significantly higher in monkeys immunized with formalin-killed cells. The serum antibody titers were also higher in the cell wall immunized animals. The caries scores were however not given.

In the attempts using enzyme preparations, glucosyltransferase has been the most common one, being used as an antigen followed by glycosidic hydrolases and fructosyltransferase. The first ever attempt to immunize with enzymes, reported (Hayashi et al 1972) very significant reductions in mean caries scores with glucosyltransferase and to a lesser extent (not significant) with glycosidic hydrolases. The immunized rats also had higher serum antibody titers compared with non-immunized rats. The protection or lack of protection was not however uniform in all the rats. In the next attempt using glucosyltransferase, (Bowen, Cohen & Colman 1975) the results showed that the monkeys were not protected against dental caries. Though the sera of immunized animals contained antibodies to glucosyltransferase
and exhibited variable degrees of inhibition of enzyme activity their plaque samples showed significantly more Strep. mutans then non-immunized animals. Then Bahn, Cummings and Hayashi (Bahn, Cummings & Hayashi 1976) reported increases in serum antibody titers which inhibited enzyme activity but the caries scores or bacterial counts were not reported. In another investigation on monkeys positive results were reported (Bahn, Shklair & Hayashi 1977). Along with inhibition of enzyme activity, a reduction of 68.3 per cent in carious lesions with glucosyltransferase, a 62.4 per cent reduction with fructosyltransferase and 57.4 per cent reduction with glycosidic hydrolyses was reported. Similar results were reported in hamsters (Smith, Taubman & Ebersole 1979). In this case a reduction in the colony forming units of Strep. mutans was also reported in the immunized animals. In a recent investigation using purified glucosyltransferase (Russell & Colman 1981) significant increases in the serum antibody titers was reported. However no difference was seen in the caries scores between the immunized monkeys and the non-immunized monkeys. It is thus possible that results of reduction in caries scores shown by some other studies could have been due to some factor present in the crude enzyme preparations and not due to the enzymes.

The purified antigen I/II (Lehner, Russell & Caldwell 1980) from Strep. mutans showed a significant rise in serum antibody titer and a significant reduction in caries scores. The colony forming units of Strep. mutans were also lower in the immunized monkeys. However the salivary antibody titers failed to show a significant increase.

Passive immunization with immunoglobulins (Sweeney, Shaw & Child 1967; Lehner 1978) failed to show any positive results.

The intraperitoneal and intravenous routes of immunization will in all likelihood not be acceptable for vaccination in the human population. All the same, these routes have not produced any better results than the subcutaneous or submuuous routes
of immunization. The subcutaneous route and the submucous route has produced comparable results as far as the reduction of caries and the rise in serum antibody titers are concerned (Lehner, Challacombe & Jill 1975, 1976; Bowen, Cohen & Colman 1979). However most studies indicate that the submucous route and the intraductal route gives rise to higher salivary antibody titers (Lehner, Challacombe & Jill 1975; Emmings, Evans & Genco 1975, 1976; Challacombe & Lehner 1980) compared with the subcutaneous route. It is interesting to note that when only one gland was immunized (Emmings, Evans & Genco 1975) salivary antibodies were only stimulated in the immunized gland.

Due to the wide range of schedules applied by various investigators, it is difficult to compare them. At the same time only one comparative study has been carried out. In most cases a number of injections (varying from one to five) are given over a short period of time followed by one or more booster doses. In others regular injections are given eg. monthly. However very little difference in caries reduction was seen when the animals were immunized 5 times or 2 times (Lehner, Jill & Challacombe 1977). If immunization is discontinued the levels of serum and salivary antibodies fall (Emmings, Evans & Genco 1975). With re-immunization the antibody titers rise again. In the longest study reported (Bowen, Cohen & Colman 1975; Cohen, Colman & Russell 1979) the monkeys were vaccinated 8 times over a period of 3 weeks, followed by 4 injections over a 2½ year period and a single injection was given almost 3 years later. The animals were very well protected against caries over a nine years period.

