On the Evaluation of Artificial Feeding

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The nutritional value of different diets can be analyzed in two ways: (1) by detailed study of the chemical composition of the nutrient in question and (2) by study of the effect of the nutrient upon the consumer. In the case of infants, these studies are relatively simple, for the main constituent of their diet, milk, is a homogenous foodstuff.

Hitherto the effect of various infant diets has been studied mainly by recording their effects on the infant's weight, frequency of stools and the like. But, as more has been learned about the complicated chemical structure of milk, it has become apparent that this method does not give enough information. In this paper we shall give a brief survey of some aspects of the chemical structure of milk proteins and also describe plans for a study to determine how human and cow's milk affect other conditions such as blood chemistry, antibody formation, and osseous development.

CHEMICAL ASPECTS

Milk proteins are considered to be of high nutritional value. Animal experiments have shown that they are better than other proteins in causing an optimum gain in weight during the growth period. This is true of the total proteins from both human and cow's milk. Only egg proteins are more effective in this respect, and they too are meant for nutrition at an early stage of life.

In every kind of milk the protein consists of a complex system, including casein and its subfractions, the albumins and globulins of the whey fraction, and a number of other proteins, some of a poorly defined nature. Casein is the most interesting of these, as it is a type of phosphoprotein found only in milk. The protein stores in the egg also contain a large amount of phosphoproteins. These substances, consequently, are a common component of the most valuable nutritional proteins.

The proportion between casein and total whey protein is very different in human and cow's milk. Human milk contains only about 0.5 per cent casein, whereas cow's milk contains about 3 per cent. Even when cow's milk is diluted with an equal volume of water—still a common way to prepare milk for infants—it contains about three times as much casein as human milk. In more concentrated mixtures the difference is still greater. In some of the modern cow's milk preparations this difference is eliminated.

If the only way protein contributed to nutrition was by supplying the essential amino acids, this difference would play no part, for milk proteins all contain about the same amount of amino acids, and no matter what sort of milk protein was used, adequate amounts of essential amino acids would be supplied. However, there are many facts which indicate that proteins not only serve to convey the essential and other amino acids, but also have more specific tasks connected with specific features of their chemical structure. Thus it is known that the immune globulins in cow's colostrum protect the newborn calf against various infectious diseases and that the casein plays a part in the absorption of calcium and phosphorus. Differences in chemical structure and in biologic properties are therefore of great interest, especially when comparing corresponding proteins in human and cow's milk.

If, to begin with, one studies the enzymatic digestion of the total proteins in the milk, one makes the puzzling observation that the proteins in cow's milk are broken down much

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faster than the proteins in human milk. Figure 1 shows the rate of hydrolysis in the infant's stomach.[1] As is seen there, very little of the human milk protein is split up. This difference in digestion of the proteins of cow's and human milk is partly due to the fact that the casein of human milk is much more difficult to hydrolyze than the casein of cow's milk.[2] This, in turn, is because a part of the casein molecule is resistant to the proteolytic enzymes in the alimentary tract. This part of the molecule is found as a peptide mixture after the casein is digested with pepsin and trypsin and has the amino acid composition shown in Table I. The reason for the resistance to enzymatic

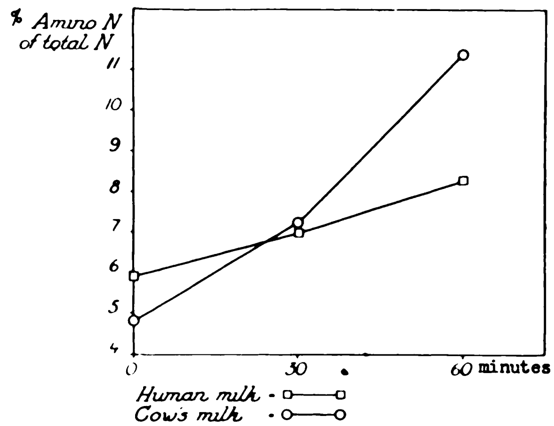

Fig. 1. *In vivo* digestion of cow's and human milk (in the infant's stomach). From: Berfenstam, R., Jagenburg, R., and Mellander, O.: *Acta Paediatrics* 44: 348, 1955.

