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EFFECTS OF MILK ON ENAMEL SOLUBILITY

M. E. WEISS and B. G. BIBBY
Eastman Dental Dispensary, Rochester, New York, U.S.A.

Summary—A reproducible method for comparing the effects of treatment with milk products on the acid solubility of enamel surfaces is described. Using this method it was found that treatment with cows' milk reduced solubility by more than 20 per cent, regardless of whether it was raw or pasteurized whole or skim milk.

Reconstituted whole and reconstituted skim milk gave similar reductions. Similar depressions of solubility followed treatment with cream or whey. Butter gave low and inconsistent reductions. No difference was found in the solubility effects of reconstituted dry skim milk obtained from twenty-three locations in the United States. Tests showed that the protective agent in milk reacts rapidly with the enamel and resists washing. That it is protein in nature is indicated by the finding that the full solubility reduction is given by treatment with casein solutions and mostly removed by washing with a protein solvent.

INTRODUCTION

PUBLISHED reports leave doubt as to whether milk promotes or retards dental caries. Indications of the former possibility are found in observations that its prolonged use in nursing bottles increases decay in infants (FASS, 1962; GOULD, 1963; ROBINSON and NAYLOR, 1963), and in the high cariogenicity of some skimmed milk animal diets (FOLK and MCCLURE, 1954). Reasons for the opposite view can be found in clinical findings (Canadian Dental Association (Research Committee), 1958; Sprawson, 1932; ROBERTS *et al.*, 1938), in the effect of milk and milk products on animal caries (SHAW, ENSFIELD and WOLLMAN, 1959) and in the demonstration (ANDLAW, 1960) that *in vitro* enamel decalcification produced by fermenting foods is reduced by the addition of milk powder.

Because one study (NIZEL and HARRIS, 1950) has predicated that geographic factors were responsible for differences in the production of caries on milk and corn diets and since we (KHANNA and BIBBY, 1964) have also found that the place of origin modified the enamel decalcifying effects of cereals, it seemed important to consider geographic influences in any study of the caries-related properties of milk. As a start, it was decided to investigate the effects of milk on enamel solubility.

EXPERIMENTAL METHOD

Several experimental approaches were made before an acceptable method for estimating the effects of milk on enamel solubility was found. Although comparisons of weight changes in slabs of young bovine enamel consistently showed reductions of solubility, the results were not satisfactorily reproducible between experiments. This was also true when phosphate loss from granular enamel was used as the measure of enamel dissolution.

The method which was finally found to suit our purpose was a "window" high-speed rotation procedure previously used by Healy (Unpublished) in our laboratories. This permitted exposure of the same area of "window" enamel to repeated decalcifications and treatments so that it could serve as its own control. After approximately 500 preliminary trials, using different volumes of buffer, pH, exposure times, and four different types of milk, the method given below was adopted and found to give highly reproducible results.

Test procedure

Roughly circular blocks of enamel supported by dentine, approximately $\frac{3}{8}$ in. in dia., were cut under water from the labial halves of bovine incisor crowns. These were cemented in acrylic resin to the ends of 3-in long acrylic rods. To eliminate extraneous deposits and the ridges frequently present on bovine incisor enamel, the surfaces were then carefully polished with silicon carbide paper, beginning with 200 and finishing with 600 grit. A circular piece of masking tape was fixed in the centre of the polished enamel and the block covered to the periphery of the tape with nail varnish. After the varnish had dried the masking tape was carefully removed, leaving exposed enamel surface of approximately 0·049 in^2 or 31·6 mm^2. The blocks were thoroughly washed, numbered and stored in distilled water under refrigeration. No preservatives were employed and the blocks were kept wet, except during brief periods of rod cementation and "window" preparation.

To measure the effects of the various milk media, two decalcification runs (A and B) were first made on each block; this was followed by treatment (T) with the milk media and then a post-treatment decalcification run (C). The difference between B and C indicated the amount of solubility reduction given by T. Tests were always run in triplicate.

