NUTRITIONAL EVALUATION OF SOME INDIAN INFANT FOODS

A THESIS
SUBMITTED TO THE KURUKSHETRA UNIVERSITY
FOR THE DEGREE OF
Doctor of Philosophy
IN THE FACULTY OF DAIRYING, ANIMAL HUSBANDRY
AND AGRICULTURE

By
V.Indira

NATIONAL DAIRY RESEARCH INSTITUTE
(I.C.A.R.)
KARNAL (Haryana) INDIA
1985

NUTRITIONAL EVALUATION OF SOME INDIAN INFANT FOODS

A THESIS

SUBMITTED TO THE KURUKSHETRA UNIVERSITY
FOR THE DEGREE OF
Doctor of Philosophy
IN THE FACULTY OF DAIRYING, ANIMAL HUSBANDRY
AND AGRICULTURE

By
V. Indira

NATIONAL DAIRY RESEARCH INSTITUTE
(I.C.A.R.)
KARNAL (Haryana) INDIA

1985

DEDICATED

To my father

Mr. V. Venkataramayya

And

To my friend

Dr. Satinder Bajaj

Dr.A.D.Deodhar,M.Sc.,Ph.D.

NATIONAL DAIRY RESEARCH INSTITUTE
(I.C.A.R.)
KARNAL - 132001, INDIA

Dated 8th May, 1985.

This is to certify that the work reported in this thesis entitled " NUTRITIONAL EVALUATION OF SOME INDIAN INFANT FOODS " was carried out by Ms. V.Indira as per requirements for the DEGREE OF PHILOSOPHY in the Faculty of Dairying, Animal Husbandry and Agriculture, Kurukshetra University, Kurukshetra under my guidance and that no part of this has been submitted for any other degree or diploma.

I further certify that such help or source of information as has been availed of in this connection is duly acknowledged by her.

A.D.Deodhar

(A.D.DEODHAR)

ACKNOWLEDGEMENTS

The author wishes to avail this opportunity to express her deep sense of gratitude and indebtedness to Dr.A.D.Deodhar, S-3, Biochemistry Section for the untiring interest, valuable suggestions, critical appreciation and encouragement shown during the course of present investigation and in the preparation of this manuscript.

The author expresses gratitude to the members of the Advisory Committee, Dr.V.D.Mudgil, Senior Scientist, Dairy Cattle Nutrition & Physiology Division and Dr.K.M.Narayanan, Senior Scientist for giving valuable advise.

The author wishes to express sincere thanks to Dr.R.Balachandran, S-3, Dairy Technology Division, Dr.K.K.Singhal, S-2, DCN & P Division, Dr.T.Prasad,S-2, DCN & P Division, Dr.Bhopal Singh,S-3 of DES & M Division and Dr.(Mrs) Aruna Chhabra,S-2 of DCN & P Division.

The author expresses gratitude to Dr.J.S.Sindhu, and Dr.Darshan Lal,Scientists S-1 of Dairy Chemistry Division for the assistance given during the course of this investigation.

Sincere thanks are due to Dr.R.Nagarcenkar,Director and Dr.I.S.Verma, Joint Director, for providing necessary facilities to carry out the present study.

Thanks are due to Mrs.Nisha Kant Chopra, S-1 of DCN & P Division and Miss Manpal Sanhotra,Ph.D.Scholar of Biochemistry Section for rendering valuable help.

Financial help in the form of NDRI Senior Fellowship is duly acknowledged.

V. Indira

(V. INDIRA)

9th May 85

C_O_N_T_E_N_T_S

-o-o-o-o-

LIST OF TABLES

LIST OF FIGURES

-o-o-o-

LIST OF ILLUSTRATIONS

LIST OF APPENDICES

-o-o-o-

INTRODUCTION

INTRODUCTION

Over seventy percent of the world population lives in technologically less advanced countries in the world. A sizable segment of this is comprised of infants and pre-school children. Various factors such as poverty, low agricultural productivity, low level of literacy reflect in a poor nutritional status of majority of infants and children (Parpia, 1972). It is during this period of rapid growth during infancy and early childhood, malnutrition is most conspicuous in reflecting in high morbidity as well as mortality and probably leaving physical and psychological scars in the later life among those who survive. Merits of human milk in infant feeding have been universally recognised. It has been well established that nutrients are available in sufficient quantity in the first three months of life, provided the mother can supply about 800 ml of her milk. However, mothers quite often fail to secrete adequate quantity of milk for various reasons such as (i) physical inability of the mother either due to disease, malnutrition or both, (ii) lack of privacy to breast feed the baby, deemed necessary by modern conventions, (iii) employment away from home, (iv) emotional barriers like worry and tension adversely affecting the let down reflex, and (v) availability of economically feasible and highly advertised infant foods in the market.

A healthy newborn infant receiving adequate nutrition would double its weight in six months and triple it by the twelfth month. Venkatachalam et al. (1967) observed that the growth performance of poor Indian infants was not as satisfactory as that of well nourished Indian or American infants even during the first few weeks. With such a striking failure to successfully nurse a baby, a mother naively resorts to artificial feeding with either milk from domesticated milch animals or infant foods. It is of prime importance, therefore, that the food given to a baby is as perfect as science and technology can make it.

The question then arises, as what qualities and milk components are specifically suited to the baby's needs, which should be emulated in designing substitute milk feeds and further could the prevalent recipe be improved?

Although a comparison of the chemical composition of milks from domesticated animals with human milk reveals the adequacy of the former to meet the nutritional requirements of infants, the paucity of unadulterated clean milk, ignorance on the part of mothers about suitable modification and supplementation of milk, besides economic constraints, renders milk far from ideal for infant feeding in developing and under developed countries.

In developed countries, there are now host of preparations on sale as dried powders or liquid formulae

that vie with each other as being best possible food for babies. These preparations are either humanised formulae simulating breast milk composition or adapted ones tailored to meet the infant body requirements during a specific age period. This is often accomplished by inclusion of several nutrients such as lactose, whey proteins or essential fatty acids in lieu of normal milk constituents.

Several studies conducted on infant food formulae based on cow milk show that the levels of protein and fat content ranged between 11 to 29 percent and 13 to 25 percent, respectively. While determining these, utilisation of milk proteins and fats by infants was the criterion in comparison to these nutrients available from human milk.

It was observed by Barness et al. (1957) that the daily amino acid requirements expressed as per kg body weight were met by 1.6 g proteins in human milk as against 2 g proteins from cow milk. Likewise, the quantity as well as quality of the fat is determined by taking into account essential fatty acid requirement for the baby. The use of various sugars like sucrose, maltose or glucose have been adopted in order to simulate artificial formulae with human milk in respect of sweetness. Similarly, fortification with iron and vitamins is done to meet the body requirements of these nutrients.

Several feeding trials conducted on these formulae showed that the protein utilisation was identical to that of human milk as evident from nitrogen balance studies and growth promoting abilities of these preparations. Similarly observations were made in respect of hematological picture. Fats fortified with vegetable oils in order to meet essential fatty acid needs, employed in these preparations were found to be similar to human milk fat in respect of their absorption when fed to infants (Barness et al. 1957, Widdowson, 1965).

Though it would appear that most of infant formulae are ideal in sustaining optimal growth among infants, serious limitations in respect of high solute content, particularly when cow milk is used as the base, cannot be over-looked, since this would put excessive load on premature kidneys and further leading to excessive level of blood sodium causing hypernatraemia (Taitz, 1973). The mechanism which influenced the volume of milk intake has been identified as the thirst impulse. Babies receiving infant foods may tend to develop extra cellular osmolality responsible for sensation of thirst. This lead to a reduction of urinary volume which reduced water loss on one hand and increased intake due to thirst on the other, as the mother responded to continuous demand. These processes depend for their efficiency on the ability of the mother to d i s t i n g u i s h between hunger and thirst and the capacity of the infant kidneys to conserve water.

Besides the aforesaid, heat treatment has been recognised to affect the structural changes in the milk proteins (Mauron et al. 1955, McDonald, 1966). The major ingredient skim milk used as the base for the most infant formulae is further subjected to either spray drying or roller drying process. Differences in the bioavailabilities especially of essential amino acids from such preparations, because of the variation in duration and intensity of heat treatments during drying, would influence the protein nutritional quality of the preparation based on these.

In India preparations based on cow and buffalo milk are widely adopted for infant feeding. Though the gross chemical composition of these preparations have been reported to be fairly identical and within the prescribed limits, little is known regarding content of various essential nutrients and also the extent to which the available nutrients meet the nutritional needs. Information on these lines would be further desirable in view of different kinds of additives used in preserving milk and also the nature of processing treatments, before it finds use in such formulation.

A knowledge on these aspects would thus be immensely useful to assess the extent to which such preparations are adequate to meet infant needs, and if these confront any hazards.

In thelight of the aforesaid, a study is proposed to critically evaluate certain popular infant foods currently available in the market in respect of:(i) nutrient levels in various infant foods, (ii) nutritional quality of protein and fat in these preparations, (iii) characterization of carbohydrate and inorganic constituents, that make up different preparations in relation to osmolarity, and (iv) their capacity to fulfil various nutritional needs.

-0-0-0-0-

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The nutritional significance of milk in infant nutrition has been well recognised for quite some period. Milk of any given species is best suited for the nutrition of the newborn of that species. By this standard, human milk is the most appropriate and essential nutritional requirement for infants. A healthy newborn infant receiving adequate nutrition from the mother would double its weight in six months and triple the same in twelve months.

However, several factors like physiological, social, economical constraints, etc, often result in the inability of the mother to successfully nurse the infant. Such situation often compels mothers to resort to artificial feeding, using either milk of other species or commercially available infant food formulae. Considerable information has been obtained on these aspects in the past few decades.

An attempt has been made in this section to review the same and presented under following subtopics : (i) attributes of human milk, (ii) milk yield, (iii) etiology of failure of the breast feeding practice, (iv) chemical and biological evaluation of infant food formulae, (v) determination of the nutritional adequacy, and (vi) limitations of bottle feeding.

Attributes of Human Milk

Certain characteristics of human milk and advantages of breast feeding practices are unequivocal. These include availability of milk at any time at desirable temperature and in the aseptic form. Breast milk costs little extra, provided maternal nutritional requirements are adequately met.

Several studies have been conducted to understand the chemical composition of human milk in many developing as well as developed countries,and also to ascertain its adequacy to fulfil nutritional needs. A comparison of proximate analyses in different studies is given in Table I.

It would appear from the same that irrespective of plane of maternal nutrition in different parts of the world, the proximate composition of human milk was fairly uniform.

The crude protein content varies between 1.06g/ 100 ml for Indian and American subjects (Belavady and Gopalan, 1959; Macy and Kelly, 1961) and 1.41 g/100 ml for Australian (Wardlaw and Dart, 1926) and Bantu subjects (Arvidsson et al., 1954).

Small variations in the contents observed could be attributed to several factors such as diurnal variation, variation in sampling (Hytten, 1954), stage of lactation and ethnic differences (Peters, 1953).

Table I : Comparison of nutrients in human milk reported in various countries.

Constituents (expressed per 100 ml)	Indian			USA	Australian	U.K.	Nigeria		New Guinea	
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
Total solids g	12.12	-		12.9	13.74	13.9	12.8	10.08	10.8	-
Fat g	3.42	4.5		4.54	4.95	4.78	3.90	2.36	3.8	3.59
Lactose g	7.51	7.15		6.8	6.43	6.95	7.10	7.34	5.0	-
Protein g	1.06	1.2		1.06	1.41	1.16	1.35	1.01	1.10	1.20
Mineral ash g	0.16	-		0.20	0.18	-	0.20	0.17	0.18	-
Calcium mg	34.2	42.0		34.4	-	29.9	28.7	-	25.8	-
Phosphorus mg	34.7	35.0		51.2	-	-	-	-	-	-
Sodium mg	22.1	22.0		17.2	-	-	-	-	-	-
Potassium mg	34.7	35.0		51.2	-	-	-	-	-	-
Magnesium mg	2.6	2.65		3.5	-	-	-	-	-	-
Vitamin A I.U.	70.0	-		20.1	-	15.3	-	-	-	1.33
Vitamin B_1 ug	15.4	-		14.2	-	18.3	-	-	-	19.4
Vitamin B_2 ug	17.2	25.63		37.3	-	25.5	-	-	-	21.4
Vitamin C mg	2.6	3.64		5.2	-	3.2	2.9	-	-	3.2
Pantothenic acid	-	161.2		-	-	-	-	-	-	-
Vitamin B_{12} ug	-	0.1138		-	-	-	-	-	-	-

(a) Belavdy and Gopalan(1959);
(b) Karmarker et al. (1959)
(c) Deodhar and Ramakrishnan(1960)
(d) Macy and Kelly (1961)
(e) Wardlawand Dart (1926)
(f) Kon and Mawson(1950)
(g) Arvidsson et.al.(1954)
(h) Venkata chalam (1952)
(i) Peters,(1953)
(j) Gunther and Stanier(1952).

The heterogenity of milk proteins plays a crucial role in determining its digestibility in the infant stomach and consequently the biological value. The ratio of casein to whey proteins was about 1 : 1.5 in the case of normal human milk and was shown responsible to impart soft curd and result better protein digestion as compared to milk from other species (Macy and Kelly, 1961).

Casein is a heterogenous mixture of several components viz, α_{S1}-, β and K-caseins. In human milk, it is reported to contain 0.4 percent casein, mostly as β casein, with some quantities of K-casein. However, αS_1- casein fraction is not present in appreciable quantity, whereas bovine milk casein contains 4 to 5 percent αS_1- casein fraction. This has been shown to influence curd tension and digestibility (Ganguli, 1979). Besides this, whey proteins are richer in tryptophan and S-amino acid contents as compared to casein (Delany, 1976). As regards amino acid profile of human milk protein, though values reasonably conform F.A.O. standard amino acid pattern (FAO, 1965), there appeared markedly high levels of cystein in human milk protein as compared to those from milk of other species (Macy and Kelly, 1961). Apparently this is the provision, nature has made to overcome the incomplete development of transulferation pathway in the infant liver, during early life (Rassin et al., 1977). Likewise, taurine content was reported to be higher in the breast milk as compared to cow milk as

evident from 33.7 u mol/100 ml in human milk whereas 1.0 u mol/100 ml in cow milk (Gaull et al., 1977).

The fat content of human milk has been reported to vary between 2.36 g/100 ml in Chimbu tribe in Africa (Venkatachalam, 1962) to 4.95 g/100 ml in Australian subjects (Wardlaw and Dart, 1926). Such variation could be due to variation in sampling procedure used during milk collection. Though this level was close to that observed in cow milk, human milk fat was shown to have higher linoleic acid content as evident from 8.03 percent reported by Macy and Kelly (1961), than cow milk (1.5 %).

As regards lactose, the contents varied between 5.0 g/100 ml in New Habrides subjects (Peters, 1953) to 7.5 g/100 ml in Indian subjects (Belavady and Gopalan, 1959). Such differences were attributed by Peters (1953) to genetic factors. Calcium content of human milk varied between 25.8 mg/100 ml in New Hebreads (Peters, 1953) to 42 mg/100 ml in Indian subjects (Karmarkar et al., 1959). Such difference could be partly ascribed to the difference in the dietary intake of calcium in the observations by Karmarkar et al. (1959).

Phosphorus content varied between 11.9 mg/100 ml in Indian subjects (Belavady and Gopalan, 1959) to 14.1 mg/100 ml in American subjects (Macy and Kelly, 1961). The phosphorus content in milk has been reported to depend on the dietary intake.

Like milk from other species, human milk is poor in its iron content. However, iron in human milk was found to be better absorbed and no iron deficiency anaemia was observed till the age of 3 months (McMillan et al., 1976).

The copper content of human milk ranged between 0.017 mg/100 ml (Rajlakshmi and Srikantia, 1976) and 0.043 (Vauhan et al., 1976). The copper in breast milk was also reported to be better absorbed due to the presence of copper binding ligands (Walravens, 1980).

Zinc content was found to vary between 0.112 mg/100 ml (Rajlakshmi and Srikantia, 1976) to 0.53 mg/100 ml (Macy and Kelly, 1961). Such variation may be due to variation in the dietary intake of zinc of mothers. Zinc in human milk was better absorbed due to presence of zinc binding ligands and specific low molecular weight proteins that enhanced intestinal absorption of this trace element (Walravens, 1980; Cosin and Smith, 1979).

Sodium content of human milk was found to range between 17.2 mg/100 ml in American subjects (Macy and Kelly, 1961) and 22.1 mg/100 ml in Indian subjects (Belavady and Gopalan, 1959). Potassium content varied between 34.7 mg/100 ml in Indian subjects (Belavady and Gopalan, 1959) and 51.2 mg/100 ml in New Hebride subjects (Peters, 1953). Chloride content is about 43 mg/100 ml as reported by Macy and Kelly (1961). These three elements together with proteins, contribute toward the solute load of milk.

Sodium, potassium and chloride contents in animal milks are higher and, in turn, exhibited higher solute load. Zeigler and Fomon (1971) computed the solute load for human and cow milk to be 79 mOsm and 221 mOsm respectively. Consequently, more water intake becomes necessary to clear such ex-cessive solute load when the baby is maintained on cow milk, failing which hypernatraemic condition would develop and could even prove fatal.

Various workers have reported vitamin contents of human milk in different countries. A comparison of values obtained is given in Table I. It could be seen that the vitamin contents were lower in the case of Indian and Bantu women as compared to American. Apparently the vitamin contents in milk reflected the dietary intake by the mothers in the respective countries.

In their studies on the dietary intake of vitamins and milk content of these nutrients among Indian subjects, Deodhar and Ramakrishnan (1960) observed significant correlation between these two. The correlation for riboflavin, nicotinic acid, ascorbic acid and thiamine content in milk with those in the diet were 0.54, 0.47, 0.65 and 0.35 respectively and were statistically significant.

Unlike milk from other species, human milk contains sizable amount of vitamin C. As can be seen from Table I, the content of this vitamin was about 3 to 4 mg/100 ml. Though dietary vitamin C intake is low, in certain studies

it was observed that vitamin C secreted in milk was much larger than the maternal intake of this vitamin(Rajlakshmi et al., 1964).

Human milk is a highly complexfluid and contains numerous enzymes. However, no clear evidence was obtained so far to ascribe to them any specific metabolic role.

Several enzymes involved in the metabolism of carbohydrates, protein and lipids were reported to be present in human milk by several workers. These include acid phosphatase (Heyndrickx, 1962), alkaline phosphatase (Stewart et al., 1958), protease (Heyndrickx, 1963), ribonuclease (Bingham and Zittle, 1964), aspartic transferase (Heyndrickx, 1962), alanine amino transferase (Heyndrickx, 1962), amylase (Heyndrickx, 1971), amylase (Heyndrickx, 1962), glucosephosphate isomerase(Heyndrickx, 1962), glucoronidase (Kiermeirner et al., 1969), malate and lactate dehydrogenases (Deodhar et al., 1964), lactose synthetase (Andrews, 1969), aldolase (Heyndrickx, 1962), arylesterase (Heyndrickx, 1963) and lipase (Klee and Klee, 1970).

Apparently,the plane of maternal nutrition influences certain enzymes. Karmarkar et al. (1963) found that dietary fat intake upto 72 g/day increased the levels of lipase, esterase and alkaline phosphatase, but decreased the acid phosphatase activity in the breast milk. Sklanunu-Zurukzoglu et al. (1965) reported the administration of vitamins A, B_6, B_{12}, C and K to lactating women, did not

have any influence on the level of glucose-6-phosphate dehydrogenase. Similarly Deodhar et al. (1964) demonstrated daily vitamin intake had no effect on xanthine oxidase, lactate and malic acid dehydrogenase activities in human milk. Belavady (1960) however, noted that protein supplementation of 3 g/day for four weeks increased alkaline phosphatase and xanthine oxidase activities in human milk.

Though, as mentioned earlier, milk enzymes have not been ascribed any specific role in the metabolism of newborn, recent studies by Harvel and Olivecrona (1974) and Hall and Muller (1980) identified two forms of lipases : (a) activated by serum; and (b) activated by bile salts. It was further shown that there was predominance of bile acid activated lipase in the breast milk and suggested their significance in the lipid metabolism of infants.

Besides factors of nutritional significance in milk, considerable information has been accrued on certain unique immunological characteristics of human milk. Several protein components such as immunoglobulins IgA, IgG, IgM, lactoferrins, lysozyme interferron, non-specific factors such as thymus dependent T, lymphocytes, phagocytes, bifidus factor, complement component and a substance inhibiting the growth of staphylococci etc have been identified in the recent years (Chandra, 1978).