TABLE I

Amino Acid Composition of Phosphopeptides from Cow's Casein

	%
Aspartic acid	4.5
Threonine	2.5
Serine	12.2
Glutamic acid	18.5
Proline	1.4
Glycine	1.1
Alanine	1.1
Valine	2.9
Isoleucine	6.0
Leucine	1.6

hydrolysis is that serine is esterified with phosphoric acid. The phosphorus content is about 7 per cent. This resistance to proteolytic enzymes of the phosphorylated peptides sets up the conditions for direct absorption of these compounds.

As mentioned, much more of the phosphorus in human milk casein is recovered in peptide form than in cow's milk casein.[2] However, there is about twice as much phosphorus in cow's milk casein. It is not yet clear whether there are any significant nutritional differences between the peptide residues of human and cow's casein.

Since the phosphoric acid residues of the serine peptides permit a complex formation of metals, what is absorbed probably consists of compounds with the metals present in the alimentary tract. The physicochemical basis for this complex formation has been studied by Österberg.[3] The calcium salt of the peptides contains not less than 10 per cent calcium in soluble form and it is quite possible that it is used as a source of mineral for ossification of the bones.[4,5] Calcium is not the only mineral which is bound. There is more calcium than there are other minerals in milk, therefore, the calcium salts predominate, especially in cow's milk. Because of this, there is a risk that the other metals, for example, iron, are displaced from the peptide carrier. This may favor the development of anemia in artificially fed infants. Even other minerals which occur in small quantities, e.g, cobalt, may be displaced from the peptides. There is much less risk of this when human milk is given, because of its lower content of all minerals, especially calcium.

This absorption of phosphopeptides may also be of interest from another point of view. It is known that protein metabolism proceeds to a large extent via phosphorylated amino acids and peptides. It seems reasonable to assume that these processes are facilitated if certain amino acids or peptides are given in phosphorylated form.

In this connection it should be mentioned that Johansson and Svennerholm[6] demonstrated large differences between the protein-bound carbohydrates in human and in cow's

TABLE II

Carbohydrate Content of Human and Cow Caseins (Values Given in Per Cent of Dry Weight)[6]

Casein	Hexos-amine	Hexose	Sialic acid ($C_{11}H_{21}$-$NO_{10}.H_2O$)	Total carbo-hydrates
Human	1.32	1.98	0.76	4.03
Cow	0.18	0.24	0.39	0.80

casein (Table II). These components are of interest, especially in relation to the microbial flora in the intestine, and should be considered in the light of recent observations of György and his co-workers.[7–9]

NUTRITIONAL ASPECTS

If the total amount of some of the most important nutrients given the infant during the first six months is calculated, it is seen that there are distinct differences between breast and bottle feeding. This is shown in Table III where the data for cow's milk are based on the use of half-strength milk with 5 per cent sugar added. Thus 540 g of casein, for example, is obtained in the human milk, as opposed to 2,400 g in the cow's milk formula. The difference would have been still greater if a more concentrated formula had been used. Added to this are the aforedescribed differences in the quality of the two forms of milk. Since these differences both in quantity and quality are particularly large in the protein component, it is obvious that in biologic research one must pay particular attention to the functions that are directly connected with the supply of protein. This was done in planning the field study now to be described.

TABLE III

Comparison between Total Amount of Various Nutrients Supplied by Human and Cow's Milk

	Breast feeding (135 liters of breast milk during 6 months) g	Cow's-milk feeding (160 liters of half-milk mixtures during 6 months) g
Calcium	45.9	89.6
Phosphorus		
Total-P	18.9	73.6
Casein-P	2.2	19.2
Total protein	1,620	2,720
Casein	540	2,400
Whey protein	1,080	320

FIELD STUDY

In this connection, only a brief presentation of the organization of the study will be given as well as some data pertinent to the development of the skeleton. Some preliminary results were presented at the International Pediatric Congress in Copenhagen in July 1956.

It was found that Northern Sweden offered the best conditions for the investigation. The town of Kiruna and the rural districts of Töre and Råneå near the town of Boden were chosen. From June 1953 to December 1954, 402 newborn children were registered in these places. Before they were born, information was obtained about the family situation, about the physical condition of the mother and other members of the family, their food habits, living quarters, and the like. After the infant was born, it was carefully supervised by specially appointed nurses and every one or two months the infant had a medical check-up at the local welfare clinic. Thorough medical supervision was maintained until the child was one year old. After that the examinations were less frequent. Careful notes of all data relevant to the health situation were made in special records.

