SECTION V

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TERMINOLOGY

The following abbreviations are used for expressing the results obtained in this work:

Hydrogen ion concentration is expressed as units of pH, thus:
'... pH of 5.58' or '... change of 1.2 pH units ...'

The acid content of a food-saliva-enamel mixture is expressed as titratable acidity. The titratable acidity was recorded as the volume of 0.1M sodium hydroxide solution which was required to titrate 10 ml. of the food-saliva-enamel mixture to pH 7.0, thus: 'titratable acidity was 1.5 ...' (i.e. 1.5 ml. of 0.1M sodium hydroxide was used to bring the pH of the food-saliva-enamel mixture to pH 7.0). The abbreviation 'T.A.' is used to represent the terms 'titratable acidity', 'titratable acidity value' or 'titratable acidity unit(s)' where appropriate, thus: '... change was 3.5 T.A.', or '... to a lower T.A.'

Enamel dissolution is expressed as the amount of enamel dissolved in a food fermentation experiment, measured in milligrams. Since the original enamel addition to the 50 ml. of food-saliva mixture was 100 mg., the enamel dissolution value also represents the percentage of enamel added which has dissolved, thus: '.. enamel dissolution of 15 mg.' (i.e., 15 mg. of the 100 mg. added at the start of the experiment has dissolved).

When a product is marketed under a registered brand name, it is indicated by the letter "R", thus: Sweetaddin R, Teflon R.
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During the preliminary developmental work leading to the experimental techniques used in this study, a wide range of carbohydrate foods was tested. These experiments were conducted at the Eastman Dental Center, Rochester, N.Y., in 1960*, on foods available locally. In general, the technique used in these tests was similar to that used in the later tests on Australian foods, with the following variations:— the saliva donor panel consisted of three people (because of difficulties in assuring continuity of saliva supplies), and separate beakers were used for measurement of enamel dissolution and titratable acidity (because of difficulties associated with titration sample size). In addition, no replicate experiments were conducted, except in the case of sucrose.

The eight replicate sucrose fermentations provided some indication of the experimental variables inherent in the method. These variables were sufficiently large to preclude rigorous comparisons between individual U.S. foods. For the same reason, and also because the experimental conditions were different, these results should not be compared with the Australian food test results. However, it is clear from examination of the data presented in Table XV that all the U.S. foods tested behaved differently from sucrose (See Section III). It will also be apparent later in this work that the results obtained from single tests on the U.S. foods are in many cases essentially similar to those obtained with

* In conjunction with Dr. B.G. Bibby and Dr. D.J. Beck
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comparable Australian food tested using the later technique, even though these similarities cannot be evaluated statistically. For these reasons, and because they provided a useful guide for the selection of Australian foods to be tested, comparisons of individual U.S. foods were made.

These preliminary results for the 35 U.S. foods are expressed in table form, and also compared with the U.S. sucrose results (Tables I-VI and XV).

TABLE I: BEVERAGES GROUP

Four beverages were tested, undiluted, and also as a mixture of 25 ml. each of the beverage and saliva, with 100 mg. of enamel added in each case. Beverages were the only foods tested in this way, as it was found that the small amount of carbohydrate contained in 5 ml. (about 5 gm.) of a beverage was not sufficient to allow acid production to proceed to any significant extent. The results in Table I are not therefore directly comparable with those of the other foods tested.

From Table I, it can be seen that the beverage pH changed little after hour 2, whether mixed with saliva or not. The initial pH of the saliva-beverage mixture was higher than that of the beverage alone, but the difference almost disappeared as the experiments progressed. With all beverages, the pH was within the range 4.0 - 4.8 for most of the experimental period.

The titratable acidity varied slightly with the undiluted carbonated beverages (no saliva), in the early part of the experiments,
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falling slightly from hour 0 to hour 1, except for Coca Cola\textsuperscript{R}, which remained unchanged throughout. After this initial fall, titratable acidity for the undiluted beverages remained almost unchanged, except with orange juice, where a slight T.A. increase was observed.

In the beverage—saliva mixtures, titratable acidity was lower at hour 0, probably because saliva is a weaker buffer than most beverages. As fermentation progressed, T.A. increased two or three times, except for orange juice, where a slight increase only was found at hour 24.

Enamel dissolution for the carbonated beverages was lower at the beginning, and higher at the end of the experiments, for the beverage—saliva experiments, in comparison with the undiluted beverages, except for orange juice, where the undiluted beverage caused more dissolution throughout. In general, dissolution with the beverages was comparable with that obtained using simple acetate buffers of similar pH and molarity, except for orange juice, which was much higher. (See Appendix B, 'Group of Curves'). The extensive dissolution expected with acidulated, carbonated beverages was not observed.

TABLE II: SUGARS GROUP

Seven sugars were tested. The initial pH of the sugar—saliva mixtures was slightly alkaline with all except the light and dark molasses, where it was slightly acid. The pH fell rapidly in all cases, to between 4.0 and 5.0 by hour 4, but with sucrose and dextrose, the pH fall was slower. The hour 24 pH values were all
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between 4.3 and 4.8.

By hour 2, titratable acid was produced with all sugars except dextrose, the less refined sugars giving the highest values. Thus honey, light molasses and brown sugar produced up to twice, and dark molasses up to three times the T.A. of sucrose, the differences being maintained, but decreasing, as the experiments progressed. Dextrose T.A. remained low throughout.

Enamel dissolution was high for honey and sucrose at hours 2 and 4, in comparison with the other sugars, though these differences were less pronounced at hours 6 and 24. By hour 24, all sugars exhibited about the same enamel dissolutions, except for light and dark molasses, which were lower, especially the latter. There was no obvious correlation between pH, titratable acidity and enamel dissolution.

TABLE III: FLOURS GROUP

Thirteen flours were tested. The initial pH of all flours except potato flour was slightly alkaline, but fell in all cases to 6.0 or less by hour 2. After hour 4 all flours had pH levels of 5.0 or less, and by hour 24, all pH levels were between 4.1 and 4.8. There was no apparent relationship between pH and degree of refinement of the flours.

Titratable acidity values were very high throughout the experimental period for wheatgerm and bran, and high for rye flour. Except for potato flour and potato starch, which had high and low T.A. respectively, the acid production appeared to be related to degree
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of refinement of the flour, and was in the range 1.1 - 6.7. Thus the
two wheat flours, plain white and enriched white, and cornstarch, had
low T.A.’s (2.8) in comparison with the whole grain flours (3.2 - 4.0).
Of the three rice flours, wild rice, a completely unrefined grain, had
higher T.A. at hour 6 (3.7).

Enamel dissolution values were generally low for the whole
grain flours, such as bran, rye flour, brown rice flour and wild rice
flour, in comparison with the refined flours, especially at hour 24.
High hour 24 enamel dissolution was obtained with potato flour and
starch, cornstarch and polished rice flour. Of the two wheat
flours, plain white flour enamel dissolution was high at hour 2, 4
and 6, while enriched white flour enamel dissolution was high at hour 24.
There was no obvious correlation between pH, titratable acidity and
enamel dissolution.

TABLE IV: CEREALS GROUP

Three cereals were tested. With all three the initial pH was
7.0, and fell rapidly to about 5.0 by hour 2, slowly falling to 4.5
by hour 24.

The titratable acidity values were low in comparison with
most other foods tested, rising slowly with Corn Flakes\textsuperscript{R} and
Shredded Wheat,\textsuperscript{R} and more slowly still with puffed rice.

Enamel dissolution was roughly comparable with that of most
of the flours at hours 2, 4 and 6. At hour 24, enamel dissolution
was about 40 mg. for Corn Flakes\textsuperscript{R} and puffed rice, but only 22 mg.
for Shredded Wheat\textsuperscript{R}. Though the pH and titratable acidity changes
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with Corn Flakes\textsuperscript{R} and Shredded Wheat\textsuperscript{R} were similar, the enamel dissolution in the latter was much lower in the later stages of the experimental period.

TABLE V: BREAD AND BISCUITS GROUP

Three breads and four biscuits were tested. The pH fell rapidly to about 5.0 by hour 2 with all except whole wheat bread, where the pH fell less rapidly. By hour 6, the pH was about 4.7 for the breads and 4.5 for the biscuits, remaining more or less unchanged to hour 24 for the breads but falling slightly to about 4.4 for the biscuits.

Titratable acidity was about 1.5 by hour 2 for the biscuits, rising slowly to about 2.5 by hour 24. With the whole wheat and whole grain breads, the hour 2 T.A. was lower, while with white bread the hour 2 T.A. was higher, in comparison with the biscuits. The hour 6 T.A. of breads were comparable with those of biscuits, except for whole wheat bread, which was 50% higher. By hour 24 all bread and biscuits T.A. were comparable, about 2.5, considerably lower than those of the flours group. An interesting feature of some of the breads and biscuits was the fall in T.A. between hour 6 and hour 24, and was especially noticeable with whole wheat bread. The only other foods tested which exhibited this effect were bran, rye flour, wheat flour and wild rice flour.

Enamel dissolution values were similar to those obtained with flours and cereals, except for whole wheat bread and Fig Newton.\textsuperscript{R} Enamel dissolution for the whole wheat bread was very low, and
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similar to that of dark molasses, while that of Fig Newton\textsuperscript{R} resembled those of potato flour and corn starch.

TABLE VI: SWEETS GROUP

Three sweets were tested. With all three the initial pH was slightly alkaline, falling rapidly to about 5.0 with chocolate-covered coconut and caramel by hour 2, and to this same level with milk chocolate by hour 4. The pH continued to fall with milk chocolate and chocolate-covered coconut to about 4.6, where it remained until hour 24. The caramel pH fell more slowly, to 5.0 at hours 4 and 6, and 4.8 by hour 24.

Titratable acidity rose to about 2.8 with chocolate-covered coconut and caramel by hour 6, increasing slightly by hour 24. The milk chocolate T.A. rose more slowly, but reached about the same level as did chocolate-covered coconut and caramel by hour 24.

Enamel dissolution was low in the early stages of the experiment with coconut, but very high, 61 mg., by hour 24. Milk chocolate enamel dissolution was low throughout, and comparable with that of dark molasses, while that of caramel was even lower, and comparable with that of beer and whole wheat bread.

In general, it can be said that for the foods tested, the rate of change of pH during fermentation was comparable, with a rapid fall in the first two hours, and a much slower fall thereafter. Lowest pH values were obtained with orange juice, Coca Cola\textsuperscript{R}, wheat germ and yellow corn meal. The hour 24 values for all foods were in the range 4.0 – 5.0. This range represents a tenfold difference in
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hydrogen ion concentration, and thus a pH of 5.0 with one food could not be said to be comparable to a pH of 4.0 with another. But in simple buffer systems, at buffer molarities comparable with those of fermented food-saliva mixtures, enamel dissolution at pH 4.0 is only about twice that at pH 5.0. (See Fig. 18). Furthermore, it is apparent that at the buffer molarities shown in Figure 18, dissolution at a given pH is half the dissolution at a pH level one unit lower. If the food-saliva mixtures were behaving as simple buffer systems, the enamel dissolutions should not differ by a factor of more than two, and foods with hour 24 pH values differing by less than one pH unit should, on this basis, show 'comparable' enamel dissolutions. With many foods, this behaviour was not observed, and the hydrogen ion effect alone could not be advanced to explain the difference.

Titratable acidity values ranged from zero to 6.0. Some foods had a low T.A. by hour 24 (0.0 - 2.5). They were: orange juice alone, orange juice and saliva, 7 UP\textsuperscript{R}, Coca Cola\textsuperscript{R} alone, beer alone, beer and saliva, dextrose, potato starch, puffed rice, white bread, whole grain bread and crackers. With some foods, the T.A. increased by 50% or more from hour 6 to hour 24. These were: 7 UP\textsuperscript{R} and saliva, Coca Cola\textsuperscript{R} and saliva, all the 'sugars' group, polished rice flour, Corn Flakes\textsuperscript{R}, Shredded Wheat\textsuperscript{R} and Saltines.\textsuperscript{R} The other foods had higher T.A. throughout the experimental period, and some, generally the less refined foods, had very high T.A. (4.0 or more), especially at hour 24. They were: brown sugar, light
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molasses, dark molasses, maple syrup, wheatgerm, bran, polished rice flour and yellow corn meal.

Enamel dissolution values were very low (25 mg. or less) at hour 24 for Coca-Cola\textsuperscript{R} alone, beer alone, beer and saliva, dark molasses, bran, rye flour, brown and wild rice flours, Shredded Wheat\textsuperscript{R}, whole wheat bread, milk chocolate and caramels. Enamel dissolution was very high (more than 50 mg.) at hour 24 for orange juice alone, orange juice and saliva, 7 Up\textsuperscript{R} and saliva, Coca-Cola\textsuperscript{R} and saliva, brown sugar, wheat flour (enriched), potato flour, potato starch, corn starch, polished rice flour, Fig Newton\textsuperscript{R} and chocolate-covered coconut. Certain foods stood out as having low pH and high titratable acidity throughout the experiment, but low enamel dissolution. They were beer, light and dark molasses, wheatgerm, bran, rye flour, graham flour, brown rice flour, yellow corn meal, Shredded Wheat\textsuperscript{R}, milk chocolate and caramels. There was no consistent correlation between enamel dissolution and pH or titratable acidity values.

When 5 gm. of a food are mixed with 50 ml. of distilled water, the amount of hydroxyl or hydrogen ion addition needed to achieve a given pH rise or fall will be large if the food has a high inherent buffer capacity, and small if the food has a low inherent buffer capacity. Similarly, the amount of hydroxyl or hydrogen ion addition needed to achieve a given pH rise or fall in 50 ml. of saliva will be large if the saliva has a high inherent buffer capacity, and small if the saliva has a low inherent buffer capacity.
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capacity. The inherent buffer capacity of the saliva used in all food fermentations was constant, and when 5 gm. of a high inherent buffer capacity food is added to 50 ml. of this saliva, the total buffer capacity of this mixture should be higher than the total buffer capacity of a mixture of 50 ml. of this saliva and 5 gm. of a low inherent buffer capacity food. The buffer capacity of a mixture of food and saliva should therefore vary according to the inherent buffer capacity of the food itself, and should be approximately equal to the combined inherent buffer capacities of the food and the saliva. If so, it should be possible to calculate the buffer capacity of a fermenting food-saliva mixture from a knowledge of the inherent buffer capacities of the food and the saliva, but only if the buffer capacity of the food-saliva mixture does not change as a result of the fermentation process.

The inherent buffer capacities of each test food were determined by titrating 5 gm. of each food with 50 ml. of distilled water (using 0.1M hydrochloric acid), to its hour 24 fermentation value. The values obtained are listed in Table VII, in increasing order of buffer capacity, together with the value obtained when 50 ml. of saliva was titrated in the same manner, from its initial pH to a pH of 4.0. From these figures it is possible to infer that, for example, if 5 gm. of brown sugar (inherent buffer capacity in 50 ml. of water in range 0.2 - 0.6) is added to 50 ml. of saliva (inherent buffer capacity in range 1.2 - 1.6) then the amount of hydrochloric acid necessary to lower the pH of the mixture from 7.0
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(the pH of a brown sugar–saliva mixture) to 4.4 (the pH after 24 hours of fermentation) should be about 2.2 (i.e., 0.6 + 1.6).

However, from the actual brown sugar fermentation experiment, the amount of titratable acid present in a brown sugar–saliva mixture after 24 hours fermentation is, from Table II, 5.6. It would take 5.6 ml. of a 0.1 M, completely dissociated base or acid to change the pH of 10 ml. of the fermented mixture from pH 4.4 to 7.0, or 7.0 to 4.4, respectively.* After 24 hours fermentation, the mixture has more than twice the buffer capacity of the same mixture at hour 0 (i.e., an unfermented food in saliva). All other foods tested exhibited a similar effect. Whether this increase in buffer capacity following fermentation is because of organic acid production alone, or a combination of this effect and changes in the nature of the food as a result of the fermentation is discussed subsequently.

From the above and Table VII, it can be stated that 50 ml. of saliva has a higher inherent buffer capacity than 5 gm. of a test food in 50 ml. of water (except in the case of wheat germ). Furthermore, after 24 hours of fermentation, a food–saliva mixture is a stronger buffer than an unfermented food–saliva mixture, and acid production during fermentation cannot be inferred from a knowledge of the pH change and the inherent buffer capacities of the food and the saliva.

* Provided 'salt' and 'dilution' effects are neglected.
RESULTS II  AUSTRALIAN FOODS

Forty seven Australian foods were tested using a modified experimental technique incorporating the improvements necessitated by analysis of the results obtained in the preliminary series of tests on U.S. foods. The results are expressed graphically, for ease of interpretation, the foods being arranged in groups, according to type. The graphs for each food group have been constructed from mean values for the three variables measured, (pH, titratable acidity and enamel dissolution) plotted against time.

The data from three replicate experiments on each food were used to obtain the mean values. The decision to employ three experiments on each food was made following a statistical analysis of the data from the eight U.S. sucrose tests, and the three tests on each of the Australian sugars. As has already been mentioned in Section III, some improvement in statistical reliability could be expected with more than three experiments on Australian foods. But this procedure would not be justified, if large and statistically valid differences in fermentation behaviour between foods could be demonstrated using only three replicate experiments.

The method used to determine whether significant differences existed between foods was based on the 't' test, as follows: the means and standard errors for three determinations of experimental values were calculated, and the significance of any differences between the means determined by calculation of the relevant values
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for 't', and reference to statistical tables.* Using this technique, it is possible to determine the probability, P, that any observed difference between means could occur by chance, in a large number of such comparisons.** Selection of the particular P value used to indicate significance depends on the nature of the experiment, and in most biological studies, a P value of 0.05 is commonly used.*** In this study, a comparison was regarded as being statistically significant when the P value was less than 0.05.

Three of the Australian foods were selected to illustrate the application of this procedure in determining whether statistically significant differences in their fermentation behaviour existed. The comparison is shown in Figure 9, where the mean hour 0, 1, 2, 3, 4, 5, 6 and 24 pH, titratable acidity and enamel dissolution values for honey, molasses and sucrose are compared. The occurrence of statistically significant differences between means is indicated by the appropriate horizontal bar crossing the vertical black line.

It can be seen from Figure 9 that throughout the experiment

* Using the formula \( t = \frac{\text{difference between means}}{\sqrt{\text{S.E.}_1^2 + \text{S.E.}_2^2}} \) and tables for values of 't' for four degrees of freedom, and specified levels of significance.

** The larger the value of 't', the smaller is the probability that the observed difference could occur by chance.

*** At this level of significance, the observed difference will occur by chance in five out of 100 trials.
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no significant differences in mean pH values were observed for the three sugars.* Similarly, the titratable acidity values were not significantly different, except at hours 3, 4 and 5, where molasses titratable acidity was higher. But molasses enamel dissolution was significantly lower than that of honey and sucrose at hours 4, 5, 6 and 24, and sucrose enamel dissolution was significantly higher than that of molasses, but significantly lower than that of honey, at hour 24. The difference in hour 24 enamel dissolution between honey and molasses is highly significant.

Because of the large number of foods tested, and the large number of experimental values obtained in each test, comparisons between foods and presentation of statistical data could not be presented effectively on a single table. The foods have therefore been separated into six groups, and the results expressed graphically, the graphs for each individual food in each group being colour-coded. Statistical data are not included on these graphs, but are presented in the text where appropriate. The colour plates are arranged as follows: the pH changes over 24 hours are shown in the upper left-hand section of each plate. The changes in titratable acidity (expressed as ml. of 0.1M sodium hydroxide per 10 ml. of the mixture) are shown on the right half of each plate. Enamel dissolution changes over 24 hours (expressed as mg. of enamel

* The different pH levels for molasses at hour 0 and sucrose at hours 2, 3 and 4 were of borderline significance.
dissolved per 100 mg. added) are shown on the left half of each plate.

Many of the differences in fermentation behaviour were large, while the standard errors were usually small, and in these cases, a high level of significance can be ascribed to such differences. Table XVI lists the mean hour 6 and hour 24 values for pH, titratable acidity and enamel dissolution for all the Australian foods tested, together with the standard error values calculated from the three relevant replicate experimental values. A level of significance can be ascribed to any observed differences between these mean values for any two foods, using the data on Table XVI to calculate the relevant 't' value, and statistical tables to determine a P value for four degrees of freedom. Where a difference between mean values is small, or a difference between standard errors large, the 't' test may indicate that the difference between means is not significant. This is not to say that a difference does not exist. More replicate experiments may reduce the calculated standard errors and the resultant 't' value sufficiently to show that the observed difference between means, while not significant on the basis of a small number of replicate experiments, is significant when a large number of replicate experiments has been conducted.

It was not feasible to demonstrate the many possible comparisons between foods in tabular form, and to ascribe levels of significance to such comparisons. Observations and comparisons are therefore made in a general way, and where appropriate, are assigned a level of significance. The convention used is 'significant' or 'S',
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where the P value is less than 0.05, and 'highly significant' or 'H.S.'
where the P value is less than 0.01. Where the P value is slightly
greater than 0.05, the term 'borderline significance' or 'B.S.' is
used, and for all other values of P, the term 'not significant' or
'N.S.' is used.

Plate I illustrates the results obtained with the 'beverages'
group. Six beverages were tested. In this group, 25 ml. of each
beverage was mixed with 25 ml. of pooled saliva. This was the only
food group so tested, all other groups consisting of 5 gm. of food
in 50 ml. of saliva. The reason for the adoption of this procedure
has been discussed in connection with the preliminary series of food
tests.

Initial pH was high with ice cream, chocolate milk* and milk,
and low with orange juice, the latter having the lowest initial pH
of all the foods tested. Orange juice pH remained unchanged
throughout the experimental period. Pilsener and Coca Cola\textsuperscript{R} initial
pH was about 5.5, rising in the first two hours to 6.0, then falling
to 5.0 from hour 4 to 6. Ice cream, milk and chocolate milk pH
remained substantially unchanged, with an hour 24 pH value of about
5.0, the same as that of orange juice. In the same period, Coca
Cola\textsuperscript{R} pH fell to 4.5, and ice cream and chocolate milk pH to 4.2.
This was the widest group variation in pH found for all foods, except
for the flour group, where similar, but more constant variations were

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found.