In a study on various immune factors such as immunoglobulin, lysozyme, lactoferrin, Lonnerdal et al. (1976) and Ogra et al. (1980) found higher levels for IgG, IgA

and IgM in colostrum and lower levels for lysozymes and lactoferrin, than those observed in normal mature milk.

The advantage of breast feeding infants over artificial feeding in respect of producing better resistance to infecting agents was evident from the studies conducted by Chandra (1978). It was observed that in respect of infectious diseases like gastro-enteritis, respiratory infections, septicemia and ear infections, breast fed infants were more resistant than bottle fed ones.

Gyorgy (1955) isolated bifidus factor and in 1962 found staphylococcal factor from human milk which conferred immunity against diarrhoea in infants.

Milk Yield

Attempts have been made to ascertain the adequacy of breast milk in meeting nutritional needs of infants by determining the milk yield together with the nutrient contents of breast milk.

Belavady and Gopalan (1956) observed the milk yield of 400 to 500 ml per day among malnourished women in Andhra Pradesh. Somewhat higher yield of 660 ml was reported by Rajalakshmi and Ramkrishnan (1964) among Gujarati women from economically less previleged class. Similar values, in the range between 515 and 750 ml were reported by Someswara Rao et al. (1959), Lonnerdal et al. (1976) and Jelliffe and Jelliffe (1978).

According to Lonnerdal et al. (1976) the yield was low in the early stage and increased progressively as lactation advanced. Similar large variations in milk yield between 427 to 922 ml/day were reported by Jelliffe and Jelliffe (1978) among African and South Asian population groups. Such variations could be ascribed to various factors.

The methodology adopted in different studies as well as physiological and nutritional status of mother, could influence the yield, as reported in these studies. While Belavady and Gopalan (1959) and Rajalakshmi et al. (1964) recorded the yield on the basis of weight difference before and after breast feeding the infant, other workers used manual expression technique. Such influence of different techniques used to record milk yield, was evident from the observation reported by Bown et al. (1982) who found 7 percent higher values when milk was expressed using a breast pump as compared to weighment method.

As regards the nutritional influence, a comparison of data on milk yield for subjects in developed and developing countries, show a marked superiority of mothers from western countries (Lonnerdal et al., 1976; Bown et al., 1982) over those in developing countries like India (Someswara Rao, 1959; Jelliffe, 1978, Forsum and Lonnerdal, 1980). It would appear that the plane of nutrition does influence the lactation performance of mothers. In another study, Belavady and Gopalan (1959) observed that supplementation of maternal diet with proteins, significantly enhanced milk yield in the case of Indian subjects having low protein intakes.

As regards the effect of plane of nutrition on composition of milk, as pointed out earlier, though the proximate composition remained unaltered, micro-nutrient contents were decisively dependent. Besides points mentioned above, the physical limitations of the mother such as sore nipples, painful breasts, possibly unhygienic conditions as a result of lack of antenatal care were reported to exist among poor Indian and Bangladeshi women (Ghosh, 1977; Huffman et al., 1980).

Also it is only when the mother takes the initiative to breast feed the child, that the let down reflex becomes effective. Some mothers, in the first few days, find that milk does not flow properly, stop breast feeding and resort to bottle feeding. On the other hand, the physiological cause of discontinuing breast feeding is identified as ensuing pregnancy which causes strain and often illness to the mother (Ghosh, 1977).

Etiology of Failure of Breast-feeding Practice

Though merits of human milk and consequently breast feeding practices are evident from the aforesaid, mothers often fail to successfully breast feed babies for a variety of reasons that range from nutritional inadequacy to social factors.

Insufficient privacy in large families which is a great inconvenience to the mother is another reason for discontinuing breast feeding. Also when the mothers are employed away from home, they resort to bottle feeding.

Misconception about the harmful effects of breast feeding practices, have been reported to be responsible to discourage mothers (Jelliffe, 1968; Rubini et al. (1959).

Other factors identified to be effective were advertisements in which a fat baby shown on the containers often mislead the mother to resort to bottle feeding ! Even the health workers, till recently believed in the bottle feeding to be superior to breast feeding and gave wrong advice to mothers (Ghosh, 1977).

Such failures as mentioned above, whether physical, physiological, psychological etc. leave mothers with no other option but to resort to artificial feeding. Wherever possible, practices of adapting milk from domesticated animals after suitable modifications or else commercially available infant food formulae, are made use of.

Methods of infant feeding in the subtropical and tropical regions vary infinitely. These differ from country to country, state to state, district to district and town to town and also known to be influenced by racial and religious beliefs and taboos. Rural people who rear milch animals resort to animal milk feeding. In several Middle East countries as well as those in the Indian Sub continent, where prolonged breast feeding is practised, buffalo, goat or cow milk have been reported to be adopted for infant feeding (Jelliffe and Jelliffe,1978).

Animal milk hardly plays any part in infant feeding among economically less previleged ones, because of its prohibitive cost.

It is through experience that these people have come to realise that young infants face difficulty in digesting untreated cow or buffalo milk. This is mainly due to the higher protein and buffer salts content of most animal milks. The large proportion of casein of the total proteins present in animals milks, is responsible for a dense curd in the stomach which is not digested by proteolytic enzymes. Besides this, these animal milks contain fat content higher than human milk.

In order to aid proper utilization, animal milk is subjected to simple treatments such as boiling, dilution etc. during infant feeding practices.

Milk is diluted with water in the ratio of 1 : 1, 2 : 1 or 3 : 2 depending on the age of the infant. Younger the infant, higher will be the dilution desired and vice versa (Jelliffe and Jelliffe, 1978).

In India diluted milks are further boiled with either 'ginger' or 'ajwayan' or 'sonf' to make it easy for the child to digest. The boiling treatment itself is reported to lower the curd tension of milk (Jelliffe and Jelliffe, 1978; Parpia, 1972).

Very often the use of liquid milk with appropriate modification is not practicable. This is because of several reasons such as not so easy availability, poor storage and

transportation of milk, etc. Besides this, modification done at domestic level could often prove to be risky, because of unsanitary and unhygienic environments, non-availability of potable water, especially in under developed countries. This lead to the development of infant food formulae based on milk solids and taking into consideration nutritional needs of infants.

Chemical Composition

With an accent on the development of ideal infant food formulae, innumerable preparations based partially or solely on partially skimmed cow milk, have been developed by food scientists and technologists in many countries. Depending on, whether these are starting formulae or follow-up formulae, these are formulated, keeping in mind the nutritional needs of the specific age group of infants. However, these often vary in their composition on the basis of the model adopted during their formulation. A comparison of some infant food formulae available in the local(Indian) market and those adopted in other countries, is given in Table II. (Illustration I)

Nutritional survillence study on various food formulae prevalent in several European countries was conducted by Widdowson (1964) and Fomon (1974). These included Oster milk and Cow and Gate (U.K.), Similac and Humana (Holland), Pelargon (Italy), Probana, Lofenalac, Natramijen, $S-_{14}$, $S-_{29}$ (U.S.A.). Among several Indian preparations notably Amul, Glaxo, Lactogen, Vijaya and

Table II : Nutrient composition of some Indian and foreign infant foods.

Nutrient (expressed per 100 g)	INDIA				
	* Amul	** Glaxo	** Lactogen	*** Vijaya spray	*** Lactodex
Protein g	22.0	22.0	21.6	22.6	14.5
Carbohydrate g	50.0	50.0	51.6	50.0	72.5
Fat g	18.0	18.0	19.0	19.0	5.4
Vitamin A IU	1500	1500	1520	1500	2000
Vitamin D IU	400	400	420	600	300
Calcium g	1.0	1.0	0.770	1.0	1.0
Phosphorus g	0.80	0.80	0.60	0.80	0.80
Sodium m Eq	13.0	13.0	13.0	13.0	13.0
Potassium m Eq	20.0	20.0	20.0	20.0	20.0
Iron mg	4.0	4.0	4.0	4.0	4.0

(Table II Contd.)

* Bindra and Deodhar (1980)
** Udani, et al.(1970).
*** as on labels

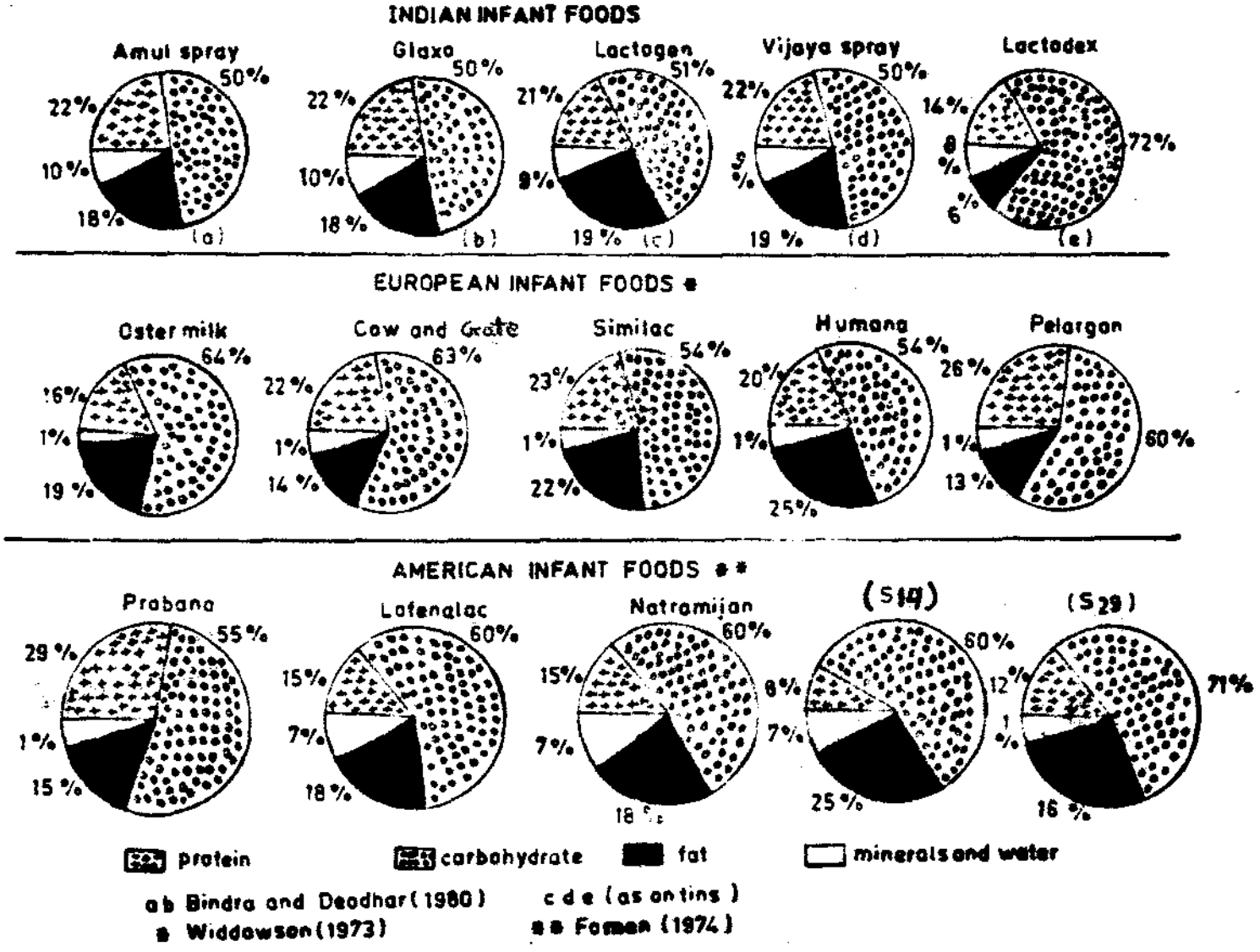
Table II. Contd.

Nutrient (expressed per 100 g)	U.S.A.*					European countries**				
	Probana	Lofenalec Mead	Nutramigen Mead	S_{-14} Wyeth	S_{-29} Wyeth	Oster milk (U.K.)	Cow and Gate (U.K.)	Similac (Holland)	Humana (Holland)	Pelargan (Italy)
Protein g	29.4	15.4	15.4	7.7	11.9	16.8	22.7	23.3	20.8	26.5
Carbohydrate g	55.3	60.9	60.0	59.7	70.7	64.0	63.0	54.0	54.0	60.0
Fat g	15.4	18.9	18.5	25.9	16.1	19.2	14.3	22.7	25.2	13.5
Vitamin A IU	3000	3000	3000	3000	3000	-	-	-	-	-
Vitamin D IU	600	600	600	600	600	-	-	-	-	-
Calcium g	1.10	0.95	0.95	0.437	1.46	0.84	0.90	0.71	0.63	0.69
Phosphorus g	0.88	0.66	0.66	0.33	1.77	0.69	0.70	0.40	0.30	0.41
Sodium m Eq	20.0	21.0	14.0	-	-	20.0	20.0	13.0	11.0	11.0
Potassium m Eq	37.0	29.0	27.0	-	-	28.0	28.0	8.0	15.0	21.0
Iron mg	1.5	12.7	12.7	7.7	7.7	11.0	5.0	9.5	9.0	4.2

* Fomon (1974)
** Widdowson (1974)

ILLUSTRATION I

The aproximate nutrient composition of Indian European and American infant foods



Lactodex, barring Amul and Vijaya, rest were based on cow milk. Amul and Vijaya were based on technology based on the use of buffalo milk.

A comparative data on the proximate analysis of these preparations are given in Table II. It was seen that the protein content in these preparations varied from 16.8g/100g is Oster milk to 26.5 g/100 g in Pelargon (Italy) in the case of European preparations. In the case of American preparations, the protein content was 7.7 g/100 g in the case of $S-_{14}$ and 29.4 g/100 g in the case of Probana. In the Indian preparations, the values ranged between 14.5 g/100 g (Lactodex) to 22.0 g per 100 g in the rest of the preparations. (Illustration I).

In recent years, a deviation in the preparation of these infant foods in European countries and U.S.A. was followed by switching emphasis on the use of whey as one of the basic constituents, providing whey protein in an attempt to correct casein to whey protein ratio. The mineral concentration was maintained similar to breast milk by using whey obtained by multichamber electrodialysis technique.

As regards carbohydrates, the content in European products varied between 54 g/100 g in Humana and Similac to 64 g/100 g in Oster milk and Cow and Gate. In American preparations, the values ranged between 55.3 g/100 g(Probana) and 70.7 g/100 g in the case of $S-_{29}$. In the case of Indian preparations, the values ranged between 50.0 g/100 g in the case of Amul, Glaxo, Lactogen, Vijaya and 72.5 g/100 g in the case of Lactodex.

In the European preparations Nan, Humana, Oster milk and Cow and Gate only lactose was used, whereas in Pelargon lactose along with Sucrose was used.

In the products from U.S.A. in Probana, dextrimaltose was used, in Lofenalac whereas starch and corn syrup was used in Natramijan and sucrose as well as starch was used in $S-_{14}$, $S-_{29}$.

In the Indian preparations, sucrose was used in Amul, Glaxo, Lactogen and Vijaya spray, and dextrimaltose in Lactodex, besides lactose present in the milk.

As regards fat, the content in European preparations varied between 13.5 g/100 g in the case of Pelargon and 25.2 g/ 100 g in the case of Humana. In preparations from U.S.A., fat content varied between 15.4 g/100 g in the case of Probana to 25.9 g/100 g in the case of $S-_{14}$. In Indian preparations, fat content varied between 6.5 g/100 g in the case of Lactodex to 19.0 g/100 g in the case of Lactogen and Vijaya.

Widdowson (1965) found that in Pelargon a combination of coconut oil 95% & corn oil 5% was used besides animal fat. Similarly, Humana and Nan consisted a mixture of animal and vegetable fat. However, Oster milk and Cow and Gate contained only milk fat. The ratio of two fats used was adjusted to resemble the fatty acid profile in human milk.

In American preparations such as Probana, Lofenalac, Natramijan, $S-_{14}$ and $S-_{29}$, corn oil was used. In Indian preparations no vegetable fat was used.

As for the content of vitamins and minerals, it was found that the vitamin A content ranged between 2000 I.U. and 3000 I.U., vitamin D was 600 I.U./100 g, calcium was between 0.437 and 1.46 g/100 g, phosphorus was 0.33 g/100 g, sodium was between 14 mEq and 21 mEq/100 g and potassium was between 27 and 37 mEq/100 g, iron between 1.5 mg and 12.7 mg/100 g in the products from U.S.A. whereas the vitamin A content ranged between 1500 I.U. and 2000 I.U./ 100 g, vitamin D between 400 I.U. and 600 I.U., calcium between 0.77 g and 1.0 g/100 g, phosphorus between 0.6 g and 0.8 g/100 g, sodium 13 mEq/100 g, potassium 0.20 mEq/ 100 g and iron 4 mg/100 g in various Indian products.

Biological Evaluation

Several approaches made in determining the protein quality of these preparations included determination of growth rates in infants maintained on these preparations nitrogen balance study on infants as well as experimental animals and hematological picture.

In an attempt to assess the quality of infant food 'Amul' based on buffalo milk preparation, Misra (1959) fed twentyfive infants between 0 - 24 months and recorded growth rates over the period of three months. A comparison

was made with other commercially available preparations namely Glaxo and Lactogen. A group maintained exclusively on the breast milk was also included as a control. It was observed that the quality of milk proteins from Amul was similar to that from other preparations. A comparison of growth rates observed in the case of normal and marasmic children in all the age groups showed slightly higher but non significant gains in body weight among marasmic infants as compared to normal. Possibly,relatively higher ratio of protein to calories (in the light of caloric requirements of marasmic children) could have limited optimal utilisation of dietary proteins, in this study.

In order to evaluate the quality of Lever baby food, a buffalo milk based infant formula, Udani et al. (1970) conducted a study on infants aged between 0 - 16 weeks, maintained on the formula diet for the period of 12 weeks. Daily gain in weight and height were recorded. The analysis of the data revealed that the average gain in weight was in the range for normal infants, but the average gain in height was not comparable to normal standard for heights. It was concluded that the infant food met the nutritional requirements barely marginally.

Pelargon, the acidified milk was tested by Carbo (1959) on 25 babies aged 23 to 86 days for 90 to 203 days. A control group received partially skimmed milk. After three months, the weight gain on acidified milk was 25.64 g/day while for skimmed milk, it was 22.28 g/day. After another three months, the gains were 20.71 g/day and

16.20 g/day respectively. It was inferred that acidified milk was better utilised than ordinary skimmed milk.

De Vitali (1965), on the other hand, evaluated the acidified milk (Pelargon) by giving it as a supplement to human milk. It was reconstituted at 7 % total solids in the first week and at 15 % total solids in the second week. It was found that none of these showed intolerance and the babies made highly satisfactory progress. The average weight gain was 25.4 g/day which was within normal limits.

Nan, an infant food, having casein to lactalbumin ratio adjusted similar to human milk, was tested on 300 premature and 180 normal infants by Staemler (1969), with a control group comprised of breast fed infants. It was observed that a daily weight gain between 20 to 30 g achieved, was similar to that in the control group. Besides this, there were no infections. It was concluded that Nan could be used in place of breast milk when necessary.

Milasan, an infant food containing vegetable fat was tested on 146 infants. It was found that the average gain in weight was 31.14 g/day. Further, it was seen that only 6.9 % fell sick during the observation period, implying the preparation to be satisfactory (Seiffert, 1969).

Nitrogen Balance Studies

Though cow and buffalo milks contain more protein in comparison with human milk, their superiority could be inferred only from better retention of nitrogen.

Studies have also been reported on the nitrogen balance among infants maintained on the test formula and comparing N retention with those receiving breast milk.

Barness et al. (1957) studied the quality of cow milk based infant formula by comparing the nitrogen balance in human infants with that observed on cow milk and human milk. All diets were isocaloric and isoproteinous. Infants received these preparations in a switch over design fashion for the period of 9 to 15 days. It was observed that nitrogen retention was 54 % in the case of human milk whereas on cow milk and cow milk based preparations, it was 51 and 48 %, respectively. These differences were, however, non significant.

Fomon (1960) tested an infant food formula based on cow milk having proximate composition similar to human milk. It was found that the mean daily intakes of nitrogen by infants fed formula in the various age groups of 8 - 45 days, 46 - 90 days, 91 - 136 days and 137 - 182 days were 320, 279, 248, 233 mg/kg, respectively as compared to infants fed pasteurized human milk with 383, 296, 274 and 240 mg/kg N intake respectively. It was seen that retention of nitrogen by infants fed formula was below the regression line calculated from data pertaining to infants fed human milk,underlining the superiority of human milk.