In making the first (A) decalcification run, three blocks attached to acrylic rods were mounted in the chucks of 1/40 h.p. electric motors so that they could be lowered through a hole in the cover into 30 × 63 mm bottles containing 20 ml 0·1 M acetic acid/sodium acetate buffer pH 4·0, which were kept at 37°C by means of circulating water jackets. After placing plastic covers over the whole assembly to maintain constant temperature and humidity, the mounted blocks were rotated at 3350 rev/min for 15 min. The amount of dissolved enamel was determined by making phosphorus analysis of the buffer using the method of KITSON and MELLON (1944). On occasions, calcium determinations were also made on aliquots of the buffer using KOULOURIDES' (1958) modification of Schwartzenbach's method. After washing the enamel blocks by spinning in distilled water for 15 sec and drying with tissue paper, the second (B) decalcification was made in the same way as before (A). A similar 15 sec washing was given and then the blocks were rotated for 15 min in 20 ml of the milk media (T), instead of the buffer. After washing the blocks for one minute while spinning, with an additional rinse from a water bottle, the third decalcification run (C) was given in the same way as previously. Sometimes a fourth decalcification (D) was added.

In testing whether the protective effect of milk products was associated with protein deposits on the surface trisodium phosphate was selected as a washing agent.

This choice was made on the basis of its use in the dairy industry for cleaning milk residues from machines and containers. In addition, Kjeldahl tests in our laboratory had shown that all of the protein taken up on enamel powder from milk could be recovered in phosphate washings.

When the runs were completed, the varnish edge of the "window" was examined for separation from the enamel. Thereafter, the varnish covering was removed, the enamel surfaces repolished and new windows prepared in the same way as before, so that they could be used again for a new series of runs. Six or more new surfaces and windows, which were recorded in order as o, a, b, c, etc., surfaces, could be used before unusual solubility changes appeared. Estimates of the thickness of enamel removed during the decalcification periods were made by calculating, from the phosphate present in the buffer, the volume of enamel removed and dividing by the area of the enamel "window."

Milk

The fresh whole milk, skim milk and cream were obtained either from the local milk collecting and processing firm or for some special tests directly from a dairy. Dryskim milk and dry whole milk samples were obtained through the co-operation of milk processors in different parts of the country, chosen as far as possible to represent regions of low, high and medium caries prevalence. For test purposes 9·7% mixtures of the dry skim milks and 12·9% of the dry whole milks were made up in distilled water.

RESULTS

Table 1 compares the amounts of phosphate dissolved in 170 first (A) and second (B) decalcification runs made on seventy-six blocks and tends to indicate that greater differences appear when the re-prepared deeper enamel surfaces (d and e) are used rather than the early a and b windows prepared from the more superficial layers of enamel. Thirty-five filtrates of A, B and C decalcifications which were analyzed for calcium as well as phosphorus gave a Ca:P ratio of $2{\cdot}08 \pm 0{\cdot}047$ s.d. The mean thickness of enamel removed was 16 μ and varied from 10 μ to 20 μ.

A comparison of the effects of different milk media (Table 2) shows that there is

TABLE 1. COMPARISON OF BLOCK SOLUBILITY IN "A" AND "B" RUNS ON SUCCESSIVE ENAMEL SURFACES

Surfaces	(*n*)	μg PO_4 per ml in used buffer Runs "A" (Av.)	Runs "B" (av.)	% Dif.
(o)	38	35·6	36·2	+1·7
(a)	54	36·9	37·6	+1·9
(b)	37	37·5	39·5	+5·3
(c)	29	39·1	40·7	+4·1
(d)	8	39·1	42·7	+9·2
(e)	4	39·0	43·2	+10·7
	170	37·3	38·6	+3·5

TABLE 2. AVERAGE COMPOSITION OF MILK AND SOME MILK DERIVATIVES AND THEIR EFFECT ON ENAMEL SOLUBILITY

Treatment agent	Number of blocks	Mean % solubility reductions	Average % Composition* Water	Fats	Proteins	Lactose	Minerals
Pasteurized fresh milk	9	22·1	87·1	3·9	3·4	4·9	0·7
Raw fresh milk	3	24·3					
Reconstituted dry whole milk	3	23·9					
Reconstituted dry skim milk	3	22·5	90·5	0·2	3·5	5·0	0·8
Cream	5	26·5	72·5	20·0	2·9	4·0	0·6
Whey	3	27·0	93·0	0·3	1·0	5·1	0·6
Commercial butter	6	5·5	15·5	81·0	0·6	0·4	2·5