Titratable acidity varied greatly, being low throughout the 24 hour period with Coca Cola\textsuperscript{R} and Pilsener, low initially but rising rapidly between hours 2 and 6 for ice cream, milk and chocolate milk, and high throughout for orange juice. For the remaining 18 hours, Pilsener T.A. increased slightly, orange juice T.A. increased about 25\%, while chocolate milk and milk T.A. doubled, and ice cream T.A. almost tripled. Ice cream hour 24 T.A. was the third highest of any of the foods tested.

Enamel dissolution was very low indeed throughout the 24 hour period for ice cream, chocolate milk and milk, and comparable with the dissolution in control experiments where no food was added. Dissolution was slightly greater, rising slowly and steadily with Coca Cola\textsuperscript{R} and Pilsener, that of Coca Cola\textsuperscript{R} being slightly greater than that of Pilsener, up to hour 6. By hour 24, Pilsener enamel dissolution had doubled to about 12 mg., while that of Coca Cola\textsuperscript{R} had increased five times, to about 36 mg.. Enamel dissolution for orange juice was very high in the first two hours, decreasing gradually, and by hour 6, was seven times that of the other beverages. By hour 24, further dissolution had taken place, leaving only 30\% of the enamel undissolved. This enamel dissolution, 70 mg., was the fourth highest of all the foods tested.

The exact composition of the beverages was not available, but the carbohydrate content of Coca Cola\textsuperscript{R} is sucrose, and stated by the manufacturer to be 10-11 gm. per 100 ml. This beverage is apparently
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acidulated to about pH 4.0 by phosphoric acid. In the beverage tests, 50 ml. of the Coca Cola®-saliva mixture contained 2.5 gm. of sucrose, or half that of 50 ml. of the sucrose saliva mixture. In neither test was all the sucrose metabolised during the 24 hours of fermentation.

Plate II illustrates the results obtained with the 'sugars' group. Eight sugars and one sugar substitute were tested. The substitute, Sweetaddin®*, is sweetened with calcium hexahydrobenzene sulfamate, its bulk being made up by the addition of 'highly purified mannite'**, so that it can be substituted for sucrose, weight for weight, in domestic use. Golden Syrup®* is a refined form of treacle. The pH changes were similar for all sugars except Sweetaddin®, and fell steadily from an initial pH of between 7.0 and 8.0 to 4.8-5.5 by hour 3, and 4.5-5.0 by hour 6. There was little change for the next 18 hours, and by hour 24, the pH range for all sugars was 4.3-4.7. Sweetaddin® pH stayed between 6.0 and 7.0 for the first six hours, and fell to 5.8 by hour 24.

For nearly all sugars, except Sweetaddin®, titratable acidity rose steadily in the first six hours to about 2.0. Molasses T.A. increased slowly in the first two hours, then rapidly, and was about 10% higher than the others from hour 4 to hour 6. Dextrose T.A. was lower than all other sugars from hour 4 to hour 6. By hour 24, the

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* Orbit Chemicals, Sydney, Australia.
** Akin to Sorbitol.
*** Colonial Sugar Refining Company, Sydney, Australia.
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T.A. for all sugars was the same, about 4.0, and double the hour 6 value. Sweetaddin™ T.A. was negligible for the first six hours and slight at hour 24, and was the lowest of all foods tested.

Enamel dissolution was very low for Sweetaddin™, and even lower than that obtained with ice cream and milks. For all other sugars, there was a slow increase in enamel dissolution to about 3 mg. by hour 2. This slow increase continued with brown sugar, Golden Syrup™, treacle and molasses, up to about 5 mg. by hour 6. This slow increase continued with treacle and molasses, to 8 and 12 mg. respectively by hour 24. Brown sugar and Golden Syrup™ dissolution had increased to about 30 mg. by hour 24. With the remaining four sugars, there was a more rapid enamel dissolution after hour 2, to about 14 mg. by hour 6. By hour 24, dextrose and sucrose enamel dissolution increased to about 47 mg. Glucose syrup enamel dissolution was higher still, at 65 mg., and honey was highest of all the sugars at 74 mg. These four sugars had higher enamel dissolutions than any of the beverages except orange juice, throughout the experimental period.

Plate III illustrates the results obtained in the 'flours' group. Five flours, two meals and two grain products were tested. The initial pH ranged from 6.5 for oatmeal to 8.5 for corn flour. In the first two hours, the pH fell by almost 2 units for all 'flours' except oatmeal, which changed little. In the next two hours, there was a further fall in pH of from 0.5 to 1 pH unit for all flours, except oatmeal, which fell 1.5 pH units. By hour 6, there was a
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Further slight fall to two levels, the first to about pH 5.5 with
wholemeal, wheat, corn and rice flours, and the second to about 4.4
for wheatgerm, bran, rye flour, oatmeal and maize flour. By hour 24,
there was a further fall in pH with all flours, the two-level pattern
being maintained. The fall between hours 6 and 24 was about 0.5 of a
pH unit with the higher pH level group. With the lower pH level
group, this fall was about 0.2 pH units, except for wheatgerm and
bran, where the fall was 0.5 pH units.

Titratable acidities were high with the flours. The most
rapid rise in T.A. in the first three hours was obtained for
wheat meal and wheatgerm to 3.5 and bran to 4.7. In this same time
period, the slowest rise in T.A. was obtained with wheat and corn
flours, to about 1.2, the others being about 2.0 by hour 3.

A steady increase in T.A. from hour 3 to hour 6 was maintained
for bran, rye, rice and maize flours, but a slower increase was
obtained with wheat meal, wheatgerm and corn flour. Oatmeal
differed from the others in the 'flours' group in that its T.A.
increased considerably, from 1.8 to 5.0, from hour 3 to hour 6. The
others showed much smaller increases over the same time period. In
comparison with the hour 6 T.A., the hour 24 T.A. was twice as high
for all 'flours' except bran, rye flour and oatmeal, where it was
three times as high, and wheatgerm, where it was four times as high.
Wheatgerm, bran, rye flour and oatmeal had the highest hour 24 T.A.
(9.5 - 13.0) of all foods tested. The only other food in this T.A.
range was ice cream, 11.0.
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Enamel dissolution with the flours showed little variation in the first two hours, being in the range 1 - 4 mg. By hour 4, wheatmeal, rye, wheat, rice and maize flour dissolution had reached about 10 mg., bran, cornflour and oatmeal about 5 mg., and wheatgerm about 4 mg. In the next two hours enamel dissolution had almost doubled with all flours, the highest being rye and wheat flour, at about 18 mg., the lowest being wheatgerm and bran, at about 8 mg. In the next 18 hours there was a great increase in dissolution, about three times with rye and wheat flour, oatmeal and maize, four times with rice flour, five times with wheatmeal and corn flour, eight times with bran, and eleven times with wheat germ. Wheatmeal, wheatgerm and bran had the highest hour 24 enamel dissolutions of all foods tested.

Plate IV illustrates the results obtained with the 'bread and biscuits' group. Three breads and four biscuits were tested. The white bread was a standard, Australian type, sliced, wrapped sandwich loaf, soft and fine in texture, with a soft crust, and made from 70% extraction flour. The brown bread was similar in all respects to the white loaf, except that it was made from a 90% extraction flour. 'Vogel' is the name applied to a fancy black bread, made according to a Swiss formula, very heavy and moist, and containing 'kibble' wheat, and is made from a 90%, or high

* Sieber's Bakery Pty Ltd., Sydney, Australia.
** Kibble wheat is specially softened whole wheat grains.
RESULTS

Extraction flour. 'Shortbread' biscuits are a sweet, soft biscuit made from wheat, and containing a large amount of fat (shortening) of which at least 50% is butter. 'Sao' is a soda cracker-type biscuit, an unsweetened, unshortened dry biscuit, made from a leavened dough. 'Lemon Slice' is a light, soda cracker-type biscuit with a sweet filling, consisting of 40% fat and 50% sucrose and 10% milk solids, plus flavouring. 'Ryvita' is a thin, hard, dry, wafer-type biscuit, made from a paste of whole grain rye flour. Other companies make this type of biscuit under other names, such as By Crisp, Vita Wheat or Rye King.

The initial pH for all the 'breads and biscuits' group was about 7.0, falling rapidly to pH 5.2 with white bread and Ryvita, and less rapidly to 5.8, for the others, by hour 2. By hour 4 the pH for all breads and biscuits had fallen to 4.7, and remained almost unchanged up to hour 6. By hour 24, the pH for all breads and biscuits was in the range 4.2 - 4.4.

Titratable acidity was similar for all breads and biscuits except for Lemon Slice and Sao, and rose steadily from 0.5 at hour 1 to 2.5 at hour 6, and then slowly to between 4.8 and 5.7 by hour 24. Lemon Slice T.A. was about 50% higher than the others in the 'bread and biscuits' group at hours 1, 2 and 3, but rose more rapidly from hours 3 - 4, to 5.0, remaining unchanged to hour 6.

* William Arnott Pty.Ltd., Sydney, Australia.
** The Ryvita Co. (Aust) Pty.Ltd., Sydney, Australia.
RESULTS

Lemon Slice\textsuperscript{R} T.A. did not increase as rapidly as did the others for the next 18 hours, and by hour 24, was 6.6, about 1 unit higher than the others. Sao\textsuperscript{R} T.A. rose steadily for the first six hours, but more slowly than the others, to about 1.8 by hour 6. Between hour 6 and hour 24, there was a slow increase to 3.7, lower than the others, and about half that of Lemon Slice\textsuperscript{R}. A feature of the 'bread and biscuits' results was the lack of extremes in either pH or titratable acidity changes.

The 'bread and biscuits' group enamel dissolution changes could be divided into two categories. Enamel dissolution increased steadily to about 5 mg. for white and brown bread, shortbread and Sao\textsuperscript{R} biscuits in the first category, and to about 3 mg. for Lemon Slice\textsuperscript{R}, Vogel\textsuperscript{R} bread and Ryvita\textsuperscript{R}, in the second category, in the first three hours. Enamel dissolution increased with the first category over the next three hours, to about 18 mg. by hour 6. Lemon Slice\textsuperscript{R} and Ryvita\textsuperscript{R} enamel dissolution increased slowly over the same period to about 8 mg., less than half that of the first category. Vogel\textsuperscript{R} bread enamel dissolution from hour 3 to hour 6 was higher than that of Lemon Slice\textsuperscript{R} and Ryvita\textsuperscript{R}, but not as high as the enamel dissolution values for the first category. Considerable enamel dissolution took place between hours 6 and 24, the highest hour 24 value being with white and brown bread, and shortbread, about 55 mg., the lowest being with Ryvita\textsuperscript{R}, 26 mg.

Plate V illustrates the results obtained with the 'cereals' group. This group includes five proprietary breakfast 'cereals', and
RESULTS

two proprietary savouries, potato crisps* (thin potato slices cooked in oil) and Cheese Twistees** (a carbohydrate based, cheese-flavoured savoury). Also included in this group is rolled oats*** (a breakfast porridge) and peanut butter****. The proprietary breakfast cereals were similar in appearance to the corresponding products available in the U.K. and the U.S.A. Rice Bubbles**** may be called Rice Crispies**** in some parts of the world, and Frosties***** (sugar coated corn flakes) may be sold under different trade names outside Australia. The initial pH range for the 'cereals' group was 6.6 - 7.0, and fell steadily to 4.8 by hour 3 for all except potato crisps, which had a more rapid initial fall. From hour 3 to hour 6, there was a much slower fall to about 4.6, except for Corn Flakes***** and Rice Bubbles*, which fell to 4.2 in the same period. By hour 24 there was a further slight fall in all cases, the range of pH values at this time being 4.0 (for Rice Bubbles*) to 4.5 (for peanut butter).

Titratable acidity values varied extensively throughout the 24 hours, and increased rapidly for all cereals in the first three hours, and then slowed over the next three hours. Highest T.A. in the first six hours was obtained with potato crisps and peanut butter, which had twice the T.A. of the lowest, Corn Flakes* and Frosties*,

* Smiths Potato Crisps (Aust) Pty.Ltd., Sydney, Australia.
** General Foods Corp. Pty. Ltd., Sydney, Australia.
*** Uncle Toby's Oats, Clifford Love & Co.Ltd., Sydney, Australia.
**** Eta Foods Pty.Ltd., Sydney, Australia.
***** Kellogg (Aust) Pty.Ltd., Sydney, Australia.
RESULTS
the other cereals lying between these extremes. The ranges of T.A. were 0.5 - 2.0 at hour 2, 1.5 - 3.0 at hour 4 and 1.7 - 4.0 at hour 6. The hour 24 values varied from 2.7 to 7.1, and could be divided into two distinct categories, the first in which there was less than 35% increase from hour 6 to hour 24, with peanut butter, Corn Flakes\textsuperscript{R} and Rice Bubbles\textsuperscript{R}, while the remainder were in the second category, where enamel dissolution increased 50 - 100% over the same period.

Enamel dissolution varied extensively, from low throughout for Weetbix\textsuperscript{R*}, Corn Flakes\textsuperscript{R} and Rice Bubbles\textsuperscript{R}, to high throughout for potato crisps. At hour 2, enamel dissolution for all cereals was in the range 1 - 3 mg. Dissolution increased steadily to between 4 and 7 mg. by hour 4, and 6 and 11 mg. by hour 6, for all 'cereals' except Frosties\textsuperscript{R} and potato crisps, which showed a higher dissolution. Hour 6 dissolution was 15 mg. for Frosties\textsuperscript{R} and 25 mg. for potato crisps. From hour 6 to hour 24, the enamel dissolution for all cereals increased 1 - 3 times, the hour 6 positions being maintained, except for Corn Flakes\textsuperscript{R} and Cheese Twistees\textsuperscript{R}. The increase was three and one half times with Corn Flakes\textsuperscript{R}, and more than four times with Cheese Twistees\textsuperscript{R}.

Plate VI illustrates the results obtained with the 'sweets and fruits' group. This group included three dried fruits, two of which were grown and packed in Australia. The third, dates, were an import

* Sanitarium Health Food Co., Sydney, Australia.
RESULTS

from North Africa. Glacé cherries are cherries preserved in syrup. The bananas used were fresh and ripe. The caramels* were similar in flavour and texture to 'Kraft' caramels of U.S. manufacture, and contained a small quantity of milk solids. The milk chocolate** contained 30% fat (25% coco fat, 5% milk fat) 40% sucrose, and the remainder lactose and milk protein.

The pH changes occurring in the 'sweets and fruits' group varied greatly. Initial pH varied from 6.5 to 7.5, falling steadily to 4.9 by hour 3 and 4.5 by hour 6 for dates, caramels, bananas and milk chocolate. The pH fall with glacé cherries was similar to the above up to hour 2, but fell less rapidly thereafter, and was nearly 0.5 of a pH unit higher at hours 4 and 6. The pH change with raisins was much more rapid right from the start of the experiment, being a full pH unit lower than most of the 'sweets and fruits' group at hour 1, but the rate of fall of pH decreased thereafter, pH 4.5 being reached by hour 6. Dried apricot pH fell extremely rapidly to 4.2 by hour 1, and stayed unchanged for the remainder of the experiment. This was the most rapid fall of all the foods tested. The hour 24 pH values for sweets and fruits were all in the range 3.9 - 4.2, except for glacé cherries and bananas, at 4.5.

Titratable acidity was generally high for most sweets and fruits,
RESULTS

very high for dried apricots and milk chocolate, especially the latter, but low for glace cherries. Except for glace cherries, apricots and milk chocolate, hour 1 T.A. for sweets and fruits was about 1.0, and increased steadily to 2.5 by hour 6, and by more than 100% to between 5.4 – 6.6 by hour 24. With glace cherries, T.A. was about 0.8 at hour 1 and increased only slightly by hour 6, to 1.0. By hour 24, this value had increased to 3.2, slightly higher than the bananas hour 24 value, but considerably lower than all the others. From hour 1 to hour 4, milk chocolate T.A. increased rapidly, and over this time period, was very similar to the T.A. values obtained with chocolate flavoured milk and ice cream. From hour 4 to 6, milk chocolate T.A. remained unchanged, but nearly doubled by hour 24 to give the highest value of all sweets and fruits. Dried apricot T.A. was extremely high, 3.5 by hour 1, rising slightly to 4.5 by hour 6. With the exception of bran, this was the highest titratable acidity found in all the foods tested over this time period. However, there was little T.A. increase with dried apricots from hour 6 to the end of the experiment, the hour 24 value being 5.7. Bananas T.A. was the same at hour 6 and hour 24, the only food tested whose T.A. did not increase from hour 6 to hour 24.

Enamel dissolution was extremely variable for 'sweets and fruits', and at hour 2, ranged from 2 – 5 mg., except for dried apricots, where it was 25 mg. By hour 3 there was little increase seen, except for raisins, to 6 mg. and bananas to 12 mg. Up to hour 6, enamel dissolution increased steadily for all sweets and fruits.
RESULTS

except bananas, where it increased rapidly, and milk chocolate and glacé cherries, where it increased slowly. Apricots enamel dissolution was extremely high for the first six hours, and second only to orange juice. By hour 6, enamel dissolution was about 5 mg. for milk chocolate and glacé cherries, 30 mg. for apricots and bananas, and about 11 mg. for the remainder. The rankings were changed markedly by hour 24. Milk chocolate and glacé cherries enamel dissolution had increased six times to 30 mg., but despite the increase, this was still the lowest value recorded for 'sweets and fruits'. Raisins, dates and caramels enamel dissolution increased more than five times, to between 55 and 65 mg. Apricots and bananas dissolution increased less than 50% to 54 mg.
RESULTS (OBSERVATIONS AND COMPARISONS)

In the various groups of carbohydrate foods tested, there was a general lack of conformity of enamel dissolution when considered with respect to pH and titratable acidity changes. A good example of this is the 'sugars' group, the results of which have been expressed in three separate graphs to demonstrate the effect.

The range of pH changes for the 'sugars' group over the 24 hour experimental period is shown in Figure 10. The initial pH's for the sugars were in the range 6.5 - 7.8, but by the first hour, the range was already less than one pH unit, and by hour 4 less than 0.5 of a unit*. This small range of pH was maintained up to hour 24.

Titratable acidity values for the eight sugars are shown in Figure 11. Acid production was similar for all sugars, increasing steadily from about 1.0 at hour 3 to about 4.0 at hour 24, except for molasses, which had a significantly higher titratable acidity than all the other sugars at hours 4, 5 and 6.** After hour 6, molasses acid production slowed, its hour 24 T.A. falling within the range for the other sugars. There were no significant differences in hour 24 T.A. for any of the sugars.

The enamel dissolution which would be expected from a simple acetate buffer system, the pH and titratable acidity of which varied

* However, the range at hour 2 was greater, because of the differing rates of pH change with different foods.
** Figure 9 demonstrates these differences with molasses, in comparison with honey and sucrose.
RESULTS (OBSERVATIONS AND COMPARISONS)

in exactly the same way as they did in the fermented sugar-saliva mixtures can be assessed from the results of the experiment described in Section IV. The enamel dissolution in this simple buffer* was identical with that obtained for a fermenting sucrose-saliva mixture, for the first six hours, and this result was also obtained when the simple buffer was made up with saliva instead of with water. The hour 24 enamel dissolution for the simple buffer system was lower than that of the sucrose-saliva mixture, but this result could be attributed to the difficulty of duplicating the sucrose fermentation pH and titratable acidity changes over a 24 hour period in a simple buffer system.

It is also possible to compare the enamel dissolution taking place in the sugar-saliva mixture with that of a simple buffer system of comparable pH and titratable acidity using the 'group' of curves described in Appendix B. This 'group' of curves was drawn using data obtained from a two hour decalcification experiment, in which the pH and titratable acidity remained constant (Fig. 18). In the food fermentations, pH and titratable acidity remained relatively stable from hour 3 to hour 6, the values being in the range 4.6 - 5.5 for pH and 0.8 - 2.5 for T.A. At these pH and T.A. levels, enamel dissolution with a simple acetate buffer is about 10 mg. or a little less, after two hours, and if the sugar-saliva mixtures were behaving as simple buffers, about 10 mg. of enamel

* Where pH and T.A. values were matched to those of the sucrose-saliva mixture by appropriate hydrochloric acid and sodium hydroxide additions at regular intervals.
RESULTS (OBSERVATIONS AND COMPARISONS)

dissolution would be expected by hour 6. However, the 'group' of curves indicates that some dissolution takes place even with higher pH and lower T.A., and some dissolution could be expected to have taken place from hour 0 to hour 3. Thus if the sugar–saliva mixtures were behaving as simple buffers, an enamel dissolution of 12 - 15 mg. could be anticipated. A similar result could be expected with molasses, since its slightly higher titratable acidity is offset by its higher pH between hours 3 and 6.

The enamel dissolutions obtained with the 'sugars' group are shown in Figure 12, and it can be seen that only the dextrose and sucrose values agreed with the predicted enamel dissolution value based on simple acetate buffer experiments. Both the honey and the glucose syrup enamel dissolutions appeared to be higher at hour 6, but the differences are not significant. The hour 6 dissolutions for brown sugar, Golden Syrup, treacle and molasses are much lower than the sucrose value, and about one third of the predicted dissolution. These differences are significant.

It was not feasible to compare the hour 24 dissolution values obtained with the sugars with comparable simple chemical buffer systems, but because sucrose behaves as a simple buffer for the first six hours of fermentation, it is probable that it does so for the remainder of the experimental period, and can thus be used to simulate a simple buffer system to be used as a baseline against which to compare the results of food fermentation tests. On this basis, the four sugars which exhibited less than the predicted enamel
RESULTS (OBSERVATIONS AND COMPARISONS)

dissolutions at hour 6 maintained this behaviour at hour 24, with brown sugar and Golden Syrup dissolution lower than that of sucrose (N.S. and B.S.), treacle dissolution lower still (H.S.) and molasses dissolution only 15% that of sucrose (H.S.). At the other end of the scale, dextrose dissolution was slightly higher than that of sucrose (N.S.), glucose syrup dissolution higher (B.S.) and honey dissolution higher still (H.S.).

