THE DECALCIFICATION POTENTIAL OF FOODSTUFFS

GILLINGS
Some of the literature relevant to the food-dental caries relationship was reviewed, and the significance of in-vivo and in-vitro tests of this relationship defined.

The development and application of a laboratory method for assessing the decalcification potential of foods was described. Food-saliva-enamel mixtures were fermented, and the dissolution produced compared with that occurring in simple buffer systems, to determine whether the presence of a food in a buffer system produced by fermentation influenced dissolution.

Radioactive tooth enamel powder was incubated with pooled saliva and the test food, under controlled conditions. At regular intervals, pH was recorded (by electrode), titratable acidity measured (by micromethod) and enamel dissolution assessed (by radiochemical means). The enamel dissolution assessments were free of the complications of excessive diminution of the experimental volume, and interfering ions from the food-saliva mixture.

In general, it was found that refined (processed) foods produced less titratable acid than did raw, unprocessed foods, though the pH changes were comparable. Enamel dissolution occurring with test foods could not be predicted solely from the pH or titratable acidity changes observed. Thus a large decrease in pH level, and
extensive titratable acid production through fermentation resulted in high enamel dissolution with some foods, but low enamel dissolution with others. The degree of dissolution could not be related to a food's inherent buffer capacity, but was related to its inherent acid content.

It was concluded that some foods do not behave as simple buffer systems when fermented, and such foods have properties which affect enamel dissolution independently of the changes in pH and titratable acidity. The low dissolution values for some foods (milk products, and some unrefined cereals and sugars) correlated with the cariogenicity of these foods in human and animal diets. Thus, this in-vitro food test could be employed to identify foods of low or minimal cariogenicity. The hypothesis that refining of crude cane juice successively increases its decalcification potential was supported.

The appendices discussed enamel powder preparation, dissolution of unirradiated and irradiated enamel, radiochemical considerations in dissolution studies, and the effect of a food additive on enamel dissolution under the food test conditions.
THE DECALCIIFICATION POTENTIAL OF FOODSTUFFS

A RADIOCHEMICAL ASSESSMENT OF TOOTH ENAMEL DISSOLUTION IN FERMENTING FOOD–SALIVA MIXTURES

By

Barrie R.D. Gillings

Submitted in partial fulfilment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Supervised by Noel D. Martin

Department of Preventive Dentistry
Faculty of Dentistry
University of Sydney

1967
ACKNOWLEDGEMENTS

An investigation of this type is never an individual effort. The assistance of many people is always necessary, and before the bulk of this work could be completed, a laboratory had to be established, equipment installed, and in some cases, constructed in the workshop. This was achieved through the co-operation of the maintenance personnel of the Dental Hospital in Sydney, Mr. A. Boundy, Mr. L. Blackler and their staffs. The author thanks them for their unfailing help when called for.

The co-operation of the Director of the Institute of Dental Research, Mr. R. Harris, is gratefully acknowledged.

The initial work on the development of the methods used in this project was carried out in conjunction with Dr. D. Beck, now of the University of Otago Dental School, a true friend and respected colleague. His assistance in some preliminary experiments was most welcome.

Loyal support and assistance with the laboratory procedures was provided by Miss C. Dodd. Her painstaking and thorough work is gratefully appreciated. The author would also like to thank Mr. B. Rumble for assistance in preparation of some of the photographic illustrations, and Mrs. E. Gillings, Miss C. Dobinson and Miss A. Graham for the final typing of the manuscript.

The dogged determination of the panel of saliva donors deserves the highest praise. Over a three year period, they
produced 16 litres of saliva, without which the experiments could never have been conducted. Thanks are also due to Miss J. Rance of the Soil Science Department, University of Sydney, and personnel of the C.S.I.R.O. Division of Coal Research, for assistance with calcium and phosphate analyses, and Dr. N. Stevenson, of the University of N.S.W. for help in x-ray diffraction analyses.

The author offers sincere appreciation to Dr. B. Bibby for the inspiration and encouragement he provided at the initial stages of this work, and to Professor N. Martin, for his guidance, advice, indulgence and constructive criticism, without which the work would never have reached completion.

Service irradiations of the tooth enamel used in this study were provided by the Australian Atomic Energy Commission, on a grant from the Australian Institute of Nuclear Science and Engineering.

The greatest appreciation of all is due to Mrs. Margaret Gillings, for her continued encouragement, tolerance and patient understanding during the last five years.
# TABLE OF CONTENTS

**Volume I, Text.**

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section I.</td>
<td>General Introduction</td>
<td>1.</td>
</tr>
<tr>
<td>Section II.</td>
<td>Review of the Literature</td>
<td>13.</td>
</tr>
<tr>
<td>Section III.</td>
<td>Development of the Experimental Method</td>
<td>100.</td>
</tr>
<tr>
<td>Section IV.</td>
<td>Experimental Method</td>
<td>129.</td>
</tr>
<tr>
<td>Section V.</td>
<td>Preliminary Results, I: U.S. Foods</td>
<td>147.</td>
</tr>
<tr>
<td></td>
<td>Preliminary Results, II: Australian Foods</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beverages</td>
<td>160.</td>
</tr>
<tr>
<td></td>
<td>Sugars</td>
<td>164.</td>
</tr>
<tr>
<td></td>
<td>Flours</td>
<td>166.</td>
</tr>
<tr>
<td></td>
<td>Bread and Biscuits</td>
<td>167.</td>
</tr>
<tr>
<td></td>
<td>Cereals</td>
<td>169.</td>
</tr>
<tr>
<td></td>
<td>Sweets and Fruits</td>
<td>171.</td>
</tr>
<tr>
<td></td>
<td>Observations and Comparisons</td>
<td>173.</td>
</tr>
<tr>
<td>Section VI.</td>
<td>Discussions</td>
<td>191.</td>
</tr>
<tr>
<td></td>
<td>Conclusions</td>
<td>224.</td>
</tr>
<tr>
<td>Section VII.</td>
<td>Summary</td>
<td>232.</td>
</tr>
<tr>
<td>Section VIII.</td>
<td>Bibliography</td>
<td>241.</td>
</tr>
</tbody>
</table>

**Appendix A.** Preparation of Powdered Tooth Enamel  
i-vii.

**Appendix B.** Dissolution of Apatites in Acid Solutions  
i-xvii.

**Appendix C.**  
I. Radioactive Tooth Enamel  
II. Radiochemical Determinations  
i-xxv.  
i-xi.

**Appendix D.** Calcium Sucrose Phosphate  
i-iv.

**Appendix E.** Composition of Foods  
i-iv.
### TABLE OF CONTENTS (continued)

#### Volume II. Illustrations.

<table>
<thead>
<tr>
<th>Illustration</th>
<th>Relevant Section in Text</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>Section III</td>
<td>107.</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>109.</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>111.</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>112.</td>
</tr>
<tr>
<td>5</td>
<td>&quot;</td>
<td>113.</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>120.</td>
</tr>
<tr>
<td>Figure 7</td>
<td>Section IV</td>
<td>130.</td>
</tr>
<tr>
<td>8</td>
<td>&quot;</td>
<td>134.</td>
</tr>
<tr>
<td>Figure 9</td>
<td>Section V</td>
<td>161.</td>
</tr>
<tr>
<td>10</td>
<td>&quot;</td>
<td>177.</td>
</tr>
<tr>
<td>11</td>
<td>&quot;</td>
<td>177.</td>
</tr>
<tr>
<td>12</td>
<td>&quot;</td>
<td>179.</td>
</tr>
<tr>
<td>13</td>
<td>&quot;</td>
<td>188.</td>
</tr>
<tr>
<td>Figure 14</td>
<td>Appendix A</td>
<td>v.</td>
</tr>
<tr>
<td>Figure 15</td>
<td>Appendix B</td>
<td>i.</td>
</tr>
<tr>
<td>16</td>
<td>&quot;</td>
<td>ii.</td>
</tr>
<tr>
<td>17</td>
<td>&quot;</td>
<td>xi.</td>
</tr>
<tr>
<td>18</td>
<td>&quot;</td>
<td>xiii.</td>
</tr>
<tr>
<td>Figure 19</td>
<td>Appendix C</td>
<td>x.</td>
</tr>
<tr>
<td>20</td>
<td>&quot;</td>
<td>xi.</td>
</tr>
<tr>
<td>21</td>
<td>&quot;</td>
<td>xiii.</td>
</tr>
<tr>
<td>22</td>
<td>&quot;</td>
<td>xviii.</td>
</tr>
<tr>
<td>Colour Plate I</td>
<td>Section V</td>
<td>164.</td>
</tr>
<tr>
<td>II</td>
<td>&quot;</td>
<td>166.</td>
</tr>
<tr>
<td>III</td>
<td>&quot;</td>
<td>167.</td>
</tr>
<tr>
<td>IV</td>
<td>&quot;</td>
<td>169.</td>
</tr>
<tr>
<td>V</td>
<td>&quot;</td>
<td>171.</td>
</tr>
<tr>
<td>VI</td>
<td>&quot;</td>
<td>173.</td>
</tr>
</tbody>
</table>

continued...
## TABLE OF CONTENTS (continued)

<table>
<thead>
<tr>
<th>Illustration</th>
<th>Relevant Section in Text</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table I.</td>
<td>Section V</td>
<td>150.</td>
</tr>
<tr>
<td>II.</td>
<td>&quot;</td>
<td>151.</td>
</tr>
<tr>
<td>III.</td>
<td>&quot;</td>
<td>152.</td>
</tr>
<tr>
<td>IV.</td>
<td>&quot;</td>
<td>153.</td>
</tr>
<tr>
<td>V.</td>
<td>&quot;</td>
<td>154.</td>
</tr>
<tr>
<td>VI.</td>
<td>&quot;</td>
<td>155.</td>
</tr>
<tr>
<td>VII.</td>
<td>&quot;</td>
<td>158.</td>
</tr>
<tr>
<td>VIII.</td>
<td>&quot;</td>
<td>187.</td>
</tr>
<tr>
<td>Table IX.</td>
<td>Section VI</td>
<td>202.</td>
</tr>
<tr>
<td>X.</td>
<td>&quot;</td>
<td>205.</td>
</tr>
<tr>
<td>Table XI.</td>
<td>Appendix D</td>
<td>iii.</td>
</tr>
<tr>
<td>Table XII.</td>
<td>Appendix E</td>
<td>i.</td>
</tr>
<tr>
<td>XIII.</td>
<td>&quot;</td>
<td>i.</td>
</tr>
<tr>
<td>Table XIV.</td>
<td>Section III</td>
<td>122.</td>
</tr>
<tr>
<td>XV.</td>
<td>&quot;</td>
<td>123.</td>
</tr>
<tr>
<td>Table XVI.</td>
<td>Section V</td>
<td>163.</td>
</tr>
</tbody>
</table>

**Transparent Overlay:**
(pocket, inside back cover of Column II.)

SECTION I

GENERAL INTRODUCTION
GENERAL INTRODUCTION

Dental caries is an extremely prevalent disease, effective control of which would benefit communities by saving many man-hours of work, and expense in repairing the damage it causes. In civilised societies, the disease is almost universal, and in Australia, even young children are often severely affected, so that already at 12 years of age, one third of their teeth are carious.\(^{11}\) A similar situation is found in Great Britain, New Zealand, Scandinavia and the United States,\(^{212, 92, 46, 168}\) despite the presence in these countries of extensive dental manpower, availability of dental advice and vigorous efforts in dental health education.

In primitive societies, dental caries is not nearly so prevalent, and in some geographically isolated areas, it is almost non-existent.\(^{13, 259}\) The significance of this fact in relation to dental caries can be seen if such groups are exposed to a civilised dietary. When geographical barriers are broken, and trading activities in foodstuffs commence, dental caries prevalence increases dramatically.\(^{266}\)

The introduction of dietary restrictions in civilised groups can produce the reverse effect, as for example in time of war, when the problem of supply often results in the rationing of certain foods. A typical example was the greatly reduced incidence of dental caries in Great Britain during World War II.\(^{211}\) While there were, no doubt, a number of factors concerned, such as decreased frequency of eating, a more balanced diet, and bread made from high extraction flours, studies suggest that probably, restricted consumption of
INTRODUCTION

refined carbohydrates was the most important. (184, 208)

These general observations have a practical application, and a measure of caries control in susceptible individuals can be achieved by the adoption of a diet where all forms of refined carbohydrates are excluded, and milk, meat and egg products are consumed in larger quantities. (143) Carbohydrate requirements in such diets are supplied as fruits, vegetables and whole grains, either raw, or cooked without the addition of refined carbohydrates.

The efficacy of diets devoid of refined carbohydrates in reducing dental caries is difficult to demonstrate in individual cases, but with groups, the benefits become obvious, as the Hopewood House and Vipelholm studies have shown. (121, 190) Such diets can control rampant caries, but have little appeal to the average Australian adult.

Most dentists are well aware of the undesirable dental effects of refined carbohydrates, and often advise their patients to avoid sweets, biscuits, cakes, carbonated beverages, white breads and sweetened spreads for this reason. Unless very strongly motivated, a patient finds this advice extremely difficult to follow, since the foods he is advised to avoid are inexpensive, pleasant to eat, satisfy the hunger rapidly, are often attractively packaged, and are cleverly and extensively advertised. So extensive is this advertising that dentists, who have a professional contact with less than one quarter of the population anyway, (12) cannot hope to change the dietary habits of the community by giving such advice. Even if dentists could
INTRODUCTION

convince patients or the community, it is unlikely that those income groups which would benefit most from the advice could afford to follow it. (67)

Why an unrefined diet should be less destructive to the teeth than a refined diet has not, as yet (1969), been fully explained. The subject is receiving attention from workers all over the world, in particular, from Bibby in Rochester (U.S.A.), Darling in Bristol (U.K.), Jenkins in Newcastle (U.K.), McClure in Bethesda (U.S.A.), Nizel in Massachusetts (U.S.A.), Shaw in Harvard (U.S.A.), Sognnaes in Los Angeles (U.S.A.), Strålfors in Umeå (Sweden), and, more recently, a research group in Zürich. It is becoming increasingly evident that outwardly similar foods can behave differently in the laboratory, and probably also in the mouth, and that some unrefined carbohydrates are not as cariogenic as some refined carbohydrates. It is not possible to state that all refined carbohydrates are cariogenic and all unrefined carbohydrates are not.

While complete agreement has not yet been reached on the exact nature of the carious process, most authorities do agree that the initial lesion occurs when oral micro-organisms utilise carbohydrates and produce organic acids, which decalcify the enamel, thereby causing changes which can be demonstrated histologically, histochemically and radiographically. Contributing factors, such as race, oral hygiene, saliva, tooth position, oral flora, food, the enamel quality and so on, may affect the rate at which these organic acids decalcify the tooth surface. (34, 38, 106, 136, 181, 247)
INTRODUCTION

If it were possible to keep the effects of the above factors constant, one might expect that the more acid resulting from a food in the mouth, the more rapid or severe would be the decalcification. It should follow then, that those foods which produce most acids when metabolised by oral micro-organisms should be the most damaging to the tooth. Since it is known that populations consuming unrefined carbohydrates have low caries experience when compared with populations consuming refined carbohydrates, it should also follow that the unrefined carbohydrates will produce less acids than the refined carbohydrates, under the same experimental conditions.

But if this is not the case, then one would have to conclude that unrefined carbohydrates possess qualities, either physical, chemical, or both, which are not possessed by the refined carbohydrates, and these qualities modify the action of the acid on the tooth surface, or the tooth surface itself, or, alternatively, that the presence of acid at the tooth surface is not the prime cause of the carious lesion.

Numerous studies have demonstrated the importance of acids in dissolving enamel, and initiating lesions which closely resemble dental caries, using in vitro techniques, while in-vivo studies by Bowen and his co-workers on monkeys have shown 'clear evidence for the production of substantial quantities of acid' at the tooth surface, which they stated 'strongly suggests its participation in the development of the carious lesions observed'. In the light of these studies, it is most unlikely that
INTRODUCTION

acids are not involved in the production of caries.

But it is difficult to investigate the cariogenicity of foods, and the effects of acids produced from specific foods on teeth in human subjects, for a number of reasons. First, dental caries is a highly complex process, which can be modified by a large number of variables not under the control of the investigator (see above). A specific food would therefore have to be tested on a large population group, so that the effect of these variables could be minimised. Secondly, any group used for testing a particular food must, of necessity, consume a variety of other foods, and these foods could themselves introduce variables, or initiate caries. This possibility is now under intensive investigation since the recent suggestion that certain oral micro-organisms may metabolise some carbohydrates in such a way as to produce extracellular polysaccharides, which contribute to the bulk and adhesiveness of plaque. \(^{(47, 48, 61, 304)}\)

Furthermore, it would be most difficult to organise a group willing to limit carbohydrate intake to one type only, to the exclusion of all others, particularly if it is, for example, blackstrap molasses. Thirdly, to obtain an assessment of the test food's effects on dental caries incidence, the investigation would have to continue over many months to allow any new carious surfaces to appear. These requirements make such experiments expensive, and difficult to organise.

Despite difficulties, large scale investigations of the effects of diet on caries have been carried out on humans, mostly
INTRODUCTION

in institutions, with significant results. In general, the number of foods so tested has been limited, and extensive testing of a variety of additives is virtually precluded.

Some of the objections cited above can be eliminated by testing the caries-producing potential of foods on animal populations, such as rats, hamsters or monkeys. The work is tedious and relatively expensive, and although some variables are eliminated because of the uniform nature of the sample, the possibility of variation in caries-susceptibility in successive generations, particularly in the rat, may make interpretation of results in long-term tests difficult. Once again, the numbers of foods tested must be limited.

It is not surprising that only limited efforts have been made in testing foods in-vivo, and it is unlikely that the enormous range of carbohydrate food types will ever be investigated in this manner. In comparison with other caries control measures, in particular the fluoride ion, in water supplies, as a surface application to teeth, or in dentrifices, this basic approach to dental caries research, diet modification as a caries control measure, is being somewhat neglected. This is unfortunate, since from a public health viewpoint, significant findings concerning the behaviour of carbohydrate foods in the mouth, and proper utilisation of this knowledge, could be of enormous benefit to the community.

When he advocates the avoidance of certain types of food as
INTRODUCTION

a caries control measure, the dentist is in the negative position of recommending substitutes which he thinks may be less cariogenic, but which he knows are often less palatable. Even if dentists were to agree (and they do not at the moment), on what constitute non-cariogenic carbohydrate foods, and advise their patients accordingly, it is unlikely that this advice would be heeded.

This approach to dietary control of caries can at best have very limited success, especially since the dentist has direct contact with but a small percentage of the population. An alternative approach might be to ensure that the carbohydrate foods are as harmless to the teeth as possible, by modifying or making additions to foods. To be acceptable to the manufacturer and the consumer, such treatments should not affect unduly the taste, odour or appearance of the food, its behaviour in storage, during or after processing, and particularly, its cost.

For psychological reasons, the modifications or additions should be natural, rather than chemical, and be as free as possible from the stigma of adulteration. The first steps in such a program would be to determine why unrefined foods behave as they do, and whether their caries-inhibiting properties can be incorporated in a refined product, with none of the disadvantages already mentioned. Should this approach prove impractical, the addition of some substance with a caries-reducing effect might be an alternative, but this would be less desirable, as it would be construed by some as 'chemical' adulteration. In either event,
INTRODUCTION

such a program would necessitate the exhaustive testing of large numbers of foods and additives, to identify the least cariogenic forms of carbohydrate, and the most effective additives.

The most desirable way to test foods or additives is by feeding them to humans under closely controlled conditions, but this is impractical for testing large numbers of foods or additives. Laboratory animals can be used, but this approach is still expensive when many foods are to be tested, and the validity of the clinical application in humans, of findings with animals, is questionable.

If, however, foods could be effectively tested using a practical in-vitro laboratory method, then it would be possible to investigate the relationship between acid production, pH and enamel dissolution with various foods. It would also be possible to compare the enamel dissolutions caused by different foods, and the effects of various additives on these dissolutions. Positive findings could then be used as the basis for selection of the more promising foods or additives for subsequent in-vivo testing on human populations.

This thesis describes the development and application of an in-vitro laboratory test of the ability of a food-saliva mixture to produce acid and dissolve tooth enamel. Because some of the laboratory procedures used embody techniques comparatively new to dental research, references to their use in dental literature are rare. Accordingly, development of the method is dealt with in greater detail than would otherwise be the case.
INTRODUCTION

A departure from conventional techniques for assessing enamel dissolution was the use of radioactive, bovine enamel powder. The assessment of the ability of a given food to dissolve tooth structure was determined by following the appearance of radioactivity in a fermenting mixture of the food and saliva, a most convenient approach, since accurate enamel dissolution figures could be obtained regardless of the food's calcium or phosphorus content. There are, however, objections to this approach. Bovine tooth enamel differs somewhat from human enamel, and its irradiated form is less soluble than its stable form. Furthermore, the solubility of the irradiated form is, in the initial stages of dissolution, higher when measured by chemical means (by calcium or phosphate analysis), than when measured by radiochemical means. The results of this study cannot therefore be applied directly to the in-vivo situation.

The use of the radiochemical method in this work, rather than chemical or gravimetric techniques, can be justified if the former enables a much more extensive investigation to be carried out, and if dissolution curves using the method are qualitatively similar to those obtained by conventional analytical techniques. The simplicity and ease of application more than justifies the use of the radiochemical method with respect to the first condition. To establish that the second condition was fulfilled also, sections of this work were devoted to a detailed consideration of the process by which tooth enamel is rendered radioactive, and an
INTRODUCTION

extensive examination of the behaviour of this radioactive enamel in acid buffer systems of various pH's and molarities. This work was necessary to establish that, although the radiochemical method did not provide enamel dissolution curves which were similar quantitatively to those obtained by other techniques, qualitatively, the curves were similar. It is not suggested that the method described in this work duplicates conditions in the human mouth. The aim was to develop a relatively simple and reproducible technique for assessing the ability of oral micro-organisms to metabolise carbohydrates and produce acids, to assess the acid production by pH measurement and titration, and to estimate the enamel dissolution resulting from the action of the acids produced. The rationale behind this approach is that for caries to occur, regardless of the complicating factors of tooth surface, plaque, trace elements, consistency of foods, micro-organism species, food buffering capacity and so on, acids must be produced. Furthermore, these acids must be capable of dissolving the tooth enamel. If then, a food is shown by the test to produce little decalcification, in comparison with other foods, it could be expected to be of low cariogenicity in-vivo, in comparison with other foods, regardless of the quantity or type of acid it produces when metabolised by oral micro-organisms.

