APPENDIX C

II: RADIOCHEMICAL DETERMINATIONS

PRACTICAL ASPECTS OF RADIOCHEMICAL DETERMINATIONS

A radiochemical technique was used in this work because it enabled the investigator to study the rate at which powdered tooth enamel dissolved in a complex mixture, without recourse to chemical separations, and because quite small changes in dissolution rate could be detected using very small experimental sample volumes. Tooth enamel powder was irradiated with neutrons, providing a radioactive label, uniformly distributed throughout the particles. When this labelled powder was added to a food-saliva mixture, any dissolution of the particles which took place was accompanied by the appearance of radioactivity in the liquid phase of the mixture. The enamel dissolution rate could then be calculated by determining the amount of radioactivity in successive small samples, and comparing these values with the amount of radioactivity present in a known amount of tooth enamel. It is fortuitous that the principal radioactive constituent produced on neutron bombardment of tooth enamel is P^{32}, a radioactive element which in several respects is ideal for use in food fermentation experiments.

In order to compare radioactivity measurements, it is essential that the standard and experimental sample count-rates be obtained under identical conditions. With radioactive elements of short half-life (one day or less), count rate determinations made at different times can not be compared directly, because some decay will take place during the time interval between determinations. This time interval between determinations can be considerable for

RADIOCHEMICAL DETERMINATIONS

materials with a longer half-life, (two weeks or more), and still permit direct comparison of count-rates, but must be short for short half-life materials, unless corrections for decay are employed. Thus the count-rates obtained with two samples of identical activity and short half-life(one day or less) will differ, since the second sample will have a lower activity by the time the first has been counted, and will then give a lower count-rate. This lower count-rate might be interpreted as indicating that the second sample is less active that the first, which is not the case, but appears so because of radioactive decay. If both samples were counted simultaneously, the count-rates would be identical.

In the food tests, count-rate determinations were not made as each sample was obtained, but were performed at the end of the experiment, samples being counted in a group, to reduce to a minimum the time intervals between determinations. This procedure greatly reduced the effects of decay on the count-rate determinations. To ascertain whether the decay taking place over the one hour period required to count all the samples in a group necessitated any corrections, the radioactive decay of tooth enamel was investigated.

Each radioactive element has a specific rate of decay, and the count-rate of a given sample at any time can be determined from an experimentally derived decay curve (Appendix C, fig.19)or by calculation, using the following formula:

RADIOCHEMICAL DETERMINATIONS

$N_t = N_o\ e^{-\lambda t}$ where N_t is the count rate at time t

N_o is the count rate at time zero

λ is the decay constant of the element

t is the time

e is the base of the natural logarithm

This formula can also be expressed as:

$N_t / N_o = \frac{1}{2}^n$, or $N_t = N_o\ 0.5^n$

where n is the number of half lives elapsed in time t, (i.e., t/half life, (T/2)

For P^{32}, this formula indicates that a sample with a count-rate of N at time zero will count 0.9527 N after 24 hours, 0.9880 after 6 hours and 0.9970 at 1 hour. Thus when a series of samples are counted within one hour, the last sample counted will indicate a count-rate which is lower by 0.3% than it would have been if counted first. This difference was considered to be insignificant. In those instances where samples for an experiment had to be counted at time intervals greater than one hour, the appropriate corrections were employed.

In this work, the actual count-rates determined were not absolute in the sense that all disintegrations taking place were detected, as the detection systems used were not 100% efficient. Of the two detection systems utilised for enamel dissolution experiments, the scintillation crystal system was found by experiment to be about 20% efficient, and the Geiger Müller system to be less

than 10% efficient. (Detection systems of 100% efficiency are not usually employed in routine radiochemical work).

The efficiency of a system depends on the type of detector, the type and thickness of the detector casing, or the 'window' through which the radiation must pass, the path taken by the radiation, the materials surrounding the detector, and the position or 'geometry' of the radioactive sample relative to the detector. The actual value of the detector efficiency was not critical in the radiochemical calculations used in this work, since absolute disintegration rates were not required. It was necessary, however, to ensure that the standard and experimental samples were counted with identical efficiency, to allow comparisons to be made, and this was the procedure adopted for all count-rate determinations.

An important consideration in count-rate determinations is the 'resolving time' of the detection system used. A system with a long resolving time may influence the observed count-rate by failing to count some of the radiations. Enamel dissolution samples gave count-rates varying from background only (about 200 counts/100 secs.) up to a maximum sample count-rate of about 100,000 counts per 100 secs. With low count-rates, both detection systems used were capable of counting nearly all radiations entering the detector. With high count-rates, a system with a short resolving time will count radiations more efficiently than a systen with a long resolving time. Any corrections which may be necessary to allow

for such resolving time effects are given by the following formula:

$$R = \frac{R_o}{1-R_o\ t}$$

where R is the true count-rate (per sec.)

R_o is the observed count-rate (per sec.)

t is the resolving time (in seconds), for the detection system used.

It can be seen that the higher the resolving time and the higher the count-rate, the lower will be the observed count-rate in comparison with the true count-rate. In the Geiger Müller detector system used in this work, resolving time was of the order of 100 μsec., but with the scintillation detector system, it was less than 5 μsec.

If 100,000 particles enter the detector in 100 seconds, the resolving time effect is such that with the G.M. detector, only 90% of these particles will be counted, while with the scintillation detector system, 99.5% will be counted. For this reason the scintillation detector was used in all cases where active samples were being counted, to avoid the necessity for resolving time corrections.

The background radiation in the laboratory varied according extraterrestrial conditions and to the amount of radioactive material held in stock in the laboratory, and ranged from 30 to 300 counts/100 sec. In some radiochemical work this would be considered high and would have to be reduced by shielding the detector. In this work the level of background radiation was not a problem, since the sample activity was so high in comparison. Nonetheless, background

was determined before the samples were counted, and subtracted from the count-rates obtained.

Corrections for backscatter were not necessary in this work, since all samples were counted on planchets of the same material, in this instance, stainless steel. Allowance for this effect would have to be made if, for example, aluminium planchets were used for some samples and stainless steel planchets for others, as it was found by experiment that samples counted 10% higher on stainless steel planchets.

Some of the food-saliva mixtures yielded counting samples containing considerable amounts of dissolved food and tooth enamel, which remained on the planchet after the counting sample was dried. There was a possibility that this material could absorb some of the radiation which would otherwise reach the detector, in a process called 'self-absorbtion', and thus produce counting errors. In this work, self-absorbtion effects were evaluated experimentally by depositing successive 100 µl. samples of a food-saliva mixture onto a planchet, and determining a count-rate after each addition. It was found that the count-rate obtained after each successive addition increased in proportion to the amount added, until more than ten such additions had been made. Thereafter, successive additions were not accompanied by a proportional increase in count-rate. This point marked the onset of self-absorbtion effects. If self-absorbtion effects were pronounced in some foods and not in others, valid count-

rate comparisons would not be possible without appropriate corrections. Self-absorbtion effects were not detected with any of the food-saliva mixtures, even with a tenfold increase in sample size. This would not necessarily be the case with beta emitters of low energy, such as Ca^{45}, which are very susceptible to self-absorbtion effects. Because of its very high beta particle energy, 1.71 Mev., P^{32} was not susceptible to self-absorbtion effects in this study, and corrections were not required.

The accuracy of all determinations of enamel dissolution rests in the first instance on the accuracy of the micropipettes used to measure the 100 µl. samples, in this instance guaranteed by the manufacturer to have less than 1% error. However, the accuracy of each determination also depends on the accuracy of the count-rate obtained. Because each disintegration is a random occurrence, the values of successive separate determinations of a decay rate often differ slightly, the range of values for such a series of determinations having a Gaussian distribution. The standard deviation for an individual count-rate can be derived statistically. The theoretical standard deviation is given by the formula:

$$\sigma = \sqrt{rt} \doteq \sqrt{n}$$

where σ is the standard deviation

r is the average number of counts per unit time interval

t is the counting time for one observation

n is the number of counts for one observation

RADIOCHEMICAL DETERMINATIONS

Typical count-rates for food fermentation experiments were in the range 1,000 to 50,000 counts per 100 secs., for small and large amounts of dissolution respectively. Each determination was made three times, to check the resetting of the counter decades. Improper resetting was indicated when one count-rate was appreciably higher than the other two, and when this occurred, a fourth count-rate determination was made. Typical variations from the mean for three count-rate determinations were $\pm$ 40 counts for low activity samples, and $\pm$ 200 counts for high activity samples. These results agree well with the theoretical values for one standard deviation with counts of 1,000 and 50,000, which are $\pm$ 32 counts and $\pm$ 223 counts respectively. Expressed another way, if we apply the formula $\sigma = \sqrt{n}$ to a sample count-rate of 1,000 counts/unit time, the true count-rate will lie in the range 1,000 $\pm$ 32 (error 3.2%) in 68 of 100 determinations. At a higher level of confidence, say, two standard deviations, the true count-rate will lie in the range 1,000 $\pm$ 64 (error 6.4%) in 95 out of 100 determinations. The corresponding ranges for a sample giving a count-rate of 50,000 counts / unit time are $\pm$ 223 (error 0.04%) at the P.10 level, and $\pm$ 446 (error 0.08%) at the P.05 level.

These results illustrate the greatly increased accuracy of 100 second P^{32} count-rate determinations with high activity samples. Since in the food experiments low count-rates were obtained only when little enamel dissolution had taken place, the lack of accuracy with low count-rates was of little practical significance

in evaluating the results. However, with P^{32}, after two months or so, when considerable radioactive decay has taken place, even a high enamel dissolution would result in a low count-rate, and such low activity enamel was discarded, even though some compensation could be achieved by counting samples for a longer period. This procedure was not used because of the increased time involved, and the unfavourable sample count/background count ratio. The above discussion should not be taken to imply that for accurate count-rate determinations, a sample with a high count-rate must be obtained. The standard deviation calculations are based on the number of counts recorded, not the count-rate, and a large number of counts can be recorded from a low activity sample counted for a long time equally as well as a high activity sample, counted for a short time, provided background and decay effects are not significant.

For all but the very low enamel dissolution figures, the P^{32} count-rate determinations in food fermentation experiments have an accuracy better than 1% at the 95% confidence level, and equal or exceed the accuracy of the pipettes used. Counter error and statistical reliability corrections were not therefore employed, as errors arising from these sources were much less than 1%.

It is evident from the above discussion that the higher the level of radioactivity used in a food experiment, the more accurate will be the results, and the greater will be the sensitivity of

detection for small amounts of enamel dissolution. But large amounts of radioactive material pose a health hazard, require more stringent precautions in use, and are more difficult to store and to dispose of at the conclusion of an experiment. The enamel used in this work had been irradiated to produce an activity which was sufficiently high to permit small amounts of enamel dissolution to be detected, but not so high that storage and use posed problems. The amount of radioactivity present in the tooth enamel was calculated experimentally from its disintegration rate. Two weeks after irradiation, a 1.0 mg. sample had a count-rate of 200,000 per 100 secs., the detector efficiency being about 20%. This means that the number of disintegrations actually taking place in the sample is about 10,000 per sec. at this time, and indicates an activity of 2.7×10^{-7} curies.* One gram of this tooth enamel therefore contains 0.27 millicuries. Several grams of this enamel could be held in stock and used routinely in a Class B isotope laboratory without special precautions.

A 0.27 millicurie sample consisting of pure P^{32}, with no other phosphorus species present (called 'carrier free' P^{32}), is a very small amount of material indeed (about 10^{-9} of a gram). Therefore, in the irradiated tooth enamel sample, the ratio of radio-

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* One curie is the activity present when disintegrations occur at the rate of 3.7×10^{10} per second.

RADIOCHEMICAL DETERMINATIONS

active P^{32} atoms to stable P^{31} atoms is about 1 to 10^9, and the P^{32} atoms are very much in the minority in comparison with the P^{31} atoms. This fact is of considerable significance in the calculation of enamel dissolution by radiochemical determination, when determined by the appearance of P^{32} in the liquid phase of food-saliva mixtures, and is discussed in Appendix B.

APPENDIX D

CALCIUM SUCROSE PHOSPHATE

CALCIUM SUCROSE PHOSPHATE

In Section I of this work it was suggested that a measure of caries control in cariogenic diets might be achieved by the addition, to cariogenic foods, of substances which reduce enamel dissolution. One of the aims of this study was to develop an in-vitro technique for assessing the ability of additives to reduce enamel dissolution. The results of the food fermentation tests indicated that some food-saliva mixtures did not dissolve enamel to the extent expected from a consideration of their basic composition. No attempt was made to isolate and test the substances responsible for the effect.

The presence in solution of calcium and phosphate is known to influence the dissolution rate of tooth enamel in acid buffers,(104,105) and two classes of food tested contain calcium and phosphate in appreciable amounts. In the 'sugars' group, molasses has an ash content of 10%, and this ash contains about 10% - 15% calcium and 2% - 5% phosphorus. In the 'beverages' group, standard whole milk and chocolate milk contain about 0.15% calcium and 0.25% phosphorus, and ice cream mix contains 10% milk solids.

After hour 4 in a food test, enamel dissolution with unrefined sugars is lower than for refined sugars, the extent of the effect being related to the degree of refinement. As can be seen from an examination of Table XIII, each step in the refining process removes more of the calcium (and phosphorus) content of the sugar. The low enamel dissolution in unrefined sugars can thus

be related to the high calcium and phosphorus content. Enamel dissolution was very low throughout the food tests for the three milk-based foods, that for ice cream being the lowest of all foods tested. It is possible that part of this effect could also be attributed to a high calcium and phosphorus content. These food test results support the suggestion that additives capable of releasing calcium and phosphorus in the mouth would merit testing for caries-reducing effects in diets.(147,233)

One such additive is calcium sucrose phosphate. This substance has been employed experimentally as a food additive, and the results of the first year of a clinical trial have been reported.(129) Specially prepared calcium sucrose phosphate* was added to the sugar, flour, sweetened spreads, tinned fruit, bread and biscuits used by the children on the test diet. After one year, the average reduction in dental caries was in the range 1 - 2 surfaces per child, for age groups 5 - 8, 9 - 12 and 13 - 17 years, in comparison with children in the control groups. A similar, but less pronounced result was obtained at the end of the second year of the trial, suggesting that calcium sucrose phosphate reduces caries when incorporated in human diets.(130)

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* Colonial Sugar Refining Co. Ltd., Sydney, Australia.

CALCIUM SUCROSE PHOSPHATE

Because calcium sucrose phosphate contains calcium and phosphorus, it was decided to duplicate the fermentation test for sucrose, but with calcium sucrose phosphate added, (at the concentration used in the clinical trial), to assess its effect on enamel dissolution, pH and titratable acidity. Samples of the clinically tested material were not available, so a sample of calcium sucrose phosphate was prepared.* The product was a readily soluble white powder with an astringent taste. Sucrose containing 1% of this material was tested in a fermentation experiment, using the techniques as described in Section IV. The experiment was performed three times.

The results, together with those for sucrose alone, and molasses alone, are shown in Table XI. It is evident that pH and titratable acidity changes with these three foods are similar, but that enamel dissolution is high for sucrose, low for molasses, and very low for sucrose containing 1% calcium sucrose phosphate. In comparison with the sucrose-calcium sucrose phosphate mixture, enamel dissolution for molasses is twice as high at hour 2 and four times as high at hour 24, and for sucrose, twice as high at hour 2 and fifteen times as high at hour 24.

In this experiment, calcium sucrose phosphate, added at a concentration of 1% by weight,greatly reduced the enamel

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* Using the techniques described in Australian patent No. 259269, 1962.

dissolution of a fermenting sucrose-saliva-enamel mixture, without affecting the pH or titratable acidity changes. Therefore, conclusions based on results from the in-vitro testing of other additives which may reduce dental caries in a manner similar to calcium sucrose phosphate may not be valid if pH and titratable acidity only are recorded. Enamel dissolution must also be determined, since the in-vitro effect of calcium sucrose phosphate is to reduce enamel dissolution without affecting pH and titratable acidity. It follows that additives which possess the calcium sucrose phosphate property of reducing enamel dissolution in a food fermentation test may also demonstrate a clinical reduction in dental caries.

APPENDIX E

COMPOSITION OF FOODS

COMPOSITION OF FOODS

Identical foods are often known under different names, and in different countries the food name may not indicate its composition. Tables XII and XIII list the common name, manufacturer or brand name and description of each food tested, together with the approximate composition or technical description where it is available and pertinent.

Many of the 'sugars' group of foods represent different levels of refinement of sugar cane juice. The refining process lowers the water, organic and ash content, as well as removing the non-sucrose carbohydrate components, by degrees, at each level of refinement. Thus molasses is the treated residue of cane juice, after the crude sugar has been crystallised out. It contains about 30% sucrose, 15% reducing sugars, 10% ash, 20% water, and the remainder a complex mixture, the exact composition of which is unknown. Molasses also contains various forms of calcium sucrose phosphate*. Molasses ash content is 10% of the wet weight, and contains 10-15% calcium and 1-5% phosphorus.

Treacle and Golden Syrup^{R} are made from two different grades of mother liquor remaining after crystallisation of sucrose from a crude sugar solution. The mother liquor is then refined and

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* Personal Communication, Research Chemist, Colonial Sugar Refining Co. Ltd., Sydney, Australia.

evaporated. Treacle has an ash content of 4.5% and Golden Syrup[R] 3%, on a wet-weight basis, and both contain calcium and phosphorus. The amount of calcium and phosphorus present is proportionally less than that of molasses on an ash basis, since a step in the treacle and Golden Syrup [R] refining process employs 'bone char', which absorbs some of the calcium and phosphorus.

Brown sugar is the unwashed, crystallised sucrose obtained from an inferior quality mother liquor, while sucrose (domestic white sugar crystals) is crystallised from good quality mother liquor, and is washed. Brown sugar has an ash content of about 1%, and a calcium content of .0001% in contrasts to sucrose, with an ash content of less than .01% and a calcium content of .000015 - .000040%.

Glucose syrup is the result of 'converting' a highly refined slurry of starch granules, and the exact composition depends on the conditions of the 'conversion', and whether acids or enzymes are used. A general purpose glucose syrup (medium conversion syrup, M.C.Y.[*]) has a dextrose equivalent[**] of about 40 (and contains about 18% dextrose, 13% maltrose, 12% trisaccharides and 57% higher saccharides. A medium conversion syrup was the

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* Wheat Industries (Aust) Pty.Ltd., Sydney, Australia.

** Dextrose Equivalent: a measure of the reducing sugar content of a sweetener, calculated as dextrose, and expressed as percent of the total dry substance.

COMPOSITION OF FOODS

food tested in this study. Glucose syrup is known in the U.S. as corn syrup. The raw material for syrup production can be any form of starch granule, wheat starch being most frequently used in Australia. The name 'cornstarch' is defined in Australian food regulations as starch made from either corn or wheat.

In identifying the various flours tested, an attempt was made to follow conventional flour-mill terminology. This is generally based on the food regulations of the country, and is often confusing. The terminology in this work is defined as follows: A 'meal', or 'whole meal', is the product of the milling or crushing of whole grains, with nothing removed. A starch is the ultimate end-product of refining, where all of the milled grain has been removed save the starch granule itself. A 'flour' is a partly refined meal, and contains the starch granules of the grain, plus other constituents, such as proteins, fibre, bran and germ, in various concentrations.

Thus whole wheat meal consists of about 12% protein, 20% sugars, 1.5% ash, 2% fat, 12% water , the remainder being starch granules, fibre and germ. Most of the fibre (bran) and germ are removed in the manufacture of 'white' flour, and the final product consists of about 12% protein, 2% sugars, 0.5% ash, 0.5% fat and 12% water, the remainder being starch granules. The germ removed during the process consists of about 24% protein, 8% fat, 4% fibre, the remainder being carbohydrates and water.

COMPOSITION OF FOODS

In Australia a whole wheat meal is generally called wholemeal flour. The white flour commonly used in bread and biscuit manufacture represents about 70% of the original whole grain, 30% being removed during refining as fibre (bran) and germ. Such flour is called a 70% extraction flour. If in the particular refining process used, less bran and germ are removed, the resulting flour will have a higher extraction rate, 75% or more.

ILLUSTRATIONS

ILLUSTRATIONS

FIGURES, COLOUR PLATES

AND TABLES

FIGURES

COLOUR PLATES

TABLES

FIGURE 1

DETERMINATION OF THE EFFECT OF PARTICLE SIZE ON FILTRATIONS, AND AMOUNT OF APATITE ADDED ON DEGREE OF DISSOLUTION IN WATER AT pH 7.0.

Tricalcium phosphate (Victor)^{R} was irradiated, 200, 100, 50 and 25 mg. samples weighed out, suspended in 10 ml. of distilled water at pH 7.0 and shaken for four minutes. Samples were then taken by glass frit, and dissolution calculated (cross-hatching). The samples were re-suspended, centrifuged for 10 minutes, samples taken by micropipette direct from the supernatant, and dissolution calculated (vertical shading). The samples were re-suspended, centrifuged for 10 minutes, samples taken by glass frit and micropipette, and dissolution calculated (diagonal shading).

Dissolution of tricalcium phosphate (Victor)^{R} took place at pH 7.0, and the glass frit sampling technique did exclude from the sample fine particles not centrifuged down. The extensive dissolution demonstrated here may be apparent, rather than real, as the tricalcium phosphate was very fine in texture, and may have contained particles smaller than the 4 u pores of the glass frit. The dissolution observed was directly related to the amount of apatite added, demonstrating a relationship between enamel dissolution and the surface area of apatite exposed to the solution.

SOLUBILITY OF VICTOR TRICALCIUM PHOSPHATE IN WATER AT VARIOUS POWDER WATER RATIOS

10ml WATER (DIST.) AT pH 7·0 (GLASS FRIT PORE SIZE 4μ)

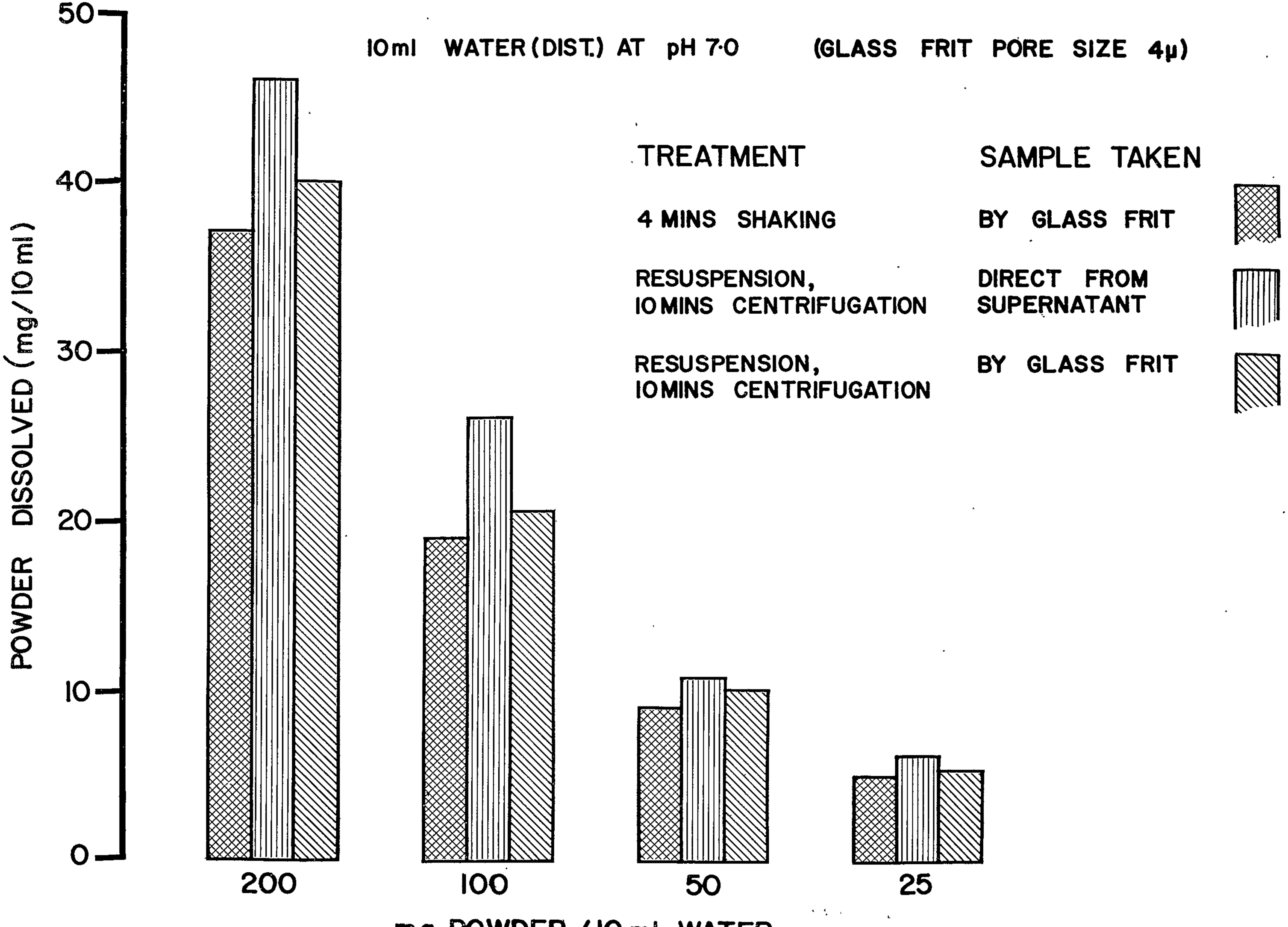

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FIGURE 2

DETERMINATION OF THE EFFECT OF AMOUNT OF UNWASHED BOVINE ENAMEL (200-240 and 120-200 MESH SIZE) ADDED ON DEGREE OF DISSOLUTION IN WATER AT pH 7.0.

Unwashed bovine enamel (200-240 and 120-200 mesh size) was irradiated, and 200, 100, 50 and 25 mg. samples weighed out, suspended in 10 ml. of distilled water at pH 7.0. and shaken for 4 minutes. Samples were taken by glass frit and micropipette and dissolution calculated (cross hatching). The samples were re-suspended, centrifuged for 5 minutes, samples taken direct from the superatant liquid and dissolution calculated (vertical shading).