Widdowson (1965) tested human milk formula S-26 and SMA on 30 full term normal healthy baby boys of 5 - 7

days age. It was observed that retention of nitrogen from breast milk was identical to that from other formulae. However, at a later stage, retention was somewhat lower in the human milk than other preparations. The percentage retentions in the first week on human milk and formulae were similar. However, at one month stage, the percentage retention in case of human milk was higher. It was evident that human milk proteins were superior to formulae protein.

Plemert et al. (1965) evaluated on infants three milk preparations, viz (i) 'Ki-Na' containing 1.8 g protein/ 100 ml, (ii) 'Ki-Na' containing 2.4 g protein, and (iii) acid milk 'Citrosan' containing 2.6 g protein/100 ml. These milks provided 3, 4 and 4.8 g of protein per kg/day and 116, 116 and 134 KCal per kg per day.

Net protein utilisation was 55, 52 and 52 % and biological value was 57, 54 and 53 %. It was concluded that acidification, extra protein were unnecessary.

Stamper et al. (1974) evaluated five Yugoslavian preparations viz, acidified milk (Laktacid), full cream milk (Laktovit-1), humanised milk (Lactomil 2), partly humanised milk (Nektarmul) and Bebiron-S_{26}, a humanised milk preparation, for premature infants. Protein content in these preparations ranged between 2.12 g to 3.6 g/100 ml. When fed to babies, the nitrogen retentions were on average 87.3, 79.2, 59.9, 59.5 and 42.0 % of intake and the biological value were 93.5, 84.3, 68.4, 65.0 and 49.3 %.

The pattern of biological value implied that acidified milk 'Laktacid' was superior to other milks having a retention percentage of 87.3 and a biological value of 93.5. It was further observed that retention of nitrogen increased upto a certain level (3.25) beyond which further increase in protein level showed decrease in nitrogen retention.

Johnston et al. (1961) determined optimum level of protein calories in the diet by studying nitrogen balance in male infants. The contribution of calories from proteins in the total feed ranged between 8 to 25 %. It was observed that the nitrogen retention per unit body weight was higher in the range of 13 to 20 %. protein calories, than at lower levels. Though there was further increase in nitrogen retention when protein calories contributed over 25 %, this had overall adverse effect on the calorie intake by infants.

Daniel et al. (1968) assessed nutritive value of infant food prepared from buffalo milk using albino rats, containing 10 %, 12.5 % and 15 % protein and fortified with D L - methionine. It was observed that the mean weekly growth rate of rats receiving diet with 10 % protein, 20 % fat and fortified with D L - methionine was of the same order as those obtained with milk foods containing higher levels of proteins upto 26 %. The milk food containing 10 % protein without D L - methionine fortification promoted significantly less growth than the ones with 12.5 to 28 % protein. The protein efficiency ratio of proteins from

milk food fortified with D L - methionine was significantly higher (PER = 4) than that of the unfortified milk food at 10 % level of protein in the diet (PER = 3.3).

Henry and Kon (1953) found that spray dried or freeze dried milk fortified with 2 % cystine resulted in about 5 percent rise in biological value. However, supplementation with D L - methionine caused still more elevation of the biological value.

Fomon (1960) evaluated an infant formula based on cow milk and vegetable fats, by conducting feeding trials on eight full term normal infants. The formula had the proximate composition similar to that of breast milk with 60 calories/100 ml. It provided 7 % of calories as protein from curd milk, 50 % from the mixture of vegetable oils and 43 % from lactose. Observations made on certain blood constituents namely, total blood proteins and urea nitrogen showed that the mean total protein content in blood serum was 5.57 g and blood urea nitrogen 6.0 g/ml. These values were identical with those reported by other workers for infants given pasteurized human milk. It was inferred from these observations that the preparation was identical with human milk.

In a study on a group of 15 infants, Natelson et al. (1955) observed the effect of varying casein to whey protein ratio in different formulae, on their body. It was found that the urea nitrogen was more in a group of higher casein to whey protein ratio and low in breast milk receiving group. Likewise, plasma proteins level was high in low

casein group. Hematocrit declined slightly until 32nd day and increased subsequently. It was higher in the breast fed group.

Raiha et al. (1976) studied the effect of varying the casein to whey protein ratio in cow milk based infant formulae by feeding healthy infants, with infants receiving breast milk served as control. On analysis of blood, it was found that formulae with higher protein levels were responsible for higher levels of total serum proteins and urea nitrogen, in comparison to the control group. Further, metabolic acidosis was more frequent and severe especially in infants receiving casein predominant mixtures than whey protein predominant ones.

Keeping in mind the limitation of milk proteins with respect to sulfur amino acids, Pop et al. (1977) supplemented cow milk based preparation with 50 % sugar and cystine and compared the protein quality with breast milk by conducting feeding trials on infants. It was observed that the weight gain was better in the formula fed group. On the other hand, data on the blood cystine levels indicated that cystine was higher in breast milk fed than those given formula feed. Similar observation was made in respect of methionine supplementation.

Misra (1959) evaluated Amul, a buffalo milk based preparation by feeding it to marasmic and normal infants. It was found that the hemoglobin levels in marasmic cases varied from 7.0 to 12.0 g/100 ml and in the normal group

from 9.0 to 12.5 g/100 ml. Total serum protein content in marasmic infants ranged between 3.7 to 5.6 g/100 ml with an apparent increase in the globulin level. The serum albumin and globulin levels varied from 0.63 g to 2.5 g and 2.07 to 4.0 g/100 ml, respectively. After feeding Amul formula, levels were markedly altered. Total proteins rose to 4.2 to 6.8 g/100 ml with a concomitant shift in the existing albumin-globulin ratio. Likewise, the serum albumin level increased 2.83 to 4.0 g percent, and globulin level decreased to 1.37 to 3.7 g percent. In normal subjects, the level of total proteins varied from 6.0 g percent to 7.35 g percent. The albumin level varied from 3.03 to 3.39 g percent and the globulin level from 2.61 to 3.37 percent. The levels of total proteins, albumin and globulin were elevated to a limited extent after feeding 'Amul', the average value for total protein being 6.53 g percent, for albumin 4.06 g percent and for globulin 2.40 g percent. It was concluded that 'Amul' restored the low protein status of blood and was successful in overcoming protein malnutrition.

Schmidt (1968) determined serum amino acid profile in 16 healthy babies, at 2 h interval after feeding either adapted cow's milk formula with 1.4 to 1.7 g percent protein, 2/3 sweetened milk with 2.0 to 2.2 of protein, 2/3 acidified milk with 2.3 to 2.5 g percent protein or human milk. It was observed that on feeding preparation with protein contents more than 1.9 percent, the serum amino acid - Nitrogen level ranged between 6 to 8 mg/100 ml.

Besides protein quality assessment, studies have also been conducted to ascertain the adequacy of various formulations to fulfil the nutritional requirements in respect of several other nutrients.

As mentioned earlier, infant food formulae have been developed in which milk fat is partially or totally replaced by one or more vegetable fats. This was done keeping in view two aspects, namely (a) fulfilment of infant needs for polyunsaturated fatty acid (PUFA), (b) improving the fat absorption, and (c) simulation with human milk fat.

Widdowson (1965) studied two infant formulae, namely S_{26} and SMA, common in European countries and compared their fat utilisation with thatfrom breast milk. The composition of fat in these formulations was so adjusted by appropriately mixing vegetable fats such as oleo oil, coconut oil, soybean oil, corn oil and soybean lecithin as emulsifier, so as to resemble the net fatty acid composition of human milk fat. On feeding these formulae to 5 to 7 days old infants, it was observed that though the fat intake in the breast fed group was relatively lower, there was lower fecal excretion of fat resulting in about 92 percent absorption. On the other hand, the fat intake was considerably higher in groups receiving artificial formulations. However, there was simultaneously markedly higher fecal excretion of fat resulting in net decreased absorption. When these formulations were tested on four to six weeks

old infants, it was observed that though the pattern of fat retention was more or less similar which was 77.9 percent for S_{26} and 74.7 percent for SMA preparations. The absorption was 91.5 percent and 87.6 percent for S_{26} and SMA preparations, respectively, as compared to 96 percent in the case of breast milk. Possibly, with the advancing age there was improved digestibility among infants, that apparently reflected in better utilisation of fat from infant foods.

According to Matson (1964), fat absorption depended on the positional distribution of certain fatty acids in a triglyceride molecule. It was observed that the pancreatic lipase, specially hydrolysed, fatty acids esterified in 1 and 3 positions in the triglyceride, with no hydrolysis of the majority of fatty acids esterified in the 2-position.

Vignetti and Sbraccia (1958) studied the fat absorption from humanised cow milk, when given to 20 premature infants. In this study, infants received either (a) human milk, or (b) humanised cow milk preparation 'Pentolac' alongwith human milk, in equal amounts, or (c) pentolac alone. It was observed that Pentolac was well tolerated, with no digestive disturbances. The mean percentage of fat absorbed was 89.9, 92.7 and 92.0 respectively.

Heinz et al. (1966) fed healthy term infants exclusively one of the following four artificial feeds for the period of 7 to 10 days, namely, Pelargon, Alete N-1,

Pomil and Alete S-1. It was observed that absorption of total fatty acids from the milk lipids ranged from 79 to 89 percent as compared to about 85 percent for human milk. Further, absorption of short and long chain saturated fatty acids ranged between 85 and 99 percent. Proportions of saturated fatty acids in the acidified dried milk preparation Pelargon and Alete N-1 were about 57 percent, in Pomil and Alete F-1, both adopted fresh milks, the first dried and the second condensed, 38 to 55 percent, while in human milk it was 46 percent. Percentages of absorption in infants fed on them were respectively 69, 69, 69, 70 and 46.

Tomarelli et al. (1968) ascertained the superior absorption of human milk fat by conducting absorption studies on young male rats. It was reported that the superior absorption was related to the high proportion of palmitic acid in the 2-position in a triglyceride molecule. On testing several fats, it was found that high absorption of human milk fat (94.6 %) was equalled by a fat blend of a similar fatty acid composition with a high content of 2-palmitoyl-triglyceride (96.3 %).

Lohr et al. (1965) studied the effect of feeding different milk preparations in 3 to 8 months old infants, on fat absorption. These included (a) 2/3rd condensed milk, (b) acidified milk, (c) low protein milk with 12 % linoleic acid, (d) a moderate protein milk 10 % linoleic acid. Blood

samples were analysed for total lipid contents at different intervals after feeding. Another group of five infants was given human milk with 8.5 % linoleic acid while other group of five got cow milk preparation containing upto 40 percent linoleic acid. It was observed that in all the infants, blood lipid content reached the optimum level between 2½ and 3 h interval. The increases in total lipid contents were about 20 to 30 percent over the fasting values. When cow's milk was diluted to two thirds or half, the 4 h values were almost back to normal, but when either modified cow milk or the human milk was given, the values were still about 15 percent above the fasting value . On this basis, it was suggested that cow milk feed for infants should be so modified as to cause a rise in blood lipids comparable to that produced by human milk.

Williams et al. (1970) performed metabolic balance studies on 55 healthy male and female infants to find the absorption of calcium, phosphorus and fat from four adapted cow milk preparations, while a group receiving human milk served as control. It was observed that absorption and retention of calcium was much higher in infants receiving human milk fat than those given adapted cow milk preparation with low stearic and palimitic acid but high oleic acid contents.

The importance of linoleic acid in the infant nutrition was demonstrated by Hansen et al. (1958) and Wiese et al. (1958) who fed infants a skimmed milk formula containing less than 0.1 percent calories derived from linoleic acid. It was observed that there was reduction in the plasma concentration of linoleic and arachidonic acid associated with an increase in the concentration of triene : tetraene ratio. Further-more, there was a characteristic dermatitis and dry scaly thickened skin.

Naismith et al. (1978) investigated the effect of low linoleic acid intake in bottle fed infants. It was found that triene : tetraene ratio in serum seldom exceeded 0.4 in infants receiving cow milk. It was concluded that the linoleic acid requirement could be fulfilled if 0.55 percent of total calories were furnished by the formula.

Goalwin and Pomerange (1962) estimated serum cholesterol in bottle fed and breast fed babies. The bottle fed were also given fruit as a supplement at 12 weeks as well as cereals and meat in the subsequent period. It was observed that the cholesterol level rose to 188.2 mg/ 100 ml serum in breast fed infants and 127.2 mg/100 ml serum in bottle fed. Those who were given cereals and other solids, the cholesterol rose to 172.6 mg/100 ml serum at 16 weeks. It was observed that breast fed infants had a higher level of cholesterol in the early months of life which may have significance in later life.

Caruso and Ciliberto (1970) estimated serum cholesterol in three groups of infants receiving either (a) cow milk with vegetable fat replacing all milk fat; (b) acidified whole milk; or (c) auxolac. The blood cholesterol was estimated initially and at 30 and 60 days of feeding. The cholesterol levels were 158, 142 and 92.3 mg/100 ml,in the acidified milk group the values were 128, 113.7 and 100.4 mg/100 ml and in the Auxolac given group, the values were 148, 131 and 108.5 mg/100 ml. The cholesterol values in all the groups decreased at a different rate and the difference was significant, and that the vegetable fat fed group had the lowest level at 60 days.

Carbohydrates

During early infancy when the diet is primarily comprised of breast milk or formula, carbohydrate usually supplies 35 to 55 percent of total calories in the feed and lactose generally accounts for a significant portion of this. For breast fed infants as well as those receiving various commercially prepared formulae, lactose is the principle carbohydrate consumed.

Anderson et al. (1972) studied the utilisation of various carbohydrates either individually or when these formed one of the constituents of infant food formulae. Observations were made on 3 days old normal infants in respect of rise in blood glucose level at 30 minutes interval over the period of 3 h after oral administration

of the feed. It was observed that the maximum rise in blood glucose occurred at 60 minute interval when glucose alone was given. Similar rise, but of lesser magnitude, was observed in respect of maltose and dextrimaltose supplementation. Corn starch, on the other hand, failed to raise blood glucose level significantly at any time interval. Similar trend was observed when formulae comprising these carbohydrates were given. A small rise of about 4 mg/100 ml was seen when either carbohydrate free formula or the one with starch as the carbohydrate source, was given. It was further observed that the blood glucose levels declined rather slowly when the sugar was given along with infant food formula.

Husband et al. (1970) studied digestion of starch in new born infants, in which infants were given either 10 percent starch solution or 10 percent glucose solution by stomach tube. The samples of gastric contents collected after 30 min were analysed for the residual starch, while blood glucose levels were determined at 30, 60, 90 and 120 min intervals after the feeding. It was found that starch meal was emptied slowly from the new born stomach, which could be due to low secretion of pancreatic amylase, leading to slow hydrolysis of starch. There was a rise in blood glucose after starch meals after 90 minutes, in contrast to 30 minutes in the case of glucose feeding, and a slow but sustained rise following starch meal.

In a study on rats, Jarvis (1930) studied the effect of various sugars on the body composition. Weanling albino rats were divided into three groups. One group was given control diet with glucose as a source of carbohydrate while other two groups received either sucrose or lactose. It was observed that sucrose fed rats averaged 11 percent gain in weight over the control, while lactose fed rats averaged 5 percent below the control. When six rats from each group were analysed for body composition, it was observed that there were more total solids in the tissues of lactose fed rats than the control or sucrose fed ones. There was less water content in the lactose fed rats. Possibly more nitrogen, calcium and phosphorus were retained in lactose receiving group.

Kleinbaum (1967) studied the utilisation of various carbohydrates incorporated in the feed, by conducting 10 day balance experiments on healthy infants. These were estimated in stools of 87 healthy infants of 14 days to 6 months old, given test feeds consisting of diluted(2 : 1) cow milk with 2 percent 'Maizana', a corn product. The milk contained 2.3 % protein, 2.5 % lipid and 10 g % carbohydrate providing 75 KCal energy per 100 ml. The carbohydrates tested were lactose 3.2 %, sucrose 5 % and maize starch 1.8 g %. The daily intake of milk was below 1000 ml. Polysaccharide not utilized, was computed from the difference between the glucose contents in stools

determined before and after hydrolysis. It was observed that during the first month of life, hardly any polysaccharide was utilised. However, in the second and third month, 21.7 and 48.4 % and during 3 to 6 months after birth, 77.3 % starch was utilised. Further, a group of 5 infants was given equal amounts of diluted cow milk and breast milk with 2 % Maizana or 10 % rice gruel which raised the intake of polysaccharide to 25.5 g. It was observed that the utilization of these polysaccharide was complete even during first month of life. This was attributed to membrane digestion of starch with amylase present in the human milk.

Southgate and Barrett (1966) studied the intake and excretion of carbohydrates by feeding 20 baby boys aged 2 weeks. In a balance study for 3 day duration, out of 20 babies, ten were given breast milk while rest were given two preparations having either lactose or sucrose. It was observed that the percent absorption was 96.9 in respect of breast milk and 99.9 in respect of cow milk with added lactose or sucrose. It was inferred that the unabsorbed lactose from breast milk was responsible to provide acidic environment in the lower part of the intestinal tract and prevented growth of infective organism.

Jarret and Holman (1966) tested two sugars namely, lactose and sucrose on two groups of preterm infants of 13 to 17 days age. After 4 h, the blood glucose levels were determined at 30, 60 and 90 min after the meal. It was observed that glucose tolerance was similar in both

groups. The rise in blood glucose level was non significant when sucrose was given as compared to that obtained when lactose was given.

As regards the influence of carbohydrate on calcium absorption, Wasserman and Comar (1959) observed that absorption of calcium by rats was not enhanced by administration of sugars such as glucose, fructose, galactose and sucrose possibly because these were rapidly absorbed from the intestinal tract. On the other hand carbohydrates such as lactose, dextrimaltose, which were absorbed slowly, enhanced calcium absorption.

Vaughan and Filer (1960) observed that although the mechanism of calcium absorption was not clearly understood, the favourable effect of lactose related to its relatively slow absorption thus providing appropriate amounts of lactose in the lower intestinal tract promoting calcium absorption. Absorption of magnesium and strontium too, were probably influenced by factors similar to those, affecting absorption of calcium.

Utilization of Certain Major and Trace Elements

Shaw (1976) conducted a calcium balance study on eleven preterm and two full term infants given either human milk, full cream cow milk, half skimmed cow milk or SMA formula. It was observed that infants fed these three, retained on an average 27, 31, and 27 percent of the daily

calcium intake whereas those receiving breast milk retained 54 and 52 percent of calcium. Thus human milk was found superior to cow milk and cow milk based formulae. In this study the fat absorption from cow milk and cow milk based formulae was estimated and was 59 and 61 percent, respectively and in the case of breast milk 84 and 87 percent indicating that fat absorption from human milk was better and promoted better calcium absorption.

In another balance study on one week old and four week old infants, Widdowson (1965) observed that the percentage calcium retention was 45.2 for human milk, but only 6.2 for the formula S_{26} and 4.9 for SMA in the first week. In the case of 4 weeks old infants, the retention was somewhat higher being 49.3 % for breast milk, 36.7 % for S_{26} and 10.4 for SMA. It was reasoned that babies failed to absorb fat of cow's milk as well as human milk which further reflected in calcium retention. Besides this, excess phosphorus also possibly hindered the absorption of calcium.

Bartlop and Oppe (1970) studied the effect of Ca/P ratio in cow milk based formula on calcium retention. The ratio was adjusted between 0.74 - 1.8 by adding calcium as calcium gluconate to cow milk based formulae. It was observed that when the ratio was identical to human milk, calcium and phosphorus uptake from cow milk formula and human milk were similar. It was further observed that higher phosphorus content in cow milk preparation produced

increased plasma phosphorus concentration, but decreased plasma calcium concentration. It was suggested that increasing the calcium level may prevent the hypocalcaemic effect of cow's milk but would give a milk of high mineral content.

In another study, Fomon et al. (1963) studied calcium uptake from milk preparation with added demineralised whey in an attempt to simulate Ca/P ratio to that in human milk. It was found that the general retention of calcium and phosphorus per unit body weight, for such preparation was similar to human milk.

Likewise, Williams et al. (1970) studied the effect of kind of fat on calcium retention by using different types of vegetable fats along with cow milk preparations, with human milk as a control. It was observed that a vegetable fat blend with low stearic and palmitic acid, but high oleic acid and with the overall fatty acid composition resembling that of human milk fat, resulted better retention of calcium than from preparations of other vegetable fats.