Feeding: The mothers were allowed to choose between breast and bottle feeding. In 26 per cent of the cases, the infants were nursed at the breast exclusively for at least six months. If weaning began earlier the mother's milk was supplemented by a formula of one part cow's milk and one part water, 5 per cent sugar and 1 per cent wheat flour. This formula gave about 550 calories per liter, and contained about 1.7 per cent protein, 1.5 per cent fat, 8 per cent carbohydrate, and 0.4 per cent minerals. The infant was allowed to get 600 to 700 g a day when it was one month old and afterward the amount was gradually increased until it reached 800 to 1,000 by the time the infant was three months old. When it was four months old, crushed rusks were added to one or two of the feedings. In one part of the series the food was prepared from fresh cow's milk and in the other from a dry milk preparation giving the same composition of the mixture used.

The diet was also supplemented as follows: From the age of one week onward 10 drops of a vitamin-preparation were given, which provided 2,500 units of vitamin A and 1,000 units of vitamin D a day. This dose was doubled at three months. At three months 25 mg of ascorbic acid were given a day, together with fruit juice or an infusion of rose-hips (a source of ascorbic acid). Strained baby foods were introduced at four months. During the period of the investigation no pressure was exerted on the mothers to breast feed the infants. On completion of weaning, at seven to eight months, all groups were receiving the same amount of milk, corre-