Dissolution of bovine enamel in water at pH 7.0. was slight, and the glass frit sampling technique did exclude from the samples fine particles not centrifuged down. There were more such fine particles in the 200-240 mesh powder than in the 120-200 mesh powder. With both powders, the dissolution observed bore a direct relationship to the amount of powder added.

SOLUBILITY OF UNWASHED BOVINE ENAMEL IN WATER AT VARIOUS POWDER WATER RATIOS

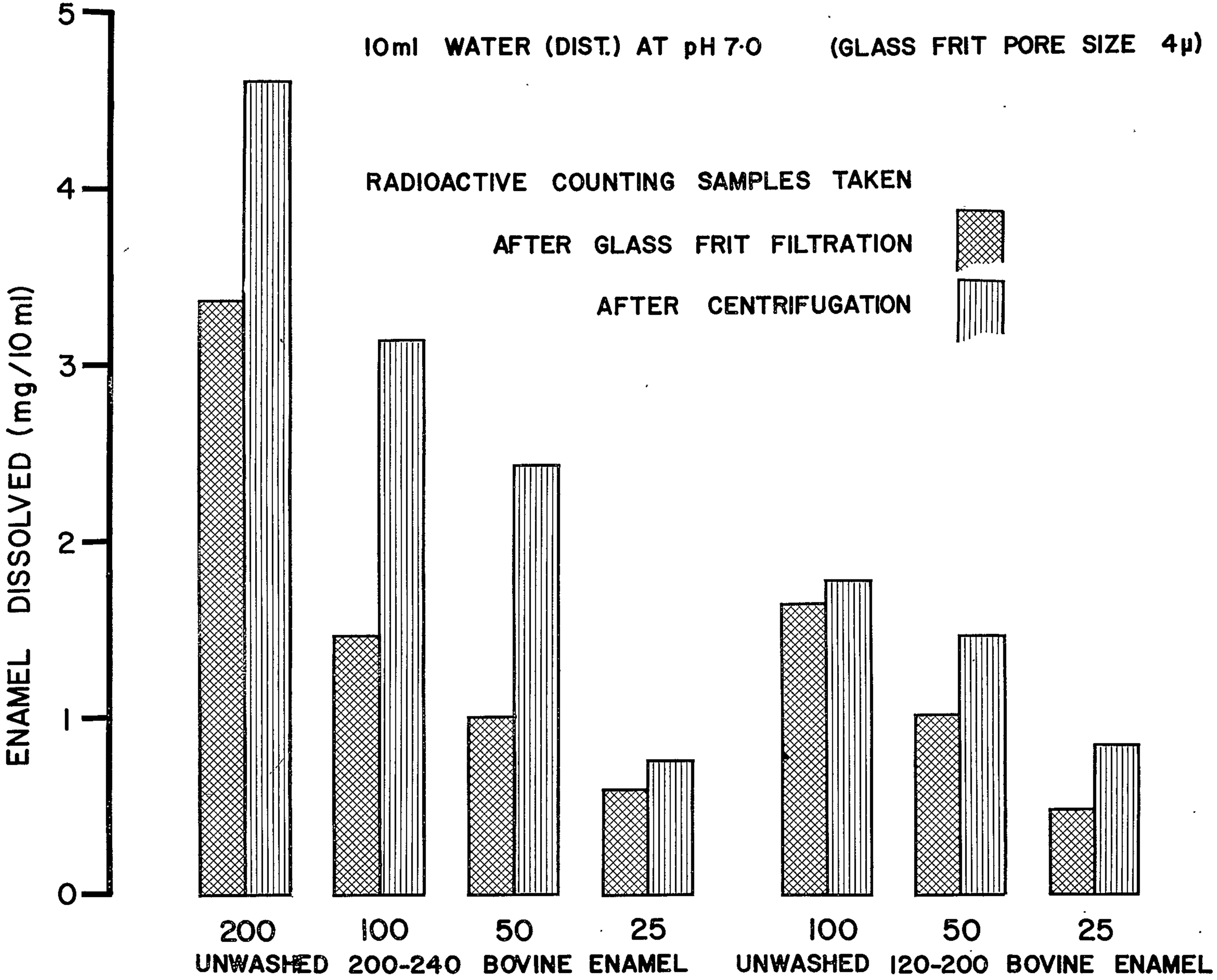

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FIGURE 3

COMPARISON OF DISSOLUTION RATES OF FOUR DIFFERENT APATITE SAMPLES IN 0.1M ACETATE BUFFER AT pH 4.5.
(USING RADIOCHEMICAL METHOD DATA FROM FIG. 15.)

Samples of tricalcium phosphate (Victor)^{R}, unwashed 120-200 mesh bovine enamel, washed 120-200 mesh bovine enamel and washed 120-200 mesh human enamel were irradiated, 50 mg. samples of each weighed out, added to 25 ml. of 0.1M acetate buffer at pH 4.5 and shaken at room temperature for 1½ hours and allowed to stand for 3 days. Samples were taken at intervals by glass frit and micropipette, dissolution calculated (by the radiochemical method) and plotted against time.

The tricalcium phosphate and unwashed 120-200 mesh bovine enamel dissolved more rapidly and to a greater extent than the washed 120-200 mesh bovine and human enamels. The latter two exhibited similar dissolution behaviour throughout the experiment.

COMPARISON OF DISSOLUTION RATES OF FOUR APATITE SAMPLES

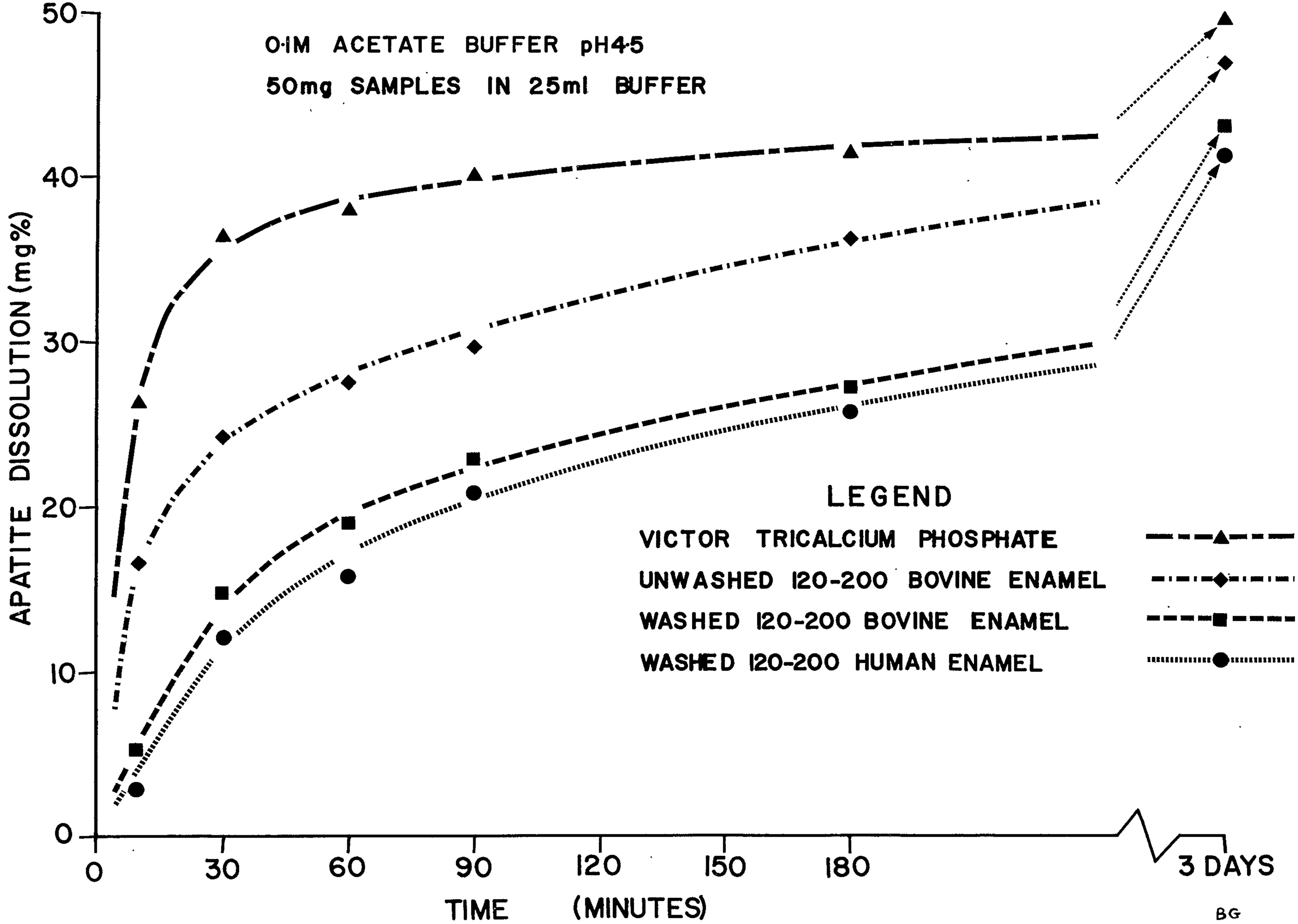

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FIGURE 4

DETERMINATION OF THE SOLUBILITY OF WASHED, IRRADIATED 120-200 MESH BOVINE ENAMEL IN SALIVA.

A mixture of 50 ml. of saliva and 100 mg. of washed, irradiated 120-200 mesh bovine enamel powder was stirred at a constant rate at 37°C for 24 hours. The pH and titratable alkalinity changes were recorded, and the enamel dissolution changes calculated at intervals. These changes were plotted against time. The experiment was performed twice.

The pH and titratable alkalinity changed little throughout the experimental period. No acid production took place. The slight enamel dissolution observed (2-5 mg.) indicates that a food-saliva mixture which does not produce acid could be expected to exhibit a very low dissolution.

SOLUBILITY OF WASHED 120-200 BOVINE ENAMEL IN SALIVA

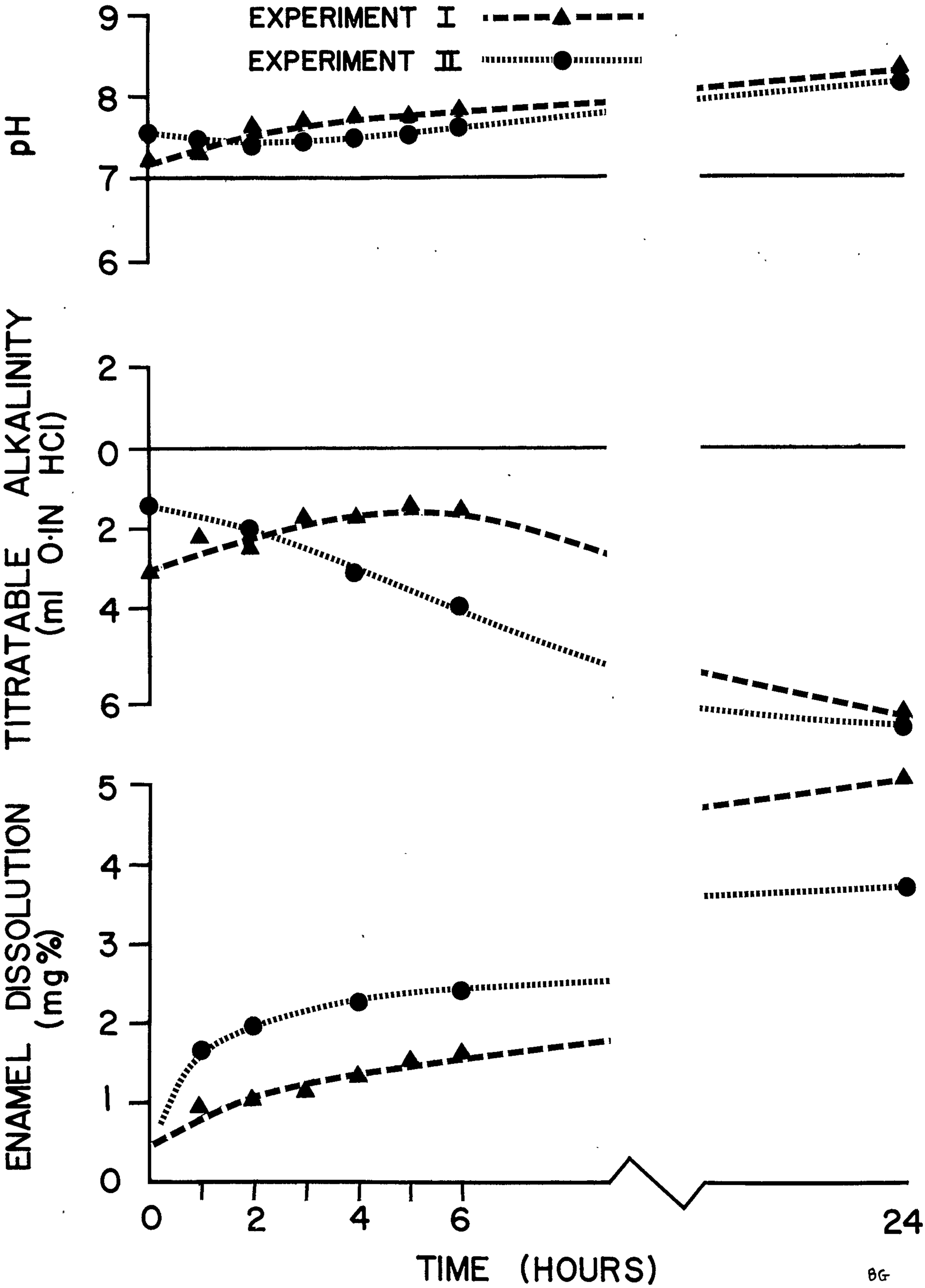

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FIGURE 5

EFFECT OF EXPOSURE TO SALIVA ON THE SOLUBILITY OF UNWASHED 200-240 MESH BOVINE ENAMEL IN 0.1M ACETATE BUFFER AT pH 5.0.

Two 250 mg. and two 50 mg. samples of irradiated, unwashed 200-240 mesh bovine enamel were weighed out, and a sample of each suspended in 10 ml. of pooled saliva, and 10 ml. of pH 7.0 distilled water. The 250 mg. suspensions were shaken at 37°C for 1 hour, the 50 mg. suspensions shaken at 37°C for 24 hours. All samples were then centrifuged at 2,000 rpm for 5 minutes, and the superatant liquid discarded. The samples were then washed by resuspension in distilled water at pH 5.0 and centrifugation, as before. The washing procedure was repeated. The samples were then re-suspended in 10 ml. 0.1M acetate buffer at pH 5.0, and shaken at 37°C for 9 hours (250 mg. samples). Glass frit enamel dissolution samples were taken at intervals, and enamel dissolution plotted against time. Saliva-soaked sample values are indicated by stars, water-soaked values indicated by dots. The enamel dissolution curves for 1 hour saliva soaking and 1 hour water soaking are shown by the long dot-dash lines and short dot-dash dark lines respectively, and for the 24 hour saliva and 24 hour water soaking by the wide dashed and narrow dashed lines, respectively.

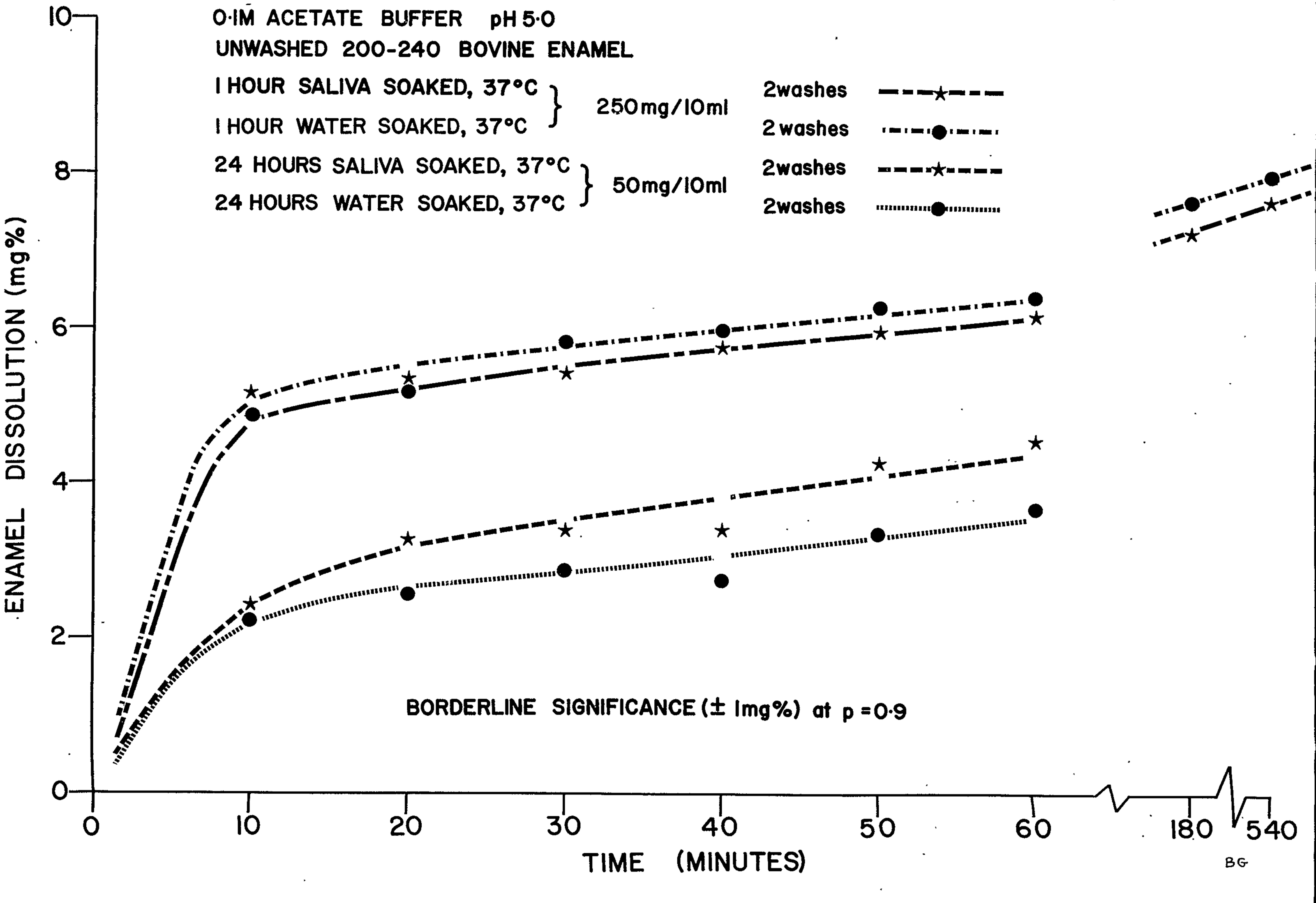
The two sets of dissolution curves were not comparable quantitatively, because of the differing amounts of enamel used in each case. Exposure of enamel powder to saliva for 1 hour did not change its solubility in acetate buffer. There appeared to be a slight increase in solubility after exposure to saliva for 24 hours. The increase was of border-line significance. (Enamel dissolution error for all samples $\pm$1.0 mg.% at p 0.9, using the radiochemical method).

EFFECT OF SALIVA ON BOVINE TOOTH ENAMEL SOLUBILITY

0·1M ACETATE BUFFER pH 5·0

UNWASHED 200-240 BOVINE ENAMEL

1 HOUR SALIVA SOAKED, 37°C	250mg/10ml	2washes	---★---
1 HOUR WATER SOAKED, 37°C		2 washes	-·-●-·-
24 HOURS SALIVA SOAKED, 37°C	50mg/10ml	2washes	---★---
24 HOURS WATER SOAKED, 37°C		2washes	····●····



BORDERLINE SIGNIFICANCE (± 1mg%) at p = 0·9

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FIGURE 6

DETERMINATION OF THE BUFFER CAPACITY OF 22 INDIVIDUAL SALIVA SAMPLES BY TITRATION WITH 0.1N HCl.

Stimulated saliva from 22 individuals was collected, and 10 ml. samples titrated from initial pH to pH 3.5, at room temperature, titration volumes being read at intervals of 0.5 of a pH unit. A similar titration was performed on a 10 ml. sample of the pooled saliva used in all food fermentation experiments. Titration volume was then plotted against pH change for all samples. To simplify the figure, individual values only are shown at each pH level, to eliminate the confusion which would result if 23 individual curves were plotted. (The curves differed considerably in shape, and crossed frequently). The mean HCl volume at each pH level for the 22 samples is shown by the black dots. The range of titration volumes at each pH level is shown by the heavy vertical black lines, and the individual values by the light horizontal bars. The titration curve for the pooled saliva sample is shown by the close-dashed line.

The initial pH of the pooled saliva sample corresponded closely to the mean initial pH of the salivas from 22 individuals . The titration values for the pooled saliva sample also corresponded closely to the mean titration values of the salivas from 22 individuals, at all pH levels except 4.0 and 3.5. This figure illustrates that saliva samples vary extensively in their buffer capacities and that the pooled sample used in the food tests is representative of saliva in general.

TITRATION OF 22 SALIVA SAMPLES FROM INITIAL pH TO pH 3·5 USING 0·1N HCl

TITRATION VOLS. READ AT 0·5 pH UNIT INTERVALS, COMPARED WITH TITRATION OF POOLED SAMPLE USED IN FOOD FERMENTATION TESTS

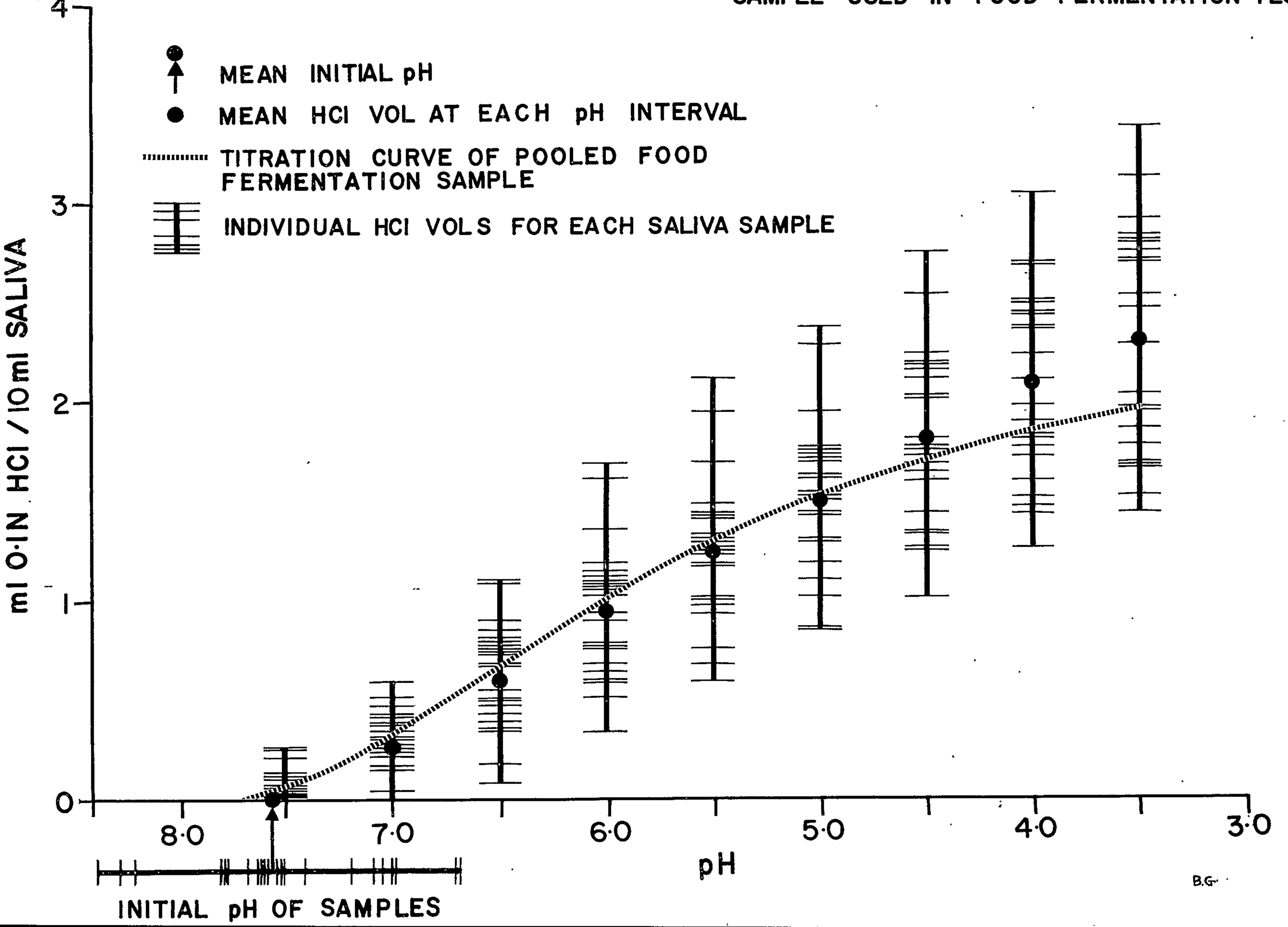


FIGURE 7

THE APPARATUS USED IN THE FOOD FERMENTATION EXPERIMENTS.

1. The jacketed beaker in place of the magnetic stirrer table, showing the rubber bung, thermometer and glass and reference pH electrodes. Water at 37°C was fed to and from the jacket by the rubber tubes.

2. Top left. Glass frit gas dispersion tube of 4 micron pore size, used to obtain filtered enamel dissolution samples by aspiration, using a vacuum pump (not shown).

   Bottom right. Flat stainless steel planchet, 1" in diameter.