The pH, titratable acidity and enamel dissolution values for sucrose can be used to emphasise the lack of conformity of enamel dissolution with pH and titratable acidity in the other food groups tested.* Food–saliva mixtures exhibiting pH and titratable acidity changes similar to those of sucrose, and similar enamel dissolution values as well, can be said to be behaving as simple buffer systems, while those foods whose enamel dissolutions are significantly lower than sucrose, despite similar or lower pH and/or similar or higher titratable acidity cannot be behaving as simple buffer systems, and have some property which is suppressing enamel dissolution.

When the sucrose overlay is superimposed on the 'beverages' graphs (Plate I), it can be seen that the pH for sucrose fell more rapidly in comparison, and was lower than all beverages at hour 3,

* To facilitate this comparison, a removable transparent overlay showing the pH, titratable acidity and enamel dissolution curves for sucrose, to the same scale as the colour plates, is provided in the pocket on the inside of the back cover of the illustration section of this work.
RESULTS (OBSERVATIONS AND COMPARISONS)

though the hour 6 and hour 24 chocolate milk and ice cream pH levels were lower (S.). Sucrose titratable acidity was lower than those of all the beverages (B.S. - S.), except for Coca Cola and Pilsener between hours 2 and 24. The T.A. was significantly lower at hour 6 for Coca Cola and at hours 6 and 24 for Pilsener. Sucrose hour 24 T.A. was about half that of all beverages except Coca Cola and Pilsener. Sucrose enamel dissolution was higher than all beverages except orange juice, throughout the experimental period. However, the lower dissolution for Coca Cola is not significant.

Chocolate milk, with pH changes similar to sucrose and titratable acidity about twice that of sucrose, should have exhibited a somewhat higher enamel dissolution, especially after hour 6. Its enamel dissolution was about 10% of the sucrose value (H.S.). Milk and ice cream had extremely high titratable acidities, 5 - 10 ml., though the intermediate pH changes were 0.5 - 1.0 units higher than sucrose. From the 'group' of curves, the expected enamel dissolutions up to hour 4 agree well with the experimental values, but thereafter, the enamel dissolutions were considerably lower than would be expected if they were behaving as simple buffers. Ice cream, in particular, with a very low hour 24 pH value, 0.5 of a unit lower than that of sucrose, and twice the titratable acidity, should have exhibited an enamel dissolution somewhat higher than sucrose at hour 24 if the fermented ice cream-saliva mixture was behaving as a simple buffer. The actual value was 2 mg., compared with 46 mg. for sucrose (H.S.). Coca Cola and Pilsener behaved as
RESULTS (OBSERVATIONS AND COMPARISONS)

simple buffers with respect to enamel dissolution, up to hour 6, as can be seen by reference to the 'group' of curves.

With the sucrose overlay superimposed on the 'flours' graphs (Plate III), it can be seen that wheatgerm, bran, rye flour and maize flour pH changes were all lower than that of sucrose after hour 3 (B.S. - S.), and most of the flours group titratable acidities were higher, particularly those of wheatgerm, bran and rye flour (H.S.). Because pH was lower and T.A. higher than sucrose for the 'flours', the enamel dissolutions for the above flour-saliva mixtures should have been higher than that of sucrose, if the mixtures behave as simple buffers. This was the case throughout the experiment for rye and maize flours. With wheatgerm and bran the hour 24 enamel dissolution was very high, twice that of sucrose (H.S. and S.). The extensive dissolution could be explained on the basis of a low pH of about 4.1 and a high T.A. (more than 10) for a long period, 15 hours or more. Yet both wheatgerm and bran had enamel dissolutions lower than sucrose up to hour 6, considerably lower than would be expected if these mixtures were behaving as simple buffer systems (S.). On the other hand, wheat and rice flours had pH changes higher than sucrose by 0.5 – 1.0 pH units throughout the experiment (H.S.), and comparable T.A., yet had higher enamel dissolutions than did sucrose. Wholemeal flour should be considered in a class of its own. It had a higher pH curve (0.5 – 1.0 pH units) (H.S.), but its T.A. was twice that of sucrose (H.S.). Now the 'group' of curves indicates that in simple buffer systems, twice as much dissolution occurs at
RESULTS (OBSERVATIONS AND COMPARISONS)

pH 4.0 as at pH 5.0, while enamel dissolution increases only slightly when T.A. is doubled. On this basis, wholemeal flour enamel dissolution was greater than would be expected if it were behaving as a simple buffer system. This effect was shown, to a lesser extent, by wheat, corn and rice flours.

With the sucrose overlay superimposed on the 'bread and biscuits' graphs (Plate IV), it can be seen that the pH level fell more rapidly with some, but less rapidly with most, in comparison with sucrose. But by hour 6 the pH levels were all similar to that of sucrose. By hour 24 the pH for the 'bread and biscuits' group was about 0.3 of a unit lower (B.S. – H.S.).

Except for Sao \textsuperscript{R} biscuits, the titratable acidity values were slightly higher (B.S.), but with Lemon Slice \textsuperscript{R}, the T.A. was 2 – 3 times higher (H.S.). These values would suggest enamel dissolutions slightly higher than that of sucrose, and most of the breads and flours followed this pattern, though white bread enamel dissolution was a little high. Lemon Slice \textsuperscript{R}, Vogel \textsuperscript{R} bread and Ryvita \textsuperscript{R}, on the other hand, exhibited lower enamel dissolutions than would be expected if the food-saliva mixture were behaving as a simple buffer, particularly Ryvita \textsuperscript{R}, throughout the 24 hours, and Lemon Slice \textsuperscript{R} up to hour 6. It is of interest to note that the sucrose and Sao \textsuperscript{R} biscuit pH and titratable acidity changes were similar, as were the enamel dissolutions, except between hours 4 and 6.

With the sucrose overlay superimposed on the 'cereals' graphs (Plate V), it can be seen that up to hour 3, the sucrose pH fell more
RESULTS (OBSERVATIONS AND COMPARISONS)


RESULTS (OBSERVATIONS AND COMPARISONS)

Enamel dissolutions for the sweets and fruits were comparable to, or less than sucrose up to hour 6, with the exception of dried apricots and bananas. At hour 24, the lower dissolution observed with milk chocolate and glace cherries was significant, but the higher dissolution observed with the others of this group was not (N.S. - B.S.). The particularly high enamel dissolution with dried apricots early in the experiment is readily explained on the basis of the pH and titratable acidity changes. It is possible to assess the enamel dissolution which could be expected over the first six hours using the 'group' of curves. Thus apricot pH, 4.2 and T.A., 4.0, from hour 1 to hour 6, would suggest an enamel dissolution of about 30 mg. by hour 3, if the system behaved as a simple buffer, and this was almost exactly the experimental value obtained. Bananas pH change was almost identical with that of sucrose, and its titratable acidity only slightly higher, yet its enamel dissolution was twice as high for the first six hours, and considerably higher than would be expected if the mixture was behaving as a simple buffer. The remaining sweets and fruits showed enamel dissolutions consistent with their pH and T.A. changes, except in the case of milk chocolate.

The enamel dissolution with milk chocolate was lower in comparison with sucrose throughout the experiment (S. - H.S.). Up to hour 3, this result could, perhaps, be attributed to its higher pH over this period. But from hour 4 to hour 24 its pH was lower than sucrose, and its titratable acidity twice that of sucrose. These conditions should have resulted in a higher enamel dissolution, rather
RESULTS (OBSERVATIONS AND COMPARISONS)

than the 50% lower dissolution which was observed (S. - H.S.).

Analysis of the food fermentation results and comparison of enamel dissolution values with those of simple buffers of comparable pH and titratable acidity indicates that many food-saliva mixtures can be considered as simple buffer systems with regard to enamel dissolution. There were, however, foods tested which did not behave in this way. These could be considered in two distinct groups, the first, which produced more dissolution than would be expected, and the second, which produced less.

Classification into these two groups can be made by comparison of results with the simple buffer system 'group' of curves over a short time period (2 hours or less), and assuming average values for pH and titratable acidity. Comparisons of results against the sucrose curves over longer periods (6 hours) can be made on the basis that sucrose was shown by experiment to produce an enamel dissolution curve which corresponded to that obtained with a simple buffer of corresponding pH and T.A. changes.

With these limitations in mind, the foods which appeared to produce more dissolution than would be expected if they behaved as simple buffers throughout the experiments are: honey, glucose syrup, wholemeal flour, wheat flour, rice flour, corn flour, white bread, potato chips, Frosties and bananas.

The foods tested which appeared to produce less dissolution than would be expected if they behaved as simple buffers throughout the experiments are: brown sugar, Golden Syrup, treacle, molasses,
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Sweetaddin\textsuperscript{R}, ice cream, milk, chocolate milk, wheatgerm, bran, maize, oatmeal, Lemon Slice\textsuperscript{R}, Vogel\textsuperscript{R} bread, Ryvita\textsuperscript{R}, Cheese Twistees\textsuperscript{R}, rolled oats, All Bran\textsuperscript{R}, peanut butter, Weetbix\textsuperscript{R}, Corn Flakes\textsuperscript{R}, Rice Bubbles\textsuperscript{R}, glace\' cherries and milk chocolate. Wheatgerm and bran showed the effect in the first six hours only. The most pronounced effects in this group were obtained with ice cream, milk, chocolate milk, treacle, molasses and milk chocolate.

Following the procedures used with the foods tested in the preliminary part of this section, the buffer capacity of each Australian food was tested by titration with hydrochloric acid from the initial $\text{pH}$ with 5 gm. of each food in 50 ml. of distilled water, down to the $\text{pH}$ value obtained after 24 hours of food--saliva fermentation. The results are listed in Table VIII, and, in general, correspond with those for the U.S. foods, as listed in Table VII. In some cases, those foods which exhibited a low enamel dissolution in the food tests also had a high inherent buffer capacity, suggesting that these two qualities were related. However, other foods which exhibited a high enamel dissolution in the food tests also had a high inherent buffer capacity. In addition, some foods with a low inherent buffer capacity produced very little enamel dissolution, and it is not therefore possible to predict enamel dissolution behaviour on the basis of a food's inherent buffer capacity.

The initial buffer capacity of a food--saliva mixture is dependent on the inherent buffer capacity of the food and the saliva. The food fermentation results indicate that the $\text{pH}$ level reached is
RESULTS (OBSERVATIONS AND COMPARISONS)

is related to the titratable acidity production. This relationship varied with time, and from hour 1 to hour 3, the pH fall generally bore a direct relationship to the corresponding increase in titratable acidity with all foods. However, from hour 5 to hour 24, the pH rarely fell by more than 0.5 of a unit, despite a great increase in titratable acidity.

This finding supports the observation made by others that the buffer capacity of a food-saliva mixture increases as a result of fermentation, and suggests that the rate of this increase is not constant. To further investigate this phenomenon, acetate buffers of 1.0, 0.5, 0.3, 0.1 and 0.05 molar strength, and various pH levels in the range 3.5 - 6.0 were prepared in the conventional way*, or by sodium hydroxide additions to a stock acetic acid solution of the appropriate molarity (See Section IV). The amount of 0.1 N sodium hydroxide required to titrate 10 ml. of each of these buffers to pH 7.0 was then recorded. The results, expressed graphically, are shown in Figure 13. These curves can be used to determine the extent of pH change which would occur following the addition of 0.1 N acid or base to a simple acetate buffer of a specific molarity. The buffering characteristics of a fermenting food-saliva mixture can be measured against this curve, to determine the molarity of fermented food-saliva mixtures, when considered as simple acetate buffers.

* Using stock acetic acid and sodium acetate solutions of the appropriate molarity, in the proportions shown in buffer tables to produce the required pH.
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On this basis, the food-saliva mixtures tested had hour 6 and hour 24 pH-titratable acidity relationships indicating buffer capacities equivalent to simple acetate buffers in the range 0.05 - 0.3 molar. Thus, for example, at hour 6, the 'bread and biscuits' group all had a pH of about 4.5 and a titratable acidity of about 2.5 (See Plate IV). This point, when plotted on the acetate buffer titration graph, indicated that these foods had a buffer capacity equivalent to that of a simple acetate buffer with a molarity of about .05. A similar result was obtained when the hour 24 pH and titratable acidity values were plotted. This was not the case with orange juice, Coca Cola, wheatgerm, bran, rye flour, wheat flour, oatmeal, rolled oats, All Bran, dates, caramels and glacé cherries. With these foods, the pH change accompanying the titratable acidity increase from hour 6 to hour 24 was less than would be the case if these food-saliva mixtures were behaving as simple acetate buffers. This result indicates that the buffer capacity of these foods is higher at hour 24 than at hour 6. A similar effect was observed, but to a lesser extent, with some of the other foods, but the remainder behaved as simple acetate buffers from hour 6 to hour 24.

This experiment demonstrates that the mechanism responsible for the increase in buffer capacity already discussed is not identical for all foods. With some, there is no increase in buffer capacity after hour 6, while with others, especially those mentioned above, the increase continues up to hour 24. This finding further demonstrates that acid production during fermentation cannot be inferred from a
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knowledge of the pH change and the initial buffer capacity of the food and the saliva.
SECTION VI

DISCUSSION AND CONCLUSIONS
DISCUSSION

The experimental methods used in this study were an attempt to improve on existing techniques for measuring the enamel dissolution taking place as a result of the fermentation of carbohydrate foods. Earlier techniques, where enamel dissolution was assessed by weight loss, do not allow the rate of enamel dissolution to be assessed, and so prevent detailed study of enamel dissolution in a fermenting food. Furthermore, there is a possibility that the small weight loss involved may be difficult to measure, if deposition of calcium salts or other substances on the enamel occurs. With radioactive tooth enamel, the determination of enamel dissolution rates is possible even in the presence of foods or solutions already containing calcium and phosphorus. In addition, because only small experimental samples are required, many determinations can be made without diminishing the experimental volume excessively. In these respects, the radiochemical method is an improvement over those used previously.

Preliminary studies indicated that saliva, being a biological material, is unpredictable, and gives variable experimental results. The use of a pooled sample from several donors in food experiments was shown to reduce this variability. Despite this precaution, variations in replicate experiments on a food were still observed. Analysis of the U.S. and preliminary Australian results indicated that in order to demonstrate very small differences in fermentation behaviour, a large number of replicate experiments on each food would be required, because of this variation. But application of the 't' test for significance indicated that with the sugars tested,
DISCUSSION

significant differences between fermentation results could be shown with only three replicate experiments on each food. This procedure was adopted in tests on the other Australian foods, as less experimental work was necessary. However, it is likely that in many of the experiments where significant differences in behaviour were not established, such differences did exist, but could not be demonstrated in the absence of further replicate experiments.

The techniques used to obtain titratable acidity values using a microtitration technique were found to be convenient, and to decrease considerably the reduction in experimental volume when successive samples were taken. Despite this precaution, some volume diminution did take place, and enamel dissolution figures should be interpreted with this in mind, as the experimental mixture becomes slightly more concentrated with respect to enamel powder as samples are taken.

The method used to assess enamel dissolution was based on the appearance of radioactivity in the solution. For this technique to be valid, the amount of radioactivity appearing in solution must be directly proportional to the amount of enamel dissolved. Following a detailed investigation of this process, it was evident that radioactive enamel is less soluble, per se, than is unirradiated enamel, and that furthermore, the radioactive portion of the irradiated enamel appears less readily in solution than the non-radioactive portion. This phenomenon prevents direct comparison of the enamel dissolution values obtained in this work with those obtained by chemical or gravimetric techniques. But it was also established
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experimentally that enamel dissolution rates assessed radiochemically were comparable qualitatively with the rates assessed chemically, and did allow valid comparisons of enamel dissolution rates in a series of food tests. This lack of quantitative correlation with chemical determinations of enamel dissolution was more than offset by the radiochemical method advantages of small sample size, convenience and lack of interference from the various test foods.

Efforts were made to develop a food test which approximated intra-oral conditions, but this was not practicable. Of the three sets of experimental values determined in the food tests, there was a direct in-vivo similarity only with respect to the degree of pH change observed. In the food tests, after six hours fermentation, the pH was in the range 4.2 - 5.7 for all foods, and compared favourably with in-vivo plaque or carious cavity pH levels observed by others after the ingestion of carbohydrates. (70, 76, 167, 222, 283) However, the in-vivo pH levels observed by the above workers were reached soon after the ingestion of the carbohydrates (usually within minutes), while several hours elapse before this low pH level is attained in food fermentation tests. Thus, on a time basis, the intra-oral conditions are not comparable with those of the food test.

The consistency of the food-saliva mixture of the food tests is viscous with most solid foods, but watery with others, and with all beverages. With foods of the latter type, the food-test mixture probably approximates the conditions present in the mouth when such foods are eaten. However, many solid foods adhere to the teeth, and
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this property is obviously of considerable importance in producing
caries, and foods which do not readily adhere to the teeth would be
less likely to produce caries than those which do. Other factors,
such as the presence or absence of plaque, the effect of the saliva,
micro-organism population and growth, and the nature of the enamel
surface will also affect the processes taking place subsequently, and
these factors were impossible to allow for in the food tests. The
fermenting food–saliva–enamel mixture may not, therefore, represent
the intra-oral food–plaque–tooth surface relationship.

However, all foods, with the exception of Sweetaddin®,
resulted in the production of acid in the food tests. Any cariogenic
food must produce acids in the mouth, and although the specific
intra-oral factors mentioned above are not accounted for in the food
tests, the effects of a food–saliva mixture itself, its fermentability,
and the acids so produced, are assessed. In these respects, the food
test can be related to the in-vivo conditions. The relationship does
not, however, permit direct transfer of in-vitro findings to the in-
vivo situation, as in the mouth, the initial carious attack takes
place under a covering of plaque. This covering can be regarded as a
reservoir of food debris available for utilisation by micro-organisms,
a source of the organic acids produced by this utilisation, and a
diffusion barrier against the passage of organic and inorganic materials
from the tooth surface to the mouth, and vice versa. In food–saliva–
powdered enamel mixtures, the diffusion barrier represented by the
plaque is absent, and any secondary effect which this barrier may
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represent under in-vivo conditions will be absent. Nevertheless, the primary effect of acids produced by fermentation, dissolution of the enamel, is measured by the food test, and by implication, so also is any effect exerted by components in the food which might, in-vivo, be capable of diffusing into plaque and influencing enamel dissolution.

The experimental conditions of the food tests were deliberately arranged to exaggerate the enamel dissolution taking place, in comparison with the in-vivo situation. The enamel dissolution values must therefore be regarded as arbitrary units, and cannot be related directly to cariogenicity. Thus food A, with half the enamel dissolution of food B, cannot be regarded precisely as having half the cariogenicity of food B. But foods exhibiting low or negligible enamel dissolutions in a food test would have less decalcification potential in human diets, and assessment of cariogenicity could be made with greater certainty with such foods. On the other hand, with foods exhibiting high enamel dissolutions, the degree of dissolution may not indicate cariogenicity directly, because of the various factors not allowed for in the food test, and because of differing degrees of susceptibility to caries in humans. These differences in susceptibility result from the complex in-vivo factors already mentioned, which cannot be duplicated experimentally, and the food test must be considered primarily as indicating non-cariogenic, rather than cariogenic foods.

No attempt was made in this study to identify the acids produced as a result of fermentation. The micro-organisms responsible for acid
production are those present naturally in saliva and salivary sediment, and it is likely that the overall micro-organism population will change with changes in the pH and acidity of the food–saliva mixture. Steinkraus and his co-workers have identified the major acids produced in an oral streptococci–cereal mixture as lactic and acetic acids. (267) Neuwirth and Baerger (225) identified lactic and pyruvic acids in fermented glucose–saliva mixtures, and Drucker and Melville (75) identified lactic acid as the major end products of carbohydrate utilisation by oral streptococci. Gilmore and Poole identified acetic, propionic and lactic acids as the products of glucose fermentation in in-vitro tests of in-vivo deposited plaques. (99) Gray and his co-workers have shown, as have many others, that the enamel dissolution taking place in a mixture of such organic acids is primarily a function of the availability and concentration of hydrogen ions, regardless of the source. (104) This is true only in the absence of any chelating and anion effects associated with the particular organic acids produced, but these effects will be small in comparison with the effect of pH at the levels reached during food–saliva fermentation. Thus the identification of the organic acids produced in fermenting food–saliva mixtures is of little relevance with respect to the enamel dissolution taking place in the food tests.

In the early stages of a food fermentation, titratable acidity production was reflected in the pH changes, and in the first few hours, titratable acidity increased as the pH decreased. But from hour 6 to hour 24, titratable acid usually doubled, and this increase was not
accompanying the pH fall which would be expected from a consideration of the inherent buffer capacities of the food and the saliva. This result indicates that the buffer capacity of a fermenting food-saliva mixture increases as fermentation progresses. Whether this increase in titratable acidity is the result of production during fermentation of organic acids which do not dissociate completely, or whether the 'inherent buffer capacity' of the food itself also changes during fermentation was not determined. In an in-vivo situation, an increase in the inherent buffer capacity of a food might have an effect on its cariogenicity, but complex analytical procedures would be required to demonstrate any such increase.

Comparison of the titratable acidity curves for the test foods indicates that unrefined foods generally produce more acid than refined foods, and must therefore be more readily utilised by oral micro-organisms. This suggests that the unrefined foods would result in lower pH values. This effect was rarely observed, because the unrefined foods also have a greater inherent buffer capacity, and must accordingly produce more acid than the refined foods to achieve an equivalent pH change. As a result, the pH change curves for the food groups were often remarkably similar, despite considerable differences in titratable acidity.