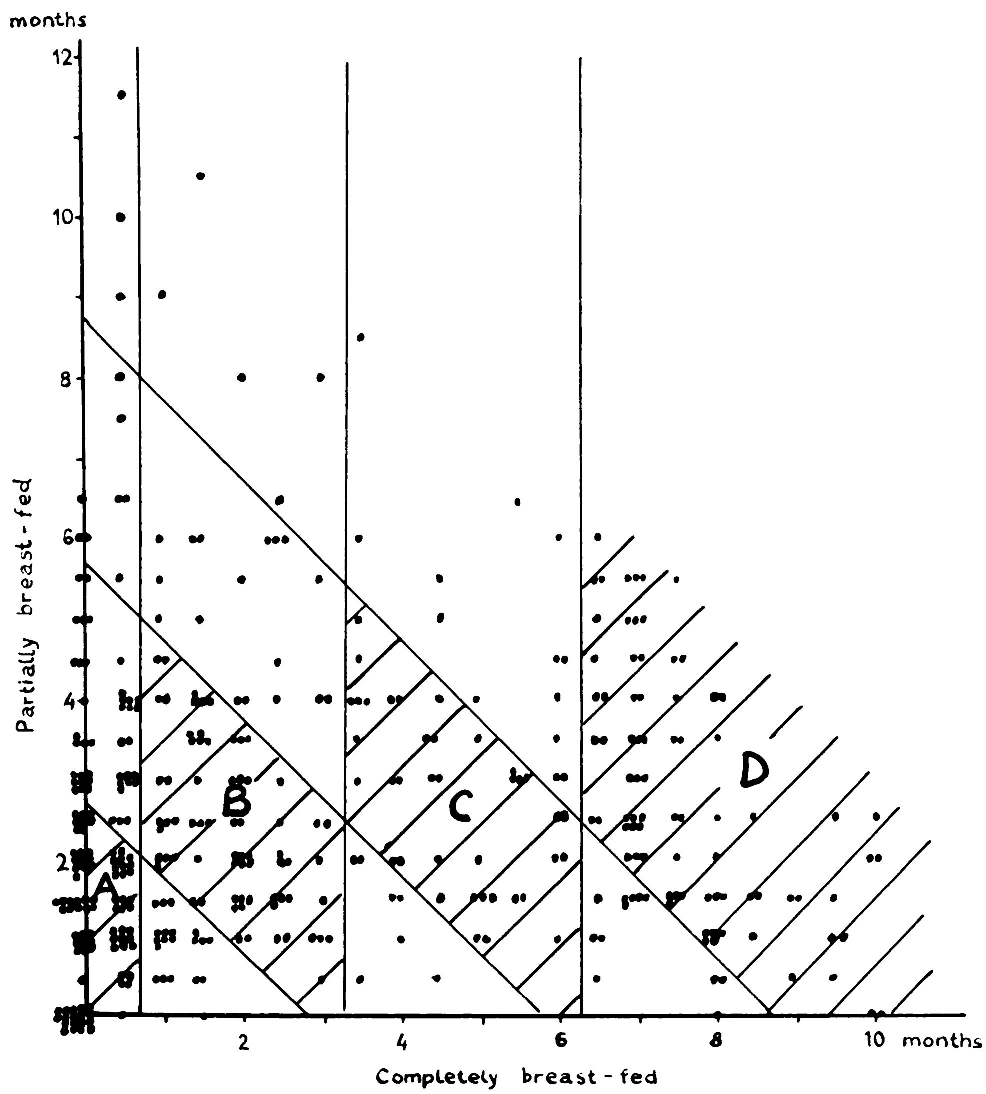

Fig. 2. Presentation of the whole material with respect to the period of complete and partial breast-feeding for each single subject. Each dot represents one child. Classification into groups A, B, C, and D (hatched areas) comprising 274, out of a total of 402 children.
Group A (weaned early) includes 88 children.
Group B (intermediate group) includes 69 children.
Group C (intermediate group) includes 38 children.
Group D (weaned late) includes 79 children.

sponding to 0.6–0.7 liter of cow's milk per day.

Immunization schedule: BCG vaccination was done during the first week of life, and the tuberculin reaction tested at the age of three and seven to eight months. Combined vaccination (whooping cough, diphtheria, and tetanus) with 1 ml of vaccine was done at the age of three, four and one-half, and six months of age and smallpox vaccination generally between the ages of four and one-half and six months. In a limited group a vaccination against influenza virus was also included.

Special Examinations: In addition to the regular medical supervision of the children special examinations were done on the following three occasions: (1) at birth and at age seven or eight months, chemical and serologic analysis of blood, (2) at birth, roentgenographic study of the skeleton, and (3) at the age of two and one-quarter to three years (chemical and serologic analysis of blood and roentgenography of skeleton in half the cases and detailed examination of the teeth in the whole series).

Results: For further analysis, the series was divided into four groups, according to the duration of complete and partial breast feeding (Fig. 2).

The weight gain in the various feeding groups is given in Figure 3. The children with prolonged breast-feeding increased more rapidly in weight during the first three months but later on more slowly than the children weaned

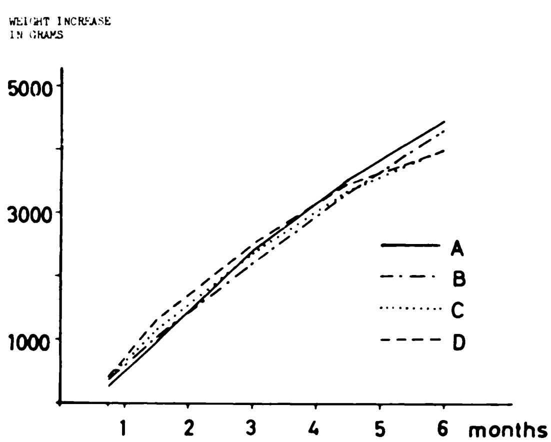

Fig. 3. Weight gain during the first six months of life. Classification into four different groups of feeding (*cf.* Fig. 2).

early. A complete report of the study will be presented elsewhere.

In view of the differences in the supply of proteins and minerals in breast feeding as compared with those in cow's milk feeding, we shall focus on a few observations concerning the development of the skeleton during the first year of life. This was judged partly by the growth in height and partly by the number of osseous centers seen in the roentgenogram. In addition, the amount of calcium, phosphorus, and alkaline phosphatase in the blood in the various feeding groups was determined. The condensed data are presented in Table IV. In the first place a comparison was made between two extreme feeding groups (A and D) but the findings in the other groups (B and C) are also referred to. It is evident from the results presented in Table IV that the children weaned early (group A) were significantly taller than those weaned late (group D).

The centers of ossification were evaluated according to Elgenmark.[10] This technic involves counting all the centers of ossification in the arm and leg of one side of the body. The results of this procedure in the series now reported indicate that the appearance of the centers of ossification was hastened in group A (those weaned early) compared with group D (those weaned late).

No definite case of rickets was encountered in this investigation, which shows that the health supervision and administration of supplementary vitamins was carried out faithfully.

Serum calcium, phosphorus, and alkaline phosphatase levels were determined at seven to eight months. The different feeding groups had similar calcium levels. The values for serum phosphorus and alkaline phosphatase, on the other hand, are significantly lower in group D as compared with the other groups (Table IV).