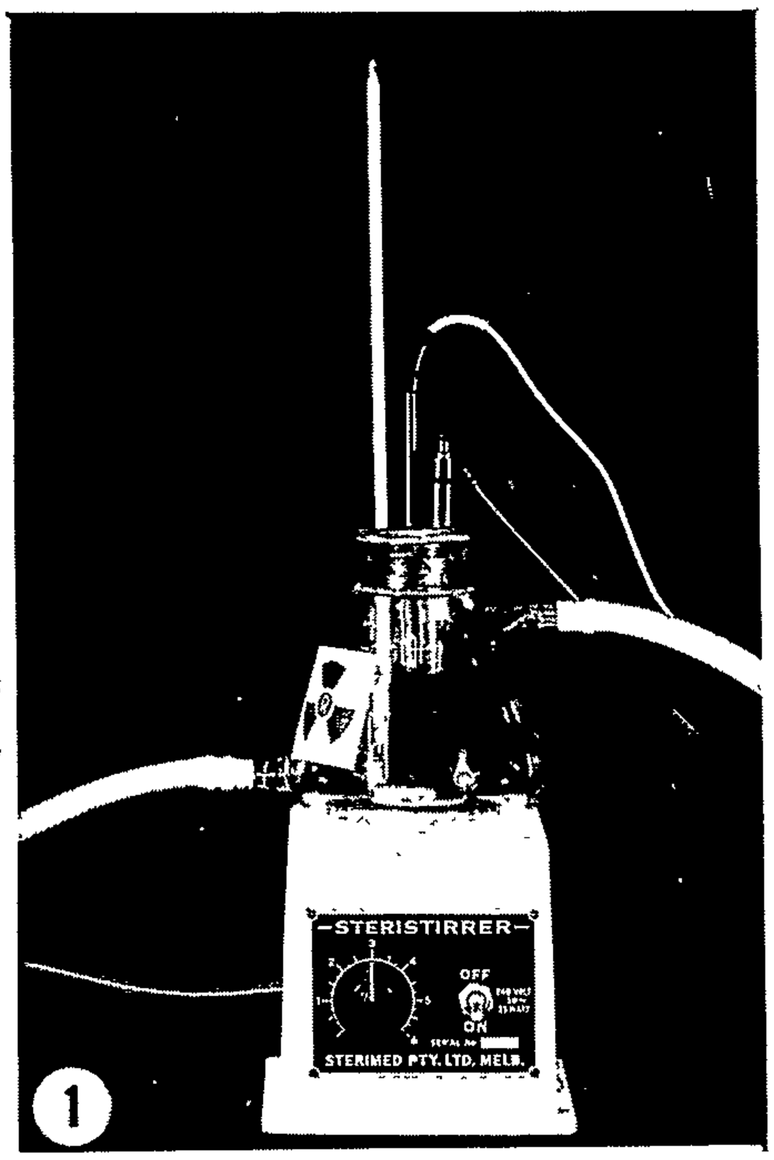
   Middle. Control pipette and micropipette, used to obtain enamel dissolution samples.

3. Left. Ekco type 620 lead-shielded G.M. tube holder and type 619 sample oven.

   Middle. Ekco N530G automatic scaler.

   Right. Nuclear Chicago Model D 47, 2 pi gas-flow detector.

   Extreme left. Ekco type N 664A scintillation counter.



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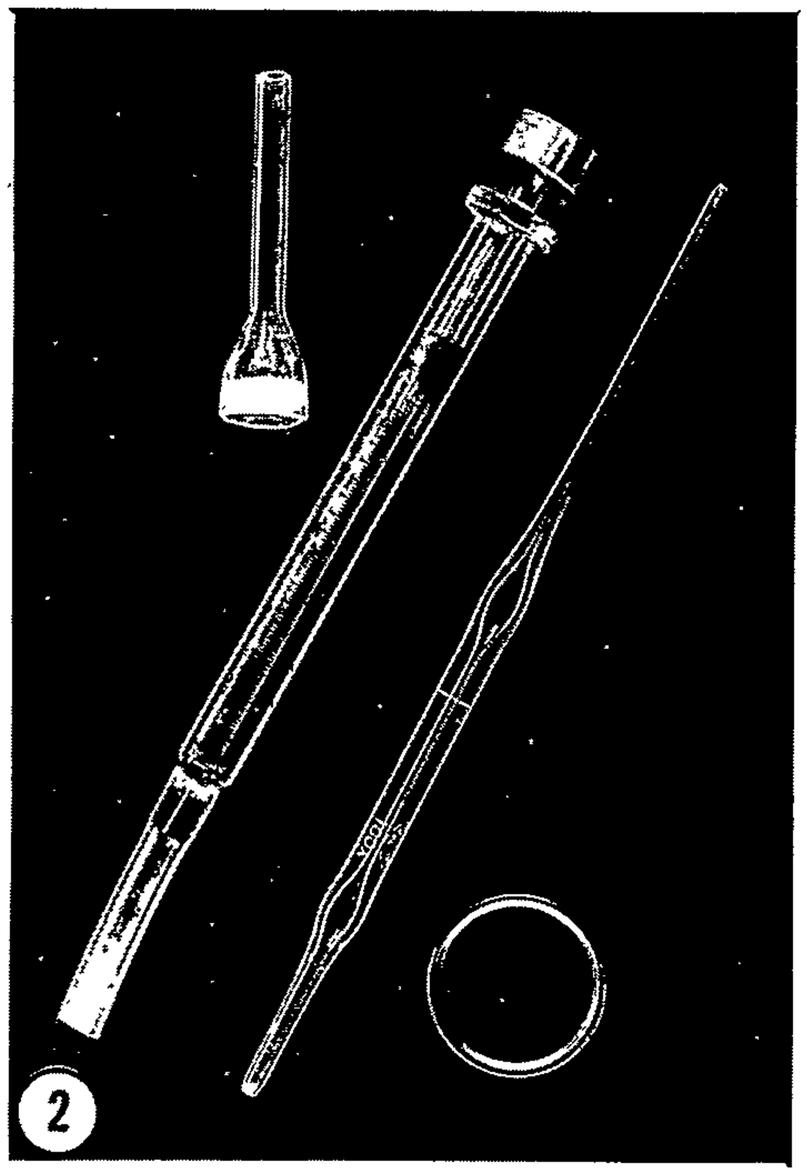
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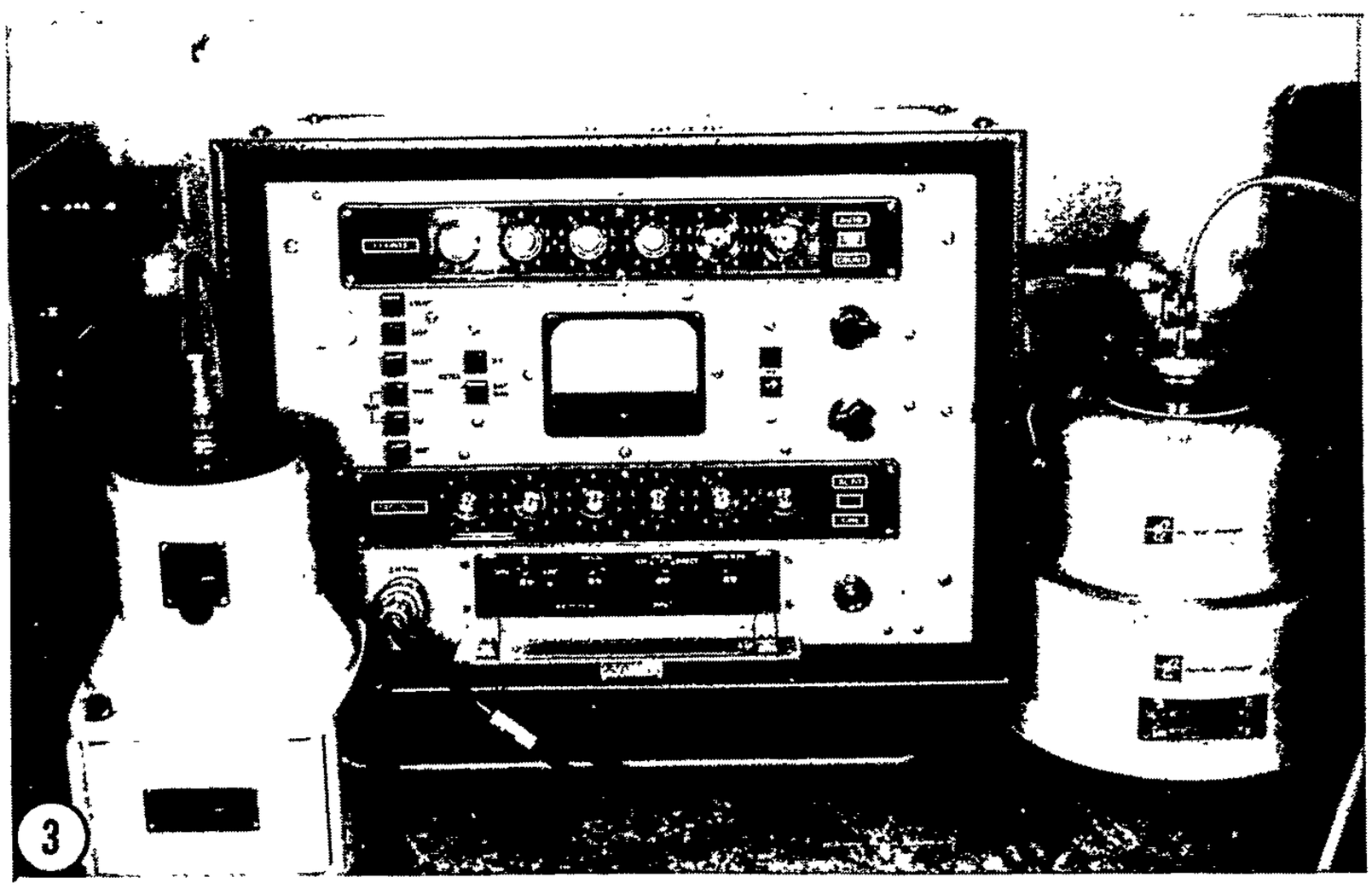
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FIGURE 8

TITRATION APPARATUS USED IN THE FOOD FERMENTATION EXPERIMENTS

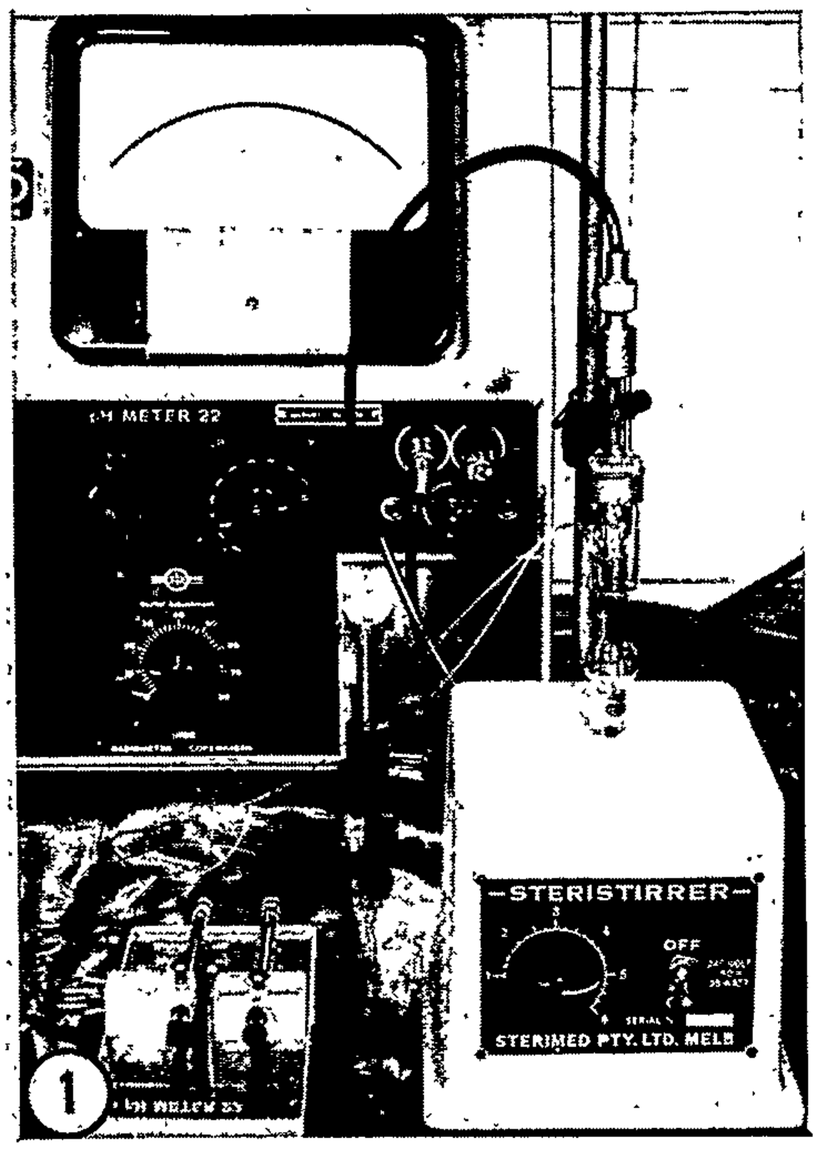
1. Rear. Radiometer Model 22 pH meter.

   Left foreground. Hamilton 500 and 250 µl. gas chromatograph syringes used to supply titration solution to the titration tube.

   Right foreground. The combined pH electrode, titration tube and clamp, fixed centrally over the magnetic stirrer table. The ceramic magnet can be seen in the bottom of the titration tube.

2. Close-up view of the combined pH electrode and titration tube, showing the fine-bore high density polyethylene tubes entering below the surface of the liquid being titrated. Note that the bulge in the titration tube allows addition of titration solution without causing solution to be drawn up between the sides of the electrode and the titration tube by capillarity.

3. Close-up view of the gas chromatograph syringes, hypodermic needles and polyethylene tubes.



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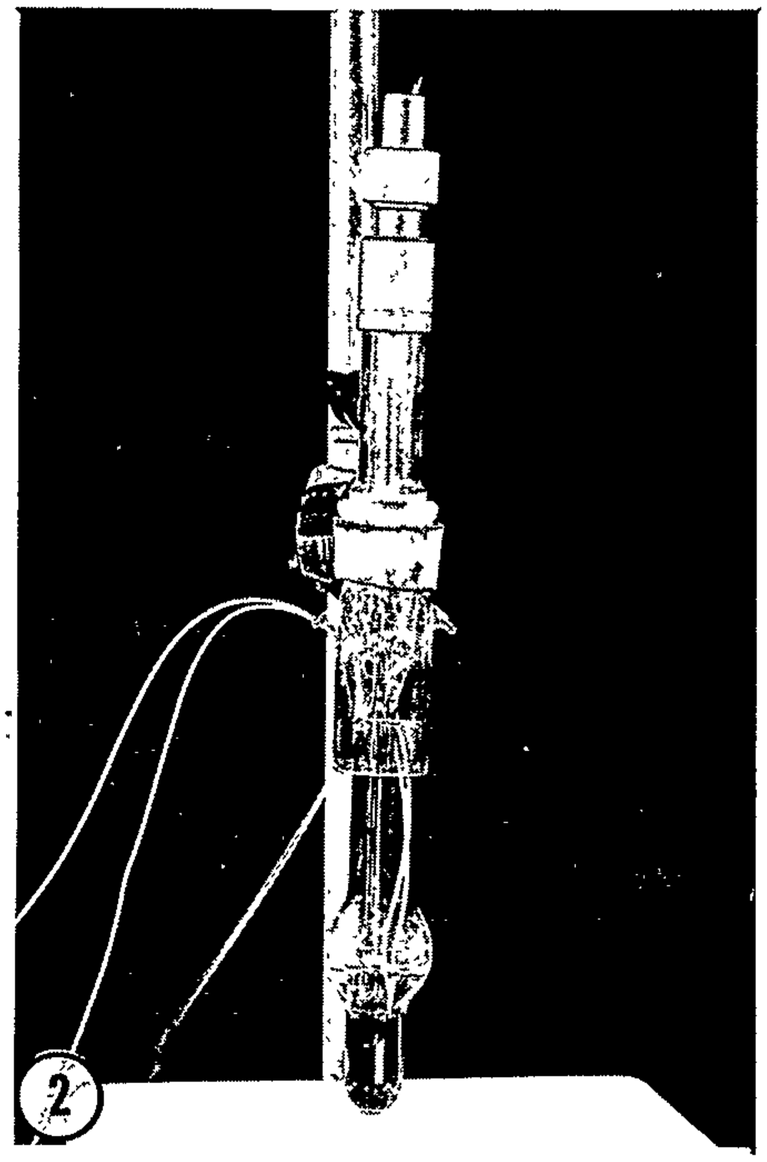
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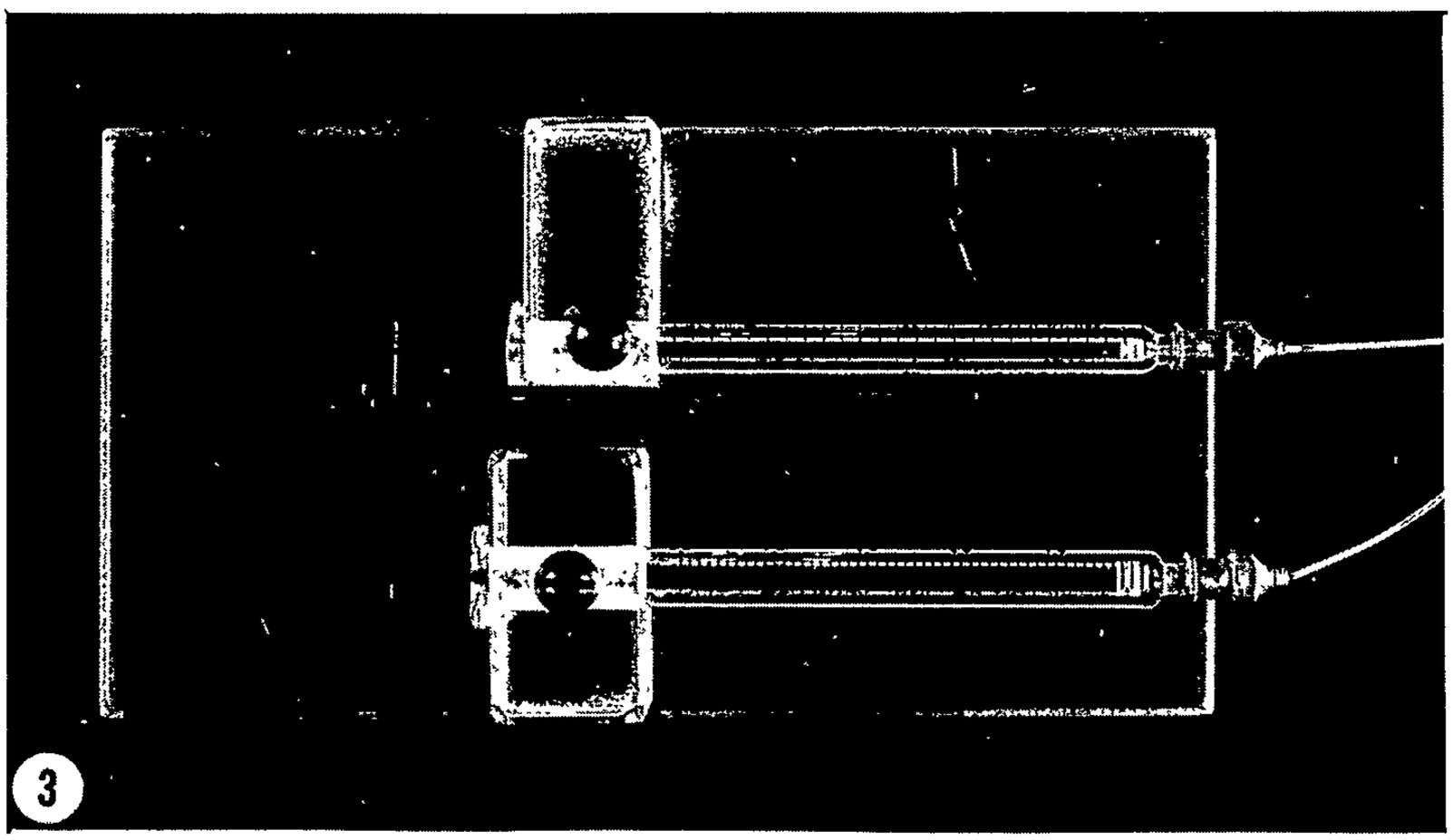
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FIGURE 9

TYPICAL RESULT FOR THREE 24 HOUR FERMENTATION EXPERIMENTS ON HONEY, MOLASSES AND SUCROSE, SHOWING THE pH, TITRATABLE ACIDITY AND ENAMEL DISSOLUTION CHANGES.

The upper graph shows mean pH, the middle graph mean titratable acidity and the lower graph mean enamel dissolution changes occurring in a 24 hour food fermentation of honey (wide-dashed lines), molasses (solid lines) and sucrose (close-dashed lines), for three replicate experiments. The range for $\pm$ two standard errors for some of the experimental values is indicated by the appropriately coded bars bounding the thin, solid, vertical lines.

There were no significant differences in pH throughout the experiments for the three sugars (expect for molasses at hour 0, and sucrose at hour 2, both of borderline significance). Apart from minor differences, it can be said that the pH changes for honey, molasses and sucrose were basically similar.

The titratable acidity values for the three sugars were also basically similar throughout the experiments, except at hours 3, 4 and 5, where molasses titratable acidity was significantly higher than the corresponding values for the other two sugars. This difference disappeared as the experiment progressed, and it can be said that apart from minor variations, the titratable acidity changes for honey, molasses and sucrose were similar.

However, the enamel dissolution figures for molasses were significantly lower than those for honey and sucrose at hours 4, 5, 6 and 24, and at hour 24 the enamel dissolution figures for the three sugars were significantly different from each other.

Thus significant differences in fermentation behaviour between foods can be demonstrated using statistical analysis of the results obtained from three replicate experiments, and this was the procedure adopted in the tests on all Australian foods.

FOOD FERMENTATION

HONEY, MOLASSES and SUCROSE
(means of three experiments)

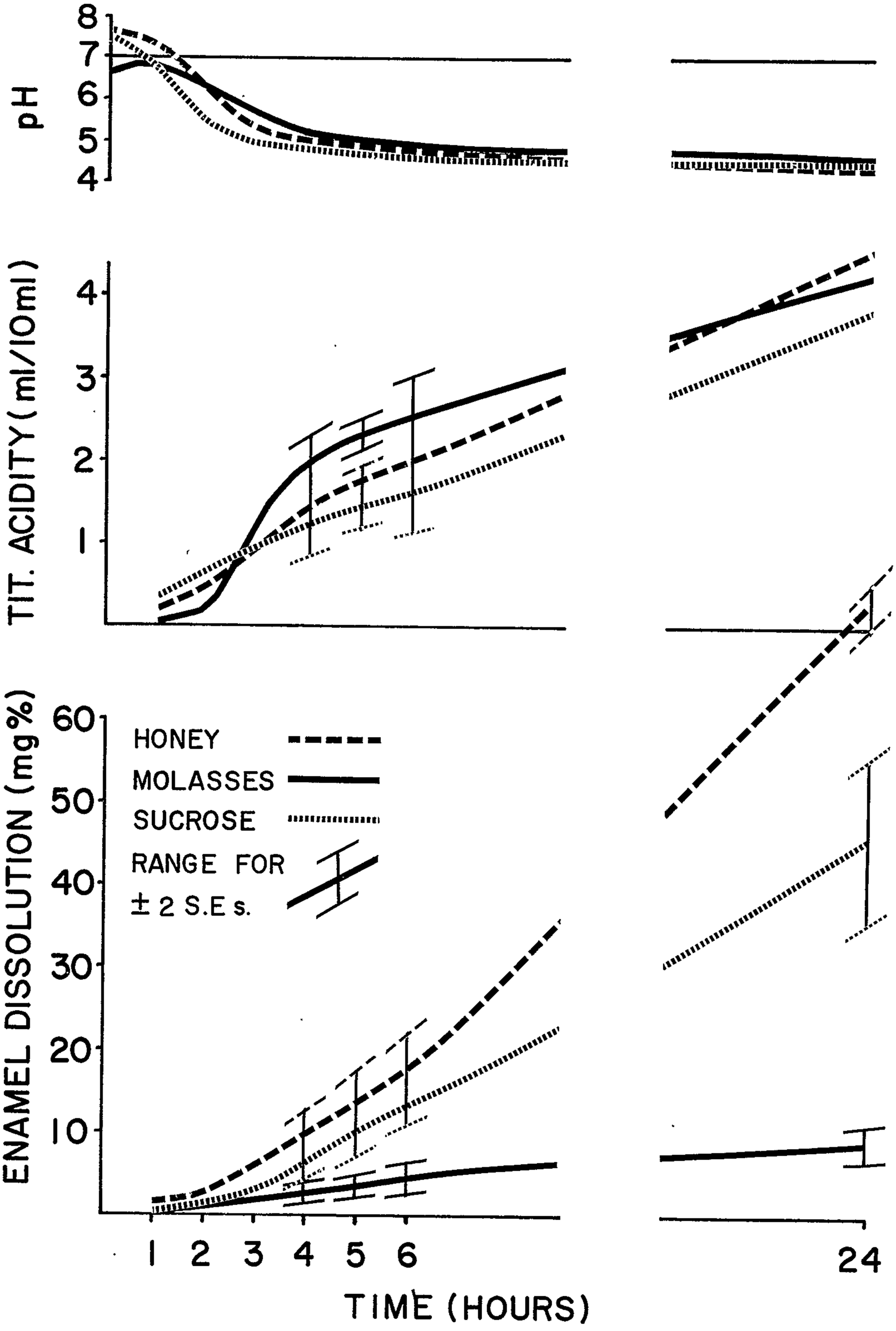

FIGURE 10

THE RANGE OF MEAN pH VALUES OBSERVED DURING 24 HOUR FERMENTATION OF FOODS IN THE 'SUGARS' GROUP (EXCLUDING SWEETADDIN)^{R}.

