# THE INFLUENCE OF DIET ON THE ASCORBIC ACID REQUIREMENT OF PREMATURE INFANTS

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The purpose of this report is to present data, obtained on premature infants, demonstrating that one of the factors determining the need for ascorbic acid is the level of protein intake. It is proposed that this factor plays a rôle in the greater requirement of artificially fed babies, as compared to breast-fed infants, for supplements containing this vitamin.

Long before actual data were available as to the daily total ascorbic acid requirement of the newborn infant, or as to the amount of the vitamin present in human and other milks, it was the common clinical observation that when scurvy occurred in infancy it was in the artificially fed baby. It was assumed that the protection of the breast-fed infant against the disease could be attributed to the antiscorbutic activity of breast milk. This opinion was confirmed by experimental evidence, which has been well summarized in a recent review by Smith (1). The evidence includes the important findings of Selleg and King (2) who showed that the actual amount of ascorbic acid received by an infant ingesting an adequate volume of breast milk from a mother whose diet is adequate in this factor closely approaches the amount which other observers have determined to be the approximate daily requirement of the newborn infant. Determinations of blood plasma ascorbic acid levels by Braestrup (3), Mindlin (4, 5), Snelling (6) and others in babies of various ages, but particularly in the first weeks of life, reveal that these levels are on the average much lower in formula-fed than in breast-fed infants. Moreover, Mindlin (5) demonstrated a definite relationship between the plasma ascorbic acid level of the infant and the amount shown to be present in the mother's milk. However, Mindlin also found (4, 7) that even with supplements of 20 to 75 mgm. of ascorbic acid daily, formula-fed infants had lower levels, determined 16 hours after the last vitamin C dose, than did breast-fed infants receiving no supplements, and Braestrup (3) observed that daily dosages of 20 mgm. of ascorbic acid had a slow effect in raising the plasma levels in babies given boiled cow's milk. These observations are only partially explained on the assumption that the ascorbic acid requirement of the breast-fed and the formula-fed infant is the same, and that the former receives all or part of it in milk whereas the latter, whose feeding is prepared by heating in the presence of air, receives practically none. The supposition that the total daily requirement of infants receiving cow's milk is actually higher than that of infants receiving human milk is more completely consistent with the findings of the authors cited.

The suggestion that the daily protein intake, which is usually at least twice as high with cow's milk formulas as with human milk, might play a rôle was made by Levine, Gordon, and Marples (8) as a result of observations on the amino acid metabolism of infants. They demonstrated the excretion of incompletely oxidized derivatives of phenylalanine and tyrosine in the urine of premature infants whose diet contained a relatively high protein intake (5 grams or more per kilogram per day) and of full-term infants to whom these amino acids were administered when no vitamin C supplements were being given, and they further observed that sufficiently large doses of ascorbic acid diminished or abolished the excretion of the derivatives (8, 9, 10). These results indicate that vitamin C plays an important rôle in the intermediary metabolism of aromatic amino acids, an interpretation consistent with the findings of Sealock and his colleagues (11, 12) that scorbutic guinea pigs also excrete derivatives of tyrosine and phenylalanine, and that the feeding of tyrosine increases the ascorbic acid requirement of these animals. Ley (13), in connection with clinical observations in the newborn, made the suggestion that the individual infant's requirement for this

vitamin may be related to activity and to intermediary metabolism.

As a practical means of investigating the relationship between diet in infancy and vitamin C requirement, two groups of premature infants were studied, one of which received boiled human milk and the other cow's milk formulas. By means of a modified Kadji test (14) the ascorbic acid stores of both groups following saturating doses of the vitamin were determined.