The differences observed in height, in the number of osseous centers and in certain blood chemical data are mostly small in absolute values but are nevertheless statistically significant. It would seem reasonable to assume that they are correlated with an increased rate of deposition of calcium and phosphorus in infants reared on cow's milk formulae.

TABLE IV

Average Height and Number of Centers of Ossification at Seven and One-half Months. Average Serum Calcium, Serum Phosphorus, and Serum Alkaline Phosphatase at Seven to Eight Months of Age. The Series is Divided into Four Groups, According to the Mode of Rearing (A, Extreme Cow's-Milk Group; D, Extreme Breast-Milk Group)

	Total number examined	Feeding groups (of Fig. 2)				
		A	B	C	D	Difference
						D − A
Height, cm	274	70.15	69.77	69.07	69.05	−1.10†
Number of osseous centers	274	14.0	13.25	13.05	13.05	−0.95†
						D − (A and B + C)
Serum calcium, mg/100 ml	112	10.25	10.35	10.30	10.45	0.15
Serum phosphorus, mg/100 ml	136	6.19	6.28	6.39	5.75	−0.51‡
Serum alkaline phosphatase, units/ml	131	18.54	18.43	19.02	16.31	−2.29†

† Significant at the 1%-level.

‡ Significant at the 1‰-level.

A more detailed presentation of the material is given in a paper by Vahlquist, Mellander, and Wickslund.[11]

The fact that infants weaned at an early stage grow and show skeleton maturation at a slightly increased rate does not imply that they are superior in other respects. In forthcoming papers it will be demonstrated that the formation of antibodies against diphtheria and whooping cough is much the same irrespective of the type of feeding, but that the combined incidence of upper respiratory infections, otitis, and diarrhea is significantly higher among the babies weaned early (group A) than among the babies weaned late (group D). It would seem, therefore that the nutritional value of a food can be assessed correctly only by detailed and careful clinical and biochemical studies. The field study discussed above is a step in this direction.

SUMMARY

The supply of various nutrients differs greatly, quantitatively as well as qualitatively in breast-milk feeding as compared with cow's milk feeding. Even if a cow's milk formula is modified to imitate breast milk in its gross chemical properties it is not necessarily superior to other more traditional formulae.

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REFERENCES

1. Berfenstam, R., Jagenburg, R., and Mellander, O.: Protein hydrolysis in the stomachs of premature and full-term infants. *Acta Paediat.* 44: 348, 1955.
2. Mellander, O.: On chemical and nutritional differences between casein from human and from cow's milk. *Upsala Läkaref. Förh. Ny Följd* 52: 107, 1947.
3. Österberg, R.: Metal and hydrogen-ion binding properties of O-phosphoserine. *Nature* 179: 476, 1957.
4. Mellander, O.: Importance of the casein phosphopeptide calcium salts. II. Peroral calcium dosage in infants. *Upsala Läkaref. Förf. Ny Följd* 55: 247, 1950.
5. Mellander, O., and Isaksson, B.: The physiological importance of the caseinphosphopeptide calcium salts, I. Intravenous and

peroral calcium dosage in animal experiments. *Upsala Läkaref. Förh. Ny Följd* 55: 239, 1950.

6. Johansson, B., and Svennerholm, L.: The content of carbohydrates in caseins from different species. *Acta Physiol. Scand.* 37: 324, 1956.
7. György, P.: Hitherto unrecognized biochemical difference between human milk and cow's milk. *Pediatrics* 11: 98, 1953.
8. György, P., Norris, R. F., and Rose, C. S.: Bifidus factor, variant of lactobacillus bifidus requiring special growth factor. *Arch. Biochem.* 48: 193, 1954.
9. Zilliken, F., Braun, G. A., and György, P.: "Gynaminic Acid" and other naturally occurring forms of *N*-acetylneuraminic acid. *Arch. Biochem.* 63: 394, 1956.
10. Elgenmark, O.: The normal development of the ossific centres during infancy and childhood. *Acta Paediat.* 33: (Suppl. 1), 1946.
11. Vahlquist, B., Mellander, O., and Wickslund, H.: Breast milk and cow's milk in infant feeding. Skeletal development during the first year of life. *Ann. Paediat. Fenn.* (to be published).