The shaded area represents the range of mean pH values observed in the food fermentation tests of eight 'sugars' over 24 hours. Initial pH varied from 6.5 to 7.8, but by hour 1, the range was less than one pH unit, and by hour 3, less than 0.5 of a pH unit. This small range was maintained up to hour 24.

pH CHANGE – SUGARS

(range of means)

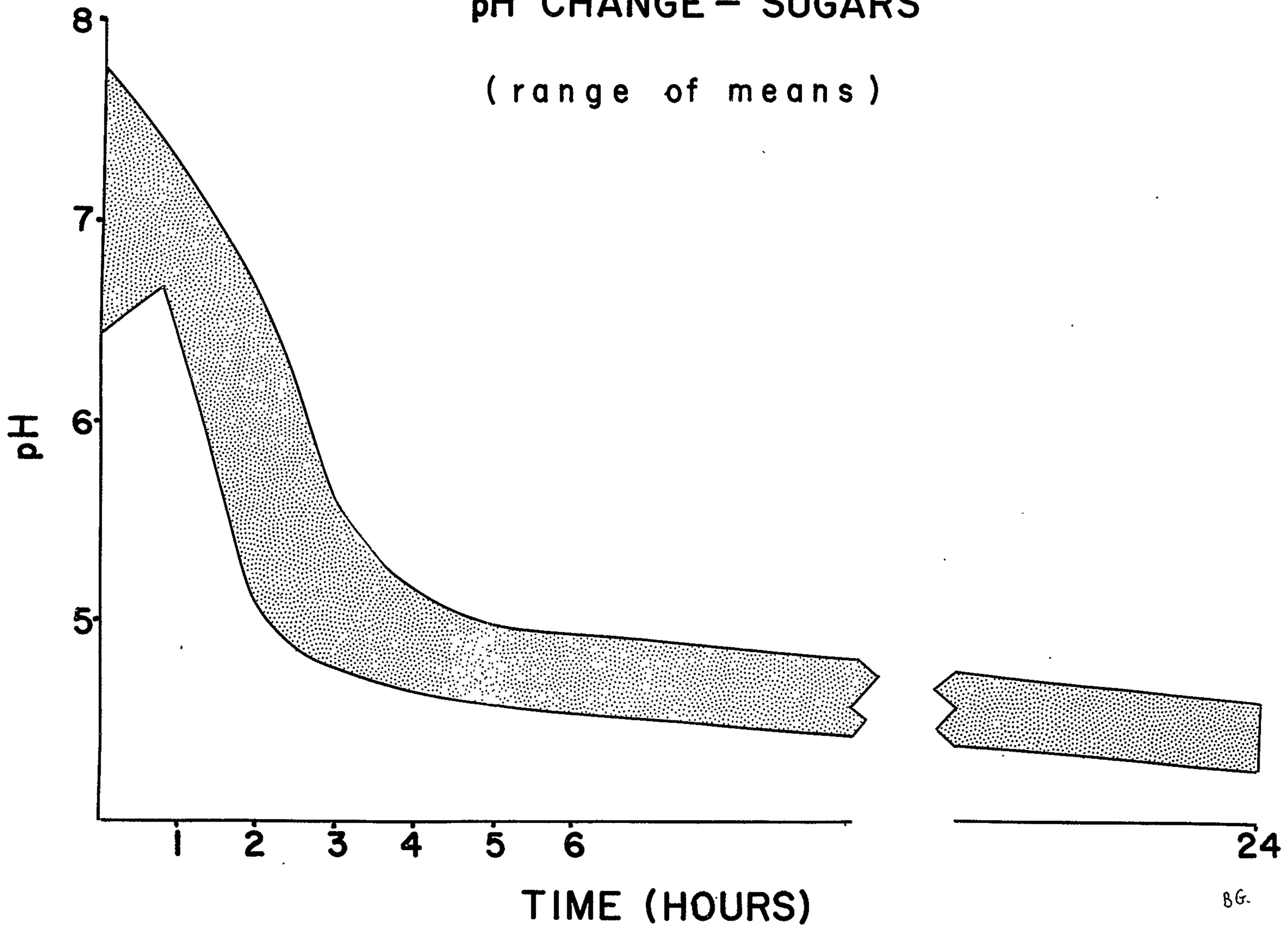

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FIGURE 11

THE RANGE OF MEAN TITRATABLE ACIDITIES OBSERVED DURING 24 HOUR FERMENTATION OF FOODS IN THE 'SUGARS' GROUP (EXCLUDING SWEETADDIN).®

The shaded area represents the range of mean titratable acidity values observed in the food fermentation tests of eight 'sugars' over 24 hours. Except for molasses, the titratable acidity for all 'sugars' increased steadily from about 1.0 at hour 3 to about 4.0 at hour 24. From hour 3 to hour 6, molasses titratable acidity increased more rapidly than the other 'sugars', but less rapidly thereafter, its 24 hour value falling within the range for the others.

TITRATION VOLUME – SUGARS

(range of means)

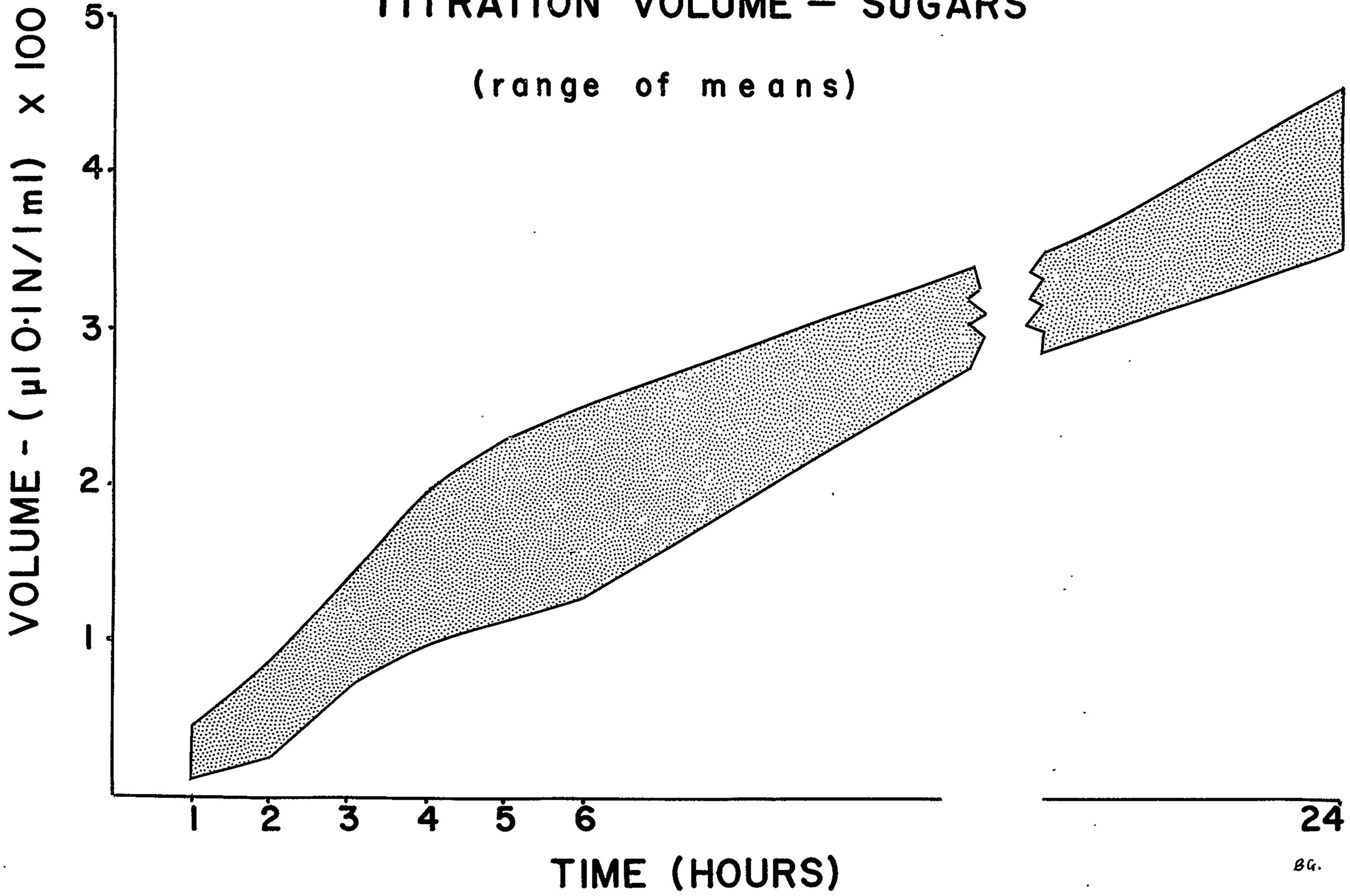

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FIGURE 12

MEAN ENAMEL DISSOLUTION VALUES OBTAINED DURING 24 HOUR FERMENTATION OF FOODS IN THE 'SUGARS' GROUP (EXCLUDING SWEETADDIN)[R].

The mean enamel dissolution curves observed in the food fermentation tests of eight 'sugars' are shown by the various dot and/or dash lines. (Note that four patterns only are used, the same code being used for honey and brown sugar, glucose syrup and Golden Syrup[R], etc. The various curves are matched according to the hour 24 values, in the order shown, with honey (wide dot-dash line) highest, and molasses (close dot line) lowest).

Up to hour 3, the enamel dissolutions for all sugars were similar. After hour 3, however, the sugars could be divided into two separate groups with respect to enamel dissolution behaviour. In the group composed of honey, glucose syrup, dextrose and sucrose, enamel dissolution increased rapidly, while in the other group, composed of brown sugar, Golden Syrup[R], treacle and molasses, the enamel dissolution increased slowly. The differences in enamel dissolution between these two groups were of borderline significance at hour 4, but were highly significant by hour 6.

Enamel dissolution increased up to hour 24, the hour 6 rankings being maintained, except for sucrose, which dropped below glucose, and for molasses, which dropped below brown sugar, Golden Syrup[R] and treacle. The differences in hour 24 enamel dissolution, as shown in this graph, are significant, except in the following cases: honey-glucose syrup, glucose syrup-dextrose, glucose syrup-sucrose, dextrose-sucrose, dextrose-brown sugar[R], sucrose-brown sugar, and brown sugar-Golden Syrup.

On the basis of pH and titratable acidity changes, the second group (unrefined sugars) produced less dissolution than would have been expected had the food-saliva mixtures been behaving as simple chemical buffers.

ENAMEL DISSOLUTION — SUGARS
(means of 3 expts.)

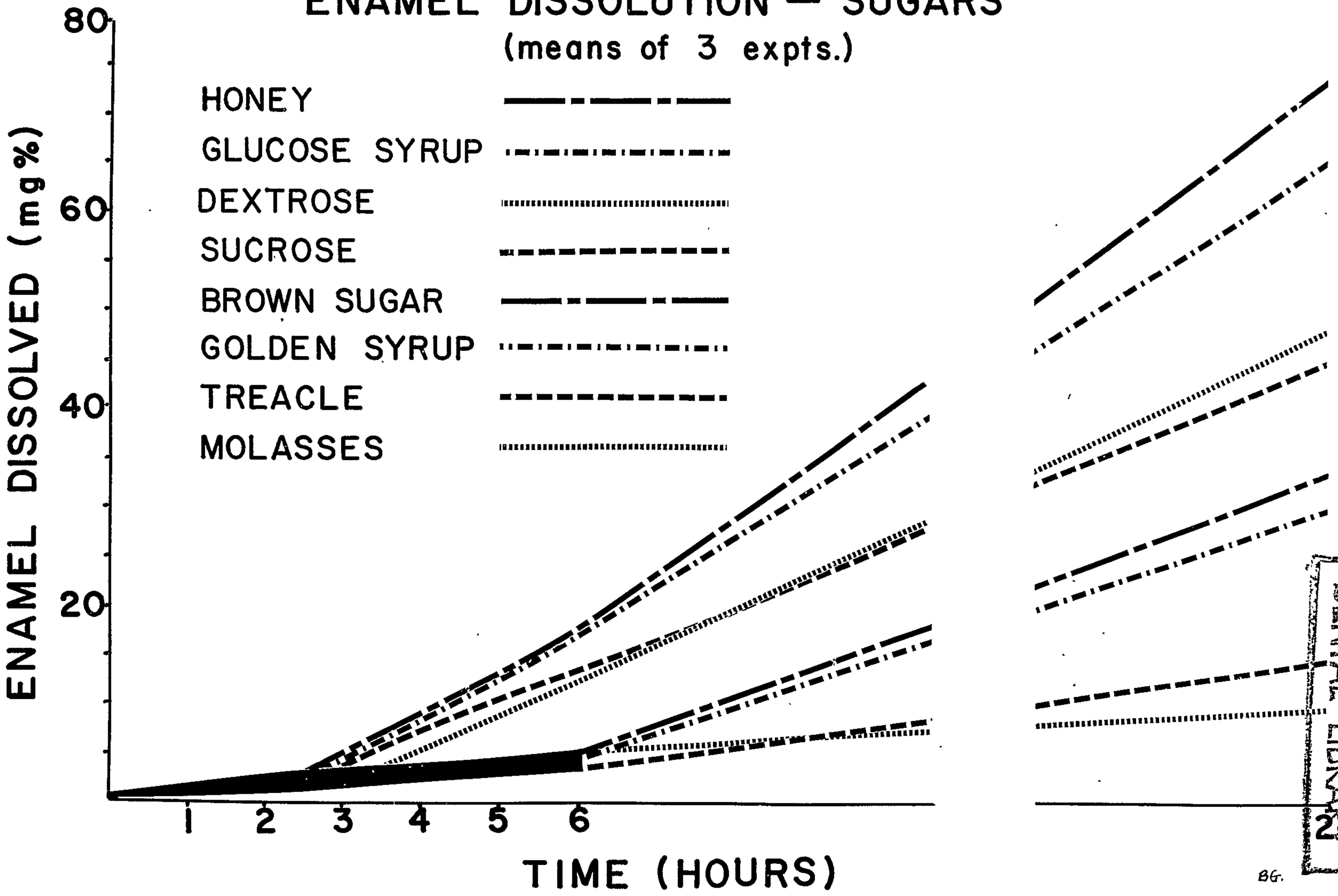

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FIGURE 13

TITRATION OF ACETATE BUFFERS OF pH LEVELS IN THE RANGE 4.0 - 6.0 AND DIFFERENT MOLARITIES TO pH 7.0, USING 0.1M SODIUM HYDROXIDE.

Acetate buffers of 1.0, 0.5, 0.3, 0.1 and 0.05 molarity and pH levels in the range 4.0 to 6.0 were prepared, and the amount of 0.1 M sodium hydroxide required to titrate each buffer to pH 7.0 recorded. These quantities were plotted against the initial pH of the appropriate buffer, and each point so plotted indicates the titratable acidity of a buffer of a specific pH and molarity. Lines joining like points permit the estimation of the titratable acidity that would be obtained from a titration to pH 7.0 of simple buffers of other pH levels and molarities.

This graph can thus be used to estimate the molarity of a food-saliva mixture, the pH and titratable acidity of which is known, if such a mixture was considered to be behaving as a simple chemical buffer.

It should be noted that the dashed lines joining like points in this graph do not represent titration curves, which are of a different shape, because of dilution effects as the titration proceeds. This graph should be considered as a table of buffer capacities, or titratable acidities, for simple acetate buffers of various pH levels and molarities.

Using this table it was possible to demonstrate that the titratable acidity of all food-saliva mixtures increases during fermentation. With some foods, there was no increase after hour 6, while with others, especially orange juice, Coca Cola®, wheat germ®, bran, rye flour, wheat flour, oatmeal, rolled oats, All Bran®, dates, caramels and glacé cherries, the buffer capacity at hour 24 was higher than at hour 6.

TITRATION OF ACETATE BUFFERS, OF MOLARITIES 1·0, 0·5, 0·3, 0·1, AND 0·05, AND INDICATED pH, (10ml ALIQUOTS) TO pH 7·0, USING 0·1N NaOH

BROKEN LINES INDICATE VOLUME OF 0·1N NaOH REQUIRED FOR NEUTRALISATION OF 1·0, 0·5, 0·3, 0·1 AND 0·05 MOLAR BUFFERS AT ANY pH FROM 3·5 TO 7·0

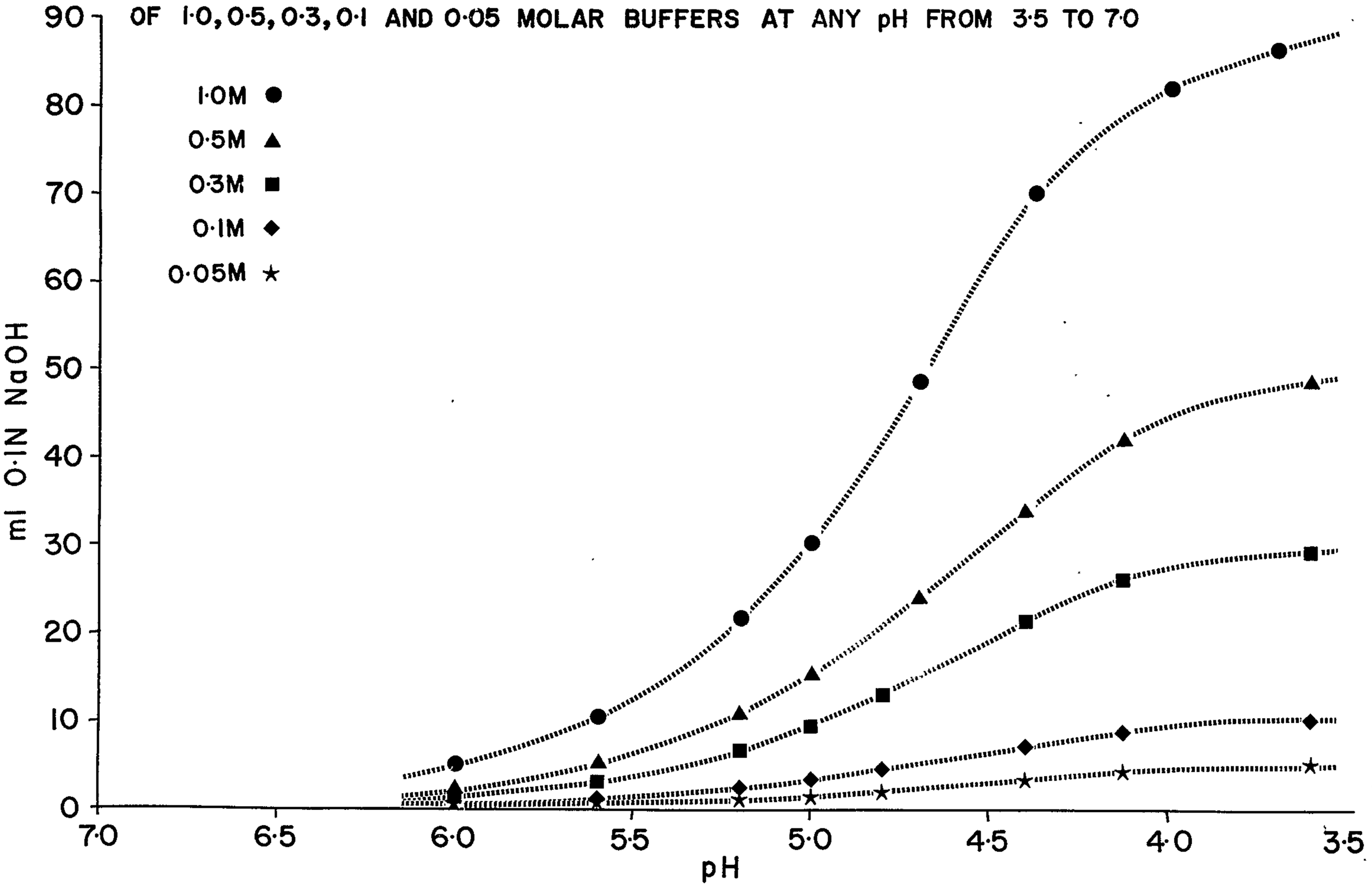
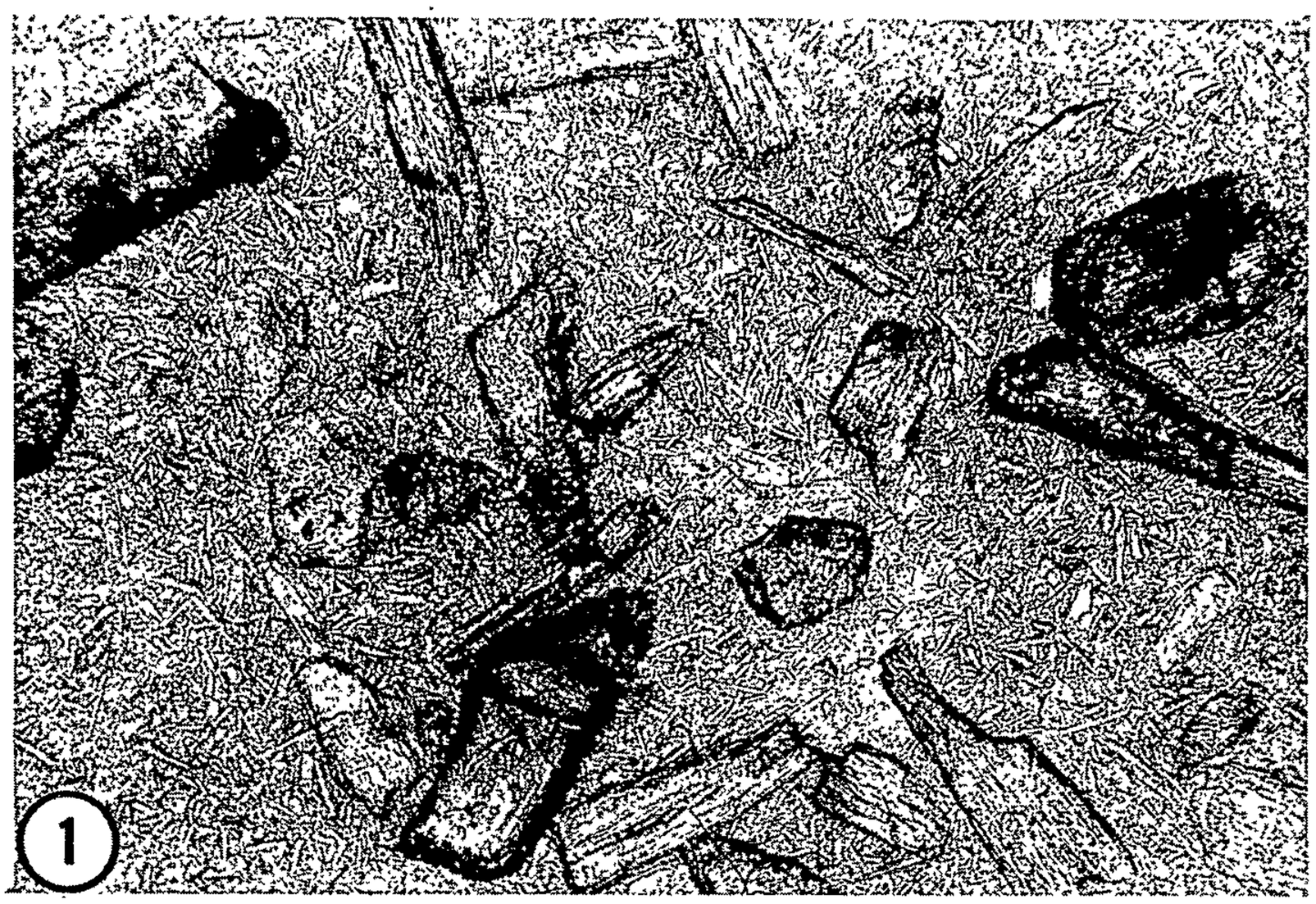


FIGURE 14

120-200 MESH BOVINE ENAMEL POWDER, BEFORE AND AFTER WASHING

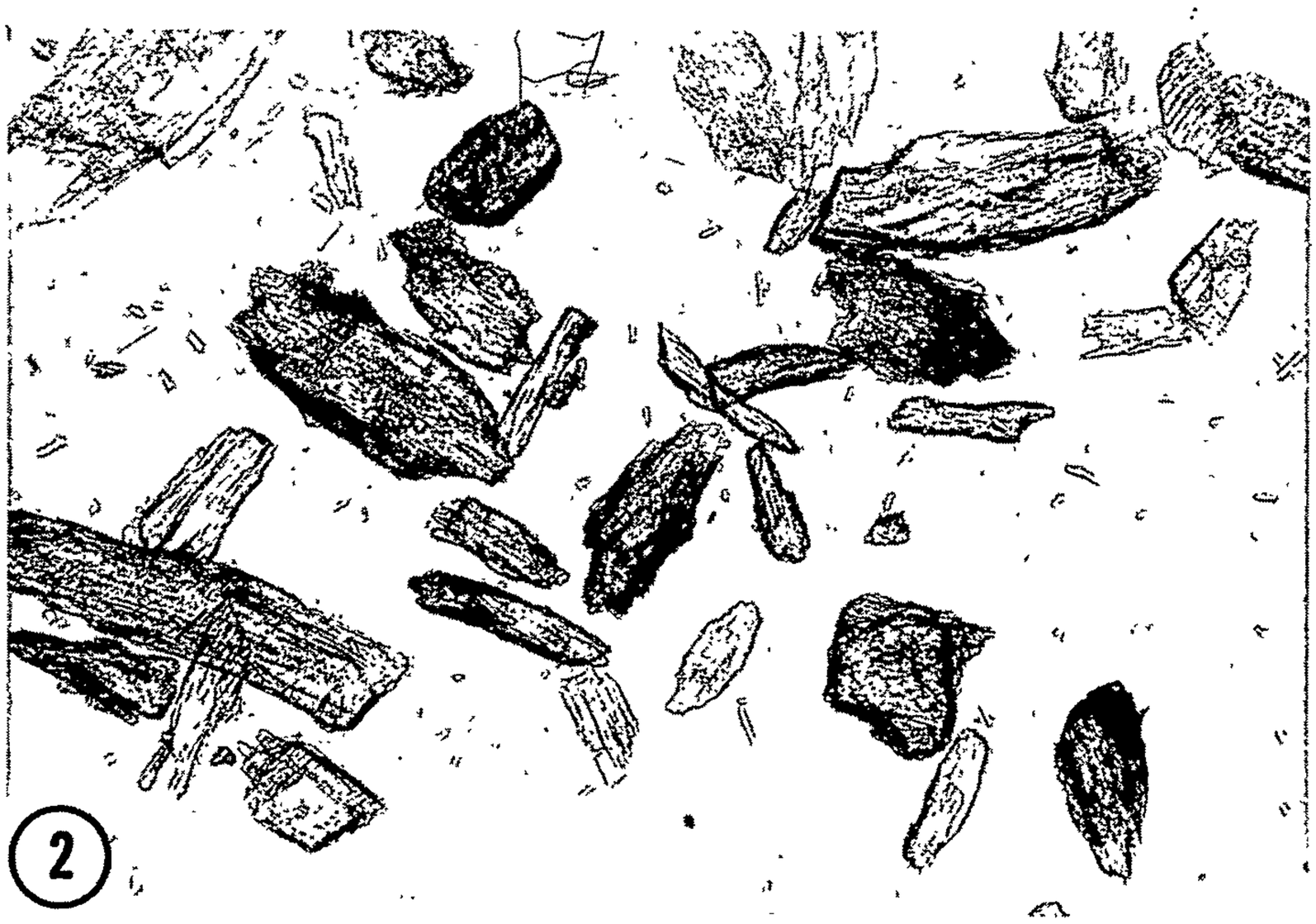
1. Microscopic appearance of the fraction of crushed enamel blocks which passed a 120 mesh sieve, but was retained on a 200 mesh sieve, before washing. Note that this fraction contains very small enamel particles (brash) which should have passed through the 200 mesh sieve.