Broken cells and cell walls seems to be the best antigen for the immunization against caries. At the same time it has been reported that certain antibodies stimulated in response to these antigens are cross-reactive with heart tissues (van de Rijin 1976; Hughes 1979). Attempts should be made to break down the cell walls to derive antigens which will give rise to antibodies that will render protection against caries but will not react with heart tissue.
It should also be noted that caries in human can be caused by a wide range of organisms. Most of the studies carried out so far have attempted developing vaccines against Strep. mutans or other single strains. It is felt that attempts should be made to develop broad spectrum vaccines which would give rise to antibodies against a wide range of organisms, capable of producing acid and giving rise to carious lesions.

Except for the salivary antibody titer, the submucous and subcutaneous routes of immunization seems to produce similar results. However it is important to realize that it would be very difficult to explain how a rise in serum antibody titer, without a rise in salivary antibody titer could bring about a reduction in caries. Though it has been noted earlier in the text that small amounts of serum along with antibodies do seep into the oral cavity through the oral tissues especially the gingival crevice, these would definitely not reach significant levels to inhibit bacterial activity to an extent of bringing about a caries reduction. It is thus felt that a significant rise in salivary antibody titer is a must if the reduction in caries is to be logically explained by the immunization process. The secretory IgA antibodies are produced locally by plasma cells in the salivary glands and the submucous tissues. Hence, it is easy to understand why the submucous and intraductal routes of immunization produce higher antibody titers.

The schedules of immunization seem to indicate that a limited number (2 to 3) of initial injections, followed by a number of booster doses spread over a prolonged period of time will render adequate and continous protection.

The results of immunization against caries have been most encouraging in animals. However very little research has been done from the safety point of view. Hence, taking the immunization process from the experimental stage to a full clinical trial in human beings cannot be taken lightly. This is more so since dental caries is neither dangerous nor fatal. Though
the risk of immunization are rare, they have been widely pub-
lished. These elements of risk would have been admissible for
preventing fatal or severely disabling diseases, but will not
be acceptable for preventing what many consider as a minor
ailment.
Dental caries have been known to have occurred in ancient times but it only reached epidemic levels since the human race adopted a diet rich in carbohydrates and sugar. Though there are no recorded dental surveys before the 19th century epidemiological surveys carried out on skulls show that over the 2000 years between the beginning of the Iron Ages and the end of the Middle Ages there were no major changes in the prevalence of caries. The prevalence of caries remained uniformly low compared with that found later.

In the last two centuries the prevalence of caries has been on the increase. At the same time native populations, such as Australian aborigines, New Zealand Maoris and Eskimos, on their native diet had low prevalence of caries until they were introduced to the European-type diet. The extent of the problem varies in different countries and in different social economic groups.

This is of great concern for everyone, as unlike other diseases which have been brought under control with the advancement of civilization, dental caries has been on the increase as standard of living and nutrition have improved.

In U.S.A. almost everyone suffers from dental caries to some extent before reaching adulthood. Australia spent about $285,000,000 in the financial year 1976 - 1977, for dental health services, and the expenses are going up every year. Due to the enormous extent of the problem and tremendous cost involved the only way to solve the problem is by prevention.

Up to date some of the preventive measures applied are outlined below.

Oral hygiene: Caries prevention can be achieved to some extent by proper toothbrushing and use of dental floss. The aim
should be to remove all plaque. This if carried out once a
day efficiently is more effective than if carried out ineffec-
tiently after every meal.

**Fluorides:** To date fluoride therapy is the single most eff-
ective method for caries prevention. Fluorides stimulate
larger crystal formation, converts hydroxyapatite to fluoro-
apatite, promote remineralization, interfere with microbial
metabolism and inhibit plaque formation. Fluorides aid in
prevention of caries both when used systemically and when
applied topically. If an optimal level of fluoride is inges-
ted by the mother during pregnancy and the child through the
years of tooth formation, about 50% of the caries can be pre-
vented. The usual means of supplying fluorides to the popula-
tion is through the domestic water supply, while some coun-
tries have attempted fluoridation of milk and salt. If fluo-
ride is not available by these means it is recommended that
fluoride supplements be taken. Topical fluoride when used
along with systemic fluorides have an additive effect. Sodium
fluoride, Stannous fluoride and Acidulated Phospho-fluoride
are some of the common ones in use.