TABLE I

*Ascorbic acid storage in premature infants on different intakes of protein*

INFANTS RECEIVING HUMAN MILK

| Name | Sex | Birth weight | Protein average | "Saturation dose" of ascorbic acid | | Day of test | | Days elapsed between saturation and test | Test dose | | Plasma ascorbic acid level | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total | Days | Age | Weight | | | Route | Before test dose | 4 hours after test dose |
| | | *kgm.* | *grams per kgm. per day* | *mgm.* | | *days* | *kgm.* | | *mgm.* | | *mgm. per 100 cc.* | |
| J. H. | F | 2.38 | 2.6 | 800 | 4 | 16 | 2.60 | 6 | 100 | Intramuscular | 0 | 1.6 |
| B. U. | F | 2.12 | 2.7 | 900 | 5 | 13 | 2.41 | 5 | 100 | Intramuscular | 0.3 | 1.3 |
| H. R. | M | 2.34 | 2.7 | 900 | 5 | 19 | 2.52 | 7 | 100 | Intramuscular | 0 | 1.7 |
| A. S. | F | 2.13 | 2.7 | 900 | 5 | 17 | 2.24 | 7 | 100 | Intramuscular | 0.5 | 3.5 |
| R. Pl. | F | 2.00 | 2.8 | 800 | 4 | 26 | 2.44 | 6 | 200 | Oral | | 2.6 |
| J. R. | M | 2.33 | 2.7 | 800 | 4 | 16 | 2.48 | 7 | 200 | Oral | | 1.4 |
| R. Pu. | M | 1.71 | 2.7 | 800 | 4 | 15 | 1.98 | 7 | 200 | Oral | | 1.8 |
| C. C. | F | 2.28 | 2.7 | 800 | 4 | 22 | 2.56 | 7 | 200 | Oral | | 1.5 |
| D. C. | F | 2.09 | 2.6 | 800 | 4 | 19 | 2.33 | 7 | 200 | Oral | | 1.9 |
| A. C. | F | 1.99 | 2.7 | 800 | 4 | 19 | 2.17 | 7 | 200 | Oral | | 2.0 |
| P. G. | M | 1.98 | 2.6 | 800 | 4 | 21 | 2.45 | 7 | 200 | Oral | | 1.9 |
| C. B. | F | 1.84 | 2.7 | 800 | 4 | 16 | 2.13 | 6 | | | 0.8 | |
| F. D. | F | 1.75 | 2.7 | 900 | 5 | 20 | 2.03 | 7 | | | 0.1 | |
| | | | | | | | | | | | Average | 1.9 |

INFANTS RECEIVING COW'S MILK FORMULAS

| Name | Sex | Birth weight | Feeding | | "Saturation dose" of ascorbic acid | | Day of test | | Days elapsed between saturation and test | Test dose | | Plasma ascorbic acid level | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Protein | Type of milk* | Total | Days | Age | Weight | | | Route | Before test dose | 4 hours after test dose |
| | | *kgm.* | *grams per day per kgm. body weight* | | *mgm.* | | *days* | *kgm.* | | *mgm.* | | *mgm. per 100 cc.* | |
| T. M. | M | 1.80 | 5.9 | PWM Olac | 900 | 5 | 18 | 2.11 | 7 | 100 | Intramuscular | 0 | 0.5 |
| M. S. | F | 2.13 | 5.0 | EM | 900 | 5 | 20 | 2.66 | 7 | 100 | Intramuscular | 0 | 1.0 |
| C. M. | M | 1.79 | 4.6 | EM | 900 | 5 | 19 | 2.06 | 5 | 100 | Intramuscular | 0.2 | 1.4 |
| P. V. | F | 1.66 | 4.7 | EM | 800 | 4 | 19 | 2.16 | 6 | 200 | Oral | | 1.2 |
| P. Mo. | F | 2.16 | 6.1 | Alacta | 800 | 4 | 19 | 2.68 | 6 | 200 | Oral | | 0.6 |
| P. Mc. | F | 2.27 | 4.8 | EM | 800 | 4 | 23 | 2.69 | 7 | 200 | Oral | | 0.3 |
| C. T. | F | 1.45 | 6.1 | Olac | 800 | 4 | 45 | 2.02 | 7 | 200 | Oral | | 1.6 |
| C. L. | F | 2.23 | 6.1 | Alacta | 800 | 4 | 19 | 2.69 | 7 | 200 | Oral | | 0.2 |
| S. M. | M | 2.31 | 4.7 | EM | 800 | 4 | 25 | 2.53 | 7 | 200 | Oral | | 0.4 |
| P. J. | F | 1.99 | 4.9 | EM | 800 | 4 | 15 | 2.28 | 7 | 200 | Oral | | 0.1 |
| T. Z. | M | 2.05 | 4.7 | EM | 800 | 4 | 15 | 2.34 | 7 | 200 | Oral | | 1.5 |
| C. G. | F | 1.85 | 7.0 | Alacta | 800 | 4 | 18 | 2.21 | 6 | | | 0.2 | |
| F. T. | M | 2.35 | 5.9 | Alacta | 900 | 5 | 19 | 2.53 | 8 | | | 0.1 | |
| | | | | | | | | | | | | Average | 0.8 |

* Abbreviations PWM and EM refer to powdered whole milk and evaporated milk, respectively.