2. Microscopic appearance of the fraction shown in 1. above, after five washings in water. Note that the washing procedure has removed nearly all the brash.



1

100 μ



2

FIGURE 15

COMPARISON OF CHEMICAL AND RADIOCHEMICAL DISSOLUTION RATES OF FOUR DIFFERENT APATITE SAMPLES IN 0.1 M ACETATE BUFFER AT pH 4.5.

Samples of tricalcium phosphate (Victor)^{R}, unwashed 120-200 mesh, washed 120-200 mesh bovine enamel, and washed 120-200 mesh human enamel were irradiated, 50 mg. samples of each weighed out and added to 25 ml. of 0.1M acetate buffer at pH 4.5. and shaken at room temperature for $1\frac{1}{2}$ hours, and allowed to stand for three days. Samples were taken at intervals by glass frit filtration, and enamel dissolution calculated by a chemical method (calcium analysis) and the radiochemical method (appearance of P^{32} in solution). The solid lines indicate the enamel dissolution rates as calculated by chemical analysis, and the close-dashed lines indicate the enamel dissolution rates as calculated by radiochemical analysis.

The dissolution curves as calculated by the two methods, are similar in shape, but differ quantitatively, for each of the four apatite samples. The radiochemical method gave lower values for apatite dissolution in an acetate buffer with synthetic, bovine and human apatite. Both the chemical and radiochemical methods gave higher dissolution values for tricalcium phosphate and unwashed bovine enamel than for washed bovine and washed human enamel. The radiochemical enamel dissolution curves for washed bovine and washed human enamel were similar (see fig. 3). Dissolution continued with all four apatites after $1\frac{1}{2}$ hours, the increase after three days being considerable with the bovine and human enamels, but not so great with the tricalcium phosphate.

EFFECT OF IRRADIATION ON SOLUBILITY OF FOUR APATITE SAMPLES

0·1M ACETATE BUFFER pH 4·5

APATITE DISSOLUTION RATE DETERMINED BY: —CHEMICAL METHOD (Ca ANALYSIS)

—RADIOCHEMICAL METHOD (COUNT RATE)

APATITE DISSOLUTION (mg %)

VICTOR TRICALCIUM PHOSPHATE

WASHED 120-200 BOVINE ENAMEL

UNWASHED 120-200 BOVINE ENAMEL

WASHED 120-200 HUMAN ENAMEL

0 30 60 90 3 DAYS

TIME (MINUTES) ⟶

BG

FIGURE 16

COMPARISON OF THE DISSOLUTION RATES OF IRRADIATED AND NON-IRRADIATED SAMPLES OF WASHED 120-200 MESH BOVINE ENAMEL POWDER , AS CALCULATED BY CHEMICAL METHODS (CALCIUM ANALYSIS AND PHOSPHATE ANALYSIS) AND THE RADIOCHEMICAL METHOD (P^{32} ANALYSIS).

A sample of washed 120-200 mesh bovine enamel was prepared, and a portion irradiated. Weighed 100 mg. samples of both the non-irradiated and irradiated powder were then placed in two separate beakers, 50 ml. of a 0.1 M acetate buffer at pH 4.0 added, and the mixtures agitated at 37°C for 2 hours. Enamel dissolution samples were taken at intervals from both beakers, for calcium and phosphate analysis, and from the radioactive enamel beaker only for radiochemical analysis. Enamel dissolution was then calculated (assuming a Ca/P ratio of 2.07.) by both calcium and phosphate analysis, for both non-irradiated and irradiated enamel, and by P^{32} count rate for the irradiated enamel, and the results plotted against time, to give five dissolution-rate curves; 1. Non-irradiated enamel by Ca analysis (close dotted line and dots). 2. Non-irradiated enamel by PO_4 analysis (wide dashed line and squares). 3. Irradiated enamel by Ca analysis (close dot-dashed line and triangles). 4. Irradiated enamel by PO_4 analysis (wide dot-dashed line and diamonds). 5. Irradiated enamel by P^{32} analysis (solid line and stars).

In this experiment, enamel dissolution appeared to be slightly greater when based on calcium analysis than when based on phosphate analysis, for both non-irradiated and irradiated bovine enamel. By both methods, irradiated enamel was less soluble in acetate buffer than was non-irradiated enamel.

The radiochemical method indicated a lower irradiated enamel solubility than did either chemical method, yet recovery with both the chemical and radiochemical methods was almost 100% when all enamel was dissolved by HCl addition at the conclusion of the experiment. This result indicates that there is a difference in the way P^{31} and P^{32} of irradiated enamel dissolve in acetate buffer solutions.

COMPARISON OF DISSOLUTION RATES OF IRRADIATED AND NON-IRRADIATED WASHED 120-200 BOVINE ENAMEL, (Ca/P RATIO 2·07) 0·1M ACETATE BUFFER pH4·0

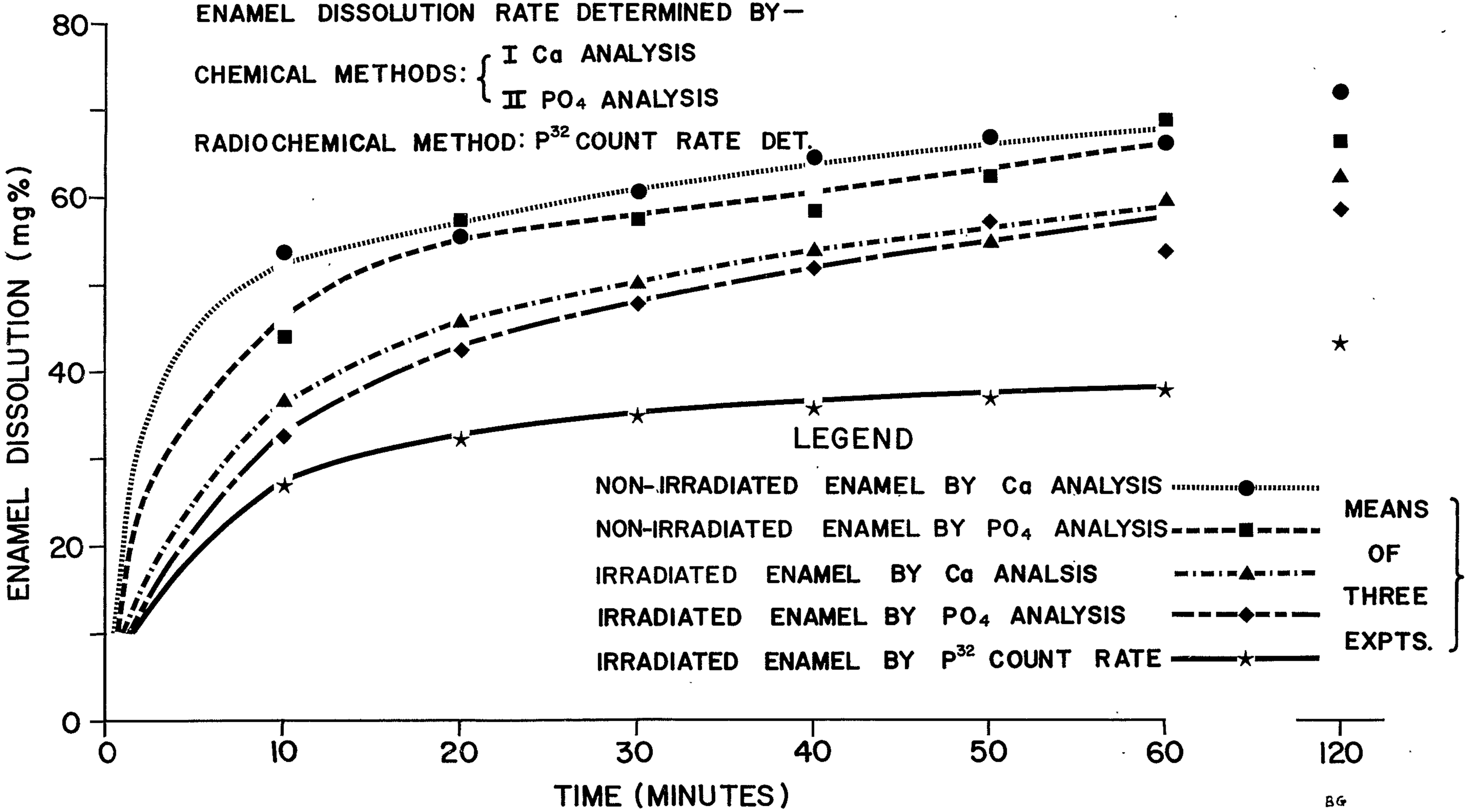


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FIGURE 17

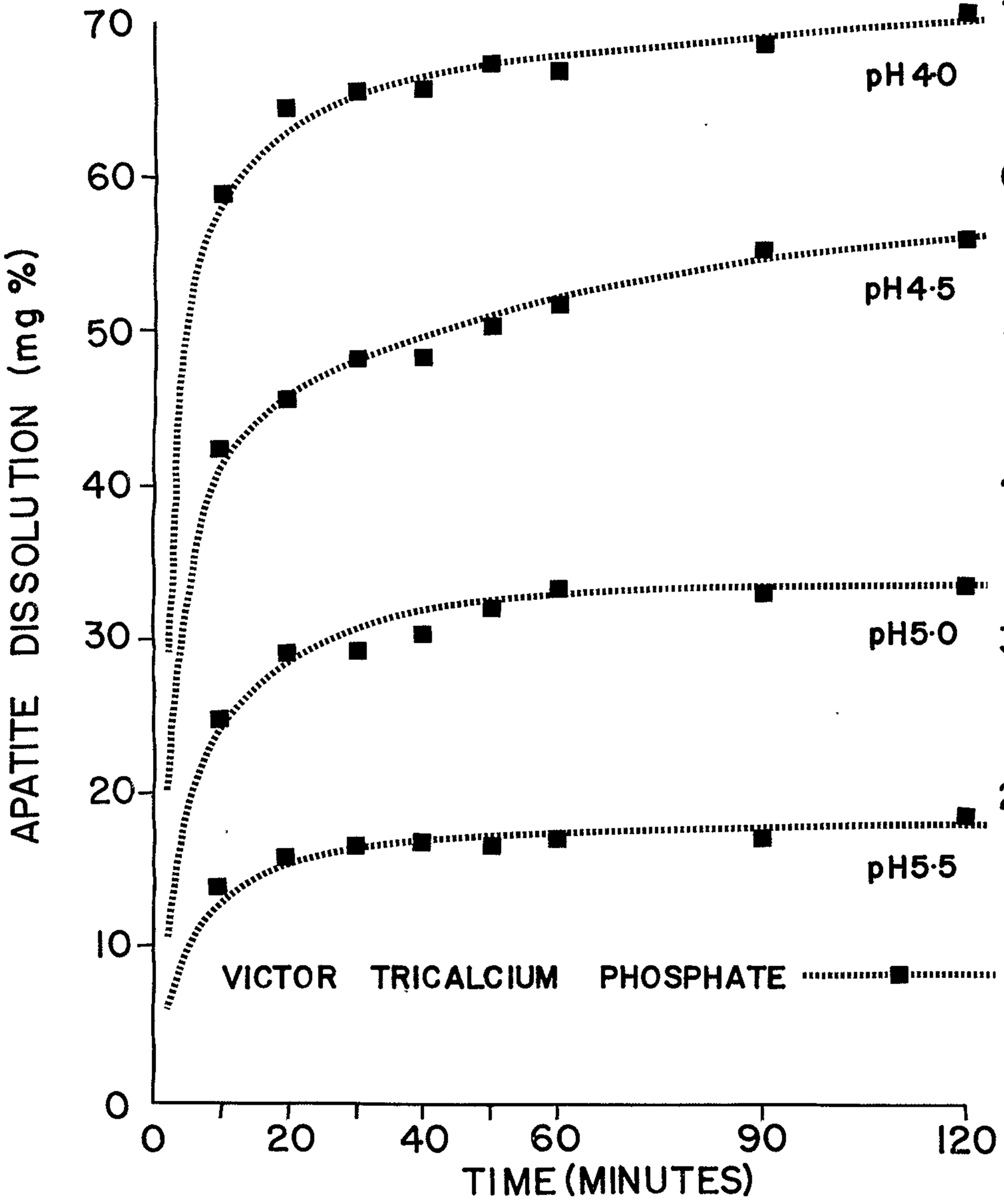
DISSOLUTION RATES OF APATITES IN 0.1M ACETATE BUFFERS OF DIFFERENT pH's.

Samples of tricalcium phosphate (Victor)^{R}, unwashed 120-200 and unwashed 200-240 mesh bovine enamel powders were irradiated, 50 mg. of each added to 25 ml. of 0.1M acetate buffers of pH 4.0. 4.5. 5.0. and 5.5. and agitated at room temperature for 2 hours. Glass frit samples were taken, and dissolution calculated by the radiochemical method and plotted against time. The left-hand curves (close-dashed lines and squares) show the dissolution rates for tri-calcium phosphate at each pH. The right-hand curves show the dissolution rates for unwashed 200-240 mesh bovine enamel (wide-dashed lines and triangles) and for unwashed 120-200 mesh bovine enamel (solid lines and circles) at each pH.

For all three apatite samples, the amount of dissolution varied inversely with the pH, a decrease in pH of one unit almost doubling the dissolution observed. Dissolution was higher with the finer materials, and took place more rapidly. Equilibrium was approached after two hours with all three apatite samples.

COMPARISON OF DISSOLUTION RATE OF SYNTHETIC AND NATURAL APATITE

0·IM ACETATE BUFFER AT pH 4·0, 4·5, 5·0 AND 5·5



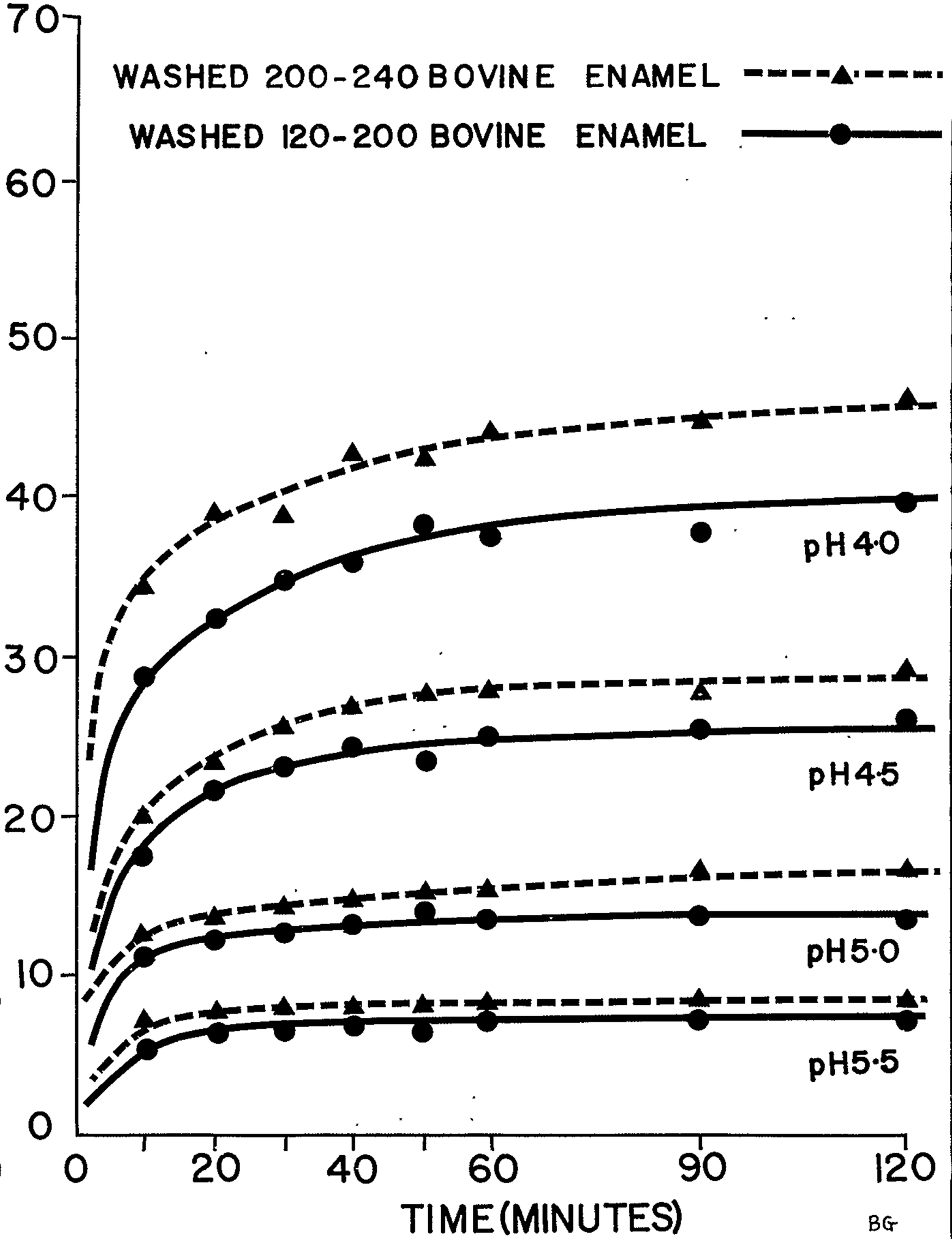


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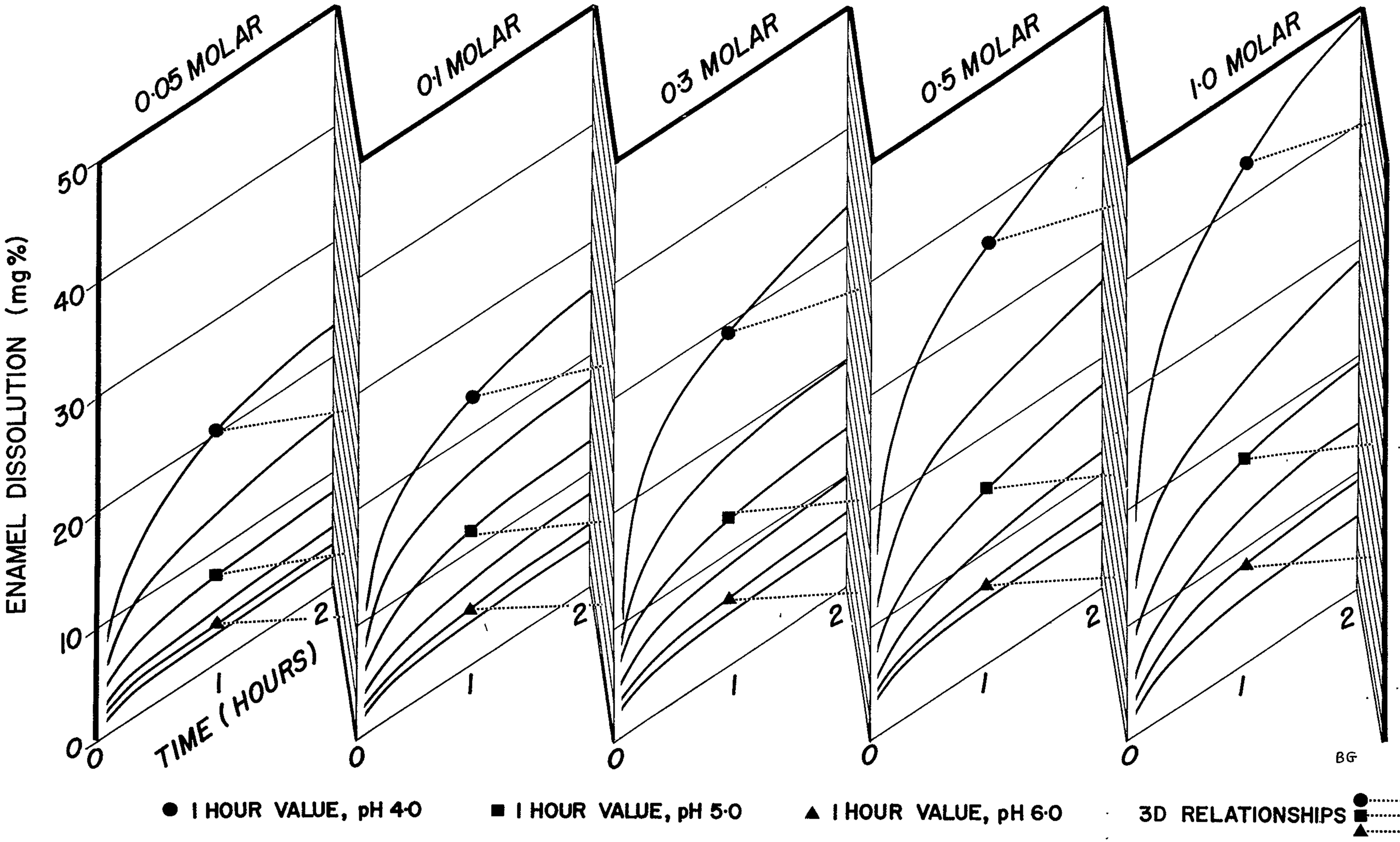
FIGURE 18

DISSOLUTION RATE CURVES FOR BOVINE ENAMEL POWDER IN ACETATE BUFFERS OF DIFFERENT pH's AND MOLARITIES.

A sample of washed 120-200 mesh bovine enamel powder was irradiated, 100 mg. amounts weighed out and added to 50 ml. of acetate buffers of pH 6.5, 6.0, 5.5, 4.0, 4.5 and 4.0 and 0.05, 0.1, 0.3, 0.5 and 1.0 molar, under identical conditions of agitation (magnetic stirrer) and temperature ($37^{o}C$). For each experiment, 100 µl. glass frit samples were taken at 10 minute intervals for the first hour, and again at $1\frac{1}{2}$ and 2 hours, and enamel dissolution at each time interval calculated, using the radiochemical method. These values were then plotted against time, on a composite graph arranged to demonstrate, three dimensionally, the relationships between enamel dissolution rate, acetate buffer pH and molarity.

The 'group' of curves indicates that a decrease in pH, an increase in titratable acidity, or both, increased the amount of dissolution, and the rate of dissolution. The 'group' of curves also indicates that a twenty-fold increase in buffer molarity (0.05M to 1.0M) at a given pH, almost doubles enamel dissolution, and a fall in pH of one unit, at a given molarity, also almost doubles enamel dissolution. To make these relationships more evident, the hour 1 enamel dissolutions at each buffer molarity are shown for pH 6.0 5.0 and 4.0 (black triangles, squares and dots respectively). When these points are joined (dotted lines) it can be seen that the above relationships apply for the whole range of buffer systems investigated, but that dissolution is proportionally more at low pH and high molarity.

120-200 BOVINE ENAMEL DISSOLUTION IN ACETATE BUFFERS: 0·05, 0·1, 0·3, 0·5 AND 1·0 MOLAR
pH 6·5, 6·0, 5·5, 5·0, 4·5 AND 4·0



● 1 HOUR VALUE, pH 4·0 ■ 1 HOUR VALUE, pH 5·0 ▲ 1 HOUR VALUE, pH 6·0 3D RELATIONSHIPS

FIGURE 19

DECAY PLOTS OF FOUR IRRADIATED APATITE SAMPLES.

Samples of tricalcium phosphate (Victor)^{R}, unwashed and washed 120-200 mesh bovine enamel and washed 120-200 mesh human enamel were irradiated, dissolved in HCl, 10 mg. samples of each plated out onto stainless steel planchets, and count rates determined, at intervals, for 2 months. These rates, expressed as counts/min/mg. were plotted on semi-logarithmic paper against time (solid lines and black dots).

The decay rates obtained were identical for each of the four samples, and indicated a half-life of 14-15 days, suggesting the presence of P^{32}.

DECAY PLOTS OF FOUR APATITE SAMPLES

(IRRADIATED IN THERMAL NEUTRON FLUX OF 10^{11} η/sq cm/sec FOR 12 HOURS)

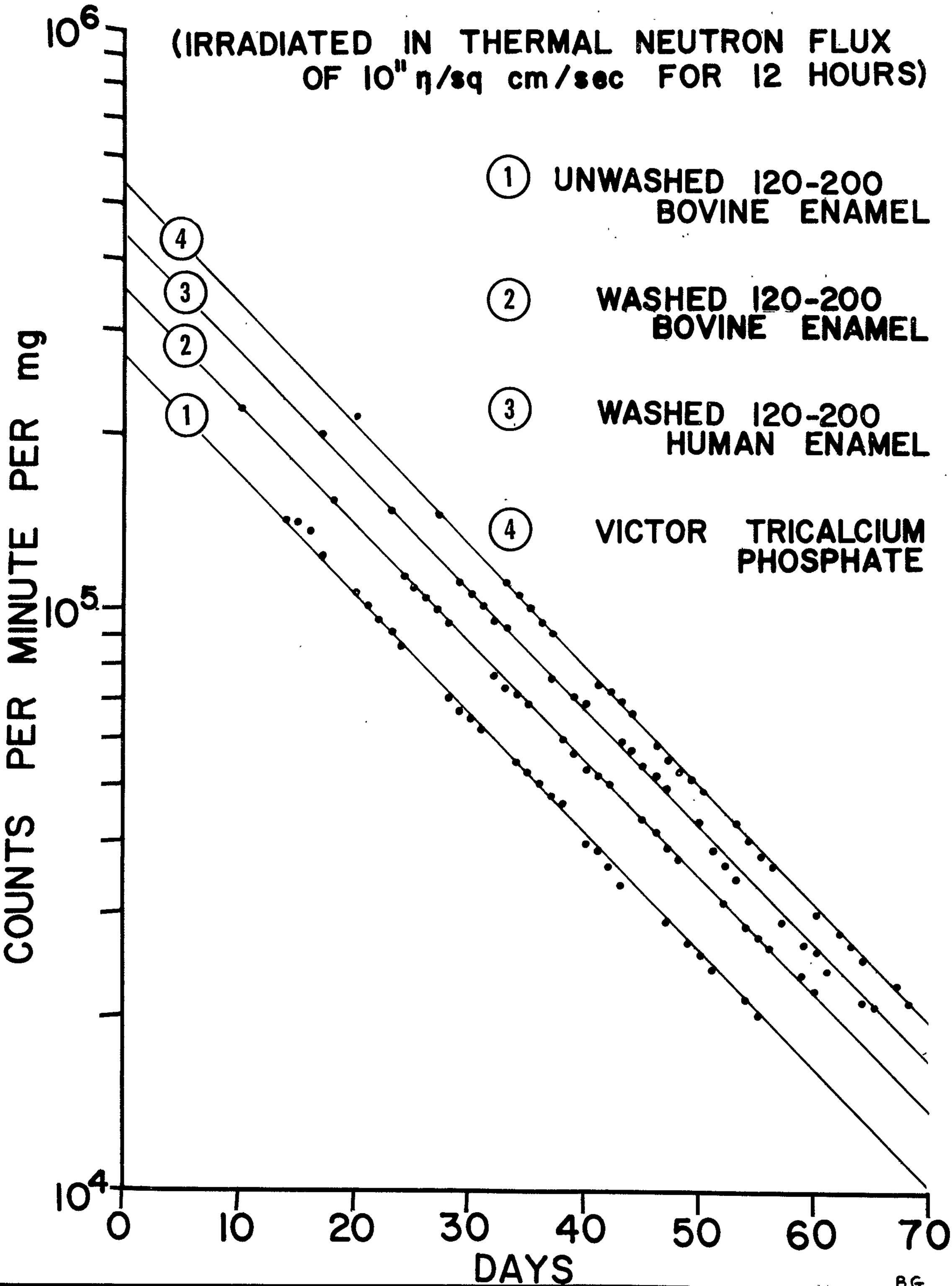


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FIGURE 20

LONG-TERM DECAY PLOT OF IRRADIATED BOVINE TOOTH ENAMEL.