The similarity in pH fall for food fermentations despite variability in inherent buffer capacity and titratable acid production deserved further investigation. The effect appeared to be a self-limiting process, in which, regardless of the suitability of the medium
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for growth of micro-organisms, or its buffer capacity, the pH of food-saliva mixtures at equilibrium was between 4.0 and 5.0. If the acid production were primarily the result of fermentation by aciduric organisms, the pH could be expected to fall below 4.0, but on the other hand, the majority of organisms in stimulated saliva are streptococci, which are not aciduric, even though they are capable of lowering the pH of a medium to about 4.0. It is likely that in food-saliva mixtures, complex growth patterns occur, especially in long term experiments, and as acids are produced and the pH level falls, growth of organisms which are not aciduric slows down, or even ceases, while the aciduric species flourish. This process has been suggested by Gilmour and Poole\(^{(99)}\) and Poole and his co-workers\(^{(240)}\) to occur in plaque, and if they are correct, the food test may, apart from diffusion considerations, be an adequate model of any long-term micro-organism metabolism changes taking place in plaque, such as might be the case following frequent ingestion of food, or accumulation of plaque matrix or food debris on the teeth.

However, it has been shown that plaque pH can fall very rapidly in the presence of sugars\(^{(192, 292)}\). Under these conditions, changes in micro-organism growth patterns would be minimal, as even under optimum growth conditions, the doubling time would be 50 minutes or more. In this respect, the pH changes occurring in food-saliva mixtures do not simulate the rapid pH changes occurring in plaque.

Andlaw\(^{(4, 5)}\), Jenkins and co-workers\(^{(146 - 151)}\), Osborne and co-workers\(^{(233)}\) and others have assessed the ability of various food-
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saliva mixtures to dissolve tooth enamel in-vitro, and suggested that some foods contain protective factors which have the effect of decreasing enamel dissolution. Andlaw's results are of special interest here, since his experimental techniques were similar to those used in this study, except in the duration of the experiment (6 hours rather than 24) and in the nature of the enamel (blocks rather than powder). Andlaw measured enamel dissolution with various foods and extracts of foods, by loss of weight of the enamel blocks, the weight loss on which he based his findings being about 5%. The results obtained in the present work agree well with Andlaw's six hour enamel dissolution values qualitatively, but the weight losses are considerably larger, being in the range 5 - 20% by hour 6, and up to 90% by hour 24. This is to be expected, in view of the larger enamel surface exposed to the mixture. However, some of the hour 24 values in the present study do not follow the trends indicated by the hour 6 values. It is possible that some enamel dissolutions taking place in the present food tests after hour 6, when pH and titratable acidity had reached equilibrium, would not be recorded by Andlaw's test. This fact might be of significance in understanding the basic mechanism taking place in-vivo if foods are in contact with the teeth for long periods.

The above workers found that, in general, decreased or negligible enamel dissolution is obtained with foods containing appreciable amounts of calcium and phosphorus, and with some unrefined sugars, flours and cereals. These results are in agreement with the author's findings.

If the findings of the present study are compared with the
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observed cariogenicity of similar foods in animals and humans, some
indication of the clinical significance of the food test results could
be obtained. Although many foods are commonly believed to be cariogenic,
there has been little experimental evidence to support this belief,
either in animals or humans. However, Stephan(271) has recently
tested the ability of different types of human foods to induce caries
in rats, using two basic diets, one cariogenic*, the other non-
cariogenic, ** to both of which the test foods were added. The foods
tested were classified into five groups according to the caries
scores observed. 'Group A' foods produced less caries than a control
diet. 'Group B' foods produced the same caries as control diets, and
'Group C' foods produced more caries than control diets. 'Group D'
foods produced moderate, and 'Group E' severe caries. Stephan also
recorded scores for erosion, as distinct from caries, with the test
foods. He also tested several other cariogenic rat diets, *** in
comparison with a non-cariogenic rat diet, and on these results,
concluded that many of the human foods he tested were as cariogenic
to the rat as standard cariogenic rat diets. It is likely that the
increased caries scores Stephan observed when certain foods were
incorporated in the rat diets would also be observed when the same
foods were included in human diets under similar conditions.

* Stephan Basic Diet 580
** Stephan Basic Diet 581
*** Keyes Diet 1503, Stephan Diets 585 and 593
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Stephen's results provide a standard against which the food test enamel dissolutions can be compared, to determine whether the latter can indicate the cariogenicity of a food. Table IX shows Stephen's groupings for some of the foods he tested, together with the hour 6 and 24 enamel dissolutions obtained in the present work for comparable Australian foods. Included in Table IX are the foods found by Stephan to cause erosion, together with the foods found in the present study to dissolve tooth enamel even before any appreciable acid production by fermentation.*

The 'Group A' (reduced caries) foods in Stephen's study were sorbitol, peanuts and whole milk. The hour 6 and hour 24 enamel dissolutions for comparable Australian foods, Sweetaddin, R (a sorbitol-based sweetener), peanut butter and whole milk were very low, in comparison with the other foods tested. Thus a low enamel dissolution with these foods in the food test correlates well with Stephen's 'Group A' (reduced caries) foods.

The 'Group B' (minimal caries) foods in Stephan's study were dried apricots and orange segments (though orange drink was included in Group E). Both the hour 6 and hour 24 enamel dissolutions for Australian dried apricots and orange juice were very high, indicating a high in-vivo decalcification potential, and it might be concluded from this result that these foods would be cariogenic. Stephan

* When this effect was observed, the food was classified as causing in-vitro erosion, because of the inherent acidity of the food.
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classified these foods as minimally cariogenic, and on this basis there is a negative correlation of the food-test results with Stephan's assessments of cariogenicity. But the food-test results of these two foods indicated that they caused in-vitro erosion (see above), and Stephan also classified these foods as causing erosion. In this instance, the enamel dissolution observed in the food test, while not providing correlation with Stephan's caries results, certainly agrees with his findings with respect to erosion. The food test results with Coca Cola\textsuperscript{R} do not agree with Stephan's findings with respect to erosion, and this is probably a result of the 50% saliva content of the food-saliva mixture tested. The low buffer capacity of Coca Cola\textsuperscript{R} is such that when mixed 50/50 with saliva, the initial pH is near 7.0, and could not therefore cause erosion in the food tests. When tested without saliva, in-vitro erosion did occur.

In Stephan's Groups C,D and E (moderate-severe caries foods), there was some correlation of the food-test enamel dissolution values with cariogenicity, in that the hour 6 and hour 24 values with these foods were high (range at hour 6, 11 - 28 mg., at hour 24, 42 - 62 mg.) in comparison with other foods tested, such as unrefined sugars and cereals (range at hour 6, 4 - 12 mg., at hour 24, 8 - 32 mg.) provided the Coca Cola\textsuperscript{R}, potato crisp and milk chocolate food-test results are excluded. These three food-test results did not correlate with those of Stephan's study. The Coca Cola\textsuperscript{R} result has already been discussed. A possible explanation for the lack of correlation with potato crisps and milk chocolate may lie in differences in the overall
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composition or preparation of the U.S. and Australian forms of these two foods, or perhaps oral clearance factors in the former and high sucrose content in the latter.

The foods tested by Stephan did not include unrefined sugars and cereals. These two foods produced generally low enamel dissolutions in the food tests, higher than Stephan's 'Group A' foods, but considerably lower than the foods in his 'Groups C', 'D' and 'E'. On the basis of the excellent correlations obtained with Stephan's 'Group A' foods, it is possible that unrefined sugars and cereals would also have given low or minimal caries scores in his experiments.

From these comparisons with Stephan's results, it may be concluded that a food which produces little or no enamel dissolution in a food test is non-cariogenic, or may even have a suppressing effect on cariogenicity, when included in a rat diet. On the other hand, high food-test enamel dissolution does not necessarily indicate high cariogenicity of a food in human diets, because of the various factors involved in caries susceptibility in humans, which cannot be allowed for in food fermentation tests.

When a human caries study was used as a standard against which to compare enamel dissolution values as an indicator of cariogenicity, certain correlations were observed. In the extensive Vipeholm study\(^{(121)}\), all groups consumed a basic diet, and various test groups consumed different forms of carbohydrate added to the basic diet. In those test groups consuming sucrose in solid form
DISCUSSION

and solution, or sweetened bread, at meals, or chocolate between meals, moderate caries scores were observed. In those test groups consuming toffees or caramels in large amounts between meals, high caries scores were observed. Table X compares the mean number of new carious surfaces per person per year, for years 4 and 5, when the test diets were consumed, in comparison with the enamel dissolutions obtained in the food tests for comparable Australian foods.*

On the basis of the comparison of food-test enamel dissolutions with Stephan's rat caries scores, it appears that an enamel dissolution of about 12 mg. at hour 6 and 30 mg. at hour 24 represents the borderline between non-cariogenic foods and cariogenic foods (See Table IX). When this property is assessed on the basis of food-test enamel dissolutions (with the possible exception of milk chocolate), all the Vipeholm test foods would be cariogenic. Table X shows that all the test foods of the Vipeholm study were cariogenic to humans. However, the significant finding of the Vipeholm study was the great increase in caries incidence following the consumption of sugars between meals. This factor could not be taken into consideration by the food test. Nonetheless, the food test results show some correlation with the Vipeholm findings.

* Toffees as such were not tested in the present study. This food is made by various processes, based on glucose syrups. Accordingly, Table X compares the Australian food test results for glucose syrup with the Vipeholm results for toffees.
DISCUSSION

The comparison of the food-test milk chocolate result with the Vipeholm study is of interest. The between-meals chocolate ration for the Vipeholm subject was 65 gm., and equivalent to about 30 gm. of sucrose. The between-meals caramels and toffees rations were equivalent to about 40 gm. and 70 gm. of sucrose, respectively. Thus the higher caries scores with caramels (2.2 carious surfaces per person per year) and toffees (I, 3.6 and II, 4.5 carious surfaces per person per year) in comparison with milk chocolate (1.4 carious surfaces per person per year) could be inferred from the lower enamel dissolution figures with milk chocolate in food tests, since the sucrose contents of the three additions, while not identical, are roughly comparable. The calcium and phosphorus content of caramels and chocolate is about 0.2% by weight, while that of bread is less than 0.01%, and that of toffees, nil. The Vipeholm results suggest a possible role for these substances in caries control, since the caries incidence with the chocolate and caramels was lower than that with toffees.

These comparisons indicate that some foods causing caries in animals and humans also produce moderate to high enamel dissolutions in food tests, while those few foods tested which failed to produce caries, or even reduced caries, also produced low enamel dissolutions in food tests. It would appear that the fermentation test gives a general, qualitative indication of cariogenic potential, but not of actual cariogenicity, in human diets. It does, however, give a definite indication of absence of cariogenic potential, and
would therefore indicate those foods which could be non-cariogenic in human diets.

In a recent study, Brudevold and his co-workers suggest that the surface treatment of enamel by agents other than fluoride which reduce the rate of acid solubility of enamel have not been demonstrated clinically to reduce caries. Fluoride exerts its effect "by influencing the alternating process of demineralisation and reprecipitation occurring in the carious lesion." Related observations by Napper and Smyth on enamel in-vitro indicate that the rate of enamel solubility in un-stirred systems is influenced solely by the fluoride ion, other agents influencing dissolution only in the presence of 'forced convection'. If these contentions are true, then the lowered caries incidence observed with some foods in the Stephan and Vipenheim studies must therefore be the result of a more complex process than simple enamel dissolution. Nonetheless, a food which produces no enamel dissolution in an in-vitro food test should exhibit a minimal cariogenic effect in-vivo, regardless of the mechanism involved.

The influence of the surrounding environment on enamel dissolution has been extensively investigated, and it is now well established that a great variety of organic and inorganic phosphates, calcium salts and certain inorganic ions are capable of reducing enamel dissolution (See Section II). Napper and Smyth have investigated various phosphates, including calcium sucrose phosphate, under closely controlled conditions, for their ability to influence
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Enamel dissolution. (221) They found that in unstirred systems, dissolution was mass-transport controlled, and the phosphates tested altered "neither the dissolution rate in these systems nor the hydroxyapatite solubility". But "when forced convection (stirring)* was sufficiently vigorous" ion disengagement from the kink sites ** on the crystal surface appeared to be rate-determining, and "the addition of organic and inorganic phosphates to such systems reduced the dissolution rate by a factor of from 2 to 8". The reductions were ascribed to the adsorption of the added phosphate anions at 'kink sites', with consequent retardation (poisoning) of the ion disengagement step. They stated that in stirred systems, fluoride in relatively high concentration exerted the above effect, as well as reducing hydroxyapatite solubility.

When calcium sucrose phosphate, a known 'kink site' poison, was added at a level of 1% to sucrose in a food fermentation test, a greatly reduced enamel dissolution was observed, and at levels even lower than 1%, has inhibited the in-vitro subsurface decalcification of enamel, and recent studies have shown that the incorporation of 1% calcium sucrose phosphate in human diets has reduced dental caries. (128 - 130) There may be many other such

* Author's addition.

** No surface is smooth at the atomic level, and will often exhibit 'dangling bonds', which are most common at edges, levels and kinks on the crystal surface. These areas of increased activity are called 'kink sites'.
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substances, often called 'protective factors', some of which are no doubt present naturally in non-cariogenic carbohydrate foods, and certainly are present in sugar cane juice and molasses.\(^{56}\) It should be possible to utilise this effect, and inhibit dental caries by adding such substances to cariogenic foods.\(^{19, 149 - 151, 233}\)

Many studies have been conducted on animals, and in-vitro, to evaluate the cariostatic effects of 'protective factors', either present naturally in foods, or added as supplements. Some of these have been reviewed in Section II, and the conclusions drawn from them will now be discussed in relation to the findings of the present study. Any observations made must of necessity be speculative, since in-vivo factors are not assessed in decalcification potential tests, and the in-vitro studies reviewed were generally conducted under different experimental conditions.

From the results obtained for Beverages (Plate I) there seems little doubt that the low enamel dissolutions observed for chocolate milk, milk and ice cream could be associated with the high calcium and phosphorus content of these foods. Bibby and Averill,\(^{21}\) Dawes and Shaw\(^{68}\) and Nizel and Harris\(^{230}\) suggest possible mechanisms whereby such an effect might result, the most likely in this instance being an addition to 'the acid resistance of the enamel surface'\(^{21}\) as the other possibilities suggested require in-vivo mechanisms not assessed in food-saliva mixtures. There is also the possibility that the effect is the result of a milk protein deposit on the particles of enamel. This effect has been noted by
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Weiss and Bibby,\(^{(296, 297)}\) Pearce and Bibby\(^{(236)}\) and Jenkins and Ferguson\(^{(148)}\) in vitro. An additional possibility is that the fat content of the milks and ice cream suppresses enamel dissolution, but the evidence for such a mechanism is not well established as a surface effect and is based on in-vivo studies, where many other factors may be operating.\(^{(59, 107, 119, 194, 245)}\) The clinical effectiveness of milk and milk products in reducing caries has, however, been well established, in studies by Green,\(^{(108)}\) McClure,\(^{(195)}\) and Shaw.\(^{(252)}\) Thus in this food group, a low enamel dissolution does correlate with low cariogenicity in animal diets.

From the results obtained for sugars (Plate II and Figs. 9-12) there appears to be a relationship between enamel dissolution and the degree of refining of sugar cane juice, and this relationship is apparently independent of pH or titratable acidity changes. The existence of such a relationship in in-vitro tests has been suggested by Osborne\(^{(233)}\) and verified by Jenkins and his group,\(^{(146)}\) Soni and Bibby\(^{(265)}\) and Thanik and Bibby.\(^{(288)}\) A similar effect in animal experiments has been reported by Strålfor, who showed that in rats, crude sugars were less cariogenic than refined sugars. König and Mühlemann found that Strålfor's results could be partly attributed to a particle size effect.\(^{(173)}\) But Strålfor also demonstrated a caries reduction with unrefined sugar dispersed in cooked foods and solutions, so his conclusion that unrefined sugars can have an anticariogenic effect in animals is probably valid.

Jenkins and his co-workers were able to demonstrate in-vitro
that the lowered enamel-dissolution effects shown by the crude forms of sugar could be reduced, or even eliminated if the calcium in the crude sugars was removed, and suggested that the strong buffering ability of these sugars contributed to, but could not completely account for the effect. (146) In the present study, the low enamel dissolution with the unrefined sugars cannot be explained on a titratable acidity basis, as the titratable acidities for all the fermented sugar-saliva mixtures were of the same order, and should have resulted in enamel dissolutions of the same order, if the mixtures behaved as simple buffer systems.* The alternative explanation, that the crude sugars contained 'anticariogenic factors' removed during refining, seems more likely in these experiments.**

There is a pronounced reduction in enamel dissolution with sucrose containing calcium sucrose phosphate, in comparison with sucrose alone (See Appendix D), and various forms of calcium sucrose phosphate are known to be present in crude cane juice and molasses (See Appendix E). The low dissolution observed with crude sugars may be due to the presence of this material, but it is also possible in view of the similar low dissolution observed with milk and milk

* A high 'inherent buffer capacity' did, however, generally correlate with a low enamel dissolution.

** In this context, honey should be regarded as naturally occurring mixture of glucose and fructose, with a relatively small mineral and vitamin content, in comparison with crude sugars.
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products, that the effect is a result of high calcium and phosphorus content, acting not as a buffer, but at the enamel surface, as suggested by Nizel and Harris (230) and Bibby and Averill (21).

From the results for Flours (Plate III) it can be seen that with the exception of milk, milk products and a few cereals, flours as a group provide a better medium for the growth of salivary micro-organisms than the other foods tested, as evidenced by the high titratable acidities produced by them when fermented. This is probably a direct consequence of the growth factors present in flours, and as might be expected, titratable acidity with the more refined flours is lower, and the corresponding fall in pH level smaller. Enamel dissolution with the flours was, up to hour 6, comparable with that of sucrose, and somewhat higher than that of most other foods, but at hour 24, the flours generally produced high to very high dissolution. Only two of the Flours group of foods, bran and wheat germ, showed any significant reduction in dissolution, and then only from hours 3 to 6.

The high dissolution with the flours does not agree with the observations of other investigators (4, 5, 115, 146, 149, 233, 263) who demonstrated that in fermentation studies, unrefined flours dissolve less enamel than do refined flours. In the present study it was shown that the flours tested do differ in their fermentation behaviour, and that bran and wheat germ cause less dissolution, but that the effect was transient, and disappeared after 24 hours fermentation. Apart from this observation, the present study failed
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to demonstrate the pronounced effects that unrefined flours have shown in the other studies referred to.

This result cannot be explained on the basis of Jenkins' suggestion that uncooked flours may permit phytases to break down phytates present in such flours, thereby increasing the organic phosphate content of the mixture, and lowering enamel dissolution from mass action effects. On this basis, the unrefined flours tested in the present study, which were uncooked, should have shown a lower enamel dissolution than the refined flours. From the results of other investigators, there seems no doubt that unrefined flour and grain products contain substances which can be demonstrated to decrease the solubility of tooth enamel. Such an effect was demonstrated in this study, but only with bran and wheat germ at hour 6. On the other hand, the enamel dissolution for wholemeal flour was found to be higher than that for white flour, which does not support the general conclusions of other workers. It must, therefore, be concluded that the conditions of the food test used in this study were such that the dissolutions resulting from the extensive acid production and low pH levels with the unrefined grain products were sufficient to overcome the effect of such substances, except when the effect was pronounced.

In this study, all the flours tested caused considerable dissolution, more than that of most of the other foods tested, particularly after 24 hours fermentation. This finding would imply that these flours would have a considerable cariogenic potential,
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regardless of the degree of refining, particularly if they were retained on the teeth for a prolonged period.

But many animal caries experiments have shown that flours have a low or minimal cariogenicity.\(^{(109, 111-113, 172, 174)}\) This suggests that an appropriate conclusion from the Flours group of food tests would be that although such foods are readily utilised by microorganisms to produce acids, and cause considerable enamel dissolution in-vitro, this result cannot be used to infer that they have a high cariogenicity in animal caries studies.

There was very little variation in the results for Bread and Biscuits (Plate IV) in comparison with that observed for other food groups. The overall performance of this group in the tests was similar to that of sucrose, and with the exception of Lemon Slice\(^ R \), Vogel\(^ R \) bread and Ryvita\(^ R \) biscuits, these food–saliva mixtures can be considered to behave as simple buffers. The three exceptions produced less enamel dissolution than would be expected.

The Bread and Biscuits consist of various cooked forms of flour. Cooking did not facilitate utilisation by salivary microorganisms, or enhance enamel dissolution, in comparison with the uncooked flours tested. This result differs from that obtained by Grenby who found that cooked wheat starch (with a protein content less than 0.5\%) produced more acid, and more enamel dissolution than the same starch, uncooked.\(^{(111)}\) This result is probably because the cooking ruptured the starch granules and made them more available for metabolism. The closest equivalent food tested in the present study
DISCUSSION

was cornflour* with the same low protein content as Grenby's starch. The cornflour produced the lowest titratable acidity of all the flours, and is a poor substrate for micro-organism metabolism. Thus, in this context, Grenby's starch must also be considered a poor substrate, and the differences in dissolution he observed as a result of cooking may not have been apparent had he tested less highly-refined flours, as the acid production with the latter would have been higher and may have masked any cooking effects. The lowered dissolution obtained with brown bread in comparison with white bread, as observed by Jenkins (146), was shown in the present study, but only at the borderline level of significance.

Animal experiments assessing the cariogenicity of cooked flour and starch products show that such foods are generally of low or negligible cariogenicity, and Grenby found little caries when either uncooked or cooked wheat starch was fed to rats. (111) A similar result was obtained by Green and Hartles. (109) White and wholemeal flours and breads have also been found by others to have low cariogenicity in animal diets. (112, 113, 172, 174) On the basis of in-vivo studies, it must be concluded that the decalcification potential for Breads and Biscuits is high, but not as high as for uncooked flours, and that this high decalcification potential does not indicate a high cariogenicity, because of the other factors

* The Australian cornflour used in the tests was probably wheat starch (See Appendix E).
involved in the carious process.

The low enamel dissolution observed for Vogel\textsuperscript{R} bread and Ryvita\textsuperscript{R} biscuits, in comparison with other cooked flour products, suggests a similar conclusion to that offered to explain the low enamel dissolution for bran and wheat germ, in comparison with other uncooked flours. Perhaps the cooking process has lowered the acid production through fermentation to such a level that the effect of any 'protective factors' they might contain is demonstrated. This possibility would however, have little clinical significance, because of the generally low cariogenicity shown for cooked flour products in animal experiments, whether refined or unrefined.

