

ADDENDUM TO APPLICATION (DECEMBER 1966) BY COLONIAL SUGAR
REFINING CO. LTD. TO THE NATIONAL HEALTH AND MEDICAL RESEARCH
COUNCIL FOR APPROVAL OF CALCIUM SUCROSE PHOSPHATES AS FOOD
ADDITIVES.

February 1968

The utility of calcium sucrose phosphates as food additives for the inhibition of dental caries

It is the purpose of this report to serve as an addendum to an application for approval of calcium sucrose phosphates as food additives for the inhibition of dental caries which was lodged with the Food Additives Committee of the National Health and Medical Research Council in January 1967. Fifteen copies of this application together with copies of all references cited in the application are in the hands of the N.H.M.R.C.

The application dealt in detail with such aspects as the chemistry, manufacture, composition, specifications, analytical methods, pharmacology, toxicology and use in foods of calcium sucrose phosphates, as well as the evidence which was available at that time on the utility of calcium sucrose phosphates as cariostatic agents.

Work to demonstrate the utility has been continuing. In particular the clinical trial to assess the cariostatic effect in the teeth of children of calcium sucrose phosphates as additives in certain carbohydrate foods, was commenced in early 1965 and will be completed in April 1968.

A detailed statistical analysis and final report on the total three years results of this trial will be forwarded to the Secretariat of the N.H.M.R.C. by the beginning of August 1968.

This present addendum summarises the evidence for the utility of calcium sucrose phosphates as cariostatic food additives which is contained in a number of reports and publications which are available at the present time (February 1968). This evidence comprises data and conclusions based on :

- (a) in vitro experiments
- (b) animal experiments
- (c) the human clinical trial to date (two years).

Fifteen copies of the following reports and publications are forwarded with this addendum. (The only exception is reference 6 of which we have limited numbers. Five copies of this are forwarded).

1. Brady, B.H.G., Napper, D.H., and Smythe, B.M.
"Dissolution kinetics of hydroxyapatite",
Nature, 212, 77-78, Oct. 1966.
2. Lilienthal, B., Bush, Elizabeth, Buckmaster, M.,
Gregory, G., Gagolski, J., Smythe, B.M., Curtin, J.H.,
and Napper, D.H.
"The cariostatic effect of carbohydrate phosphates in
the diet", Aust. Dent. J. 11, 388-395, Dec. 1966.
3. Napper, D.H. and Smythe, B.M.
"The dissolution kinetics of hydroxyapatite in the
presence of kink poisons", J. Dent. Res. 45, 1775-1783,
Nov.-Dec., 1966.
4. Harris, R., Schamschula, R.G., Gregory, G., Roots, Miriam,
Beveridge, J.
"Observation on the cariostatic effect of calcium
sucrose phosphate in a group of children aged 5-17
years", Aust. Dent. J. 12, 105-113, April 1967.
5. Curtin, J.H., Gagolski, J., and Smythe, B.M.
"A food additive for the control of dental caries",
Food Technology in Australia 19, 508-513, August 1967.

6. Madow, W.G.
 "The analysis of data obtained in a dental caries trial involving calcium sucrose phosphate", Private Report by Stanford Research Institute, U.S.A.
 S.R.I. Project AM-5648 Sept. 1967.
7. Brady, B.H.G., Napper, D.H., and Smythe, B.M.
 "The effect of additives on the rate of dissolution of hydroxyapatite in unstirred acid buffers", Paper accepted for publication in J. Dent. Res. Jan. 1968.
8. Lilienthal, B., Napper, D.H., and Smythe, B.M.
 "The hardening and softening of human tooth enamel", Paper accepted for publication in Aust. Dent. J., Jan. 1968.
9. Harris, R., Schamschula, R.G., Gregory, G., and Beveridge, J.
 "The cariostatic effect of calcium sucrose phosphate in a group of children aged 5-17 years", Aust. Dent. J., Feb. 1968 (in the press).

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Papers 1, 3, 7 and 8 comprise data from in vitro experiments. Paper 2 summarises animal experiments. Papers 4 and 9 contain the results of the first two years of the clinical trial. Paper 6 is an independent statistical analysis by Stanford Research Institute of the two year results of the clinical trial: Paper 5 is a general paper outlining the project as a whole.

We submit that the data contained in these publications, considered collectively, conclusively point to the utility of

calcium sucrose phosphates as cariostatic food additives.

However, we await the results of the third year of the clinical trial before the evidence of the three years is finally presented for consideration by the National Health and Medical Research Council.

Clinical Trial

It will be realized that a clinical trial of the type required in this study is unique. To demonstrate that an agent when added to a range of processed carbohydrate foods is capable of reducing significantly the incidence of dental caries in consumers of such food, necessitates that the major part of the diet of the trial population is under control. To obtain population groups in which all subjects are willing to accept such a measure of dietary control is not a simple task. One of the few such groups in Australia of sufficient size and population stability over three years of a trial are children living in welfare institutions in the environs of Sydney. Earlier surveys had shown the impracticability of obtaining such groups from private boarding schools and indeed from similar welfare institutions in the Melbourne area. The latter are not suitable because of the high turnover of children in these institutions. (50% per annum). Even in the Sydney area it has been found that the number of children who will have been receiving the treated foods for three years will be about 40% of the original population of 619 in the test group.