**Modify the diet:** Attempts have been made to encourage the
masses to reduce the consumption of sugar and refined carbo-
hydrates. Sweets to be taken should be taken at meal times
followed by toothbrushing. Snacks inbetween meals are to be
avoided as far as possible.

Except for water fluoridation, the rest of the means of caries
prevention involves a great deal of motivation, cooperation
and work on the part of the dentist and his patients. Getting
the total population to maintain good oral hygiene or to
change their dietary habits are things easier talked about
than done. At the same time to get people to take the daily
dose of fluoride if the water is not fluoridated or to carry
out home regimes of self applied fluorides is no easy task.
Though the cost of all the preventive measures discussed is
not much it could be quite taxing for some developing and under developed countries. At the same time if all preventive measures are to be applied to the entire population, not many countries at present have the required qualified manpower to carry it out. However if all these measures are applied we will still not be assured of total protection against caries. So the search for a cheaper, simpler and more effective means of protection against caries goes on. In the medical field a number of vaccines and anti-serums have been introduced for protection against specific diseases which are of microbial origin. Hence, it is feasible to assume that a similar approach is very likely to produce results in the dental field.

The aims and objectives of this thesis are to trace the history of attempts to immunize against caries, to review some of the methods of immunization against caries and to evaluate and compare various means of immunization against caries.

Beginning from 4000 B.C. many theories have been suggested for the etiology of dental caries, mainly the tooth worm theory, Humors theory, Vital theory, Chemical theory and the parasitic theory. Then in 1889 W.D. Miller put forward the acidogenic or chemo-parasitic theory. According to this theory acid was produced by metabolism of dietary carbohydrates by oral bacteria. The acid in turn caused demineralization of the mineral phase of the teeth followed by the breakdown of the organic matrix. Later two more theories, the proteolytic theory and the proteolytic chelation theory were suggested but the validity of these two theories is questionable due to the lack of experimental evidence and supporting data. On the other hand Miller's theory has built up much support and a great deal of experimental evidence has been documented in its favor. Though the basic concept is now well understood, it is necessary to look into the various factors that take part in producing the varying patterns of caries attack in different individuals.

Teeth:—Except for gross developmental disturbances or micro-structural defects on the enamel surface the degree of mineral-
ization is not a contributing factor in the etiology of caries. Fluorides at optimal levels renders the teeth more resistant to acid attack. There is also some evidence that a higher concentration of carbohydrates in the enamel content is related to lowered solubility to acid. Morphologically pits and fissures are more prone to caries and so too are crowded and overlapped teeth.

**Saliva:** A high rate of flow of saliva and saliva of low viscosity aids in washing away food debris and possibly helps by diluting the acids formed. The bicarbonate, carbonic acid and phosphate in saliva acts as buffers to neutralise the acids. The saliva also has antibacterial factors such as lysozyme, lactoperoxidase and immunoglobulins (Ig). The main Ig in saliva is the secretory Ig A which is synthesized by immunocytes in the salivary glands. These antibodies may lyse bacterial cells, may prevent adherence of the cells to the tooth surface or may coat the bacterial cells and interfere with their metabolism.

**Dental plaque:** Plaque is the soft, non-mineralized, bacterial deposit which forms on teeth. The bulk of dental plaque consists of bacteria embedded in an organic matrix. The matrix is in part derived from salivary glycoproteins and in part from microbial extracellular polysaccharides. This plaque allows the diffusion of carbohydrates into it from the oral fluid, which are taken up by plaque organisms and acid is liberated resulting in a drop in pH. At the same time the complex structure of plaque prevents the acid from being easily washed away from the inner layers of plaque by oral fluids thus allowing for prolonged action of the acid on the tooth surface to cause demineralization.