The low enamel dissolution for Lemon Slice\textsuperscript{R} is difficult to explain. Since the biscuit portion of this food is similar to Sao\textsuperscript{R}, which has a fermentation behaviour similar to that of sucrose, and since the sweetened filling contains a large amount of sucrose, it could be expected to produce an enamel dissolution similar to that of sucrose. But Lemon Slice\textsuperscript{R} titratable acidity was higher than that of sucrose, and its enamel dissolution lower at hours 4, 5 and 6. Since the sweet filling of this biscuit contains hydrogenated fat and powdered milk, it is possible that the low dissolution observed for this food is a result of the presence of either or both of these constituents, as it has been shown that some oils and fats are cariostatic in animals, and that milk proteins can attach themselves to the enamel surface.\textsuperscript{(59, 107, 119, 194, 236, 296, 297)}

The results obtained with Cereals (Plate V) show generally more
DISCUSSION

pronounced falls in the pH level and higher titratable acidities, in comparison with sucrose, and it might be expected that slightly higher enamel dissolutions than that for sucrose would be observed, if these food saliva mixtures were behaving as simple buffers. But most of the dissolutions were lower than expected, especially Corn Flakes\textsuperscript{R} and Rice Bubbles,\textsuperscript{R} and this group of foods could be said to have a low decalcification potential. This finding is in good agreement with the results obtained in-vitro by Soni and Bibby,\textsuperscript{(263)} but not such good agreement with those of Thanik and Bibby.\textsuperscript{(288)} Possible reasons for these inconsistencies are suggested by Khanna and Bibby\textsuperscript{(158)} who showed differences in fermentation behaviour between cereals of different geographic origin, and de Crousaz and his co-workers,\textsuperscript{(69)} who showed that the decalcification potential of cereals was partly dependent on their inherent buffer capacities.

Animal caries experiments using whole grain products often show that the husk or raw grain has an anticariogenic effect.\textsuperscript{(45, 199, 205, 289, 293)} The results obtained in the present study indicated a lower decalcification potential for the Cereals Group of foods, and since these cereals are made from relatively unrefined grains, it might be concluded that the low food-test decalcification potential correlates with low cariogenicity in animals. But a rigorous comparison is not possible, because of the lack of animal experimentation on the cariogenicity of these types of cereal.

The high titratable acidity and high enamel dissolution observed for potato crisps in the present study indicates a high cariogenic
DISCUSSION

potential for this food. This finding does not agree with Stephan's results for potato chips which were found to be of low cariogenicity in his experiments. (271) This lack of correlation could be interpreted as indicating that in Stephan's experiments, potato crisps possessed a 'protective factor', that the oil used to cook them exerted a protective effect, or that they were not retained on the teeth. There have been no published reports supporting the first possibility, and the second possibility has not been well established in-vitro, or in animal studies. The last possibility would appear to be the most likely explanation.

The higher decalcification potential with Frosties\textsuperscript{R} in comparison with Corn Flakes\textsuperscript{R} is of interest. The only difference between these foods is the presence of a sugar coating on the Frosties\textsuperscript{R}. Frosties\textsuperscript{R} enamel dissolution and titratable acidity resemble those of sucrose much more closely than those of Corn Flakes\textsuperscript{R}, and there is little doubt that the cariogenicity of Frosties\textsuperscript{R} would be higher than that of Corn Flakes\textsuperscript{R} because of their sucrose content. Notwithstanding the conclusion that the food test cannot be used to infer cariogenicity because of high enamel dissolution, this result suggests that an increase in the decalcification potential of a specific food as a result of some addition or modification could indicate an increased cariogenic potential.

The results for Sweets and Fruits (Plate IV) show that as a group, these foods exhibit considerable variation in fermentation behaviour. The decreases in pH levels are greater, and the titratable
DISCUSSION

acidities are higher than those for sucrose, but dissolution for this
group of foods is basically similar to that observed in simple buffer
systems, except for bananas and milk chocolate. The pattern of pH
change for apricots and raisins is atypical, and is probably related
to the presence of acids in these foods before fermentation, and the
rapid release of these acids in the early stages of the experiments,
additional to the acids produced by fermentation. The extremely high
enamel dissolution observed for apricots in the initial stages of
the fermentation is almost certainly a result of this early release
of acid, and correlates well with the rapid pH fall and with Stephan's
finding of erosion (but not caries) with this food in his studies.
The high enamel dissolution with bananas could be explained by
proposing that bananas contain a factor which enhances the enamel-
dissolving effects of the acids produced, or that some especially
damaging acid is produced during fermentation, as suggested by Jenkins\(^\text{(151)}\)
and Khanna and Bibby.\(^\text{(158)}\)

No evidence of a reduced enamel dissolution with caramels was
found in this study. This is not in agreement with the results of
Andlaw,\(^\text{(5)}\) Soni and Bibby\(^\text{(263)}\) and Thanik and Bibby.\(^\text{(288)}\) If the
caramels tested by these workers are the same brand* tested by the
author in his studies on U.S. foods**, the higher Australian caramel
dissolution is probably because of their much lower content of milk

\* \text{Kraft}^R

\** At the Eastman Dental Center, Rochester, N.Y.
DISCUSSION

products.

The low enamel dissolution observed for milk chocolate supports the findings of Strålfors, who demonstrated that cocoa, its various components, and other chemically related substances were cariostatic in hamsters.\textsuperscript{(276–281)} There is also some evidence from the Vipeholm study that chocolate between meals is less cariogenic than caramels or toffees between meals (See Table X). On the other hand, Kinkel and Cremer believe that Strålfors' findings are due to the mineral content of cocoa,\textsuperscript{(161)} and Ishii and his co-workers showed that in rats the ad libitum feeding of biscuits containing chocolate was more damaging to the teeth than other types of biscuit.\textsuperscript{(141)} This lack of agreement, coupled with the findings of the present study that the milk chocolate dissolution was low, but not as low as those of both chocolate milk and milk\textsuperscript{*} suggests that the cocoa content of milk chocolate may be of less importance in reducing dissolution than the milk fat, lactose, milk protein and mineral content. It could also be surmised from the results of Ishii and his co-workers that milk chocolate, which has a high sucrose content, may be cariogenic in-vivo because the high cariogenicity of the sucrose has masked any of the anticariogenic properties which Strålfors has demonstrated for cocoa, and which are inferred from the low decalcification potential shown by milk chocolate in the food tests.

\textsuperscript{* Which were almost identical, despite the cocoa content of the former.}
DISCUSSION

The possibility that foods which have been demonstrated to contain 'protective factors' owe their protecting effect to antibacterial substances, as suggested by Taketa and Phillips, was not supported in this study. Many of the foods shown to have a low decalcification potential, and observed to have low cariogenicity in in-vivo experiments had a high titratable acidity, indicating considerable micro-organism growth. The low cariogenicity of such foods must then be because of enamel solubility effects, rather than antibacterial effects.

The low enamel dissolution observed in this study for some foods could have been due in part to the presence of trace elements, especially fluorides, but this possibility cannot be evaluated, since no trace element analyses were performed. In those low decalcification-potential foods which were shown in animal experiments to be of low cariogenicity, any fluoride effects were probably not involved, as in most animal caries experiments, care is taken to ensure that the diets are of low fluoride content, or that the control and test diets are comparable with respect to fluoride content. The latter situation would also apply to other trace elements, but not to a specific test food containing a trace element highly effective in reducing enamel dissolution.

Studies on the anticariogenic properties of trace elements

* Such as molybdenum, vanadium, selenium, iron, manganese, etc.
have been conducted, but molybdenum is the only one to date which has been demonstrated to have a significant effect. Jenkins has reviewed many of these studies, and concluded that the effect with molybdenum has been reasonably established for humans.\(^{(152)}\) Some of the animal experiments he reviewed showed a caries reduction with molybdenum, but others did not, at high and even toxic levels. Although molybdenum might have been responsible for the low enamel dissolution in the food tests, this is unlikely.\(^{(150, 151, 153a)}\)

In the light of recent work, a significant factor in the development of caries is the possibility that the cariogenicity of foods can be enhanced by sucrose. It seems clear that the high cariogenicity of sucrose is the result of extracellular polysaccharide production from it in the plaque. The part that other fermentable carbohydrates play in conjunction with sucrose in the carious process has not been fully established, but it is not difficult to propose mechanisms whereby previously non- or minimally-cariogenic foods could exhibit increased cariogenicity in the presence of a glutinous plaque produced by sucrose. It is feasible that with the foods shown to have high decalcification potential in this study, but low cariogenicity in animal studies, part of the explanation lies in absence of this specific sucrose effect. But for cariogenicity to be demonstrated in-vivo, the food responsible must produce acid. It follows then that foods of low decalcification potential could be expected to be of low cariogenicity, even in the presence of sucrose.

The author believes that analysis of the food tests described
DISCUSSION

here, and comparison of the results with those obtained by others in-vitro and in-vivo indicates that certain classes of carbohydrate-containing foods, namely milk and milk products, and some unrefined sugars, cereal products, flours and grains have the effect of reducing enamel dissolution in a fermenting food-saliva mixture, and that this effect would be reflected in the cariogenicity of such foods if fed to humans under carefully controlled conditions. It is further believed that the effect could not be predicted on the basis of the ability of such a food to produce a low pH or a high titratable acidity, or both, when fermented with saliva. The author believes that the major value of the food test, the development and application of which has been described, is that it permits the prediction of non-cariogenicity, or minimal cariogenicity, in those foods or food-additive combinations where negligible or low enamel dissolutions are observed.
CONCLUSIONS

There is general agreement among investigators of the relationship between food and dental caries that when foods are utilised by oral micro-organisms, acids are produced, and that it is the reaction of these acids with the enamel which initiates the carious process. But many other factors are also involved, and in their studies individual investigators often reach different specific conclusions, because of difficulties in evaluating the effects of these factors. A review of the literature shows that the food–caries relationship can be studied epidemiologically in human populations, but the effects of specific foods can be studied only under special circumstances. Animal investigations do permit the control of many experimental variables, and can be employed in studying the effects of specific foods, but the findings may not be applicable to the human situation, because of biological differences. In–vitro studies of specific foods enable the investigator to study one particular aspect of the food–caries relationship, the effect of acids produced from foods on tooth structure, and to assign a decalcification potential to such foods. But transfer of this information to humans cannot be made directly, because the oral factors involved modify the potential cariogenicity of a food, as inferred from its decalcification potential. For this reason, the decalcification potential of a food and its cariogenicity in human diets may not be identical.

However, the presence of acids at the tooth surface is an essential step in the production of dental caries, and even if all the other possible caries–promoting conditions are present, caries will not
result if these acids are absent. Furthermore, even if these acids are present, whether as a constituent of the food, or the result of fermentation, caries may still not occur, if the food in some way inhibits the dissolution of tooth structure, or the tooth itself has an inherent resistance to caries. A decalcification-potential test cannot evaluate the tooth or mouth factors which may, subsequent to acid production, enhance or limit the carious process, but it can assess acid production and extent of enamel dissolution in the presence of acid and food, and thus is of considerable importance in studies of the relationship between food and dental caries. In addition, such tests at present offer the only rapid means of identifying foods with little or no cariogenic potential, or food additives with anticariogenic potential.

This study was undertaken to develop a test of the decalcification potential of foods which would be simple and reproducible, and record the important changes taking place during fermentation. The test was then applied to various types of food, in an attempt to gain a better understanding of the relationship existing between pH, acid production and enamel dissolution. In developing this test, efforts were made to eliminate some of the difficulties and limitations inherent in techniques used by others. In brief, these are:—difficulty in standardising the test materials, limitation as to the extent of sampling possible during the experiments, and uncertainties in assessing the extent of decalcification taking place.

It was shown that standardisation of the food-saliva mixtures
CONCLUSIONS

was possible if pooled saliva, provided by a large panel of saliva donors, was employed, but that some variability was still encountered in the results, despite this precaution. It was then shown that this disadvantage could be offset by conducting replicate experiments. Three replicates gave mean experimental values which were significantly different with many foods, and enabled differences in fermentation behaviour between foods to be demonstrated.

It was also shown that frequent determinations of titratable acidity and enamel dissolution values could be made throughout the experiments, without altering the experimental conditions unduly, by employment of micro-titration and radio-chemical techniques. In this way it was possible to observe the manner in which the changes occurring during fermentation take place throughout the experiment.

Some investigators experienced difficulty in detecting enamel dissolution in food–saliva mixtures using chemical analysis, because of interference from the calcium and phosphate content of the food or the saliva, and the often relatively small amount of dissolution taking place under some experimental conditions. It was shown that the radiochemical technique used in this study permitted frequent and precise assessment of enamel dissolution in such mixtures, with the added advantage of being independent of any calcium or phosphate release or binding occurring in the mixture as a result of fermentation.

The buffer capacity of a food–saliva mixture was shown in this study to increase during fermentation, because of the inherent buffering effects of the organic acids so produced. Changes in total buffer
CONCLUSIONS

capacity may be due in part also to changes in inherent buffer capacity
as a result of fermentation. These findings show that enamel
dissolution as a result of fermentation cannot be inferred from
changes in pH or titratable acidity alone.

In broad terms, the conclusions of this study were based on
comparisons between the enamel-dissolving ability of fermented food-
saliva mixtures and simple chemical buffer systems of equivalent pH
and molarity. In order to make these comparisons, it was necessary
to investigate the enamel dissolution occurring in a range of simple
buffer systems, under similar experimental conditions, and to determine
the titratable acidities of such buffer systems. The data from these
investigations were used to construct graphs which facilitated these
comparisons, and, when considered together with the other experimental
variables recorded, led to the conclusions which follow.

In general, it can be concluded that the carbohydrate foods
tested, and probably all carbohydrate-containing foods are capable
of producing considerable amounts of acid when allowed to ferment with
saliva under the food-test conditions. The rate of acid production
was similar for most foods, and reflects the growth pattern of the
micro-organisms. There was a rapid production of acid from hour 2 to
hour 4, slowing considerably between hour 4 and hour 6, and reaching
a stable level by hour 24. Between hour 6 and hour 24, there was often
a considerable increase in acid production, but despite this increase,
the pH of the mixture decreased only slightly. This indicates that
the buffer capacity of the food-saliva mixture increased as fermentation
CONCLUSIONS

progressed because of the inherent buffering effects of the organic acids so produced, and the additional possibility that the fermentation process could change the buffer capacity of a food, independently of this acid production. Thus acid production in a food–saliva mixture cannot be estimated from its pH and initial buffer capacity.

When foods were considered with respect to refining, an unrefined food usually had a higher inherent buffer capacity, caused a lower pH fall, and produced much more titratable acid than its refined counterpart. Thus when different groups of food–saliva mixtures are compared, they may have identical pH change curves, yet different titratable acidity curves, and vice versa.

When powdered enamel was added to a fermenting food–saliva mixture, the resulting dissolution was a function of both the pH and the titratable acidity of the mixture, but was not dependent on pH fall or titratable acidity rise alone. There was no evidence of a critical pH level for enamel dissolution in fermenting food–saliva mixtures. With some foods or food–additive combinations, enamel dissolution over a 24 hour period was so low as to be negligible, and with other foods, so high as to almost completely dissolve the enamel powder. Some foods with high enamel dissolutions were those foods commonly thought to be, and in some cases demonstrated to be cariogenic, while most foods with low or negligible enamel dissolutions were foods which have been shown to be slightly cariogenic, or non–cariogenic, with some exceptions, and it can be stated that a low decalcification potential for a food correlated with low cariogenicity in animal and
CONCLUSIONS

human experiments, with some notable exceptions. There was positive correlation of enamel dissolution with in-vivo erosion.

When used to assess the effect of the food additive calcium sucrose phosphate, at a 1% level in sucrose, the food test indicated that this substance greatly reduced the enamel dissolution. When considered with the demonstrated caries-reducing effect of calcium sucrose phosphate, this result suggests that if a low enamel dissolution is produced when a food or food-additive combination is tested, it can be reasonably anticipated that the food or food-additive combination additive will be less cariogenic in a clinical trial.

The general conclusions reached as a result of this study are:

1. When carbohydrate foods were fermented with saliva and tooth enamel, acids were produced, there was a decrease in the pH of the mixture, and enamel was dissolved.

2. The pH changes taking place were basically similar for all carbohydrate foods, the pH level after 6 hours fermentation lying between about 5.0 and 4.0.

3. A further 18 hours fermentation resulted in an increase of about 100% in acid production, but only a slight fall in pH level.

4. The amount of titratable acid produced during fermentation varied greatly with different foods, and was, in general, high with unrefined and low with refined foods.

5. The inherent buffer capacity of a food was generally high with
CONCLUSIONS

unrefined, and low with refined foods.

6. The buffer capacity of a fermenting food-saliva mixture increased as fermentation progressed.

7. The amount of enamel dissolution which occurred in a fermenting food-saliva mixture was not directly dependent on the pH or titratable acidity of the mixture.

8. Some fermenting food-saliva mixtures caused considerable enamel dissolution, others slight dissolution. The degree of enamel dissolution was generally low for foods containing appreciable amounts of calcium and phosphorus, and for some unrefined sugars and cereal products.

9. There was no evidence of a critical pH level for enamel dissolution in acetate buffers or fermenting food-saliva mixtures.

10. The products of sugar cane juice showed increased enamel dissolution, and often decreased titratable acidity in the food tests, as the degree of refinement increased.

11. The degree of variation in replicate food fermentations was sufficiently small to permit differences in fermentation behaviour to be demonstrated on the basis of three replicate experiments.

12. A low enamel dissolution in the food test indicates low cariogenicity. But a high enamel dissolution does not necessarily indicate a high cariogenicity in human diets, because the food test does not take into account all the factors
CONCLUSIONS

involved in caries susceptibility.

13. The major value of the food test developed in this study is that it permits the identification of foods or food additive combinations which may reduce dental caries.

14. Calcium sucrose phosphate inhibits enamel dissolution when added to sucrose at a level of 1%.
SUMMARY

Much work has been and is being carried out on the aetiology, control and prevention of dental caries, but little of this effort has been directed to the study of the deleterious effects of various foods on the enamel of erupted teeth. Foods are a fundamental link in a complex chain of reactions occurring in dental caries, and deserve more research effort than they have received in the past.

It is now well established that some diets are less conducive to the development of dental caries than others, and lowered caries rates can often be observed in groups whose racial, cultural or economic situation results in a caries-limiting dietary pattern. Such conditions can be deliberately arranged in human populations. Thus wartime diets often change the caries experience dramatically, and effective control of caries has often been achieved in institutionalised populations by intentional dietary modification.

Investigation of the effects of additions to human diets on dental caries has not been extensive, because of the experimental difficulties involved. Animal experiments have been carried out, and while this approach minimises some of the experimental difficulties, results are often conflicting, and may not be transferable to the human subject.

In general, sucrose increases caries, while some unrefined grains and grain products reduce caries, when added to animal diets. Raw and cooked starches both have a low cariogenic potential in animal diets. Phytates, calcium and sugar phosphates and some oils reduce caries in rodents.
SUMMARY

In-vitro tests for assessing decalcification potential of foods have little relevance to in-vivo caries production, because of the tooth and mouth variables which are eliminated in such tests. They do, however, have some relevance to the food-caries relationship, as dissolution through acid production is basic to the carious process. Most observers find that foods which appear to reduce enamel dissolution are unrefined, and have a high buffer capacity, and many demonstrate evidence of extractable 'protective factors' in such foods. Some suggest incorporation of these factors in foods as a possible caries control measure.

A factor not assessed by most in-vitro studies is the effect of plaque. Recent plaque studies indicate that sucrose enhances plaque formation, through the formation of an extracellular polysaccharide. Some have suggested enzymic or antigenic methods to reduce this formation. Although extracellular polysaccharides may prove in the future to be of considerable significance in the carious process, present evidence suggests caution, since caries can occur in the absence of sucrose. Although caries will not occur in the absence of plaque, in-vitro studies, which do not allow for its effects, are still relevant to the food caries relationship, as the carious process must involve mineral loss from the tooth, and it is this loss which is assessed by in-vitro decalcification potential studies.

This thesis describes an attempt to develop a laboratory method for assessing the decalcification potential of foods by measuring the dissolution produced when a food-saliva-enamel mixture is fermented.
SUMMARY

under controlled conditions. The results are also considered in the light of the food–saliva–decalcification relationship, in the hope that further application of the technique might lead to a better understanding of the basic mechanisms involved in caries control through alteration of the composition of foods.

The method employed differs from others in that radioactive tooth enamel was incubated with pooled saliva samples and the food under investigation. Controlled conditions of temperature and agitation were maintained for 24 hours, and pH and titratable acidity recorded at regular intervals. Enamel dissolution was measured at the same intervals by determining the amount of radioactivity appearing in solution as a result of the action of the mixture on the radioactive enamel powder. Accurate assessments of dissolution were thus possible without changing the experimental conditions unduly by excessive diminution of the experimental volume, and without the complication of interfering ions of the food–saliva mixture.

All foods were tested under identical experimental conditions, and valid comparisons of the properties of individual foods in the test could be made. It was found that under the experimental conditions employed, foods produced characteristic and reproducible pH, titratable acidity and enamel dissolution curves.

Although considerable effort was directed to methods for standardising the test, some variation in replicate experiments was encountered. Statistical analysis of the U.S. and preliminary Australian results indicated that significant differences in fermentation behaviour
could be demonstrated when three replicate experiments were conducted on each food, and this procedure was adopted in the Australian food tests.