If the conclusions reached in this work agree with those obtained by other workers, using different experimental procedures, the objections discussed above may not be of great significance, and
INTRODUCTION

this food fermentation test, with its inherent advantages of simplicity and reproducibility, may be of value in the large-scale testing of foods and food additives for their ability, when fermented with saliva, to decalcify tooth enamel.
SECTION II

REVIEW OF THE LITERATURE
REVIEW OF THE LITERATURE

Caries in Humans

From the earliest times, observers have commented on the effects which foods may have on the teeth. Aristotle stated that 'figs and soft sweets damage the teeth because small particles adhere between the teeth where they very easily become the cause of putrefactive processes'. (35) In the Book of Jeremiah, (154) the Lord said "... every man that eateth the sour grapes, his teeth shall be set on edge". As a general rule, however, ancient and medieval man was comparatively free of dental caries. Mummery (220) observed that the skulls of ancient man were comparatively free of caries, and investigations of Australian Aborigines, (227) American Indians, (241) Bantu, (266) Bushmen, (53) Eskimos, (237) Maoris, (238) Melanesians, (256) and Zulus (282) indicate that similar freedom from caries is also seen in primitive groups. A consistent finding in these investigations was that the diets of these groups, while not necessarily well-balanced nutritionally, were invariably unrefined, and none used the refined* (processed) foods (e.g. flours and sugars) so common in contemporary Western

* Refined, or processed foods are foods which have been submitted to processes which remove parts of the food which were present naturally, to improve appearance, palatability or storage and handling characteristics.
LITERATURE REVIEW

civilisations. It is now accepted that any groups consuming unrefined foods only, will have a low caries prevalence.

Further support of this conclusion is seen when explorers, missionaries or traders associate with isolated groups, and provide them with ready access to various processed foods. Several investigators have reported on the increase in caries which then results, even with groups with such differing food habits as the Australian Aborigine, (60) Bantu, (232) Eskimo (237) and Maori. (238) Sognnaes (258) and Hoye (140) have demonstrated that it is not only native races which show this effect, and that some isolated communities of Europeans also have very little caries. Davies (66) has compared the diet and dental caries in isolated communities, and found that in those communities with unrefined diets, there was generally little caries, despite differences in the types of foods consumed, the types of soil on which the foods were grown, and the methods of food preparation.

Fergusson (84) found lower caries rates in children attending 'jungle' schools, in comparison with those attending 'town' schools in Samoa, as did Gault (93) in Fiji, who stated that there was little doubt that the dental condition of individuals living remote from the towns was better. Sinclair and her co-workers (256) have investigated native groups in New Guinea with low caries experience, and concluded that "the main difference between the general diet of the New Guinea people as a whole and that of white people in urbanised societies lies in the complete absence from the former of
LITERATURE REVIEW

soft, sweet food made from refined (denatured) ingredients (e.g., white flour and white sugar)."

Barmes\(^{(10)}\) has reported on New Guinea tribes living in close proximity, but with entirely different caries experience. There is a possibility that this difference is related to dietary factors, as there is some correlation between soil acidity and caries, suggesting a trace element effect.\(^{(246)}\) Ludwig and others have reported a similar situation in the two New Zealand cities of Hastings and Napier.\(^{(193)}\) During an extensive dental examination of children in these two apparently similar cities, only 12 miles apart, differences of from 20 - 50% in caries prevalence were found. These differences could not be explained on the basis of rainfall, hours of sunshine, socio-economic conditions, race, water or milk supply, or dietary fluoride. It was hypothesised that the difference might lie in the vegetables grown in Napier on land exposed by an earthquake. Comparisons of the mineral contents of vegetables from the two cities indicated that those from Napier had a generally higher content of trace elements and investigations are still in progress. Jenkins has reviewed many of these studies.\(^{(152)}\)

The variations often observed in the caries prevalence of city and country dwellers can also be observed in different ethnic groups living in the same locality. Davies\(^{(66)}\) has reviewed the reported effects of social customs and habits on oral disease, and Kau\(^{(157)}\) has shown differences in caries
prevalence between Hawaiians of Japanese, Polynesian and Caucasian extraction. Similar results were obtained by McCombie and Chua\(^{(201)}\) for young adult Chinese, Malays and Indians in Malaysia. Klein\(^{(166)}\) states that these patterns are generally preserved because of tendencies to marriage within the ethnic group. For this reason, the caries pattern of the daughter (and of the daughter's husband), often follows that of the mother. Harris\(^{(132)}\) has observed that foods are often selected for cultural, rather than nutritional reasons, and suggests this as an additional factor in preserving caries patterns in different ethnic groups.

Jenkins reported that the caries incidence was high in Ancient Greece and Rome, but fell after the collapse of the Roman Empire, only to rise again in about 1000 A.D. and has continued to rise since then.\(^{(151)}\) He then reviewed many studies on the caries experience of civilised and primitive people of more recent time, and concluded: 'there can be no doubt whatever that a westernised diet is universally associated with a higher average caries rate than are most primitive diets, and there is a strong presumption that refinement of food is a factor in caries, although this cannot be regarded as fully proved'.

Planned, large-scale experiments to investigate the relationships between food and caries are rare. However, dietary restrictions and modifications which are introduced in time of war have often been shown to affect the caries prevalence, and for World War I, decreased caries was observed in Gothenburg by
LITERATURE REVIEW

Bensow, (18) Rjukan by Ramm, (242) Shropshire by Wheatley, (300) Offenbach by Wimmernauer, (502) and reviewed by Krohn and Pedersen. (184)

Wheatley attributed the wartime improvement to "restrictions and modifications of food ... affecting principally sugar, bread and milk". Ramm stated that the children "had not the access to sweets, and lived on coarse bread made from unbolted flour", while Bensow suggested that the caries decrease was a result of "the limited access to sugar, pastry and chocolate."

Reductions in dental caries were also observed for World War II, in Oslo by Grythfeldt, (116) London by Mellanby and Coumoulos, (211) Dalarna by Moland, (216) Als by Skunke, (257) Gothenburg by von Sneidern, (294) Norway by Toverud (290) and reviewed by Alexander. (2) More recently, Marthaler has compared the caries rates observed in Scandinavia, Switzerland and New Zealand, before, during and after World War II, (208) and Jenkins has reported on the distribution of caries in prehistoric, classical and modern times. (151) Mellanby and Coumoulos considered that the lowered caries rates were due to "changes in feeding habits ... the cheap milk scheme ... the wartime food policy ... the addition of calcium carbonate to bread" and other factors.

In summarising much of this work, Krohn and Pedersen (184) stated that "generally speaking, the use of sticky flour and sugar products is regarded as especially harmful", and that "fermentable carbohydrates seem to be of primary importance." They further concluded that "we still lack exact knowledge as to whether the
LITERATURE REVIEW

positive or the negative side of the dietary changes in this direction is the most essential," and "what we do lack is any proof that will enable us to categorically establish one of the factors mentioned as responsible for the increased rate of decline in caries incidence ..."

Marthaler observed that the teeth formed during the period of sugar restriction and high extraction flour consumption did not appear to have increased caries resistance after the restrictions were eased, and that populations living primarily on starchy foods but consuming little sugar have "conspicuously low caries rates". (208)

Both Sognnaes (260) and Toverud (290) noted the decreased consumption of sugar during World War II, and Wilska (301) elaborated on this theme, pointing out that in Belgium, England, Holland, Scandinavia and the U.S.A., where sugar consumption is high, caries is also high. On the other hand, Sognnaes favoured "an indirect favourable influence on the development and maturation of the teeth" as an explanation of the effect. Marthaler could find no evidence to support this explanation. (208)

Takeuchi (287) studied the relationship between sugar consumption and caries incidence in the first molars of over 7,000 Tokyo school-children, and Takahashi (285) analysed the results statistically. The conclusions were that as annual sugar consumption rose, so did the annual caries incidence, with both males and females, in upper and lower first molars. These findings
LITERATURE REVIEW

have been supported by a number of workers who suggest that mono- and di-saccharides are more effective than polysaccharides in promoting caries in animals, (32, 112, 113, 170, 171, 172, 248, 252) while König has provided further support in his statement that 'bread and other baked goods of white and wholemeal flour do not impair dental health very much unless they are garnished with sweet spreads or supplemented with sugar.' (174)

A well-known and extensive investigation of the effects of carbohydrate on caries is the Vipeholm study. (121) Gustafsson, in discussing this study in relation to previous clinical studies in this field, defined three experimental designs:

1. Uniform groups from children's homes and dental clinics were selected, and their carbohydrate consumptions assessed. Observations made were then analysed for any relationship between caries activity and carbohydrate consumption.

2. Groups similar to the above were selected, and their carbohydrate consumption increased or decreased, under controlled conditions, and observations analysed, as above.

(In some cases, control and test groups were selected from the same experimental material).

3. Clinical examinations were used to locate subjects with rampant caries. The carbohydrate intake was then restricted for varying lengths of time, during which any
LITERATURE REVIEW

changes in caries activity were recorded. Gustafsson discussed the previous clinical studies under three headings:

Approach (a): Caries activity in association with unregulated carbohydrate intake.

Approach (b): Caries activity in association with regulated carbohydrate intake.

Approach (c): Reduction of carbohydrate in the tentative treatment of caries. \(^{(121)}\)

Approach (a) has been used by Breese, \(^{(33)}\) Brodsky, \(^{(37)}\) Collett, \(^{(54)}\) Collins and his co-workers, \(^{(55)}\) Koehne and Bunting, \(^{(169)}\) and Westin and his co-workers. \(^{(299)}\) In general, these studies indicated that high refined carbohydrate consumption and between-meals eating was conducive to dental caries.

Both Boyd \(^{(29, 30)}\) and Toverud and co-workers \(^{(291)}\) used approach (b) in studying individuals on restricted diets because of diabetes (low carbohydrate diet) or coeliac disease (high sugar diet). They found low caries in the groups on restricted carbohydrate diets. Jay and his co-workers \(^{(142)}\) fed candy without restriction to some of a group of institutionalised children, and stated "the consumption of large amounts of candy points in the direction of increased dental caries." Boyd, \(^{(31)}\) King \(^{(160)}\) and Mack \(^{(203)}\) have also reported on studies where children were fed increased carbohydrates, but the findings were inconclusive.

Some investigators have used approach (c), diet modification,
LITERATURE REVIEW

in an attempt to achieve caries control. Becks,\(^{(16)}\) Becks and co-workers,\(^{(15)}\) Collins and co-workers,\(^{(55)}\) Forshufvud and co-workers\(^{(87)}\) Howe and co-workers,\(^{(139)}\) Jay and co-workers,\(^{(142)}\) and Kitchin and Permar\(^{(163)}\) have all reported on studies where the carbohydrate consumption was restricted. In most of these studies, caries was reduced.

In 1963, Froesch and his co-workers reported on individuals suffering from hereditary fructose intolerance (H.F.I.), an inborn error of metabolism which produces severe nausea if individuals suffering from the disease consume foods containing fructose or sucrose, while carbohydrate foods which do not contain these substances are well tolerated.\(^{(89)}\) The two cases reported by Froesch and his colleagues were stated to be remarkably free of caries, in comparison with their relatives. Marthaler,\(^{(208)}\) Newbrun\(^{(226)}\) and Sullivan\(^{(284)}\) have also observed low caries experience in subjects suffering from this disease and it is evident that the simple act of eliminating sucrose and fructose from the diet in these people is sufficient to decrease dental caries experience.

A long term investigation of a group of children living under rigidly controlled conditions of diet is provided by the Hopewood House study, a type (c) investigation. In a series of papers,\(^{(101, 126, 190)}\) the dental condition of this group, and other observations, are reported for the period 1952 – 1966. Hopewood House is a Youth Welfare home, originally at Bowral,
LITERATURE REVIEW

N.S.W., but now with annexes in other country areas. The original group studied was 81 boys and girls, aged 4–9 years. In 1961, 52 of the original group still lived at the home.

The children were well cared for, and led a healthy outdoor life. Kindergarten schooling was provided at the home, the older children attending various local schools. The diet at the home was notable not only for the almost complete absence of refined carbohydrates, but also because much of the food was eaten uncooked. Those children eating meals at school took them in the classrooms, away from the other children, though this precaution did not entirely prevent the occasional consumption of refined foods.

In December of each year, the children were assembled, and it was during this time that most of the survey material was collected. This material was extensive, and covered dental condition, lactobacillus counts, diet, physical health and orthodontic status. The authors concluded that the children were slightly smaller than average, with slightly more stammering, goitre and heart murmurs, but had less sickness than average. Prevalence of thumbsucking was higher than normal, but adequate occlusions were more frequent than in a corresponding group of institutionalised children on an average diet. Oral hygiene was not good, and 75% had gingivitis. Dental caries experience in the group was negligible, the annual increment in the number of lesions being about 10% of the average for Australian children of
LITERATURE REVIEW

comparable age. At the beginning of the study, 63 of the children were caries-free, and 32 were still caries-free four years later.

The diet did not contain any meat or refined sugar. White flour, as bread, was eaten occasionally. Vegetables constituted the major part of the diet, and milk, cream, eggs, butter, cereals and dried and preserved fruits were eaten fairly regularly. Dietary carbohydrate was supplied mainly in the form of lentils and whole grain cereals, while wheatgerm, nuts, vegetable extracts and vitamins were regular additions. Sweetening of foods was provided infrequently, and then only in the form of honey or treacle. The diet was nutritionally adequate.

The extremely low caries prevalence in the children of Hopewood House, in comparison with that of average Australian children of comparable ages, serves to illustrate that caries control can be achieved by dietary means. The diet employed would be unlikely to appeal to many, despite its economy and nutritional adequacy. There is evidence to suggest that these children did not continue with the diet after they left the home. (127)

The Vipoholm study (121) is another example of a type (c) investigation, where a large number of subjects were used to investigate the effects on dental caries of various carbohydrates, when added to a basic diet. The Vipoholm hospital is a large mental institution, with over 1000 patients, geographically, but not demographically representative of the Swedish population. It is organised into twelve separate departments, in each of which
LITERATURE REVIEW

the patients are isolated, and under constant supervision. The main purpose of the study was oriented towards dental caries, but many supplementary studies were also undertaken.

The dental research aims of the study were to answer the following questions:

"A. Whether, and if so, how, caries activity as studied under controlled conditions is influenced:

(a) by the ingestion, at meals, of refined sugar with only a slight tendency to be retained in the mouth (non-sticky form),

(b) by the ingestion, at meals, of sugar with a strong tendency to be retained in the mouth (sticky form, sugar-rich bread),

(c) by the ingestion, between meals, of sugar with a strong tendency to be retained in the mouth (sticky form, sweets, etc.).

B. Whether, and if so, how, dental caries activity is influenced by the omission of a variable proved to be capable of increasing caries activity.

C. Will any new carious defects arise if the consumption of sugar is reduced as far as is practically possible?"(121)

The six year period of the experiment was divided into approximately one year of preparation, when examination techniques were standardised and assessed, followed by a year of nutrition studies, where various supplements were added to the basic diet. The following four years were divided into two experimental periods.
LITERATURE REVIEW

In the first, (years three and four) an 1,800 calorie basic diet was provided, which was nutritionally adequate, but deficient in calories. Supplementary foods were added to this basic diet, to make up a 3,000 calorie complete diet. This same experimental procedure was used in the second experimental period (years five and six), except that a 2,700 calorie basic diet was used, and supplementary foods were added to make up a 3,000 calorie complete diet.

Over 400 patients were used as subjects, divided into one control and six experimental groups. Not all inmates could be used, as they had to consent to the experiment, be free of diseases requiring special diets, and have at least ten teeth. Caries scores were made on the Moulage system, and results analysed statistically. Checks were made on the amount of food not consumed by the subjects, with occasional spot checks on the amount of food consumed by individual subjects, and each subject’s eating habits were discussed with supervising staff at main dental examinations. Between-meal supplements were supplied to the patients at four fixed times each day, though patients chose the amounts they wished to eat on each occasion, and some swallowed the supplements whole.

The six experimental groups were constituted as follows: Control, Sucrose, Bread, Chocolate, Caramel, Toffee I and Toffee II groups. For one year, all diets were a standard 3,000 calories, with 350 gm. of carbohydrates at meals. For the next two years, the basic 1,800 calorie diet (containing 130 gm. of carbohydrate)
LITERATURE REVIEW

was supplied to all groups. The test foods were added to each basic diet to make up the 3,000 calories of the complete diet. In the Control group, the supplement was in the form of margarine. In the Caramel and Toffee groups, the supplements were supplied for consumption between meals. In the last two years of the experiment, similar techniques were used, with changes in the supplement, and in some cases, replacement of carbohydrate with margarine or sucrose solution.

Evaluation of the results was detailed, thorough and also subjected to statistical analysis. The subjects were geographically representative of the Swedish population, but highly selective with regard to sex, age and mental capacity. Not only were the subjects mentally defective and of high average age, but were mostly males, with poor oral hygiene, and a low caries experience at the beginning of the trial. These factors were considered in evaluating the findings, and it was concluded that in comparison with non-institutionalised Swedish adults, the subjects were more resistant to dental caries. It can therefore be assumed that the findings are understated, rather than overstated, with respect to the general Swedish population.

The general findings of the study were that "dental caries increased when the consumption of sugar in sticky form was introduced" and "decreased when such consumption was stopped". This "... constitutes convincing evidence that sugar increases dental caries activity in man". Also, "when sugar was consumed in
LITERATURE REVIEW

solution at meals, up to more than twice the Swedish consumption, no increase in dental caries activity was observed", and that "sugar exerts its caries-promoting effect locally in the mouth ..."
The following conclusions were suggested:

1. The consumption of sugar can increase caries activity.
2. The risk of sugar increasing caries activity is great if the sugar is consumed in a form with a strong tendency to be retained on the surfaces of the teeth.
3. The risk of sugar increasing caries activity is greatest if the sugar is consumed between meals in a form in which the tendency to be retained on the surfaces of the teeth is pronounced, with a transiently high concentration of sugar on these surfaces.
4. The increase in caries activity under uniform experimental conditions varies widely from one person to another.
5. Increase in caries activity due to the intake of sugar-rich food disappears on withdrawal of such foodstuffs from the diet.
6. Carious lesions may continue to appear despite the avoidance of refined sugar, maximum restriction of natural sugars and total dietary carbohydrates."(121)

A criticism sometimes levelled at the Vipeholm study is that the subjects were a special group of adults of below normal intelligence, living under institution conditions, and for these reasons, the findings may not be applicable to normal adults and
LITERATURE REVIEW

children. However, the Vipeholm conclusions with respect to between-meal eating have been confirmed in a study by Weiss and Trihart, (298) who studied the eating habits and caries experience of 783 rural and small town pre-school children in West Tennessee. Dental examinations were carried out on the subjects, and the parents questioned as to the types of foods eaten by the children, and the frequency of eating between meals. The foods eaten were predominantly sweet and sticky, and based on carbohydrate. When the caries experience was plotted against frequency of between-meal eating, it was found that the mean def scores for the children increased progressively from 3.3 for no between-meal snacks to 4.8 for 1, 5.7 for 2, 8.5 for 3 and 9.8 for 4 or more between-meal snacks per day, thus confirming that one of the conclusions of the Vipeholm study was valid for normal children.

The Vipeholm, and other similar studies serve to illustrate that while the composition of a food is related to its cariogenicity, the physical form of the food, and the pattern of eating of the individuals consuming it also affect is cariogenicity. Thus the cariogenicity of a food cannot be assessed solely on the basis of its decalcification potential, and because cariogenicity can vary according to how a food is consumed, the experimental conditions in the clinical evaluation of such foods must minimise these effects, or take them into account in interpreting the results.

Contemporary Western civilisations often exhibit moderate to high caries prevalence, and many investigators have used approach (c),
LITERATURE REVIEW

modification of the diet, not by changing the dietary pattern, but by adding various substances to the food, to influence caries. The most extensive of these have concerned the effect of addition of fluoride to drinking water and foods, and this approach to caries control has been proved to be effective. Another addition which has been tested is phosphate, and of all the various forms of phosphate tested on animals and shown to reduce caries, only three, dicalcium phosphate, sodium acid phosphate and calcium sucrose phosphate appear to have been tested on human populations. A formidable difficulty in such studies is locating and organising suitable experimental groups. Most commonly, homes and institutions provide the subjects, but isolated or primitive communities have been used, despite geographic inaccessibility.

Strålfors (275) studied the effects of dicalcium phosphate addition to the sugar, bread and flour eaten by over 2,000 schoolchildren aged nine years, over a two year period. He found a 50% reduction in caries in the first year, and a 40% reduction in caries in the second year. However, the fluoride content of the dicalcium phosphate addition was high in the first year, and this factor could well have influenced the effects observed, and cast some doubt on the validity of the results. Ship and Mickelsen (255) performed a similar experiment on over 700 schoolchildren in eight boarding schools in North and South Dakota. Half ate ordinary bread, the other half ate bread containing 2% dicalcium phosphate, but no difference in caries incidence was found over a three year
LITERATURE REVIEW

period. Averill and Bibby (8, 9) also tested the addition of dicalcium phosphate to food, using as their subjects schoolchildren in New York and Brazil. The New York groups were fed sugar and flour supplemented with 2% dicalcium phosphate, over a two year period. No pronounced caries-reducing effect was observed. However, during the two years, the age distribution of the groups changed markedly, because of subjects leaving the schools, which complicated the interpretation of the results.

In the second trial, two isolated rubber plantation towns in the Amazon basin, Fordlandia and Belterra, were selected on the basis of high caries prevalence and a controllable food supply. The diet consisted primarily of a carbohydrate, (mandioca), which provided 75% of the caloric intake of the groups. The diet contained much less than the recommended minimums for minerals, especially calcium. Baseline dental examinations were made, and despite difficulties concerning dispersion of the supplement in the coarse, granular mandioca, and problems of taste, a 2% dicalcium phosphate addition was made to the Fordlandia mandioca supplies, the other township, Belterra, serving as a control. Approximately 500 children from each town were examined, in the age range 7 - 17. Three dental examinations were made during the experiment, using trained personnel and standardised procedures. Blood samples and wrist radiographs were taken, salivary flow rates measured, and saliva calcium and phosphorus levels determined.

After 30 months, 40% of the initially sound teeth and 17% of
LITERATURE REVIEW

the initially sound surfaces of the teeth of the Fordlandia children had become carious, compared with 45% and 21% in Belterra. The authors stated that "no dramatic reduction in caries resulted", but that despite the fact that this was the third time that a dicalcium phosphate supplement had failed to produce a significant caries reduction in humans, "other phosphates ... may be more effective in humans, as they are in animals."(9)

A more recent study of the clinical effectiveness of a phosphate supplement to the diet is that of Stookey and his co-workers.(273) Five hundred children, selected at random from residences in Bloomington, Indiana were dentally examined, and assigned to control and test groups, balanced according to dental age and past dental caries experience. The children in both groups were then issued with four types of pre-sweetened breakfast cereals, those in the test groups receiving cereals identical with those of the controls, except that the test cereals contained 1% sodium acid phosphate. At the end of two years, repeat dental examinations revealed that children consuming the phosphate-enriched cereals had significantly less caries than did the children consuming the non-phosphated cereals. In similar studies on 527 adults between the ages of 16 and 62 and South Bend, Indiana,(273a) statistically significant reductions in dental caries were observed. In the first of these, the reduction (DMFS) was 40% and 20% for the two independent examiners. In the second, the reductions were 57% and 24% for DMFT and DMFS after six months, and 42% and 22%
LITERATURE REVIEW

after one year. The significance of these studies is that for the first time in any clinical study, a reduction in caries in adults was demonstrated by incorporation of a phosphate in the diet.