## PROCEDURE AND METHODS

### *Subjects and diets*

The subjects were 26 premature babies whose birth weights and other significant data are tabulated in Table I. Except for the formulas and ascorbic acid,[1] none of the infants received other food or medication except 5 per cent glucose on the first day or two of life, and a concentrate of vitamins A and D beginning usually about the seventh day. No infants who required transfusions were included. One group of infants received pooled human milk obtained chiefly from mothers of infants on the ward and prepared by boiling for 20 minutes in the formula room at the hospital. Formulas of cow's milk of isocaloric value but of varying composition were given to the second group, some being prepared from evaporated milk, some from Olac,[2] a mixture having the proportions of whole cow's milk but in which the butter fat is replaced by olive oil, and some from Alacta,[2] a preparation of cow's milk from which more than half of the fat has been removed. All of the latter formulas contained added sugar and all were boiled. Vitamin C analyses were made on occasional samples and will be discussed later.

### *Ascorbic acid administration*

Observation on each infant was begun when he or she reached an age and state of vigor at which he was able to take a full formula providing about 120 to 125 calories per kilogram of body weight per day. The average age was 10 days. In order to saturate the tissues with ascorbic acid, 800 to 900 mgm. were given in divided doses over a period of 4 to 5 days. The vitamin doses were given by mouth in every instance except in 4 infants in whom it was decided to obtain a check on the fact that these enormous doses actually did "saturate" the body. In these 4 infants the final dose was an intramuscular one of 100 mgm. ascorbic acid which was followed by a test similar to that of Kadji, Light and Kadji (14). The results are recorded in Table II.

During an interval of 5 to 8 days following the last dose of ascorbic acid, the infant was given no additional source of the vitamin. The diet during this interval, as well as during the days when the "saturation" doses of vitamin C were given, is shown for each infant in Table I, the figures for protein intake representing the average daily amount for the period. On the test day, ascorbic acid was given, either 100 mgm. intramuscularly in the gluteal region or 200 mgm. by mouth, between 7:30 and 9:30 a.m. Four hours later a venepuncture was performed to obtain blood for the determination of plasma ascorbic acid. In addition, in all of the infants who were to receive the intramuscular test dose, a "fasting" plasma level was obtained just before the injection.[3]

TABLE II

*Test for saturation on day after four successive daily doses of 200 mgm. of ascorbic acid*

Test dose, 100 mgm. intramuscularly.

| Subject | Feeding | Plasma ascorbic acid level | | |
|---|---|---|---|---|
| | | 18 hours after last oral dose | 4 hours after test dose | Increase |
| | | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *mgm. per 100 cc.* |
| B. U. | H. M. | 0.5 | 2.8 | 2.3 |
| F. D. | H. M. | 0.4 | 2.6 | 2.2 |
| H. R. | H. M. | 1.2 | 3.0 | 1.8 |
| C. M. | E. M. | 0.8 | 3.5 | 2.7 |

### *Chemical methods*

The method for the blood plasma ascorbic acid determinations was essentially that of Mindlin and Butler (15), using the Evelyn photocolorimeter. The method was modified by the omission of cyanide, as this has been shown to be unnecessary by Friedman, Rubin, and Kees (16). Special care was taken to avoid hemolysis of the blood and to chill the samples soon after drawing, as recommended by Snelling and Jackson (17), and Heinemann and Hald's precaution (18) of checking the pH of the metaphosphoric acid reagents by frequent titration was followed. In many instances it was undesirable or impossible to withdraw from the small veins of premature infants sufficient blood to yield 2 cc. of plasma on centrifugation. After a preliminary study of the micromethod described by Mindlin and Butler (15), it was decided that more accurate results could be obtained by a modification of the macromethod, using smaller amounts of plasma, precipitating them with metaphosphoric acid reagent and water in the proportions used in the macromethod, and diluting the filtrates as necessary with 0.22 N metaphosphoric acid before making the colorimeter readings. In agreement with Heinemann and Hald (18), it was found that readings so obtained on an original sample of 0.8 cc. or more of plasma were entirely satisfactory. In a few instances in which turbid filtrates were obtained, the modification suggested by Bessey (19) was used.

Milk samples were analyzed for ascorbic acid by a modification of Bessey's method (19). No ascorbic acid

[1] Prior to the "saturation" doses of ascorbic acid listed in Table I, the following infants had received a number of daily doses of 25 mgm. each: infants H. R., C. C., M. S., P. M., C. T., and S. M. received, respectively, 1, 2, 1, 5, 17, and 7 doses. Infant R. Pl. received 25 mgm. daily on the eighth to eleventh days of life and 200 mgm. daily on the twelfth through the fifteenth days.