A sample of irradiated bovine tooth enamel was dissolved in HCl, 10 mg. plated out onto a stainless steel planchet, and count rates determined at intervals for 7 months. The rates, expressed as counts/100 secs/0.1 mg., were plotted on semi-logarithmic paper (black dots).

The resulting plot (solid lines)is typical of a mixture of two radioactive species with different half-lives. Determined graphically, the half-lives appeared to be about 15 days and 100-200 days (by calculation, 14.5 and 174 days) uncertainty in the latter half-life being because of a low count rate, which precluded a more accurate determination. The half-lives of the two radioactive species thought to be present in the samples are, P32: 14.5 days, Ca45: 160 days. These values are in excellent agreement with the experimentally determined values.

BOVINE TOOTH ENAMEL

RADIOACTIVE DECAY CURVE

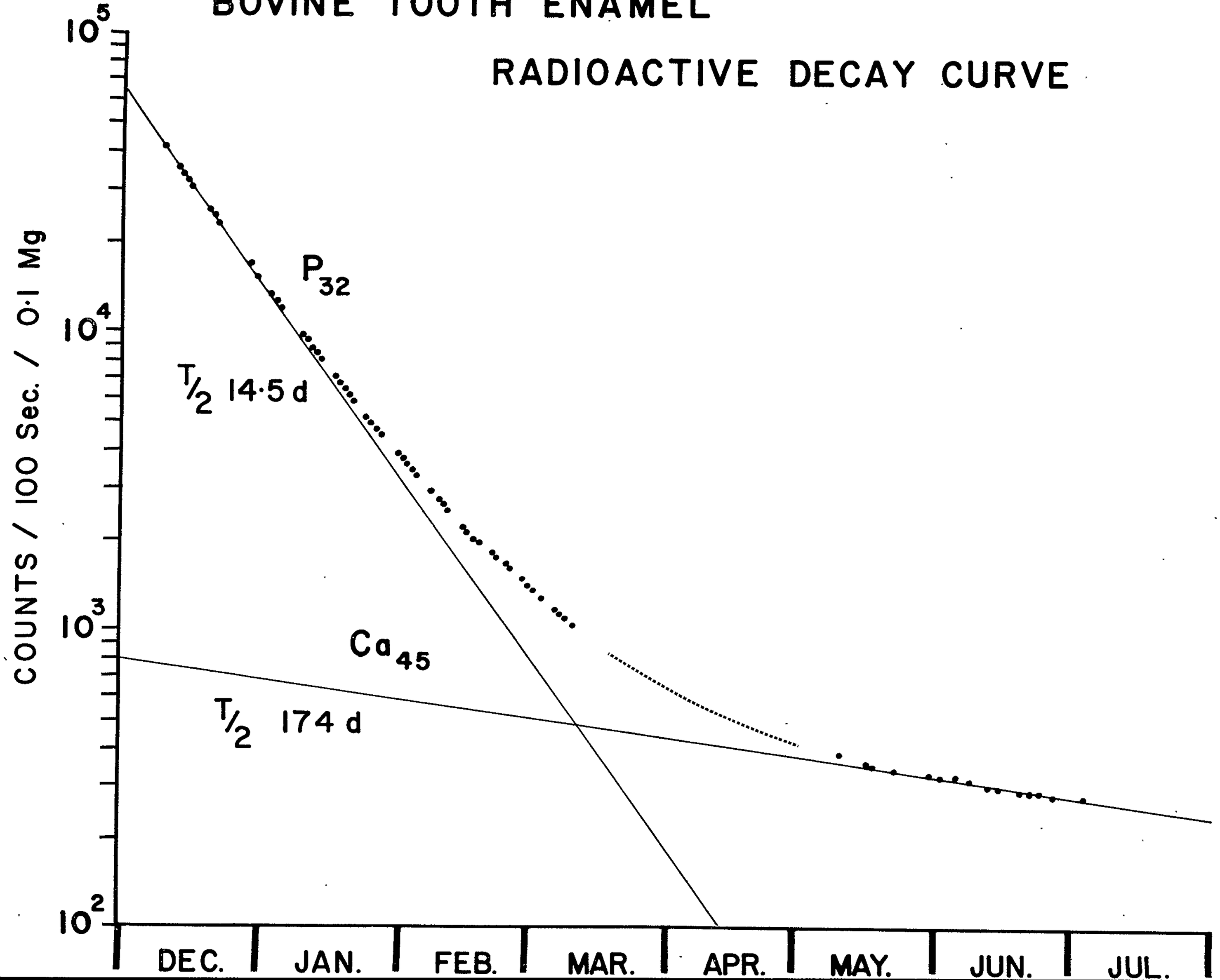


FIGURE 21

PRINCIPAL NUCLEAR TRANSFORMATIONS TAKING PLACE DURING AND AFTER IRRADIATION OF APATITES.

A P31 nucleus captures a neutron, emits a gamma ray (the capture gamma) and becomes an 'excited' P32 nucleus, which is radioactive.

At some indefinite future time, the 'excited' P32 nucleus becomes stable S32 by emitting its excess energy in the form of an electron (beta particle) whose energy lies in a specific range of values, from very low up to a definite maximum (the E_{max}), in this case, 1.71 Mev. The S32 nucleus has more positive charge than the P32 nucleus, and acquires an electron from its surroundings to complete its orbital shells, and thus gain electric neutrality. The rate at which P32 decays to S32 depends on the half-life, in this case 14.5 days.

A similar process takes place with Ca44. A Ca44 nucleus captures a neutron, emits a gamma ray (the capture gamma) and becomes an 'excited' Ca45 nucleus, which is radioactive.

At some indefinite future time, the 'excited' Ca45 nucleus becomes stable Sc45 by emitting its excess energy in the form of an electron (beta particle), whose energy lies in a specific range of values (E_{max}, 0.25 Mev.). The Sc45 nucleus has more positive charge than the Ca45 nucleus, and acquires an electron from its surroundings to complete its orbital shells, and thus gain electric neutrality. The rate at which Ca45 decays into Sc45 depends on the half-life, in this case 160 days.

(Note that in this legend, the correct superscript notation, thus: P^{31}, is not used, because of limitations of space).

$$\left[\begin{array}{l} {}_{15}P^{31} + {}_{0}n^{1} \rightarrow {}_{15}P^{32} + \gamma \quad \text{(ACTIVATION)} \\ {}_{15}P^{32} \rightarrow {}_{16}S^{32} + {}_{-1}e^{0} \quad (1{\cdot}71\ \text{Mev} \quad T/_2\ 14{\cdot}5\ \text{D}) \end{array}\right.$$

$$\left[\begin{array}{l} {}_{20}Ca^{44} + {}_{0}n^{1} \rightarrow {}_{20}Ca^{45} + \gamma \quad \text{(ACTIVATION)} \\ {}_{20}Ca^{45} \rightarrow {}_{21}Sc^{45} + {}_{-1}e^{0} \quad (0{\cdot}25\ \text{Mev} \quad T/_2\ 160\ \text{D}) \end{array}\right.$$

% ABUNDANCE	P^{31} : 100 ,	Ca^{44} : 2·1
CAPTURE CROSS SECTION (BARNS)	" ·029,	" ·013

BG

FIGURE 22

X-RAY DIFFRACTION ANALYSIS OF THREE APATITE SAMPLES, IRRADIATED AND NON-IRRADIATED.

Samples of bovine and human enamel powder and tricalcium phosphate (Victor)^{R} were prepared, and a portion of each irradiated. An irradiated and non-irradiated sample of each apatite was then submitted to x-ray diffraction analysis, using copper radiation, a nickel filter, 35Kv. at 30ma. and a θ-2θ scan.

In none of the three irradiated samples was there appreciable displacement of the characteristic apatite peaks as a result of the irradiation. This indicates that any change in crystal structure that may have occurred as a result of irradiation involves less than ten percent of the total sample.

THREE APATITE SAMPLES, IRRADIATED AND NON-IRRADIATED

X-RAY DIFFRACTION ANALYSIS
PHILIPS PW1060 35Kv 30ma
NICKEL FILTER
COPPER RADIATION (K_α)
αI 1·5405 αII 1·5443
θ-2θ SCAN

NON-IRRADIATED		IRRADIATED	
BOVINE	(1)	BOVINE	(2)
HUMAN	(3)	HUMAN	(4)
VICTOR Trical. Phos.	(5)	VICTOR Trical. Phos.	(6)