The fact that the supplements did not consistently reduce caries in the human clinical studies reported here demonstrates the difficulties involved in assessing the cariogenicity of foods and food-supplement combinations on human subjects, even when only one food or food-supplement combination is being studied. It is therefore unlikely that all the many potentially cariogenic forms of carbohydrate will ever be tested clinically on humans. However, it is possible to test a range of human foods on animal populations. Stephan\(^\text{(271)}\) has conducted a study on the effect of various human foods on rat caries, when they are incorporated in standard non-cariogenic and cariogenic rat diets, under controlled conditions. His findings are discussed in detail in Section VI of this work, where they are compared with the results obtained in the present study.

A study of particular interest is that of Harris and his co-workers\(^\text{(128)}\) on the effect of a calcium sucrose phosphate dietary supplement on caries in children. Calcium sucrose phosphate was first prepared in Germany in 1910 by Neuberg and Pollak\(^\text{(223)}\) and was used at that time as a substitute for calcium gluconate. The composition of this material varies according to the method of preparation, and the form of calcium sucrose phosphate used by
LITERATURE REVIEW

Harris and his group was specially prepared* for the study.

About 1,000 children living in various institutions in N.S.W. were selected as test subjects. Following dietary surveys and baseline dental examinations, the subjects were divided into matched test and control groups. Both the control and test groups consumed their usual diet, but the test group diet was modified by the addition of 1% by weight calcium sucrose phosphate to the sugar, flour, sweet spreads and biscuits, and slightly less than 1% to the bread and tinned fruit. The estimated daily intake of calcium sucrose phosphate per child was 4.3 gm.

The results at the end of the second year of the study indicated that the calcium sucrose phosphate addition demonstrated a cariostatic effect. They stated: "the average reduction in dental caries per child 5 - 8 years of age is 1.42 surfaces, 9 - 12 years of age is 1.97 surfaces and 13 - 17 years of age is 1.25 surfaces", but indicated that the results must be viewed only as a progress report.

In subsequent publications, Harris and his co-workers confirmed the caries-reducing effect of calcium sucrose phosphate (129, 130). Some difficulty was experienced by the investigators in evaluating the results because of "increased imbalance in age and decreased numbers in the groups after three years". These

* Colonial Sugar Refining Co.Ltd., Sydney, Australia (See Appendix D).
LITERATURE REVIEW

factors precluded placing a statistical significance on caries reduction figures obtained from the experimental group members from a fluoridated area. However, they felt that their results were consistent with overall benefit for fluoride and an additional benefit for the calcium sucrose phosphate additive. They also stated that: "in the age groups 9 - 13 years there are reductions for the Treatment group of

DMF teeth 15.3%
DMF surfaces 17.9%
DMF proximal surfaces 29.5%

and that the medical investigations showed no differences in the physical status and general health between the children of the Control and Treatment groups.

CARIES IN ANIMALS

The trials reported on human populations were often preceded by studies using animal populations, the majority of which concerned the cariostatic effect of fluorides, calcium salts and phosphates, but other substances, either applied to the teeth, or incorporated in the food or drinking water of the animals, have also been studied. These substances may be broadly classified into two groups: the first: substances which do not occur naturally in foods at the levels tested, and the second: substances which occur naturally in foods, or are added during preparation for consumption.

Fluoride could be classified as belonging to the first
LITERATURE REVIEW

group, although many foods, especially seafoods, often have a high natural fluoride content. The literature on the well-known cariostatic effect of dietary fluoride is voluminous, and is not considered in this work. Calcium salts and phosphates also belong to this group, and are discussed later in this review.

Antibacterial substances can also be considered in the first group. Mühlemann and his co-workers classify such substances into two main categories, those with antibacterial-antienzymatic effects, and those combining these effects with effects on enamel solubility. They refer to 69 other studies describing the cariostatic effect of various antibacterial substances in animal experimentation, and point out the difficulty of comparing the data, because of variation in the experimental conditions. They found that in Osborn-Mendel rats, antibiotics effective against gram positive organisms were effective as caries inhibitors, while the anti-mycotics, chemotherapeutics and disinfectants generally had little or no cariostatic effects. The same group also explored the possibility that a high salivary amylase content could degrade starch, and thereby increase its cariogenicity. No caries increase was observed when amylase was added to the diet, suggesting that saliva with a high amylase content would not enhance caries.

Many investigators have tested substances which could be classified in the second group. They are discussed under the following headings: simple carbohydrates, cereals and cereal
products, minerals, cocoa, milk and milk products, fats and oils, and human foods.

Simple Carbohydrates

Rosebury and Karshan demonstrated in 1939\(^{(244, 245)}\) that sucrose added to ground rice or corn diets in rats tended to increase caries, and in 1949, Shafer\(^{(250)}\) found that in otherwise identical diets containing 61% corn-starch, glucose or sucrose produced caries in hamsters, the severity of which was roughly in proportion to the solubility of the carbohydrate tested. Johansen\(^{(155, 156)}\) also reported that in the hamster, an increase in the amount of sucrose in the diet accelerated the rate of initiation of carious lesions, and speeded the progress of existing lesions. Ockerse and de Jager fed vervet monkeys different diets, and found that the one containing white bread and sugars caused significantly more caries than the one containing brown bread, raw vegetables and fruit.\(^{(231)}\)

Wynn and his co-workers found that two diets containing the same amounts of sucrose but differing in other respects did not initiate caries to the same extent, even when all the other experimental variables were controlled.\(^{(306)}\) In subsequent studies, this group varied the salt, yeast and vitamin contents of the diets, in an effort to determine the reasons for the difference in cariogenicity of these two diets, without success.\(^{(307)}\) They suggested that further work was necessary "to solve this perplexing problem". They continued the study, varying the vitamin and yeast
portions of the diets, and were once again unsuccessful in establishing a reason for the difference.\(^{(308)}\)

König commented on some anomalies in rat caries experiments, and in an extensive and carefully controlled series of experiments, investigated the cariogenicity of no sugar, fine sugar and coarse sugar, no corn, fine corn and coarse corn diets, with and without masticatory function, in Osborne-Mendel rats.\(^{(170)}\) Some difficulty was experienced with differing growth patterns, possibly stemming from food preferences in the animals. He found less sulcal lesions with corn, and less initial lesions with coarse particle diets. On the corn diets, non-function resulted in less caries, but on the sugar diets, non-function resulted in more caries. In discussing his results and those of others who investigated the relative cariogenicity of coarse and fine particle diets, he suggested that the duration of the experiment may be important vis-à-vis impaction of food into the fissures, progression of fissure lesions, force of mastication, and cuspal fractures. These findings emphasise the difficulty of comparing animal caries experiments in different laboratories.

Grenby stated that rats vary in their susceptibility to caries, and that changes in environment may alter the caries response. He found that when the sucrose portion of the Stephan 580 diet was replaced by glucose, the caries incidence decreased, and was reduced even further when raw wheat starch was used.\(^{(110)}\)

Miller employed a finely-ground rice diet to eliminate
LITERATURE REVIEW

particle size effects, and studied caries incidence following variations in the mineral salt and fat-soluble vitamin content of rat diets.\(^{(213)}\) Increasing the mineral salt content of the diet decreased caries, but varying the vitamin content had little effect. However, when part of the rice in the diet was replaced by sucrose, caries increased.

At this stage it was concluded by most investigators that sucrose was cariogenic. But caries susceptibility in experimental animals was not always reproducible, and it was thought that special diets were necessary to preserve a uniform caries susceptibility. However, as a result of a breeding problem in their laboratories, Shaw and Griffiths were obliged to feed their caries-susceptible Hunt-Hoppert rats a natural diet in place of the special semi-purified diet they thought essential for preserving the caries susceptibility of this strain.\(^{(253)}\) Despite this change, the anticipated decrease in susceptibility was not observed, nor was it observed with two commercially available natural diets.

By this time, some of the factors responsible for lack of reproducibility were understood sufficiently to permit the conduct of experiments comparing the cariogenicity of different foods under controlled conditions. Krasse implanted cariogenic streptococci in hamsters fed standard laboratory diets and Keyes diet 2000, both ground and powdered, and also with diets where the lactose or the sucrose was replaced by glucose.\(^{(182)}\) Few organisms were recovered from animals on the standard diets, but many with the 2000 diet.
LITERATURE REVIEW

He concluded that lactose favours, and sucrose greatly favours implantation of cariogenic streptococci in the hamster, and that substitution of either of them with glucose does not. In a subsequent paper, he reported the caries activity in hamsters on these diets, and found a high caries incidence with sucrose, and a very low caries incidence with glucose. (185)

From the above results, it appeared that the form of carbohydrate in the diet could be important in the carious process. Grenby noted that cooking swells and may rupture the starch granules of wheat flour, and might perhaps facilitate enzyme attack, or make the cooked starch more adherent to the teeth. When he tested this possibility in rats, he found very little caries with either the cooked or the raw starch, provided sucrose was absent. (111)

However, his in-vitro decalcification tests showed that cooking increased the ability of a fermented starch mixture to decalcify enamel. He concluded that despite its lack of inherent cariogenicity, starch might help bring sugars into close contact with the teeth, thereby enhancing the carious attack.

Strålfors has reported on the cariogenicity of many substances, and in one study tested diets containing refined and unrefined sugars. (279) He found that brown sugar and treacle "induced a much lower caries activity than pure sugar" (i.e. sucrose). Although calcium phosphate added to brown sugar increased the cariostatic effect, he concluded that the brown sugar and treacle effects were not due to their inherent phosphates.
LITERATURE REVIEW

ccontent, and furthermore, the factor or factors responsible were not destroyed by cooking, as coffee cakes containing the brown sugar also exerted an anticariogenic effect. He suggested fractionation of unrefined sugars to identify the substance responsible.

These observations by Strålforö were further investigated by König and Mühlemann, who suspected a particle size or feeding preference as an explanation for his results. With both programmed and free feeding, they found a high caries incidence with fine sucrose, and a low caries incidence not only with samples of the coarse brown sugar studied by Strålforö, but also with coarse refined sugar, suggesting a cleansing action of the coarse particles as the explanation for the low caries rates he observed. This explanation would not, however, be valid for Strålforö findings of lowered caries rates with the coffee cakes made from brown sugar, and with treacle in solution, unless it was a food preference effect.

The effect of sugars and sugar substitutes on dental caries in hamsters and rats was studied by Frostell and his colleagues, who found smooth surface and fissure caries with sucrose. But with dextrose, fructose, sorbitol, dextrose–fructose, maltose, starch and hydrogenated starch there was less plaque accumulation, less active progression of caries, and little or no new caries. An important observation in this study was that "the combination of fructose and glucose in amounts equal to those present in sucrose did
not have the pathogenic potential of the disaccharide", and
foreshadows subsequent suggestions that extracellular dextrans,
which are formed from sucrose (but not glucose or fructose), under
the action of certain streptococci, may play an important role in
the carious process.

In 1967, Green and Hartles studied the relative cariogenicity
of raw starch, cooked starch and sucrose, in various combinations,
in the rat. (109) Animals consuming the sucrose diet had most
caries, and those consuming the uncooked starch diet, least caries.
The authors suggested that their results indicated that "it is
not necessarily the quantity of carbohydrate in a diet or merely
the ease with which it can be converted into acids which governs its
cariogenicity", and that "it emphasises the possible importance of
interaction between carbohydrates."

König has summarised the results of his own studies and those
of others, and concluded that sucrose is highly cariogenic, while
starches are not, and that the slight differences in cariogenicity
observed between refined and unrefined flours are negligible from
a practical viewpoint, "compared with the highly destructive effect
on teeth of frequent and excessively high intake of sugar." (174) He
also stated that bread and other baked goods "do not impair dental
health very much unless they are garnished with sweet spreads or
supplemented with sugar".

Grenby has also reviewed many animal caries experiments. (113)
He refers to the use of fine diets, to avoid mechanical tooth damage,
and high sucrose diets fed to caries susceptible strains of animal, to obtain reproducible caries scores. He indicted sucrose and glucose as the main cariogenic agents, and states: "cereal products seem to be less cariogenic than sugar". He also reported experiments where he fed wheat starch, other wheat products and sucrose to rats, and found that wheat starch was non-cariogenic, and that white or wholemeal wheat flour and bread were of low cariogenicity, unless the diet also included sucrose. Experiments on the starch and gluten components of bread led him to suggest that flour and cereal products, although far less cariogenic than sucrose, may promote caries by adhering to teeth, thereby facilitating the action of sucrose.

Cereals and Cereal Products

Numerous animal studies have demonstrated that simple sugars, especially sucrose, increase caries when included in the diet, but studies have also been conducted to determine whether some cereals exert a specific anti-cariogenic effect. Buttner and Muhler reported that cariogenic diets containing 5% and 10% oat hulls reduced the incidence of caries when fed to Sprague-Dawley rats. They suggested that the anticariogenic factor responsible was a nitrogenous base conjugated with palmitic acid.

Dodds tested wheat, corn, rice and oats, and proprietary cereals made from them, for anti-cariogenic effects when fed to rats. Littermates were not used, and weight gains on the different diets were not comparable, despite attempts to equalise
LITERATURE REVIEW

the protein contents. She conducted further experiments on litter-
mated animals, and obtained inconsistent results with respect to
smooth surface and fissure caries, which she suspected were a
result of differing food particle size and hardness. She did
state, however, that there was no indication that the processing of
the cereals increased their cariogenicity.

Vogel and his co-workers conducted experiments on oat hulls,
oat hull extracts and fatty acids, and found that the hulls reduced
caries significantly in the cotton rat, while the fatty acids did
not.\(^{(293)}\) The extracts were also anti-cariogenic, but not as
much as the hulls themselves. Madsen and Edmonds found a
cariostatic effect with oat hulls, even when fed for only 10 days
to weanling rats, the reduction persisting to the end of the 98 day
experimental period.\(^{(205)}\) They offered no explanation for the
effect.

Further data on oat hulls were provided by McClure, using
two cariogenic diets, 585, producing mainly fissure caries, and
374, producing mainly smooth surface caries, in the white rat.\(^{(199)}\)
He found a significant inhibition, even when the hulls were finely
ground. From his own studies and those of others he concluded that
neither the physical form nor the calcium and phosphate contents of
the diets correlated with the caries inhibition, that a caries-
protective factor may be removed from certain cereal foods during
refining, and that this factor may be an organic phosphate. He also
stated that he found a sugar phosphate (fructose 1 - 6 diphosphate)
to be highly cariostatic, and suggested that an effective
cariostatic agent "might be isolated as a natural constituent of
certain cereal grains or seed hulls, or in the by-products of
sugar refining."

Further studies designed to identify the factor or factors
responsible for the cariostatic effect of oat hulls were conducted
by Thompson and his colleagues. They summarized their studies
from 1958 to 1964 on the effect of a number of different organic
compounds on caries in the cotton rat, including amino and
phenolic acids, polyphenols and antioxidants. Some compounds
reduced caries, some had no effect, and one increased caries. The
authors warned of the dangers of extending the results beyond the
experimental conditions under which they were obtained, and listed
the following factors as being important in evaluating animal
caries studies:

a. type of caries being studied
b. species and strain of animal used
c. length of the assay period
d. method of administration
e. type of diet used

Despite the apparent difficulties involved in obtaining
similar results in different laboratories, it has been achieved.
Konig and Grenby have evaluated the cariogenic potential of
wheat grain fractions and sucrose mixtures in laboratories in
Zurich and St. Albans, using different strains of animal, different
LITERATURE REVIEW

basic diets and different methods of scoring caries. The conclusions from the two sets of experiments were in general agreement that diets containing sucrose "gave rise to a high incidence of caries, while wheat starch, white or wholemeal flour, bran and fine offals were all of low cariogenicity." They suggested that sugar may be able to penetrate the plaque better than starch, and any acids produced from it less easily washed away. Grenby has reported separately the results of further experiments using white and wholemeal flour and bread, and verified that the caries observed was related to the sucrose content of the diet\(^{112}\), rather than the flour or bread content. He did not observe any difference between white and wholemeal flour and bread, none of which was cariogenic under his experimental conditions. Grenby also studied the growth and breeding patterns of the two strains of animal used in the König and Grenby study, when fed high sugar diets in the same laboratory, and found strain differences in caries susceptibility.\(^{114}\) He concluded that "susceptibility (or resistance) to dental caries is an inheritable characteristic, which can be modified by environmental influences."

Ishii and his co-workers have evaluated the effect of ad libitum feeding to rats of breads with cheese or jam, plain biscuits, sweetened biscuits and chocolate-containing biscuits, in producing caries.\(^{141}\) The bread and cheese was of low cariogenicity, but the bread and jam of high cariogenicity. Biscuits made with white flour and sucrose were more cariogenic than those made with wholemeal flour.
LITERATURE REVIEW

and sucrose, the latter being more cariogenic than sucrose-free biscuits. Both the biscuits containing chocolate were highly cariogenic. They concluded that both sucrose content and physical properties of the foods were responsible for the effects they observed, and that the fat content of the milk chocolate failed to influence its cariogenicity.

There appear therefore, to be reasonable grounds for assuming that some cereals at least can influence the caries pattern in animals, but that the effects can be masked by the effects of sucrose. It is apparent also that when comparing animal caries experiments, the different experimental conditions must be taken into account.

Minerals

Of all the foods and food additives tested on animals for their effects on caries, calcium and phosphorus compounds appear to be the most intensively studied. In 1931, Klein and McCollum reported an increase in caries in the rat when the phosphate content of the diet was reduced.\(^{(164)}\) This finding was confirmed by Agnew and co-workers,\(^{(1)}\) Blackberg and Berke,\(^{(23)}\) Klein and Shelling,\(^{(165)}\) Rosebury and Karshan\(^{(244)}\) and Shelling and Asher.\(^{(254)}\)

In 1953, Nizel and Harris were able to demonstrate extensive reductions in hamster caries when the mineral content of the diet was doubled by the addition to it of the 'natural ash' of the diet.\(^{(228, 229)}\) McClure reported a significant reduction in caries induced in rats by a wheat cereal diet, when it was fortified with
milk ash or similar additions, and Wynn and his co-workers were able to show a decrease in rat caries as the phosphorus content of the diet was increased. McClure has also reported experiments on the effect of addition to the diet of a variety of sodium and calcium phosphates, sodium phytate, calcium lactate and calcium gluconate on rat caries. He found that all the compounds except calcium gluconate exerted a cariostatic effect, and suggested that the rat, being coprophagic, could recirculate faecal phosphates through the oral cavity, and thus complicate interpretation of the results if applied to the human situation. Strålfors has also demonstrated experimental caries reduction in hamsters by the addition of tribasic sodium phosphate to the diet.

Many other workers have conducted similar trials, using a variety of laboratory animals and a wide range of diets and calcium and phosphorus additions. Nizel and Harris have summarised the results of 100 of these trials, and in only 10 of these was it reported that the experimental addition failed to reduce caries. In most of the others, the reductions observed were about 40%, though the range was extreme. Nizel and Harris stressed that the development of experimental caries is influenced by the species, strain and age of the animal, the oral flora, diet, water supply, length of the experiment, housing conditions, and methods for assessing the caries scores. They also reported that in none of the trials "did the phosphate supplement produce an increase in dental
LITERATURE REVIEW

caries" that "higher cariostatic effects were reported when caries was scored in terms of smooth surface caries than as pit and fissure caries", and that "in most of the experiments the phosphate was cariostatic even though it was added to diets already nutritionally adequate in phosphorus content. Nizel and Harris also added "it is remarkable that ... the conclusion is essentially unanimous that phosphates have significant cariostatic properties when added to the diets of rodents, ... despite the wide diversity of experimental conditions employed."

Other reports on animal experimentation have been published since this review by Nizel and Harris. In 1964 Dodds and Lawe reported a study designed to establish the reasons why sodium hydrogen phosphate is cariostatic, but calcium hydrogen phosphate is not, in wheat diets. (74) McClure had suggested that the sodium chloride content of the diet might be the factor responsible for the inconsistency, and this suggestion was tested by Dodds and Lawe using two forms of phosphate and eleven diets. They concluded that in their experiments, the cariogenicity of high cereal diets was related to low sodium content.

Gustafson and his colleagues investigated the effects of calcium salts on hamster caries, (120) and found, unlike McClure, (197) that calcium gluconate was cariostatic, and furthermore, that calcium chloride was even more effective. They also stated that it is doubtful whether calcium carbonate has any caries-inhibiting effect, once again differing from McClure. They concluded that "in
LITERATURE REVIEW

cariogenic diets with low mineral content, the level of Ca is the
determining factor in the development of caries."

McClure has also reported cariostatic effects with ammonium
hydrogen phosphate, beta glycerol phosphate, fructose 1-6 diphosphate
and sodium phytate. Of particular interest is the caries
reduction with the sugar phosphate, since a similar sugar phosphate
has since been shown to have an anticariogenic effect in
humans. He concluded that "further evaluation of the
cariostatic potential of phosphates, organic and inorganic, for the
control of human caries warrants serious consideration, with
emphasis on appraisal of the 'protective factor' presumably lost
during the refining of sugar and in the processing of certain factors."

In 1965, Harris and his co-workers attempted to clarify the
rather confused picture appearing from more than 100 experiments
evaluating more than 20 different phosphate compounds, by testing
three different phosphate radicals, in various proportions, but
with the sodium content of the test diets held constant. A
doubling of the phosphorus content of a cariogenic diet exerted a
cariostatic effect. Trimetaphosphate was the most strongly
cariostatic, followed by metaphosphate, then orthophosphate. They
found that when fed together, the cariostatic effects were additive
except with trimetaphosphate, which exerted a synergistic effect.
No explanation was offered for the mode of action of these compounds.

In a further study, Harris and co-workers investigated
possible pathways for the cariostatic effects of phosphates by
comparing the effect of potassium dihydrogen phosphate when added directly to the diet, or embedded in lard with a melting point above mouth temperature. \(^{(133)}\) The unexpected results were that the diet containing lard was more cariogenic than the control diet, but that the diet containing phosphate embedded in lard had a cariostatic effect. They felt that the suggested cariostatic effect of fats is a result of replacement in cariogenic diets of less cariogenic or even cariostatic foods, rather than the effect of fats. To explain their results they suggested that because it was solid at mouth temperatures, lard could occlude fissures, and thus set up 'areas of cariogenesis', and if containing phosphates, set up 'areas of cariostasis'. On the other hand, they stated that it was also possible that the lard slowed down the rate of systemic absorption of phosphate sufficiently to permit a greater total retention. This 'attenuated absorption' theory they advanced as a possible explanation for their previously reported finding \(^{(250)}\) that the cariostatic effectiveness of five types of sodium phosphate "was inverse to their water solubility."