* From: HARROW, B. 1944. *Textbook of Biochemistry*, p.132. Saunders, Philadelphia.

very little difference between the solubility reducing effects of raw fresh milk, pasteurized fresh milk, reconstituted dry whole milk or reconstituted dry skim milk. Cream and whey give slightly greater reductions. A lack of a proportionate relationship to the fat content of the treatment agents is obvious. It also can be seen that as much solubility reduction is produced by the low protein whey as by the milks which contained three times as much.

Although the initial plan was to use fresh milk for the geographic comparison, our inability to demonstrate that its effects on enamel solubility differed from those of reconstituted dry milks and practical considerations of transportation made it appropriate to use dried skim milk for this purpose.

Table 3 compares the effects on enamel dissolution of reconstituted dried skim milk from twenty-three different geographical areas. It is quite apparent that no significant differences exist between them, neither is there any indication of any relationship between the solubility reductions and the caries prevalence in the region from which the milks were obtained.

To cast some light on the nature of the reaction between milk and enamel, comparisons of treatment and washing times were made (Table 4), using reconstituted dry skim milk as the treatment agent. It appears that the solubility reduction occurs quickly, is not much increased by longer treatment and that it is not easily removed by washing.

Because the comparison of milk products (Table 2) indicated that greater solubility reductions were associated with the high protein milk media, a series of tests were run to indicate whether other components of milk in concentrations comparable to those normally present had solubility effects comparable to those of milk protein. The decalcification and treatment times, 15 min, were the same as in previous tests. The results given in Table 5 indicate that casein produces the only significant solubility reduction.

As a test of whether the solubility reduction was associated with protein deposits

TABLE 3. REDUCTIONS OF ENAMEL SOLUBILITY PRODUCED BY RECONSTITUTED DRY SKIM MILK FROM DIFFERENT GEOGRAPHICAL AREAS

Sample	Locality	State	Number of blocks	Mean % reduction	s. d.	Approx. relative caries prevalence
1	Andover	Mass.	9	19·26	7·9	High
7	Seattle	Wash.	3	20·33	4·48	,,
8	W. Springfield	Mass.	3	25·6	4·15	,,
9	St. Albans	Ver.	6	22·56	4·1	,,
11	Payette	Idaho	3	22·96	1·42	Medium
12	Idaho Falls	,,	12	19·57	6·65	,,
14	Meridian	,,	3	20·86	2·25	,,
15	Jerome	,,	3	21·93	2·99	,,
2	Springfield	Mo.	9	21·07	7·1	,,
3	Fosston	Minn.	3	23·9	4·1	,,
4	Mountain Lake	,,	3	24·2	3·05	,,
5	Pine City	,,	3	21·7	4·95	,,
6	Whitehall	Wis.	12	20·16	7.0	,,
10	Shawano	,,	3	23·56	0·74	,,
13	Louisville	Ky.	9	19·95	6·48	,,
17	Barron	Wis.	5	21·74	5·65	,,
18	Sauk City	,,	4	24·6	2·1	,,
16	Fresno	Cal.	8	18·36	6·59	,,
19	Willows	,,	3	22·63	2·65	,,
20	Fernbridge	,,	3	22·4	1·65	,,
21	Muenster	N. Texas	6	19·65	4·69	Low
22	Rusk	S. Texas	3	23·33	1·26	,,
23	LeGrange	S. ,,	3	23·86	4·22	,,
		Average of:	119	21·16	5·5	

TABLE 4. EFFECT OF VARIATIONS IN TREATMENT ("T") AND WASHING TIMES ON SOLUBILITY REDUCTION
Agent: Reconstituted dry skim milk

"T" time (min)	Wash time (min)	Blocks	% Reduction
1	1	a	19·8
		b	21·8
2	1	a	19·2
		b	15·2
3	1	a	24·1
		b	21·1
5	1	a	19·1
		b	21·2
		c	22·7
15	1	119*	21·16 ± 5·5 s.d.
15	5	1	23·7
15	10	1	25·9
15	15	1	23·8

* From Table 3.