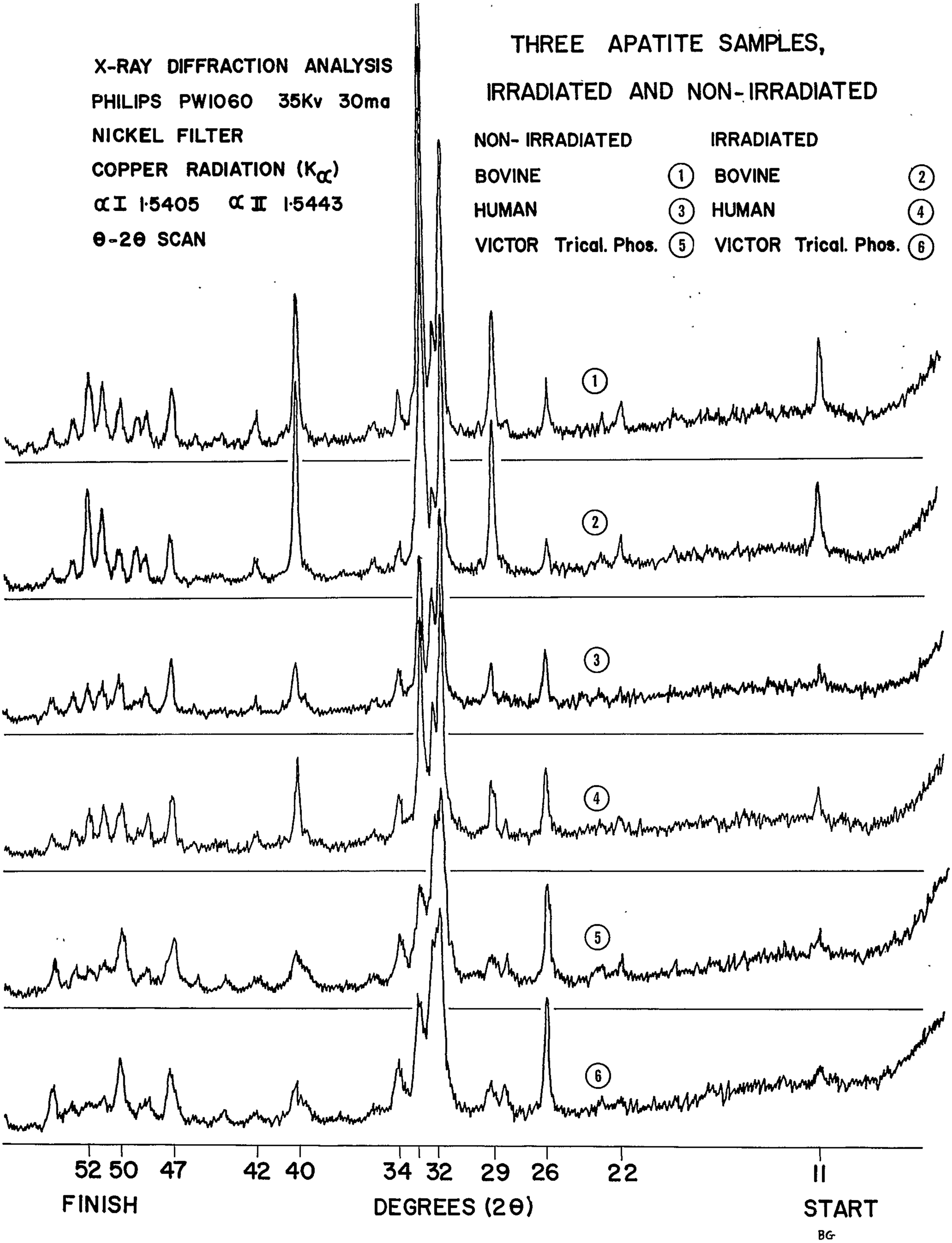


PLATE I

'BEVERAGES' GROUP

FOOD FERMENTATION BEVERAGES

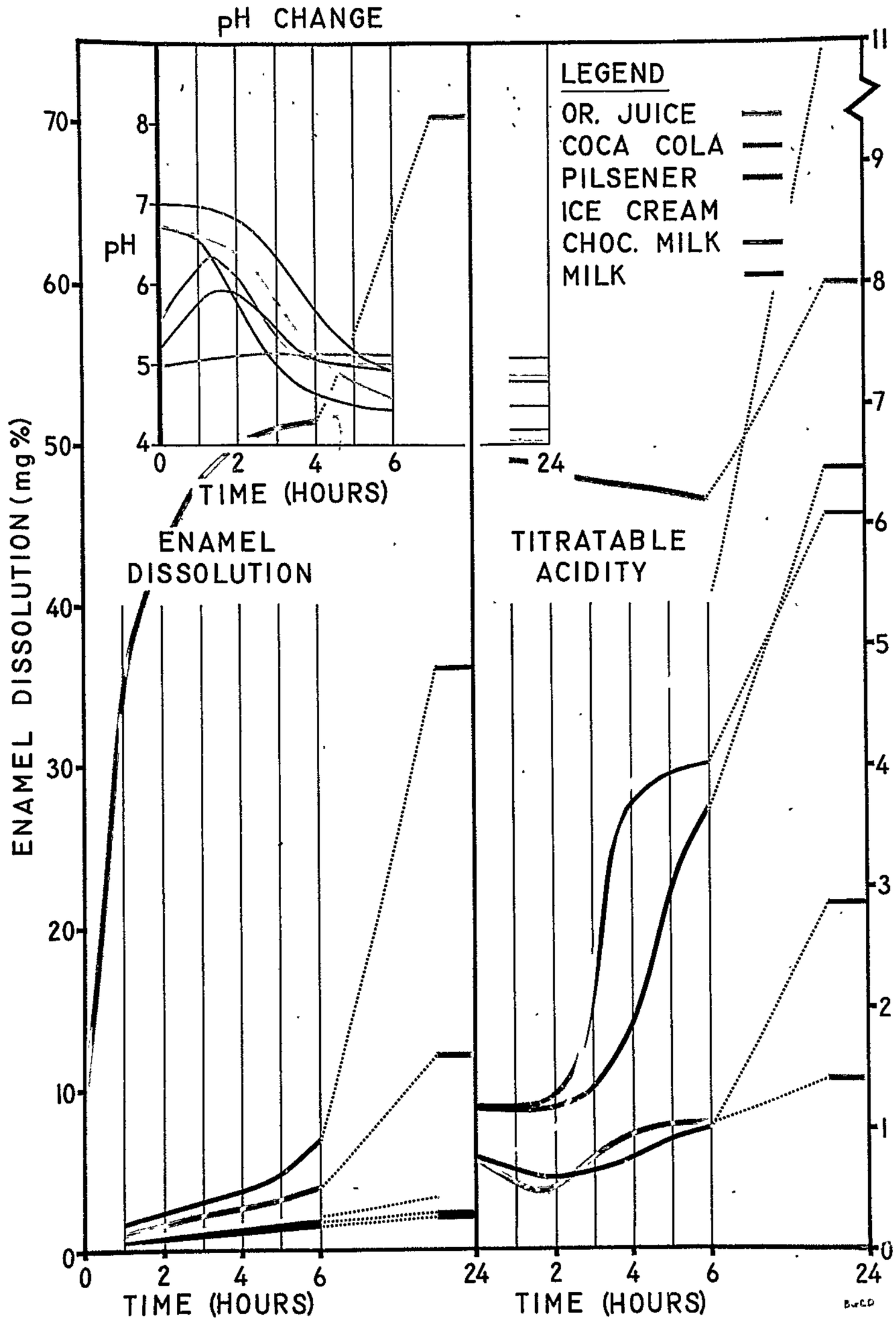


PLATE II

'SUGARS' GROUP

FOOD FERMENTATION SUGARS

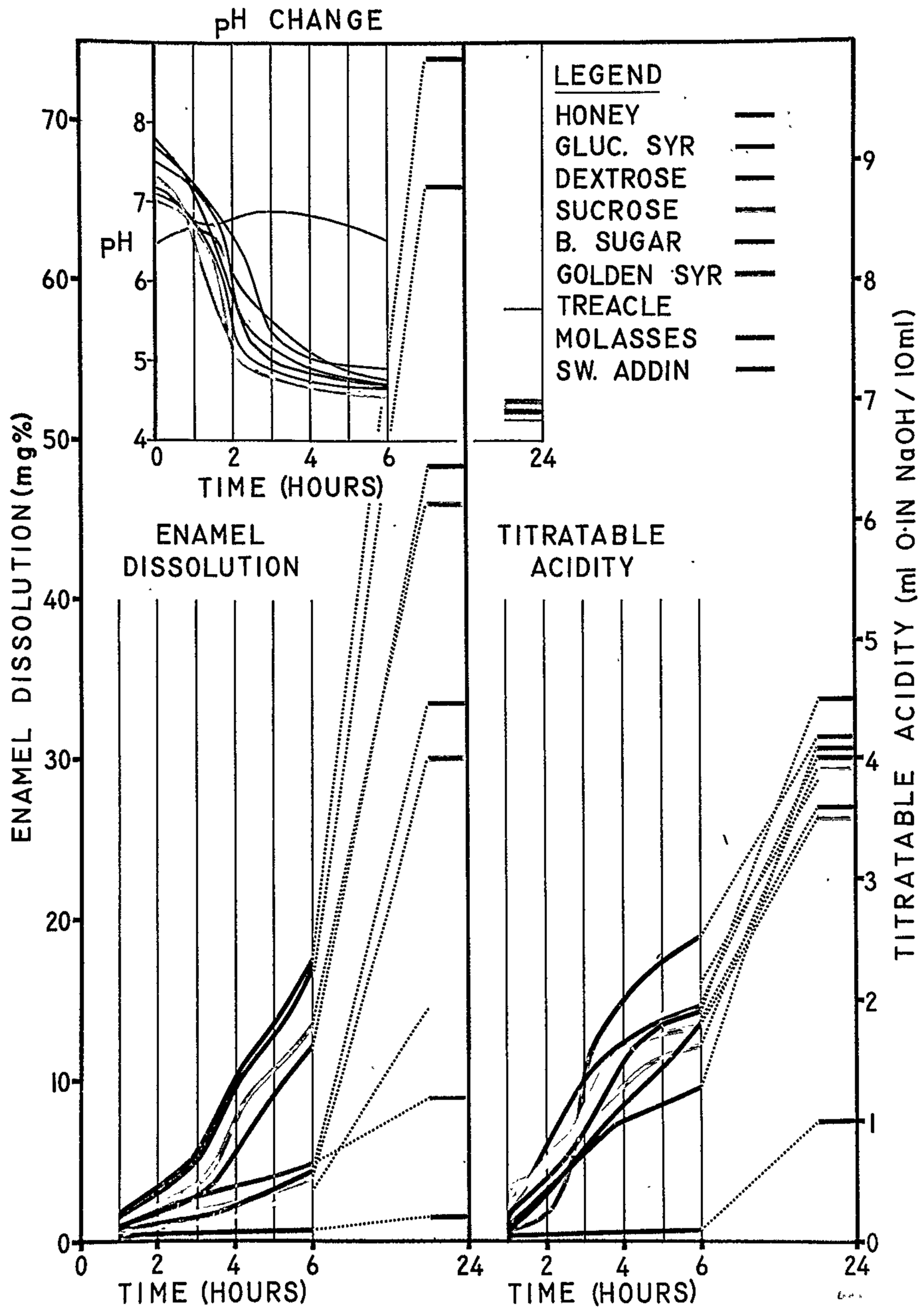


PLATE III

'FLOURS' GROUP

FOOD FERMENTATION FLOURS

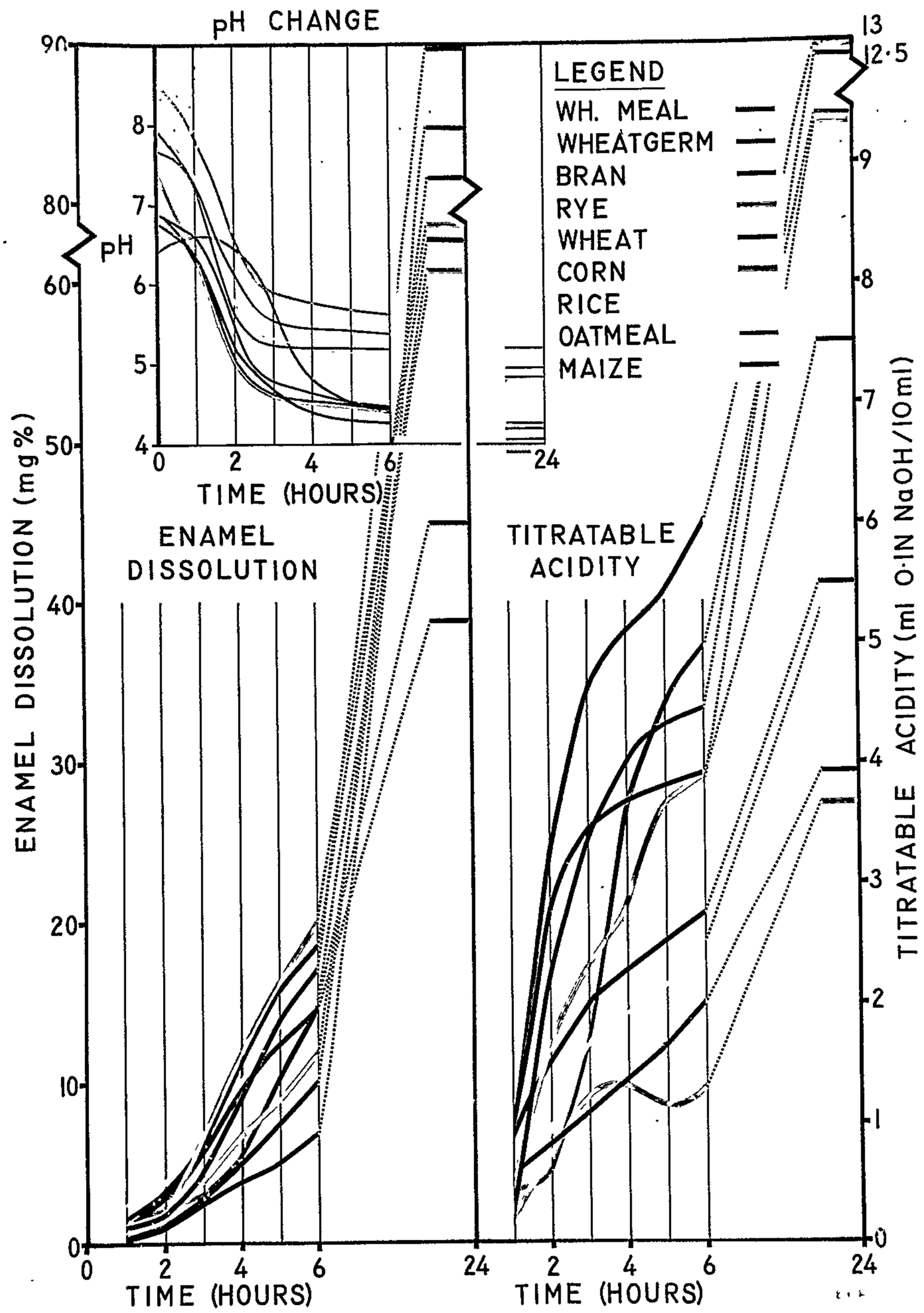


PLATE IV

'BREAD AND BISCUITS' GROUP

FOOD FERMENTATION

BREAD
BISCUITS

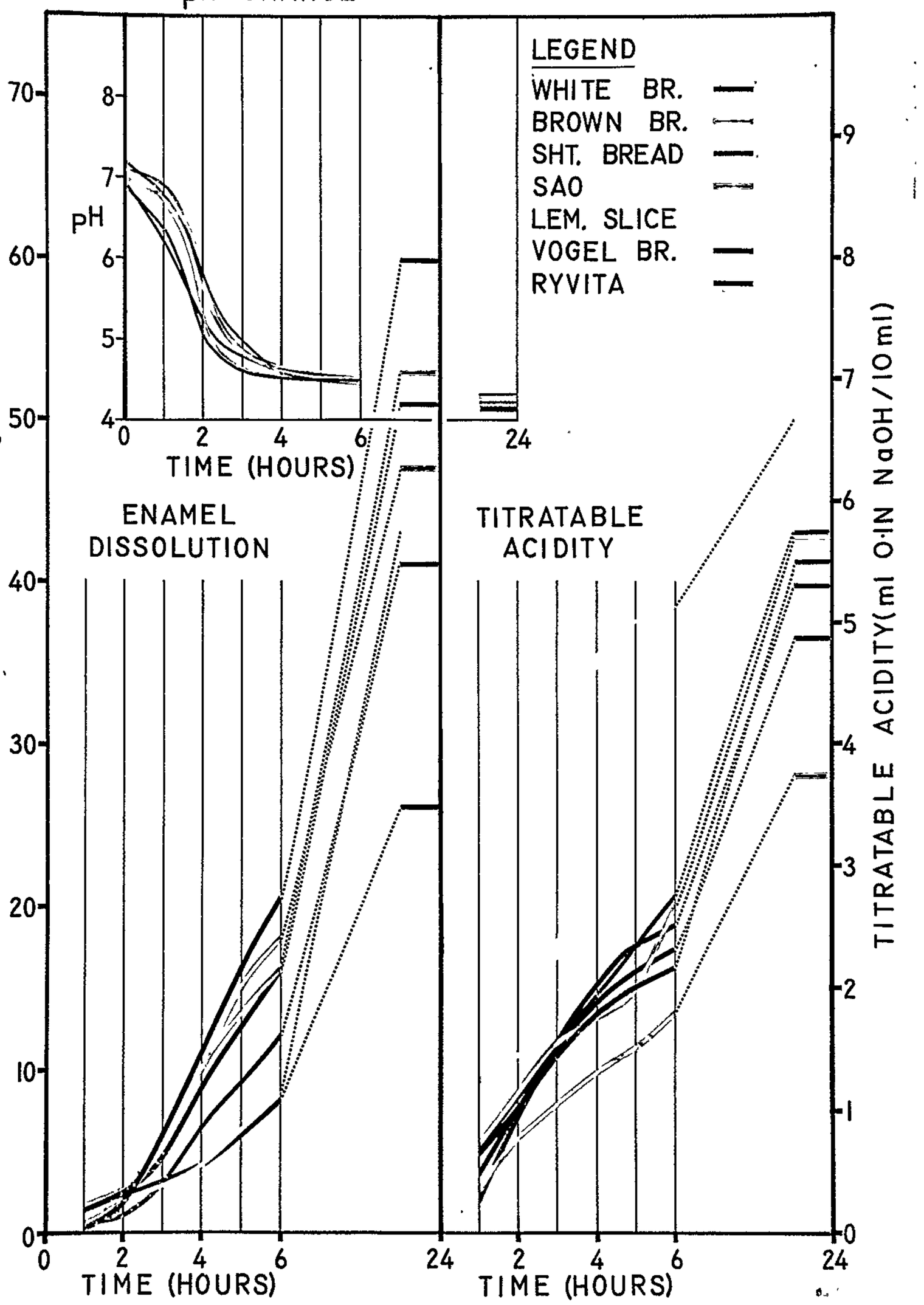


PLATE V

'CEREALS' GROUP

FOOD FERMENTATION CEREALS

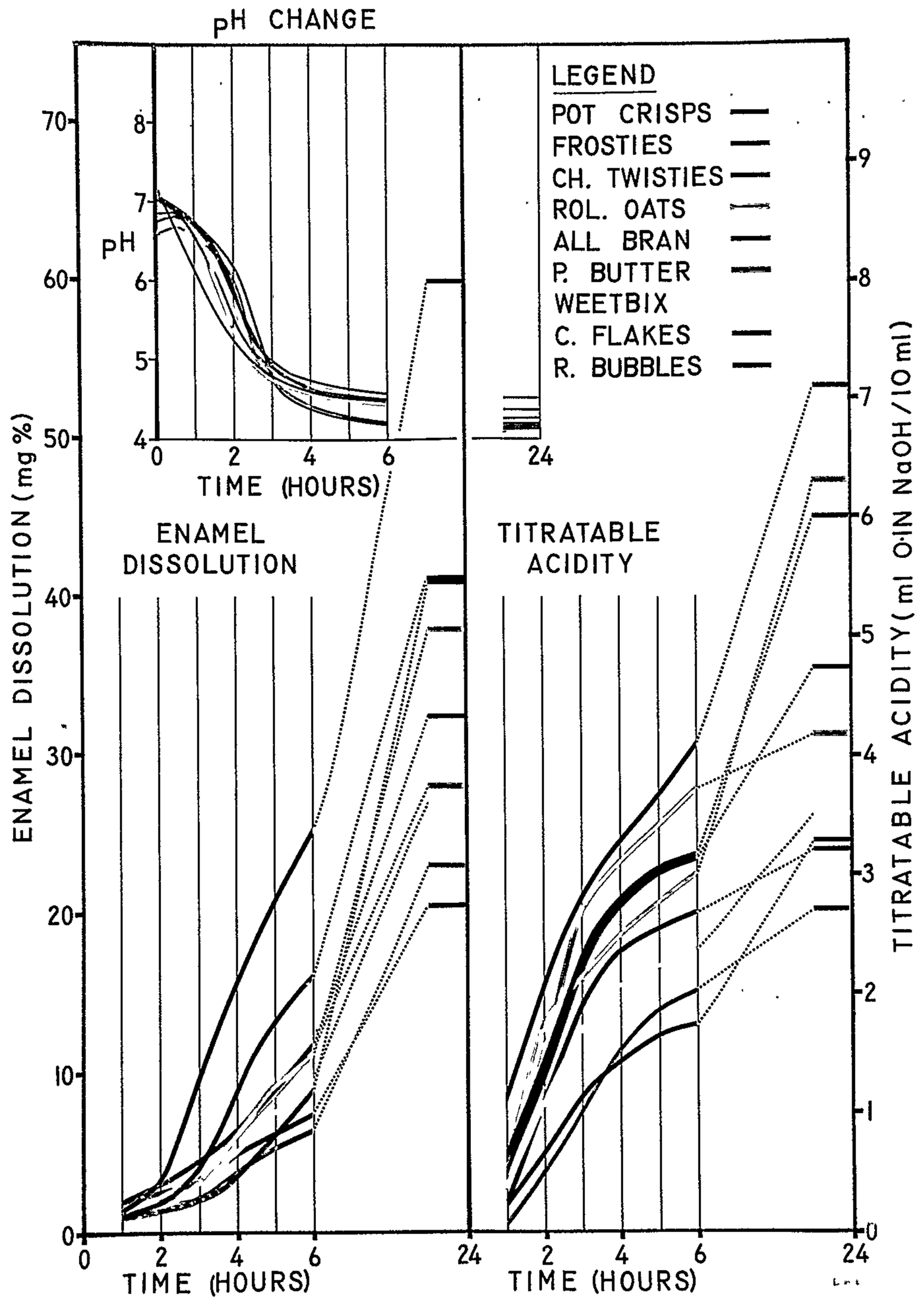
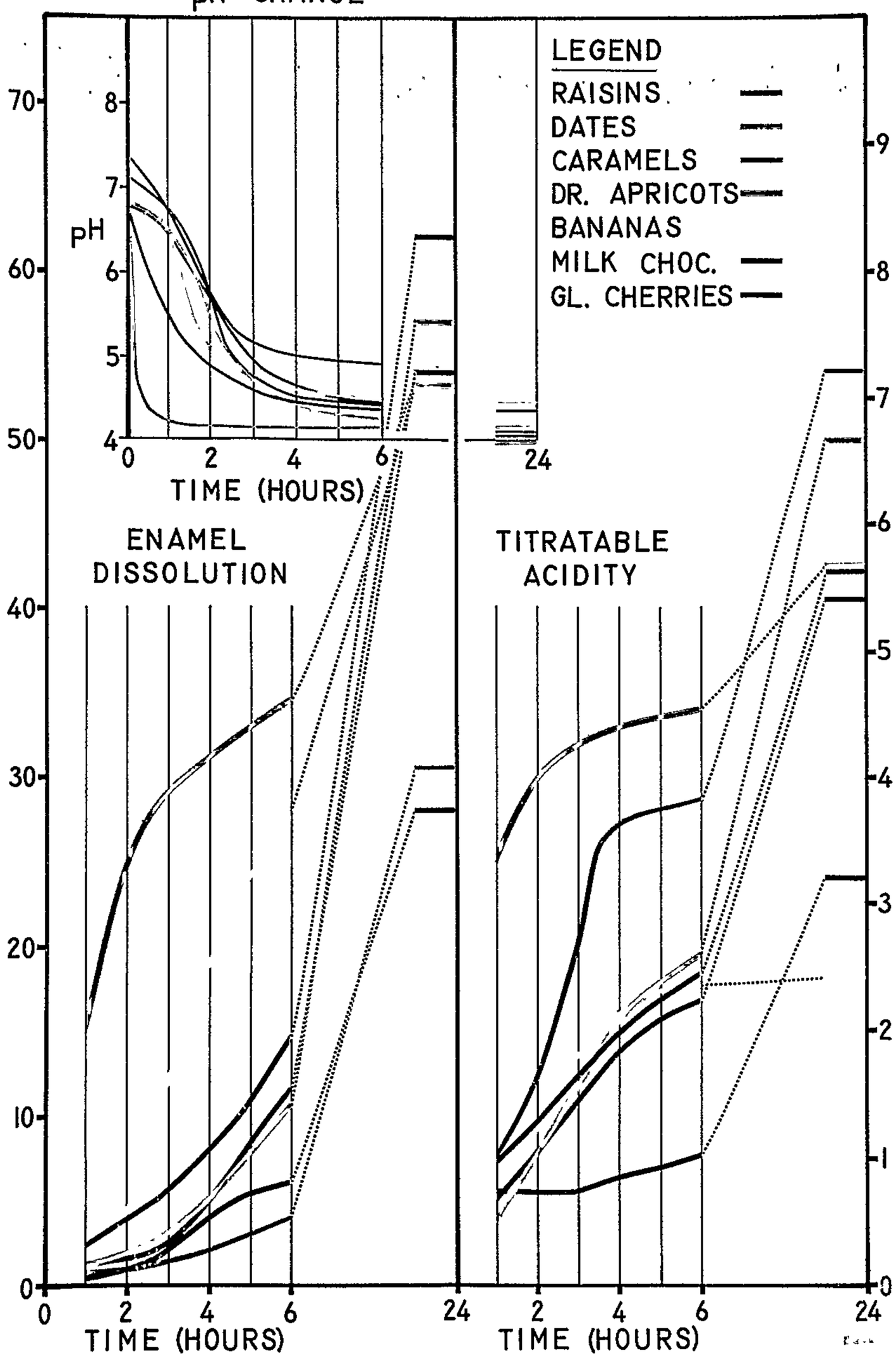


PLATE VI

'SWEETS AND FRUITS' GROUP

FOOD FERMENTATION SWEETS FRUITS



TABLES I - VI

U.S. FOOD TESTS

These tables list the food, the hour 0, 2, 4, 6 and 24 values for pH and titratable acidity (expressed as ml. of 0.1M NaOH required to titrate a 10ml. food saliva sample from its pH at that hour to pH 7.0.) and enamel dissolution (expressed as mg. of enamel dissolved in a mixture of 5gm. of the food, 50ml. of pooled saliva and 100mg. of washed radioactive 120-200 mesh bovine tooth enamel, agitated at a constant speed, the temperature being maintained at 37°C.)

In Table I only, 50ml. of each beverage was tested alone, without saliva, and also as a mixture of 25ml. of the beverage and 25ml. of saliva, the other experimental conditions being identical with the above.

TABLE I

BEVERAGES (U.S.)

FOOD	pH					TITRATABLE ACIDITY					ENAMEL DISSOLUTION				
HOUR	0	2	4	6	24	0	2	4	6	24	0	2	4	6	24
ORANGE JUICE (50ml.)	4.1	4.4	4.4	4.4	4.0	1.3	1.3	1.3	1.5	1.8	0	58	65	62	75
ORANGE JUICE (25ml.+25ml.saliva)	4.3	4.5	4.5	4.5	4.2	0.6	0.5	0.5	0.5	0.6	0	34	40	45	61
7 UP (50ml.)	4.1	4.5	4.5	4.7	4.7	1.6	1.3	1.3	1.3	1.3	0	26	27	28	32
7 UP (25ml.+25ml.saliva)	5.2	4.8	4.5	4.5	4.7	0.9	1.0	1.7	2.3	3.7	0	9	16	22	53
COCA COLA (50ml.)	4.0	4.6	4.6	4.6	4.2	0.9	0.9	0.9	0.9	0.9	0	19	19	20	26
COCA COLA (25ml.+25ml.saliva)	5.6	4.6	4.4	4.4	4.0	0.8	1.0	1.4	1.9	3.0	0	6	17	24	58
BEER (50ml.)	4.3	4.7	4.7	4.7	4.7	1.5	0.9	0.8	0.8	0.8	0	8	9	11	12
BEER (25ml.+25ml.saliva)	5.5	5.1	4.8	4.7	4.7	0.8	1.0	1.4	1.5	2.1	0	3	7	9	20

TABLE II

SUGARS (U.S.)

FOOD	pH					TITRATABLE ACIDITY					ENAMEL DISSOLUTION				
HOUR	0	2	4	6	24	0	2	4	6	24	0	2	4	6	24
HONEY	7.4	5.0	4.8	4.7	4.6	-	0.9	1.7	2.0	3.3	0	6	20	30	44
DEXTROSE	7.5	6.5	5.0	4.8	4.6	-	-	0.4	0.6	1.2	0	2	6	15	42
SUCROSE (mean of 8)	7.5	6.0	4.9	4.8	4.3	-	-	1.5	1.7	2.8	0	4	16	23	41
BROWN SUGAR	7.0	5.3	4.7	4.6	4.4	-	0.8	1.6	2.3	5.6	0	2	12	22	55
MOLASSES (light)	6.9	5.2	4.7	4.6	4.8	-	0.7	2.0	3.2	6.0	0	3	9	20	32
MOLASSES (dark)	6.6	5.5	4.8	4.7	4.6	-	1.8	3.8	3.9	4.6	0	3	6	12	22
MAPLE SYRUP	7.4	5.8	5.0	4.7	4.6	-	0.7	1.6	1.8	4.2	0	2	7	13	38

TABLE III

FLOURS (U.S.)

FOOD	pH					TITRATABLE ACIDITY					ENAMEL DISSOLUTION				
HOUR	0	2	4	6	24	0	2	4	6	24	0	2	4	6	24
WHEAT GERM	7.0	5.2	4.7	4.6	4.1	-	3.5	5.9	6.8	6.7	0	7	11	15	39
BRAN	7.0	5.3	4.7	4.6	4.7	-	3.8	7.5	8.0	5.8	0	3	6	11	23
RYE FLOUR	7.0	5.0	4.5	4.4	4.5	-	2.6	4.0	4.7	3.4	1	4	12	18	25
WHEAT FLOUR (Plain)	7.0	5.0	4.8	4.7	4.7	-	1.7	2.8	3.2	2.9	2	9	28	37	45
WHEAT FLOUR (enriched)	7.5	5.0	5.0	4.9	4.6	-	1.8	3.0	3.1	2.8	0	7	18	21	55
GRAHAM FLOUR	7.2	4.7	4.5	4.5	4.5	-	2.1	3.1	3.1	3.2	0	7	18	23	28
POTATO FLOUR	6.6	5.1	4.7	4.7	4.8	0.5	2.5	3.7	3.4	3.4	0	8	21	31	65
POTATO STARCH	7.0	6.0	5.0	4.9	4.3	-	0.6	0.9	1.1	1.1	1	2	8	11	67
CORN STARCH	7.3	5.0	4.7	4.6	4.0	-	1.0	1.9	2.1	2.5	0	5	20	28	62
POLISHED RICE FLOUR	7.3	5.3	4.7	4.5	4.2	-	1.4	2.4	2.4	4.5	0	3	17	22	61
BROWN RICE FLOUR	7.0	5.9	4.8	4.8	4.3	-	1.3	2.8	2.6	3.3	0	2	9	14	24
WILD RICE FLOUR	7.0	5.3	4.8	4.6	4.5	-	1.4	3.0	3.7	3.0	0	3	11	16	24
YELLOW CORN MEAL	7.0	5.0	4.8	4.7	4.0	-	2.2	3.8	4.1	4.2	0	9	18	23	33

TABLE IV

CEREALS (U.S.)

FOOD	pH					TITRATABLE ACIDITY					ENAMEL DISSOLUTION				
HOUR	0	2	4	6	24	0	2	4	6	24	0	2	4	6	24
CORN FLAKES	7.0	5.2	4.8	4.6	4.5	0.4	1.4	1.5	1.7	3.0	0	7	19	30	44
PUFFED RICE	7.0	5.0	4.6	4.5	4.5	-	1.2	1.9	1.7	2.0	0	4	15	25	40
SHREDDED WHEAT	7.0	5.0	4.7	4.7	4.5	-	1.6	2.3	1.8	3.1	0	6	13	16	22

TABLE V

BREAD AND BISCUITS

FOOD	pH					TITRATABLE ACIDITY					ENAMEL DISSOLUTION				
HOUR	0	2	4	6	24	0	2	4	6	24	0	2	4	6	24
WHITE BREAD	7.1	4.8	4.6	4.6	4.7	-	1.9	2.5	2.5	2.0	0	10	28	36	41
WHOLE WHEAT BREAD	7.0	6.4	5.0	4.8	4.8	-	0.8	2.4	3.4	2.7	0	2	5	10	13
WHOLE GRAIN BREAD	7.0	5.0	4.9	4.8	4.6	-	1.0	2.2	1.9	2.1	0	5	17	20	31
SALTINES	7.4	4.9	4.6	4.4	4.3	-	1.5	1.5	1.4	2.8	0	5	18	24	37
CRACKERS	7.1	5.0	4.6	4.6	4.5	-	1.2	2.3	2.7	2.0	0	7	21	28	42
SHORTBREAD	7.5	5.0	4.7	4.6	4.4	-	1.4	2.3	2.6	2.5	0	5	15	23	33
FIG NEWTON	7.4	4.9	4.6	4.5	4.4	-	1.5	2.2	2.3	2.5	0	8	21	30	58

TABLE VI

CONFECTIONERY (U.S.)

FOOD	pH					TITRATABLE ACIDITY					ENAMEL DISSOLUTION				
HOUR	0	2	4	6	24	0	2	4	6	24	0	2	4	6	24
MILK CHOCOLATE	7.1	6.8	5.0	4.6	4.5	-	0.2	1.6	2.3	3.0	0	2	4	12	20
CHOCOLATE-COVERED COCONUT	7.2	5.2	4.8	4.6	4.5	-	1.4	2.2	2.6	2.8	0	5	13	19	61
CARAMELS	7.2	5.3	5.0	5.0	4.8	-	1.6	2.7	2.9	3.2	0	2	6	6	11

TABLE VII

INHERENT BUFFER CAPACITY U.S. FOODS

This table lists the inherent buffer capacity of the foods in Tables I-VI. Inherent buffer capacity of a food is the term used in this work to describe the amount of 0.1M of HCl (in ml.) required to lower the pH of 10 ml. of a mixture of 5 gm. of food and 50ml. of water to the pH attained by 5gm. of the same food and 50ml. of saliva after 24 hours of fermentation.

The inherent buffer capacity of pooled saliva is the term used here to describe the amount of 0.1 M HCl. (in ml.) required to lower the pH of 10ml. of the saliva from its initial pH (about 7.5) to 4.0.

TABLE VII

INHERENT BUFFER CAPACITY OF TEST FOODS (U.S. FOODS)

Volume of 0.1M HCl. (in ml.) required to change pH of 10 ml. of a mixture of 5 gm. of food and 50 ml. of water from initial value to hour 24 fermentation value.

FOOD	VOLUME
ALL BEVERAGES: ORANGE JUICE, 7 UP, COCA COLA, BEER (Note that the inherent buffer capacities of the beverages alone was NIL, by this test, as their pH's were low initially. As used in the food tests (25 ml. of beverage and 25 ml. of saliva) the inherent buffer capacities of the mixtures were about half that of saliva alone).	NIL (i.e. initial pH similar to hour 24 value).
HONEY, DEXTROSE, SUCROSE, WHEAT FLOUR, POTATO STARCH, CORN STARCH, CORN FLAKES, PUFFED RICE, WHITE BREAD, WHOLE WHEAT BREAD	0-0.2 ml./10 ml. (0-1 ml./50 ml.)
BROWN SUGAR, MAPLE SYRUP, WHEAT FLOUR (ENRICHED), GRAHAM FLOUR, POLISHED RICE FLOUR, WILD RICE FLOUR, YELLOW CORN MEAL, SHREDDED WHEAT, WHOLE GRAIN BREAD, SHORTBREAD, FIG NEWTON, MILK CHOCOLATE, CHOCOLATE-COVERED COCONUT, CARAMELS	0.2-0.6 ml./10 ml. (1-3 ml./50 ml.)
LIGHT MOLASSES, DARK MOLASSES, BRAN, RYE FLOUR, POTATO FLOUR, BROWN RICE FLOUR, CRACKERS, SALTINES	0.6-1.2 ml./10 ml. (3-6 ml./50 ml.)
WHEAT GERM	2.8 ml./10 ml. (14 ml./50 ml.)
50 ml. POOLED SALIVA pH 7.5 to pH 4.0	1.2-1.6 ml./10 ml. (6-8 ml./50 ml.)

TABLE VIII

INHERENT BUFFER CAPACITY, AUSTRALIAN FOODS

This table lists the inherent buffer capacity of the foods in Plates I–VI. Inherent buffer capacity of a food is the term used in this work to describe the amount of 0.1M HCl (in ml.) required to lower the pH of 10 ml. of a mixture of 5 gm. of food and 50ml. of water to the pH attained by 5gm. of the same food and 50ml. of saliva after 24 hours of fermentation.

The inherent buffer capacity of pooled saliva is the term used here to describe the amount of 0.1M HCl (in ml.) required to lower the pH of 10ml. of the saliva from its initial pH (about 7.5) to 4.0.

TABLE VIII

INHERENT BUFFER CAPACITY OF TEST FOODS (AUSTRALIAN FOODS)

Volume of 0.1M HCl. (in ml.) required to change the pH of 10 ml. of a mixture of 5 gm. of food and 50 ml. of water from initial value to hour 24 fermentation value .

FOOD	VOLUME
ORANGE JUICE, COCA COLA, PILSENER, DRIED APRICOTS, SWEETADDIN (Note that the inherent buffer capacities of the beverages alone was NIL, by this test, as their pH's were low initially. As used in the food tests (25 ml. of beverage and 25 ml. of saliva) the inherent buffer capacities of the mixtures were about half that of saliva alone).	NIL (i.e. initial pH similar to hour 24 value).
HONEY, DEXTROSE, SUCROSE, GLACÉ CHERRIES, FROSTIES, WHEAT FLOUR, CORN FLAKES, RICE FLOUR, GLUCOSE SYRUP	0-0.2 ml./10 ml. (0-1 ml./50 ml.)
WHITE BREAD, RAISINS, BROWN SUGAR, RICE BUBBLES, SAO, BANANAS, BROWN BREAD, LEMON SLICE, RYVITA, VOGEL BREAD, CARAMELS, SCOTCH FINGER, WEETBIX, DATES	0.2-0.6 ml./10 ml. (1-3 ml./50 ml.)
MILK CHOCOLATE, MAIZE, TREACLE, GOLDEN SYRUP, ROLLED OATS, FINE OATMEAL, ALL BRAN	0.6-1.2 ml./10 ml. (3-6 ml./50 ml.)
CHEESE TWISTEES, POTATO CRISPS, PEANUT BUTTER, WHOLEMEAL FLOUR, WHEAT GERM, MOLASSES, MILK, RYE FLOUR, BRAN	1.2-2.4 ml./10 ml. (6-12 ml./50 ml.)
CHOCOLATE MILK, ICE CREAM	2.2-4.4 ml./10 ml. (11-22 ml./50 ml.)
50 ml. POOLED SALIVA pH 7.5 to pH 4.0	1.2-1.6 ml./10 ml. (6-8 ml./50 ml.)

TABLE IX

COMPARISON OF RAT CARIES SCORES (STEPHAN) FOR HUMAN FOODS (I) WITH FOOD TEST RESULTS FOR COMPARABLE AUSTRALIAN FOODS (II).

The left-hand side of this table(I)lists some of the human foods tested by Stephan, and his assessment of their ability to induce caries and erosion in rats.

The right-hand side of this table(II)lists the hour 6 and hour 24 enamel dissolution values obtained in the food tests for comparable or similar Australian foods, and whether they caused in-vitro erosion (i.e. enamel dissolution in the absence of acid production by fermentation).

Also listed in this table are the hour 6 and hour 24 food test enamel dissolutions for sucrose containing 1% Calcium sucrose phosphate, and for unrefined sugars, cereals and some grains.

TABLE IX

COMPARISON OF RAT CARIES SCORES (STEPHAN) FOR HUMAN FOODS (I) WITH FOOD TEST RESULTS FOR COMPARABLE AUSTRALIAN FOODS (II)

I			II			
TEST FOOD	STEPHAN GROUP	EROSION	TEST FOOD	ENAMEL DISSOLUTION Hour 6	ENAMEL DISSOLUTION Hour 24	EROSION
Sorbitol Peanuts Whole milk	A Reduced caries		Sweetaddin Peanut butter Whole milk	1 11 2	2 28 3	
Dried apricots Oranges	B Minimal caries	Dried apricots Oranges	Dried apricots Orange juice	34 52	53 71	Dried apricots Orange juice
Soda crackers Cracked wheat bread Potato chips	C Slight caries		Sao biscuit Vogel bread Potato Crisps	18 12 25	47 42 60	
Whole wheat bread Corn starch White bread	D Moderate caries		Brown bread Corn flour White bread	16 12 21	53 61 60	

Continued..