Dawes and Shaw also attempted to identify the mechanism of action of dietary phosphates in reducing caries. \(^{(68)}\) They analysed the salivary flow rates and calcium phosphate and bicarbonate content of the saliva and serum of rats fed a cariogenic diet, alone and containing sodium hydrogen phosphate and sodium phytate. Both additives reduced caries, but the control and experimental saliva and serum calcium and inorganic phosphorus
LITERATURE REVIEW

values were similar, suggesting a local, rather than a systemic effect. They suggested a plaque buffering effect, a high local concentration of phosphate ions, or a remineralisation of early lesions as possible mechanisms for the caries reduction. They cited the low inorganic phosphate content of rat saliva in comparison with human saliva as a possible explanation for the effectiveness of phosphate supplements in controlling rat caries, and their comparative ineffectiveness in controlling human caries.

However, phosphates have been shown to reduce caries in humans. Stookey and his colleagues have reported an anticariogenic effect with phosphated cereals in humans, (273) and the cereals they used were also tested by them on rats. (272) In the rat studies, they employed four sugar-coated cereals, and a variety of unsugared cereals, with and without a sodium dihydrogen phosphate addition. They found that caries was reduced significantly "in every instance in which the phosphate was added to the different cereals, irrespective of whether or not they were presweetened, and no harmful effects to the rats resulted from the phosphate addition."

There appears to be little doubt that many calcium and phosphorus compounds can reduce caries in rodents, even when added in comparatively small amounts, and at low levels these additions have little effect on the health of the experimental animals. Experiments designed to determine the mode of action of these compounds in reducing caries have been inconclusive. Nizel and Harris
LITERATURE REVIEW

state that the effect "appears to be largely a local action on the
tooth, either as the phosphates pass through the mouth, or as they
return to the mouth in the saliva", and that "it appears to be
exerted on the structure of the enamel, possibly by an initial
demineralising and subsequent remineralising process, producing
changes in morphology as well as surface lustre." They also
stated that "the adhesiveness or retentiveness of the phosphates
on the surfaces or in the pits and fissures of the teeth seems
important."(230)

Bibby and Averill suggest that the mechanism is unlikely to
be simple, and theorise:

(a) "Phosphates become incorporated in the plaque, and buffer
acids that are formed ... or "provide common ions that retard
tooth decalcification."

(b) "Phosphate at the tooth surface could add to the acid
resistance of the enamel surface, or contribute to its
recalcification" ...

(c) "Phosphates may influence the types of acids elaborated,
producing greater percentages of types with lower
decalcifying ability."

(d) "The increased phosphorus in the food could result in a
saliva with a higher phosphorus content, thus strengthening
buffering and common ion effects, and resulting in a
prolonged recalcifying effect on the enamel surface."(21)

Few animal caries experiments have been conducted on minerals
LITERATURE REVIEW

other than calcium and phosphates, although some studies on the effect of molybdenum in the diet have been reported. Jenkins has reviewed the subject, and concluded that on the basis of human epidemiological studies and some animal experiments, molybdenum may have an anticariogenic effect when present in the diet at low levels. (152) The results of the animal experiments were difficult to compare because the complicated chemistry of molybdenum made it difficult to establish which forms of molybdenum were used in the experiments. In addition, some of the molybdenum concentrations in the diets were very high, and possibly unpalatable to the animals. These complications led him to state that the anti-caries action of molybdenum "is reasonably, but by no means conclusively established."

Cocoa

Strålfors has conducted numerous studies on the anticariogenic properties of cocoa, and in a series of papers, reported on the effect of cocoa, cocoa fat and butter, water extracts of cocoa, purine derivatives, tannin containing materials, and milk and dark chocolate, on caries in hamsters. (276–281) These studies were prompted by the Vipelholm findings for chocolate, which resulted in less caries than expected on the basis of its sugar content and oral clearance times, suggesting that chocolate might contain caries-inhibiting substances. To test this hypothesis, Strålfors fed hamsters cariogenic diets containing 20, 10, 5 and 2% whole cocoa powder, and observed caries reductions of 84, 75, 60 and 42%
LITERATURE REVIEW

respectively. He found that defatted cocoa powder was more effective than whole cocoa powder, and that 15% cocoa butter in the diet increased caries. Despite differences in the control and experimental diets, and nutritional deficiencies, and despite Kinkel and Cremers' conclusions that the mineral content of cocoa was the most likely explanation for the reduction, Strålfors concluded that his experiments "clearly point to the conception that the defatted cocoa powder contains the substance or the substances capable of inhibiting caries." He then subjected cocoa to various extraction procedures and identified two cariostatic factors in the non-fat part of cocoa, one water-soluble, the other sparingly water-soluble. Other extraction procedures, and further tests on various allied substances, including xanthine, vanillin and tannin-containing substances led him to conclude that the caries reductions with cocoa were the result of a factor or factors contained in it. His tests on milk and dark chocolate supported his conclusions, as the caries reductions he observed when these were fed to hamsters were similar to his previous results, when considered with respect to their cocoa powder content. Although this work has not been verified by others, there seems little doubt that cocoa does exert an anticariogenic effect in hamsters. When consumed as chocolate by humans the presence of sucrose may alter the picture entirely. This possibility is supported by the findings of Ishii and his co-workers that biscuits containing chocolate are cariogenic in comparison with other forms of bread
and biscuits, whether sweetened or not, for both fissures and smooth surfaces, when fed ad libitum to rats. (141)

Milk and Milk Products

In a carefully controlled experiment using two generations of littermated animals, Shaw and his co-workers studied the developmental, pre- and post-eruptive effects of milk and milk products in 600 rats. (252) The levels of addition to the diet were chosen to approximate human consumption, and all caused major reductions in dental caries, but only on a post-eruptive basis.

Green has found that otherwise identical high sucrose diets containing casein or simulated skimmed milk powder were less cariogenic than those containing skimmed milk powder. (108) The difference did not appear to be related to the protein, fat or carbohydrate content of the milk, and he suggested that skimmed milk might form a more sticky mixture in the mouth. He also suggested a taste preference for the skimmed milk, resulting in increased oral clearance times, and frequency of eating, thus promoting caries.

Fats and Oils

In 1939, Rosebury and Karshan reported experiments testing the effects of vitamin D+ corn oil, parrafin oil, olive oil, cottonseed oil, hydrogenated cottonseed oil and lard added to rice-dextrin-spinach or corn-sucrose-spinach diets, on caries in rats. (245) The vitamin D reduced caries significantly, as did the fats, but to a lesser extent. The authors suggested that free rather than bound oil was the effective factor, and that the effect "is
LITERATURE REVIEW

exerted locally in the mouth rather than systemically."

Several workers cited in this review have reported caries reductions when fat or oil is incorporated in cariogenic diets in animals. \((59, 119, 194)\) A more recent report is that of Green and Hartles, who tested vitamins and groundnut oil, separately and combined, when added to a cariogenic diet, for their effect on rat caries. \((107)\) They found that the oil was significantly cariostatic, regardless of the fat-soluble vitamin content. The absence of water-soluble vitamins also reduced caries. However, in some experiments, the animals were 'distinctly unwell' and this may have influenced the results.

The most obvious general conclusion which can be drawn from the animal caries experiments reviewed here is that such studies are difficult to standardise and control. Many of the investigators mention this problem specifically, and stress the difficulties of comparing studies in one laboratory with those in another, even when conducting similar or even identical experiments. The anomalies arise from a number of sources. The experimental animal, most commonly the rat, varies in its susceptibility to caries, according to the strain being employed. This variability can be controlled to some extent by careful inbreeding, and close attention to the manner in which the littermates are distributed among the experimental groups. Further variables arise from the housing of the animals. Rats, in particular, are coprophagic, and the caries pattern observed may differ according to the caging methods
used. The periods for which the animals are studied can also give rise to variables in otherwise identical experiments. Most experiments commence with weanling animals, and the progress of caries is studied for as short a period as 14 days, or for 120 days or longer. Since the nature of the carious attack varies with the age of the animal (because of tooth eruption and progress of lesions), comparison of experiments conducted for different periods is not always valid. In addition, there are several different methods used for scoring the caries, each placing its own emphasis on the caries picture.

A variable which caused much confusion for a number of years in animal caries experiments was the nature of the diet. Some diets were found to be cariogenic only when they included coarse particles, while others were cariogenic when finely ground. Other normally cariogenic diets were sometimes found to cause less caries when they included substances which by their detergent or abrasive action tended to keep the teeth clean, even though the substances had no intrinsic cariostatic properties. Thus some experiments designed to compare the effects of one dietary supplement with another indicated cariostatic effects which were related to the particle size of the supplement rather than the supplement itself. Similarly, diets in which the incorporation of a test substance was accompanied by removal of other dietary components essential to the good health of the test animals sometimes resulted in a caries pattern which was the result of a
change in frequency of eating and drinking, and rate of growth, because of poor appetite and health, rather than a direct effect of the test substance. In addition, some test substances are in themselves unpalatable, and cause the animal to change its eating pattern, thus altering the nature of the caries attack.

In the more recent animal caries studies, many of these variables have been controlled, or allowance has been made for them in interpreting the results, and it is now possible to draw some definite conclusions as to the effect of certain dietary supplements on rodent caries. When sucrose is included in a diet in sufficient quantity, caries will be enhanced, while if sufficient sucrose is removed from a cariogenic diet, caries will be reduced. When included in some cariogenic diets, unrefined cereals, notably oat hulls and allied substances, reduce caries, but the majority do not. Raw and cooked starches are not noticeably different in their cariogenic potential, which, furthermore, is low, in comparison with sucrose. A wide variety of calcium and phosphate compounds will reduce caries, even when incorporated in the diet at comparatively low levels, and do not generally affect the animals' rate of growth or health, even when incorporated in the diet at comparatively high levels. Conclusive evidence as to the mode of action of these compounds has not yet been definitely established in rat experiments, but it is unlikely to be a systemic effect, since caries reductions were observed even when the test and control animals exhibited similar calcium
LITERATURE REVIEW

and phosphorus levels in the saliva. Phytates and sugar phosphates have also been found to reduce rodent caries. Milk products reduce caries, the effect being post eruptive only. Cottonseed, corn and peanut oils and some fats also reduce caries. These general conclusions must be considered as applying to rodents, when the material under test is added to an otherwise cariogenic diet. The cariogenic effects may not be of sufficient magnitude to be of significance in human diets, which are usually much more varied than rodent diets.

A particularly interesting study on the effect of human foods on rat caries has been conducted by Stephan. In an extensive series of tests on over 50 foods, he obtained results which support the above conclusions in some cases, but do not in others. His findings are discussed in greater detail later in this work. In this context, his study is of interest because it represents a comprehensive analysis of the effects of many different human foods on caries in the one group of experimental animals, and while his results may not be comparable with those of others testing similar foods, for the reasons already enumerated, the caries scores with the foods he tested can be compared with each other. Another area of interest in his study is his employment of both non-cariogenic and cariogenic diets as controls. Most other animal studies employ a cariogenic diet, and assess additives for their ability to reduce caries. Stephan's results showed that very few of the foods he tested failed to induce
LITERATURE REVIEW

caries when included in his non-cariogenic diet, and it was these foods which also reduced caries in the cariogenic diet. All the other foods he tested were cariogenic when added to the non-cariogenic diet, and with two exceptions, increased caries when added to the cariogenic diet.

Many of the foods shown in Stephan's results to be cariogenic have been found by some workers to be cariostatic, and furthermore, their findings have often been verified by others, suggesting that perhaps less weight should be placed on Stephan's results, in comparison with those of the others. Yet his study clearly indicts sucrose as being cariogenic, and his ranking of foods in increasing order of cariogenicity in his tests would appear, intuitively, to be reasonable, with dry, abrasive, non-sticky and relatively insoluble foods of low cariogenicity, and sticky, sweet, readily soluble foods of high cariogenicity. It is clear that our knowledge of the food-caries relationship is far from complete, and it is equally clear that foods or additives which reduce the cariogenicity of a cariogenic diet may cause caries in a non-cariogenic diet. It might be, then, that foods or additives reported as having an 'anticariogenic' effect have, in fact, merely a less cariogenic effect than the foods they replace.

IN-VITRO DECALCIFICATION EXPERIMENTS

Some of the variables associated with human and animal experiments on foods can be reduced or eliminated by conducting in-vitro experiments designed to assess potential cariogenicity.
LITERATURE REVIEW

This is most commonly done by exposing tooth mineral or calcium phosphate to the material being tested, and measuring the dissolution occurring, either in fermented mixtures or acid buffers. In this approach the elimination of some variables is replaced by uncertainty as to the significance of the in-vitro findings when applied to the in-vivo situation, and for this reason, such studies assess the 'decalcification potential' of foods rather than their 'cariogenicity'.

To process of dissolution of tooth mineral in acids has been the subject of a number of investigations, and is discussed in Appendix B of this work. In food-saliva mixtures, the process is not always a simple dissolution, as Blackwell and Posdick demonstrated. (24) They found that sucrose at a concentration of 0.5M and 1.0M in 1.0M acetate buffer depressed the solubility rate of human enamel powder shaken for four hours at a pH 4.5, 5.0 and 5.5. They offered two explanations for the effect, one, that the sucrose increased the viscosity of the solutions, thereby depressing the solubility rate, and the other, that the sucrose was attracted to the enamel particle surfaces, thereby protecting to some extent against dissolution.

Hills and Sullivan studied enamel dissolution in citrate and lactate buffers at various pH levels, with and without calcium phosphate additions, and found no dissolution in buffers saturated with calcium phosphate in the pH range 7.0 - 4.0.(138) They concluded that Enright's 'critical pH' of 5.0, below which he thought
LITERATURE REVIEW

enamel would be dissolved in the mouth,\(^{(79)}\) was not valid for solutions saturated with calcium phosphate, and that enamel could dissolve, even at pH 7.0, if the buffer contained no calcium phosphate.

Buonocore and Sommer studied the effect of amino-acid addition on enamel in acetate, lactate, citrate and pyruvate buffers at pH 4.0.\(^{(43)}\) They found that aspartic acid reduced dissolution. Other aspects of this work were studied in detail by Koulourides\(^{(177)}\) and Apostolopoulos.\(^{(6)}\) Koulourides found that dissolution rates of enamel and dentine could be affected considerably by tribasic, less so by dibasic and not at all by monobasic organic acids. The effect could be an increase or decrease in solubility, according to the pH and the organic acid tested. He postulated a concentration of acids at the enamel surface, or a surface-bound complex, to explain his observations. Apostolopoulos studied the dissolution rates of enamel, dentine, and bone in acetate buffers in the pH range 3.5 - 5.5, and found that the dissolution rate was dependent on the mesh size of the powders, solid/solution ratio, and common ion effects. Powders with a high organic content were less soluble, especially at low pH.

Leach has studied the solubility of finely powdered enamel and dentine in acetate buffers, and noted that results obtained by others for similar types of experiments were often at variance. He studied decalcification at equilibrium, and the relationship between dissolution and amount of mineral initially present, acid strength,


**LITERATURE REVIEW**

pH and presence of fluoride. He found that equilibrium was reached rapidly, and that the solubility varied with the amount of mineral present. At fixed solid/solution ratios, solubility was related directly to the molarity and indirectly to the pH. He concluded that because solubility is dependent on several interrelated variables, "the pH of the acid-mineral solution does not necessarily indicate the dissolution that has taken place or is about to take place."

Jenkins and Smales studied the influence on enamel solubility of substances in plant products suspected of exerting a protective action against dental caries. They tested alcohol and water extracts of various plant hulls, offals and cocoa for their effect on the dissolution of tricalcium phosphate in pH 5.0 acetate buffer, and for their effect on acid production in inoculated nutrient broth- and saliva-glucose mixtures. All aqueous extracts tested reduced enamel solubility, but only aqueous pecan hull extract inhibited bacterial growth. Alcoholic extracts of oat hulls and wheat bran inhibited bacterial growth. They concluded that some unrefined foods contain substances which reduce enamel solubility, and, based on the relative solubilities of antibacterial and dissolution-reducing substances, they suggested that the caries reductions obtained with unrefined foods in animal experiments are more likely to be the result of a solubility effect than an antibacterial effect.

Grenby has also employed acetate buffers containing calcium
phosphate to assess the dissolution-reducing effects of grain extracts and associated substances. He found that ether-alcohol extracts of wheat bran and sodium phytate reduced enamel dissolution. The results with commercial phytin were difficult to assess because of the release of large amounts of calcium from the phytin, which obscured the dissolution. Calcium and phosphate precipitation were eliminated as an explanation for the effect, which Grenby suggested might involve "the absorption or interaction with phytate at the surface of the enamel or calcium phosphate particles."

Whole teeth, rather than enamel or calcium phosphate powders have also been employed to study enamel dissolution. Wynn and his co-workers found that succinic acid removed more calcium and phosphorus, and much more magnesium, from whole teeth than did lactic acid. Kostlan and Krejsa conducted similar experiments using formic acid, veronal, glycine and borax buffers containing various concentrations of sucrose and glucose, and found that these sugars did not affect demineralisation to any significant extent.

Mühlemann assessed the decalcification occurring when whole teeth were agitated in various buffers at 37°C for various intervals, using surface replica techniques, light and polarised light microscopy, and calcium and phosphate determinations. He found that agitated buffers produced surface breaks after 30 minutes. The presence of sucrose retarded the progress, but did not alter the pattern of dissolution. The addition of 1-5% gelatin resulted
LITERATURE REVIEW

in subsurface demineralisation, in place of surface etching. He stated that at low pH, and in agitated or constantly renewed buffers, etching occurs, while with high viscosity or stagnation, white spot formation results.

Koulourides and his co-workers have described techniques for rehardening the enamel of human teeth softened by exposure to 0.001M acetate buffers at pH 5.5.\(^{(178)}\) The Knoop hardness could be partly restored after 8 days exposure to metastable calcium phosphate solutions, especially in the presence of fluoride. Using these techniques, Koulourides and Reed investigated the effect of various combinations of calcium, phosphate and fluoride ions on softening and dissolution.\(^{(179)}\) They employed synthetic bacterial plaques supplied with culture media, and acetate buffers at pH levels similar to those developed in the synthetic plaques. They found that with plaque, only combinations containing fluoride protected against softening, but in the buffers, calcium and phosphate, calcium and fluoride, and calcium, phosphate and fluoride all provided protection. They discussed the difficulty of demonstrating small differences in their results, because of individual tooth variations, unless large numbers of teeth are used, or the effect under test is so strong that individual tooth variations do not influence it.

Simple buffer experiments do not take into account the effect of saliva and its variables, and many experiments have been conducted where the enamel dissolution taking place under the action
of acids produced by fermentation of foods with saliva is measured. Osborne employed this technique in 1937,\(^{(233)}\) and in subsequent studies, investigated the effect of organic and inorganic calcium and phosphorus additions on the decalcification patterns.\(^{(234)}\) His conclusions were that if flour contained "more of the calcium and phosphorus of the whole berry" caries would diminish, that if sugar "should have added to it the calcium and phosphorus of the original cane juice" a greater caries reduction would result, and that this approach "offers the best line of attack upon the disease of dental caries." These conclusions have since been supported by others.

Tullar studied the rate of acid production from sucrose and glucose crystals added to small samples of plaque containing methyl red indicator.\(^{(292)}\) He found that a pH of 4.0 - 5.0 was reached under his experimental conditions in from 8 to 281 seconds at room temperature, but found little correlation between DMF scores and the time required to reach a low in-vitro plaque pH in the 31 subjects he tested. This rapid fall in pH of plaque was also observed by Ludwig and Bibby who tested seven solid and three liquid carbohydrate foods on plaque pH in five subjects.\(^{(192)}\) With some foods the plaque pH fell rapidly, often to below 5.0 in 5 - 10 minutes, rising slowly to the resting plaque pH of 7.0 after 30 minutes. The lactic acid level in saliva generally correlated with the pH falls. In a subsequent paper, they attempted to assign caries potentials to foods, by measuring their retention and acid-producing ability when fermented with whole saliva.\(^{(192)}\) Difficulty
LITERATURE REVIEW

was experienced in measuring retention, with variations being observed from time to time, in the same and in different individuals. In addition, some foods were retained mainly on the teeth, and others on the tongue and mucous membranes. They also observed that acid production did not seem to depend on the food's carbohydrate content.

In 1959, Jenkins and his co-workers (146) discussed reports on the epidemiology of caries and the laboratory studies of Osborne and his group (233) concerning the effect of refined and unrefined carbohydrates on caries and enamel decalcification. They then described some experiments they conducted to investigate the ideas generated by Osborne and his group, quantitatively, and with some of the experimental variables controlled. The method used was to shake enamel, dentine or calcium phosphate powders with water, saliva and the food being tested, for 24 hours, and determine pH, titratable acidity and calcium and phosphate concentrations at various intervals. Jenkins and his group verified Osborne's finding that the unrefined flours, and bread made from them, dissolved less enamel or calcium phosphate than white flours or bread, when fermented with saliva. They stated that the effect could not be attributed to calcium or phosphate release from the brown flour, or to pH, acid production or buffering differences. They further found that water extracts of brown flour lowered enamel powder and whole tooth solubility, and concluded that the effect may be associated with the phytate and other organic phosphate content of
brown flour. In a further paper, Jenkins and his group reported similar studies on sucrose, three types of brown sugar, treacle, golden syrup, crude cane juice and honey.\(^{146}\) In addition, they investigated the effect of various sugar solutions on enamel dissolution in 1.0M, pH 5.0 acetate buffer. They found that fermented cane juice- and treacle-saliva mixtures both dissolved less calcium phosphate than did a sucrose-saliva mixture, a similar but smaller effect being observed with golden syrup. When the calcium content of the unrefined sugars was removed, the dissolution-reducing effect was eliminated with golden syrup, and reduced with cane juice and treacle. The brown sugars and honey actually increased dissolution in the saliva tests, but did reduce dissolution in acetate buffers. They suggested that the strong buffering ability of unrefined sugars contributed to the effect they observed, but could not account for it completely. They discussed the clinical significance of their findings and concluded that "the idea of protective substances in unrefined foods is worthy of further study", and that the experiments "suggest the possibility of the addition of protective substances to refined sugars or starchy foods."

Some difficulty was encountered by Jenkins and his group in determining enamel dissolution in molasses-saliva mixtures, using conventional chemical analysis. This difficulty arose because the initial calcium content of molasses is high, and the increase in calcium concentration as a result of enamel dissolution 'was very small' in comparison, and their calculations had to be based on
phosphate analysis alone. There is, in addition, the further complication that fermentation may release calcium or phosphate from foods containing these substances, which could lead to erroneous conclusions concerning the degree of actual enamel dissolution which had taken place.