This problem coupled with the large organizational problems and expenditure involved in supplying such numbers of children with over 70% of their carbohydrate requirements precludes the carrying out of more than one trial. (The cost of the present three year trial will be about \$A400,000).

Consequently, a trial had to be planned with care not only to ensure complete co-operation from all participating organizations and subjects, but also to ensure that results obtained were valid and were capable of analysis and assessment as to the efficacy and safety of the calcium sucrose phosphates as a cariostatic agent in the conditions of the trial. The complete responsibility for the protocol and medical and dental examinations are in the hands of independent medical, dental and statistical experts. This Company has provided the considerable background services which a trial planned in the manner described necessitates.

A single trial on a specific substance unsupported by in-vitro and animal experiments could not be considered to be proof of utility. However, in the present case, not only are the results of the trial supported by substantial evidence from these sources, but they are also supported by a large volume of in-vitro, animal and clinical evidence on the use of phosphates generally as cariostatic agents.

This evidence is discussed in detail in the papers 1-9 forwarded as part of this addendum. However it is desirable to summarise the main premises and arguments on which the claim is made that calcium sucrose phosphates are cariostatic agents.

Premises

(a)

It is generally agreed that dental caries arises initially in man by the dissolution of hydroxyapatite from tooth enamel (hydroxyapatite is a form of calcium phosphate comprising about 95% of tooth enamel) by acids from the action of microflora on carbohydrate foods present in the mouth.

It follows that any agent which can be shown by in-vitro tests to inhibit the dissolution of hydroxyapatite could well possess prophylactic cariostatic activity in man.

(b)

The onset of dental caries is signalled by sub-surface decalcification of enamel which reduces the hardness of the enamel surface as measured by conventional hardness tests.

It follows that any agent which can be shown by in-vitro tests to deposit a material into decalcified softened enamel, thereby rehardening it, could well possess both prophylactic and curative cariostatic activity in man.

(c)

As exemplified hereunder, a number of important studies on cariostatic agents have been published in which close correlation has been established either between in-vitro tests and human utility or between animal experiments and human utility or between all these methods of evaluation.

For example, the in-vitro studies by Stralfors on the inhibiting effects of calcium and phosphate ions on the dissolution of dental enamel were followed by his further studies demonstrating the reduction of dental caries in hamsters when inorganic calcium phosphate was added to the diet of the experimental animal, and by his still further studies in which the hamster experiment was translated to the human situation:

Stralfors, A. -

(i) The effect of calcium and phosphate ions on the rate of enamel dissolution. Odont. Revy 10: 367, 1959.

(ii) Inhibition of dental caries in hamsters. II.
The effect of tribasic calcium phosphate. Odont. Revy 12:
124, 1961.

(iii) Inhibition of dental caries in hamsters. X.
The effect of toothbrushing. Odont. Revy 13: 139, 1962.

(iv) Sven Tandlag Tidskrift 49: 108, 1956.

(v) The effect of calcium phosphate on dental caries
in school children. Suppl. J. Dental Res., 43, 1137-1143,
Nov.-Dec., 1964.

While it is recognised that a demonstration of the
cariostatic activity of any agent by animal experiments is not
conclusive of the cariostatic activity of that agent in man,
animal experiments are nonetheless admitted to play an important
part in predicting the likelihood of such utility.

When it has been demonstrated that a given agent
selected from a chemically homogeneous group of materials is
cariostatic in man and that this activity conforms with
predictions of its behaviour based on a variety of in-vitro
and animal experiments, and when it has been demonstrated that
other materials selected from the group behave similarly to the
given agent in the same in-vitro and animal experiment situations,
the conclusion may be drawn that all such members of the group
are cariostatic in man.

(d)

The evidence of nearly 100 separate studies "is nearly
unanimous that phosphates are effective anticaries agents in
rodents" (A.E. Nizel and R.S. Harris, Proc. Conf. on Phosphates
and Dental Caries, Suppl. J. Dental Res. 43, Nov.-Dec. 1964).

There is a growing amount of evidence that the cariogenicity of carbohydrate foods is reduced by the incorporation therein of phosphates. (S.B. Finn and H.C. Jamison, J. Am. Dent. Assoc. 74, 987-995, April 1967; G.K. Stookey, R.A. Carroll, J.C. Muhler, J. Am. Dent. Assoc. 74, 752-758, March 1967)

Argument

In paper 7 which has been accepted for publication in the Journal of Dental Research, it has been shown how calcium ions, inorganic phosphate ions and sucrose phosphate anions can each reduce the rate of dissolution of hydroxyapatite and that the greatest effect occurs when all three of these ionic species are present at the same time.