**Microorganisms:** The fact that germ free animals do not develop caries and that oral bacteria can demineralize enamel in vitro to produce caries-like lesions shows that microorganisms are a necessary part in the formation of caries. However not all organisms are cariogenic and all cariogenic organisms are
not equally virulent. Organisms capable of inducing carious
lesions include Strep. mutans (several strains), a Strep.
salivoruis strain, a Strep. millesi strain, Strep. sanguis
(several strains), a Lactobacillus acidophilus strain, a Lacto-
bacillus casei strain, Peptostreptococcus intermedius, Actino-
myces viscosus and Actinomyces naeslundi. However great emphasis
has been given to Strep. mutans by most research workers.

Diet: Carbohydrates are essential in the formation of carious
lesions. Of these the monosaccharides and disaccharides are
relatively more cariogenic than starches. This is shown by the
fact that rats fed with such a diet by gastric intubation
remain caries free while those fed orally developed carious
lesions. The incidence of caries is also related to the freq-
ueny of carbohydrate ingestion. It has also been shown that
sugars enhance the ability of Strep. mutans to colonize tooth
surface. Some cariogenic organisms also synthesize glucan and
fructan from sucrose which aid in their adhesion to the tooth
surface and act as a reserve source of carbohydrates. The cario-
genicity of a carbohydrate is however determined by the ease
with which it can be metabolised by plaque organisms to
liberate acid. A pH of 5.5 is sufficient to demineralize
enamel. However with sugar the pH can fall to as low as 4.

In short, the three principal factors, the host (mainly the
saliva and the teeth), the microflora and the substrate (i.e.
the diet) are necessary for the initiation of caries. For caries
to progress a fourth factor, time has to be taken into
consideration.

Immunity: The term immunity includes all those physiologic
mechanisms that endow the animal with the capacity to recognize
materials as foreign to itself and to neutralize, eliminate, or
metabolize them with or without injury to its own tissues.

Much of our immunity against infection is innate or inborn al-
though immunity to certain microorganisms and their toxins
develops following actual contact with the antigens in the microorganisms or their toxic metabolic by-products. In certain cases immunity can be produced by injection of the dead organism or their products. Such immunity can be transferred to a normal person through the transfer of serum of such an immune.

Immunity can be classified as follows:

I. Non-specific immunity.
II. Specific acquired immunity.
   a. active acquired immunity.
   b. passive acquired immunity.

Non-specific immunity: Non-specific immunity is often equated with natural resistance and refers to the ability of an individual to resist infections through the normally present body functions of all members of his species. It consists of the external defence system and the internal defence system. The external defence system is the skin and the mucous membranes which have antibacterial properties in their secretions. The ciliated epithelium of the respiratory tract help to get rid of any inhaled microorganisms and particles. At the same time phagocytic cells known as the alveolar macrophages roam over the surface of the mucous membrane. The major function of this cell appears to be the phagocytic destruction of inhaled objects. In the genitourinary system the periodic flushing with urine, which is normally somewhat acidic, provides protection to the mucous surfaces. The eyes are protected by the tendency to weep when objects of any size enter the eyes, and the presence of an enzyme known as lysozyme. Defence in the digestive tract is achieved in two ways. The first is through the acidity in the stomach which can be as low as pH 1.0. Next the normal flora, present in large intestines prevent the growth of other bacteria, including pathogenic organisms.

Successful pathogens that escape the external defence system and penetrate into the true physiologic interior of the host are met with the internal armory of the host which is phagocy-
Macromolecules in blood may have antiviral or antibacterial properties. Glycoproteins, transferrin and ferno are all antiviral. Beta lysin, leukin, plakin and other poorly described proteins are antibacterial.

Specific acquired immunity:— The immunity an individual develops during his lifetime is called acquired immunity. Unlike natural or non-specific immunity which is a broad-spectrum type of resistance not directed against any particular pathogen, acquired immunity is expressed most typically against a specific pathogen and develops as a result of exposure to the specific pathogen. Acquired immunity is based on the activities of three major cell types: the macrophages, the B lymphocytes and the T lymphocytes.

When a person contacts an overt clinical disease or an unrecognized subclinical illness, his body develops protective antibodies against that pathogen. A subsequent encounter with the same pathogen finds the host well prepared against reinfection. This type of immunity is classified as naturally acquired active immunity.

