a significant difference in the effect of vit. B_{12} when fed or injected. The action of ascorbic acid does not appear to be specific, since it can be replaced by glucoascorbic acid(8). It must furthermore be remembered that the natural means of receiving nutrients is by absorption from the alimentary canal. It is also well known that the intrinsic factor(9) has a definite effect on the utilization of vit. B_{12}. With these facts in mind, it is possible that the vit. B_{12} absorbed from the tract is altered in such a manner that it may then act as a precursor to a coenzyme participating in an enzyme system which converts PGA to LCF. If this is the case, it is possible that the injected vit. B_{12} is similar enough to the coenzyme to compete for the apoenzyme, thus producing the inhibition noted. However, the effect of ascorbic acid in overcoming this inhibition is more obscure.

Summary. Vit. B_{12} injected at low levels increased the PGA stored in chick liver tissue. Higher levels of injected vit. B_{12} suppressed the liver storage of PGA. Vit. B_{12} administered orally, increased the level of both PGA and LCF as measured by chick liver storage.

Vit. B_{12} injected at the level of 1 γ per day significantly inhibited the conversion of PGA to LCF in PGA-vit. B_{12} deficient chicks. This inhibition was overcome by the *in vitro* addition of ascorbic acid. The oral administration of vit. B_{12} at the level of 1 γ per day significantly increased the conversion of PGA to LCF with or in the absence of ascorbic acid, in the PGA-vit. B_{12} deficient chick.

The possible relationships and interactions of vit. B_{12}, PGA, LCF, and ascorbic acid have been discussed.

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Essential Amino Acids in Mature Human Milk.* (18689)

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The amounts of the essential amino acids in colostrum and transitional milk from healthy nursing mothers were given in a preceding paper(1). This report presents the values for the essential amino acids in whole human milk as consumed by infants of the same group of mothers after lactation was established.

From 61 healthy mothers (47 white, 14 Negro) who were successfully nursing their infants, 147 samples of milk were collected at intervals ranging from 15 to 362 days postpartum. The mothers were 18 to 42 years old and resided in the Detroit area. Five women receiving known diets varying in quantity according to size and appetite but not in quality, provided 18 samples, each sample being a composite of all milk obtained by completely emptying both breasts at regular intervals during 24 hours. Fifty-six women whose diets were self-chosen provided 129 samples, each consisting of the entire amount of milk obtainable from one or both breasts, 4-8 hours after nursing. Samples were usually collected at the mid-morning feeding period, but in some instances were not obtained until early afternoon. Preceding papers have described in detail the method of manual expression used in the collection of samples(2,3), the preparation and storage of samples(4,5),

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2. Davies, V., *Am. J. Dis. Children,* 1945, v70, 148.

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4. Macy, I. G., Williams, H. H., Pratt, J. P., and Hamil, B. M., *Am. J. Dis. Children,* 1945, v70, 135.

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TABLE I. Essential Amino Acids in Mature Human Milk.

	Content per 100 ml whole milk											
	Total solids	Total N	Leucine	Iso-leucine	Valine	Histi-dine	Lysine	Methio-nine	Phenyl-alanine	Argi-nine	Threo-nine	Trypto-phan
24-hr samples*	g	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg
Mean	12.8	204	97	61	74	25	72	12	42	45	54	20
Min.	11.4	175	72	46	48	20	53	10	35	35	43	15
Max.	13.8	240	118	86	114	30	93	16	58	64	64	26
4-8 hr samples†												
Mean	13.2	192	110	70	71	23	76	14	41	45	55	18
Min.	10.8	147	80	48	51	16	53	9	30	28	40	13
Max.	15.8	255	159	102	105	34	104	21	51	61	76	26

* Eighteen samples from 5 women obtained 46 to 316 days postpartum.
† One hundred twenty-nine samples from 56 women obtained 15 to 362 days postpartum.

the hydrolysis procedure, selection of standards, preparation of media, and technics of analysis(1,6).