[2] Manufactured by Mead Johnson and Co.

[3] The reason for the change from a parenteral to an oral test dose was the occurrence in 2 infants of a rather severe local reaction characterized by sterile necrosis and sloughing. This was attributed to the acidity of the solution and the possibility that, due to the small size of the gluteal muscles in premature infants, a portion of the dose may have inadvertently been injected subcutaneously instead of intramuscularly.

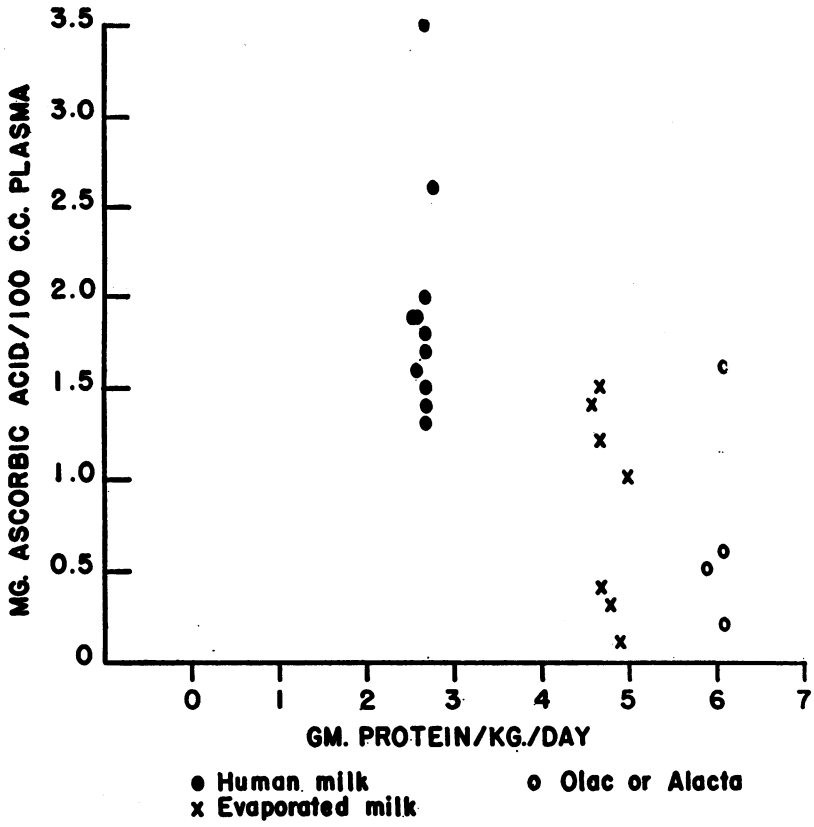


FIG. 1. RELATION BETWEEN PROTEIN INTAKE AND PLASMA ASCORBIC ACID LEVEL, 4 HOURS AFTER TEST DOSE

was found either in boiled human or boiled cow's milk.[4] A few attempts were made to use the method of Woessner, Elvehjem, and Schuette (20), which proved satisfactory except in a few instances in which a heavy turbidity could not be prevented.[5]

## RESULTS

The findings are in accord with the opinion expressed by Mindlin (7), Kadji, Light, and Kadji (14) and others, that the response to a test dose is more significant than single plasma ascorbic acid determinations. The latter show much variation even in infants of presumably the same nutritional status. For example, in Table II the "fasting" levels (18 hours after the last large dose of vitamin C) ranged from 0.4 to 1.2 mgm. per cent. Two additional plasma samples obtained from infants C. B. and C. G. under the same conditions contained 0.9 and 0.5 mgm. per cent, respectively. On the day of the final test, after 5 to 8 days without ascorbic acid, "fasting" levels ranged from 0 to 0.8 mgm. per cent (Table I, next to last column). Little significance can be attached to these values except that, as might be expected, the 5- to 8-day interval invariably resulted in a lower "fasting" level.

Plasma values at a suitable interval after a test dose (in this study, a 4-hour interval) are a more reliable index of the true state of the body with respect to vitamin C storage. This is illustrated in Table II. In babies who were fully saturated,

[4] We are indebted to Dr. Walter Golden for some of the milk analyses.