Phosphorus content of human milk was in the range of 35 mg and 51.2 mg (Belavady and Gopalan, 1962; Macy and Kelly, 1961) and 96 mg in the case of cow milk (Macy and Kelly, 1961). It was found that phosphorus content varied between 0.33 mg and 0.88 mg per 100 g in Indian and foreign products (Fomon, 1974; Bindra and Deodhar, 1980).

As regards utilization of phosphorus, Widdowson (1965) compared phosphorus retention from human milk, S_{26} and SMA using one week and four weeks old infants as experimental subjects. It was observed that the percentage retention was 86.7 %, 56 % and 45.8 % in the first week and in the fourth week percentage retentions were 70, 45.8 and 32.2 respectively. It was found that phosphorus from human milk was better absorbed than cow milk based formula.

Magnesium

Magnesium content of human milk has been reported between 2.6 and 3.5 mg/100 ml (Belavady and Gopalan, 1962; Macy and Kelly, 1961) and in cow milk 12 mg (Macy and Kelly, 1961). It was found that magnesium content varied between 0.08 and 0.13 mg per 100 g in foreign products (Fomon,1974).

As regards magnesium utilization from the infant formula, Widdowson (1965) conducted a magnesium balance study on one week and four weeks old infants, maintained on different cow milk based preparations. It was observed that in the one week old infants, the percent retention of magnesium from humanmilk was 41.2 % whereas that from S_{26} and SMA, was 34.9 and 31.7, respectively. In four week old infants, the values were 47.5, 27.5 and 30.8 for human milk, S_{26} and SMA respectively. It was evident from these results that retention from human milk was superior to cow milk based preparations. Harvey et al. (1970) estimated plasma Ca and Mg contents as a measure of the utilization of these elements in breast fed and bottle fed

infants and observed that the utilization was lower in bottle fed infants.

Sodium and Potassium

The sodium content of human milk was reported in the range of 17.2 mg/100 ml and 22.1 mg/100 ml (Macy and Kelly, 1961; Belavady and Gopalan, 1962) and much higher in cow milk i.e. 58 mg/100 ml (Macy and Kelly, 1961). As regards infant food formulae, it was found that sodium content varied between 14 and 20 mEq per 100 g in foreign infant foods (Fomon, 1974).

Considerable attention was devoted to the retention of sodium from milk as well as milk based formulae because of its contribution to the osmolar load. Widdowson (1965) studied the retention of sodium from cow milk based preparations, namely S_{26} and SMA and compared the same with human milk. It was observed that the retention of sodium was 47.6, 50.9 percent in the case of S_{26} and SMA preparations, as against only 31.6 percent in the case of human milk when administered to one week old infants. In 4 weeks old infant, the retention was found to be 55.2, 34.1 in respect of cow milk preparations, S_{26} and SMA and 49.1 % for mature human milk. It was evident that there was better retention of sodium from formulae diets in early stage of life than at later stage.

Potassium content in human milk was found in the range of 34.7 mg/100 ml and 51.2 mg/100 ml (Belavady and Gopalan, 1962; Macy and Kelly, 1961). The potassium content

of cow milk is 138 mg per 100 ml (Macy and Kelly, 1961). It was found that potassium content was in the range of 24 and 37 mEq in foreign infant foods (Fomon, 1974).

As regards potassium utilization, it was found that the retention was 60.1, 50.4 and 56.6 % for S_{26}, SMA and human milk, respectively, in the first week of life while in one month old infants, retention was 33.9 and 23.7 percent in cow milk based preparations and 43.1 percent from human milk. It was evident that in the case of younger infants, retention was better on formula diets but in older infants retention was better on human milk.

Renal Solute Load

Sodium and potassium, besides nitrogenous components like urea and acid radicals such as chloride, contribute to renal solute load. As pointed out earlier, cow milk based preparations have been reported to contain more sodium and potassium than human milk. This would further reflect in the renal solute load when administered to infants.

Fomon and Ziegler (1971) computed the solute loads for various infant formulae and compared them with human milk. It was observed that the solute load for human milk was markedly lower viz. 79 m Osm as compared to 221 and 308 for cow milk based preparations.

Trace Elements

The iron content of infant food formulae available, varied between 4.0 mg and 6.0 mg per 100 g powder,while in the preparations from other countries, it varied between 1.5 mg to 12.7 mg per 100 g(Fomon, 1974).

Farquhar (1963) studied hemoglobin level in infants during the first year and found to range between 11.7 and 13.1 g %. After giving iron supplementation, there was small increase. It was inferred that infants receiving iron supplementation had higher level of hemoglobin.

Woodruff et al.(1977) reported that daily intake of iron in breast fed infants was 2.9 mg in 3 to 6 months period and 6.52 mg in 6 to 9 months period. The bottle fed infants, on the other hand, received 5.7 mg iron in 3 to 6 months period and 7.18 mg in 6 to 9 months period. It was concluded that infants required additional iron besides that available from milk. Breast fed infants, however, appear to use iron more efficiently than artificially fed infants.

Dallman et al. (1980)conducted a balance study on infants subsisting on human milk, cow milk or cow milk based formulae. It was found that the iron absorption was 49 % from human milk, whereas only 10 and 12 % from cow milk and cow milk based formulae,respectively. The superiority of human milk in this regard was attributed

to its higher content of the iron binding protein, lactoferrin. It was further suggested that ingestion of breast milk per se upto six months may condition the intestinal mucosa in a manner that facilitated absorption and consequently there was no iron deficiency. It was observed that breast milk alone could supply iron for 6 months.

The copper content of various preparations varied between 0.08 mg and 0.65 mg per 100 g from other countries (Fomon, 1974; Dang et al., 198[illegible]).

Various studies further reflected the nutritional quality of the milk based formula obtained from them.

Soderhjelam (1959) found copper content of Swedish powdered milk to be 0.44 mg per 100 g while in the prepared liquid feed, it ranged between 0.03 to 0.70 mg/100 ml. Lower incidence of hypocupraemic anaemia among Swedish infants was ascribed either to large hepatic stores of copper or possibly overestimation of the copper requirement.

Tanaka et al. (1980) reported neutropenia sideroblastic anaemia and bone abnormalities, characteristic of copper deficiency condition, in a single girl patient who was maintained on milk formula having the content of 51 ug copper per 100 g powder. It was observed that supplementation with the oral dose of copper sulphate had salutory effect in alleviating the deficiency conditions.

These observations were further supported by Walravens (1980) who reported that prolonged administration of diets consisting of cow milk low in copper content, could be responsible for development of copper deficiency among bottle fed infants.

The zinc content of the infant foods varied between 0.33 mg and 4.55 mg per 100 g in products from abroad (Fomon, 1974; Dang et al., 1982).

Walravens and Hambridge (1976) investigated the adequacy of zinc in cow milk based formulae by feeding two formulae providing 1.8 mg per litre and 5.8 mg of zinc per litre, to two groups of male and female infants. It was observed that even low levels of zinc in the formula did not produce any skin rash or other deficiency symptoms, but higher level of supplementation improved the growth. It was further found that when zinc to copper ratio was elevated, it increased blood cholesterol level and also in mortality among infants.

Cosin and Smith (1980) investigated the cause of better absorption of zinc in human milk and found it was due to the way zinc was bound to the low molecular weight legands that facilitated its absorption. Human milk has been reported to have less zinc than that present in cow milk. Further, a high proportion of zinc was found to be associated with a relatively low molecular weight legand (molecular weight 8700). It was postulated that this

zinc binding legand was responsible for alleviating the symptoms of the genetic disorder of zinc metabolism, acrodermatitis enteropathica. The therapeutic value of human milk, in this disorder, has been recognized, in this context. Interestingly, the presence of zinc does not impair the iron binding function of lactoferrin, but in fact enhances its uptake rate.

Manganese

The manganese content in infant foods has been reported to vary between 0.03 mg and 0.199 mg per 100 g in products used in other countries (Fomon, 1974).

Though no information is available on the incidence of manganese deficiency among Indian and foreign subjects, report of higher manganse in the hair of disabled infants (Collip et al., 1983; Dang et al., 1982) have stated that there was 5 to 15 times higher manganese in infant food formulae than human milk.

Vitamins

Infant foods are, by and large, fortified in respect of vitamins in the light of requirements of infants as well as to compensate losses incurred during processing of milk.

The information given on the nutrient content in these products indicated that the vitamin A content is between 1500 IU/100 g and 2000 IU/100 g, vitamin D content is between 300 IU/100 g and 600 IU/100 g, vitamin B_1

content between 0.3 mg/100 g and 0.6 mg/100 g, vitamin B_2 content between 0.4 mg/100 g and 1.0 g/100 g and niacin content between 3.5 mg/100 g and 6.0 mg/100 g, folic acid content between 42 ug/100 g and 100 ug/100g, B_{12} content 1 ug/100 g and vitamin C content between 30 mg/100 g and 60 mg/100 g.

The vitamin content of some foreign infant foods such as Nutramigen, Lofenelac, Portagen and Pregestimil are : Vitamin A content between 2120 IU/100 g and 2820 IU/100 g, vitamin D content between 282 IU/100 g and 423 IU/100 g, vitamin B_1 content between 0.635 mg/100 g and 1.06 mg/100 g, vitamin B_2 content between 1.0 g/100g and 1.27 mg/100 g, niacin content between 8.5 mg/100 g and 12.7 mg/100 g, folic acid content between 50 ug/100 g and 70 ug/100 g, B_{12} content between 2.6 ug/100 g and 3.5 ug/100 g and vitamin C content 55 mg/100 g (Fomon, 1974).

It could be seen that in respect of vitamin A, B_1, B_2, viacin folic acid, B_{12} and vitamin C, the foreign products had higher content.

Overfeeding

There is a tendency to overfeed infants because of the wrong notion that a fat baby is a healthy baby (Taitz, 1971).

Obesity during infancy should be looked at from two angles : (i) whether it persists into late childhood and

later adult stage, and (ii) its immediate effect on the health of the infant.

Tracy and Harper (1971) conducted a survey to ascertain whether over-weight infants are more liable to respiratory infections. Of the 120 over-weight children, 47 experienced respiratory infection during the trial, whereas remaining 73 did not. At the end of the trial, out of 47 who were about 90th percentile, 19 experienced respiratory infection. On the other hand, the control group of normal weight infants (2.75) only 20 % suffered from respiratory infection, while the rest remained free from infection.

During the survey conducted on bottle fed and breast fed infants, Taitz (1971) observed about 40 % male and 37 % female infants who were bottle fed to be above 90th percentile, in contrast to 19 % among breast fed infants.

In an attempt to find relation between over-weight condition in the early stage of life with the incidence of obesity in the later stage, between 6 to 8 years, Eid(1970) studied 138 over-weight infants in first 6 months of life. These infants were re-examined at 6, 7 and 8 years. It was observed that over-weight infants continued to remain obese at respective ages.

In a study conducted by Brooke (1980) on the utilisation of calories from artificial formulae, preterm

infants were given feeds varying in energy contents. It was observed that diets providing energy in the range of 3000 KJ to over 3700 KJ per litre when administered, the retention of calories ranged between 58 - 61 %. On the other hand, at a low energy concentration of 2600 KJ/litre, the retention was slightly higher. However, as regards deposition of excess calories in the form of body fat, there was no appreciable change among these groups as evident from determination of subcutaneous fat levels by skin fold measurements. Similar reflection was seen when other criterion such as arm circumference was taken. As regards body weight gain, groups receiving higher energy contents showed slightly higher values than control groups. It was inferred that metabolic rates were higher in groups given diets rich in calories than those having normal level of 2600 KJ/litre.

Sanitation

Several workers investigated the cause and incidence of disease in artificially fed infants.

Crosse et al. (1960) investigated in three groups of infants : (i) wholly breast fed, (ii) partly breast fed, and (iii) wholly bottle fed, the occurance of various diseases. It was found that infections such as septicaemia, meningitis deep obscess, lung urine and cord infections, sticky eyes, skin infections, oral thrush, nasal discharge, were more common in totally bottle fed but least in breast fed infants.

Mellander et al. (1959) found incidence of infections such as rhinitis cough, otitis media, upper respiratory infections with pyrexia and acute diarrhoea tended to be greater in bottle fed infants between three months and one year.

Mukherjee (1959) investigated 538 infants in West Bengal of which 13.3 % were artificially fed. It was observed that diarrhoea and other infections were more frequent in the artificially fed babies.

The quality of protein depends on its amino acid profile and their biological value. Though heat treatment has been recognised to affect the structural changes in the milk proteins, little is known about their effect on the amino acid profile of milk proteins. Several investigations have been made on the influence of such treatment on the availability of amino acid from milk proteins. Skim milk powder used as a base for infant formulae is derived from milk subjected to heating during spray drying or roller drying process.

Mac Donald (1966) observed that spray drying of milk resulted in a loss of 0-4 % lysine, while drum drying of milk caused about 16 % loss of lysine. It was found that the higher loss during drum drying process was due to the milk proteins coming in contact with the drum at high temperature, over longer period. Similar finding was

reported by Rolls and Porter (1973), who recorded that the loss in the available lysine in spray drying was 3 to 10 % but between 5 to 40 % during roller drying treatment of milk. In case of methionine, the limiting amino acids in milk protein, Narayana Rao (1963) found that heating adversely affected its availability. The loss was dependent on temperature and moisture content. He further studied the effect of heat treatment on milk powder (i) in hot oven, (ii) 37°C for 10 days, and (iii) autoclaving at 121°C at 15 lb pressure for 15 min and found that 33 % to 45 % methionine was lost.

It would appear from these data that irrespective of the level of total proteins in the infant foods, the nature of heat processing treatment for milk powder preparation, would clearly influence the availability of certain essential amino acids and, in turn, the nutritional quality of such milk proteins and consequently infant food preparations.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation was undertaken to evaluate milk based infant food formulae popular in the Indian market, from nutritional standpoint. Such preparations are generally based on either cow, buffalo or mixed milks. Besides this, milks treated either using spray drying or roller drying processes are incorporated in these preparations. It was further observed, as pointed out in the earlier section, that these preparations considerably vary in their proximate composition. However, it could hardly be said with any degree of certainty about their nutritional quality, since beside the known effect of processing on their nutritional attributes, species differences in respect of milk sources are also considered to influence the nutritional quality of the product. In view of this, certain popular infant food formulae available in the market, were chosen in the study.

Statistical Design

A randomized block design as described by Cochran and Cox (1977) with three replicates was adopted to compare the nutritional qualities of six types of popular infant foods prepared with different processes and under different managemental and manufacturing conditions. Out of six

preparations chosen, two were prepared by roller drying process and remaining four by spray drying process.

Six milk based infant foods of the following brands namely, (i) Lactogen; (ii) Amul Spray; (iii) Vijaya Spray; (iv) Lactodex; (v) Amul (Roller Dried); and (vi) Glaxo (Roller Dried), were purchased from the market for the investigation.

These were selected from different regions. Thus Amul Spray and Amul Roller Dried were obtained from Anand (Gujarat State), Vijaya Spray from Hyderabad (Andhra Pradesh), Lactogen from Delhi, Glaxo from Bombay and Lactodex from Madras.

Further samples of these preparations were taken from three different batches to take into account manufacturing conditions.

These infant foods were analysed for proximate composition and detailed analysis was done for important nutrients. Besides these, studies were also conducted on the nutritional quality of milk proteins and fat absorption.

Determination of Proximate Principles

Protein, fat, carbohydrate, total ash and moisture contents were determined in various infant food samples as described below :

Protein

The protein content in test samples was determined by using conventional micro Kjeldahl digestion and distillation procedure described by AOAC (1980).

One gram sample was digested in 10 ml of conc. H_2SO_4 with the aid of a digestion mixture (K_2SO_4, $CuSO_4$, SeO_2 in the ratio of 40:8:1). After complete digestion it was diluted and suitable aliquot was taken for ammonia liberation by adding 20 ml of NaOH(40 w/v %). It was collected in 10 ml of boric acid (3 % w/v) and determined by titrating against standard 0.02N H_2So_4 using mixed indicator (methyl red and methylene blue 2 : 1) as the external indicator. The nitrogen content was converted into protein by multiplying with the factor of 6.38.

Fat

The fat content was determined by Gerber method as described in I.S.I. Bulletin (1958).

Five gram sample of the infant food was uniformly mixed in 50 ml water, from which 10.75 ml aliquot was taken in a Gerber tube with a milk pipette and mixed with 10 ml of H_2SO_4 (90 %) and 1 ml of amyl alcohol. The tube was stoppered and carefully mixed by inverting a few times. After centrifuging for about 10 min. in a Gerber

centrifuge, the reading on the stem was recorded. The percent fat content was calculated as follows :

Fat % = Reading x Dilution factor.

Determination of Iodine Number

The iodine number was determined by the method described in I.S.I. Bulletin (1968).

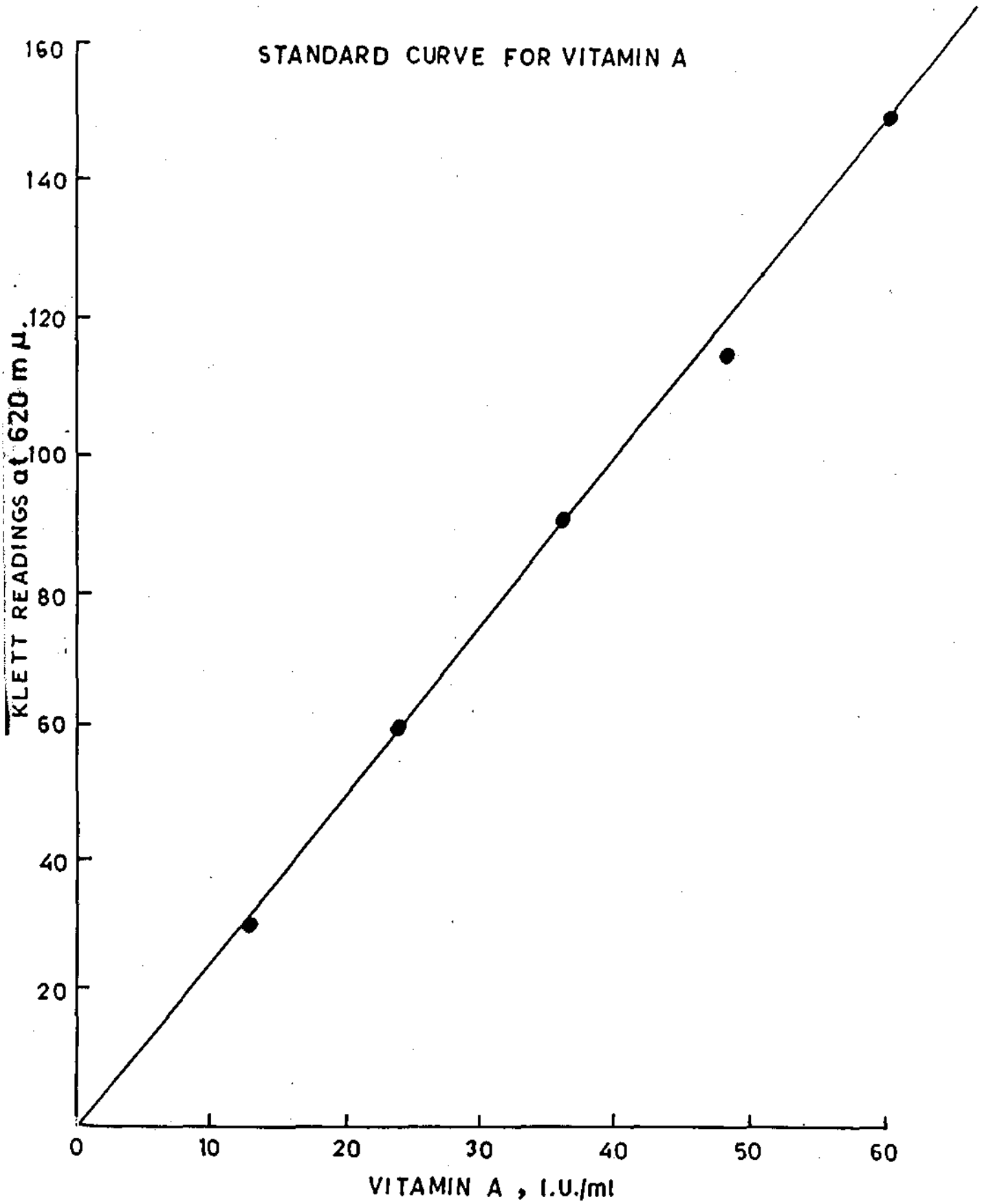
The fat in the test sample was extracted with diethyl ether (60 - 80°C) in the Soxhlet apparatus and vacuum dried. The residual ether was eliminated under nitrogen atmosphere. About 0.5 g of the extracted milk fat was weighed in a conical flask and dissolved in 15 ml chloroform. It was mixed with 20 ml of Wij's reagent (0.44 % v/v) iodine monochloride in glacial acetic acid. On keeping in the dark for one hour, 20 ml of KI (10 % w/v) and 150 ml distilled water were added to it and the contents were titrated against 0.1 N Na thiosulfate, using 2 ml of starch solution as indicator.