Lactobacillus plantarum (8014)† was used for determination of leucine, isoleucine, and valine, *Lactobacillus mesenteroides* (8042)† for histidine, lysine, methionine, and phenylalanine, and *Streptococcus fecalis* (8043)† for arginine, threonine, and tryptophan, with simultaneous determinations upon test mixtures of pure amino acids, vitamins, and salts with every series as "knowns," the percentage recovery of the amino acid in the test mixture being calculated for each assay. In addition, a sample of casein and a composite sample of mature human milk were hydrolyzed with each group of samples and assayed simultaneously. An average value was established for each amino acid of casein and human milk. Any assay in which the value for the amino acid content of the controls and duplicate samples failed to check within $\pm 5\%$ was considered doubtful and not included in the results.

Results. The volumes of the 18 individual 24-hour samples of mature milk and their contents of total solids, nitrogen, and the essential amino acids are given in Table I. The volumes of milk secreted in 24 hours varied greatly among the mothers studied and at different intervals postpartum for the same subject. The concentrations of total solids and total nitrogen were not related directly to the volumes of milk secreted. Amino acid concentrations also varied widely among subjects and at different intervals postpartum and differences in concentration did not parallel changes in contents of total solids or volumes of milk secreted.

For one subject (V.G.) 6 24-hour collections of milk obtained during one lactation period demonstrate the biologic variability which must be recognized as normal in human beings. Analytical data for the composition of human milk frequently are based upon analyses of composite samples composed of milk collected from several women, at various stages in lactation, or representing diverse intervals of milk production during the 24 hours. It is evident that such data might represent the average intake of nutriments from breast milk by a group of infants over a length of time, but would not necessarily provide an accurate estimation of the nutritive intake of a group of infants at a given time or of a single infant over any period of nursing.

Mean and range values for nitrogen and the essential amino acids in the 129 samples of milk from 56 mothers also are given in Table I. The values show quite close agreement between the mean concentrations in the 4-8 hour samples and the means for 24-hour collections of milk, supporting the validity of the analyses of the 24-hour collections as representative of the composition of mature milk from healthy nursing mothers.

Discussion. Physiologic trends are best identified from serial observations and over

6. Miller, S., and Ruttinger, V., *Arch. Biochem.*, 1950, v27, 185.

† American Type Culture Collection, 2029 M Street, N.W., Washington, D. C.

15 years ago Wilson(7) emphasized that several (longitudinal) observations on the same individual are more valuable in determining physiologic trends than single (cross-sectional) observations on a large number of individuals. For the first time data have been presented in this and the preceding paper(1) which show the patterns of essential amino acid composition in 24-hour collections of colostrum, transitional milk, and mature milk from women of the same group who were successfully nursing their infants. From this group of healthy mothers, the mean values determined for samples collected under highly controlled conditions, prepared, stored, and analyzed under identical conditions with the same microbiological technics, demonstrate that the concentrations of essential amino acids decrease rapidly during the first 10 days postpartum and reach average concentration levels in mature milk less than one-half those of early colostrum. The much higher concentrations of essential amino acids provided by colostrum during the first few days postpartum may be very important in the nourishment of the newborn infant.

The wide variation among women in a survey in which the samples were not procured from the different subjects at corresponding times during lactation‡ provided mean data (Table I) with great variation but without definite trend. However, among the 56 women who provided 4-8 hour samples were 11 who contributed a sample during each of 3 intervals postpartum: 31-68 days; 77-103 days; and 110-157 days. The results from analyses of these samples were tested by the Analysis of Variance. The variance between women was highly significant ($P \geq 99$) in every instance. The variance between intervals postpartum was significant ($95 < P < 99$) for total nitrogen, leucine, isoleucine, phenylalanine, and threonine and highly significant for histidine, lysine, methionine, and tryptophan. Only arginine and valine showed less variance between intervals postpartum than between women, and except for arginine all mean values showed decreases with time postpartum, indicating that the reduction in concentrations of essential amino acids which is rapid during early lactation(1) continues, but at a slower rate, during mature milk production.