In an attempt to overcome these and some other difficulties associated with decalcification experiments, Beck and his colleagues investigated the possibility of employing radioactive tooth enamel, where dissolution measurements could be based on the appearance of radioactivity in solution. The measurements could thus be independent of the test food's initial calcium or phosphorus content or subsequent release of calcium or phosphorus from the food or the saliva as a result of fermentation. The authors suggested that the technique may have limitations because of the possibility that the irradiating process might change the chemical form of the enamel. They point out, however, that their results "show solubilities of orders of magnitude similar to those obtained by others" and did permit multiple sampling for dissolution without excessive diminution of the experimental volume, and without interference from the calcium or phosphate content of the food or the saliva.

The possibility that irradiation of tooth enamel might alter its chemical form has been investigated by Cutress and his co-workers. They subjected bovine and human enamel, powdered and solid, and tricalcium phosphate to a neutron flux, then measured the dissolution occurring when these materials were exposed to acetate
buffers. The dissolution was measured by calcium and phosphate analysis, and by radioactive count-rate determination. They found that the irradiation converted a variable amount of orthophosphate to other forms, and that enamel dissolution determinations based on the appearance of radioactivity in solution did not always agree with those based on chemical analysis, and suggested that the non-orthophosphate forms of P$^{32}$ were less soluble than the unchanged tooth enamel.

The relationship between the amount of acid produced by salivary fermentation of carbohydrate foods and the resulting decalcification has been studied by Andlaw.$^{(5)}$ He suspended small blocks of bovine enamel in fermenting food–saliva mixtures, and measured the loss of weight of the blocks after six hours of fermentation under agitation. He also recorded the changes occurring in pH and titratable acidity during the experiment. Experiments were performed in duplicate, using three different saliva pools, and although no statistical analyses were performed, the author felt that any differences in weight loss which were maintained with each of the three pools were meaningful. On this basis, he found a lack of correlation between titratable acid production and enamel dissolution, and that the graham flour, wheat germ and wheat bran he tested exerted a protective effect against decalcification. When the ash of wheat germ or bran was added to white flour–saliva mixtures, he found that acid production increased, but enamel decalcification decreased.
LITERATURE REVIEW

Andlaw extended this work to include 27 food-saliva mixtures, using the same techniques, once again finding little correlation between enamel dissolution and acid production or pH changes.\(^{(4)}\) Several foods containing the germ, bran or hull fractions were associated with very little enamel dissolution, as were milk chocolate and caramel. He suggested protective factors in grains and milk products as the explanation for the low dissolutions he observed. He also studied the effect on enamel dissolution of the addition of wheat germ and wheat bran, untreated and ashed, and a water extract of wheat germ, alone or ashed, to fermenting flour-saliva-enamel mixtures. Increasing amounts of germ or bran ash increased acid production and decreased dissolution, and water extracts of germ, alone or ashed, reduced enamel dissolution. He concluded that inorganic protective agents were responsible. He obtained similar results with skim milk powder and chocolate, alone or ashed, and suggested that the effects might be associated with the calcium and/or phosphate content of the additions.

Soni and Bibby also studied the effect of food-saliva mixtures on tooth enamel, using a polarised light technique to assess dissolution.\(^{(263)}\) Tooth sections were fixed to glass slides, and exposed to various food-saliva mixtures for 24 hours, measurements being made of pH, titratable acidity and decalcification at hours 6, 8 and 24. They verified Andlaw's findings, stating that there was "a lack of relationship between the amount of acid formed ... and the extent of decalcification produced ..." They attributed the pronounced
LITERATURE REVIEW

decalciﬁcation seen with orange juice, carbonated beverages and jam to the initial acidity of these foods, and suggested common ion effects to explain the low dissolution observed with foods rich in calcium and phosphorus. They noted that sugars generally produced less acid than did cereal products, and that some sugars, especially natural sugars such as honey and maple syrup "fermented with particular rapidity."

Frostell studied the acid production resulting from mixtures of dental plaque and various sugars in phosphate buffers under anaerobic conditions.\(^{(90)}\) He found a high acid production for the first \(10 - 15\) minutes, but decreasing thereafter, with glucose, sucrose, fructose, maltose and starch, lower acid production with lactose, and negligible acid production with sorbitol, mannitol and rhamnose. He suggested that the non-fermenting sugars could be used as sweetening agents, to prevent acid production in the mouth, and maintained that fermentation experiments "ought to be performed at constant pH, since the activity decreases with decreasing pH." It is difficult to see why he makes this suggestion, as pH is clearly of great importance in the development of caries, and a food test which detects the pH changes occurring in fermenting food–saliva mixtures would have greater relevance to the oral situation than one which does not.

Several investigators have suggested that the 'protective factors' responsible for lowered enamel dissolution may be associated with the organic or inorganic calcium or phosphorus content of the
LITERATURE REVIEW

Thanik and Bibby analysed various carbohydrate foods for their calcium and phosphorus content, then assessed their decalcifying ability when fermented with saliva, using a modification of Andlaw's techniques. The authors stated that radioactive bovine enamel was used, but appear to have employed only gravimetric and chemical techniques to assess dissolution. They found that high acid production and low enamel dissolution occurred with foods containing large quantities of calcium and phosphorus, but "there was not a direct proportionality between either of these and the observed enamel dissolution." They observed a great increase in soluble calcium and phosphorus after incubation, "probably the result of breakdown of organically bound calcium and phosphorus after incubation," and also mentioned a lack of consistent relationship between pH and titratable acidity, supporting the idea that "different spectrums of acids are being formed from different foodstuffs." The wide differences in acid production from the different foodstuffs related best to the calcium and phosphorus content, buffering the system to a pH at which acid production could continue, but these differences could also be attributed in part to poor bacterial growth with some foods because of nutritional inadequacy, high initial acidity or high sugar content.

Khanna and Bibby carried out similar experiments on cereals from different geographical areas, using coarse-mesh radioactive enamel. The cereals were analysed for calcium, phosphorus, fluorine, nitrogen, sulphur, bromine and some heavy metals. Distinct
LITERATURE REVIEW

differences in dissolution were obtained for cereals from different geographical areas, but parallel results were not obtained for different cereals from the same areas. There was no consistent pattern of trace element or mineral content which could be related to decalcification or acid production, and the authors concluded that geographic influences are not the major factors in determining acid production and decalcification.

The effectiveness of milk in reducing enamel dissolution when fermented with saliva has been studied by Jenkins and Ferguson.\textsuperscript{(148)} They measured the in-vitro pH changes and enamel dissolution occurring in milk–saliva mixtures and in buffers, and the plaque pH changes in-vivo, following milk, lactose and glucose rinses. The pH fall with milk was less than the pH fall with lactose, in the milk–saliva fermentations. Enamel dissolution was very much less in the presence of milk, but milk had very little effect on plaque pH. They felt that the calcium and phosphate content of milk was largely responsible for the reduction in enamel dissolution, and suggested that milk could exert a protective effect against cariogenic foods.

De Crousaz and his colleagues tested 5% water suspensions of thirteen commercially available breakfast cereals, alone and with mineral supplements, for buffering power, by titration with lactic acid to pH 4.0, and measured the in-vitro pH changes when these cereals were fermented with plaque suspensions.\textsuperscript{(69)} They also recorded plaque pH changes in-vivo following ingestion of
LITERATURE REVIEW

carbohydrates, using telemetry techniques. They found that cereals with high buffering properties were those which in previous experiments (22a) dissolved less enamel when fermented with saliva, but that, in general, the cereals they tested had low buffering properties. The addition of mineral supplements increased the in-vitro buffering capacity markedly. No correlation was found between the total sugar content of the cereals and the pH fall when fermented with plaque. With no plaque present, the pH of the interdental area in in-vivo experiments did not fall appreciably when cereals were eaten, but fell immediately when plaque was present. The addition of minerals to the cereals did not appreciably alter the pH fall. (69)

As might perhaps be expected when many variables are eliminated from experiments, investigators of enamel dissolution and the decalcification potential of foodstuffs are in substantial agreement. Enamel dissolution can take place under a wide range of conditions, and is enhanced with increased agitation, large surface area, low pH, high buffer molarity, and in the absence of common ions. It can be modified by many substances, in particular some foods, food extracts and ashes, and certain organic compounds. In food–saliva–enamel mixtures, the situation is more complex. Again, most investigators report that some foods, food extracts and ashes reduce enamel dissolution, even when using quite different experimental techniques, but difficulty is encountered in some studies when the initial calcium or phosphate content of the food
or saliva is high, or when the food or saliva releases calcium or phosphate on fermentation, unless the dissolution taking place is considerable. When whole teeth provide the experimental enamel surface, individual tooth variations may obscure the result, unless the effect is pronounced.

In general, the foods which appear to reduce enamel dissolution are unrefined, and most have a high buffer capacity. Nonetheless, the buffer capacity cannot account for the effect entirely, and the degree of dissolution occurring in food fermentation tests cannot be preducted from the pH or titratable acidity changes. Many investigators employ the phrase 'protective factor' in discussing their results, and some have shown that the factor can be extracted from the food. Several suggest that the addition of the factor to cariogenic foods may assist in the control of dental caries.

THE TOOTH SURFACE

The existence of layers or deposits on teeth has been recognised for many years. In 1902, Miller reported the existence of these deposits, and the subject is still being studied today. Investigators differ in their interpretation as to the significance of these deposits in relation to dental caries, and some of these differences stem from confusion regarding the terminology used. It is only recently that much of the controversy has been resolved.

Meckel distinguishes five separate structures, Naysmith's membrane, subsurface cuticle, surface cuticle, stained pellicle and
LITERATURE REVIEW

He concluded from his studies of unerupted and erupted teeth that the subsurface cuticle was from 1-3 μ thick, the surface cuticle 0.2 μ thick and the stained pellicle 1-10 μ thick. The staining reactions of these layers suggested the presence of salivary muco- and glyco-proteins, together with some lipid, and he suggested a process of denaturation and polymerisation from the saliva, preceded by formation of a porous surface in the case of subsurface cuticle, to explain their formation. He suggested a similar process, or alternatively a lysis of structured deposits, to explain the formation of the pellicle, and stated that the deposits are very insoluble, and could act as diffusion barriers against acids, thereby reducing the solubility rate of the enamel surface.

Tooth enamel can also acquire surface deposits from sources other than the saliva. Weiss and Bibby reported that treatment of enamel with various milk products could reduce its solubility in acetate buffers by as much as 25%. Washing of the enamel surface with protein solvents after exposure to the milk products but before exposure to the buffer almost eliminated the effect, but washing with water did not. They attributed the effect to adsorption on the enamel surface of a milk protein, and suggested that under some circumstances "milk could exert a moderating effect on enamel solubility in the mouth." In a further paper Weiss and Bibby studied the effect of various proteins on enamel surface solubility. They found that casein and gastric mucin were more
LITERATURE REVIEW

effective, and egg albumin, gamma globulin, gelatin and saliva less effective than milk protein concentrates in reducing solubility. They concluded that the observed reductions were too large to be attributed to the calcium and phosphorus content of milk.

Pearce and Bibby tested eleven proteins for their ability to adsorb on the surface of powdered bovine enamel, and found that casein, gamma globulin and beta globulin were absorbed most, and serum albumin and egg albumin least. Using casein, they found that initially the adsorption rate was rapid, but slowed thereafter, and almost ceased after 60 minutes. Gelatin adsorption increased as the iso-electric point of the protein was approached, or in the presence of calcium chloride, but decreased in the presence of potassium phosphate or sodium citrate. In general, these protein treatments made the enamel surface hydrophobic, and the authors suggested that attraction of organic material to the enamel surface may modify the progress of caries, but that their experiments do not duplicate the in-vivo situation of an intact enamel surface, which has a much smaller surface area available for reaction than powdered enamel. However, protein adsorption could account for the higher nitrogen content and the lower enamel solubility in areas of initial enamel destruction.

Ericson studied the ability of hydroxyl apatite to absorb proteins and conjugated proteins from human saliva, using adsorption chromatography and disc gel electrophoresis. All salivary samples tested contained proteins and conjugated proteins which were adsorbed
strongly to apatite crystals. \(^{(80)}\) He suggested that this process may play a part in the formation of films on teeth. In a further publication, Ericson and Ericsson reported that fluorapatite had a significantly smaller adsorption capacity for salivary proteins than hydroxyapatite. \(^{(81)}\) They concluded that the subjective observation that teeth in fluoride areas are particularly clean may be a result to a lowered adsorption capacity for organic material. They also suggested that while the presence of adsorbed proteins may reduce the solubility of the enamel surface, this effect might not be clinically significant if such adsorption promotes the formation of plaque, and subsequent acid production from carbohydrates.

These studies serve to illustrate the importance of the enamel surface in the carious process, and the possibility that components of foods and saliva could exert effects independently of acid production. There seems little doubt that proteins, and, in particular, those in milk and saliva can attach themselves to the enamel surface. This aspect of enamel surface behaviour acquires considerable significance in interpreting the results of food–saliva–enamel fermentation experiments, since it is possible, if not probable, that the in–vitro enamel dissolution–reducing effects of some foods is partly a result of surface coating. But the presence of such layers in–vivo may not exert the protective effect observed in–vitro, since they may facilitate the accumulation of surface deposits originating from bacterial action and food debris.

Tooth surface deposits originating from foods have not been
LITERATURE REVIEW

studied as extensively as the deposits originating embryonically, or from the saliva. Most investigators had concentrated on 'plaque', but Armstrong has studied the 'acquired pellicle' released from the surface of extracted teeth by dilute hydrochloric acid, using hydrolysis followed by amino-acid analysis.\(^7\) He concluded that the deposits he examined were neither collagenous nor keratinous, and were probably of salivary origin, thus supporting Meckel's conclusions.\(^{210}\) He also reported that they contained "significant amounts of bacterial cell wall protein material."

The organic films described by Meckel and Armstrong were basically structureless, and can usually be demonstrated on teeth. But the existence of structure, or lack of it, is a difficult property to define, and Armstrong\(^7\) indicated the presence of micro-organisms in some of his material, at some time. When these micro-organisms are viable, the surface deposits on teeth are often referred to as 'plaque', this description being applied without rigorous definition to layers of variable thickness and composition.

Confusion still exists as to what structures are described by the word 'plaque'. Bibby points out that 20 or so names have been used to describe various uncalcified, fixed organic layers on teeth, and he favours the terms 'enamel cuticle', and 'reduced enamel epithelium' to describe layers of embryological origin, and 'acquired pellicle' and 'dental plaque' to describe layers appearing after eruption.\(^{22}\) He suggested that some of the lack of agreement in the findings of 'plaque' investigations stem from variations in
LITERATURE REVIEW

experimental material, because of differences in age, site of origin and previous clinical experience. He urged caution in accepting as fact that surface deposits are implicated in all decalcifications of the tooth surface, and further suggested that Meckel's 'subsurface cuticle' may be a defense mechanism against decalcification. Bibby stated that there is lack of precise information on the nature of plaque, its bacterial or other components, its relationship to the tooth surfaces, and its distribution in the mouth, and that "it would therefore be foolish to accept without question the various properties which are attributed to it."

But whatever the nature of 'plaque', and whatever its properties, there is general agreement that development of dental caries will not commence in its absence. However, the mere presence of 'plaque' on a tooth surface does not necessarily mean that caries will result, and at most times, in all mouths, some 'plaque' will be present. Because of its undoubted importance in the carious process, 'plaque' has been studied for many years, but it is only since the development of refined micro-analytical techniques, and improvement in laboratory equipment, that detailed studies have been possible.

Critchley and his colleagues have reviewed the histology and chemistry of dental plaque, (62) and list three mechanisms for its formation:

"a. the sticking of epithelial cells, invaded by bacteria, to the (tooth) surface, giving foci of bacterial colonies.
LITERATURE REVIEW

b. the formation, by streptococci, of extracellular polysaccharide from sucrose, and the precipitation of this material.

c. the precipitation of salivary glycoproteins by acid, by wetting and drying, or by bacterial degradation."

They state that the squamous cells and leucocytes in plaque tend to disappear as the plaque ages, because of migration, digestion by enzymes, or invasion by micro-organisms, and that in the presence of sucrose, plaque is especially heavy and gelatinous, because of its high extracellular polysaccharide content. They suggest that plaque formation may proceed in two stages, immature plaque, with an initial, non-bacterial plaque matrix, and mature plaque, where the matrix is invaded by micro-organisms, possibly followed by extracellular polysaccharide formation, and precipitation of proteins from glyco-proteins by enzyme degradation. At this stage, the plaque may be considered as having layers, with a surface accumulation of precipitated, partly degraded saliva, bacteria and food debris, a matrix of polysaccharides, and a thin, cell-free band adjacent to the enamel surface. Complex, interrelated, secondary changes can now take place, "leading to calcification in one area and decalcification in another." They concluded that:

a. bacterial plaque formation "... requires a source of glyco-protein and certain types of bacteria ..."

b. the metabolism of the plaque "is a complex of interconnected cycles, involving diet, proximity to the salivary glands and/or crevicular fluid, species of bacteria and the defense
mechanism of the host."

c. plaque "possessed some structure and organisation ..."
"different species of bacteria are separated from each other in individual colonies ..." and enzymes most effective in an alkaline environment "are present in plaque which is acid to an electrode ..." suggesting cyclic changes in the pH of the microenvironment or whole plaque."

d. micro-organisms "deep in the plaque degrade the matrix material and probably use it as a substrate", while those near the plaque surface "use saliva and dietary food."

e. for these reasons, germ free animals are a useful experimental tool, but "the use of one type of organism, and the investigation of plaque in narrow fissures where food impaction is inevitable, are not completely representative of human conditions."

Leach and his co-workers compared the carbohydrate and amino-acid composition of acquired pellicle and plaque matrix with that of saliva, and found all three to have an amino-acid composition characteristic of glycoproteins.\(^{186}\) They concluded that the pellicle was composed, to an appreciable extent, of salivary glycoproteins, and that the major carbohydrate component of plaque matrix was composed of bacterially produced dextran.

Jenkins has reviewed some of the studies on dental plaque\(^{153}\) and reached substantially the same conclusions as did Critchley and his colleagues.\(^{62}\) Jenkins concluded that there was a marked
tendency for some salivary proteins to precipitate spontaneously, and that the process was favoured by lowering the pH and raising the calcium concentration. He also concluded that although extracellular polysaccharides can increase the bulk and stickiness of plaque, they may not be essential for initial plaque formation. He stated that there was good evidence that the main sources of plaque matrix are salivary mucoids and perhaps bacterial proteins, and that at some stage in plaque formation, carbohydrate is removed from salivary mucoids.

Stephan showed in 1940 that in the presence of carbohydrate, there was a rapid drop in the pH of plaque, but was limited to the study of easily accessible teeth because of limitations in the size of the electrodes used. (268) Kleinberg and Jenkins used a miniature antimony electrode to gain access to previously inaccessible areas of the mouth, and in a detailed study, measured the plaque pH changes and salivary flow rates in fasting, non-carbohydrate meal and carbohydrate meal groups of students. (167) They found variation in pH values according to location, and that non-carbohydrate meals taken after fasting had little effect on plaque pH. But meals containing carbohydrate lowered the pH level in all areas studied, often for hours after the meal. They concluded that a low plaque pH occurs in caries-susceptible areas, and regarded the plaque as an 'environmental membrane' between the tooth and the saliva, the plaque bacteria playing a major role in calcium phosphate movement between the two.
LITERATURE REVIEW

Correlation of fermentation of food and stimulated saliva (followed by measurement of enamel decalcification) with cariogenicity implies that the mode of enamel dissolution in such mixtures resembles that taking place in plaque, if such in-vitro tests are to be relevant to the in-vivo situation. Krasse has shown that half of the facultative streptococci in saliva are streptococcus salivarius, but this organism is present in plaque only to the extent of 1% \(^{180}\). He concluded that the dorsum of the tongue supplies most of the salivary micro-organisms. This conclusion was supported by Gibbons and his co-workers, who also found that this organism comprised less than 1% of the gingival crevice population. \(^{95}\) Gibbons also noted that plaque cannot be easily washed from the teeth, and stated that investigations based on the assumption that the salivary microbial population is representative of dental plaque are open to criticism. \(^{96}\)

Carlsson has analysed the streptococcal population of teeth, saliva, tongue and mucosa in subjects consuming diets supplemented with frequent intakes of sucrose and glucose, and who refrained from toothbrushing. \(^{48}\) He found that more plaque was formed with the sucrose supplement, and that the tooth surface was the most favourable habitat for streptococcus sanguis and streptococcus mutans, but that streptococcus salivarius constituted a major part of the streptococcal flora of the tongue. He concluded that the streptococci favouring the tooth surface as a habitat probably "play an important role in the formation of the inter-microbial matrix of
plaque when sucrose is consumed." In a subsequent paper, Carlsson studied the streptococcal population of subjects who refrained from toothbrushing for three days while consuming diets containing sugars. As the experimental period progressed more streptococcus sanguis, which grow preferentially on the tooth surface, were recovered from the saliva, and he suggested that "the microbiota on the teeth may contribute as much as that of the tongue to the pool of salivary streptococci in persons with ineffective toothbrushing habits." This suggests that pooled saliva samples probably contain considerably more tooth-derived micro-organisms than would be suggested from the studies of Krasse and Gibbons.

In an effort to elucidate the role of diet on plaque formation in man, Carlsson and Egelberg fed protein-fat diets alone, and supplemented with sucrose, and basic soft diets, alone, and supplemented with sugars, to a small experimental group, for periods of seven days. They assessed plaque formation direct from photographs, and from counts of plaque cocci. They found that diets substantially free of carbohydrate formed thin, structureless plaque after a few days. The plaque was not substantially increased with glucose in the diet. Sucrose additions, however, increased plaque formation considerably. They concluded that the sucrose increased plaque formation because of the production of extracellular polysaccharides by plaque micro-organisms.

The distribution of lactobacilli in the saliva, plaque and carious dentine of caries-active monkeys has been studied by Bowen.
LITERATURE REVIEW

No more than four species were recovered from any sample, and the distribution varied in the three sources. Lactobacilli comprised 1% of the bacterial flora of the saliva, 8% of the plaque and 60% of the carious dentine. He suggested that lactobacilli are important in the aetiology of dental caries. Eastoe and Bowen studied the effect of sugars in aqueous solution and in pH 7.0 phosphate buffer on plaque pH in monkeys fed a cariogenic diet. They found that aqueous and buffered sucrose solutions lowered plaque pH to about 5.0 after 20 minutes. They concluded that their experiments provided "clear evidence for the production of substantial quantities of acid at these sites, and strongly suggests its participation in the development of the carious lesions observed in these animals."