Representative foods of various types were tested, in six food groups: 'beverages', 'sugars', 'flours', 'bread and biscuits', 'cereals' and 'sweets and fruits'. Nearly all the foods tested had an initial pH of about 7.0, which fell rapidly in the first few hours to about 5.0, then less rapidly over the remainder of the experimental period, the hour 24 values lying in the range 4.0 - 5.0, with the exception of Sweetaddin, an artificial sweetener, not readily utilised by oral micro-organisms. Titratable acidity values (expressed as ml. of 0.1M sodium hydroxide required to titrate 10 ml. samples to pH 7.0) varied greatly with different foods. With nearly all sugars, wheat, rice and maize flours, bran, most cereals, white, brown and Vogel breads, most biscuits, most fruits and with caramels, titratable acidity increased more or less uniformly to 2 ml. by hour 6, and increased to between 3 and 5 ml. by hour 24. Lower titratable acidity (less than 1.5 ml. by hour 6) was observed with cornflour, Corn Flakes, Frosties, Sao biscuits, glace cherries, pilsener and Coca Cola. These foods also showed lower titratable acidity values at hour 24. Higher titratable acidities (more than 3 ml. by hour 6) were observed with molasses, wholemeal flour, oatmeal, wheatgerm, bran, rye flour, peanut butter, potato crisps, Lemon Slice, dried apricots, milk chocolate, milk, chocolate milk, ice cream and orange juice.

Many of these foods showed very high hour 24 titratable acidity
SUMMARY

values (7.5 ml. or even higher).

Enamel dissolution (expressed as milligrams of enamel dissolved per 100 milligrams added) was low (about 5 mg. by hour 6 and 10 mg. by hour 2½) for treacle and molasses, and all beverages except Coca Cola® and orange juice. Enamel dissolution was in the range 10 - 20 mg. by hour 6, and 20 - 50 mg. by hour 2½, for sucrose, dextrose, brown sugar, oatmeal, maize, all cereals, peanut butter, potato crisps, Vogel® bread, biscuits and milk chocolate. Enamel dissolution was high (above 20 mg. by hour 6 and above 50 mg. by hour 2½) for honey, glucose syrup, all flours, but especially wholemeal flour, rye flour, bran and wheat germ, and for potato crisps, white and brown breads, bananas, raisins, dried apricots and orange juice.

In general it was found that refined (processed) foods produced less titratable acid than did raw, unprocessed foods, but that the pH changes observed were comparable. This finding was related to the lower initial buffer capacity of refined foods, in comparison with unrefined foods. The degree of enamel dissolution occurring with a test food could not be predicted solely on the basis of either the change in pH or titratable acidity, or the inherent buffer capacity of the food. Thus a large fall in pH and extensive acid production as a result of fermentation resulted in high enamel dissolution with some foods, but low enamel dissolution with others. These results did not agree with those obtained in comparable experiments using simple acetate buffer systems, in place of the food–saliva mixtures. In such simple buffer systems, a lowering
SUMMARY

of pH, a raising of titratable acidity, or both, always resulted in higher enamel dissolution.

If fermenting foods in the mouth behaved as simple buffer systems with respect to enamel dissolution, then it might be concluded that foods which have the potential to produce acids in the mouth should be avoided, if caries control is to be achieved by diet. The findings of this study suggest that such a conclusion would be invalid, because the foods themselves often have an effect on enamel dissolution independent of the pH and titratable acidity levels they produce when fermented with saliva and tooth enamel.

Some of the foods tested, notably milk and milk products, and some unrefined sugars and cereal products possess the ability to modify the action of food fermentation products on enamel, and it is likely that this property is removed by refining, in the case of sugar.

When the cariogenic potential of foods was predicted on the basis of the food-test enamel dissolution results, some correlation was obtained with animal and human experiments where comparable foods were assessed for cariogenicity. The correlation was good for those foods showing low enamel dissolution in food tests. Some foods showing high enamel dissolution in the food tests were shown to be cariogenic in animal and human diets, but unlike the low dissolution foods, the degree of cariogenicity could not be predicted quantitatively from the enamel dissolution figures. Correlation of in-vitro erosion with in-vivo erosion was good. It was concluded that the food test developed in this study could be used to identify foods and food
SUMMARY

additives which may reduce caries.

Calcium sucrose phosphate, a substance reported to reduce dental caries in humans, was found to exert a marked enamel dissolution-reducing effect when added to sucrose at the level of 1% in the food test. From this result it was concluded that food additives which reduce enamel dissolutions in the food test could exert a cariogenic effect when incorporated in human diets.

The experimental methods used in this work required the development of a new technique for preparing pure, uniform mesh-size enamel powders in large quantities. This material was irradiated by exposure to neutrons in an atomic reactor. The radioactive enamel produced permitted the use of a radiochemical method to measure enamel dissolution, with its inherent advantages over conventional chemical methods.

To establish the validity of the application of the radiochemical method, the dissolution behaviour of radioactive tooth enamel in a series of buffer systems was investigated. These experiments showed that the effect of irradiation on the solubility of enamel in acetate buffers was twofold. First, overall solubility of irradiated enamel is less than that of non-irradiated enamel. Secondly, the solubility of the radioactive portion of the irradiated enamel ($\text{P}^{32}$) is less than that of the non-radioactive portion of irradiated tooth enamel ($\text{P}^{31}$). Despite these differences, the dissolution curves obtained by the chemical and radiochemical methods were reproducible, and comparable qualitatively. It was concluded that radioactive tooth enamel can be
SUMMARY

employed to demonstrate differences in the effects of various fermenting food–saliva mixtures on enamel dissolution.

The procedures and techniques employed in this work have been described in detail. In addition, the process of irradiation, its effects on enamel, and techniques for minimising the undesirable effects were discussed, together with the factors to be considered in the selection and use of radio-isotopes in this form of investigation.
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APPENDIX A

PREPARATION OF POWDERED TOOTH ENAMEL
PREPARATION OF POWDERED TOOTH ENAMEL

In any extensive investigations of tooth enamel solubility, large amounts of tooth enamel are required. Each food fermentation test, and most of the enamel dissolution tests described in this work involved the use of 100 mg. of powdered tooth enamel. The total amount used for this work, over 20 gms., would have been most tedious to obtain using the enamel powder preparation techniques in current use, so alternative techniques were evaluated.

A common method for preparing enamel powder is that of Manly and Hodge (206) where whole teeth are crushed, and the enamel and dentine powder fractions separated in a flotation solution of high density, by centrifugation in a specially designed tube. The method gives a high yield of material, but has the attendant disadvantages of exposure of the surface of the particles to the heavy metal ions in the flotation solution, and some contamination of the enamel powder fraction with dentine, and vice versa. The surface contamination is removed by prolonged refluxing with appropriate solvents, and the efficiency of separation of enamel from dentine assessed microscopically, using the differing optical properties of enamel and dentine. For more efficient separation, the flotation procedure must be repeated. This method is unsuitable for the preparation of uncontaminated, uniform mesh-size enamel powder.

A more precise technique is to abrade an enamel surface with a high speed diamond stone, and collect the abraded dust by
POWDERED TOOTH ENAMEL

aspiration onto filters in an enclosed box. In this method the operator can be sure of the purity of the sample, but the particles are extremely fine, the work tedious in the extreme, and the yield very low. Neither method yields fractions of a uniform particle size, and if such uniformity is required, the samples obtained must be passed through a bank of sieves, with an accompanying loss should some of the particles subjected to sieving be smaller than the mesh size required.

Because of the small amount of enamel available from one human tooth, and because of the attendant disadvantages of limited supply, frequent presence of caries, and variation in chemical composition, the use of human enamel powder in this study was virtually precluded. Though it would no doubt be possible to obtain 20 gm. or more of uniform mesh-size human tooth enamel powder, and it would be desirable to use human enamel in this work, it was felt that the time necessary to obtain it would far outweigh any advantages accruing from its use. An alternative was then sought, from readily available animal material. Of the available domestic animals with suitable teeth, young cattle were selected as the source of powdered enamel. Their lower incisor teeth are very large, and apart from the roots, which differ somewhat from human teeth in shape, root apex and periodontal ligament, these teeth are practically indistinguishable in external appearance from human upper central incisors. Their enamel volume is approximately four times that of human incisors. The microscopic appearance of ground sections
of bovine enamel is similar to that of human enamel, with minor differences in structure.

As is the case in the human dentition, the permanent incisors in cattle are preceded by their deciduous counterparts, which are shed just before the permanent teeth erupt. Cattle, aged two-four years, ('yearlings'), are generally in the process of losing their deciduous teeth, and the heads of cattle of this age are readily identified and obtained at any large abattoir. The anterior segment of the lower jaw is easily removed, and will yield from two to six unerupted, or partially erupted, undamaged permanent incisor teeth.

Several hundred incisor teeth were removed from the lower jaws of cattle, by incision and elevation. These teeth were stripped of the adhering soft tissues, and stored in 70% alcohol. Stained or damaged teeth were discarded.

Two techniques for obtaining enamel-dentine separation were investigated.

It was suggested * that tooth enamel could be readily removed cleanly from the dentine by plunging the whole tooth into liquid air. The enamel is said to crack away at the dentine–enamel junction by differential expansion. This method was found to be ineffective with bovine teeth, which cracked into

* Technique advocated by B. Bibby.
POWDERED TOOTH ENAMEL

small pieces, with no evidence of enamel separation. Similar results were obtained with human teeth, except when old, dried specimens were used, where occasionally, enamel-dentine separation was achieved.

In the second method, serial, transverse sections were cut from the tooth crowns, and the enamel harvested from each section by hand. This technique provided small blocks of pure enamel. The teeth were first removed from the alcohol storage solution, and thoroughly cleaned with pumice and glycerine using a stiff bristle brush on a dental engine. The roots were then removed using a water-cooled diamond blade, and the pulp removed. The pulp tissue almost always came away in toto. The crowns were wiped dry, and fixed to the cutting table of a section machine* using soft wax. The crowns were kept moist in the subsequent procedures, to prevent the occasional fracturing which occurs in dried, bovine tooth crowns. Transverse cuts were then made at 1 mm. (1000 micron) intervals, yielding sections approximately 0.6 mm. thick. These sections were then freed of any adhering wax, washed, wiped dry and stored in petri dishes. Thin sections were not susceptible to fracture on drying. Small blocks of enamel were then cut from each section, using either a fingernail clipper or a miniature wire cutter. Cuts were made

* Bronwill Gillings Thin Section Machine. Will Corp., Rochester N.Y.
POWDERED TOOTH ENAMEL

close to the dentine-enamel junction, so that a minimum of enamel was wasted. The cutting operation was performed over a large-diameter petri dish, resting on a black background, to assist in examining each cut enamel block. Each block was checked after cutting, to guard against accidental inclusion of dentine. Any blocks containing dentine were either recut or discarded.

These dentine-free blocks of enamel were then lightly crushed in a glass mortar using a glass pestle, and the resulting powder shaken by hand, through a bank of sieves, of 120, 200 and 240 mesh * (Greenings, BSF standards) and receiver. The fraction which was retained on the 120 mesh sieve was returned to the mortar, and recrushed and resieved, the procedure being repeated until all the enamel had passed the 120 mesh sieve. On microscopic examination, each fraction was seen to consist of irregularly shaped particles of substantially uniform size, together with many much smaller particles, (brash) which would theoretically have passed the sieve retaining them. (See fig.14)

Dissolution experiments performed on these enamel powder samples showed that the brash affected the dissolution rate quite markedly, (Section III, fig.3 and Appendix B,) but that it could be removed by a series of washings. The 'pass 120, retained 200 mesh' powder fraction (120–200 mesh) was then

* Test Sieves B.S. 410/1945 N. Greenings and Sons, Ltd., Hayes, Mddx., U.K.
POWDERED TOOTH ENAMEL

suspended in distilled water, the pH of which was adjusted to pH 7.0 by the addition of a small amount of buffer,* and the coarser particles allowed to settle. The supernatant liquid was then discarded, and the sediment resuspended as before. This process was repeated. After five such washings, microscopic examination of the enamel powder showed that it was substantially free of brash. (See fig.14)

Because of the tedious nature of most enamel powder preparation techniques, figures were kept of the yield of enamel powders obtained using the method described, to allow comparisons to be made with other methods. Twelve bovine incisors yielded 180 transverse sections, weighing 20 gm. The weight of enamel blocks recovered from these sections was 5.9 gm. After crushing and sieving, the 120–200 mesh powder fraction weighed 2.9 gm., the 200–240 mesh fraction 0.9 gm. and the pass 240 mesh fraction 1.9 gm. There was a loss after washing to remove the brash of 20% for the 120–200, 50% for the 200–240 and 35% for the pass 240 mesh fractions, respectively. The laboratory time taken by one operator for harvesting, sieving and washing was four hours, the yield being more than four grams of pure, uncontaminated and uniform particle size enamel powders, over half of which was of the desired 120–200 mesh size. This washed 120–200 mesh sample

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represented about 20% of the total enamel of the intact teeth. Expressed as percent recovery of the tooth enamel actually available for harvesting, the yield was about 35%, since there is an unavoidable loss of material during sectioning. This loss decreases as the section thickness increases. At a section interval of 1mm (1000 microns), the loss is about 40%, using a diamond blade which is 12 thousandths of an inch thick. (0.3 mm.) A better yield is obtained with thicker sections, but with such sections, it is difficult to cut enamel blocks which are free from dentine. The upper limit of section thickness for satisfactory enamel block production is about 0.7 mm.

Provided adequate supplies of teeth are available, the method described is attractive because of the speed with which quantities of enamel powders can be prepared, and because the samples are known to be free of dentine. In addition, the powders obtained are uncontaminated, and not contacted by any foreign materials, except for the diamond blade, clippers, sieves, glass pestle and mortar, and the washing water. This is an important consideration where experiments could be influenced by the presence of foreign materials on the particle surface.
APPENDIX B

DISSOLUTION OF APATITES IN ACID SOLUTIONS
DISSOLUTION OF APATITES IN ACID SOLUTIONS

Recent work by Higuchi et al.\(^{(137)}\) has clarified some of the important aspects of enamel dissolution in acid buffer solutions. These workers measured enamel solubility rates and found that the reaction was diffusion controlled, and affected by both temperature and agitation. In addition, they reported that the initial rate of dissolution was a function of hydrogen ion concentration, and, in the presence of buffers, also dependent on the amount of undissociated acid, which could furnish hydrogen ions to the site of reaction at the enamel surface. Further, they found that the initial rate was decreased by the presence in solution of the reaction products, (calcium and phosphate ions,) and also all cations. To explain this effect, they suggest that the enamel surface is coated by the reaction products reprecipitating on it, either alone or with added or foreign ions.

In an effort to determine whether the enamel powder used in the food fermentation tests behaves the same way in acid buffers as the enamel examined by Higuchi et al., dissolution experiments were conducted on non-irradiated and irradiated bovine enamel powders.

In the first of these experiments, four different apatite \(R\) samples, tricalcium phosphate, (Victor), unwashed 120–200 mesh bovine enamel powder, washed 120–200 mesh bovine enamel powder and washed 120–200 mesh human enamel powder were used. The apatite samples were irradiated as described in Appendix C, and 50 mg. of
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each weighed out and placed in 25 ml. of 0.1M acetate buffer at pH 4.5. The apatite-buffer mixture was shaken at room temperature for 1\frac{1}{2} hours, and allowed to stand for three days. Enamel dissolution samples were taken by glass frit and micropipette at thirty minute intervals for the first 90 minutes, and again after three days. The samples were then submitted to calcium analysis, (EDTA titration using Erichrome indicator) and to radiochemical analysis ($P^{32}$ count rate determination). Enamel dissolution values at each time period were then calculated, using appropriate standards.

Apatite dissolution (in mg.) was then plotted against time for the four samples, (fig.15). With each sample tested, the apatite dissolution values calculated from radiochemical analyses were lower than those calculated from calcium analyses, and show that the radiochemical method yields lower values for apatite dissolution with synthetic, bovine and human apatite. The curves for chemical and radiochemical methods are similar qualitatively, but differ quantitatively.

This unexpected finding was further investigated using washed, 120–200 mesh bovine enamel powder. A quantity of this material was prepared, and a portion irradiated as described in Appendix C. Weighed 100 mg. samples of both non-irradiated and irradiated enamel powders were then placed in two separate beakers, 50 ml. of 0.1M acetate buffer at pH 4.0 added, and the mixtures agitated at 37°C for two hours. At ten minute intervals for the first hour, and again at hour 2, 1.5 ml. samples were taken from both the non-irradiated and
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irradiated enamel-buffer mixtures, for chemical analysis of calcium and phosphate. Small samples (100 µl.) were taken at the same time intervals from the irradiated enamel beaker only, for radiochemical analysis of P³².

Chemical analyses were performed on the samples. Calcium content was determined on a Hilger Atomic Absorption Spectrometer, and a Hilger Unispek®, modified according to the method of Davey (64). Appropriate dilutions were prepared using a stock solution containing 1500 p.p.m. of strontium and potassium and 1000 p.p.m. of sodium, placed in cuvettes, and read at 4227 Å. Calcium concentrations were read from a standard curve, plotted from a series of standards containing known concentrations of calcium. Recovery was better than 95%. The phosphate content of the samples was determined on a Unicam SP 600 spectrophotometer. Appropriate dilutions were prepared with a reducing agent and ammonium molybdate, placed in 1 cm. cells and read at 8300 Å. In one experimental series, phosphovanado-molybdate reagent was used (125) and the samples read on a Hilger spectrophotometer at 4200 Å. Phosphate concentrations were read from a standard curve, plotted from a series of standards containing known concentrations of phosphate. Recovery was better than 95%.

The results were expressed as mg. of enamel dissolved per 100 mg. of enamel added. Five curves were constructed from the data; i. Non-irradiated enamel dissolution, by calcium analysis; ii. non-irradiated enamel dissolution, by phosphate analysis; iii. irradiated enamel
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dissolution, by calcium analysis; iv. irradiated enamel dissolution,
by phosphate analysis; v. irradiated enamel dissolution, by radio-
chemical means, (count rate of $P^{32}$). At hour 2, concentrated HCl was
added to dissolve any remaining enamel powder, and recovery by each of
the five methods checked. This was within the range 95–102\% in all
cases, assuming a Ca/P ratio by weight of 2.06.

This experiment showed that irradiated tooth enamel was less
soluble than the non-irradiated tooth enamel, and that the radiochemical
method indicated a lower rate of solubility for the irradiated enamel
than that calculated from either calcium or phosphate analysis data.
The author could not reconcile this result with the almost 100\% recovery
of enamel with all three methods, when all the sample was dissolved,
so the same experiment was repeated twice, using the various analytical
methods previously described, to verify the first result. Similar results
were obtained, so the three sets of values for each of the five
determinations were combined. (fig. 16)

There are small differences in dissolution rates when
determined from calcium and phosphate analyses, for both non-
irradiated and irradiated enamel, the rate as determined from calcium
analysis, being slightly higher than that from phosphate analysis,
throughout the two hour experimental period.

It is apparent that the process of irradiation does change
the dissolution behaviour of tooth enamel in acetate buffer solutions.
The irradiated material was consistently less soluble throughout the
experimental period, this difference being maintained even up to hour 2,
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when the systems approach equilibrium. Although there was a slight difference in the rate of dissolution between non-irradiated and irradiated enamel in the first 20 minutes of the experiment, the curves ran more or less parallel after this, and thus it can be said that for the greater part of the experiment, the qualitative (though not the quantitative) behaviour of the non-irradiated and irradiated enamels was similar, with respect to solubility in acetate buffer.

When the radiochemical method was used to determine the dissolution rate of the irradiated enamel, the resulting curve was different from those obtained using either the calcium or phosphate analysis methods, and indicated a much lower 'enamel solubility'.

Such an effect could result if the ratio of enamel powder to solution increased as a result of excessive sampling in the experiments on which the chemical analyses were performed, but not in the experiments where radiochemical analyses were performed. But in the experiments reported here, the radiochemical samples were taken from the same experimental volume as the chemical samples, so different enamel powder/solution ratios cannot explain the effect. Such an effect could also result if the P^{32} labelling of the enamel was not uniform, but higher in the interior of the enamel particles. But, as is explained in Appendix C.I, p. xvi., the labelling of the irradiated tooth enamel is uniform, and furthermore, recovery of enamel at the conclusion of the experiment is always close to 100%, by both the radiochemical and chemical methods, when all the enamel
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is dissolved by HCl. There is no doubt that there is a difference in solubility between non-irradiated and irradiated enamel, as is obvious from the solubility curves obtained by chemical analysis. This experiment shows that there is also a difference in the dissolution of $^{31}P$ and $^{32}P$ in acetate buffer solutions.

It is well established that when non-irradiated apatite is exposed to acid solutions, some of the dissolved phase may redeposit on the surface of the undissolved phase, probably as dicalcium phosphate. It is also known that the $^{32}P$ in irradiated apatite can be in chemical forms different from tricalcium phosphate, commonly hypophosphosphate and metaphosphate. When the dissolution curves of irradiated enamel measured by the chemical and radiochemical methods are analysed in relation to these facts, there are several possible explanations for the low rate of dissolution obtained using the radiochemical technique.

If it is assumed that in dissolution experiments, the chemical methods yield accurate dissolution values and there is no surface redeposition of the dissolved phase, then it follows that the radioactive form of phosphate in the enamel, $^{32}P$, is less soluble than the stable form, $^{31}P$. But if this explanation is accepted, it must be further assumed that the stable $^{31}P$ is dissolved preferentially from around the $^{32}P$, leaving proportionately more $^{32}P$ in the undissolved phase. From the dissolution curves (fig. 16), it can be assumed that, after one hour, 60% of the $^{32}P$ must be undissolved,
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but only 40% of the P\textsuperscript{31}. As the P\textsuperscript{32} is uniformly distributed in the particles, this assumption implies that there is a meshwork of P\textsuperscript{32}-containing materials surrounding each partly dissolved enamel particle, and occupying 20% of each particle's original volume, or almost half the remaining volume. Examination of partly dissolved particles showed no evidence of such a meshwork, so this assumption is probably incorrect.

If it is assumed, as before, that the chemical methods yield accurate dissolution values, but that there is surface redeposition of the dissolved phase in both non-irradiated and irradiated enamel experiments, and that only the P\textsuperscript{31} of irradiated enamel is redeposited, then it follows that for at least some of the time, dissolution and redeposition are taking place simultaneously*.

