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carbonatées d'anesthésiques locaux constituent un progrès technique important dans le domaine de l'anesthésie régionale. Ces produits sont sûrs et il vaudrait la peine de procéder à des essais cliniques approfondis dans les principales techniques d'anesthésie par conduction.

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Effect of Preparation Technique and Storage Time on Vitamin C Content of Infant Formulas Made with Vitamin-Enriched Commercial Evaporated Milk

MARJORIE B. PHILLIPS, M.S.* and JANET M. WARDLAW, Ph.D.,+ Guelph, Ont.

IN RECENT years, there has been considerable concern among pediatricians and other interested individuals about the number of cases of scurvy appearing in Canada. Various reports have been made, two of which related to the disturbing number of admissions of children with scurvy to children's hospitals in Ontario and Manitoba.1, 2 In 1963, the Committee on Nutrition of the Canadian Paediatric Society reported on 69 cases of scurvy occurring from 1961 to 1963 in children aged 5 years and under.³ Distribution of these cases involved seven out of the ten Canadian provinces. More than half of these cases (68%) occurred in the 6- to 9-month age period, the 8th being the single month of highest incidence. It was noted that the most common formula fed to the infants was made from evaporated milk.

Recommended allowances of ascorbic acid for infants and children vary in different parts of the world, from 10 mg. per day in Great Britain to 40 mg. per day in South Africa.⁴ The Canadian Council on Nutrition considers that 20 mg. daily is sufficient to protect the infant against scurvy, and 30 mg. per day is adequate for a

normal adult.⁵ The Council also recommends that in Canada during pregnancy and lactation, the mother's allowance of 30 mg. be increased to 40 and 50 mg. respectively. The intake of the vitamin by the mother is reflected in the quantity available in breast milk, and human milk can be an important source of vitamin C to the infant. The results of analyses for vitamin C content of human milk vary considerably. Macy⁶ suggested a value of 5 mg. per 100 ml. as an average. On the basis of an average daily intake by the baby of 600 ml. of milk, this would be sufficient to provide approximately 30 mg. of vitamin C per day. Thus it is easy to understand why scurvy is seldom seen in breast-fed infants and why it is tempting to use the level of ascorbic acid intake from human milk as a practical standard.

Breast feeding is not the common practice it was in the early part of the century. Canned evaporated milk is a cheap and popular base in home-prepared infant formulas. However, vigorous processing in the plant virtually destroys any vitamin C present in the original milk. Nutritionists and health authorities have for some years been advocating reinforcement of evaporated milk with vitamin C, and in March 1964 an Order-in-Council was passed which allowed for addition of vitamin C to evaporated milk.⁷ Amendments to the existing provincial health legislation encouraged the co-operation

From the Department of Foods and Nutrition, Mac-donald Institute, University of Guelph, Guelph, Ontario. *Assistant Professor, Department of Foods and Nutrition. †Professor, Department of Foods and Nutrition.

Reprint requests to: Professor Marjorie B. Phillips, De-partment of Foods and Nutrition, Macdonald Institute, Guelph, Ontario.

of manufacturers, and by June of 1964 almost all evaporated milk in Canada was fortified with sodium ascorbate to provide 60 mg. per 15 fluid oz., or 14 mg. per 100 ml.

Enquiries as to the stability of vitamin C in evaporated milk when the product was subjected to the rigors of infant formula preparation prompted an investigation in 1965 by Pelletier and Morrison⁸ in Ottawa. Using two brands of evaporated milk enriched with ascorbic acid, it was found that while losses of the vitamin ranged from 6 to 19%, depending on the type of heat preparation or storage conditions, in no case did the level of ascorbic acid fall appreciably below that claimed on the label.

The present investigation was designed to study how the vitamin C content was affected when evaporated milk was subjected to the various heat techniques currently in use for infant formula preparations. For comparative purposes, samples were provided by The Hospital for Sick Children, Toronto,* prepared in the formula room and autoclaved. In addition, a limited number of samples of formula prepared by mothers in the home was obtained.

Experimental Procedure

Formula mixtures for testing the effects of various heat techniques were prepared in the laboratory, according to the procedures outlined in "The Canadian Mother and Child".9 Two types of evaporated milk, whole and partly skimmed, were used. For each type of milk the manufacturer[†] provided a specific batch from which samples were drawn. Each type was made up into mixtures, both with and without sugar; water to make up 6 oz. was added to the milk or milk-sugar mixtures. Replications were made from eight different tins of each type of milk. Every test bottle contained 2 oz. of milk and 4 oz. of water. Half of the samples contained, in addition, 6.3 g. of sugar, the equivalent of $1\frac{1}{2}$ teaspoons. For each of these mixtures, the following four methods of preparation were used:

Aseptic: Bottles were sterilized in boiling water for five minutes. The formula was heated in a saucepan and allowed to boil for three minutes, then poured into sterile bottles and capped.