Neff has pointed out that acid production in plaque-saliva-substrate suspensions does not constitute a very good model for the in-vivo situation, since the diffusion mechanism is not duplicated. He therefore developed an in-vivo technique for measuring acid production when various carbohydrates and carbohydrate-fluoride combinations provided the plaque substrate. At 0.9% sugar concentrations, the plaque pH fell below 5.0 in less than 10 minutes with sucrose, fructose, glucose and sweetened wholemeal bread extract. The pH fall was less rapid and less pronounced with maltose, lactose and unsweetened wholemeal bread extract, and negligible with sorbitol and raw starch. The rate and degree of pH fall with sucrose solutions decreased as the fluoride concentration was increased.
LITERATURE REVIEW

There has been evidence to show that the glycoproteins of the plaque matrix are formed from the saliva.\(^{(7, 62)}\) But it has also been shown that oral micro-organisms can synthesise large amounts of polysaccharide from sucrose, and these findings, coupled with the animal experiments showing greater plaque formation with sucrose than with glucose prompted Critchley and his co-workers to study plaque polysaccharide formation in-vitro and in-vivo, in the presence of sucrose.\(^{(61)}\) They found that the major plaque polysaccharide was dextran, with smaller amounts of levan, that both were rapidly synthesised directly from sucrose, and that they accounted for 10\% by weight of dry plaque, and 20\% by weight of the plaque matrix. The authors suggested that plaque micro-organisms can metabolise polysaccharide to acid, and because the plaque is an effective diffusion barrier, the saliva cannot neutralise this acid rapidly. For this reason sucrose is more cariogenic than glucose in animal experiments, as glucose cannot form extracellular polysaccharide.

Poole and his co-workers described a technique for depositing plaque on thin, porous membranes in-vivo, for subsequent in-vitro testing.\(^{(240)}\) Despite careful standardisation, large subject-to-subject variation in microbial population counts were observed, which could not be correlated with the subjects' dental caries experience. However, the membrane and natural plaques were statistically similar with respect to micro-organism content, and they suggested this approach for controlled plaque studies. Gilmour and Poole then employed the technique on children, incubating the
membranes obtained both aerobically and anaerobically, analysing the products of fermentation by gas chromatography, and recording the pH and titratable acidity changes. They observed three main patterns of acid production for their plaque samples, fast fermenters (pH fall of one unit in less than 5 hours) intermediate fermenters (pH fall of 0.5 – 0.9 unit in 5 hours and one unit in 11 hours) and slow fermenters (pH fall of less than 0.5 of a unit in 5 hours, followed by a pH rise). In the first two types, the aerobic pH fall was generally greater than the anaerobic fall, but variable results were obtained with some subjects. Different rates of decrease in pH level were observed, and they suggested changes in relative rates of acid production as a result of the differing biochemical behaviour of the initial microbial population of the plaque, and disproportionate growth during incubation, as an explanation for this effect. Their gas chromatograph results indicated that the pH decreases observed could not be attributed to any one of the three acids (lactic, acetic and propionic) they studied. The concentrations produced differed from subject to subject, and also on the aerobic and anaerobic sides of the membrane in the same subject. They also found that the lactic acid concentration was similar to or less than either acetic or propionic acid concentration, and suggested that this puzzling finding could be explained if some of the lactic acid formed was rapidly metabolised to yield other products.

Luoma and Luoma studied the effect of sucrose tablets, alone,
and fortified with minerals, on the fermenting and decalcifying ability of plaque \(^{(195a)}\). The test subjects sucked the tablets, and plaque samples obtained from the subjects were then fermented with saliva and irradiated enamel, the pH change and enamel dissolution being recorded. The in-vitro pH fall and enamel dissolution were less following the mineral-fortified sucrose tablets than with the sucrose tablets alone, and up to one half of the \(\text{P}^{32}\) released from the enamel could be recovered from the washed plaque. In the in-vivo tests, the plaque pH fell during the sucking of the sucrose tablets, and continued to fall after the tablets had been consumed, but with the mineral-fortified tablets, the pH did not fall during the sucking of the tablets, and fell less after the tablets had been consumed. The authors felt that such mineral supplements could have an application in lessening the cariogenicity of refined carbohydrates.

The importance of plaque in controlling the transport of calcium and phosphate between the teeth and the oral cavity has been commented upon by Jenkins, who noted that the calcium, phosphate and fluoride concentrations in plaque are much higher than those in saliva, and that little of the plaque calcium is removed by washing with water \(^{(150)}\). He stated that some of the inorganic phosphate is converted to organic forms during acid production from sugar in the plaque, but the plaque inorganic phosphate level is still higher in the plaque than in the saliva. This fact is clearly of importance in the initiation of dental caries.
LITERATURE REVIEW

There has been considerable research effort directed to the study of the carbohydrate components of plaque. Wood and Critchley (304, 305) analysed plaque extracts using acid hydrolysis and paper chromatography, and concluded that polysaccharides form a large part of plaque matrix. The possible importance of this fact in the production of caries was investigated by Gibbons and his co-workers, who isolated strains of capsule-forming streptococci from human carious lesions, and introduced them as pure cultures in gnotobiotic rats. (97) The animals were fed cariogenic diets for periods of up to 105 days, and caries scores obtained. Strains of cariogenic streptococci were also cultured with various sugars, and extracellular polysaccharide production assessed. All strains produced non-dialysable extracellular carbohydrate, which was presumed to be capsular material, and in all cases, sucrose produced more than did the other sugars tested. In addition, all the cariogenic strains produced more extracellular carbohydrate than the non-cariogenic strains. Three of the four human cariogenic streptococci induced caries in the gnotobiotic rats, and the biochemical and serological characteristics of these organisms were similar to rodent cariogenic streptococci.

Wood and Critchley continued their studies of the extracellular polysaccharides produced by cariogenic streptococci, using incubation, dialysis and analysis of various components of the organisms. (304) They concluded that the extracellular polysaccharides were composed of glucose and fructose, and present naturally in the
LITERATURE REVIEW

plaque as dextrans and levans.

Other organisms besides capsule forming streptococci can produce extracellular polysaccharide, as Hammond has shown. He studied over 40 strains of lactobacillus casei in glucose and sucrose media, and the ability of eight sugars to support lactobacillus growth and produce extracellular polysaccharide. He found that in the glucose medium, lactobacilli produced eight times the growth, four times the lactate and $1\frac{1}{2}$ times the extracellular polysaccharide in comparison with the sucrose medium. He also found that all the sugars tested produced extracellular polysaccharide, and concluded that under the appropriate in-vivo conditions, polysaccharide produced by lactobacilli could contribute to the development of plaque matrix in the absence of sucrose.

Guggenheim and his co-workers tested the ability of a cariogenic, capsule-forming streptococcus to induce caries in animals made relatively gnotobiotic using an antibiotic, and fed cariogenic diets containing five different carbohydrates. In their experiments, starch was found to be non-cariogenic, and glucose less cariogenic than sucrose, as were the other sugars tested. With all the carbohydrates except sucrose, the test organism did not thrive, and surface deposits on the teeth were slight. Furthermore, extracellular polysaccharide was formed only from sucrose and raffinose. The authors concluded that on smooth surfaces accessible to functional cleansing, "aggregation of caries-inducing plaque and its sequels seems to be dependent on the establishment of micro-
organisms producing polysaccharide of the dextran type."

In a brief report, Zinner and his co-workers described experiments where streptococci recovered from human carious lesions were found to be capable of inducing caries in germ-free rats maintained on coarse- and fine-particle cariogenic diets. The human strain employed was found to be identical morphologically, antigenically and in biochemical characteristics with streptococcal strains known to be cariogenic in hamsters and rats.

Aspects of extracellular polysaccharide production, and its occurrence in human dental plaque, have been studied by Gibbons and Banghart. They analysed the metabolic products of various cultures of known cariogenic organisms, and found that the associated extracellular polysaccharide was a dextran-like polymer. In addition, they found that the dextrans synthesised by rat, hamster and human cariogenic streptococci, and a cariogenic strain of lactobacillus acidophilus were immunologically similar, relatively resistant to bacterial degradation, and could form precipitates with saliva and adhere to enamel surfaces. The authors felt that in the light of these properties of dextran, the concept emerges of the formation from sucrose by cariogenic bacteria of a dextran which enables these organisms to form plaque "which is necessary for the production of dental caries", and that this ability is lacking in non-cariogenic organisms.

Dahlqvist and co-workers also studied the polysaccharides produced by cariogenic streptococci, two from humans and shown to be
LITERATURE REVIEW

cariogenic in hamsters, and one from hamsters. They verified the presence of extracellular and capsular polysaccharides, and concluded from their analyses that these consisted of glucans and fructans.

The biochemical and morphological aspects of extracellular polysaccharide production from streptococcus mutans were studied by Guggenheim and Schroeder, using incubated cultures, carbohydrate tests, chromatography, infra-red spectroscopy and electron microscopy. They found that polysaccharide production commenced a few seconds after the addition of sucrose to resting cell cultures, and identified it as a 1-3, 1-4 branched dextran. They suggested that the enzyme responsible is bound to the cell structure, and that the resulting polysaccharide, formerly thought to be structureless, exists in three forms, globular, homogenous and fibrillar, indicating differences in molecular arrangement.

In 1968, Carlsson reviewed seven of his own studies on plaque and streptococci, and summarised his findings as follows:-

a. On cleaned tooth surfaces, a pellicle almost free of microorganisms forms first, and is subsequently colonised in cracks on the tooth surface, and in scattered, discrete microbial aggregations.

b. Frequent sucrose consumption in soft diets causes abundant plaque accumulation.

c. Streptococcus sanguis and mutans dominate the early plaque flora, and both can synthesise extracellular polysaccharide,
but only from sucrose.

d. After frequent sucrose intake, an appreciable volume of plaque consists of an intermicrobial carbohydrate matrix.

He suggested that polysaccharide formation by streptococci plays an important role in plaque formation on teeth.

The importance of plaque microbiology and chemistry in caries has been further stressed in review papers by Fitzgerald, (86) Leach (187) and König. (175) Fitzgerald stated that the extracellular slimes of the dextran type are now considered to be a factor in the formation and adhesion of plaques to the tooth surface. Leach suggested that the major sugar components of plaque matrix are derived not from salivary mucin, but from dietary sucrose converted by bacterial enzymes to dextran and levan. König stated that "dietary sucrose favours the establishment and metabolism generally of acidogenic micro-organisms, and especially of dextran-synthesising, plaque-forming streptococci," and suggests that in general a soft diet increases food and plaque accumulation and increases caries activity. He also made the suggestion that impacted food may act as a substitute for bacterial plaque.

Carlsson and Krasse have reported on a further step in the study of extracellular polysaccharide, where they have attempted to produce an antiserum against the enzyme responsible for dextran production. (51) They reported success in obtaining, from rabbits inoculated with streptococci, antisera capable of inhibiting a streptococcal dextran-sucrase. They hypothesise that if such antisera

LITERATURE REVIEW
LITERATURE REVIEW

could reach the bacterial plaque via the saliva or gingival pocket fluid, there could be a reduced tendency for plaque formation after sucrose intake, and perhaps also "lowered incidence of caries and periodontal disease."

There seems to be little doubt that studies of plaque hold an important key to the solution of caries control in man. This is reflected in the many recent studies conducted on this subject. Apart from minor differences, most of the investigators agree that the bulk and adhesiveness of plaque is greatly enhanced by extracellular polysaccharide formation, and that sucrose encourages this formation much more than other common dietary carbohydrates. It is tempting, in the light of these findings, and the known cariogenicity of sucrose, to postulate that dextran formation in plaque is the most important factor in caries. But caries does occur in the absence of dietary sucrose, and even if dextrans and levans could be eliminated from the plaque using enzymatic or antigenic techniques, caries may still occur.

A more conservative hypothesis that sucrose makes plaque more cariogenic, and enhances the effects of other carbohydrates might be indicated in the absence of more definitive findings. This approach then leaves open the question of caries control by modification of, or addition to the diet, despite these recent developments in plaque studies. Whatever the plaque contains, minerals must transfer across the plaque-tooth interface, when caries is initiated, and any process which exerts an effect on this transfer
LITERATURE REVIEW

must be involved in the carious process. The model represented by a food–saliva–enamel mixture cannot represent the diffusion controlling action of plaque, or its undoubtedly important role in initiating the carious process, but does provide data on the combined effects of food, and the acids produced from food by fermentation, on enamel dissolution at the tooth surface, and the effects of pH change, buffer capacity and enamel solubility–controlling substances on this process.

It is evident, from this review of selected literature on the food–dental caries relationship, that primitive and isolated groups have low caries prevalence, and that this effect is consistently associated with the consumption of unrefined foods. When refined foods are introduced into these communities, caries increases, regardless of race or dietary habits.

Differences are frequently observed in the caries experience of groups living in a given geographical area, and these differences can often be attributed to different diets and dietary patterns, and may also be related to differences in the composition of the diet.

When the consumption of refined carbohydrates is reduced, fortuitously, as a result of war, or intentionally, in specific communities or experimental groups, dental caries is reduced. Such diet modification can be used as a method of caries control. If this diet is then supplemented by the addition of refined carbohydrates, the resultant increase in caries is dependent not only on the type and form of the carbohydrate added, but also on the manner in which
LITERATURE REVIEW

it is consumed. It is essential to consider these factors in interpreting the results of human dietary experiments.

The assessment of the cariogenicity of a food or food-additive combination is difficult in human groups, but clinical trials have indicated that some foods and food additives, such as fluoride and phosphates, can reduce dental caries. In some of these studies, experimental difficulties were encountered, and the results were inconclusive. To make a valid assessment of the cariogenicity in humans of a food or food-additive combination, a human clinical trial is essential, but it is unlikely that such trials will ever be conducted on more than a limited number of foods and food-additive combinations, because of the difficulties associated with long-term human clinical studies.

Experiments which assess the cariogenicity of human foods on animals do allow the investigator to test large numbers of potentially caries-limiting or non-cariogenic foods and food-additive combinations, but the findings may not be directly applicable to humans.

In-vitro studies of the decalcification potential of foods suffer from the limitation that although differences in the behaviour of foods can be demonstrated, the significance of the findings with respect to cariogenicity in either animal or human studies is uncertain. Furthermore, no assessment is made of the cariogenic effect of plaque, and its possible role in enhancing the cariogenicity of foods, and until the role of extracellular polysaccharide in the carious process is more definitively established, in-vitro
LITERATURE REVIEW

decalcification potential should be regarded as offering a means for detecting those foods which have a minimum cariogenic potential in human diets, and those substances which could influence cariogenicity in vivo. It was on this basis that the present study was undertaken.
SECTION III

DEVELOPMENT OF THE EXPERIMENTAL METHOD
DEVELOPMENT OF THE EXPERIMENTAL METHOD

A direct, and in some respects, the simplest approach to the observation of the effects of various substances on teeth is to expose the intact enamel surface of a tooth to each substance, then examine the surface, or section the tooth itself, and examine the sections. (79, 135, 217, 264) Enamel loss is assessed by microscopic examination, using transmitted light or polarised light. (262, 263) A more accurate assessment is obtained from soft x-radiographs of the sections. (122)

This direct approach is unsuitable for determining rates of enamel dissolution, since results can be obtained only at the conclusion of the experiment. Furthermore, standardisation of the experimental enamel surface is difficult to achieve, especially if series of experiments are to be compared. (59)

'Artificial mouth' techniques have been described which more closely approach in-vivo conditions. (239) The enamel surface is exposed to an environment of micro-organisms or salivary debris, and saliva or culture medium is applied to the surface, continuously or intermittently. However, the 'artificial mouth' has some of the disadvantages described above.

Continuous observation of the enamel surface and sub-surface is possible using 'edge decalcification', a refinement of the artificial mouth technique. A tooth section is mounted between glass slides in such a way that the substance under test occupies the space between the glass slides, and reacts with the exposed enamel edge of the tooth section. (78)
DEVELOPMENT OF METHOD

Changes occurring throughout the experiment can be observed, but standardisation of the test surface is still a problem if series of experiments are to be compared. It is also difficult, because of the small volumes involved, to obtain data on pH change or acid production taking place at the enamel surface. If these objections could be overcome, edge decalcification would be a most useful technique for studying the effect of a food–saliva mixture on tooth enamel.

It is generally accepted that the bacterial degradation of a food produce hydrogen ions which initiate the carious process by decalcifying tooth structure. This theory has led to indirect techniques for investigating the 'decalcification potential' of a food. In these techniques, the food's ability to produce a low pH, large amounts of acid, or both, is measured, either in the mouth or in-vitro. The logic of this approach is well supported by in-vitro experiments with simple buffer systems, where enamel dissolution is high with low pH- and high titratable acidity-buffers, and vice versa. With food tests where decalcification potential is inferred from examination of pH and titratable acidity data alone, it is presupposed that the presence of the food in the system has no effect, per se, on the mechanism of enamel dissolution. This is not necessarily true, as recent work has shown.

The pH of a food–saliva mixture indicates the activity of the hydrogen ions present, but does not indicate the total amount of
undissociated acid. The total acid must be assessed by titration or chemical analysis, and a decalcification potential test which records both pH and total acid will be more valid than those which consider only pH or total acid, alone. Since it is possible that the food itself may modify any enamel dissolution,\(^{(4, 5)}\) independently of pH or titratable acidity, enamel dissolution taking place should also be determined. When data on pH, titratable acidity and enamel dissolution are recorded, it is then possible to form definite opinions as to the action of a fermenting food–saliva mixture on tooth enamel.

When tooth enamel is dissolved by acid, the time taken to reach equilibrium depends on the experimental conditions\(^{(103)}\) (See Appendix B). The time factor thus becomes an important consideration in food tests, especially since acid production is dependent on micro–organism growth rate and total population, which take some time to stabilise.\(^{(269)}\) A fermenting food–saliva mixture will thus exhibit a lag phase with respect to both pH and titratable acidity change, and the time taken to reach equilibrium will vary according to the rate of growth of the micro–organisms. The lag phase may differ with different foods, and when series of experiments are to be compared, it is essential that continuous or periodic determinations of pH, titratable acidity and enamel dissolution are made, if valid conclusions are to be reached.

The pH changes taking place in a food–saliva mixture can be monitored continuously using a suitable electrode, but titratable
DEVELOPMENT OF METHOD

acidity can only be determined intermittently, from experimental
samples, which must be of reasonable volume for accurate results.
On the other hand, if the titratable acidity sample-volume is too
large, or samples are taken at too frequent intervals, the
experimental volume will be depleted excessively. This is undesirable,
since the continual diminution in experimental volume complicates
the interpretation of pH and titratable acidity changes, and more
importantly, as will be seen later, the degree of agitation is not
constant. In a well designed experiment, both the volume of
titratable acidity samples and the frequency with which they
are taken, should be limited, so that the depletion of the
experimental volume will not affect unduly the interpretation of
the results.

The determination of enamel dissolution has inherent
difficulties. A gravimetric approach, using small blocks of tooth
enamel which are weighed before the experiment, suspended in the
fermenting food mixture, and re-weighed at the end of the
experiment, provides a direct measure of enamel dissolution. (4, 5)
The blocks are of necessity small, and for accurate results, must be
dried to constant weight for each determination. A terminal enamel
dissolution value only can be obtained, as the blocks cannot be
dried to constant weight during the experiment. It is possible to
obtain enamel dissolution figures based on conventional chemical
analyses, but samples must be of substantial volume for accurate
assessments to be obtained. (65) Chemical analysis techniques involve
DEVELOPMENT OF METHOD

volume diminution, with the associated disadvantages already discussed in connection with titratable acidity determinations. Unless correction factors are applied, such techniques may lead to incorrect determinations of the relative rates of dissolution, when based on the appearance of calcium and phosphate in solution (See Appendix B). There are other disadvantages associated with enamel dissolution-rate determinations using chemical analyses. Early in an experiment, dissolution may be so slight that reliable figures cannot be obtained, unless the samples taken are large, or large amounts of enamel are used in each experiment. Further difficulties appear if the food being tested contains appreciable quantities of soluble calcium or phosphorus.\(^{(295)}\) At the start of the experiment, the investigator can allow for the calcium or phosphorus content of the food, but the blank (hour 0) calcium or phosphorus values will be high and tend to obscure the slight increase in calcium or phosphorus resulting from enamel dissolution. Later in the experiment, when appreciable enamel dissolution has taken place, a high calcium or phosphorus blank is not so important. However, later in the experiment, the food may be broken down to release more soluble calcium or phosphorus. Should this occur, the investigator cannot be sure that enamel dissolution figures calculated from calcium or phosphorus values (corrected for hour 0 blank values) will be correct, since the calcium or phosphorus values used may include further calcium and phosphorus released from the food. There is no simple method for overcoming this difficulty.
DEVELOPMENT OF METHOD

Much thought was given to the type of apatite mineral to be used in the food-saliva mixture. Human enamel is the material of choice, but is difficult to obtain in large quantities, and of uniform quality. But the chemical differences between various types of biological apatites are slight, and their behaviour in acid buffers is similar, suggesting that animal enamel would be a suitable substitute, especially since it is comparatively easy to obtain. Artificially prepared apatites are also readily available from various manufacturers. An evaluation of these alternative sources of apatite was carried out.

The artificially prepared apatites were found to be extremely fine in texture, and presented filtration difficulties under the proposed experimental conditions. Furthermore, previous experience with these preparations indicated that a variation in particle size could be expected with different batches. Of the common domestic animals, young cattle were selected as the most suitable source of animal enamel, since they have large lower incisors, which are easily obtainable, and provide a high yield of enamel. Apart from size, these teeth are similar to human incisors, and the enamel structure is not significantly different microscopically.

In an in-vivo situation, the area of the tooth surface which is under attack is quite small. The artificial mouth and edge decalcification techniques duplicate these conditions, but require highly refined techniques to assess enamel dissolution quantitatively, because of the small sample volume available. When enamel blocks are
used, dissolution determinations are facilitated, because of the increased enamel surface exposed to the acid. However, dissolution is generally small, and is in the milligram range, even with large blocks.\(^{(4, 5)}\) Dissolution of apatite in acids is enhanced if the surface area exposed is increased by powdering the enamel,\(^{(104)}\) and it was felt that apatite in this form would be the material of choice for addition to fermenting food–saliva mixtures. Practical consideration dictated that powders of different mesh sizes be tested to develop efficient filtration techniques for obtaining enamel dissolution samples, and to decide on the most suitable mesh size. There was a possibility that the dissolution obtained in a food fermentation would be affected by reaction of the saliva with the apatite powder.\(^{(82, 88, 247)}\) If any such reaction occurred to an appreciable extent, effects attributed to differences in foods might, in fact, be due to an effect resulting from the reaction of saliva with enamel, modified in some way by the test food. Experiments designed to evaluate these possibilities were carried out, and are described below.

To determine the effect of a very small particle size on filtration efficiency, and amount of apatite added on degree of dissolution at neutral pH, a sample of reagent grade tricalcium phosphate \(\text{Vitor}^R\) was irradiated as described in Appendix C, and 200, 100, 50 and 25 mg. samples weighed out. These were suspended in 10 ml. of distilled water, the pH of which was adjusted to 7.0
DEVELOPMENT OF METHOD

by addition of a minimum amount of buffer. The suspensions were then shaken mechanically at room temperature for four minutes, and glass-frit filtration samples taken to determine enamel dissolution, using the techniques described in Section IV. The suspensions were then centrifuged at 3000 r.p.m. in 15 ml. centrifuge tubes for ten minutes, and enamel dissolution samples taken by micropipette, direct from the supernatant liquid, and enamel dissolution determined, as before. The tubes were then reshaken and recentrifuged, and further enamel dissolution samples taken by glass-frit filtration, and enamel dissolution calculated, as before.