[5] In two human milk samples, very turbid filtrates were obtained. Prior to correction, the ascorbic acid values corresponding to the galvanometer readings were actually less than zero, but after Bessey's correction (19) was applied apparent reducing activity was present equivalent to 1.6 and 1.3 mgm. of ascorbic acid per 100 cc. If this were actually ascorbic acid, the maximum intake would have been 2.6 mgm. of ascorbic acid per kilogram per day, an amount which might conceivably aid in retention of tissue stores of the vitamin. However, it seems unlikely that ascorbic acid in reduced form could be present after 20 minutes of boiling in contact with air, especially since none was detected in milk specimens in which turbidity did not interfere.

the plasma ascorbic acid after a test dose rose to 2.6 to 3.5 mgm. per cent.

Table I shows the results after a 5- to 7-day "depletion" interval in 22 babies, half of whom received boiled human milk and the others formulas made of cow's milk. It is at once apparent that the former showed a much greater response to the test dose (Table I, last column). The values for the infants receiving human milk ranged from 1.3 to 3.5 mgm. per 100 cc. of plasma, while the others ranged from 0.1 to 1.6 mgm. per cent. The average for the former group was 1.9 and for the latter 0.8 mgm. per 100 cc. If exceptions are made of the 2 infants, R. Pl. and C. T., who happened to receive large amounts of vitamin C prior to the "saturation" dose,[1] the averages become 1.86 and 0.72 mgm. per 100 cc. for the "human milk" group and the "cow's milk" group, respectively. All of the babies fed human milk showed a plasma response to the test dose of at least 1.2 mgm. per 100 cc., while of the babies receiving cow's milk formulas only 3, one of whom was subject C. T. already mentioned, were above this level.

Figure 1 shows graphically the difference between the two groups which have been plotted so as to show the correlation between the response to the test dose of ascorbic acid and the level of protein intake. No such correlation was found with age, sex, birth weight, weight on the day of the test, or with any other variable except the type of feeding.

## DISCUSSION

The greater response to the test dose of ascorbic acid in the group of infants receiving human milk than in those receiving cow's milk formulas is interpreted as meaning that the former were able to retain a greater amount of the "saturation" doses of the vitamin in their tissues. Presumably, either something in human milk has a "sparing" effect on the vitamin or something in cow's milk calls for a more rapid use of it, or both factors play a part. The presence of ascorbic acid, as such, which may be abundant in fresh breast milk, seems almost surely eliminated by the prolonged boiling of the human milk used in this study.[5]

The hypothesis is suggested that the vitamin C requirement of infants is raised as a result of a difference in composition of human milk and of cow's milk formulas. The most striking difference is that in protein content, human milk having about 1.5 grams of protein per 100 cc., and therefore providing, in the amounts given to these premature infants, from 2.6 to 2.8 grams per kilogram per day, while the cow's milk formulas contained amounts providing from 4.6 to 6.1 grams per kilogram per day.

Linking the vitamin C requirement to the protein in the diet is consistent with the aforementioned observation of Sealock, Ziegler, and Driver (12) that guinea pigs given tyrosine in their diets require more ascorbic acid than controls, and with the findings of Levine, Gordon, and Marples (8) that the aromatic amino acids tyrosine and phenylalanine are incompletely metabolized by premature babies in the absence of supplements of ascorbic acid. Whether the vitamin plays a rôle in the oxidative processes of intermediary metabolism, or in the building of body protein, or both, is beyond the scope of this paper.

## SUMMARY

Following a standard procedure of saturation with ascorbic acid, and a subsequent period of no ascorbic acid, the response of the blood plasma ascorbic acid level to a test dose of the vitamin was used as a criterion of the degree of saturation of the tissues. Observations were made in premature infants receiving boiled breast milk or artificial formula feedings.

Eleven infants given human milk showed plasma ascorbic acid values ranging from 1.3 to 3.5 mgm. per 100 cc. 4 hours after a test dose of either 100 mgm. of ascorbic acid intramuscularly or 200 mgm. by mouth, the average value being 1.9 mgm. per cent. Eleven infants whose feedings consisted of various cow's milk formulas responded to test doses with values having a range of 0.1 to 1.6 mgm. per cent, only 3 being above 1.2 mgm. per cent; the average was 0.8 mgm. per cent.

The results are interpreted as signifying that premature infants receiving human milk retain a larger part of a "saturation" dose of ascorbic acid in their tissues than do infants given cow's milk. The hypothesis is offered that an increased daily requirement for vitamin C is related to a high level of protein intake. This explanation is in accord

with available evidence that ascorbic acid is concerned in the intermediary metabolism of aromatic amino acids.

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