The second type is artificially acquired active immunity which is carried out through the use of vaccines or toxoids. Vaccines can be prepared from killed or inactive organisms. Attenuated vaccines are vaccines composed of living organisms of low virulence. If the disease is due to specific toxins of the pathogen, toxoids are used as the immunizing principle.

Passive acquired immunity:— Passive immunity can also be acquired naturally or artificially. The former exists when maternal IgG transgresses the placental barrier or IgA received by the infant through the mother’s milk. Artificial means of receiving passive immunity relies on the injection of the gamma globulin fraction or whole hyperimmune serum into the person needing this protection.
Developments in immunization against dental caries

A crude form of immunization was practiced in India and China in ancient times where protection against smallpox was obtained by inoculating live organisms from disease pustules. Still later a similar form of immunization was practiced in the middle east where powdered scabs were applied interdermally.

This crude form of immunization reached England in the 18th century and Edward Jenner discovered that inoculation with cowpox crusts protected man from smallpox in 1773. However it was Louis Pasteur who coined the term vaccine, and developed techniques for culture of microorganisms in vitro. These cultures provided material for developing vaccines with living, heat-killed and attenuated organisms. In the years that followed many vaccines were developed against various microorganisms and their products.

Dental caries is an infectious disease and the role of microorganisms in its etiology is well documented. It is thus not surprising that the dental profession has been in search for a vaccine against caries.

The history for the search for a vaccine against caries goes back to the early part of this century. The idea was first brought up by Von Beust in 1912, when he wrote that it is "possible, even probable that the immunity to caries...... is the result of the formation of antibodies." Then in 1926 E.W. Fish considered it " worth while investigating the possible existence of an active immunity to caries in the blood stream".

In 1932, P.Jay and his associates conducted skin tests with a filtrate of Lactobacilli, on individuals to determine the relationship between the skin reactions of the individual to his susceptibility to dental caries. They noted that individuals with antibodies in their sera were immune to caries while those without antibodies had high caries score.
In the following year they failed to develop antibodies to Lactobacillus acidophilus in rats. However when rabbits were given subcutaneous injections of killed L. acidophilus, they developed antibodies. The investigation was carried further and the vaccine was tried on children. The vaccine was administered subcutaneously in 0.5 c.c. doses, one week apart for four weeks. Two children developed abscesses on their arms and their antibody titer increased from 0 to 1-640, and from 1-30 to 1-640 respectively. The children who did not have sore arms showed no appreciable change in agglutinin titer.

In the years that followed there were no attempts at immunization but there were a great deal of reports that saliva had an inhibiting effect on the growth of certain bacteria including Lactobacillus. Attempts were made to draw a relationship between the inhibiting effects of saliva and caries.

A period of nine years lapsed, before another attempt was made at immunization against caries. This time Canby and Bernier selected certain strains of Lactobacilli which would not give rise to abscess formation, but stimulate antibody formation. The vaccine from heat-killed Lactobacilli was preserved in 0.5% phenol. The vaccine was first tried on guinea pigs and rabbits. Then human volunteers were given intracutaneous injections of 0.1 c.c. The volunteers were vaccinated every 5 days for a start and later changed to three day intervals.

The results showed a drop in the number of L. acidophilus in the mouth of all vaccinated persons except one. There was also an increase in the agglutinin titer in most of the volunteers.

A follow up of the above investigation was carried out in 1944 by N.B. Williams. Vaccines were prepared from L. acidophilus of the 4a and 13c strains. A total of 33 volunteers took part, 20 in the experimental group and 13 in the control. Injections were given subcutaneously at weekly intervals for 4 weeks, one arm receiving a living suspension of a mixture of the two Lactobacilli strains and the other arm receiving a heat killed
one of the same mixture.

The results showed that only 5 or 25% of volunteers showed statistically significant reduction in the Lactobacilli count in saliva. The serum agglutinin titer was also elevated in the vaccinated volunteers.