Vitamin A

Vitamin A content in samples were determined chemically according to the method of Carr and Price(1926) as given in the I.S.I. Bulletin (1968).

Five gram sample was saponified with 7 ml of alcoholic KOH (50 %) for 30 min. On cooling, the unsaponifiable fraction was separated by extracting the saponified

Fig. 1.


STANDARD CURVE FOR VITAMIN A
KLETT READINGS at 620 mμ.
160
140
120
100
80
60
40
20
0
10
20
30
40
50
60
VITAMIN A , I.U./ml

fraction three times with diethyl ether , using 50 ml solvent for each extraction. Etherial extracts were pooled and made free from alkali by washing with adequate quantity of water. These were evaporated under vacuum. The residue obtained was dissolved in 5 ml chloroform and the known aliquot was, treated with antimony trichloride reagent. The blue colour produced was measured instantly at 620 mμ using Klett Summerson photoelectric colorimeter.

Essential Fatty Acid Content

Contents of essential fatty acids were estimated by the method described in AOCS (1959).

About 20 g sample was weighed in a beaker and the fat was extracted by diethyl ether (60 - 80°C). 11.0 g alcoholic KOH (21 % w/v) was weighed in the isomerisation tube connected to a nitrogen source. It was heated in the oil bath maintained at 180°C. After 20 min. of heating, a glass cup containing 100 mg sample fat was inserted in it. The system was heated in the oil bath for 25 min. at 180°C for isomerisation. After that it was removed and cooled with chilled water. The contents were taken in methanol and transferred to 100 ml volumetric flask. A blank was prepared in a similar manner. The absorbance was read at different wave lengths viz. 223, 262, 268, 274, 308, 315, 322, 346 nm in a ultraviolet spectrophotometer (Spectronic 21, Bausch and Lomb).

Another aliquot of 100 mg fat was dissolved in isoptane and readings were recorded at 233, 262, 268, 274, 308, 315, 322, 347 nm to account for interference due to impurities. Spectrophotometric readings were taken on both sides of the analytical wave lengths to ascertain that an optimum absorbance is recorded. A component was considered absent when a maximum was not found in the characteristic region. Wave lengths (mμ) involved in the equation are :

Dienoic	-	233	-
Trienoic	262	268	274
Tetraenoic	308	315	322
Pentaenoic	-	346	-

Calculations :

Linoleic acid $= 1.09 \times a'_2 - 0.571\ a'_3 - 0.26 + 0.002 a'_5$ g/100 g fat

Linolenic acid $= 1.10\ a'_3 - 0.88\ a'_4 + 0.31 a'_5$ g/100 g fat

Arachidonic acid $= 1.65\ a'_4 - 1.55 a'_5$ g/100 g fat.

$$a \text{ (Absorptuity)} = a' = \frac{A}{b \times c}$$

A = Observed absorbance.

b = Cell length

c = Grams of sample in a litre of the final dilution used for the absorbance measurement.

The conjugated acids were calculated from the absorptivity. The non-conjugated fatty acids were determined after isomerisation of the fat samples with alcoholic KOH. From the total conjugated fatty acids (after isomerisation), values of conjugated fatty acids determined were subtracted in the following manner :

The absorptivity at 233 mu in methanol and isoptane was deducted and it was taken as a_2'. Similarly for a_3', a_4' and a_5'

$a_2' = a_2 - a_2^*$ at wave lenth 233

$a_3' = a_3 - a_3^*$ at wave length 268

$a_4' = a_4 - a_4^*$ at wave length 315

$a_5' = a_5 - a_5^*$ at wave length 346

a^* is reading in isoptane

a is reading in methanol.

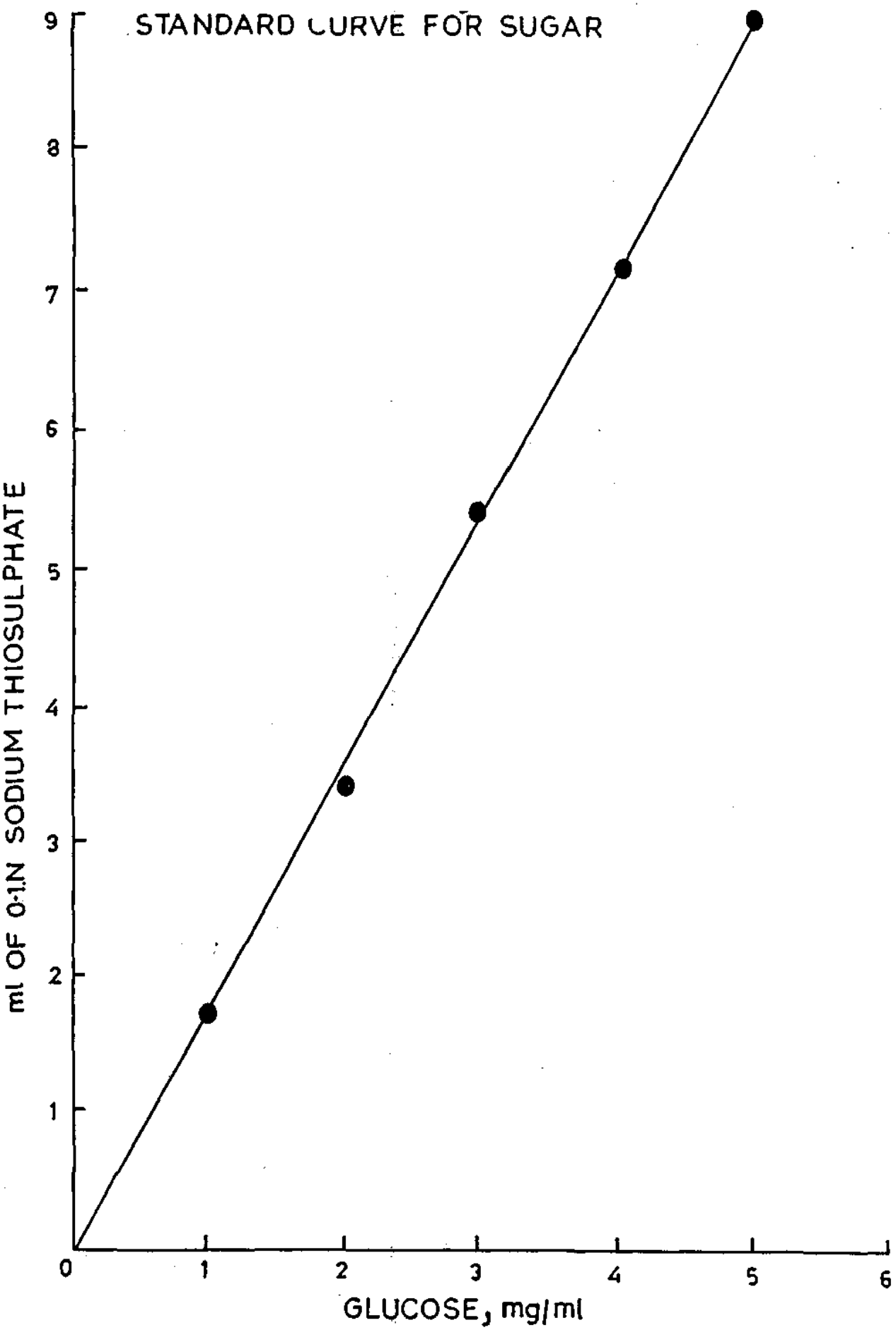
Total Carbohydrates

The carbohydrate content was determined as the total sugar content according to Hane's method as described in AOAC (1980).

Preparation of Extract

One gram of the test material was suspended in 10 ml water and deproteinized by adding 50 ml acetic acid

Fig. 2.


STANDARD CURVE FOR SUGAR
ml OF 0·1N SODIUM THIOSULPHATE
GLUCOSE, mg/ml
0
1
2
3
4
5
6
1
2
3
4
5
6
7
8
9

acetate buffer and 2 ml of sodium tungstate (12 %). It was filtered and the volume of the filtrate was made upto 250 ml.

5 ml of protein free extract was heated after adding a few drops of conc. HCl in a boiling water bath for 1 hr. On cooling under a running tap, it was mixed with exactly 10 ml of 0.1 N $K_3Fe(CN)_6$ solution and tubes were immersed in vigorously boiling water bath for exactly 20 minutes. After cooling, contents were transferred quantitatively to 150 ml Erlenmeyer flask and the unreacted $K_3Fe(CN)_6$ was titrated against 0.1 N sodium thiosulphate solution with starch KI as the external indicator.

Glucose Content

Glucose was determined enzymatically according to the method described by Slein (1963).

One gram of test sample was suspended in 10 ml of water. After deproteinizing with suitable aliquots of 0.34 M $HClO_4$ and neutralizing with K_2Co_3(73 %), the clear supernatant was saved for enzymatic determination. 0.20 ml of the supernatant was mixed with glucose oxidase (1800 units/g solid (25.2 mg %) and horse radish peroxidase 40 units/g solid (14 mg %) and sodium phosphate buffer pH (7.0) in a total volume of 5 ml. After incubating at 20 - 22°C for 40 min. the colour developed was measured by using

blue filer (436 mμ) in Klett Summerson photoelectric colorimeter. Similarly a blank and standard were prepared.

Lactose Content

Lactose was determined according to Nickerson et al. (1976).

One gram of sample was suspended uniformly in 250 ml water and deproteinized with 1 ml of zinc phosphate phosphotungistic acid reagent. On filtration, 5 ml of filtrate was neutralised with 1 N NaOH and diluted to 10 ml. The precipitate was removed by filtration through Whatman filter paper No.1 and suitable aliquot was taken for colour development after necessary dilution, so that the final lactose content was 1.0 mg/ml.

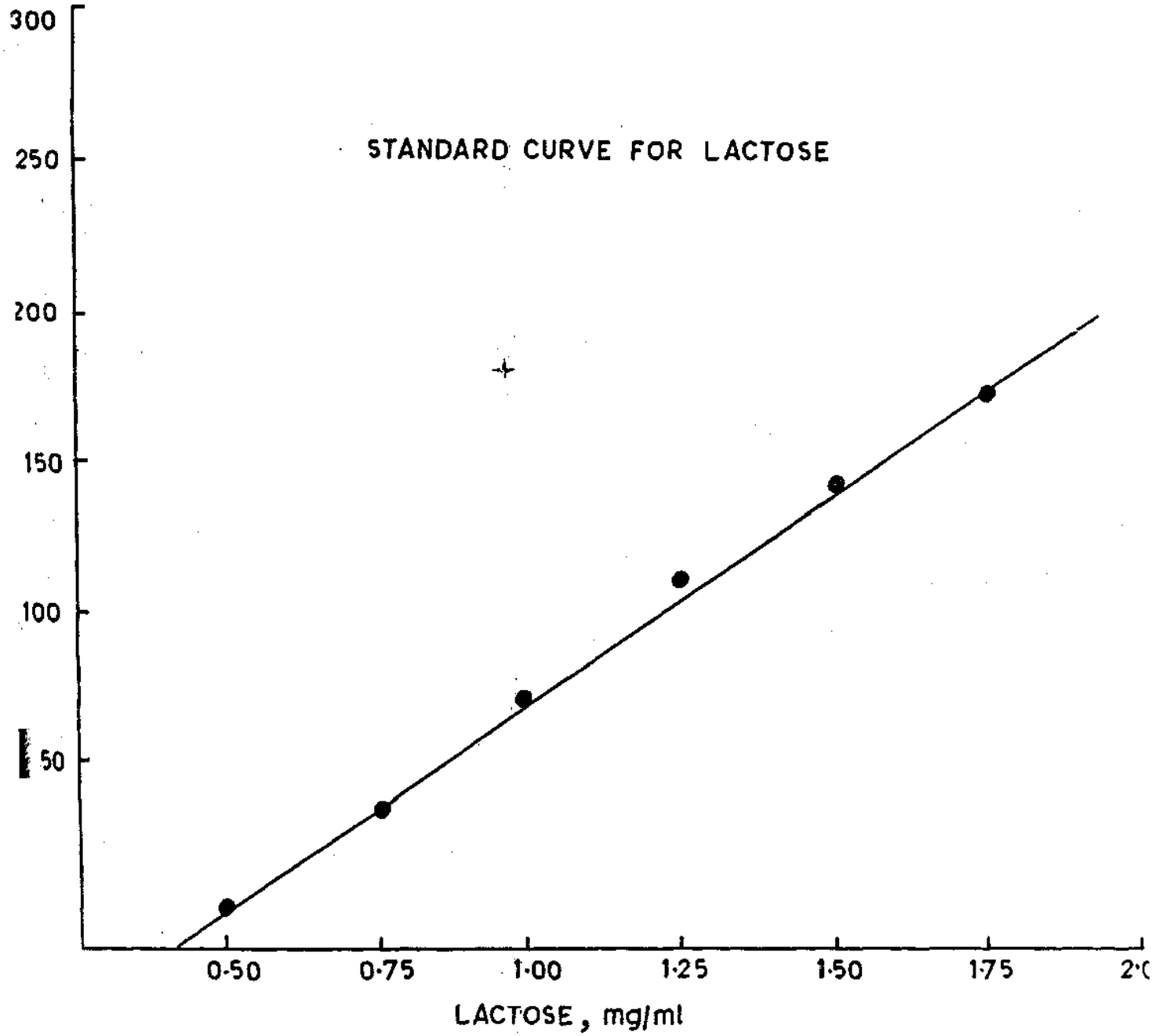
5 ml of standard solution or unknown or water(blank) was mixed with 5 ml glycine NaOH-buffer (pH 12.8) and 0.5 ml methyl amine hydrochloride (5 % w/v). This was heated in a water bath at 65°C for exactly 25 min. The reaction was stopped by cooling in a chilled water bath for 2 min. The absorbance was read in Klett Summerson photoelectric colorimeter using green filter (540 mu).

Sucrose Content

Sucrose was estimated by the method of Pantulu et al. (1982).

Five grams of sample was suspended in 100 ml water. The protein was precipitated by adding saturated solution of lead acetate. It was filtered through Whatman filter paper No.1. The filtrate was suitably diluted to contain 0.5 of sugar.
to 1 mg/ml. To 2 ml of the diluted filtrate 2 ml of 0.1 % resorcinol and 6 ml of conc. HCl were added. The final

Fig. 3


STANDARD CURVE FOR LACTOSE
300
250
200
150
100
50
0·50
0·75
1·00
1·25
1·50
1·75
LACTOSE, mg/ml

volume was made to 10 ml. The tubes were kept in the water bath at 70°C for 40 min. On cooling, the colour developed was read at 490 nm in Klett Summerson photoelectric colorimeter.

Total Ash Content

The total ash content was determined according to the procedure described in the ISI Bulletin (1968).

Five gram infant food sample was taken in a crucible and ignited in a muffle furnace maintained at 500°C for 4 h. The crucible was weighed before and after ashing and the difference in weights was taken as the ash content of the sample.

Moisture

The moisture content was determined according to the method described in I.S.I. Bulletin (1968).

Five gram sample of infant food was taken in the pre-weighed aluminium moisture dishes and dried in an oven at 100°C to a constant weight. The difference in weights, represented the moisture content of the sample taken.

Minerals

Determination of Calcium and Magnesium

Calcium and magnesium contents were determined by the method of Davies and White (1962).

One gram sample was digested with 10 ml triacid mixture ($HClO_4$: H_2SO_4 : HNO_3 1 : 3 : 9) and finally made to 100 ml. 5 ml of digested sample was mixed with 1 ml ammonium oxalate, to precipitate calcium as oxalate. The contents were mixed and made alkaline by drop-wise addition of NaOH(20 % w/v) with methyl red as the indicator. Later, dilute HCl was added till the colour changed to pink. The

tubes were kept for 4 hrs to complete the separation of calcium oxalate and the contents diluted to 10 ml with distilled water and later centrifuged at 1400 rpm for 10 min. The upper layer (A) was decanted for Mg estimation and the precipitate was used for calcium estimation.

Calcium

Precipitate of Calcium oxalate was dissolved in 0.5 ml of HCl and 1 ml of 0.05 M EDTA (NH_4Cl : NH_3). After 5 min, 1 ml of mixed NH_4Cl : NH_3 buffer(0.10 N) and 2 - 3 drops of indicator were added. This was titrated against magnesium acetate (0.015 M), till colour changed to purple red. This was corrected for blank by titrating against 1 ml of 0.05 M EDTA and 1 ml of NH_3 buffer.

Magnesium

The supernatant (A) obtained earlier, was titrated against 0.004 M EDTA using erichrome T as indicator and magnesium content was calculated by multiplying the titre value for 0.004 M EDTA with a factor of 0.09728.

Determination of Trace Minerals Content

The following trace elements, namely Zinc, Copper, Iron and Manganese, were determined. Samples were wet digested according to method of Sandell (1959).

One gram of sample was digested with tri-acid mixture ($HClO_4$: H_2SO_4 : HNO_3 1 : 3 : 9). For copper and zinc determination, volume was made to 25 ml, whereas for manganese and iron, volume was made to 50 ml.

For the determination of zinc, copper, iron, manganese etc. the readings were taken directly in the atomic absorption spectrophotometer (Pye Unicham No. SP 191), at the following wave lengths :

324 nm for copper
213.9 nm for zinc
279.5 nm for manganese
248.3 nm for iron.

The contents were calculated directly by multiplying with the dilution factor.

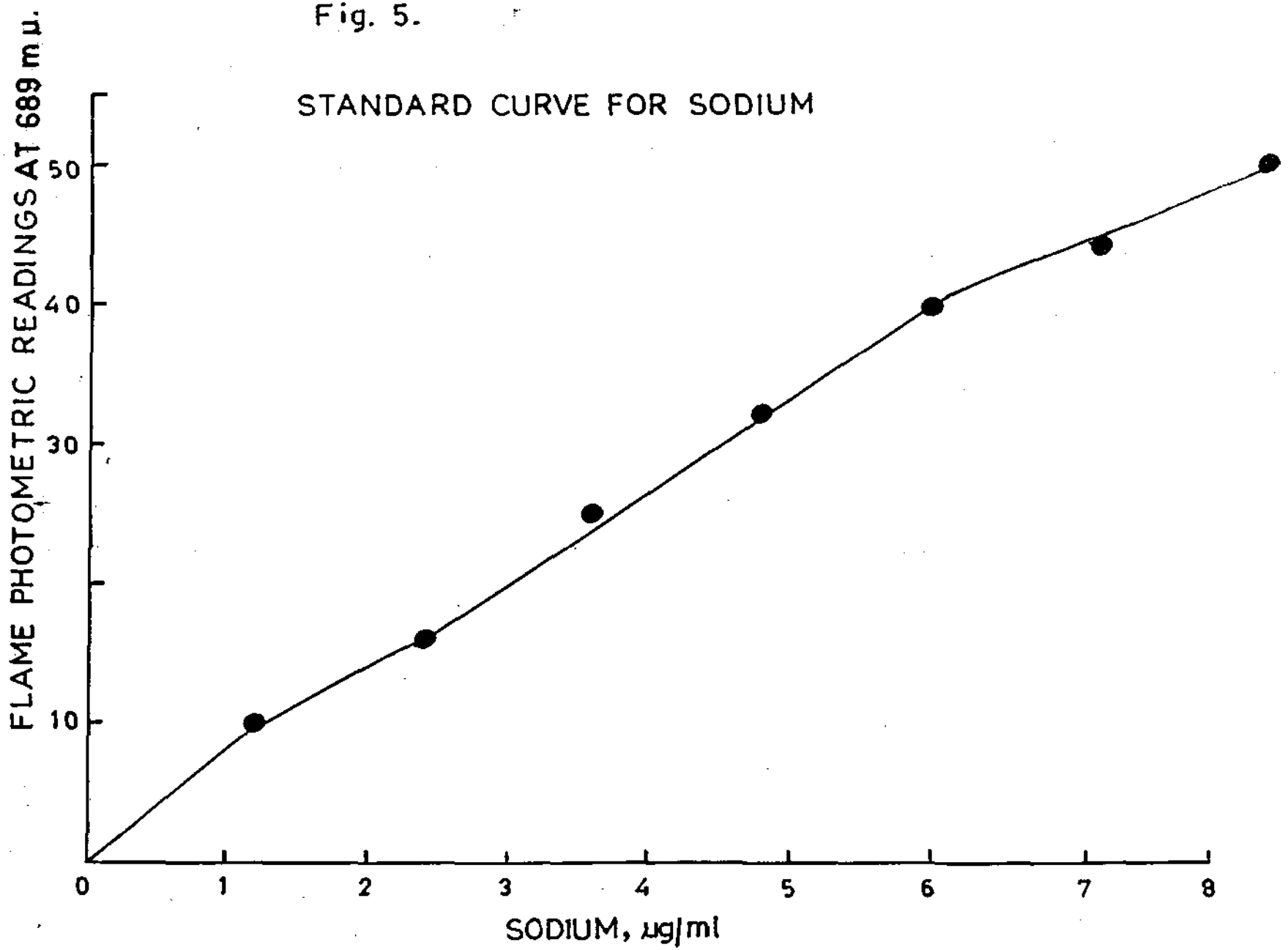
Sodium and Potassium Content

Sodium and Potassium contents were determined by flame photometric method of Have and Mulder (1957). Flame photometer (ELICO Model No.C1-22) was used in these determinations.