Terminal: Samples were heated in capped, clean bottles in a boiling-water bath for 20 minutes. Timing was started from the time water boiled.

No heating technique: Milk was placed in clean bottles, boiling water was added, and the bottles were capped.

Control: Milk was placed in clean bottles, cold tap-water was added and bottles were capped.

Samples were analyzed for ascorbic acid on the same day as the formulas were prepared, and again after 24-hour storage in the refrigerator at 40° F.

Vitamin C content of batches of evaporated whole and evaporated partly skimmed milk was determined by assaying the ascorbic acid contents of eight tins of each and averaging the figures.

The analysis for ascorbic acid was carried out by the method used by the Food and Drug Directorate, Ottawa,¹⁰ i.e., titration of ascorbic acid with 2,6-dichlorophenolindophenol on filtered acetic acid—meta-phosphoric acid extracts of the milk samples.

Home-prepared samples were collected in duplicate from homes in the Guelph-Fergus area, with the co-operation of the staff of the Wellington-Dufferin-Guelph Health Unit. Unannounced visits were made to the mother, who supplied a sample of the formula she had made for current use. In no case was any sample more than 12 hours old. Assays for vitamin C were carried out on the day of collection and again after 24-hour storage in the refrigerator at 40° F. A second collection was made from the same home approximately one week after the first, and was tested in the same way.

Results and Discussion

In Table I figures are presented for the ascorbic acid content of processed samples, expressed as the percentage retention of the contents of the tins as purchased. The latter were established as 20.8 mg. ascorbic acid per 100 ml. of whole evaporated milk, and 16.6 mg. per 100 ml. of partly skimmed milk.

Analysis of variance indicated that treatment technique and time were significant factors (p < 0.01) affecting the vitamin C retention in the formula preparations. The aseptic method markedly lowered the retention of vitamin C. The terminal method also significantly lowered the vitamin C retention, but to a lesser degree than the aseptic method. Neither the no heating nor the control treatments resulted in any appreciable loss of ascorbic acid.

Using the lowest figure obtained for vitamin C retention at 0 hours (90.2%) and at 24 hours (80.6%) in the whole evaporated milk, it is apparent that the ascorbic acid content in no

^{*}Courtesy of Dr. Elizabeth Chant Robertson and Miss Helen Cornish. †Carnation Company Limited, Toronto.

TABLE I.—PERCENTAGE RETENTION OF VITAMIN C IN EVAPORATED MILK¹ FORMULA AFTER FOUR PREPARATION PROCEDURES

	Whole evaporated milk				Partly skimmed evaporated milk				
Preparation procedure	With sugar		Without sugar		With sugar		Without sugar		
	0 hours ²	24 hours ³	0 hours	24 hours	0 hours	24 hours	0 hours	24 hours	
Terminal No heating	97.0 ± 1.2	91.6 ± 2.1	$\begin{array}{c} 90.2 \pm 2.8 \\ 96.8 \pm 1.3 \\ 97.8 \pm 0.6 \\ 98.0 \pm 1.1 \end{array}$	91.4 ± 3.0 91.0 ± 2.0	95.8 ± 1.6	86.0 ± 2.0	95.8 ± 1.7 98.2 ± 1.0	87.0 ± 2.6 89.7 ± 2.3	

¹Vitamin C content of evaporated milk before preparation procedures: whole, 20.8 mg, per 100 ml;

partly skimmed, 16.6 mg. per 100 ml. 2Not more than six hours after preparation.

³Twenty-four hours after preparation.

⁴Standard deviation.

instance fell below 18.8 mg. per 100 ml. or 16.8 mg. per 100 ml. respectively. These figures are well above the Canadian Food and Drug regulation of 14 mg. per 100 ml. Considering the comparable figures for vitamin C retention in the partly skimmed evaporated milk (88.7% and 78.6%), it appears that the processed samples provided 14.7 mg. per 100 ml. and 13.0 mg. per 100 ml. ascorbic acid for 0 hours and 24 hours respectively. Although the latter figure is slightly below the regulation, it should be borne in mind that it is doubtful an infant would receive more than one bottle of formula which had been stored for this length of time. Under these experimental conditions, a level of vitamin C below the standard would result only when partly skimmed evaporated milk was processed by the aseptic technique and stored for 24 hours.