Examination of the dissolution curves in fig. 16, for both forms of enamel, indicates that the dissolution rate exceeds the redeposition rate, except at equilibrium, when both rates are equal (or both zero). In either case, the amount of solid phase which has left the particles is higher than the curves would indicate, since some material has redeposited, the equilibrium value for dissolution being the resultant of dissolution and redeposition. The dissolution rate as measured by radiochemical means is much lower than the true

* Note that the P\textsuperscript{31} content of non-irradiated and irradiated enamel is almost identical, because the P\textsuperscript{32} in irradiated enamel is only a minute portion of the total phosphorus.
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rate, and this can only be the case if some $P^{32}$ remains on the enamel particles while the surrounding $P^{31}$ is removed, as was proposed above. If the $P^{31}$ redeposition rate is assumed to be high, the $P^{32}$ could be retained on the particle surface by this redeposition, without the necessity of postulating a $P^{32}$ meshwork. But for ions to be available for redeposition, they must first be dissolved, thereby leaving more unsupported $P^{32}$ behind on the particle surface. This assumption fails for the same reason as before, since a meshwork must still be postulated.

The only remaining assumption is that both the $P^{31}$ and the $P^{32}$ of irradiated enamel are redeposited on the partly dissolved enamel particles, and from examination of the dissolution curves (fig. 16) the dissolution rate exceeds the redeposition rate for each form of phosphorus, except at equilibrium, when both rates are equal.

For non-irradiated enamel, the redeposition rate under these experimental conditions is unknown. If it is low, any difference in the curves for enamel dissolution as calculated by phosphate analysis and calcium analysis will be slight. If the redeposition rate is high, the two curves will be quite different. This is so because the Ca/P ratio for whole enamel is about 2, while the Ca/P ratio for di-calcium phosphate, suggested as the redeposited material, is 1. When redeposition takes place, the Ca/P ratio will rise in the solution, while the surface of the enamel particles will carry a higher proportion of phosphate than the interior of the particle.
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If an enamel dissolution curve is plotted on the basis of phosphate content of the solution, the resulting curve will not represent true dissolution on a weight basis, and the same reasoning applies if the calcium content of the solution is used as the basis of the calculation. Under the conditions of these experiments, the redeposition rate must be low for non-irradiated enamel, except in the first ten minutes of the experiment, as the calcium and phosphate-based dissolution curves are similar, though that of phosphate is a little lower. For irradiated enamel, the same results are obtained. The dissolution curves calculated from calcium and phosphate values are similar, though once again, that of phosphate is generally a little lower. The values for 'tooth enamel' actually in solution (on a weight basis) in both the non-irradiated and irradiated enamel must lie somewhere between the calcium and phosphate-based dissolution curves, and it can be said that redeposition rate in each case is low, in comparison with dissolution.

It is quite evident that irradiation reduces the amount of dissolution of enamel in acetate buffers, though after 20 minutes, the rates of dissolution for both irradiated and non-irradiated enamel are comparable. There is an overall difference of about 10% in solubility as equilibrium conditions are approached. This difference in solubility can be ascribed to the changes in crystal structure which have taken place in the enamel as a result of irradiation.
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Determination of the dissolution rate of the irradiated enamel using the radiochemical method is based entirely on the appearance of radioactive phosphorus in solution, and is completely independent of the chemical form of this phosphorus. For reasons already explained, the low 'enamel dissolution' curves obtained in this way cannot be explained by proposing that the $P^{32}$ of the irradiated enamel is less soluble than the $P^{31}$. The $P^{32}$ must therefore be as soluble as, or more soluble than the $P^{31}$.

If the $P^{32}$ is as soluble as the $P^{31}$, then to explain the results obtained it must be assumed that the $P^{32}$ is redeposited at a faster rate than is the $P^{31}$. If the $P^{32}$ is more soluble than the $P^{31}$, it must be assumed that the $P^{32}$ is redeposited at a still faster rate. Either of these two mechanisms could explain the results obtained. The author believes that the $P^{32}$ of irradiated enamel is removed from the surface of the enamel particles at about the same rate as the $P^{31}$, but because its chemical form has been changed by irradiation, redeposition of the $P^{32}$ takes place more readily than the $P^{31}$, thus lowering the apparent 'enamel dissolution' values obtained using radiochemical determinations based on irradiated enamel samples.

Since the radiochemical method does not indicate a true enamel dissolution, and since the process of irradiation lowers the solubility of enamel no matter how the solubility is calculated, it might be assumed that such enamel would be unsuitable for decalcification
experiments. If however, the irradiated enamel behaves in a qualitatively similar way to non-irradiated enamel, this disadvantage is of little importance, provided the conclusions are not based on data from both non-irradiated and irradiated enamel.

Previous workers have found that when non-irradiated enamel is exposed to acetate or lactate buffers, there is an initial high rate of dissolution, which slows down as the reaction progresses, and eventually reaches an equilibrium. They also showed that a decrease in the pH of the system increased the dissolution rate, and also raised the equilibrium dissolution value. Furthermore, an increase in dissolution was obtained if the molar strength of the buffer, the surface area of the enamel or the agitation rate was increased.

In order to determine whether irradiated apatite in acetate buffer follows the behaviour of non-irradiated apatite, series of experiments were conducted. In the first of these, samples of tricalcium phosphate (Victor)®, unwashed 120–200 and unwashed 200–240 bovine enamel were irradiated as described in Appendix C. Acetate buffers of pH 4.0, 4.5, 5.0 and 5.5 were prepared, at 0.1 molar strength. 50 mg. of each of the three materials was then subjected to dissolution in 25 ml. of buffer at the four different pH's, under the same experimental conditions of agitation (magnetic stirrer) and temperature (18°C) for two hours. For each experiment, 100 µl. samples were taken at ten minute intervals for the first hour, and again at
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1\(\frac{1}{2}\) and 2 hours for each experiment. These samples were plated out onto stainless steel planchets, dried and counted using a thin end-window Geiger Müller tube, and gas flow techniques, with appropriate corrections for coincidence, background and decay. The amount of apatite present at each time interval in the stirred solutions was calculated by comparison with standard counting samples. These data were used to plot the dissolution-rate curves shown in fig. 17.

There was a very rapid initial dissolution with tricalcium phosphate, as shown by the steepness of the curves. The amount of dissolution taking place varied inversely with the pH, with almost 70 mg. being dissolved at pH 4.0, and only 17 mg. being dissolved at pH 5.5 after two hours. This material was examined microscopically, and found to be much finer than the bovine enamel powders.

The rate of dissolution with the two bovine powders was generally slower, and the total dissolution less, in comparison with the tricalcium phosphate. As before, the amount of dissolution taking place varied inversely with the pH, about 40 mg. being dissolved at pH 4.0, and only 7 mg. at pH 5.5 after two hours. There was a consistent difference in solubility between the 120–200 and the 200–240 mesh enamel powders, throughout the experimental period, the 200–240 mesh sample being the more soluble. This difference was less pronounced at the higher pH's. Equilibrium was approached after two hours, at the low pH's and in less than 30 minutes at the high pH's.

The behaviour of the irradiated synthetic and natural apatites thus conforms to the behaviour of non-irradiated apatites in acetate
buffer solutions, with respect to the effects of pH, and of surface area of the apatite, on dissolution, and with respect to the general shapes of the dissolution rate curves. It should be noted that these dissolution experiments were performed in Rochester N.Y., using unwashed bovine enamel. At this stage of the study, the presence of very finely divided enamel particles (brash) in the enamel powders was not suspected. (See Appendix A). Accordingly, these dissolution curves are not directly comparable with those depicted later, where the enamel powders were carefully washed. The latter show a considerably lower solubility, thus confirming the effect of surface area on enamel dissolution.

The second series of experiments was designed to test in detail the effect of acetate buffers of varying pH's and molar strengths on the dissolution rates of washed 120-200 mesh bovine enamel powder. Radioactive enamel powder was prepared as described in Appendix A and C. Acetate buffers of pH 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 were prepared, at molar strengths 0.05, 0.1, 0.3, 0.5 and 1.0. Equal amounts of enamel powder (100 mg.) were added to 50 ml. of acetate buffer of each pH and molarity, under the same conditions of agitation, (magnetic stirrer) and temperature, (37°C,) using a jacketed beaker, as shown in Section IV. For each experiment, 100 µl. counting samples were taken at 10 minute intervals for the first hour, and

* In conjunction with Dr. D.J. Beck
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again at 1\(\frac{1}{2}\) and 2 hours, for each experiment. These samples were plated out onto stainless steel planchets, dried and counted using a scintillation counter, with appropriate corrections for background and decay. The amount of tooth enamel present at each time interval, as shown by the radiochemical method, was calculated by comparison of the sample count rate with that of a standard sample. A 'group' of dissolution rate curves was then plotted three dimensionally for each of the various pH's and molarities used, to enable enamel dissolution relationships under varying conditions of pH and molarity to be visualised. The graph obtained is shown in fig. 18.

The 'group' of curves clearly indicates the effect of a decrease in pH and an increase in buffer molarity in increasing the rate of enamel dissolution and total dissolution at equilibrium, as calculated by the radiochemical method. The graph also indicates that a twenty-fold increase in buffer molarity, from 0.05 M to 1.0 M, almost doubles the enamel dissolution, with smaller increases being roughly proportional, while a pH change from pH 6.5 to pH 4.0 increases the enamel dissolution about seven times, for all the molarities tested. Also demonstrated is the effect on enamel dissolution at equilibrium of a change in pH of one unit. For all the molarities tested, the hour 2 dissolution value at pH 5.5 is twice that at pH 6.5, at pH 5.0 twice that at pH 6.0, at pH 4.5, just over twice that at pH 5.5, and at pH 4.0, just over twice that at pH 5.0. This effect of a decrease of approximately one pH unit in doubling the enamel dissolution applies
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for all time intervals throughout the two hour experimental period, as can be seen from the one hour dissolution values at pH 6.0, 5.0 and 4.0 for the five molarities tested. The dotted curves joining these points on the graph in a three dimensional display demonstrate this doubling of dissolution for a decrease in pH of one unit. This rule is also applicable to the other curves. However, in those tests employing a high molarity and low pH, the enamel dissolution is slightly more, proportionally, than it is for low molarity and high pH, as the diverging path of the uppermost three-dimensional relationship shows, in comparison with the other two, which are straight and more or less parallel. On a numerical basis, it would seem that in enamel dissolution experiments using acetate buffers and radiochemical techniques, large increases in buffer molarity have a small effect on dissolution rate, while a fall in pH of approximately one unit doubles the dissolution rate in the pH and molarity ranges tested. The findings do not therefore support the concept of a 'critical pH' with respect to enamel dissolution, where it is held that the dissolution rate increases rapidly below pH 5.0 \(^{(79, 270)}\)

The 'group of curves' also demonstrates the effect of changes in molarity and pH on the time taken for a given system to reach equilibrium. Equilibrium was reached quite rapidly, within fifteen minutes or so for high pH and low molarity, but it took longer to reach equilibrium over 2 hours, with low pH and high molarity, as is seen by the steepness of the curves in those experiments where the pH was lower than 5.0 and the molarity more than 0.3.
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The above experiments on enamel dissolution, as measured by the radiochemical techniques, give results which agree qualitatively with those obtained by others using gravimetric or chemical techniques.\(^{105}\) For this reason radioactive tooth enamel can be used in experiments where comparisons are made of the effects of pH change and titratable acidity on the enamel dissolution occurring in various fermenting food–saliva–enamel mixtures.

To obtain a better understanding of the effect of a fermenting food–saliva mixture on tooth enamel, the dissolution rate with the food should be compared with the dissolution rate obtained in a simple chemical system whose rates of change of pH and titratable acidity duplicate those of the food saliva mixture. This would be extremely difficult to carry out on an experimental basis. However, the 'group' of curves shown in fig. 18 indicate that equilibrium in a simple chemical system at about 0.1 M is approached within about thirty minutes for pH's of 6.5 and 6.0, about one hour for 5.5 and 5.0, and about two hours for 4.5 and 4.0. Equilibrium is approached even more rapidly at these pH's for lower molarities. In the 150 food fermentation experiments carried out in this investigation, the pH rarely went below pH 4.0, and the titratable acidity, rarely above 0.5 M. (expressed as acetate buffer equivalent). Thus in a food fermentation where the pH and titratable acidity remain substantially unchanged for an hour or so, it is possible to obtain an indication of the minimum level of enamel dissolution which ought to have taken place if the food–saliva mixture behaved as a
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simple chemical buffer. It must be a minimum level, since the predicted dissolution value would not include the enamel dissolution which had taken place before the pH and titratable acidity attained relative stability. If, under these circumstances, the enamel dissolution value in a food-saliva mixture is below that which could be expected in an equivalent simple chemical buffer, the pH and titratable acidity produced by the fermenting food-saliva mixture cannot be behaving as a simple chemical buffer. However, this reasoning cannot be applied to those situations where pH and titratable acidity are changing rapidly, since the two experimental conditions are no longer equivalent. Nor can this reasoning be applied to periods where the pH and titratable acidity are stable for longer periods than two hours, since there may be a slow increase in dissolution over the longer period, even in those cases where equilibrium appears to have been reached.
APPENDIX C

I: RADIOACTIVE TOOTH ENAMEL
I: RADIOACTIVE TOOTH ENAMEL \(3,100,162,235\)

Radioactive atoms differ from stable atoms of the same element only in the arrangement of the nucleus. The arrangement of the orbital electrons is identical for each. The nuclei of radioactive atoms are unstable, and reach stability by radioactive decay (disintegration), whereby each nucleus rearranges its internal structure, eventually reaching stability by loss of energy in the form of alpha, beta, positron or gamma emission, and sometimes combinations of these. These emissions can be detected because of their effects on surrounding matter.

If, in acquiring stability, the radioactive nucleus emits charged particles, the loss of charge of the now stable nucleus must be balanced by a rearrangement of the orbital electrons, for the atom as a whole to reach electrical neutrality. When this occurs, the atom has been transmuted into a different element, as its chemical behaviour is dependent on the number of orbital electrons.

The disintegration rate of radioactive elements is fixed, completely independent of the physical or chemical form of the element, cannot be changed by heat, cold, light, pressure, etc., and is specific for each radioactive element. This rate can be determined using suitable equipment, and because the disintegration rate is fixed, the amount of radioactivity present in a sample can be calculated from its disintegration rate. Furthermore, because even small amounts of matter contain large numbers of atoms, radioactivity measurements often provide a very sensitive method for
detecting and estimating radioactive elements at very low concentrations.

The commonly used radioactive elements can be prepared using a particle accelerator, a neutron source or a nuclear reactor. The amount of radioactivity which can be prepared rests primarily on the number of atomic interactions which it is possible to produce, and the most convenient technique for preparing radioactive elements is to bombard a stable element with neutrons. Extremely large numbers of neutrons are provided in the fission reaction, where certain radioactive elements are induced to split, and this is achieved under controlled conditions in an atomic reactor.

Neutrons are particles with no charge, but when first produced in a reactor, have very high energies, of from 5 - 10 million electron volts (5 - 10 Mev). This energy is lost, not by ionising surrounding atoms, as is the case with charged particles, but by elastic collisions with atoms, until a stage is reached where the neutrons' energies correspond roughly with the thermal energies of the reactor materials, about 0.025 million electron volts (0.025 Mev). For this reason, they are called 'thermal' neutrons, and because they have no charge, find little difficulty in entering the nuclei of atoms. When a neutron approaches the nucleus of an atom, there is, in fact, an attractive force which assists in this neutron capture. The process can take place with high energy neutrons, 0.1 Mev and above, as well as with thermal neutrons, but in the former case
RADIOACTIVE TOOTH ENAMEL

(called 'inelastic' collision), the target nucleus re-emits a neutron of lower energy, and releases the energy left behind as a result of the capture, in the form of gamma rays.

Efficient production of radioactive materials in a nuclear reactor depends on adequate supplies of 'thermal' neutrons. In reactors designed for isotope production, these 'thermal' neutrons are produced by slowing down (moderating) the fast neutrons, commonly by helium, water or graphite, and this moderating process is achieved by 'inelastic' collision. Neutron production in a reactor is expressed as the 'flux!', and will vary in different parts of the reactor. In HIFAR** reactor, the flux in the part of the reactor used for irradiating tooth enamel was about $5 \times 10^{12}$. This is a high flux, and suited to efficient isotope production.

Typical examples of irradiation reactions are shown below:

- **neutron: gamma**
  \[ _1^1\text{H} + _0^1\text{n} = _1^2\text{H} + \gamma \]

- **neutron: proton**
  \[ _7^1\text{N} + _0^1\text{n} = _6^{14}\text{C} + _1^1\text{H} \]

- **neutron: alpha**
  \[ _3^6\text{Li} + _0^1\text{n} = _1^3\text{H} + _2^4\text{He} \]

- **neutron: 2 neutron**
  \[ _{92}^{238}\text{U} + _0^1\text{n} = _{92}^{237}\text{U} + _0^1\text{n} + _0^1\text{n} \]

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* The neutron flux is the number of neutrons passing through an area of one square centimeter in one second, in a designated part of the reactor.

** High Flux Australian Reactor, Lucas Heights, Sydney.
RADIOACTIVE TOOTH ENAMEL

By far the most common of these reactions is the neutron gamma reaction, particularly with thermal neutrons, and can take place with most elements. The target nucleus captures a neutron, emitting excess energy in the form of gamma rays, (called 'capture' gamma rays), and if the gamma emission leaves the nucleus still in an excited state, the target nucleus possesses induced radioactivity, and can only reach stability by emitting further energy, in the process of radioactive decay.

The two most common systems for detecting the emission of this energy are the Geiger–Müller (G.M.) detector and the scintillation detector. Because of their differing characteristics, the G.M. detector is more efficient for detecting gamma rays. The G.M. detector is a tube containing special gases, and two electrodes connected to a high D.C. potential. An ionising radiation, on entering the tube, produces 'ion pairs'. The high D.C. potential separates these 'ion pairs', which are accelerated to the electrode of opposite charge, and in doing so, collide with other atoms of gas, forming further ion pairs, the process 'cascading' until an 'avalanche' of ions reaches the two electrodes. The time taken for this process may be 50 milliseconds or more. The sudden arrival of these ions at the electrodes causes a momentary change in potential, or pulse, across the electrodes. This pulse, which represents one disintegration, can be amplified and counted electronically. The number of disintegrations occurring in the
RADIOACTIVE TOOTH ENAMEL

sample will not necessarily correspond with the number of pulses counted, since a G.M. tube is rarely 100% efficient.

A scintillation detector is a material which converts the energy deposited in it by an ionising radiation into a tiny flash of light, or scintillation, the brightness of the flash being dependent on the amount of energy deposited. The energy deposited depends in turn on the mode of interaction of the ionising radiation with the detector material. The most commonly used scintillation material is a specially prepared crystal, the surface of which is painted white, except for one face, which is polished flat and lies in intimate contact with the photocathode of a photomultiplier tube. Any scintillation occurring in the crystal is thus reflected onto the photocathode, which then releases electrons into the photomultiplier tube according to the brightness of the scintillation. The photomultiplier tube then accelerates these electrons to the first of a series of targets (dynodes)*, causing the emission of more electrons, which are in turn accelerated to a second dynode, and so on. Thus the original scintillation causes a large number of electrons to arrive at the final dynode, resulting in a pulse, which can be amplified and counted electronically, and represents one disintegration.

The G.M. system is rugged and cheap, but is inefficient for

* Usually ten dynodes are used.
samples with a high disintegration rate (count rate), because some particles will enter the detector when an avalanche is already taking place, and will not be recorded, as they cannot produce a pulse. It follows that the longer the avalanche, the greater will be the inefficiency of counting, as more disintegrations will go undetected. Such a system is said to have a long resolving time (sometimes called 'dead' time), of 50 milliseconds (m.sec.) or more. The scintillation detector system commonly has a very short resolving time (5 m.sec. or less) but requires very stable, and therefore expensive, electronic circuits for reproducible results.

Because each radioactive species has a fixed rate of disintegration, the number of atoms which disintegrate in unit time is proportional to the total number of radioactive atoms present at that time.

Thus: \( \frac{dN}{N} = -\lambda dt \); integrating: \( \log_n \left( \frac{N_t}{N_0} \right) = -\lambda t \)

or in exponential form: \( N_t = N_0 e^{-\lambda t} \)

where \( N \) is the number of atoms
\( N_0 \) is the number of atoms at time 0
\( N_t \) is the number of atoms at time \( t \)
\( t \) is the elapsed time
\( \lambda \) is a constant (the decay constant)

specific to the isotope involved.

If the logarithm of the number of radioactive atoms present (or disintegrating, since the two are proportional) in a sample is
plotted against time, a straight line will result, the slope of the line being dependent on the decay constant, $\lambda$. If now, it is decided to calculate the time required, $T$, for the radioactivity to decay to half its initial value, (the 'half-life'), then from the above $N_t/N_0 = e^{-\lambda t}$, $\log 1/2 = -0.4343\lambda t$; or $\log 2 = 0.4343\lambda t$, and $T = \frac{0.693}{\lambda}$

This 'half-life' can be easily calculated from the decay constant, if the element is known, or conversely, can be calculated experimentally from a radioactive sample, and provide the decay constant by calculation, thus helping identify the element if the latter is unknown.

The half-life of a radioactive material can be very short, seconds or hours, or long, many thousands of years. Very short half-life materials, (1 day or less), are difficult to use experimentally because the time during which activity is present is so short, and experiments using them must of necessity be short. Determination of a disintegration rate is also difficult, since the activity of the sample is continually decreasing, and correction factors are usually required when comparing count rate determinations. Radioisotopes with long half-lives, weeks or even years, are much more convenient to use, since corrections factors are rarely necessary.

When a stable element is irradiated in a nuclear reactor, the activation taking place is, in some respects, similar to radioactive decay, but in reverse, and can be expressed in the following equation:

$$A(t) = K \sigma \text{ac. f. n.} \left(1 - e^{-\lambda t}\right) e^{-\lambda t}$$

where $A$ is the measured activity produced at time $t$.\footnote{\label{footnote}In this equation, $K$ is the number of atoms of the stable element, $\sigma$ is the cross-section for the reaction, $ac.$ is the abundance of the isotope, $f.$ is the fraction of the isotope, and $\lambda$ is the decay constant.}
\( \sigma_{ac} \) is the activation cross section for neutron capture by the material.