Preparation of Sample

About 100 mg of sample was weighed and uniformly suspended in 100 ml double distilled water during the determination of sodium content while for estimation of

Fig. 5.


STANDARD CURVE FOR SODIUM
FLAME PHOTOMETRIC READINGS AT 689 mμ.
10
30
40
50
0
1
2
3
4
5
6
7
8
SODIUM, μg/ml

potassium content, 0.1 g of sample was suspended in 500 ml of warm distilled water. The absorption was read at 589 nm in case of sodium and 767 - 769 mu in the case of potassium.

Chloride Contents

Chloride contents were estimated according to the method given by British Standards Institute(1963).

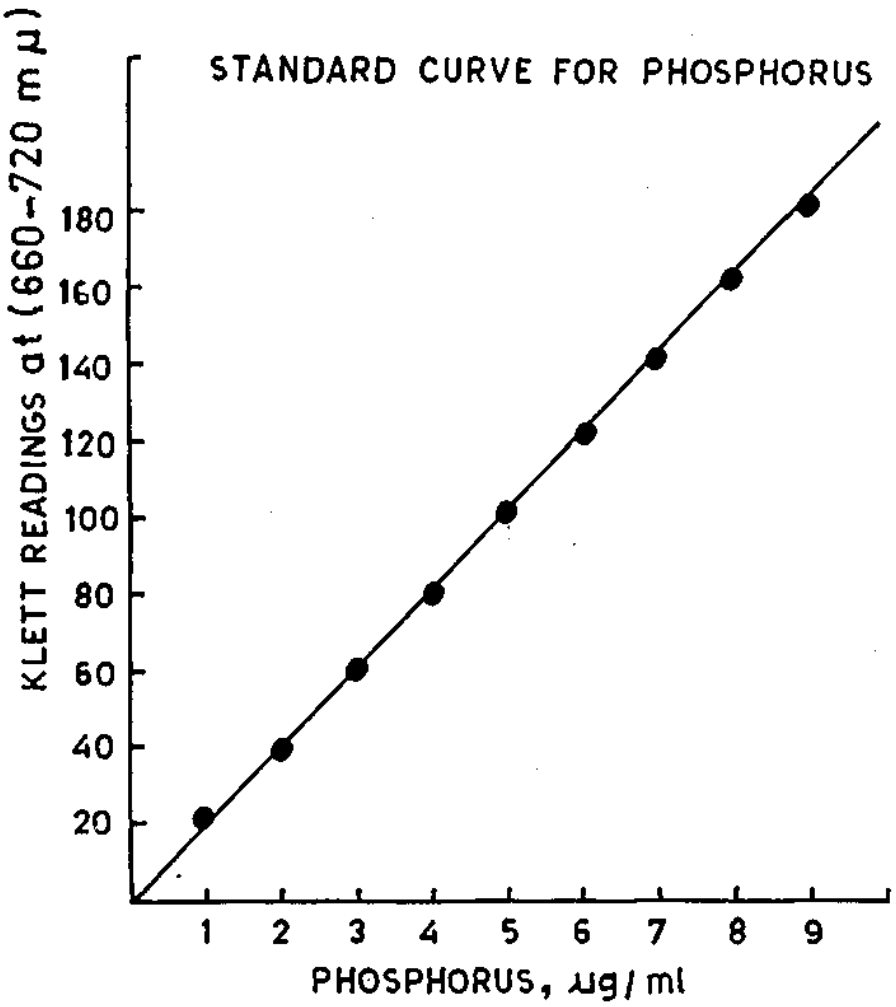
One gram of test sample was made to 100 ml, out of which 10 ml aliquot was taken in a 250 ml conical flask. This was mixed with $AgNo_3$(0.05 N) and 10 ml of HNO_3(70 % w/v). The mixture was gently boiled till pale yellow colour developed. At this stage, it was titrated against potassium thiocyanate solution (0.05 N) till a faint reddish colour persisted. The titre value obtained was multiplied by 0.001773 to obtain the amount of chloride present in the sample.

Phosphorus Content

The phosphorus content was determined by the method of Ames (1966).

The sample was digested with the aid of a pinch of digestion mixture and conc. H_2SO_4. The volume was made to 2500 ml. An aliquot of digested material was taken in a Folin tube and volume adjusted to 3 ml. 7 ml of ascorbic acid - ammonium molybdate mixture was added to each tube

Fig. 6.


STANDARD CURVE FOR PHOSPHORUS
KLETT READINGS at (660-720 mμ)
20
40
60
80
100
120
140
160
180
1
2
3
4
5
6
7
8
9
PHOSPHORUS, μg/ml

and incubated for 20 min at 45°C. On cooling, the blue colour of phosphomolybdlic acid formed, was read in Klett Summerson colorimeter using the red filter.

Fat Absorption (Biological Evaluation)

Fat absorption studies were conducted according to the method of Tomarelli et al. (1968). Adult male albino rats weighing 80 - 90 g were used in the study. Experimental diets contained 18 % fat (Table III). Animals were sensitized by maintaining them on fat-free diet for the period of 3 days. Subsequently, these were switched over to experimental diet for the period of next seven days.

During the period of 7 days of feeding, the dietary fat intake were recorded and also faeces collected for the determination of fat excretion level. The faeces were put in weighed amount of ethanol in well stoppered conical flasks.

Table III : Composition of fat free diet.

	%
Starch	478
Sucrose	200
Skim milk powder	300
Salt mixture*	20
Choline chloride*	1
Vitamin mixture*	1
Experimental fat	-
	1000

* Composition of salt/vitamin mixture was adjusted according to AOAC (1980).

As these infants foods contained 18 % fat, these were given without modification except Lactodex in which case fat was extracted and added.

5 g faeces were saponified with 10 ml of 33 % KOH (w/v) and 40 ml of ethanol (70 - 80°C) with 0.4 % amyl alcohol, the mixture was reflexed for 20 min. On cooling, it was acidied with 17 ml of 25 % HCl(w/v) and extracted with 50 ml petroleum ether. A known aliquot (25 ml) of the etherial extract was evaporated and the residue was taken in neutral ethanol. Fatty acids were titrated with 0.1 N NaOH using thymol blue as indicator. The fat absorption was calculated in terms of fatty acid composition on food and faeces.

$$\text{Fat Absorption} = \frac{\text{Fatty acid in food ingested} - \text{Fatty acid in test faeces.} - \text{Fatty acids in fat free faeces.}}{\text{Fatty acids in food ingested}}$$

The fatty acids in food and feces was calculated by applying the formula :

$$\text{Fatty acids g/sample} = \frac{A \times 284 \times 1.04 \times 2}{1000\ Q}$$

Where A = ml of 0.1 N alkali

Q = g of faeces taken for analysis.

Chemical, Microbiological and Biological Evaluation of Protein Quality

Amino Acid Analysis

Samples of infant food were analysed for their amino acid profile using Beckman Model LL9 B Amino Acid Autoanalyser.

Protein hydrolysates were prepared according to Nagasawa et al (1970). 100 mg of defatted sample containing 2.1 to 2.73 mg nitrogen was hydrolysed within 5 ml of 6 N HCl in an oven maintained at 110°C for 24 hrs in corning glass tubes sealed under vacuum. The hydrolysates were diluted to 25 ml and filtered. Two ml aliquot of the filtered hydrolysate was dried in a vacuum oven at 60°C and the residue dissolved in citrate buffer (0.1 M, pH 2.0) so as to obtain 25 to 85 µg nitrogen/ml. An aliquot of 100 ul was injected into the amino acid autoanalyser for analysis.

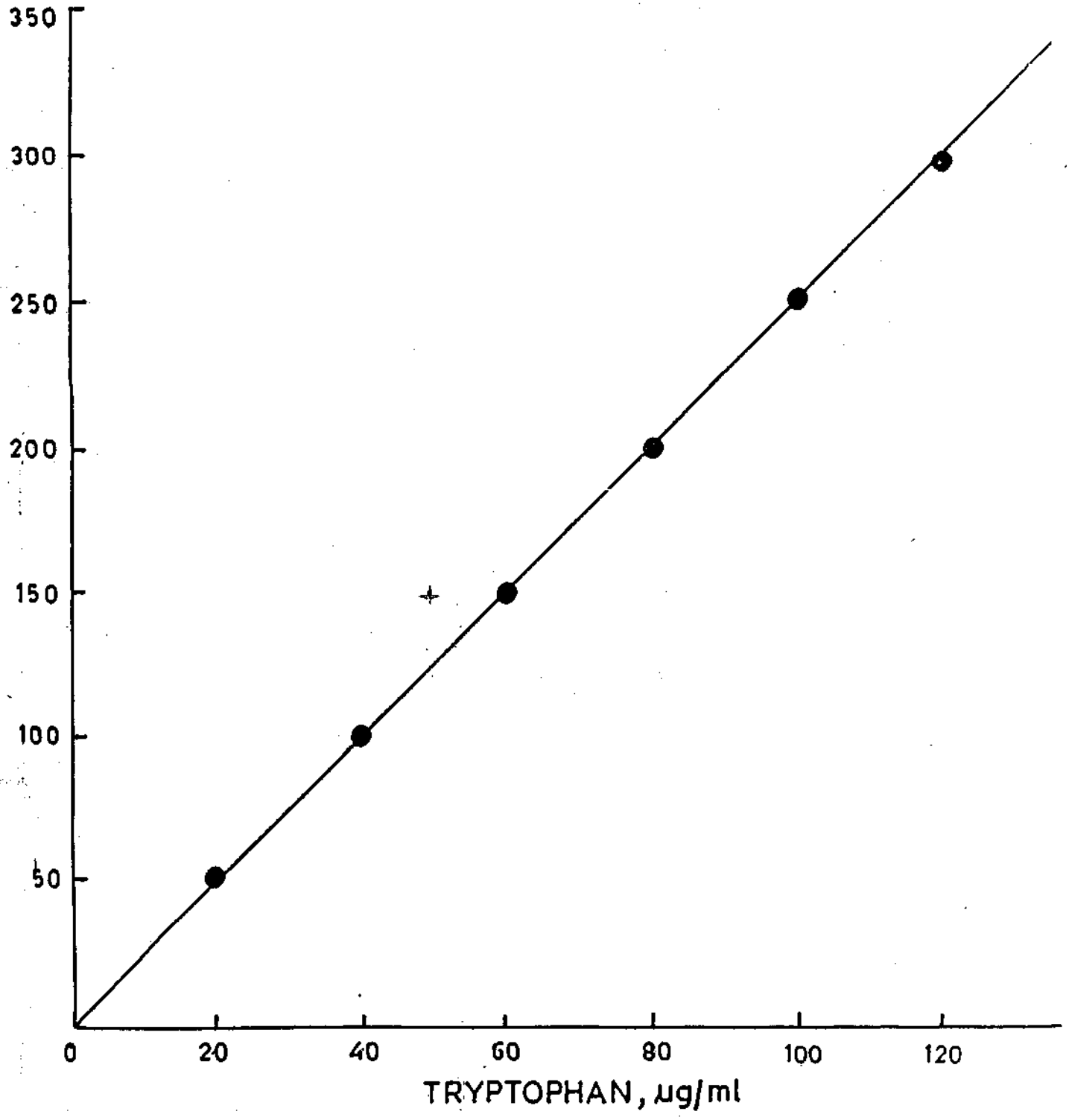
Determination of Tryptophan

The tryptophan content was determined chemically by the method of Spies and Chamber (1949).

To 50 to 100 mg of defatted material was added 10 ml of H_2SO_4 (19 N) and 1 ml of p-dimethyl amino-benzaldehyde (30 mg/ml) of sulphuric acid reagent 1 : 18 v/v. After keeping for 12 h, the solution was filtered, if necessary,

Fig. 7

STANDARD CURVE FOR TRYTOPHAN



through glass wool and the absorbance was read at 580 mu (red filter) after adding 0.1 ml of $NaNo_2$ to each sample using Klett Summerson photoelectric colorimeter.

Determination of Whey Protein

Total whey protein content was determined by the method of Rowland (1938).

One gram of test sample was uniformly suspended in 50 ml water in a beaker and warmed to 40°C. To this acetic acid(10 %)was added. The contents were mixed. After 10 min, 1 ml of 1 N sodium acetate was added. The contents were cooled and the volume was made to 100 ml. The casein was allowed to settle and filtered through Whatman filter paper No.40. 5 ml of the filtrate was digested and the nitrogen content estimated.

Determination of Curd Tension

The curd tension was determined by the modified method of Rao et al. (1964).

5 g of test sample was weighed in a beaker and the volume was made to 50 ml. The curd tension knife was placed in it and 1 ml of 0.5 percent rennet solution (Hansen's) was added rapidly to the beaker. The milk was thoroughly mixed. The beaker was placed in a thermostatically controlled water bath at 37°C and allowed to stand for a period of 3 hrs. The pan was gradually loaded

with lead shots till the curd tension knife cut its way through the curd. The weight of the lead shorts expressed in grams, was taken as a measure of curd tension.

Available Methionine

Determination of available methionine in samples was made as described by Ford (1964) using Streptococcus zymogenes MCD 592. The sample containing about 100 mg nitrogen was suspended uniformly in 10 ml of 0.05 N HCl using Potter-Elvehjem homogenizer. The uniformly suspended samples were adjusted topH 1.8 and predigested with 2 mg of pepsin (Sigma 600 units/g protein) at 56°C with frequent shaking, for a period of three hours. Subsequently the pH was adjusted to 7.2. It was appropriately diluted to 100 ml. The enzyme digests thus obtained, were used for determination.

Preparation of Reference Standard Solution

For the assay of total methionine, a solution of 20 µg concentration of L-methionine per ml in 0.46 % (w/v) NaCl, was prepared. In case of available methionine, the solution was made in water.

Procedure

Different aliquots of the test samples were taken for the determination of total as well as available methionine in triplicate at different levels as given in

Fig. 8

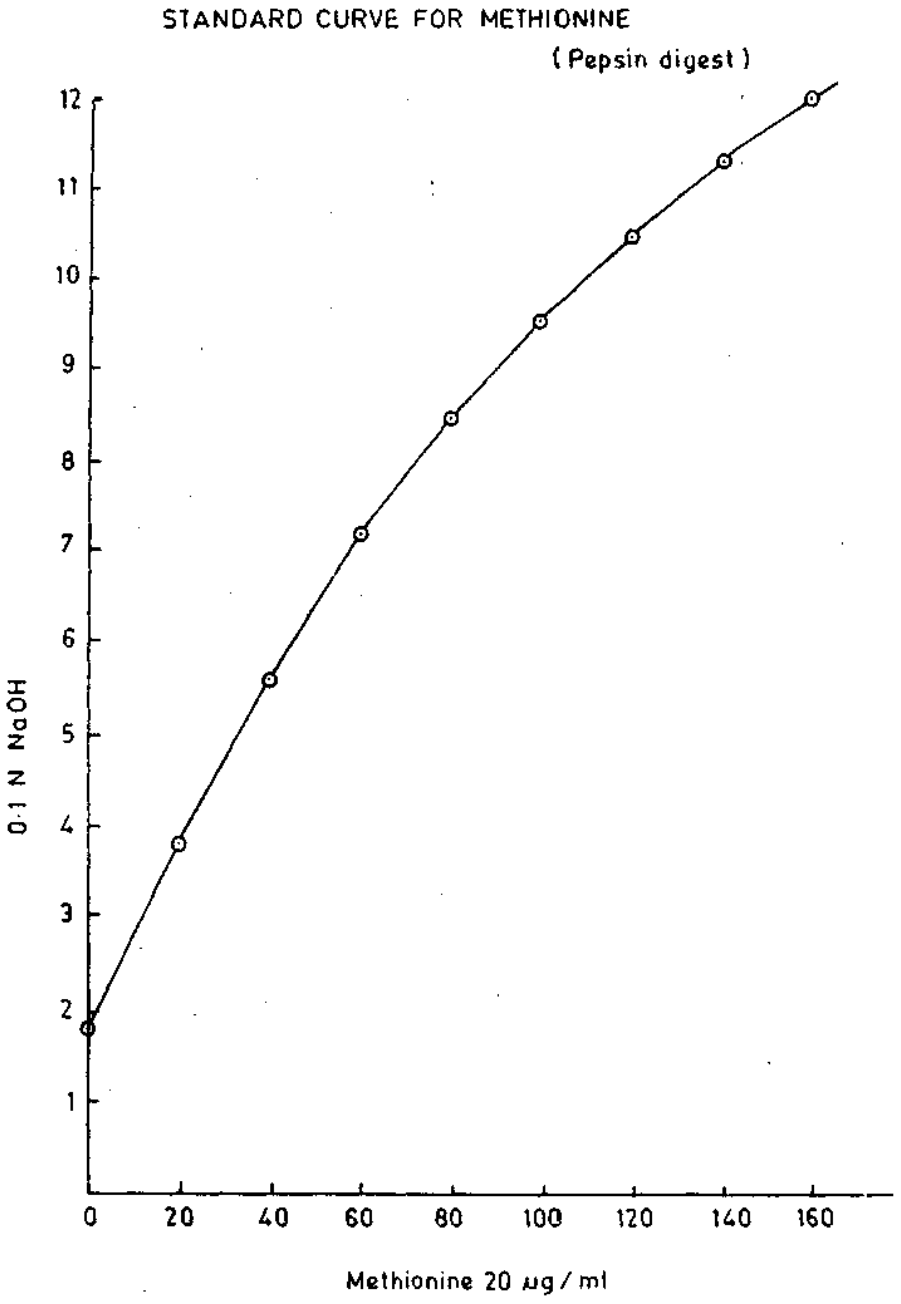

STANDARD CURVE FOR METHIONINE
(Pepsin digest)
0·1 N NaOH
12
11
10
9
8
7
6
5
4
3
2
1
0
20
40
60
80
100
120
140
160
Methionine 20 µg / ml

Table IV. The tubes were plugged and steamed for 20 minutes. On cooling, these were inoculated with the test organism Streptococcus zymogens (MCD 592) and incubated at 37°C for 48 h. The growth response was measured titrimetrically with NaOH (0.1N) using bromothymol blue as the indicator (Fig.8).

Table IV : Assay system for the estimation of total and available methionine.

Test tube No./ Reagents.	Blank	1	2	3	4	5
	ml					
Standard/Sample	-	1	2	4	6	8
Assay medium*	3	3	3	3	3	3
Distilled water	8	7	6	4	2	0
Final volume	11	11	11	11	11	11

* Appendix I.

Determination of Biological Value (BV)

Biological value was determined according to the method described by Mitchell (1923-24).

Thirty-six male albino rats weighing between 40-45 g, were taken in this experiment. These were randomly divided into six groups, with six aminals in each group and housed individually in metabolic cages made of anodized aluminium. Animals were maintained on a protain-free diet

for a period of ten days, followed by the period of ten days on experimental diets providing 10 percent protein (Table V). Animals were fed ad libitum and had free access to water. During both dietary regimes, first three days were identified as adaptation period. Urine and faeces were quantitatively collected separately and daily food intakes recorded separately. Daily food intakes recorded individually for the period of next seven days. The urine was collected over 1 N HCland 2 ml tolune in plastic bottles, while faeces were daily collected, dried in an oven and stored in glass bottles. These were analysed for nitrogen content using conventional microkjeldahl method as described earlier.

Biological value was calculated as given below :

$$\text{Biological Value (BV)} = \frac{I_n - (F_n - F_e) - (U_n - U_e)}{I_n - (F_n - F_e)} \times 100$$

where

I_n = Nitrogen intake.