Results also indicated that neither the type of milk (i.e. whole or partly skimmed) nor the addition of sugar made any marked difference to the amount of vitamin C loss produced by the various treatments or by the 24-hour storage.

Twenty-four ounces of formula is considered to be, according to "The Canadian Mother and Child", the usual daily intake for an 8-lb. baby. Using the percent retention figures for vitamin C obtained in this study, the amount of vitamin C retained in 24 oz. of formula made with evaporated milk was calculated for each preparation technique (Table II).

Even taking into account the use of the aseptic technique and storage for 24 hours (when combined, these procedures caused the greatest loss), in no case was the intake calculated to be below 20 mg. of vitamin C, the recommended intake for an infant of this size.⁵ We therefore are assured that evaporated milk, under these conditions, provided an adequate intake of vitamin C for the infant. It appeared that even under the most stringent conditions of the experiment, this batch of whole evaporated milk retained an equivalent of at least 14 mg. ascorbic acid per 100 ml. of milk.

TABLE III.—PERCENTAGE RETENTION OF VITAMIN C IN AUTOCLAVED EVAPORATED MILK FORMULA 24 HOURS AFTER PREPARATION

Whole	e milk	Partly skimmed milk			
Sugar	Plain	Sugar	Plain		
88.9	87.5	84.5	80.8		

Samples from The Hospital for Sick Children were prepared to determine the effect of autoclaving on the vitamin C content. Upon delivery to the laboratory, samples were 24 hours old, and only one assay for ascorbic acid was made on each. The results, shown in Table III, serve to emphasize that vitamin C loss occurred as a result of heat preparation, but the loss was not serious in terms of adequate availability of

TABLE II.-VITAMIN C CONTENT IN MILLIGRAMS PER 8 OUNCES¹ OF EVAPORATED MILK FORMULA (WITHOUT SUGAR) AFTER FOUR PREPARATION PROCEDURES

	Whole evap	orated milk	Partly skimmed evaporated milk		
Preparation procedure	0 hours ²	24 hours ³	0 hours	24 hours	
Aseptic. Terminal No heating Control.	47.5 ± 0.5 47.8 ± 0.3	$\begin{array}{c} 40.5 \pm 1.6 \\ 44.8 \pm 1.1 \\ 44.5 \pm 1.0 \\ 44.6 \pm 1.0 \end{array}$	$\begin{array}{c} 34.6 \ \pm \ 0.8 \\ 35.0 \ \pm \ 0.9 \\ 38.1 \ \pm \ 0.3 \\ 38.0 \ \pm \ 0.4 \end{array}$	$\begin{array}{c} 30.8 \pm 1.8 \\ 33.7 \pm 1.7 \\ 34.7 \pm 0.9 \\ 33.9 \pm 1.0 \end{array}$	

¹On the basis of 1 oz. of evaporated milk per lb. for an 8-lb. baby.

²Not more than six hours after preparation.

³Twenty-four hours after preparation.

⁴Standard deviation.

				No. oz.	Mq. vitamin C -	Supplements		Total mg.
	Preparation procedure	Age of baby	Type of milk	formula per day	per day from formula	Fruit juice	Vitamin ¹ prepn.	vitamin C per day
1	No heat	6 wks.	Whole P.S. ²	36	$\begin{array}{c} 83\\52\end{array}$		A.C.D.	113 82
2	"	3 mos. 4 mos.	Whole	24 30	$\overline{42}$	Orange	"	117
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Aseptic	9 mos. 4 mos.	"	$\begin{array}{c} 30\\24\end{array}$	84 48	Apple	"	$\begin{array}{c}149\\78\end{array}$
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	No heat Aseptic	8 mos. 2 mos.	P.S. Whole	$\begin{array}{c} 42\\ 32 \end{array}$	$\begin{array}{c} 95 \\ 59 \end{array}$	Orange	A.C.D.	$\begin{array}{c}140\\89\end{array}$
8	No heat "	$5\frac{1}{2}$ mos. $6\frac{1}{2}$ mos.	"	$\frac{24}{20}$	$\begin{array}{c} 64 \\ 19 \end{array}$	Orange 		$\begin{array}{c} 139 \\ 64 \end{array}$
10	" Terminal	$\frac{4}{4}$ mos. $\frac{41}{2}$ mos.	••	$\frac{29}{30}$	$\begin{array}{c} 60\\51 \end{array}$		A.C.D.	$\begin{array}{c} 90 \\ 81 \end{array}$
12 13	Aseptic No heat	3 wks. 7 wks.	"	$\frac{24}{24}$	$\begin{array}{c} 25 \\ 40 \end{array}$	Orange	 	$\frac{55}{115}$

TABLE IV.--VITAMIN C INTAKE OF INFANTS CONSUMING HOME-PREPARED EVAPORATED MILK FORMULA

¹Commercial preparation containing indicated vitamins. ²Partly skimmed.

the vitamin C. Loss of vitamin C ranged from 11.1 to 19.2%, with an average from all samples of 7.4%.