TABLE 5. EFFECT OF INDIVIDUAL MILK CONSTITUENTS ON ENAMEL SOLUBILITY

Treatment agent	Source	Number of blocks	% Solubility change Mean	Individual	
Lactose 5%	Eastman-Kodak Co. Rochester, N.Y.	3	− 3·2	a	− 2·4
				b	− 4·6
				c	− 2·6
Casein 2·5%	Nutritional Biochemical Corp., Cleveland, Ohio.	5	−25·3	a	−23·8
				b	−23·4
				c	−25·0
				d	−28·1
				e	−26·2
Ca 0·13%	as $CaCl_2$	3	+ 3·9	a	+ 2·6
				b	− 1·2
				c	+10·3
P 0·096%	as K_2HPO_4	3	− 0·6	a	− 1·4
				b	− 1·0
				c	+ 0·7

on the enamel, five experimental runs were made in which one of the three skim milk treated blocks from each run was washed for 5 min. in 2% trisodium phosphate after the casein treatment and then subjected to the C decalcification with the remaining two blocks. The findings given in Table 6 show that practically all the solubility reduction was eliminated.

To rule out the possibility that the solubility reduction produced by the milk treatments could be attributed to stagnation of deposits on the depressed enamel

TABLE 6. EFFECTS OF POST-TREATMENT WITH A PROTEIN SOLVENT ON ENAMEL SOLUBILITY REDUCTIONS PRODUCED BY MILK
(μg PO_4 per ml in used buffer)

Exp.	Block	Before milk treatment Decalcification "A"	Decalcification "B"	After milk treatment and "C" Decalcification Solvent post-treat.	No solvent post-treat.	% Solubility reductions
1	73c	47·5	50·3		38·2	23·8
	80c	48·2	45·9		34·4	25·0
	79c	45·3	42·6	40·3		5·4
2	64d	37·8	41·8		28·3	32·3
	68d	42·7	44·0		29·9	32·0
	66c	38·7	39·0	34·5		11·5
3	81b	40·5	42·1		31·5	25·2
	83a	39·3	39·4		29·8	24·1
	82c	40·2	40·8	39·7		2·5
4	70d	38·2	39·5		26·5	32·9
	75d	44·6	48·3		33·3	31·0
	71d	40·5	41·6	38·0		8·6
5	78b	40·3	38·7		28·2	27·1
	83b	40·5	40·2		29·4	26·8
	80d	43·5	41·0	38·3		6·6

"window" rather than to some positive sorption on the enamel, three sets of blocks were prepared so that in one of each set, the surface of the enamel was level with that of the surrounding varnish. The findings (Table 7) show that after the usual decalcification treatment sequences, the level windows showed as much solubility reduction as was produced by the usual depressed window.

TABLE 7. COMPARISON OF SOLUBILITY REDUCTIONS IN ORDINARY (DEPRESSED) AND LEVEL WINDOWS
% REDUCTION OF ENAMEL SOLUBILITY

Exp.	Ordinary (depressed) windows		Level windows
	1	2	3
1	19·3	13·4	19·4
2	26·6	26·0	28·3
3	28·0	26·7	28·6

DISCUSSION

The results presented seem to establish that, under the conditions used, milk reduces the solubility of enamel. Indeed, since because of the reported general increase in solubility in deeper layers of enamel which our figures in Table 1 support, it could be expected that our test solubility (decalcification C) would, in the absence of protective effect, be higher than the base or control solubilities (decalcifications A and B). From this it could be reasoned that the solubility reductions may actually be slightly greater than they appear to be.

The comparison of milk products of different geographic origin gave fairly uniform results and excluded the possibility of finding differences which might be associated with variations in caries prevalence. Actually, the milk giving the greatest solubility reduction (25·6%) came from West Springfield, Massachusetts, a high caries area, whereas the one which was the least protective (18·36%) came from Fresno, California, a relatively low caries area.