F_n = Faecal nitrogen on test protein diet.

F_e = Faecal nitrogen on protein-free diet.

U_n = Urinary nitrogen on test protein diet.

U_e = Urinary nitrogen on protein-free diet.

True Digestibility (TD)

From the data on nitrogen intake and faecal nitrogen excreted, digestibility coefficient was

calculated according to Mitchell (1923-24) as follows :

$$D C = \frac{I_n - (F_{e_n} - F_e)}{I_n} \times 100$$

Determination of Net Protein Utilization (NPU)

Net protein utilization was determined using young male albino rats according to Bender and Miller (1955). Animals weighing between 55 to 60 g obtained from the Small Animal House of the Institute were randomly divided into four groups, with eight rats in each group. The average body weight of animals in each group was identical. These were housed individually in anodized aluminium cages and maintained on one of the following dietary regimes for a period of ten days during which animals were fed ad libitum and their daily food intakes were recorded.

The protein content in these diets was adjusted at 10 % level by adding starch.

At the end of the experimental period, animals were exsanguinated. Incisions were made in the skull, thoracic region and body cavities and the carcass was dried in an oven at 95°C until constant weight was attained. The dried carcass was powdered with the help of hand grinder to uniform fineness and stored in a desiccator until used for further nitrogen analysis.

The net protein utilization value was calculated using the following formula :

$$NPU = \frac{B - (BK + IK)}{I} \times 100$$

where

B = Body nitrogen of the animal fed test protein diet.

BK = Body nitrogen of the animal fed on protein-free diet.

IK = Nitrogen in the protein-free diet consumed.

I = Total N intake.

Table V : Composition of protein-free and experimental diet.

Ingredients (g)	Protein -free diet %.	Lacto-gen.	Glaxo	Amul Spray Dried	Amul roller dried	Vijaya	Lacto-dex
Infant food	-	50.0	50.0	50.0	50.0	50.0	67.0
Starch	72.0	28.0	28.0	28.0	28.0	28.0	6.0
Sucrose	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Groundnut oil	9.0	3.0	3.0	3.0	3.0	3.0	8.0
Cellulose	5.0	5.0	5.0	5.0	5.0	5.0	5.0
** Salt mixture	4.0	4.0	4.0	4.0	4.0	4.0	4.0
*** Vitamin mixture	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	100.0	100.0	100.0	100.0	100.0	100.0	100.0

** Salt mixture - NaCl 139.3 g, KI 0.69 g, KH_2PO_4 389.0g, $MgSO_4$ (anhydrous) 57.3 g, $CaCO_3$ 381.4 g, $FeSO_4$. 7 H_2O 27.0g, $MnSO_4.H_2O$ 4.01g, $ZnSO_4.7H_2O$ 0.548 g, $CuSO_4.5H_2O$ 0.477g, $CoCl_2.6\ H_2$) 0.023 g (AOAC, 1980).

*** Vitamin mixture mg/100 g of ration - Vitamin A(dry,stabilized) 2000 IU, Vitamin D (dry,stabilized) 200 IU, Vitamin E(dry, stabilized) 10 IU, menadione 0.5, choline 200, P-aminobenzoic acid 10, inositol 10, niacin 4, Ca-Pantothenate 4, riboflavin 0.8, thiamin HCl 0.5, Pyridoxine HCl 0.5, folic acid 0.2, biotin 0.04, vitamin B_{12} 0.003 and glucose to make 1000. (AOAC, 1980).

Statistical Analysis

The statistical analysis of the d ata was carried out according to Snedeer and Cochron (1967).

The analysis of variation in a completely randomized block design was adopted to analyse the variation between batches of infant foods and also six varieties chosen in this study. Two way classification of analysis of variance was used to study the significance of difference between various characteristics of all the food nutrients. Analysis of variance tables were prepared and the contents of different components of six popular Indian infant foods were tested for statistical significance by 'F' test.

Whenever 'F' values were found significant, the critical difference test was applied to identify the significantly superior infant food with respect to the respective character analysed.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

This study was carried out to assess the nutritional quality of certain popular brands of infant milk food formulae marketted in the country. Though such preparations appeared comparable in respect of gross nutrient contents, as evident from the declared contents on the containers, several factors such as differences in procedures for milk processing their micronutrient content, in particular, could profoundly influence the net nutritional potential of these preparations. Six brands of infant food formulae were chosen for this study depending on their popularity as well as the processes adopted in their preparation. These were evaluated in respect of their (i) proximate composition; (ii) mineral content; (iii) protein and fat quality; and (iv) the nature of carbohydrates used. Data obtained on these aspects are presented in this section.

Proximate Composition

The proximate composition of various infant foods is given in Table 1.

It was seen from the data that except for Lactodex, all preparations showed very close compositional similarity. This was evident from the crude protein

content showing very narrow variation, between 21.50 ± 0.14 g and 21.86 ± 0.09 g; total carbohydrate content between 52.03 ±0.28 g and 52.18 ±0.26 g; fat content between 18.25 ±0.12 g and 18.5 ±0.16 g; total ash content between 3.81 ±0.12 g and 4.26 ±0.14 g; moisture content between 2.33 ±0.16 g and 2.41 ±0.12 g; vitamin A content between 2125 ±11 IU and 2365 ±62 IU per 100 g.

In the case of Lactodex, level of protein, fat, total ash and Vitamin A were significantly lower than those present in other preparations ($P < 0.01$) (Tables 2,3,4,5,6). On the other hand, total carbohydrate content was distinctly higher, accounting for about 72 % of the total contents in the formula. Such similarity was understandable in thelight of the fact that these preparations, except Glaxo, conformed ISI specifications which require any infant food formula bearing ISI mark, to contain maximum moisture 4.5 %, minimum protein 20 %, fat content between 18 - 28 %, minimum carbohydrate 35 %, ash 8.5 %, minimum vitamin A 1500 IU, vitamin D 400 IU and minimum iron 4 mg %. When compared with the proximate composition declared on containers, values obtained on actual analysis showed variation lesser than 2.5 % and could be accounted as experimental variations.

A comparison of data obtained in this study with other Indian preparations as well as those in other countries, however, revealed significant differences (Table 7). Values as high as 27 % in the case of an

Table 1 : Proximate composition of some popular Indian infant foods.

Product	Moisture	Protein	Fat	Carbohydrate	Total Ash	Vitamin A I.U./100 g
	g/100 g					
Lactogen	2.40 ± 0.12	21.50 ± 0.14	18.50 ± 0.16	52.18 ± 0.26	3.81 ±0.12	2125 ±11
Amul spray	2.36 ± 0.14	21.51 ± 0.13	18.50 ± 0.16	52.18 ± 0.26	4.25 ±0.13	2365 ±62
Vijaya spray	2.41 ± 0.12	21.86 ± 0.09	18.25 ± 0.12	52.18 ± 0.26	4.26 ±0.14	2234 ±37
Lactodex	2.33 ± 0.12	14.39 ± 1.90	5.76 ± 0.09	71.87 ± 0.37	2.64 ±0.13	1959 ±37
* Amul roller dried	2.33 ± 0.16	21.59 ±0.11	18.50 ± 0.16	52.03 ± 0.28	4.21 ±0.12	2328 ±12
* Glaxo	2.35 ± 0.14	21.59 ± 0.09	18.25 ± 0.16	52.18 ± 0.26	4.23 ±0.15	2234 ±37

Mean of six observations ±S.E.

* Roller dried products.

Table 2 : Analysis of variance for protein content.

Source	d.f.	S.S.	M.S.S.	F
Batch	1	0.042	0.042	0.332^{NS}
Treatment	5	349.61	69.92	553.24**
Batch error	5	0.082	0.0164	0.129
Sampling error	36	4.55	0.126	

NS Not significant
S.E. ±0.177
** Significant at 1 % level.
C.D. 0.3613

Table 3 : Analysis of variance for fat contents.

Source	d.f.	S.S.	M.S.S.	F
Batch	1	0.188	0.188	1.177^{NS}
Treatment	5	1056.105	211.22	1322.42**
Batch error	5	1.437	0.2874	1.79
Sampling error	36	5.75	0.159	

NS Not significant
S.E. ±0.199
** Significant at 1 % level.
C.D. 0.406

Table 4 : Analysis of variance for total carbohydrate content.

Source	d.f.	S.S.	M.S.S.	F
Batch	1	0.15	0.15	0.259^{NS}
Treatment	5	2543.29	508.658	850.79**
Batch error	5	0.32	0.064	0.110
Sampling error	36	20.79	0.577	

NS Not significant
S.E. 0.3799
** Significant at 1 % level.
C.D. 0.772

Table 5 : Analysis of variance for ash.

Source	d.f.	S.S.	M.S.S.	F
Batch	1	0.003	0.0036	0.3238^{NS}
Treatment	5	12.31	2.46	220.38**
Batch error	5	0.094	0.018	1.68
Sampling error	24	0.26	0.011	

NS Not significant.
S.E. +0.0528
** Significant at 1 % level.
C.D. 0.1091

Table 6 : Analysis of variance for vitamin A.

Source	d.f.	S.S.	M.S.S.
Batch	1	1333.29	1333.29**
Treatment	5	865468.75	173093.75NS
Batch error	5	13541.71	2708.342
Sampling error	36	23436.79	

** Significant at 1 % level.
NS Not significant
S.E. 5.05
C.D. 13.43

Table 7 : Proximate Composition of Indian and Foreign Infant Foods as Given on Labels.

Product	Moisture	Protein	Fat	Carbohydrate	Total ash	Calorie K Cal/100 g
	g/100 g					
INDIAN						
Amul spray*	4.0	24.96	18.12	44.93	1.87	442
Indec*	4.12	24.39	18.09	44.09	1.73	436
Lever baby food**	-	27.0	19.0	48.0	-	471
FROM OTHER COUNTRIES						
Oster milk(UK)***	-	16.8	19.2	64.0	-	496
Similac (UK) ***	-	23.3	22.7	54.0	-	513.6
Cow and Gate(UK)***	-	22.7	24.2	63.0	-	471.4
Portagen (USA)****	-	17.25	24.0	57.75	-	502
Probana(USA)****	-	31.5	16.5	59.25	-	502
Enfamil(USA)****	-	11.26	27.0	52.5	-	502
Present study	2.3-2.4	14.39-21.86	5.76-18.25	52.18-71.87	2.23-4.26	400-450

* Bindra and Deodhar (1980)
** Udani et al. (1970)
*** Widdowson (1973)
**** John and Lamy (1975).

Indian preparation, lever baby food (Udani, et al. 1970) and as low as 11.26 % in the case of Enfamil (USA) were found in respect of protein content. Fat contents, however, were fairly comparable in most of these preparations. In the case of a British preparation, Cow and Gate, it was 24.2 % and 27.0 % in the case of Enfamil (USA). As regards total carbohydrate contents, there was a wide variation, with values as low as 44.0 % in the case of Amul spray (Bindra and Deodhar, 1980) and as high as 64.0 % in the case of Oster milk (UK).

When nutrient contents in these preparations were expressed on the basis of their respective calorie content, it was found that the protein content ranged between 1.78 g and 4.76 g per 100 K Cal. However, fat content showed some what larger variation, between 1.45 g for Lactodex to 4.04 g per 100 K Cal in the case of other preparations and contributed 37 % of the total calories. A comparison with the specifications specified by Codex Alimentarius Commission (1976; Appendix II) showed that in respect of proteins, the contents were markedly higher. Except in the case of Lactodex, the protein contents per 100 K Cal, were well above the maximum limit of 4 g/100 K Cal, notwithstanding the fact that proteins in all the cases could be considered as good quality proteins, being derived entirely from milk. As regards vitamin A contents, these were close to the maximum limit of 500 I.U./100 K Cal in the case of almost

all preparations with both Amul preparations slightly exceeding this limit.

The moisture was in the range of 2.33 g and 2.41 g per 100 g of various preparations and was within the range specified by I.S.I. (1968).

Likewise, the total ash content was in the range of 2.64 g and 4.26 g per 100 g of various preparations which was well below the limit desired by I.S.I. (1968).

Vitamin A content was in the range of 1959 I.U. and 2265 I.U. per 100 g, and was higher than 1500 I.U. reported by Udani et al. (1970) for Lever baby food. However, these contents were much lower than 3000 I.U./100 g in Oster milk (U.K.), Cow and Gate (U.K.) and Similac (U.K.) reported by Widdowson (1973).

The pressing need of vitamin A in infant nutrition, specially in this region needs to be taken into account while assessing infant foods. This becomes imperative in the light of wide prevalence of vitamin A deficiency among pre-school children (Gopalan and Roghavan, 1961). When vitamin A content in the daily prescribed intakes of various formulae were considered, these were found to range between 2005 I.U. to 5676 I.U. at the sixth month of age. These intakes more than fulfilled vitamin A needs of infants upto first six months.

While judging the adequacy of these preparations to meet the nutritional need of pre-term infants, factors influencing the nutritional needs of the infants in this category, in order to meet the extra stress in adjusting in the environment outside the maternal body. The requirements for calories in the case of pre-term infants were reported to be 140 K Cal/kg/day as against about 120 K Cal in the case of full term infant (Shaw, 1973; Fomon, 1974). With the assumption of 2 kg body weight, it would appear that even at the recommended level of serving, these infants foods adequately fulfil calorie requirements of the body. Though at this level, protein requirements are reasonably fulfilled and do not exceed the protein requirement alarmingly high as in the case of full term infant (Fomon, 1974).

The pre-term infants requirements are, by and large, the same as the full term. Vitamin A content of the infant foods under study when computed on the basis of 140 K Cal/kg/day were found to fulfil the requirements for this vitamin.

Adequacy in Terms of Infant Requirements

A comparative account of the nutrients available per day from each preparation calculated on the basis of allowances declared on the container, together with the recommended dietary allowance by Indian Council of Medical Research (I.C.M.R.) is presented in Table 8.(Illustration II)

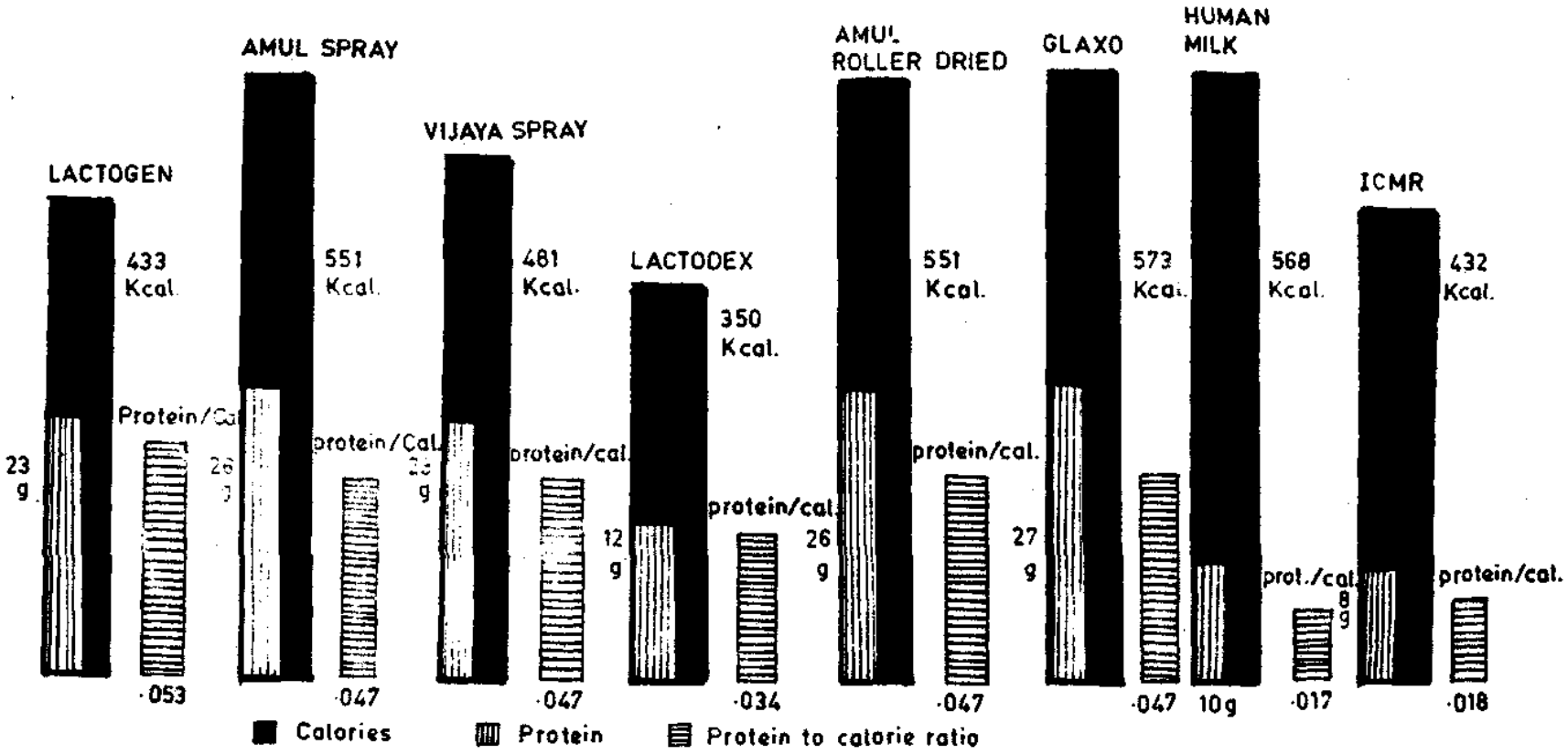
Table 8 : Daily intake of protein and calories during first six months from different infant foods.**

Product		1st month	2nd month	3rd month	4th month	5th month	6th month
Lactogen	Protein(g)	22.95	21.86	27.32	32.76	35.44	38.25
	Calorie(KCal)	433.7	516.37	619.65	687.49	742.39	802.07
Amul spray	Protein(G)	25.81	32.26	38.71	45.17	48.39	51.62
	Calorie(KCal)	551.0	688.75	826.5	964.25	1033.14	1102.0
Vijaya spray	Protein(g)	22.95	21.86	27.32	32.76	35.41	38.25
	Calorie(KCal)	481.24	454.33	572.91	687.49	742.49	802.07
Lactodex	Protein(g)	10-15	10-15	10-15	15-25	15-25	25-31
	Calorie(KCal)	285-427	285-427	285-427	427-712	427-712	712-855
* Amul roller dried	Protein(g)	25.84	32.26	38.71	45.17	48.39	51.62
	Calorie(KCal)	551.0	688.75	826.5	964.25	1033.13	1102.0
* Glaxo	Protein(g)	26.98	26.98	32.38	37.7	40.37	43.25
	Calorie(KCal)	573.96	573.96	688.75	803.54	858.64	918.35
Human milk 800 ml***	Protein(g)	9.6	9.6	9.6	9.6	9.6	9.6
	Calorie(KCal)	568.0	568.0	568.0	568.0	568.0	568.0
ICMR recommended allowances.	Protein(g)	8.25	9.89	11.96	13.59	14.72	15.64
	Calorie(KCal)	432.0	516.0	624.0	708.0	768.0	816.0

* Roller dried products.
** Reconstituted based on the instructions on tins.
*** Macy and Kelly (1961).

ILLUSTRATION II

Calorie and Protein intake in the 1st month (infant)



It was observed that whereas the calorie requirements of infants upto 6 months could be fairly met, the protein available from almost all the preparations was far more than 2,5 fold of the recommended allowance. Such, some what redundant supply of proteins may have deleterious effect in the long run. It has been established that excessive intake of proteins, particularly at the cost of calories, leads to wasteful utilisation of proteins towards meeting calorie requirements (Gopalan et al. (1981). Besides this, such excessive intake of nitrogenous compounds may possibly burden the premature kidneys in the early stage of life (Taitz, 1978). Furthermore, the cited recommended allowances have been calculated on the basis of body weights (Ghosh, 1977) which are much above the average body weight of the average Indian infants. This would further add to the already exaggerated supply of proteins in relation to actual body requirements, when compared with the amount of proteins available from 800 ml breast milk (considered to be adequate to fulfil the nutritional needs of infant upto 6 months). It would be evident that the recommendations made on the containers warrants a critical review and appropriate revision.

Protein

Dietary proteins have a primary role in supporting growth in the living organism, the phenomenon predominant in the early stage of life. This ability is, by and large, determined by their potential in providing essential amino acids in appropriate proportions which, in turn, determine their nutritional quality.