Collection of home samples was made at random from 13 homes where it was known that evaporated milk was used for infant formulas. At the time of collection of the second sample from each home, the nutritionist obtained additional information concerning related aspects of the infant's daily intake. Some of these pertinent data are presented in Table IV.

Of the 13 mothers, three used the aseptic method, one the terminal, and nine prepared their baby formulas as they required them, using no heat in their preparation.

As the samples were collected from each household on two different days, analyses were made on individual bottle contents, and the results averaged. Based on these analyses, and on the reported amount of formula consumed by the baby, it was possible to estimate the amount of vitamin C available to the infant from his formula. These figures appear in Table IV, column 5. All but one infant, whose intake was borderline, were receiving more than the 20 mg. recommended in the Canadian Dietary Standard.

It is of interest that despite the use of a milk providing added vitamins C and D, all babies were given some form of vitamin supplement, either in the form of fruit juice or commercial vitamin preparation, or both. Four ounces of vitaminized apple juice or orange juice would provide from 35 to 45 mg. of vitamin C, while the commercial preparation used would add approximately 30 mg. of the vitamin. Thus, between 30 and 75 mg. of vitamin C were added to the already more than adequate intake of these infants. The average intake of vitamin C of the babies in this study ranged from 55 to 150 mg. per day when all such sources were taken into account. It is clear that any one of several generally used infant foods provides a generous daily allowance, as judged by the Canadian Dietary Standard.

Summary The effect of various heat techniques currently in use for infant formula preparations on the vitamin C content of vitaminenriched, commercial evaporated whole and partly skimmed milks has been studied.

The following points were established: Aseptic and, to a lesser extent, terminal techniques significantly lowered vitamin C retention. Vitamin C loss was accentuated after refrigerated storage of the formula for 24 hours. Neither the type of evaporated milk nor the addition of sugar made any marked difference to the amount of vitamin C loss produced by the various treatments, or by the 24-hour storage. Even under the most stringent conditions of formula preparation, whole evaporated milk provided at least 14 mg. vitamin C per 100 ml., the amount required by Food and Drug Regulations in Canada. Partly skimmed evaporated milk contained a minimum of 14 mg. per 100 ml. when tested at 0 hours, but when aseptic technique was used this content was reduced to 13 mg. per 100 ml. after 24 hours' storage. Based on an intake of 1 oz. evaporated milk per lb. of body weight of the infants, and regardless of preparation technique and a possible 24-hour refrigerated storage of the formula, vitaminenriched, commercial evaporated milk provided enough vitamin C, according to the Canadian Dietary Standard, to meet the 20-mg. per day requirement of the infants.

Résumé Les auteurs ont étudié l'effet qu'exercent le mode de préparation et la durée de l'entreposage sur la teneur en vitamine C des formules infantiles préparées à partir de lait évaporé commercial, entier ou partiellement écrémé, et additionné de vitamines.

On a pu établir les points suivants: La technique aseptique et, dans une moindre mesure, les techniques finales réduisent notablement la rétention de la vitamine C. La perte de cette vitamine a été

accentuée par la conservation de la formule au réfrigérateur pendant 24 heures. Ni le type de lait évaporé utilisé, ni l'addition de sucre n'ont changé quoi que ce soit à la diminution de la teneur en acide ascorbique produite par les divers traitements ou par l'entreposage de 24 heures. Même dans les conditions de préparation de la formule les plus rigoureuses, le lait évaporé entier apporte au moins 14 mg de vitamine C par 100 ml, soit la dose exigée par les règlements de la Division des Aliments et des Drogues au Canada. Quant au lait évaporé partiellement écrémé, il contenait un minimum de 14 mg par 100 ml quand l'essai était fait à 0 heure. Cette teneur était cependant réduite à 13 mg par 100 ml après emploi de la technique aseptique et après un entreposage de 24 heures. Si l'on tient compte d'une ration de 1 once de lait évaporé par livre de poids corporel du nourrisson, le lait évaporé commercial additionné de vitamines apporte au nourrisson une dose suffisante de vitamine C, selon les normes alimentaires canadiennes, pour couvrir les besoins de 20 mg par jour et ce, quel que soit la technique de préparation et même en tenant compte de la période de 24 heures d'entreposage de la formule.

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