\( K \) is the efficiency of the counter used for measuring the induced activity.

\( f \) is the flux of activating neutrons.

\( n \) is the total number of target nuclei.

\( t \) is the time of exposure to the neutron flux.

\( \phi \) is the time increment between the end of the irradiation and the time at which the activity is determined.

\( e \) is the base of the natural logarithm.

It can be seen that the higher the flux, or the more material being irradiated, the more activity is produced. Also the higher the activation cross section (or likelihood that the target nucleus will capture a neutron), the more activity is produced, and the shorter the half-life of the radioactive species being produced the more rapidly will activation take place. But because radioactive decay will start the moment radioactivity is produced, the amount of activity it is possible to induce must be a balance between the rate of activation, and the rate of decay. When these two rates are in equilibrium, the material is said to have been irradiated 'to saturation', and the time necessary to achieve this state is short for short half-life materials, and long for long half-life materials. For this reason, the time of irradiation and the time of storage of a mixture of materials of different half-lives has a profound effect on the proportions of radioactivity present.
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The activation cross section is a measure of the numerical probability that a neutron will be captured by a target nucleus. It is measured in extremely small units called 'barns', and varies greatly, being as low as $10^{-8}$ barns for some elements and high as $10^{6}$ barns for others. The activation cross section of an element bears no relationship to the physical size of its nucleus.

The effects of irradiation on tooth enamel were investigated in the following experiments.

Samples of tooth enamel were sealed in silica vials, placed in aluminium irradiation cans, and inserted in HIFAR reactor in an area of uniform neutron flux, with a low thermal (but not necessarily nuclear) temperature ($70^\circ$C or less) for twelve hours. After removal, the sample exhibited both beta and gamma activity. The beta activity was filtered out using aluminium sheets, the gamma activity recorded, and the spectrum of energies analysed.

The principle gamma ray emissions from the samples were at 1.37 and 2.76 Mev., the spectrum peaks decreasing in height at a rate indicative of a 15 hour half life, indicating the presence of Na$^{24}$. This emission was so strong that it effectively masked other spectrum peaks. After a few days, when most of the Na$^{24}$ activity had disappeared, the spectrum showed peaks at 0.48, 0.88, 1.10 and 1.29

* One barn is $10^{-24}$ of a square centimetre.
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Mev., the peaks decreasing in height at rates indicative of half lives in the range 30–90 days. These peaks suggested that the majority of the gamma activity in the samples at this time was Sc$^{46}$ and Fe$^{59}$. The above-mentioned gamma emitters were found in human and bovine enamel, whether in powder or block form. When the tricalcium phosphate (Victor)$^R$ spectrum was analysed, Na$^{22}$ and Sc$^{46}$ activity only was found.**

Chemical analysis of tooth enamel (40) indicates the presence of the following: calcium, phosphorus, hydrogen, oxygen, small amounts of sodium, potassium, magnesium, zinc, chlorine, fluorine and traces of various heavy metals. Hydrogen and oxygen do not become radioactive with thermal neutrons, but many metals, particularly Na, Sc and Fe have high capture cross-sections, and these are responsible for the appreciable gamma activity found in irradiated tooth enamel.*

A week after irradiation, tooth enamel samples had lost nearly all gamma activity, but still possessed considerable beta activity. Irradiated samples of various forms of apatite mineral were counted at regular intervals for several months, and decay curves prepared, in an effort to identify the source of this beta activity (see fig.19).

* This effect is used in the technique of 'neutron activation analysis', to identify certain trace elements.

** Using the whole-body counter facility of the Australian Atomic Energy Commission, a 5" NaI crystal and a 256 channel analyser with automatic printer.
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The curves all indicated a half-life of 14–15 days, suggesting the presence of $P^{32}$. An irradiated bovine enamel sample was counted at regular intervals for a further five months, and another decay curve plotted (fig. 20). This curve indicated that irradiated bovine enamel contains two beta emitters, with half-lives of 15 days and 100–200 days * suggesting the presence of $P^{32}$ and $Ca^{45}$. The dotted portion of the decay curve represents the period when the more active component was rapidly disappearing. The data for this curve were obtained using a windowless counter, since the 100–200 day half-life component was thought to be $Ca^{45}$. This material emits particles of low energy, which are difficult to count using conventional G.M. or Scintillation detectors.

Although the half-life values obtained experimentally strongly suggested that irradiated tooth enamel contains $P^{32}$ and $Ca^{45}$, a more positive identification is achieved if the energy of the beta emission can be determined. Unlike gamma ray emitters, which have fixed gamma ray energies in a given radioactive species, beta particle emitters have beta particle energies ranging in value from low, up to a definite maximum, called the $E_{max}$. These energies can readily be demonstrated using a mass spectrograph, which reveals that most beta particles have energies about one third of the $E_{max}$ value. The beta particle $E_{max}$ can be estimated without resort to the mass spectrograph using the so-called 'Feather' analysis. *(235)*

* With samples of low activity, as in this case, half-life values cannot be determined exactly.
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With this technique, a series of count rates is obtained from a sample when increasingly thick absorbers (aluminium or lead) are placed between the sample and the detector. By plotting these counts against absorber thickness, and comparing the results with those obtained from a beta particle emitter of known energy, it is possible to determine the $E_{\text{max}}$ of the unknown beta emitter.

It is difficult to perform a 'Feather' analysis on mixtures of beta emitters, so a sample of tooth enamel was dissolved in HCl, oxalate added, and the resultant calcium oxalate precipitate recovered by filtration. The filtrate and precipitate were then subjected to 'Feather' analysis. The filtrate gave an $E_{\text{max}}$ value of 1.9 Mev., and the precipitate an $E_{\text{max}}$ value of 0.27 Mev. Positive identification of the two beta-emitting components of irradiated tooth enamel was now possible on the following basis:

Unknown I. Half life, predicted: 14.5 days; observed: 14–15 days (calculated 14.5 days)

\[ E_{\text{max}} \]

" 1.71 Mev; observed 1.9

Unknown II. Half life  " 160 days; observed 100–200 days (calculated 174 days)

\[ E_{\text{max}} \]

" 0.25 Mev; observed 0.27

and indicates P$^{32}$ as unknown I and Ca$^{45}$ as unknown II.

Knowing the end products of irradiation of tooth enamel, it is now possible to explain the sequence of events taking place during and after irradiation. There are ten calcium atoms to every six phosphorus atoms in tooth enamel. So on a numerical basis,
irradiation should produce more radioactive calcium than radioactive phosphorus. This is not the case. First, the capture cross section of calcium is half the capture cross section of phosphorus, and the phosphorus is twice as likely, therefore, to capture a neutron. Secondly, phosphorus occurs in nature as $^{31}_{P}$ in 100% abundance, and a naturally occurring sample of phosphorus contains no other forms of phosphorus, stable or radioactive. Naturally occurring calcium, on the other hand, is a mixture of $^{40}_{Ca}$, $^{42}_{Ca}$, $^{43}_{Ca}$, $^{44}_{Ca}$, $^{46}_{Ca}$ and $^{48}_{Ca}$, calcium $^{44}_{Ca}$ being only 2.1% of the total calcium. The nuclear transformations taking place in the reactor are shown in fig. 21. This figure shows how $^{31}_{P}$ becomes $^{32}_{P}$ in a neutron-gamma reaction, decaying by beta particle emission, with an $E_{max}$ of 1.71 Mev. and a 14.5 day half life to $^{32}_{S}$, while $^{44}_{Ca}$ becomes $^{45}_{Ca}$, decaying by beta particle emission with an $E_{max}$ of 0.25 Mev. and 160 day half life to $^{45}_{Sc}$. It is now apparent that if we disregard time of irradiation and the decay taking place during irradiation, the calcium activity would be about 1% of the phosphorus activity, because of its lower cross section, and much lower percent abundance.

But the half lives of the products of irradiation will also affect the activities produced. From a consideration of the neutron activation formula (see page vii), short half-life radioactive elements are produced more rapidly upon irradiation than are long half-life elements. This situation does not always obtain for very long irradiations, but is so for the twelve hour irradiation period.
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employed here. There will be an increase in radioactivity until
the rate of activation and rate of decay are in balance (a theoretical
situation which is never actually attained in practice). Practically,
saturation is said to be achieved, after irradiation for a period of
time amounting to about three half lives of the radioactive species
being produced. For short term irradiations, the activities
induced in a sample are related inversely to the half-lives of the
various elements made radioactive. When tooth enamel is irradiated,
$\text{Na}^{24}$ activity increases very rapidly, $\text{P}^{32}$ less so, and $\text{Ca}^{45}$ very much
less so. After twelve hours irradiation, $\text{Na}^{24}$ has reached saturation,
$\text{P}^{32}$ is not yet saturated, while the $\text{Ca}^{45}$ level of saturation is
very low. Six days after removal from the reactor, all $\text{Na}^{24}$ activity
has disappeared, while most of the induced $\text{P}^{32}$ are nearly all the
induced $\text{Ca}^{45}$ remains. Longer irradiation periods increase both $\text{P}^{32}$
and $\text{Ca}^{45}$ content, but cause extensive radiation damage in the
sample, and increase the expense of irradiation. Shorter periods
produce less activation, and decrease the period for which the
material remains usable for experimental work. A twelve hour
irradiation period was found to provide sufficient $\text{P}^{32}$ activity for
the procedures used in this work.

The daughter products of $\text{P}^{32}$ and $\text{Ca}^{45}$ decay are $\text{S}^{32}$ and $\text{Sc}^{45}$
respectively. These two decay products are stable, and will appear
in constantly increasing amounts as radioactive decay takes place,
though the amounts involved are minuscule, and virtually undetectable.
They will appear during irradiation, since decay will occur immediately the production of the radioactive species commences. The $S^{32}$ can capture neutrons in a neutron–proton reaction to become $P^{32}$ again, in the so-called 'yo-yo' reaction, while $Sc^{45}$ can undergo a further neutron–gamma reaction to produce $Sc^{46}$, which is both a beta and gamma emitter. The first reaction is of no practical significance, but the second, because of the high capture cross-section of $Sc^{45}$, could be detectable after irradiation. Interestingly enough, the gamma spectrum of irradiated tooth enamel shows the presence of $Sc^{46}$, which has probably been formed in this way from $Ca^{44}$.

The probability of neutron capture in an irradiation process is completely independent of the chemical form of the target nucleus and different types of apatite should exhibit similar patterns of induced radioactivity, provided their calcium and phosphorus contents are comparable. An examination of the four decay curves for four types of apatite, in fig. 19 reveals that the plots are four parallel straight lines. This indicates that the induced radioactivity is identical for each sample, and consists primarily of $P^{32}$. The graph also shows that over a two month period, the equivalent of approximately four half-lives, the count rate of a 0.1 mg. sample will decrease from about 250,000 counts/min. to about 10,000 counts/min. Activities of this order are quite sufficient for food fermentation experiments, and radioactive tooth enamel can be used experimentally
for at least two months after irradiation, without sacrificing too much sensitivity of detection.

Radioactive tooth enamel is used as a means of detecting enamel dissolution, by comparing the count rate of an experimental sample with that of a standard, containing a known amount of tooth enamel. The comparison will only be valid if the distribution of $^{32}$P in the outer parts of an enamel particle is the same as the distribution in the interior, the $^{32}$P is in the same chemical form as the $^{31}$P, and the chemical behaviour of $^{32}$P is the same as that of $^{31}$P. The chemical behaviour of an atom depends on the orbital electron configuration, which is identical for $^{31}$P and $^{32}$P. However the $^{32}$P nucleus is 1/31 (3.2%) heavier, so in a situation where this extra mass affects the movement of the atom, such as might be the case in a diffusion-controlled experiment, slight differences in rate of reaction could occur.

The uniform distribution of $^{32}$P throughout a particle can be easily demonstrated by counting various samples of the same irradiated tooth enamel from the same irradiation vial. The activity/weight ratio is invariably the same, thus showing that the labelling must be uniform on a particle basis. The labelling in individual particles will also be uniform. However, when apatite samples of three different mesh sizes were irradiated at the same flux for the same period of time, the resulting activity/weight ratio was not

* Experimentally verified, Dr. J. Robson, A.A.E.C., Lucas Heights Sydney.
identical for each, as the following figures show:

Pass 240 mesh bovine tooth enamel: $6.05 \times 10^4$ counts/100 secs/mg.
200–240 mesh " " " : $4.37 \times 10^4$ " " "
120–200 mesh " " " : $4.16 \times 10^4$ " " "

Under identical conditions of irradiation, the coarser powders acquired less activity, and when blocks of bovine enamel (4mm. x 4mm. x 1mm.) were irradiated as above, the activity was lower still. These results would appear to indicate that the activity produced in an irradiation is related to sample density, and as a consequence, the coarser particles cannot be uniformly labelled, owing to neutrons being absorbed by the outer layers of the coarser materials. But for neutrons to be absorbed in this way, the dimensions of the absorbing body must exceed the 'mean free path' of the neutrons in that body.* Even the bovine blocks are considerably smaller than the 'mean free path' of the neutrons, so neutron absorption cannot explain the phenomenon. The most likely explanation is that of 'flux depression', a property of neutrons as yet incompletely understood. It has been described as a property akin to surface tension forces in liquids, where the neutron flux can be considered as the liquid, and the target body as a non-wettable object immersed in the liquid. The result of 'flux

* 'Mean free path of a neutron is the average distance travelled by neutrons in a material before they are captured.
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depression is to produce more radioactivity in finely divided materials than in coarser materials. This explanation is supported by the activities resulting from irradiation of apatites of apatites of similar mesh size:

Unwashed bovine enamel 120-200 mesh 14164 counts/100 secs./mg.
Washed bovine enamel " 15484 " " "
Washed human enamel " 15718 " " 

An important aspect of the irradiation process is the damage to the crystal structure of apatite. The overall extent of radiation damage in apatites was assessed by submitting three irradiated apatite samples to x-ray diffraction analysis and comparing results with those of similar non-irradiated samples, as shown in fig.22. There is no appreciable displacement of the characteristic apatite peaks as a result of the irradiation, and this indicates that any change in crystal structure that may have occurred involves less than ten percent of the total sample.*

But it is obvious that there must be some damage following irradiation, as irradiated apatite is less soluble than non-irradiated apatite. There are two possible types of damage, the general type, caused by the unavoidable exposure of the samples to the ionising radiations present in all nuclear reactors, and the specific damage caused by the local effects of neutron capture.

* Diffraction analysis by Dr. N. Stevenson, Crystallographer, University of N.S.W., Sydney.
Emanations of low energy, such as beta particles and x-rays, will produce temporary ionisation, but no permanent damage, although high energy radiations, such as alpha particles, gamma rays and fast neutrons are capable of causing damage which is more or less permanent, depending on the nature of the substance being irradiated, and its subsequent treatment. In molecular compounds, any changes observed are largely because of chemical rearrangement by ionisation. In ionic compounds too, the defects arise from ionisation processes, but here the differences must be thought of as physical rather than chemical. When an atom is displaced by direct collision with the radiation, the reaction is referred to as a 'primary knock-on', and this is the predominant reaction with beta radiation. Theoretical and experimental work has shown that comparatively little energy is required to produce these effects, about 25 electron volts (e.v.), and even with quite low radiation levels, many primary knock-ons can be expected. But the displaced atoms, especially in crystalline materials, cannot move more than a few inter-atomic distances before coming to rest. Where the energy transfer has been particularly intense, there may be many defects concentrated in a small volume, and when this is the case, the nature of the defective area can be best regarded as having rapidly melted and solidified (see page xx). When irradiation takes place at room temperature, some recovery from the damage takes place immediately after the irradiation ceases, and is usually a recombination of closely
approximating atoms, but not necessarily in the same way as they were combined before irradiation. Partial to complete recovery may take several hours, or may never be achieved, unless the temperature of the material is raised.

A characteristic radiation damage effect in crystalline non-conductors is an expansion of the crystal lattice. In many materials there is a saturation point reached where about 1% of the crystal lattice is defective, regardless of the intensity of the irradiation. In a few materials, the end result of severe irradiation is a glassy, amorphous condition, called the 'metamict' state. This may be accompanied by quite dramatic changes in the chemical behaviour of the material.

As a result of the presence of ionising radiations in a reactor, a specimen of tooth enamel can be expected to change its crystalline and chemical characteristics following irradiation. There will be crystal defects caused by primary knock-ons, as well as the more extensive bunches of defects (local melting), caused by the higher energy transfers from fast and thermal neutrons, and high energy gamma rays. These defects will be more or less random in the type of atoms affected. There is no discrimination, and this type of damage will occur whether the target material is rendered radioactive or not.

When neutron capture takes place another type of damage may occur. As an example, during irradiation of apatite, $^{32}\text{P}$ is formed when a $^{31}\text{P}$ nucleus captures a neutron. Excess energy of
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this nucleus as a result of the capture (about 8 Mev.) is given off as gamma radiation (the 'capture' gamma). One, or sometimes more gamma rays are emitted by the nucleus, and depending on the direction and energy of these gamma rays, there will be a recoil of the nucleus. The energy of this recoil is generally about 1000 ev. It has already been stated that defects can be produced by about 25 ev., so this recoil energy is more than enough to displace a P\textsuperscript{32} atom from its crystal lattice, and unlike the general damage mentioned above, which is random, this capture recoil energy accompanies every P\textsuperscript{31} neutron capture. After the recoil, the P\textsuperscript{32} atom may return to its previous position, particularly if it has shared most of its energy with the atoms surrounding it. On the other hand, it may find itself trapped in some vacant space in the lattice where it would not normally be found, thus distorting the crystal. The position eventually adopted by the P\textsuperscript{32} will depend on the number, direction and energy of the emitted capture gammas, and will be entirely random spatially in relation to the crystal lattice, if the atom fails to return to its original location. This is so because the direction of approach of a thermal neutron captured by a nucleus, and the direction away from the nucleus taken by the capture gamma or gammas is completely independent of the environment.

The subsequent behaviour of the P\textsuperscript{32} atom will depend on the temperature, and the position of the atom in relation to the crystal
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lattice. Libby\(^{189}\) has suggested that in phosphates, about half the recoils result in the loss of an uncharged oxygen atom, leaving the \(P^{32}\) atom with an oxidation number of three. This change would be difficult to detect using conventional chemical analysis, since the ratio of stable to radioactive phosphorus atoms in irradiated apatite is about \(10^9\) to 1. It can be achieved relatively easily, however, using isotope dilution techniques. MacKenzie and Borland\(^{204}\), in an investigation of various forms of orthophosphate, found that about one third of the \(P^{32}\) is in a form other than orthophosphate, which agrees with Libby's theoretical calculations. These results have been duplicated by others.\(^{85,191,249}\)

But even if the \(P^{32}\) dislocations occurred with every neutron capture, the extent to which they occur in comparison with the number of stable \(P^{31}\) atoms could not disturb the overall crystal lattice of irradiated apatites. Since the solubility in acetate buffer of irradiated apatite as determined by chemical means is less than non-irradiated apatite, this difference must be as a result of the randomly occurring structure and chemical changes which occur unavoidably as a result of beta, gamma and fast neutron irradiation, and not from recoil effects.

However, the radiochemical method for determining irradiated enamel dissolution yields values still lower than those given by the chemical methods, and it is evident that this further apparent depression in irradiated enamel solubility is related to the
behaviour of $P^{32}$ in acetate buffers. A mechanism for this effect was suggested in Appendix B, and the fact that the $P^{32}$ in irradiated apatites is commonly in a form other than orthophosphates tends to support the increased rate of redeposition with $P^{32}$ suggested as a probably explanation for the effect.

The exact chemical form of the $P^{32}$ in irradiated phosphates has been the subject of considerable investigation, with authorities differing in the results obtained and the conclusions reached.\(^{(191)}\) Irradiation of apatites and tricalcium phosphates followed by chemical determination of the amount of $P^{32}$ present in forms other than orthophosphate has yielded values of from 30-50%. The chemical forms of these non-orthophosphate fractions have been identified as hypophosphate, metaphosphate, pyrophosphate and others,\(^{(85)}\) while other investigators have irradiated sodium orthophosphate to produce $P^{32}$ as orthophosphate to the extent of 90% of the total $P^{32}$\(^{(249)}\).

These variable results are most probably a reflection of the differing conditions in different nuclear reactors, (fast and thermal neutron flux, gamma activity, type of moderator, etc.) and slightly different conditions of temperature and physical form of the sample during and after irradiation. It is also quite possible that the different parts of the same reactor will produce irradiated samples with differing properties. The author has found such
differences in irradiated apatites with different reactors, even though labelling was uniform in each case. Dissolution results obtained from enamel samples irradiated in different reactors may not, therefore, be comparable quantitatively. In this work, all enamel samples were irradiated in the same part of the reactor, over the same time period, for uniformity.

Recent work on the chemical behaviour of $^{32}$P in neutron-irradiated rock phosphates indicates that the non-orthophosphate content can be greatly reduced if the material is heat treated after irradiation. Mackenzie and Borland ($^{204}$) found that after testing a variety of treatments, heating the sample for 91 hours, dry, at 500°C at atmospheric pressure reversed the effects of irradiation on the $^{32}$P. The $^{32}$P non-orthophosphate content after this treatment was only 1.6%, compared with 27.5% immediately after irradiation. In addition, they found that while some petrographic damage was caused as a result of some of experimental treatments they tested, the above technique caused no such damage.

Sellars et al. ($^{249}$) have investigated the same problem, with similar results, and express the opinion that 'the gamma-induced reactions and the neutron induced reactions have similar but not necessarily identical mechanisms.' It can be surmised that heat

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* The Oak Ridge Reactor, ORR; Nuclear Research Center Reactor, Buffalo; and HIFAR reactor, Lucas Heights.
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treatment to reduce the non-orthophosphate contamination of $P^{32}$ after irradiation may achieve a similar result for any samples containing $P^{31}$ in non-orthophosphate form as well.