# Thiamin Content of Milk in Relation to Vitamin B, Requirement of Infants\*

ELIZABETH M. KNOTT, Ph.D.

Department of Pediatrics, University of Chicago, Chicago, Ill.

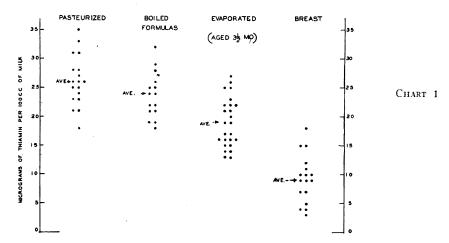
THE thiamin content of milk is im-• portant since it is the only natural source of vitamin B<sub>1</sub> available to the young infant. There are, however, complications which make thiamin assays on milk difficult. With biological assays, if the curative technic is used, it is difficult to get the depleted rats to consume the quantity of test dose that is necessary; if growth technics are employed, the basal diets must be completely adequate except in thiamin so that the resulting growth is due solely to the thiamin content of the test dose, but milk doses introduce the problem of added caloric ingestion which may affect growth. With thiochrome assays on milk the problem is also complex, since thiamin may be lost if proteins are precipitated, and the absorption and elution of the vitamin from the decalso column may not be quantitative. In our study we have employed all of these methods and are also investigating the application of the fermentation technic. The results reported here are based chiefly on growth assays, since thus far these have proved to be the most reliable.

Chart 1 compares results for pasteurized milk, boiled milk formulae, evaporated milk, and breast milk. The formulae were prepared from pasteurized milk which had been boiled for 3 minutes. The average thiamin content of these formulae was 24 micro-

grams per 100 ml. of milk, in contrast to the average of 26  $\mu g$ . obtained per 100 ml. of pasteurized milk. The boiling for 3 minutes thus had caused a loss of 8 per cent in the vitamin content. The range of thiamin for these pasteurized and boiled milks was from 18 to 35  $\mu g$ . per 100 ml. The evaporated milks tested had a slightly lower range from 13 to 27  $\mu$ g. of thiamin, with an average value of 19. The data for these evaporated milks have been calculated on the basis of reconstitution with equal parts of water to make comparisons easier between the different types of The breast milks tested came from 17 different women. The thiamin content was surprisingly low, ranging only from 3 to 18  $\mu$ g. per 100 ml. of milk, with an average of 9  $\mu g$ . There may be several explanations for the low thiamin content of human milk. In the case of one woman whose milk was tested on four different occasions the milk consistently had high thiamin except during one week when she was ill and her milk contained only 20 per cent of its previous thiamin value. Diet is undoubtedly the most important factor in influencing the thiamin content of breast milk. To support this theory was the observation of a trend toward lower values for thiamin for some of the women who had but limited knowledge of fundamental principles in nutrition. Some preliminary studies have been done with supplementary thiamin for these women, but one week of increased intake has not been enough to

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### VARIATIONS IN THE THIAMIN CONTENTS OF MILKS

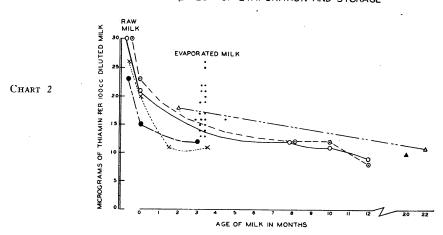


produce increased excretion of thiamin via the mammary glands. It is presumable that cow's milk is richer than human milk in thiamin because the cow can utilize thiamin which has been synthesized in her rumen.

Chart 2 shows in more detail the effect of processing on the thiamin content of evaporated milk. Raw milks and their corresponding evaporated milks were sent by special delivery direct from the factory to the laboratory and were assayed simultaneously. Destruction of vitamin due to the processing of

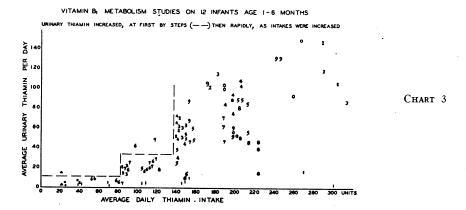
evaporated milk ranged from 23 to 35 per cent for the four lots of milk tested. Further loss of thiamin occurred during storage of these evaporated milks. The amount of destruction varied with different milks, but reached 50 per cent after 1 year for two milks, although another milk showed loss of one-half its original vitamin content in 2 months' time. This destruction upon storage is presumably more rapid during the first few months and eventually reaches an equilibrium. The rate varies for each lot of milk and is roughly indicated by

THE THIAMIN CONTENT OF MILK EFFECT OF EVAPORATION AND STORAGE



the lines connecting the separate assays on the chart. It must be noted, however, that the twenty-five commercial samples of milk representing seven different brands, in general had higher contents of thiamin than the four lots stored in our laboratory. The age of these commercial milks was approximately  $3\frac{1}{2}$  months, which is an average elapsed time between the manufacture and retailing of evaporated milk. The destruction of thiamin in evaporated milk is influenced by the degree and duration of heat during processing, the temperature during storage, and the pH

amount of thiamin excreted in the urine has been plotted against the level of intake for that metabolism period. At low levels of intake the infants excreted consistently low amounts of thiamin. At 80 units or more daily intake the thiamin appeared in the urine in larger quantities. Above 140 units of daily thiamin, the vitamin was excreted in still larger quantities, and it was apparent that more was being fed than the infant needed. From these data we might conclude that an intake of about 80 units of thiamin meets the infant's immediate needs and above this level of



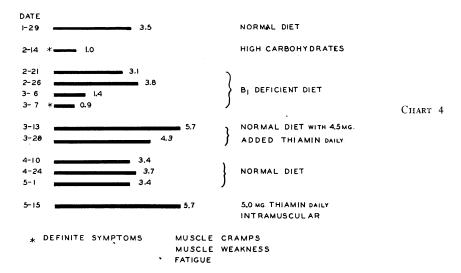
of the milk. That this destruction is a simple cleavage of the thiamin molecule into pyrimidine and thiazole was shown when one sample of old evaporated milk was assayed for pyrimidine as well as thiamin content and total values found which were identical with the vitamin B<sub>1</sub> content of the original raw milk.