After the attempt to immunize against caries in 1944 by Williams there was a sudden decline in interest in immunization against caries. There are a number of reasons that can be attributed to this decline of interest. In the years that followed it was becoming increasingly apparent that Lactobacilli did not occupy such a central role in caries as had been thought. Another reason for the decline of interest could be due to the fact that the teeth seemed immunologically to be 'outside' the body and unavailable to the antibodies in serum and the antibodies in saliva were far below the plasma levels. Yet another reason could be the diversion of interest towards fluorides as a preventive measure. For the first time fluorides were deliberately added in controlled amounts to community drinking water in Michigan, New York and Ontario, Canada in 1945.

As the years passed by it became more and more apparent that fluorides alone could not fulfill the role of a total preventive programme against caries. At the same time there were reports of increased amounts of gamma globulin in saliva, in caries resistant individuals and that antibodies could leak out of serum through the oral tissues, mainly, the gingival crevice. Of greater importance was research indicating that the principle antibodies in saliva, as in other secretions, are of the secretory IgA (S IgA) variety and that these antibodies are produced locally by plasma cells in the salivary glands and the submucous tissues. Since these antibodies were produced in response to local antigenic stimulation the amount in serum and saliva are unrelated.

After a lapse of 16 years, in 1962, Fitzgerald and Keyes
attempted to immunize Albino Hamsters against induced dental caries. They failed to protect the hamsters by using phenalised cells of Streptococcus, strain HS-6 subcutaneously or intraperitoneally. They also failed to passively immunize the animals by using anti-HS-6 rabbit sera.

Then in 1967 Sweeney failed to passively immunize rats by using gamma globulin preparation from pooled serum obtained from 250 matured stock rats. The same year Wagner successfully vaccinated gnotobiotic rats infected with Strep. faecalis. He vaccinated parenterally using homologous strain in adjuvant. He reported that the immunized animals had higher serum and salivary agglutinin titers, fewer bacteria in their saliva, and less carious lesions. In another series of experiments Wagner and Orland reported that rats vaccinated with formalin killed Strep. mutans serotype C, Strep. sanguis or L. casei in Freunds complete adjuvant remained virtually free of dental caries.

Bahn et al tried immunization with the enzyme dextranucrease in 1967. The rats were injected the enzyme extract in Freunds incomplete adjuvant intraperitoneally. The reduction of caries scores were statistically significant (P<0.001). Then for the first time Bowen attempted to immunize monkeys. He vaccinated the animals intravenously with live Streptococcus isolated from a carious lesion. 1 ml. of the vaccine was given 8 times over a period of 3 weeks followed with a single booster dose after 12 weeks. at the same time the animals were infected with homologous Streptococcus. The results showed that the immunized animals had less caries and was statistically significant at the 5% level.

The following year, in 1970, Gaffer et al reported a successful attempt to immunise hamsters with formalin killed cells in Freunds adjuvant. The animals had a 68% reduction in caries and was statistically significant at the 1% level.

Once again in 1972 Hayashi et al tried immunizing rats with
two enzymes, glucosyltransferase and glycosidic hydrolases. Rats immunized with glucosyltransferase had a 59.4% reduction in caries which was statistically significant at the 1% level, while rats immunized with glycosidic hydrolases only had a 28.3% reduction which was not statistically significant.

Then in 1973 Tanzer et al conducted a number of experiments in an attempt to immunize rats against Strep. mutans strain 6715. Rats were infected with streptomycin-resistant virulent 6715 after formalin killed cells of 6715 were injected subcutaneously from the salivary glands. There was a rise in the serum and salivary agglutinin titer. There was an apparent protection against caries which was not statistically significant at the 5% level in all the experiments.

We have seen that by the 1970's a great deal of interest has been built up once again to develop a vaccine against caries. As Sims wrote, "As before when Lactobacilli were thought to be the organisms responsible for caries, the idea of preventing or controlling the disease by immune responses is again in fashion".