Values for amino acids in human milk reported by Beach; Block; Plimmer and Lowndes; and by Williamson were summarized by Macy, Kelly, and Sloan(8). These workers collected pooled samples of milk and isolated the protein fraction, in which the amounts of the amino acids were determined chemically or microbiologically. The values presented in this communication are based on analyses of whole milk and thus total nitrogen includes non-protein nitrogen and amino acid data include the quantities of "free" amino acids present.

The average values for the concentrations of the amino acids in mature milk (Table I) and in colostrum and transitional milk(1) show a definite relationship with respective mean values for total nitrogen. The values for the same woman at different intervals in lactation and those for different subjects showed wide but not parallel variation in both total nitrogen and amino acid contents. An early paper(9) from this laboratory showed that the non-protein nitrogen content of mature human milk may vary from 25.6 to 59.2 mg per 100 ml, which could be a source of significant variation in the data for total nitrogen. Thus, the average values represent compensating variations which obscure important individual differences with respect to infant nutrition.

Summary. 1. Results are presented from analyses of 147 samples of mature human milk from healthy mothers for nitrogen and 10 essential amino acids. The samples consisted of 18 collections from 5 women of all

7. Wilson, E. B., *Proc. Nat'l Acad. Science,* 1935, v21, 633.

‡ Because only a few collections of milk were obtained within any specific interval postpartum, possible effects of age, race, parity, or season cannot be evaluated.

8. Macy, I. G., Kelly, H. J., and Sloan, R. E., The Composition of Milks, *Nat'l Res. Council Bull.* 119, January, 1950.

9. Erickson, B. N., Gulick, M., Hunscher, H. A., and Macy, I. G., *J. Biol. Chem.,* 1934, v106, 145.

milk secreted in 24-hour periods and 129 collections from 56 women of milk secreted in periods of 4-8 hours. Mean values for concentrations of the amino acids in the 2 groups of samples agreed well. 2. Wide individual variations were found among successfully nursing women and at different intervals postpartum. Mean concentrations of nitrogen and the essential amino acids in 24-hour samples of colostrum, transitional, and mature milk showed a general lowering to the levels in mature milk as lactation was established and application of the Analysis of Variance to the data for 11 women, each of whom provided a sample during the same postpartum interval of mature milk production also indicated a gradual reduction in amino acid concentrations.

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Action of *Bacillus cereus* Toxins on Chicken Erythrocytes.*† (18690)

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A preliminary survey of the action of bacterial toxins on cells indicated that the toxins of *Bacillus cereus* had little or no effect on the rate of respiration of chicken erythrocytes(1), dogfish erythrocytes, *Arbacia* or *Asterias* eggs(2). These toxins did, however, have a marked effect on the osmotic behavior of dogfish and chicken erythrocytes(3). The present investigation was undertaken to analyze these changes in more detail. Such a study might help one to understand more fully the way in which these toxins exert their *in vivo* action and might contribute to an understanding of the relationship between the structure of the cell membrane and the passage of non-electrolytes through it.

Materials and methods. General. An isolation of *B. cereus* was made from the laboratory air. The organisms were grown on agar slants and then transferred to proteose peptone broth for incubation at 37.5°C for 36-60 hr. Following filtration, the toxins were stored at 4°C, 95% humidity. Since some of the material was stored for approximately 2 years before it was used, there was considerable variation in the strength of the toxins in different experiments. Consequently, an assay value would have no meaning. It was noted, however, that the qualitative changes in the erythrocytes were the same regardless of the age of the toxins. The older toxins merely had to act on the cells for a longer time before comparable changes were observed.

Chicken and rabbit bloods were obtained by cardiac puncture and heparinized. Sterile technics were employed except during the final 5-10 minutes when measurements were being made and only data obtained from sterile stocks were considered.

Permeability. Using the apparatus previously described(4), rates of hemolysis, shrinking and swelling were measured. The experimental stock solution contained a mixture of erythrocytes and toxins while the control stocks contained cells and broth or

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† The experimental work was performed in the Department of Zoological Sciences, University of Oklahoma, Norman.

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3. Hunter, F. R., Rawley, June, Bullock, Jane A., and Larsh, Howard W., *Science*, 1950, v112, 206.

4. Hunter, F. R., *Science*, 1949, v109, 119.