In order to determine the significance of the thiamin content of these different types of milk, the metabolism of thiamin in the infant's body has been studied. Chart 3 summarizes the results of urinary excretion of thiamin when the infant has been given different levels of supplementary thiamin. Each dot on the chart represents data obtained during a 5 day period. In all, 12 healthy infants were studied for a total of 104 periods. The average daily

intake excess begins to appear in the urine. We do not know, however, whether or not 80 units represents the optimum thiamin requirement for the infant.

Another approach to the problem of requirement has been made by studying blood cocarboxylase levels. Since cocarboxylase is the functioning form of thiamin in the body cells, its estimation in blood cells should give an indication of the supply throughout the body. After several hundred determinations, during healthy as well as pathological conditions, we have concluded that about 5  $\mu$ g. of cocarboxylase per 100 ml. of blood is a normal value for health. Chart 4 shows the correlation between blood cocarboxylase, dietary thiamin, and symptoms in an adult.

# CORRELATION OF BLOOD COCARBOXYLASE WITH DIETARY THIAMIN AND SYMPTOMS IN AN ADULT



This person does not absorb thiamin well from the digestive tract, so that a normal diet gives subnormal blood levels. The 3.5  $\mu g$ . of cocarboxylase per 100 ml. of blood is not enough to protect against the sudden strain of illness, strenuous exercise, or excess carbohydrate diet. Symptoms of muscle cramps, muscle weakness, and fatigue were definite when the blood vitamin dropped to 1.0  $\mu g$ . A feeling of well-being was outstanding when blood levels had been raised above 5.0  $\mu$ g. by use of supplementary thiamin.

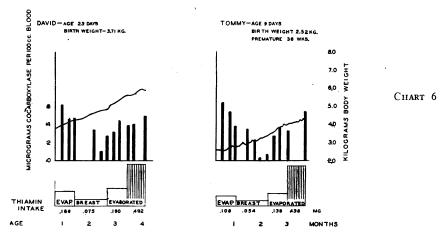
Chart 5 gives results for a few infants. In general, values tended to decrease from a level of 5 or more  $\mu g$ . shortly after birth to levels between 3 and 4  $\mu g$ . Lowest levels were found for infants receiving manually expressed breast milk. The data for two of these infants are given more in detail in Chart 6. The type of milk, amount of thiamin intake, and age of the infants are indicated across the bottom of the chart. On evaporated milk formulae the blood decreased from an initial level of more than 6  $\mu$ g. to 4.5 and 3.8  $\mu$ g. Breast

# INFLUENCE OF TYPE OF FEEDING ON BLOOD COCARBOXYLASE



\*\*INFANT ABSORBED ALL NUTRIENTS VERY EFFICIENTLY

## BLOOD COCARBOXYLASE LEVELS IN ARTIFICIALLY FED INFANTS



milk feedings for the next 5 weeks brought the values down to 1.0 and  $0.5 \mu g$ . Subsequent feeding of evaporated milk slowly caused an increase in blood cocarboxylase to 4.4 and 3.6  $\mu g$ . but the more optimum level of 5  $\mu g$ . was only reached after supplements of pure thiamin were included in the formulae. The infant whose blood responded best to his evaporated milk feedings received 190  $\mu g$ . or 63 units of thiamin daily in the milk. This level of intake is not far from the level of 80 units at which excess thiamin began to be excreted in the urine. It thus appears that, although intakes of approximately 200  $\mu g$ . in the present study could not maintain the 5  $\mu g$ . of cocarboxylase which we have come to consider optimum, the amount of vitamin B<sub>1</sub> furnished by milk formulae apparently did produce the fairly satisfactory level of 3-4  $\mu g$ . This level is adequate unless some unusual fever or metabolic strain increases the metabolic needs for thiamin. The milk thiamin for artificially fed infants can therefore presumably carry the burden of thiamin requirement for the young infant until cereals and other additional foods are included in his diet. It must be emphasized, however, that during times of stress supplementary thiamin will be desirable as a safety factor.

An additional comment is necessary concerning infants receiving breast milk. According to our asasys this type of milk cannot furnish 200  $\mu g$ . of thiamin daily. Nursing infants studied have usually had blood cocarboxylase levels between 3 and 4  $\mu g$ ., comparing well with those receiving milk formulae. On the other hand, those infants of our receiving manually expressed breast milk showed lower levels of blood cocarboxylase. It may be possible that the thiamin content of the breast milk was reduced during the 2-4 days of storage before it could be fed. We know that cleavage of thiamin occurs more rapidly in more alkaline solutions. The average pH for our breast milk samples was 8.1, in contrast to values of 6.8 for pasteurized milk and 6.2 for evaporated milk.

Further studies on the thiamin content of breast milk and its adequacy for infant feeding are now in progress at the University of Chicago.

Additional data available as this paper goes to press show breast milk thiamin values to be higher than our earlier work indicated. Average thiamin content for women succeessfully nursing young infants was 20.1 µg. per 100 ml.