TABLE IX

(Continued)

COMPARISON OF RAT CARIES SCORES (STEPHAN) FOR HUMAN FOODS (I) WITH FOOD TEST RESULTS FOR COMPARABLE AUSTRALIAN FOODS (II)

I				II			
TEST FOOD	STEPHAN GROUP		EROSION	TEST FOOD	ENAMEL DISSOLUTION Hour 6	ENAMEL DISSOLUTION Hour 24	EROSION
Caramels	E	Severe caries		Caramels	11	54	
Bananas				Bananas	28	53	
Cola			Cola	Coca Cola	7	37	Coca Cola
Dextrose				Dextrose	12	48	
Raisins				Raisins	14	62	
Dates				Dates	10	57	
Milk chocolate				Milk chocolate	4	31	
Sucrose				Sucrose	13	46	
None comparable				Sucrose + 1% calcium sucrose phosphate	2	4	
None comparable				Unrefined sugars, cereals, some grains	Less than 12	Less than 30	

TABLE X

COMPARISON OF VIPEHOLM STUDY CARIES SCORES (I) WITH FOOD TEST RESULTS FOR COMPARABLE AUSTRALIAN FOODS (II).

The left-hand side of this table (I) lists the various groups employed in the Vipeholm study, and the average number of new carious surfaces appearing per person per year, in each group, for years 4 and 5, the years during which the stated additions were made to the diets (except for the Toffee Group II, where the stated addition was made during years 2 and 3).

The right-hand side of this table (II) lists the hour 6 and hour 24 enamel dissolution values obtained in the food tests for Australian foods comparable with the test foods of the Vipeholm Study. Toffees as such were not tested in the present study. Since toffees are made from glucose syrups, the table compares the Vipeholm study results for toffees with the Australian food tests for glucose syrup.

TABLE X

COMPARISON OF VIPEHOLM STUDY CARIES SCORES (I) WITH FOOD TEST RESULTS ON COMPARABLE AUSTRALIAN FOODS (II)

I		II		
VIPEHOLM GROUPS	NEW CARIOUS SURFACES PER PERSON PER YEAR (av.of years 4 & 5)	TEST FOOD	ENAMEL DISSOLUTION	
			Hour 6	Hour 24
CONTROL GROUP (Basic diet plan)	0.7		Less than 12 mg.	Less than 30 mg.
SUCROSE GROUP (Basic diet plan plus sucrose,solid and liquid, at meals)	0.9	Sucrose	12	46
BREAD GROUP (Basic diet plan plus high sugar bread at meals)	1.5	White bread	20	60
CHOCOLATE GROUP (Basic diet plan plus sucrose liquid at meals & chocolate between meals)	1.4	Milk chocolate	4	31
CARAMEL GROUP (Basic diet plan plus caramels between meals)	2.2	Caramels	12	54

Continued..

TABLE X

(Continued)

COMPARISON OF VIPEHOLM STUDY CARIES SCORES (I) WITH FOOD TEST RESULTS ON COMPARABLE AUSTRALIAN FOODS (II)

I		II		
VIPEHOLM GROUPS	NEW CARIOUS SURFACES PER PERSON PER YEAR (av.of years 4 & 5)	TEST FOOD	ENAMEL DISSOLUTION	
			Hour 6	Hour 24
TOFFEE GROUP I (Basic diet plan plus sucrose liquid at meals & toffees between meals)	3.6	Glucose syrup	17	66
TOFFEE GROUP II (Basic diet plan plus sucrose liquid at meals & toffees between meals)	4.5 (av. of years 2 & 3)			

TABLE XI

COMPARISON OF FOOD FERMENTATION RESULTS FOR SUCROSE ALONE, MOLASSES ALONE AND SUCROSE CONTAINING 1% CALCIUM SUCROSE PHOSPHATE.

This table compares the pH,titratable acidity and enamel dissolution changes occurring at hours 0, 2, 4, 6 and 24, in the food tests for sucrose, molasses and sucrose containing 1% calcium sucrose phosphate. Each test was performed three times and the results shown are the means for three tests.

The pH and titratable acidity changes in the sucrose and sucrose plus calcium sucrose phosphate tests were similar. With molasses, the initial pH of the food-saliva mixture was lower, but this difference had disappeared by hour 4. After hour 2, molasses titratable acidity was considerably higher than the titratable acidity for both sucrose samples.

Enamel dissolution was high for sucrose, low for molasses and very low for sucrose plus calcium sucrose phosphate. The differences are especially noticeable after hour 4, where sucrose dissolution is 7, 12 and 15 times higher at hours 4, 6 and 24 respectively,in comparison with sucrose containing 1% by weight calcium sucrose phosphate.

TABLE XI

COMPARISON OF FOOD FERMENTATION RESULTS FOR SUCROSE, MOLASSES AND SUCROSE CONTAINING 1% CALCIUM SUCROSE PHOSPHATE

FOOD	pH					TITRATABLE ACIDITY					ENAMEL DISSOLUTION				
HOUR	0	2	4	6	24	0	2	4	6	24	0	2	4	6	24
SUCROSE	7.3	5.2	4.7	4.6	4.4	-	0.8	1.3	1.6	3.9	0	2	7	12	46
MOLASSES	6.5	6.1	5.1	4.8	4.5	-	0.2	2.0	2.5	4.2	0	2	3	4	8
SUCROSE + 1% CALCIUM SUCROSE PHOSPHATE	7.2	6.2	5.2	4.8	4.5	-	0.8	1.4	1.8	3.2	0	1	1	2	3

TABLE XII

COMPOSITION OF THE FOODS TESTED

U.S. FOODS

This table lists the common name, manufacturer or brand name, and description, together with comments on the approximate composition or technical description where it is available and pertinent, of the U.S. foods tested in the preliminary experiments.

TABLE XII

COMPOSITION OF THE FOODS TESTED (U.S. FOODS)

COMMON NAME	MANUFACTURER/ BRAND NAME	DESCRIPTION	COMMENTS
ORANGE JUICE	Crosse & Blackwell	Frozen, canned, concentrate.	Diluted with tap water to fresh orange juice strength
7 UP	7 UP Bottling Company	Uncoloured, lemon-flavoured, carbonated beverage	Sucrose, acidulant, CO_2, water, flavouring
COCA COLA	Coca Cola Company	Cola-flavoured, carbonated beverage	10-11 gm./100ml. sucrose, CO_2, phosphoric acid, water, flavouring
BEER	Genessee Brewing Co.	Light, Danish lager-type beer	
HONEY	Carey's crystal clear	Clear, amber, free-running, clover-type honey	Dextrose, Fructose, Minerals
DEXTROSE	Baker's	Analytical, Reagent Grade	
SUCROSE	Domino	See Table XIII	
BROWN SUGAR	Domino	See Table XIII	
LIGHT MOLASSES	Brer Rabbit	See Table XIII	

Continued..

TABLE XII

(Continued)

COMPOSITION OF THE FOODS TESTED (U.S. FOODS)

COMMON NAME	MANUFACTURER/ BRAND NAME	DESCRIPTION	COMMENTS
DARK MOLASSES	Brer Rabbit	See Table XIII	
MAPLE SYRUP	Nature's Gold Cup	Boiled, concentrated sap of Maple trees.	
WHEAT GERM	Atlantic	Part of wheat grain left after outer casing and flour extracted	Contains 24% protein, 8% fat, 4% fibre, carbohydrates, water
BRAN	Atlantic	Outer casing of wheat grain	
WHEAT FLOUR (PLAIN)	Byrd Mill	Milled wheat with bran and wheat germ removed	Contains 50% starch 12% protein, 2% sugars, 12% water, ash, fats
WHEAT FLOUR (ENRICHED)	Byrd Mill	As above, with additives to improve handling & nutritional qualities	Wheat Flour (plain & enriched) are 70% extraction flours
GRAHAM FLOUR			
POTATO FLOUR	Atlantic	Flour from whole potatoes	
POTATO STARCH	Richters	Starch granules from potatoes	

Continued..

TABLE XII

(Continued)

COMPOSITION OF THE FOODS TESTED (U.S. FOODS)

COMMON NAME	MANUFACTURER/ BRAND NAME	DESCRIPTION	COMMENTS
CORN STARCH	Argo	Starch granules from corn	
POLISHED RICE FLOUR	Atlantic	Flour made in laboratory from whole grains of polished rice	
BROWN RICE FLOUR	Riceland	As above, but made from rice retaining part of outer casing	
WILD RICE FLOUR	Nokomis	As above, but made from a special, unpolished rice, grown in North America	
YELLOW CORN MEAL	Byrd Mill	Milled whole corn	
WHITE BREAD	Pepperidge Farm	Soft,sliced,wrapped sandwich loaf, with soft crust	Made from 70% extraction flour
WHOLEMEAL BREAD	Pepperidge Farm	As above, but made from wholemeal flour	
WHOLEGRAIN BREAD	Monks	Heavy,moist bread, made from milled whole wheat grains	

Continued..

TABLE XII

(Continued)

COMPOSITION OF THE FOODS TESTED (U.S. FOODS)

COMMON NAME	MANUFACTURER/ BRAND NAME	DESCRIPTION	COMMENTS
SALTINES	Keebler	Dry, leavened, unbrowned unsweetened, salt-dusted, soda-cracker type biscuit	
CRACKERS	Ritz	Leavened, browned, unsweetened, oil-sprayed, soda-cracker type biscuit	
SHORTBREAD	Lorna Doone	Sweetened biscuit	Contains fats and oils (50% butter)
FIG NEWTON	Nabisco	Sweetened biscuit, covering a soft, dried-fig filling	
MILK CHOCOLATE	Hershey	Plain, light-coloured chocolate	Cocoa powder, cocoa fat, sucrose, milk, solids, milk fat
CHOCOLATE-COVERED COCONUT	Peter Paul Mound Bar	Soft, sweet candy bar	Desiccated coconut & sugar binder, covered with milk chocolate
CARAMELS	Kraft	Soft, brown toffee	Glucose, sucrose, milk solids, butter fats

TABLE XIII

COMPOSITION OF THE FOODS TESTED

AUSTRALIAN FOODS

This table lists the common name, manufacturer or brand name, and description, together with comments on the approximate composition or technical description, where it is available and pertinent, of the Australian foods tested in this study.

TABLE XIII

COMPOSITION OF THE FOODS TESTED (AUSTRALIAN FOODS)

COMMON NAME	MANUFACTURER/ BRAND NAME	DESCRIPTION	COMMENTS
ORANGE JUICE	Berri	Frozen, canned concentrate	Diluted 1:3 with water
COCA COLA	Coca Cola Co.	Cola-flavoured, carbonated beverage	See Table XII
PILSENER	Reschs	Light Danish, lager-type beer	
ICE CREAM	Peters	Vanilla-flavoured ice cream	10% milk solids, 10% butter fat, 15% sucrose, alginate
CHOCOLATE MILK	Nestles Quik	Milk to which Quik has been added to taste	Chocolate powder, containing fine sucrose
MILK	N.S.W. Dairy Farmers	Pasteurised, conforming to appropriate British Standards	78.5% non-fat solids, 3.2% butter fat. 4-5% lactose, 3-4% protein, 0.3-0.4% Ca & P minerals
HONEY	Smith's Capilano	Brisbane clear, amber, free-running	See Table XII
GLUCOSE SYRUP	Maize Products	Inverted grain starch, (called Corn Syrup in U.S.)	80% sugars (dextrose, maltose, tri- and higher saccharides)
DEXTROSE	B.D.H.	Analytical Reagent grade	
SUCROSE	C.S.R.	Refined, granulated sugar	0.01% ash, 15-40 ppm. Ca.

Continued..

TABLE XIII

(Continued)

COMPOSITION OF THE FOODS TESTED (AUSTRALIAN FOODS)

COMMON NAME	MANUFACTURER/ BRAND NAME	DESCRIPTION	COMMENTS
BROWN SUGAR	C.S.R.	Unwashed, crystallised product of inferior quality mother liquor	1% Ash, 100 ppm Ca.
GOLDEN SYRUP	C.S.R.	Different levels of refinement and evaporation of mother liquor	3% Ash
TREACLE	C.S.R.	remaining after crystallisation from crude sugar solution	4% Ash
MOLASSES	Blue Label,	Treated, evaporated residue of cane juice after crude sugar has been crystallised out	30% Sucrose, 15% reducing sugars, 20% water, 10% ash, (10-18% Ca, 2-5%P), calcium sucrose phosphate
WHOLEMEAL FLOUR	Nugrist	Milled Whole wheat grains	See Text (Appx.E)
WHEAT GERM	Nugrist	As for U.S.	See Text (Appx.E)
BRAN	Nugrist	As for U.S.	See Text (Appx.E)
RYE FLOUR	Nugrist	Milled Whole rye grains	
WHEAT FLOUR	Sydney Flour Millers	Milled wheat with bran and wheat germ removed	See Text (Appx.E)
CORN FLOUR	Wades		

Continued..

TABLE XIII

(Continued)

COMPOSITION OF THE FOODS TESTED (AUSTRALIAN FOODS)

COMMON NAME	MANUFACTURER/ BRAND NAME	DESCRIPTION	COMMENTS
RICE FLOUR	Nugrist	Milled whole grains of polished rice	
OATMEAL	Nugrist	Milled whole oats	
MAIZE FLOUR	Nugrist	Milled whole corn grains	

TABLE XIV

U.S. AND AUSTRALIAN FOODS

RANGES, MEANS, STANDARD ERRORS AND RANGE FOR ± TWO STANDARD DEVIATIONS FOR REPLICATE SUCROSE EXPERIMENTS.

This table compares the hour 6 and hour 24 experimental pH, titratable acidity and enamel dissolution values obtained for replicate experiments on U.S. sucrose and Australian sucrose. For U.S. sucrose, eight replicate experiments were performed, and for Australian sucrose, three. For the U.S. sucrose tests, the range, mean, standard error and range for ± two standard deviations are shown for each value, while for the Australian sucrose tests, the range mean and standard error are shown.

The ranges for the experimental values obtained in the U.S. and Australian tests were of the same order, and the means were similar, except for enamel dissolution at hour 6. U.S. sucrose experimental values had lower standard errors, in comparison with Australian sucrose experimental values, except for enamel dissolution at hour 6.

The ranges shown for ± two standard deviations for U.S. sucrose were used in conjunction with the values shown in Tables I-VI for the other U.S. foods to determine which of these values were significantly different from those of U.S. sucrose, as shown in Table XV.

TABLE XIV

U.S. FOODS AND AUSTRALIAN FOODS

Ranges Means Standard Errors and Range for ± two Standard Deviations for replicate sucrose experiments.

U.S. SUCROSE, 8 REPLICATES

	pH		TITRATABLE ACIDITY		ENAMEL DISSOLUTION	
HOUR	6	24	6	24	6	24
RANGE	4.7-4.9	4.5-4.7	1.1-2.1	2.4-3.1	22-39	33-45
MEAN	4.8	4.3	1.7	2.8	23	41
STD. ERROR	.03	.03	.14	.05	1.8	1.6
RANGE ± 2 STD. DEVS.	4.64-4.96	4.14-4.46	0.9-2.5	2.5-3.1	12-33	32-50

AUSTRALIAN SUCROSE, 3 REPLICATES

	pH		TITRATABLE ACIDITY		ENAMEL DISSOLUTION	
HOUR	6	24	6	24	6	24
RANGE	4.5-4.8	4.3-4.5	1.1-1.9	2.9-5.1	11-14	37-55
MEAN	4.6	4.4	1.6	3.9	12	46
STD.ERROR	0.09	0.06	0.25	0.64	0.9	5

TABLE XV

U.S. FOODS

COMPARISON OF THE RESULTS FOR U.S. SUCROSE (MEAN OF EIGHT REPLICATE EXPERIMENTS) WITH THOSE FOR THE OTHER U.S. FOODS (SINGLE EXPERIMENTS).

This table compares the significance of differences between pH, titratable acidity and enamel dissolution values obtained for the U.S. foods (Tables I-VI) and the corresponding mean U.S. sucrose values (Table XIV). The eight replicate experiments on U.S. sucrose were used to establish a range of $\pm$ two standard deviations for each mean sucrose value. Any U.S. sucrose experimental value lying beyond this range would occur by chance in less than five out of 100 trials. Thus any individual experimental value for U.S. foods which lies beyond this range is significantly different from the corresponding U.S. sucrose value, at the P.05 level.

In this table the individual experimental values for the U.S. foods which are not significantly different from the corresponding U.S. sucrose mean values are designated '0', those which are significantly higher '+' and those which are significantly lower, '-'.

All the U.S. foods differed in at least one of the six experimental values recorded for each food, and most differed in at least three. Furthermore, with some foods the values were higher, and with others lower than the corresponding sucrose values, showing that all U.S. foods differed from sucrose, even in the absence of replicate experiments, and suggesting that some also differed from each other in their fermentation behaviour.

TABLE XV

U.S. FOODS

Significant differences between U.S. food test values and sucrose (P.05)

0: not significant, +: significant(higher), -: significant (lower)

FOOD	pH		T.A.		E.DISS.	
HOUR	6	24	6	24	6	24
OR.JUICE & SALIVA	-	0	-	-	+	+
7UP & SALIVA	-	0	0	+	0	+
COCA COLA & SAL.	-	-	0	0	0	+
BEER & SALIVA	0	+	0	-	-	-
HONEY	0	+	0	+	0	0
DEXTROSE	0	+	-	-	0	0
SUCROSE	0	0	0	0	0	0
BROWN SUGAR	-	0	0	+	0	+
LT. MOLASSES	-	+	+	+	0	0
DK. MOLASSES	0	+	+	+	0	-
MAPLE SYRUP	0	+	0	+	0	0
WHEAT GERM	-	-	+	+	0	0
BRAN	-	+	+	+	-	-
RYE FLOUR	-	+	+	+	0	-
WHEAT FLOUR PLAIN	0	+	+	0	0	0
WHEAT FLOUR ENRICHED	0	+	+	0	0	+
GRAHAM FLOUR	-	+	+	+	0	-
POTATO FLOUR	0	+	+	+	0	+

Continued .....

TABLE XV

(Continued)

Significant differences between U.S. food test values and sucrose (P.05)

0: not significant, +: significant (higher), -: significant (lower)

FOOD	pH		T.A.		E.DISS.	
HOUR	6	24	6	24	6	24
POTATO STARCH	0	0	0	-	-	+
CORN STARCH	-	-	0	0	0	+
POL. RICE FLOUR	-	0	0	+	0	+
BR. RICE FLOUR	0	0	+	+	0	-
W. RICE FLOUR	-	+	+	0	0	-
YELLOW CORN MEAL	0	-	+	+	0	0
CORN FLAKES	-	+	0	0	0	0
PUFFED RICE	-	+	0	-	0	0
SHR. WHEAT	0	+	0	0	0	-
WHITE BREAD	-	+	0	-	+	0
WH. WHEAT BREAD	0	+	+	0	-	-
WH.GRAIN BREAD	0	+	0	-	0	-
SALTINES	-	0	0	0	0	0
CRACKERS	-	+	+	-	0	0
SHT. BREAD	-	0	+	0	0	0
FIG NEWTON	-	0	0	0	0	+
MILK CHOC.	-	+	0	0	0	-
CHOC. COV. COCONUT	-	+	+	0	0	+
CARAMELS	+	+	+	+	-	-

TABLE XVI

AUSTRALIAN FOODS

MEANS AND STANDARD ERRORS FOR HOUR 6 AND HOUR 24 pH, TITRATABLE ACIDITY AND ENAMEL DISSOLUTION VALUES (THREE REPLICATE EXPERIMENTS).

This table records the means and standard errors for the hour 6 and hour 24 pH, titratable acidity and enamel dissolution values for all the Australian foods tested.

This table was used, where indicated, to ascribe a level of significance to any comparisons made between the fermentation behaviour of two foods, or groups of foods. The statistical method employed was based on the 't' test, where values for 't' were calculated according to the formula:

$$t = \frac{\text{difference between mean}_1 \text{ and mean}_2}{\sqrt{(S.E._1)^2 + (S.E._2)^2}}$$

Statistical tables showing values of 't' for four degrees of freedom, at specified levels of significance, were then used to determine whether the observed difference was significant.

A difference between means was designated 'significant' or '(S.)' if the P value was less than .05, and 'highly significant' or '(H.S.)' if less than .01. For P values slightly higher than .05, the term 'borderline significance', or '(B.S.)' was used. For all other values of P, the term 'not significant' or '(N.S.)' was used.

A level for P of less than .05, commonly used in biological studies, indicates that the observed difference could occur by chance in less than five out of 100 trials, and was the level of significance employed in this study.

TABLE XVI

AUSTRALIAN FOODS

M: mean of three replicate experiments, S.E.: standard error

FOOD	pH		TIT. ACIDITY		ENAMEL DISS.	
HOUR	6 M S.E.	24 M S.E.	6 M S.E.	24 M S.E.	6 M S.E.	24 M S.E.
ORANGE JUICE	5.1 .02	4.8 .05	6.2 .17	8.0 .55	52 .9	70 1
COCA COLA	5.0 .09	4.5 .08	1.0 .09	2.8 .57	7 5	36 1
PILSENER	5.0 .02	5.1 .08	1.0 .01	1.4 .07	4 .7	12 2.5
ICE CREAM	4.5 .03	4.0 .02	5.4 .99	11.0 .09	2 .2	4 .6
CHOC. MILK	4.4 .02	4.2 .02	4.0 .15	6.1 .21	2 .6	3 .2
MILK	4.9 .07	4.8 .03	3.6 .12	6.4 .66	2 .2	3 .2
HONEY	4.7 .03	4.3 .03	1.9 .09	4.5 .23	18 2	74 .9
GLUCOSE SYRUP	4.9 .2	4.5 .03	1.8 .25	3.6 .61	17 5	66 9
DEXTROSE	4.7 0	4.3 .03	1.3 .07	4.1 .62	12 1	48 7
SUCROSE	4.6 .09	4.4 .06	1.6 .25	3.9 .64	12 .9	46 5
BROWN SUGAR	4.7 .03	4.4 .06	1.9 .16	4.0 .48	4 .6	34 .9
GOLDEN SYRUP	4.5 .03	4.4 .03	1.8 .08	3.5 .44	4 .6	30 5
TREACLE	4.8 .07	4.6 .03	2.1 .36	3.8 .10	3 .4	15 1.7
MOLASSES	4.8 .06	4.5 .03	2.5 .22	4.2 .50	5 .6	9 .9
SWEETADDIN	6.5 .03	5.7 .06	0.2 .02	1.0 0	1 0	2 0
WHOLE MEAL F.	5.4 .01	4.9 .03	3.9 .76	7.5 .07	17 .2	90 2
WHEAT GERM	4.5 .05	4.1 0	4.5 .23	12.5 1.63	7 .5	85 3
BRAN	4.3 .13	3.9 .01	6.0 .22	13.0 .62	10 1	82 9
RYE F.	4.5 .06	4.2 .06	3.9 .79	9.3 .31	20 2	64 1

Continued .....

TABLE XVI

(Continued)

AUSTRALIAN FOODS

M: mean of three replicate experiments, S.E.: standard error

FOOD	pH				TIT. ACIDITY				ENAMEL DISS.			
HOUR	6		24		6		24		6		24	
	M	S.E.	M	S.E.	M	S.E.	M	S.E.	M	S.E.	M	S.E.
WHEAT F.	5.2	0	4.9	.05	2.0	.20	3.9	.48	18	.3	63	7
CORN F.	5.6	.06	5.2	.01	1.3	.16	3.7	.35	12	1	61	3
RICE F.	5.2	.03	4.8	.06	2.5	.34	5.3	.46	16	1	60	5
OATMEAL F.	4.5	.11	4.3	.01	5.0	.12	9.5	.57	15	1	45	2
MAIZE F.	4.5	.04	4.2	.05	2.7	.12	5.5	.06	15	.3	39	2
WHITE BREAD	4.5	.02	4.2	.02	2.2	.33	5.5	.71	21	2	60	2
BROWN BREAD	4.4	.02	4.2	.02	2.7	.49	5.7	.63	16	1	53	2
SHORT BREAD	4.5	.10	4.2	.02	2.5	.51	5.3	.72	16	5	51	4
SAO BISCUIT	4.5	0	4.3	.03	1.8	.23	3.7	.26	18	.2	47	.6
LEM.SLICE BIS.	4.6	.05	4.3	.02	5.1	.68	6.7	1.19	8	3	43	8
VOGEL BREAD	4.5	.02	4.2	.02	2.7	.09	5.7	.03	12	.2	42	1
RYVITA BISCUIT	4.5	.06	4.3	.13	2.3	.17	4.8	.58	8	2	26	5
POTATO CRISP	4.5	.02	4.2	.03	4.1	.23	7.1	.02	25	2	60	3
FROSTIES	4.6	.03	4.4	.05	1.7	.10	3.3	.09	16	1	42	4
CHEESE TWISTIES	4.5	0	4.2	0	3.2	.39	6.0	.26	8	.3	41	1
ROLLED OATS	4.5	.02	4.1	.03	3.0	.39	6.3	.40	12	.5	38	3
ALL BRAN	4.5	0	4.3	.03	3.2	.09	4.7	.11	12	1	32	3
PEANUT BUTTER	4.5	.03	4.5	.07	3.7	.12	4.2	.38	11	.2	28	.6

Continued .....

TABLE XVI

(Continued)

AUSTRALIAN FOODS

M: mean of three replicate experiments, S.E.: standard error

FOOD	pH		TIT. ACIDITY		ENAMEL DISS.	
HOUR	6 M S.E.	24 M S.E.	6 M S.E.	24 M S.E.	6 M S.E.	24 M S.E.
WEET BIX	4.6 .02	4.3 0	2.4 .15	3.5 .11	9 .4	27 1
CORN FLAKES	4.2 .04	4.1 .02	2.0 .21	2.7 .17	6 0	23 2
RICE BUBBLES	4.2 .03	4.0 .05	2.7 .17	3.2 .25	7 .6	21 1
RAISINS	4.4 .08	4.1 .06	2.4 .21	5.7 .45	15 2	62 5
DATES	4.3 .03	3.9 .03	2.6 .13	6.7 .22	11 1	57 3
CARAMELS	4.4 0	4.1 .03	2.2 .20	5.4 .51	12 .3	54 8
DRIED APRICOTS	4.2 .04	4.2 .02	4.5 .52	5.7 .95	35 .6	53 3
BANANAS	4.5 .03	4.5 .16	2.3 0	2.4 .29	28 .4	53 .9
MILK CHOC.	4.3 .03	4.0 .04	3.8 .2	7.2 .64	4 .6	31 .7
GLACÉ CHERRIES	4.9 0	4.4 0	1.0 .02	3.2 .24	6 .3	28 .7
SUCROSE + Ca SUC.PHOS.	4.8 .07	4.5 .08	1.8 .23	3.2 .89	2 .4	3 .8