Our findings that the solubility reduction develops quickly and, as was shown in the tests with level windows, that it is not associated with sedimentation or stationary contact with the enamel, seem to indicate that the reaction between a milk constituent and the enamel is of an active sort. That it produces a strong attachment seems to be shown by the persistence of the solubility reductions after active washing. Such a reaction is in line with the adsorption of proteins on hydroxylapatite or calcium phosphate which is used in the calcium column separation of protein from other materials.

That the solubility reducing factor is a milk protein is shown by the failure of other milk constituents to produce comparable changes in enamel solubility, by the ability of casein to produce a full measure of solubility change and the effect of the protein solvent in almost completely restoring the enamel to its earlier solubility level. At this time nothing more than speculation can be offered regarding the manner by which milk constituents such as casein protect enamel against the action of acid. That more

than a sedimentation or loose deposit on the tooth surface is involved is indicated by experiments which showed the persistence of reduced solubility after vigorous washing, including that on the level "windows". The failure of such water washing to remove the solubility depressing agent is in keeping with the general inability of solvent or suspending agents to remove materials which were absorbed from them. That adsorption on the enamel was implicated in the binding of casein to the enamel was indicated by the adsorption isotherm given in tests in our laboratory (personal communication). Whether such putative adsorption occurs in selected areas having specific surface configurations at the atomic level, or whether it takes place uniformly over the whole enamel surface, is not known.

Whether the solubility effects of milk which we have demonstrated have any significance in respect to human or animal caries cannot be decided at this time. However, the findings may be taken to indicate that under some circumstances at least, milk could be assumed to exercise a moderating effect on enamel solubility in the mouth.

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Résumé—Une méthode reproductible pour comparer les effets d'un traitement par des produits laitiers sur la solubilité dans les acides de la surface de l'émail est mise au point. En utilisant cette méthode, il a pu être démontré que le traitement avec du lait de vache réduit la solubilité de plus de 20%, qu'il s'agisse de lait entier, pasteurisé ou écrémé.

Des laits entier et écrémé, reconstitués, ont donné des réductions du même ordre de grandeur. Des diminutions de solubilité identiques ont été obtenues avec de la crème ou du petit lait. Le beurre donne des réductions peu élevées et peu nettes. Aucune différence dans les effets sur la solubilité n'est constatée avec du lait écrémé en poudre, provenant de 23 localités différentes des Etats Unis. L'expérience a montré que l'agent de protection du lait réagit rapidement avec l'émail et résiste au lavage. Il s'agit d'une protéine ainsi que le démontre le fait que la réduction totale de solubilité est obtenue à l'aide de solutions de caséine et qu'elle est presque totalement abolie par un lavage à l'aide d'un solvant des protéines.

Zusammenfassung—Es wird eine reproduzierbare Methode beschrieben, um den Einfluss von Milchprodukten auf die Säurelöslichkeit der Schmelzoberfläche zu vergleichen. Mit Hilfe dieses Verfahrens wurde gefunden, dass die Anwendung von Kuhmilch die Löslichkeit um mehr als 20% reduzierte, unabhängig davon, ob es rohe oder pasteurisierte Voll- oder Magermilch war.

Wiederhergestellte Voll- bzw. Magermilch ergab ähnliche Reduktionen. Gleichartige Verminderungen der Löslichkeit fanden sich nach Behandlung mit Sahne oder Molke. Butter zeigte geringe und uneinheitliche Reduktionen. Hinsichtlich der Löslichkeitswirkungen von wiederhergestellter Trocken-Magermilch aus 23 Orten der Vereinigten Staaten wurde kein Unterschied gefunden. Die Versuche zeigten, dass der schützende Wirkstoff in der Milch schnell mit dem Schmelz reagiert und dem Abwaschen widersteht. Dass es seiner Natur nach ein Protein ist, geht daraus hervor, dass die volle Löslichkeitsreduktion durch Behandlung mit Kaseinlösungen gegeben ist, und dass sie durch Waschen mit einem Eiweisslösungsmittel fast vollständig beseitigt wird.

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