In the recent years due to the greatly elevated interest and a better understanding of the etiological role of Strep. mutans in caries, a greater number of studies have been carried out than ever before. In the process a lot of duplication have been going on. The basic theme however remains the same and the antigens used in most of the studies have been from Strep. mutans or its products. The methods of vaccine preparation include formalin killed cells, heat killed cells, whole live cells, broken cells or cell walls and enzyme preparations of glycosidic hydrolases, glucosyltransferase and fructosyltransferase. The experimental animals have been rats or monkeys. There have been a wide range of dosages and an equally broad range of schedules been employed. The schedule usually follows a similar pattern of a number of injections over a short period of time, followed by one or more booster doses after a prolonged period. The
routes of vaccination employed include subcutaneous, submucosal, intravenous, intraperitoneal and intraductal.

The results though varied have been very promising. Most of the studies carried out have reported reduction in caries, some of which have been statistically significant at the 1% level. Most of them have also reported elevation in the serum and salivary agglutinin titers which have been significant at varying levels. A few have also reported a reduction in the colony forming units of Strep. mutans in the immunized animals, while others have reported no difference between the immunized and non-immunized animals.

The feasibility of immunization against caries will depend on certain criteria before it can be acceptable as a preventive measure in human beings. The vaccine should render adequate protection against caries. The antigen used should stimulate antibody formation which in turn should inhibit microbial activity. The most important criteria is that the vaccine should be safe for vaccination in human beings.

Though most of the antigens used have given positive results, such as reduction in caries or elevation in the serum and salivary antibody titers in some experiment or other, broken cells or cell walls have definitely produced far better results than the rest. The enzymes when used as crude preparations have shown reduction in caries but not when used in the purified form. Hence it is possible that it is not the enzymes but some other factor in the crude preparation that renders protection against caries.

The intraperitoneal and intravenous routes of immunization are not likely to be acceptable for vaccination in human subjects. At the same time these routes have not produced any better results than the subcutaneous or submucous routes. The subcutaneous and submucous routes of immunization have given similar results as far as the reduction in caries and the rise in
serum antibody titers are concerned. However the submucous route have constantly given rise to higher salivary antibody titers. At the same time it is necessary to realize that it would be very difficult to explain how a rise in serum antibody titer, without a rise in salivary antibody titer could bring about a reduction in caries. Though small amounts of serum do seep into the oral cavity, these would definitely not reach significant levels. Hence, it is felt that a rise in salivary antibody titer is necessary to explain the reduction in caries by immune response. This would indicate that the submucous route is best suited for immunization against caries.

A wide range of schedules for immunization have been applied by various investigators. These schedules indicate that a limited number (2 to 3) of initial injections, followed by a number of booster doses spread over a prolonged period of time will render adequate and continues protection.

The results of immunization against caries have been most encouraging. However it has also been reported that some of the antigens used are cross reactive with heart tissues. Though the risks of immunization are rare they have been widely published. These elements of risk would have been admissible for preventing fatal or severely disabling diseases, but will not be acceptable for preventing what many consider as a minor ailment.
9 CONCLUSION

The extent of the caries problem and the various means of caries prevention were discussed. The next two sections were included to give the reader a basic understanding of the etiology of caries and a basic knowledge of immunity.

The history of immunization against caries has been traced and some of the recent methods of immunization against caries reviewed. These methods of immunization have been compared and the following conclusions drawn.

(1) Broken cells or cell walls are best suited for immunization against caries.

(2) The submucous route of immunization is most likely to produce results.

(3) Two or three initial injections followed by a number of booster doses spread over a prolonged period of time will assure continuous protection against caries.

It is also suggested that further research be carried out in the following areas.

(1) To determine the various antigenic components of cell walls and to differentiate the antigens responsible for protection against caries and those components that cross react with heart tissue. Attempts should then be made to separate these antigenic components.

(2) To carry out comparative studies between the submucous and subcutaneous routes of immunization.

(3) To determine how protection against caries is brought about with rises in serum antibody titer in the absence of salivary antibodies.

(4) To carry out comparative studies using different schedules to determine the best schedule for immunization against caries.
The history of immunization against caries have been traced and the recent methods of immunization reviewed. The various methods of immunization have been compared and evaluated. Certain conclusions have been drawn and suggestions for future research made, thus fulfilling the aims and objectives set.
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