The results of this experiment are shown in Figure 1. It is evident that even at pH 7.0, dissolution takes place, the extent of which is dependent on the amount of tricalcium phosphate powder added, regardless of the technique used to obtain the experimental sample. These results are in accord with the findings of Gray and his co-workers, (104) and indicate that the degree of dissolution is dependent on the surface area exposed to the solution. These results also indicate that the use of reagent-grade tricalcium phosphate as a source of apatite in food fermentations would not be advisable because of the extensive dissolution taking place at pH 7.0. It is further evident that direct sampling of the supernatant liquid following centrifugation gives higher apparent enamel

DEVELOPMENT OF METHOD

dissolution values than is obtained by glass-frit filtration, thus demonstrating that the glass-frit filtration does exclude from dissolution samples, fine particles which are not centrifuged down. The degree of exclusion is not extensive however, especially when a comparison of the second glass-frit samples is made with those taken directly. The lack of correlation between the two glass-frit samples for each powder addition can be ascribed to the dissolution taking place during the two resuspension and centrifugation procedures.

A similar experiment was then conducted using unwashed, radioactive, bovine enamel powders of 200-240 and 120-200 mesh sizes. Weighed 200, 100, 50 and 25 mg. amounts of the 200-240 mesh powder and 100, 50 and 25 mg. amounts of the 120-200 mesh powder were prepared, and added to tubes containing 10 ml. amounts of distilled water at pH 7.0, as before. Two enamel dissolution samples were taken from each tube, the first by glass-frit filtration, the second by micropipette, direct from the supernatant liquid, following centrifugation at 3000 r.p.m. for five minutes.

The results of this experiment are shown in Figure 2. Once again, the increased dissolution taking place with an increase in surface area exposed is demonstrated, with the highest dissolution being obtained with the greatest enamel powder additions, the lowest with the least. The overall dissolutions obtained with both bovine enamel powders were much lower than those obtained in comparable experiments using reagent grade tricalcium phosphate, regardless of the sampling methods used. This was to be expected, in view of
DEVELOPMENT OF METHOD

the extreme fineness of the latter material. However, a marked difference was evident in comparable experiments using the two different mesh-size bovine powders, when the two sampling methods, glass-frit filtration, and direct, after centrifugation, were compared. The dissolution values obtained for the 100, 50 and 25 mg. powder additions were the same for both mesh sizes, when the samples were taken by glass-frit filtration. After centrifugation, and with the samples taken by micropipette direct from the supernatant liquid, the calculated enamel dissolution values were generally higher with the 200-240 mesh powder than with the 120-200 mesh powder, and both sets of values were higher than those obtained from glass-frit filtration samples.

These results suggest that the amount of dissolution taking place at pH 7.0 is similar for both 200-240 mesh and 120-200 mesh enamel powders, provided dissolution samples used for calculation are taken by glass-frit filtration. When samples are taken direct, there is an apparent enamel solubility difference. This difference is probably due, in part, to undissolved, fine particles being included in the sample because of the sampling technique used. This conclusion was supported by the microscopic appearance of samples of unwashed enamel powder (See Appendix A). The unwashed sample contains many very fine particles which should have passed through the sieve used to obtain the given mesh-size sample. Samples of both mesh-size powders were then washed in distilled water at pH 7.0, and the larger particles allowed to settle. The supernatant liquid,
which contained suspended powder, was discarded, the enamel powder sediment resuspended and the process repeated. Samples of the supernatant liquid were taken by glass-frit filtration after each washing. The amount of enamel dissolved was lower with each successive washing, and was minimal after five washings. Microscopic examination of the powder after the five washings revealed that nearly all the fine particles evident in the unwashed powder had been removed. These findings indicated that for filtration efficiency and low blank dissolution values, washed enamel powders were to be preferred in food fermentation tests.

To compare the dissolution rate of bovine enamel powder with that of human enamel powder, the results obtained in separate dissolution experiments on four different irradiated apatite samples (Appendix B and Figure 15) were combined on the one graph (Figure 3). This graph shows the solubility rates, as obtained by the radiochemical method, for tricalcium phosphate (Victor\textsuperscript{R}), unwashed and washed 120-200 mesh bovine enamel and washed 120-200 mesh human enamel, in 0.1M acetate buffer of pH 4.5. The tricalcium phosphate and unwashed bovine enamel dissolved more rapidly than the washed bovine and human enamel. The latter samples exhibited similar dissolution behaviour under these experimental conditions throughout the experimental period. On the basis of its dissolution rate, and similarity in dissolution to human enamel, it was decided to use washed 120-200 mesh bovine enamel for all food fermentation experiments. The tricalcium phosphate and unwashed bovine enamel
DEVELOPMENT OF METHOD

powder were rejected because of their initial high dissolution rate, and because they were much more soluble than human enamel.

The experiments described above indicate that some enamel dissolution can be expected, even in neutral solutions. Since the aim of the food fermentation tests was to compare enamel dissolutions obtained in various food-saliva-enamel mixtures as a result of acid production, an experiment was conducted to determine how much dissolution could be expected in the total absence of food and acid. A 100 mg. sample of washed 120–200 mesh bovine enamel powder was stirred with 50 ml. of pooled saliva at 37°C for 24 hours, and changes in pH, titratable acidity and enamel dissolution recorded at regular intervals, using the procedures described in Section IV. The experiment was repeated, and the results are shown in Figure 4.

In each case, the pH remained relatively stable at about 7.5 for the first six hours, and rose to 8.0 by hour 24. Titratable alkalinity was low in each case, and paradoxically, lower in experiment II for a higher pH. This was attributed to a fundamental difference in the nature of the saliva, since the experiment II saliva panel included donors replacing two regular donors, unavailable owing to illness. Enamel dissolution rose very slowly in each case. Dissolution was slightly higher at hour 6, and slightly lower at hour 24, in experiment II. These results indicate that in food fermentation experiments, an enamel dissolution of 1–5 mg. can be expected when no acid production takes place, and any dissolution in
DEVELOPMENT OF METHOD

excess of this in a food test must be as a result of the addition of a food to the saliva.

A freshly exposed enamel surface is more reactive than a tooth surface which has been exposed to the oral environment, the difference being attributed to the presence of foreign elements or an organic layer in the latter case. (72, 83, 207, 236, 297). The following experiment was conducted to determine the effect of an exposure to saliva on the solubility of bovine enamel powders. Two 250 mg. and two 50 mg. samples of radioactive 200-240 mesh bovine enamel were weighed out. One 250 mg. sample was suspended in 10 ml. of pooled saliva, the other 250 mg. sample was suspended in 10 ml. of distilled water at pH 7.0, and both suspensions were shaken at 37°C for one hour. The suspensions were then centrifuged at 2000 r.p.m. for five minutes, and the supernatant liquid discarded. The enamel samples in each tube were then washed by resuspension in distilled water at pH 5.0, and centrifugation, as before, and the supernatant liquid discarded. This washing procedure was repeated. The enamel samples in each centrifuge tube were then resuspended in 10 ml. of distilled, de-ionised water (pH 5.0), and shaken at 37°C for nine hours. Glass-frit enamel dissolution samples were taken at ten minute intervals for the first hour and again at hours 3 and 9. This experiment was repeated with the 50 mg. enamel samples, except that dissolution samples were taken at ten minute intervals, for one hour only. Enamel dissolution was then plotted against time (Figure 5).
DEVELOPMENT OF METHOD

The two sets of dissolution curves shown are not comparable quantitatively, because of the differing amounts of enamel used in each case. Exposure of the enamel powder to saliva for one hour does not change its solubility in water at pH 5.0. When the exposure period is extended to 24 hours, there appears to be a slight increase in solubility. This increase is of border-line significance and it can therefore be concluded that exposure of a bovine enamel powder to saliva does not appreciably change its dissolution characteristics in acetate buffers.

The experiments just described provided answers to the questions of filtration technique, type and form of apatite, and effects of experimental conditions on enamel dissolution. The filtration technique was shown to be effective in excluding undissolved particles under the experimental conditions described, but no consideration was given to the efficiency of filtration after considerable dissolution had taken place, when small enamel particles might be present. This situation was not encountered, since smaller particles dissolve before larger ones, because of their greater surface/volume ratio. In enamel dissolution experiments, as the small particles become even smaller, their rate of dissolution increases rapidly, and after appreciable dissolution has taken place, the remaining undissolved particles are quite large.

In food fermentation experiments, it is essential that the micro-organisms be able to metabolise the food and produce acids.
DEVELOPMENT OF METHOD

Ideally, the experiment should duplicate the conditions of the mouth, but the oral microbiota are in a constant state of flux throughout the day, especially at the tooth surface, when foods are consumed.\(^{44}\) Exact duplication of these changing conditions in an in-vitro test would be difficult to achieve. Some investigators have employed pure cultures of micro-organisms in the interests of reproducibility.\(^{94}\) But the types of acids produced in the oral cavity under natural conditions may vary according to the type of food being consumed, and the predominant oral micro-organisms may also vary according to the type of food available to them.\(^{202, 243}\)

In order to evaluate the feasibility of using a pure culture of organisms as the fermenting medium, pooled saliva was centrifuged for 30 minutes at 3000 r.p.m., yielding a substantially micro-organism- and debris-free liquid, which was then inoculated with various pure cultures of known titre. Sucrose was then added, the mixture fermented, and the pH and the titratable acidity changes recorded.

Acid production did take place, and a low pH was produced, but the pH and titratable acidity remained unchanged for from six to ten hours, before any changes were recorded. This pronounced lag phase occurred even with concentrated inocula, and was attributed to a slowing of the growth rate while the organisms acclimatised themselves to the new conditions.\(^{265}\) This lag introduced practical difficulties, since laboratory personnel could not be present on a 24 hour basis, and the technique was discarded.
DEVELOPMENT OF METHOD

In the oral cavity, foods are usually in intimate contact with the tooth surface. This situation would best be duplicated in-vitro with a thick paste of food and saliva, in a bath of saliva.\(^{(214)}\)

But under these experimental conditions, it would be extremely difficult to assess accurately the pH and titratable acidity changes taking place at the tooth surface, owing to uncertainties concerning the uniformity of the mixture, and the possibility of uneven concentration gradients. Practical considerations require a fluid food mixture, which can be agitated uniformly, and from which samples can be easily taken. Uniform agitation is important, since the degree of agitation has a significant effect on the time required for enamel dissolution to reach equilibrium.

The temperature of the oral environment is normally about \(37^\circ C\). The experimental conditions should duplicate this situation, not only for consistency, but also because microbial action proceeds much more rapidly at this temperature than it does at room temperature. An elevated temperature causes evaporation, so experiments lasting more than a few hours must be conducted in a closed vessel, to prevent volume changes. Nevertheless, changes in volume must occur throughout the experiment, when experimental samples are withdrawn from the food–saliva mixture. The reduction of experimental volume as a result of this sample-taking must be held to a minimum, or the consistency of the mixture may change, and thereby alter the conditions of agitation. This is undesirable, for the reasons stated above.
DEVELOPMENT OF METHOD

Stimulated human saliva was selected as the liquid medium for the tests, and as the source of micro-organisms for the fermentation process. But saliva is an unsatisfactory experimental material if reproducible results are to be obtained, since its composition and micro-organism content differ in different individuals, and change from day to day, even in the same individual.\(^{(44, \, 245)}\) Several methods of collection were investigated, and several techniques employed, in an effort to obtain a saliva which would yield reproducible results. Saliva collected from individuals who chewed small wax blocks to stimulate salivary flow and dislodge debris from the teeth, tongue and mucous membranes was found to be most satisfactory regarding micro-organism content, but was found to be contaminated by small particles of wax, which were difficult to remove. Soft, uncoloured, gum-rubber bands, 3" x \(\frac{3}{4}\)", were tried in place of the wax.\(^{(71)}\) Using this technique, saliva flow was stimulated almost as effectively as with the wax, debris was removed, and wax contamination was eliminated. When saliva collected in this way was tested for acid production, wide variability was found with individual donors, and even from the same donor, on different days. Food fermentations using such salivas could be expected to suffer from a lack of reproducibility. A saliva donor panel was then assembled, consisting of from eight to ten regular donors, who each produced a fixed volume (15 ml.) of saliva at a fixed time each day prior to a food test. These samples were pooled, and the mixture stirred until the sediment was evenly distributed, before use (See
DEVELOPMENT OF METHOD

Section IV).

The quantity of saliva employed in all tests was 50 ml. This amount was easily produced by the saliva-donor panel on a daily basis, but was at the same time sufficient to allow the taking of several titration and enamel dissolution samples without decreasing unduly the experimental volume. It was found that this volume could contain 5 gm. of any of the foods tested and still remain sufficiently fluid to be stirred easily, and for experimental samples to be taken. Larger additions of food resulted in a food-saliva mixture which was often too viscous for stirring and sample taking.

No attempts were made to test foods on the basis of their carbohydrate content alone. The foods tested varied from a pure chemical, such as dextrose, to substances containing fibres, non-carbohydrate materials and water, in greatly differing amounts (See Appendix E). Since the foods were always present in a large excess over the requirements of the micro-organisms, it was felt that considerations of stirring and sampling were more important, and these are described in Section IV.

The measurement of pH alone does not give an indication of the total amount of acid in a food-saliva mixture, because the acids produced will vary in their dissociations, and the different foods will vary as to their buffer capacities. In food-saliva mixtures, an assessment of acid content can be measured by titration. If a completely dissociated alkali, such as sodium hydroxide is used
DEVELOPMENT OF METHOD

to titrate a food-acid mixture from a low pH back to a standard end-point, the amount of hydrogen ion neutralised will represent the amount of hydrogen ion which would have to be added to the same food-acid mixture to lower its pH from the standard end point to the original low pH. This figure is termed 'titratable acidity' in this work, and includes all acids dissociated at pH 7. These acids will not necessarily be dissociated at the low pH. To perform a titration of this type, a reasonable sample size is required. In the preliminary series of food tests, two beakers, each containing 50 ml. of food-saliva-enamel mixture were used, one for enamel dissolution determination, the other for titratable acidity determination. Titration sample size was 10 ml. and the results are open to the criticism that while enamel dissolution figures were obtained under virtually unchanging experimental conditions, those for titratable acidity were obtained from an experimental volume which decreased by 10 ml. when each sample was taken. These changing experimental conditions were occasionally reflected in different pH and titratable acidity values for the two beakers. A method was therefore developed for titrating small samples, which allowed the investigator to take both enamel dissolution and titratable acidity samples from the one container without diminishing the experimental

* The titratable acidity of a food-saliva mixture after fermentation is not the same as its inherent buffer capacity (See Section IV).
DEVELOPMENT OF METHOD

volume excessively.

The reproducibility of a given food fermentation can be established experimentally, by performing separate identical experiments, and this is the procedure which was adopted in this work. Any lack of reproducibility would indicate a difference in acid production by the micro-organisms. But this would be the case only if the buffering capacity of the saliva or food was the same in each separate experiment. The inherent buffer capacity of a food is constant for any given food sample, but the saliva sample could well vary in this property. This possibility was tested by collecting stimulated saliva samples from 22 young adult males with complete or nearly complete natural dentitions, and titrating 10 ml. samples of each with 0.1M hydrochloric acid from initial pH to pH 3.5, at room temperature, titration volumes being read at intervals of 0.5 of a pH unit. A similar experiment was also conducted on a 10 ml. sample of the pooled saliva used in food experiments. Titration volumes were plotted against pH, and are shown in Figure 6. To eliminate the confusion which would result if individual curves for each of the 22 individuals were plotted, individual values only are shown at each pH interval, as the curves differed considerably in shape, and crossed frequently. The mean hydrochloric acid volume added to reach each pH level was then calculated for each pH interval, as well as the mean initial pH. The titration curve obtained from the pooled saliva sample used in the food tests is shown on the same graph, by the close dotted line.
DEVELOPMENT OF METHOD

It can be seen that the pooled saliva initial pH corresponded closely to the mean initial pH of the 22 individuals, and except for slight differences at pH 4.0 and 3.5, corresponded also to the mean pH values at each sample interval. This result indicates that the pooled sample of saliva used for the food fermentation tests was similar to a random sample of 22 different salivas, when they are considered as a pooled sample. The panel of 8–10 saliva donors provides a pooled saliva sample which is representative of saliva in general, and would be unlikely to vary excessively in buffer capacity from day to day. Figure 6 also demonstrates the great variation in buffer capacity and initial pH for salivas from different individuals,\(^{(145)}\) indicating the desirability of using pooled, rather than single-donor saliva samples in food fermentation experiments, to eliminate variables from these sources.

But even when pooled saliva was used in the food tests, replicate experiments did not yield identical results. This effect, probably a result of variations in the micro-organism population of the pooled saliva samples, complicates the interpretation of food test data. When a number of replicate food tests have been conducted on a given food, it is possible to analyse the data, and assess the reproducibility of the method. If the reproducibility is satisfactory, it might then be possible to reach valid conclusions when they are based on comparisons of data obtained from small numbers of replicate experiments on other foods. If, however, there is a lack of reproducibility, more replicate experiments on these
DEVELOPMENT OF METHOD

foods may have to be conducted, to establish that any observed
differences between food test results did not occur by chance.

An indication of the reproducibility which could have been
expected from a number of experiments on each of the U.S. foods
tested in the preliminary study was provided by the eight replicate
U.S. sucrose fermentations. The ranges, means standard errors and
range for \( \pm \) two standard deviations for the hour 6 and hour 24
values of pH, titratable acidity and enamel dissolution for these
replicate fermentations are shown in Table XIV. It can be seen
that one experiment only on each U.S. food would not necessarily
suffice for valid comparisons between U.S. food tests to be made.
For this reason, it is not possible to make rigorous direct
comparisons of U.S. foods with each other, as, with the exception
of sucrose, only one test was performed on each.

But it is possible to compare directly the results of
individual U.S. food fermentations with the U.S. sucrose
fermentations, and when this was done, it was clear that the results
obtained with some foods were quite different from those obtained
with sucrose. This direct comparison was possible because the
eight replicate experiments on U.S. sucrose provided means and
standard deviations for the experimental values obtained, and it
could then be stated that any experimental value lying beyond the
range of \( \pm \) two standard deviations from a given mean would occur by
chance in only five out of 100 trials. Thus any experimental values
obtained for the other U.S. foods which lay beyond this range can be
DEVELOPMENT OF METHOD

said to be significantly different at the P. 05 level.

The experimental values obtained for the U.S. foods are shown in Tables I-VI, and the hour 6 and hour 24 values for pH, titratable acidity and enamel dissolution shown in these tables are compared with the corresponding U.S. sucrose mean values, in Table XV. Those values which are not significantly different from the corresponding sucrose mean are designated "0", those which are significantly higher "+" and those which are significantly lower "-".

It can be seen that in comparison with sucrose, all the U.S. foods tested differed in at least one of the six experimental values recorded in Table XV, and most differed in at least three of these values. Furthermore, with some of these foods, the significantly different experimental values were higher than the corresponding sucrose values, while with others they were lower, suggesting that even in the absence of replicate experiments, real differences in fermentation behaviour between U.S. foods did exist. Since it was the aim of the preliminary study to determine whether such differences existed, and because improvements were to be incorporated in the food test before the commencement of the detailed study, replicate experiments were not required to achieve the aim of the preliminary study, and were not conducted.

For the Australian food tests, there remained the problem of determining the minimum number of replicate experiments which would be required to show significant differences in behaviour between foods and groups of foods. The ranges and means shown for the eight
DEVELOPMENT OF METHOD

replicate U.S. sucrose tests suggested that a small number of replicate experiments would suffice. Three replicate Australian sucrose experiments were then conducted, and the results compared with those for the eight U.S. sucrose experiments, to test this possibility (See Table XIV).*

The table shows that the ranges for the experimental values recorded were of the same order and that the means were similar in comparison with the differences shown in Table XV for many of the U.S. foods.** As might be expected, the eight replicate experiments conducted on U.S. sucrose did result in smaller standard error values, in comparison with the three replicate experiments conducted on Australian sucrose.*** It was considered that, while the smaller standard errors obtained from eight replicate experiments might be necessary to demonstrate significant differences in fermentation behaviour when such differences were small, three replicate experiments would be sufficient to demonstrate larger differences, provided the standard errors values obtained from three replicate experiments were not large.

Three replicate experiments were then conducted on all the

* Such a comparison is not strictly valid for titratable acidity, as the conditions under which it was determined were not identical in the U.S. and Australian tests.

**Except for enamel dissolution at hour 6.

***Except for enamel dissolution at hour 6.
DEVELOPMENT OF METHOD

Australian sugars, and the results compared. The standard errors obtained from the data were then employed to determine whether any of the differences observed were statistically significant. The results of such a comparison, between honey, molasses and sucrose are shown in Figure 9, and are discussed in Section V. Here it is sufficient to state that the standard errors for some of the mean values from three replicate experiments on each of these sugars were sufficiently small to enable definite and statistically reliable differences in fermentation behaviour between these three foods to be demonstrated.

Since it was the aim of the Australian study to detect large differences in the fermentation behaviour between different foods, it was decided, from the results of the fermentation comparisons shown in Figure 9, that three replicate experiments on the Australian foods would be sufficient to demonstrate any large differences in fermentation behaviour, and permit statistically valid comparisons between foods to be made.

The inherent buffering properties of the foods tested were determined using 5 gm. of food and 50 ml. of water. Hydrochloric acid of 0.1M was used to titrate the food-water mixture from its initial pH to the experimental hour 24 pH value of the actual food fermentation experiment. These values were obtained to determine whether the enamel dissolution observed in food fermentation experiments was in any way related to the inherent buffer capacity of the food, and are shown in Tables VII and VIII.
DEVELOPMENT OF METHOD

For reasons already discussed, it was not possible to obtain accurate enamel dissolution figures when they were based on conventional calcium or phosphate analyses. In the search for a suitable alternative, the technique employed by Grabenstetter and his colleagues to study tooth wear as a result of toothbrushing was investigated. \(^{(102)}\) He irradiated whole teeth, and assessed tooth wear during brushing by estimation of the amount of radioactivity released from the teeth.

In conjunction with Beck and co-workers, the author irradiated powdered tooth enamel and enamel blocks, to determine whether this radioactive labelling technique could be used to assess enamel dissolution. \(^{(14)}\) It was found that the technique produced tooth enamel uniformly labelled with \(^{32}\)P. The appearance of \(^{32}\)P in solution was then used to assess the amount of enamel dissolved, by comparison of the radioactivity in the experimental sample with that of an appropriate radioactive enamel standard. This technique overcame the difficulties discussed in connection with chemical analyses, since it was completely independent of the calcium or phosphorus content of the test food or saliva. This approach possessed the added advantage of providing accurate results with very small experimental samples, thereby limiting excessive diminution of the experimental volume.