In another paper, namely 3, it has been shown how various organic phosphate ions (including glucose-6-phosphate, glucose-1-phosphate, fructose 1:6-diphosphate and sucrose phosphate) and condensed inorganic phosphate ions can markedly reduce the rate of dissolution of hydroxyapatite by a mechanism involving their adsorption on the surface of the hydroxyapatite crystal.

It is evident from the information in these two papers that any one or a combination of the ionic species calcium, inorganic phosphate and organic phosphate could well possess cariostatic activity in man when administered in a suitable carrier, such as a foodstuff.

On the basis of this information, it can thus be predicted that agents likely to prove cariostatic in man are for example: soluble calcium salts (such as calcium nitrate), soluble inorganic phosphates (such as sodium or potassium

phosphates), and soluble organic phosphates (such as various sugar phosphates). The greatest cariostatic effect would be expected from an agent comprising soluble calcium and soluble phosphate ions, either of inorganic and organic type.

It has been pointed out in two papers, 2 and 5, that the inorganic calcium phosphates themselves are insoluble, or become sparingly soluble in aqueous systems, and these agents are thus not in a form best adapted to assert their cariostatic properties.

The essential advantages of the calcium salts of sugar phosphates is that, unlike those of the inorganic phosphates or other investigated organic phosphates, they are generally very soluble in water and would thus allow the inhibiting effect of both calcium and phosphate ions to be realised in the one agent. This has been borne out by the in-vitro experiments referred to above and by the experiments with rats referred to in paper 2.

In the course of the research project which developed from these earlier findings, it was further found that some calcium salts of sugar phosphates could be made containing appreciable quantities of inorganic calcium phosphates. As pointed out in paper 7, calcium sucrose phosphate made according to the method described in German patent No. 247,809 (filed February 3, 1910) does not contain appreciable amounts of inorganic phosphate. However, calcium sucrose phosphate made by the modified process described in our original application to the N.H.M.R.C. can contain as much as 15% by weight of inorganic calcium phosphate and surprisingly this phosphate is not insoluble. The solubilization has been shown to be due to

a complex interaction with the calcium sucrose phosphate.

As would be expected from the evidence outlined in paper 7, these complex associations, of calcium sugar phosphates and inorganic calcium phosphate, are even more effective in inhibiting the dissolution of hydroxyapatite than calcium sugar phosphates per se. This follows because they contain soluble calcium ions, inorganic phosphate ions and sugar phosphate ions, each of which inhibits hydroxyapatite dissolution.

Complex associations of calcium sugar phosphates and inorganic calcium phosphate were found to have yet another advantage over both inorganic phosphates per se and sugar phosphates per se. In another paper, namely 8, it has been shown that calcium ions or inorganic phosphate ions when added to water (as distinct from saliva) do not rehardèn softened human tooth enamel. Sugar phosphate ions alone also do not produce significant rehardening. However, when all three ions, calcium, inorganic phosphate and sugar phosphate are present together in soluble form a significant degree of rehardening is obtained.

For the reasons:

- (i) that sucrose is the most readily available sugar,
- (ii) that it is more economical to manufacture complex associations of sugar phosphates and inorganic calcium phosphate than sugar phosphates per se, and
- (iii) that theoretical grounds suggest that such complex associations must be more effective than sugar phosphates per se,

the clinical feeding trial in humans on which the utility of calcium sucrose phosphates is finally predicated have been carried out on a calcium sucrose phosphate agent containing about 15% of solubilized inorganic calcium phosphate.

The degree of dental caries inhibition which has been observed in this clinical feeding trial^{4,6,9} must, however, be attributable essentially to the calcium sucrose phosphate component of the agent. This follows because, at the level of the total agent used in the trial (1% by weight of the carbohydrate diet), the level of the inorganic calcium phosphate component is less than 0.15% by weight of the overall diet. From the evidence shown in Table 5 of paper 2, as well as from the conclusions to be drawn from the general review of Nizel and Harris (ibid), it is obvious that this level of inorganic calcium phosphate by itself cannot be responsible for producing the obtained degree of dental caries inhibition which has been demonstrated in the clinical feeding trial. Clearly therefore, it has been established by this trial that calcium sucrose phosphates (no less than complex associations of calcium sucrose phosphates and inorganic calcium phosphate) have cariostatic utility in man.

The sucrose phosphates belong to a chemically homogeneous group of materials and there is no evidence that any of them has a pattern of behaviour in the in-vitro and animal experiments which have been described above which is materially different from that of any other member of the group.

The results of the clinical feeding trial thus conclusively bear out the evidence accumulated from the noted wide range of laboratory tests and lead to the conclusions that calcium sucrose phosphates generally and complex associations thereof with inorganic calcium phosphate have cariostatic utility in man.

The Colonial Sugar Refining Company Ltd.

per General Manager
February 1968.