But for the appearance of radioactivity in solution to provide an accurate assessment of the amount of tooth enamel dissolved, radioactive enamel should dissolve in a manner identical with that of
DEVELOPMENT OF METHOD

non-irradiated tooth enamel in acid solutions. The radioactivity appearing in solution should also be proportional to the amount of tooth enamel actually dissolved. These requirements were not met in the food tests. The reasons, their implications and the justification for using radioactive enamel are discussed at length in Appendices B and C.

In any food fermentation, there is a slow but continuous change in pH and titratable acidity, both of which achieve stability only after a considerable time period has elapsed. It is difficult to decide at what stage the most significant enamel dissolution takes place, early in the experiment or at its conclusion, so far as application of results to the in-vivo situation is concerned. Since enamel dissolution also takes a considerable time to reach equilibrium under a given set of experimental conditions, the dissolution of enamel powders in prepared acetate buffers was investigated, to provide a baseline to which acids produced by food fermentation could be compared, especially in the early stages of an experiment, where pH and titratable acidity changes were likely to be so rapid as to prevent enamel dissolution from reaching equilibrium within a given time interval. The dissolution experiments were carried out using simple acetate buffers at various pH levels and molar strengths, and from these results, a 'group' of curves was constructed. These curves enabled the investigator to compare the enamel-dissolving ability of a fermenting food-saliva mixture with that of a simple chemical system of similar pH and molarity,
DEVELOPMENT OF METHOD

where no food was present. It was by comparisons of food fermentation experiments between themselves, and between a food fermentation experiment and pure buffer experiments where pH and buffer molarity conditions were comparable, that the conclusions reached in this work were obtained.

In an additional experiment, acetate buffers of various molarities were titrated from pH 4.0 to pH 7.0, using sodium hydroxide. The amount of sodium hydroxide addition necessary to achieve a given pH change was then plotted against pH, and the resulting curves could be used to indicate the degree of pH change which would result in a simple buffer system of various molarities, when acid or base was added (Section V and Figure 13). These curves enabled the investigator to compare the buffer capacity of a simple acetate buffer with the buffer capacity of an equivalent fermented food—saliva mixture.
SECTION IV

EXPERIMENTAL METHOD
EXPERIMENTAL METHOD

The experimental method used in this investigation consisted of determining, at frequent intervals, the pH and titratable acidity of a stirred mixture of pooled saliva, bovine enamel powder and the food being tested, and assessing the amount of enamel which dissolved in this mixture. Tests on each food were conducted three times, and the mean values recorded in graphical form for purposes of comparison.

Two weeks before a series of tests, several grams of washed, 120-200 mesh bovine enamel powder were prepared (See Appendix A) and sent to a nuclear reactor* for irradiation. After twelve hours of irradiation, the enamel was shipped back to the laboratory and stored for one week. The silica irradiation vial was then cut open in a fume cupboard, and a 100 mg. sample of the radioactive powder weighed out, to an accuracy of ± 1.0 mg. on a single pan balance.**

The sample was then dissolved in a minimum amount of concentrated hydrochloric acid and the volume made up to 100 ml. with distilled water, in a volumetric flask. A 100 μl. sample of this solution was transferred to a 1", flat, stainless steel planchet using a micropipette, aspiration to the gauging mark being effected by a control

---

* HIFAR Reactor, Lucas Heights, Sydney, Australia.
** Mettler H 16 T.E. Mettler, Zurich, Switzerland.
METHOD

pipette \* (See Fig. 7, 2) The planchet was then placed under a heat lamp, the sample spread using a wetting agent, and evaporated to dryness. Count rates for the sample were determined at intervals, to establish a decay curve, and thus verify that the activity detected was that of P\textsuperscript{32} (See Appendix C II). This sample was used as a radioactive enamel standard for all food fermentations of a given series, to provide a count rate for the P\textsuperscript{32} contained in a 0.1 mg. sample of radioactive tooth enamel.

On the afternoon of the day before a food fermentation experiment, each member of a panel of 8 to 10 saliva donors was provided with a 6" x 3/4" stoppered test tube and half of a large, sterilised 3" x 3/4" gum rubber band. Saliva donors were all adult males with complete or nearly complete natural dentitions, who chewed the rubber bands and deposited the accumulated saliva in the tubes until about 15 ml. had been collected by each. Collection was timed to precede the afternoon tea break. The saliva samples were then placed in a beaker and stored overnight in a freezer. On the morning of the experiment, the pooled saliva was thawed at room temperature and a Teflon\textsuperscript{R}-covered 1" stirrer bar added to the beaker. The beaker was then placed on a magnetic stirrer table ** for ten minutes, to distribute the salivary sediment evenly, prior to use.

\* Micro-syringe No. 0010, Hamilton Co., Whittier, California, U.S.A.
\** Steristirrer, Sterimed Pty.Ltd., Melbourne, Victoria, Australia.
METHOD

A jacketed beaker was used for all food fermentations. It consisted of a double-walled glass vessel, with connections for carrying heating water to and from the jacket (See Fig. 7, 1). Water circulation and heating were provided by rubber hose connections to and from a thermostat-controlled heater and pump* immersed in a water bath. In this way the contents of the jacketed beaker were maintained at 37°C. A large rubber bung, carrying a pH electrode, reference electrode, thermometer and stoppered access hole was then fitted to the beaker, the pH electrode checked for potassium chloride level, and appropriate connections made to a pH meter.** (See Fig. 7, 1). The pH meter was then adjusted to indicate pH, using buffers at pH's of 9.2, 7.0 and 4.0.*** The electrodes were washed with distilled water between buffer changes, and stirring of buffers was achieved by placing the jacketed beaker on a magnetic stirrer table, and adding a Teflon-covered 1'' stirrer bar to the buffer solution. During pH adjustment, the stirrer was stopped, to obtain stable readings. The stirring speed for all operations using the jacketed beaker was set by rheostat adjustment at 150 r.p.m.

The jacketed beaker and electrodes were then cleaned and dried, the apparatus assembled, and 50 ml. of the saliva, (at room

* Thermomix, B. Braun, Melsungen, Germany.
** Model 22, Radiometer a/s, Copenhagen, Denmark.
*** 'Soloid' Buffer Tablets, Burroughs Wellcome, London, U.K.
METHOD

temperature) added, and stirred. While the saliva was warming, 5 gm. of the test food, and 100 mg. of radioactive enamel powder were weighed out. When the saliva in the jacketed beaker had reached a temperature of 37°C, the weighed food was added to the beaker and stirred. When foods were in the form of large particles, e.g., Rice Bubbles® and breads, the test food was crushed or otherwise divided into small pieces, before being added to the saliva. Sticky foods, such as molasses, or solid foods such as caramels, were added to 50 ml. of saliva in a separate small beaker before being added to the jacketed beaker, to prevent portions of the test food from adhering to the electrodes or sides of the jacketed beaker, and thus not mixing. This small beaker was removed from the jacketed beaker when the food had dissolved in the saliva. With all foods tested, the final consistency was quite fluid, and easily stirred at 150 r.p.m.

When the food and saliva were completely mixed, the 100 mg. of radioactive enamel powder was added, care being taken to ensure an even distribution, with none remaining on the exposed parts of the electrodes or thermometer, or the sides of the beaker. Immediately all the enamel had been added, the pH of the mixture was read, and samples taken for titratable acidity and enamel dissolution determinations.

Titratable acidity samples were obtained using a glass 1.0 ml. tuberculin syringe, modified by removal of the adapter tip, square and flush with the 0.0 ml. mark, and fitting metal extensions
METHOD

to the glass plunger and body. In this way, a 1.0 ml. sample could be taken from all food-saliva mixtures, regardless of consistency, without blockage, and could be ejected completely into small titration tubes, modified by providing each with a bulge in the middle of the tube. (See Fig. 8, (1) and (2)).

The microtitration apparatus consisted of a combined, miniature pH electrode, held by a clamp above the surface of a magnetic stirrer table. (See Fig. 8, (1) and (2)). The titration tube carrying the sample was placed on the table, the pH electrode inserted, and the tube held in place by it. Stirring was achieved by placing a small cube of ceramic magnet material in the tube, and running the magnetic stirrer at maximum speed. The size of the bulge in each titration tube was such that with the miniature electrode in place, 1.0 ml. of liquid in the tube completely covered the recording surfaces of the electrode. This bulge also allowed a further 1.5 ml. of solution to be added without causing solution to be drawn up between the sides of the electrode and titration tube by capillarity. Without this modification, some of the sample was prevented from mixing completely with the titration liquid, and led to erratic titrations.

The titration electrode was connected to the pH meter when required, and pH determined indirectly from the millivoltage reading, by reference to a graph of millivoltage plotted against pH, using buffers of pH 9.2, 7.0 and 4.0. Titration solution was supplied to the titration tube from a 500 µl. and a 250 µl. gas
chromatograph syringe, each equipped with a hypodermic needle attached to fine bore, high density polyethylene tubing. This tubing was inserted in the titration tube so that the titrant was discharged well down in the solution, near the ceramic magnet stirrer. (See Fig. 8, 1 2 and 3).

The titration solution consisted of approximately 0.1 M sodium hydroxide, standardised by colourimetric titration against a 0.1 M potassium hydrogen phthalate standard solution, using phenolphthalein indicator. Care was taken to ensure that when the syringes and tubes were filled with titration solution, all air bubbles were excluded. Titratable acidity was always expressed as the volume of 0.1 M sodium hydroxide required to return the pH of a given volume of the sample to 7.0. Correction factors were used for the calculation when the sodium hydroxide solution used was not exactly 0.1 M. With all foods tested, the initial pH (at hour 0) was close to 7.0. When the initial pH was above 7.0, no titration was performed, and when just below, and also when very small amounts of titration solution were required, the 250 µl. titration syringe was used. Later in each experiment, when appreciable acid production had taken place, the larger volumes of titration solution required were supplied by the 500 µl. syringe. The amount of titration liquid used was obtained by subtracting the final volume reading from the initial volume reading.

When titrating certain food-saliva-enamel mixtures, usually those with large amounts of insoluble matter present, it was found
METHOD

that the end-point of the titration was not stable, and tended to return to a slightly lower pH when left for a few seconds. In such cases, the final titration value was not read until this tendency had ceased, by adding more titration solution, and waiting until a stable pH 7.0 reading was obtained.

Enamel dissolution samples were taken by aspiration through small glass-frit gas dispersion tubes of 4 micron pore size, cut so as to allow the insertion of a long, thin 100 µl. micropipette far enough to reach the glass frit. (See Fig. 7, 2). Aspiration was achieved through a rubber tube connecting the glass-frit tube to a water-operated vacuum pump, and which incorporated a glass T-piece, one arm of which was left open. With the stopper of the access hole in the rubber bung removed, the glass-frit tube was lowered into the food-saliva-enamel mixture, and the vacuum pump turned on. The operator’s finger was used to close the open arm of the glass T-piece, causing a fall in the air pressure inside the frit tube. The external air pressure thus forced the sample through the glass frit. When the required amount of filtrate, about 0.15 ml., had been aspirated, the finger was removed, equalising the pressure and stopping the filtration process. In this way, fine control of the filtration process was obtained, without back-flow from the pump or boiling of the filtrate, and allowed rapid control in those mixtures which were very easily filtered.

With some foods of very fine consistency, such as the flours, the filter pores tended to block during filtration. In such cases
METHOD

the stirring was stopped a minute prior to sampling, to allow the food to settle, leaving a clear zone at the surface of the mixture. This precaution usually allowed a filtrate to be obtained without blockage. In those few cases where it did not, a 1.0 ml. sample of the mixture was removed and centrifuged for a few minutes before filtration. When a filtrate had been obtained, the remaining solution was shaken by hand and returned to the jacketed beaker, to limit experimental volume diminution.

A 100 µl. pipette was now inserted into the frit tube and a sample aspirated to the gauging mark using a control pipette (See Fig. 7, 2) ejected onto a previously labelled stainless steel planchet, and spread and dried, as for the standard sample of tooth enamel. Planchets were kept in a small carrier until all the enamel dissolution samples for the experiment had been collected.

All count rate determinations on the sample planchets and the enamel standard were made within one hour, on the day following the experiment. Two types of detector were used. One was a gas flow detector,* operated in the Geiger Müller region, either windowless or with an ultra-thin window. The other was a scintillation counter.** (See Fig. 7, 3). The former had a counting chamber with 2π geometry, and was used for calibration and checking, and for some samples with

* Model D47, Nuclear Chicago Corp., Chicago, Ill., U.S.A.
** Model N530G, Ekco Electronics Ltd., Southend-on-Sea, Essex, U.K.
low count-rates. The latter was used for nearly all enamel dissolution experiments, because of its very short resolving time. Each was operated in conjunction with a six decade scaler, to count the pulses. (See Fig. 7, 3). Samples were counted for 100 seconds, under identical conditions, provision being made for background radiation and resolving time corrections where appropriate. Each count-rate determination was made three times.

Count rates were sufficiently high to ensure statistically valid results (See Appendix C II), and from these rates, the amount of $P^{32}$ in an experimental sample could be derived by comparison with the count-rate of the standard sample, by simple arithmetical calculation, thus:

\[
\frac{\text{Corrected count rate of experimental sample}}{\text{Corrected count rate of standard sample}} = \frac{P^{32} \text{ in sample}}{P^{32} \text{ in enamel standard}}
\]

If the standard sample count rate is $S$ counts/100 sec./mg., and the experimental sample count rate is $E$ counts/100 secs., the experimental sample contains the amount of $P^{32}$ present in $E/S$ mg. of radioactive tooth enamel. Since this experimental sample was 0.1 ml., or 1/500 of the total experimental volume, the $P^{32}$ in the liquid phase of the food-saliva mixture is $500 \times E/S$ of the total

METHOD

P\textsuperscript{32} added\textsuperscript{*}. The value 500 E/S will thus be enamel dissolution, in milligrams, provided the P\textsuperscript{32} appearing in solution indicates the amount of enamel dissolution which has taken place.

During the fermentation of carbohydrates by oral microorganisms, lactic acid is generally regarded as one of the principal organic acids which is formed\textsuperscript{(75, 267)}. This suggests the use of lactate buffers as appropriate standards against which to compare the buffering capacity and enamel dissolution potential of fermented food-saliva mixtures, and for studying enamel dissolution in simple chemical systems of various pH levels and molarities. In this study, acetate buffers were used for this purpose rather than lactate buffers, for the following reasons:

a. The enamel dissolution taking place in buffer systems is primarily a function of the pH, and to a lesser extent, the molarity of the system\textsuperscript{(103, 105, 221)}. The source of these hydrogen ions is not important, and provided any ion effects on enamel dissolution are disregarded, the hydrogen ions could be supplied by lactic acid, acetic acid, or even hydrochloric acid, without affecting the experiment. On this basis, then, there is no particular advantage to be gained

\textsuperscript{*} Note that this is not strictly correct, since the experimental volume is 50 minus 6 ml. at hour 24, 50 minus 5 ml. at hour 6, etc., because of the titration samples taken. Correction factors were not employed, since volume diminution was the same for all experiments.
b. During most 24 hour food-saliva fermentations, the pH of the mixture falls from just above 7.0 to about 4.0. Any simple chemical buffer system used for studying the enamel dissolution which takes place at a given pH and molarity should maintain the pH and molarity, even if extensive enamel dissolution takes place, so that interpretation of the results is not complicated by changing experimental conditions during the experiment. In buffer systems, the zone of effective buffer action varies with concentration, and for concentrations of about 0.1 M, this zone lies within about ± one pH unit of the pK for the buffer used. Thus the effective buffer range for the acetate buffers used in this study is about pH 3.6 - 5.6, in comparison with the range for corresponding lactate buffers of about 2.9 - 4.9. Thus acetate buffers are to be preferred in the simple chemical buffer dissolution experiments conducted in this study, as their effective buffering range at the molarities tested matches more closely the pH range observed in fermenting food-saliva mixtures.

c. Any buffer system used to study enamel dissolution should exhibit minimal chelating effects, so that the interpretation of the results for dissolution is not complicated by these effects. Since acetate ions have weaker chelating effects
METHOD

than lactate ions, acetate buffers are to be preferred.

Some of the acetate buffers used in this study were prepared in the conventional way, using stock acetic acid and sodium acetate solutions of the appropriate molarity, in the proportions indicated in standard buffer tables to produce the required pH. Where a buffer with a pH other than those listed in such tables* was required, an alternative method of preparation was employed. Stock acetic acid solutions were titrated to the desired pH using sodium hydroxide, and diluted to the required molarity, with appropriate pH adjustments as required.

The term 'buffer capacity' is used in this study to describe the ability of a solution to resist a change in pH when acid or base is added. This property, as assessed for mixtures of food and water, is the 'inherent buffer capacity' of the food. When the food is mixed with saliva, the resulting buffer capacity of the mixture can be assessed by adding the 'inherent buffer capacity' of the food to the buffer capacity of the saliva. (See Section III and Tables VII and VIII). A food–saliva mixture of high 'buffer capacity' requires the addition of more acid than does a food–saliva mixture of low 'buffer capacity' to achieve the same fall in pH. In an unfermented food–saliva mixture, the 'buffer capacity' can be assessed by measuring the hydrogen ion addition necessary to achieve a given fall

* Acetic acid – sodium acetate mixtures with a pH of 6.0 or higher are not effective buffers, but are referred to as buffers in this study, for the sake of uniformity.
METHOD

in pH. Provided 'salt' and 'dilution' effects are ignored, the pH of this mixture can be restored to its previous level by an equivalent hydroxyl ion addition. On this basis, then, the 'buffer capacity' of a food–saliva mixture could be assessed by measuring the pH change resulting from the addition of either acid or base.* Thus it might be assumed that the buffer capacity of an unfermented food–saliva mixture, as measured by acid titration to a low pH, will be identical to its buffer capacity after fermentation, as measured by base titration from this low pH to its unfermented pH.

This will be the case only if the pH fall as a result of fermentation was achieved through production of completely dissociated acids, but the acids previously mentioned as being the principal products of fermentation are organic, and only partly dissociated at low pH. Hence the amount of base required to restore the pH of a fermented food–saliva mixture to its initial unfermented pH must be greater than the amount of completely dissociated acid required to lower the pH of the unfermented food–saliva mixture to the pH level reached after fermentation. There is yet another complication in determining the buffer capacity of food–saliva mixtures. It is possible that food–saliva mixtures are modified in some way during fermentation, becoming thereby stronger or weaker buffers, independently of the acid production.** Thus the buffer

---

* Provided the acid or base used is completely dissociated.
** This would occur if, for example, the 'inherent buffer capacity' of a food changed as a result of fermentation.
capacity of an unfermented food–saliva mixture could not be precisely
determined by titratable acidity measurements after fermentation, as
this value was always found to be greater than its unfermented buffer
capacity. Nor could the organic acid production as a result of
fermentation be accurately determined from titratable acidity
measurements, unless it was known that the food–saliva mixture had
not changed its buffer capacity during such fermentation.

It is possible to predict the extent of the enamel dissolution
which should take place in a food–saliva mixture, if this mixture
behaves as a simple chemical buffer, by referring to the family of
curves for dissolution obtained from experiments on simple chemical
buffer systems. (See Fig. 18). To make such a prediction, the
'effective molarity' of the food–saliva mixture, considered as a
simple buffer system, must be determined.

In this study, the 'effective molarity' of a food–saliva
mixture was estimated by comparing its 'titratable acidity' with
the 'titratable acidity' of simple chemical buffers of known pH and
molar strength. The graph shown in Figure 15 was used to facilitate
such comparisons, and was prepared by recording the quantity of
0.1M sodium hydroxide required to change the pH of acetate buffers
of 1.0, 0.5, 0.3, 0.1 and 0.05 molarity, and pH levels in the
range 4.0 – 6.0, from these pH levels to 7.0. Each point shown on
the graph thus indicates the titratable acidity of a given buffer
of a specific pH and molarity, and the lines joining like points
permit the estimation of titratable acidity for simple buffers of other
METHOD

pH levels and molarities.* Similarly, the family of curves shown in Figure 13 can be used to estimate the molarity of food-saliva mixtures, if they behaved as simple chemical buffers when titrated, provided the pH and titratable acidity of such food-saliva mixtures is known.

The buffer capacity of a food alone, without saliva (inherent buffer capacity), was determined by titration against 0.1M hydrochloric acid. A 5 gm. sample of each food was added to 50 ml. of distilled water, and the pH taken. This pH was invariably lower than that of 5 gm. of the food in 50 ml. of saliva. The food-water mixture was stirred and hydrochloric acid added until the pH of the mixture was the same as the pH of a similar food-saliva mixture after 24 hours fermentation. The hydrochloric acid volume was here termed the buffer capacity of the food alone (inherent buffer capacity) for the pH range mentioned.** A similar titration was performed on 50 ml. of pooled saliva, from the initial saliva pH of 7.5 to pH 4.0, for comparison purposes (See Tables VII and VIII).

A separate 24 hour enamel dissolution experiment was conducted using a simple buffer system where the pH and titratable

* It should be noted that the dashed lines shown in Figure 13 do not represent titration curves, but rather a table of buffer capacities, or titratable acidiities, for acetate buffers of various pH levels and molarities. Titration curves for buffers give rise to curves of a different shape, because of dilution effects as the titration proceeds.

** With cornflour, this initial pH was less than 4.0. This food does not, therefore, have an 'inherent buffer capacity' in this context.
acidity changes were arranged to follow those occurring in the sucrose food tests. This experiment was designed to determine whether a simple buffer system which varied in its pH and molarity in the same way as a test food would result in a similar enamel dissolution. Sucrose was chosen as the food providing the pH and titratable acidity changes to be copied, as it has no inherent buffer capacity, and has the simplest structure of the common carbohydrate foods.

At the conclusion of each food test, all glassware and apparatus were thoroughly cleaned, soaked in chromic acid where appropriate, then checked to ensure that there was no residual radioactivity. All glass-frit tubes and micropipettes were thoroughly cleaned by aspiration of chromic acid, strong sodium hydroxide, water, alcohol, then acetone, in that order, and pH electrodes were thoroughly washed and wiped. The used food-saliva-enamel mixture was stored in an unbreakable container, with phenol added to retard decomposition, until six months after irradiation. The activity present was then such that the contents could safely be disposed of.

All laboratory personnel were supplied with radiation badges, which were read at two-week intervals to check the level of exposure to radiation, and the laboratory was surveyed at weekly intervals to detect and clear any accidental contamination or spillage, and to ensure that working conditions were safe at all times. Two incidents occurred during the five years of the project, each a spillage, one of enamel powder, the other of a food-saliva-
enamel mixture. Decontamination in each case was straightforward, and easily achieved.