The proximate composition of infant food formulae presented in the earlier section, serves only limited purpose. It was found that when administered as liquid formula, the nutrient contents were fairly identical and the level of certain nutrients, especially protein, was markedly higher than protein in the human milk. It was, however, not clear whether the differences in the processing of milk as well as in the nature of carbohydrates affects the nutritional quality of proteins or fats. Although feeding trials on human babies would have been ideal, certain ethical considerations preclude detailed well controlled studies on certain aspects of protein utilization. Similar views have also been expressed earlier by different workers who suggested the use of either rats (Fomon, 1974; Hamosh, 1979) or new born piglets (Gurr, 1981). Hence in this study, growing albino rats were used for the evaluation pf protein quality and fat absorption according to conventional methods described by Miller and Lachance (1975) and Tomarelli et al. (1968). Data are presented in this section.

Heat processing often leads to decrease in the protein quality, especially through the loss of lysine duing Maillard reaction (Fomon and Owen, 1962b; Heyns, 1965). In the case of milk proteins, other processing treatment such as homogenization, has also been reported to influence digestibility as well as amino acid availability although the overall amino acid profile of milk proteins are rated to be good (Mehta, 1981). Besides this, proteins from human milk have been shown to have better biological value than milk proteins from other species(Kon and Mawson, 1950). In this study, products were chosen on the basis of processes, viz. roller drying or spray drying used to obtain the milk since these differences could profoundly influence the protein quality (Mauron et al., 1955; Narayana Rao et al. 1963).

Experiments were conducted to study the amino acid profiles of proteins in different preparations, curd tension as the indicator of protein digestibility and biological evaluation of proteins in these foods to see, if they reveal any deleterious effect.

Casein to Whey Protein Ratio & Curd Tension

In the present study, the ratio of casein to whey proteins was also determined in view of its known influence on the curd tension and, in turn, implied digestibility. Besides this, it was shown by several workers that such ratio profoundly influenced the biological value of the preparation (Delaney, 1976; Singh, 1983). This appeared

possible in the light of known superiority of whey proteins over casein in respect of their sulfur amino acid contents (Forsum and Hambreus, 1972). These would partly help in overcoming the limitation of casein in respect of these amino acids. Whey protein content was determined by precipitation of casein and determining protein content in the whey fraction. Data are given in Table 9. It was seen that casein contents in all these preparations were about 16 g/100 g, except lactodex which had 11 ± 0.06 g/100 g (Table 11) ($P<0.05$). However, these values accounted for about 78 % of the total protein content in these preparations. The non-casein nitrogen fraction was not further differentiated into protein and non-protein fractions since the non-protein nitrogen was reported to comprise only small fraction of total nitrogen in milk (Ganguly, 1964). The whey protein contents ranged between 4.8 ± 0.02 to 5.34 ± 0. 12/100 g in various preparations, except Lactodex. The ratio of casein : whey proteins in these preparations, was found to be above 3, with the maximum ratio observed 3.47 for Lactogen. This ratio closely resembled to that for cow or buffalo milk, rather than human milk.

Such higher ratio warrants further efforts to lower it so as to match the human milk. In this context, Raiha et.al. (1976) showed that infant formulae with casein as the predominant fraction lead to metabolic acidosis, with markedly higher blood urea level among infants maintained on such formulae. This will undoubtedly contribute towards

Table 9 : Casein to whey protein ratio of Indian infant foods.

Product	Casein (g/100g)	Non-casein protein (g/100g)	Casein/ Non-casein (g/100g)
Lactogen	16.70 ±0.001	4.80 ±0.028	3.47 ±0.028
Amul spray	16.26 ±0.001	5.25 ±0.113	3.09 ±0.001
Vijaya spray	16.61 ±0.070	5.25 ±0.059	3.16 ±0.020
Lactodex	11.00 ±0.060	3.39 ±0.067	3.24 ±0.001
* Amul roller dried	16.25 ±0.050	5.34 ±0.120	3.04 ±0.040
* Glaxo	16.33 ±0.010	5.26 ±0.068	3.10 ±0.020

* Roller dried products.

Mean of six observations ±S.E.

Table 10 : Analysis of variance for whey protein.

Source	d.f.	SS	MSS	F
Batch	1	0.01	0.01	0.01^{NS}
Treatment	5	23.075	4.615	53.79*
Batch error	5	0.033	0.0067	0.079
Sampling error	36	3.088	0.0857	

NS - Not significant

* Significant at 5 % level

SE - 0.146

CD - 0.296

renal solute load. According to Edelman et al.(1960), consumption of such formulae caused more thirst and further enhanced the demand for more water intake. It would thus appear that such formulae are unlike human milk in respect of biological value. To overcome the possible hazards of excessive renal load, it may become essential to ensure more water intake than recommended dilutions. Although this ratio has been reported by Schultz and Ashworth,(1974) to influence the curd tension, data obtained in this study did not support such inference in this regard. Actual curd tensions were, therefore, determined for these preparations and data are given in Table **11**.

The curd tension in different formulae were determined by using feed reconstituted according to given instructions. It was observed that these were markedly low ranging between 2 in the case of Lactodex to 6 in the case of Amul spray and Vijaya spray. Though these preparations were based on either cow or buffalo milk, the curd tension was decisively lower for these preparations than those observed for cow and buffalo milk. It should be noted, however, that these values were higher than the curd tension of zero reported for human milk (Abu Dawood and El Sawaf, 1977). Similar low curd tensions, 3.5 and 5.5 for roller dried and spray dried products, were reported earlier by Swaminathan and Parpia (1968). In a study reported by Abu Dawood and El Sawaf(1977) for several European preparations, SMA, Eledon, Similar, Babiron, curd tensions were almost zero and idential to human milk. Such

Table 11: Curd tension values of some Indian infant foods under study.

Human Milk	Cow Milk	Buffalo Milk	Lactogen	Amul Spray	Vijaya Spray	Lactodex	Amul* Roller Dried	Glaxo*
0.0	23.0	44.0	4.0	6.0	6.0	2.0	5.0	5.0

* Roller dried products.

Average of six observations.

lowering of curd tension when compared with untreated cow and buffalo milk could be possible because of several processing treatments, the milk undergoes before it is converted into infant foods. These treatments include heating, homogenisation as well as inclusion of additives such as sodium bicarbonate. All these have been reported to lower the curd tension of milk (Abu Dawood and El Sawaf, 1977). The calcium content of these milks was between 100 mg/100 ml and 214 mg/100 ml in comparison to 117 mg/100 ml in the case of cow milk. The calcium content, too, influenced the curd tension as reported by Dean and Welsch (1934) and Abu Dawood and El Sawaf (1977).

Amino Acid Composition

Nutritional quality of protein depends on certain factors such as essential amino acid contents, digestibility as well as biological availability of certain essential amino acids, notably those which are limiting in the diets of populations. Processing, particularly heat processing of milk, has been shown to influence the protein quality, since conditions adopted for drying milk preparation during roller and spray drying markedly differ in respect of temperature. Drying conditions on rollers were more severe than in spray chamber. It is possible that there was less available lysine in roller dried than in spray dried powder (McDonald, 1966).

Data on amino acid composition of proteins in various formulae expressed as g/16g N, are presented in Table 12.

Table 12 : Amino acid composition of some Indian infant foods.

Amino acid	Lacto-gen (a)	Amul spray (b)	Vijaya spray (c)	Lacto-dex (d)	Amul roller dried (e)	Glaxo (f)
Lysine	8.00	8.40	8.72	7.68	7.46	6.56
Histidine	2.70	2.46	2.36	3.24	2.80	2.44
Arginine	3.64	3.04	4.96	5.00	4.68	4.50
Threonine	5.18	5.74	5.24	4.44	5.78	5.08
Valine	3.06	4.12	3.60	4.66	3.56	3.30
* Methionine	2.46	2.42	2.24	1.96	2.20	2.21
Isoleucine	4.00	4.04	2.74	4.24	3.98	3.26
Leucine	8.50	8.66	8.00	9.80	8.32	9.46
Tyrosine	4.28	3.74	4.80	3.75	3.34	3.50
Phenylalanine	5.06	5.84	5.28	5.60	3.80	4.30
**Tryptophan	1.45	1.45	1.45	1.33	1.45	1.45
Aspartic acid	8.60	7.20	6.06	6.30	6.52	6.50
Serine	4.34	5.02	5.38	6.14	4.18	4.42
Glutamic acid	16.48	16.14	16.70	23.0	17.12	20.20
Proline	4.94	5.38	5.52	6.14	4.50	5.60
Glycine	1.68	1.50	1.70	2.00	1.64	1.28
Alanine	3.02	3.10	2.60	3.02	2.56	2.44

Expressed as g/16gN

a,b,c,d - Spray dried products

e,f - Roller dried products

* Estimated microbiologically by the method of Ford (1964).

** Estimated chemically by the method of Spies and Chamber (1949).

The range for various essential amino acids, expressed as g/16g N, was found to be as follows : lysine 6.56 (Glaxo) to 8.72 (Vijaya spray), histidine 2.36 (Vijaya spray) to 3.24 (Lactodex), arginine 3.0 (Amul spray) to 5.0 (Lactodex), threonine 4.44 (Lactodex) to 5.78 (Amul roller dried), valine 3.0 (Lactogen) to 4.66 (Lactodex), methionine 2.02 (Lactogen) to 3.20 (Vijaya spray), isoleucine 2.74 (Vijaya spray) to 4.24 (Lactodex), tyrosine 3.34 (Amul roller dried) to 4.80 (Vijaya spray), phenylalanine 3.8 (Amul roller dried) to 5.84 (Amul spray), tryptophan 1.33 (Lactodex) to 1.45 (rest other preparations). By and large, the amino acid contents were identical.

As regards contents of non-essential amino acids, there were small to moderate variations among different preparations.

In these experiments infant food formulae were chosen on the basis of difference in the processes adopted during milk powder preparations viz, spray drying and roller drying. Such differences in respect of temperature and duration of heat treatments were reported earlier to influence the level of available lysine as well as methionine in these treated milk products (Mauron et al., 1955, Naragyana Rao et al., 1963, Cheftel, 1979). A comparison of amino acid profile in spray dried (Table 12 columns a, b, c, d) and roller dried (Table 12, columns e, f) showed

that, in general, the level of total lysine content was about 10 % lower in roller dried preparations. Similarly, considering phenylalanine and tyrosine together, were found to be lower in roller dried preparations. However, there was no marked difference in respect of other amino acids.

The amino acid profile observed in the present study was compared with that for cow and buffalo milk proteins (Table 13). It was seen that except for valine, isoleucine and leucine, content of all other essential amino acids were fairly comparable. The concentration of these three amino acids were markedly low in the infant foods. Though little is known about the destruction of these amino acids during processing, observations made in this study suggested such possibility and warrant further investigation.

The decrease in valine content could be significant as it was shown to be second limiting amino acid at least in the case of human milk proteins (Bodwell, 1967).

As mentioned earlier, levels of available lysine and methionine have been shown to decrease as a result of heat processing during powder making. The total content of these amino acids would not give true picture of the amino acid availability from these foods. Attempt was made to determine the levels of available methionine.

Table 13 : Comparison of amino acid composition of protein in infant foods, cow and buffalo milk.

Amino acid	Infant foods	Cow milk	Buffalo milk*
Lysine	7.80 (6.56-8.72)	7.67	7.67
Histidine	2.66 (2.36-3.24)	2.66	2.03
Arginine	4.30 (3.04-5.00)	3.44	3.13
Threonine	5.24 (4.44-5.78)	4.38	4.70
Valine	3.29 (3.06-4.66)	6.26	5.95
Methionine	2.28 (2.02-3.20)	2.56	2.66
Isoleucine	3.74 (2.74-4.24)	5.32	5.17
Leucine	8.78 (8.00-9.80)	9.40	10.02
Tyrosine	3.87 (3.34-4.80)	4.70	-
Phenylanine	4.97 (3.80-5.84)	5.01	4.23
Tryptophan	1.45 (1.33-1.45)	1.47	1.41

Expressed as g/16g N.

* I.C.M.R. (1981).

Values in parentheses are lowest and values.

Available Methionine Content

In this study only the level of available methionine was determined since it is the limiting amino acid for milk proteins. Although reports available showed moderate losses of available lysine during Maillard reaction. This would not lower the levels of lysine derived from these infant foods even upto 30 % loss. The quantity of lysine still available far exceeds the lysine requirements of the infant upto 6 months.

The total content and the availability of methionine was estimated using microbiological method of Ford (1964). The values are given in Table 14. It was found that in the case of Lactogen the availability was 69.92 %, Amul spray 78.1 %, Vijaya spray 79.47 %, Lactodex 81.2 %, Amul roller dried 66.4 % and Glaxo 69.7 %. The amount of available methionine was 9 % less in roller dried than spray dried infant foods.

Estimated Daily Intake of Amino Acids

Based on the prescribed daily consumption of infant foods, estimated intake of different amino acids from these were computed using data on amino acid contents (Table 12) and compared in the light of recommended amino acid intakes (Table 15). During these computations, the body weights at Ist and 6th month of life were assumed to be 3.6 kg and 3.8 kg, respectively, as declared on the containers.

Table 14 : Total and available methionine content of some Indian infant foods.

Infant food	Total methionine	Available methionine	% loss of methionine
	g/100g protein		
Lactogen	2.46 ±0.01	1.72 ±0.01	30.08
Amul spray	2.42 ±0.03	1.89 ±0.001	21.90
Vijaya spray	2.24 ±0.14	1.78 ±0.02	20.53
Lactodex	1.96 ±0.10	1.59 ±0.08	18.80
* Amul roller dried	2.20 ±0.01	1.46 ±0.11	33.60
* Glaxo	2.21 ±0.01	1.54 ±0.08	30.30

* Roller dried products.

Mean of six observations ±S.E.

Table 14-A : Analysis of variance for methionine.

Source	d.f.	S.S.	M.S.S.	F
Batch	1	0.03	0.0300	37^{NS}
Treatment	5	0.62	0.1220	152*
Batch error	5	0.12	0.0240	30
Sampling error	12	0.03	0.0008	

NS Not significant
* Significant at 5 % level
SE 0.022
CD 0.044

From the comparison presented in Table 15, it was observed that for the Ist month, intakes of various amino acids from different preparations ranged as follows : lysine 1047 mg (Lactodex) and 22.17 mg (Amul spray), histidine 460 mg (Lactodex) and 694 mg (Amul roller dried), arginine 710 mg (Lactodex) and 1188 mg (Amul roller dried), threonine 624 mg (Lactodex) and 1515 mg (Amul spray), valine 633 mg (Lactogen) and 1087 mg (Amul spray), methionine 418 mg (Lactogen) and 617 mg (Amul roller dried), isoleucine 602 mg (Lactodex) and 1066 mg (Amul spray), leucine 1391 mg (Lactodex) and 2497 mg (Amul roller dried), tyrosine 820 mg (Lactodex) and 1108 mg (Vijaya spray), phenylalanine 795 mg (Lactodex) and 1541 mg (Amul spray), tryptophan 205 mg (Lactodex) and 382 (Amul spray and roller dried).

Similarly in the 6th month, the variation in the intakes was as follows : lysine 2094 mg (Lactodex) and 4432 (Amul spray), histidine 848 mg (Lactogen) and 1296 mg (Amul spray), arginine 1144 mg (Lactogen) and 2376 mg (Amul roller dried), threonine 1248 mg (Lactodex) and 3030 mg (Amul spray), valine 962 mg (Lactogen) and 2174 mg (Amul spray), methionine 635 mg (Lactogen) and 1234 mg (Amul roller dried), isoleucine 1049 mg (vijaya spray) and 2132 mg (Amul spray), leucine 2673 mg (Lactogen) and 4994 mg (Amul roller dried), tyrosine 1236 mg (Glaxo) and 1974 mg (Amul spray), phenylalanine 1222 mg (Glaxo) and 3084 mg (Amul spray), tryptophan 410 mg (Lactodex) and 764 mg (Amul spray and roller dried).

Table 15 : Estimated daily intake of essential amino acids from some Indian infant foods*

Amino acid	Lactogen		Amul spray		Vijaya spray		Lactodex		Amūl roller dried**		Glaxo**	
	Ist Month	6th Month	Ist Month	6th Month	Ist Month	6th Month	Ist Month	6th Month	Ist Month	6th Month	Ist Month	6th Month
Lysine	1656	2517	2217	4432	2014	3343	1047	2094	1731	3462	1805	2400
Histidine	558	848	649	1296	545	904	460	920	694	1288	677	900
Arginine	753	1144	802	1604	1146	1900	710	1420	1188	2376	1132	1505
Threonine	1072	1629	1515	3030	1210	2008	624	1248	1335	2670	1398	1859
Valine	633	962	1087	2174	831	1379	661	1322	871	1742	861	1145
Methionine	418	635	549	1098	466	773	317	634	617	1234	493	655
Isoleucine	828	1258	1066	2132	632	1049	602	1204	860	1720	943	1254
Leucine	1759	2673	2286	4512	1848	3067	1391	2782	2497	4994	2013	2677
Tyrosine	885	1345	987	1974	1108	1839	820	1640	950	1700	1236	1236
Phenylalanine	1047	1591	1541	3084	1219	2023	795	1590	1135	2270	1222	1222
Tryptophan	300	456	382	764	334	554	205	410	382	764	365	465

* Computed on the basis of weights given on tins. Expressed as mg/day.
** Roller dried products.

These data were further compared with the amino acid profile present in certain American preparations responsible to support the optimal growth for infants (Fomon and Filer, 1967), as well as intakes of amino acids required to support optimal growth in infants as described by Holt and Snyderman, (1967) (Table 16). Such comparison revealed substantially higher intakes of all essential amino acids in the present study. These were as high as five fold in some cases.

Even after admitting the losses for methionine and lysine (upto 20 % in view of the reported cases), the estimates amounts of these amino acids still available were over 3 fold higher in all preparations except Lactodex in which case it was nearly twice more than the recommended allowance of Holt and Snyderman (1967).

Essential Amino Acid Index (EAAI)

The essential amino acid indices were calculated according to the method of Oser (1959), on the basis of 11 essential and semi-essential amino acids and are given in Table 17. The indices were found to be as follows : Amul spray 76.6, Vijaya spray 77.8, Lactodex 77.2, Amul roller dried 70.0 and Glaxo 75.6.

The values for roller dried preparations were slightly lower than those obtained for spray dried preparations. This suggested that there was only slight difference in the protein quality.

Table 16 : Comparison of estimated amino acids intakes required to support optimal growth in infants.

Amino acid	Present study (mg/day)		Holt and Synderman(1967) (mg/day)		Fomon and Filer (1967) (mg/day)		Human milk (mg/day)
	Ist Month	6th Month	Ist Month	6th Month	Ist Month	6th Month	
Lysine	2045	3041	370	700	579	1094	631
Histidine	701	1025	122	231	100	190	255
Arginine	1143	1657	-	-	-	-	375
Threonine	1425	2074	313	591	417	998	435
Valine	967	1454	378	682	334	632	465
Methionine	499	883	162	304	208	394	149
Isoleucine	821	1436	428	309	252	476	496
Leucine	1965	3450	824	1559	579	1094	781
Tyrosine	946	1622	-	-	-	-	345
Phenylalanine	1109	1963	324	612	450	850	345
Tryptophan	3250	568	79	149	61	115	165

Table 17 : Essential amino acid index (EAAI) of some popular Indian infant foods*

Amino acid	Amino acid in egg protein	Lactogen			Amul spray			Vijaya spray		
		Amino acid in infant food (mg/g)	Egg ratio	Log of egg ratio**	Amino acid in infant food (mg/g)	Egg ratio	Log of egg ratio**	Amino acid in infant food (mg/g)	Egg ratio	Log of egg ratio**
	b	a			a			a		
Lysine	450	500	1.1111	2.0000	525	1.1666	2.0000	545	1.2111	2.0000
Histidine	131	168	1.2824	2.0000	153	1.1673	2.0000	147	1.1221	2.0000
Arginine	400	227	0.5678	1.7536	190	0.4750	1.6767	310	0.7760	1.8893
Threonine	306	323	1.0555	2.0000	358	1.0555	2.0000	329	1.0686	2.0000
Valine	456	191	0.4188	1.6212	257	0.5635	1.7581	225	0.4934	1.6928
Methionine	203	126	0.6428	1.8075	130	0.6632	1.8216	126	0.6428	1.8102
Isoleucine	500	250	0.5000	1.6990	252	0.5040	1.7024	171	0.3420	1.5340
Leucine	575	531	0.9234	1.9652	541	0.9408	1.9731	500	0.8695	1.9390
Tyrosine	325	267	0.9962	1.9983	233	0.8961	1.9390	300	1.2875	2.0000
Pheynlalanine	350	316	0.8753	1.9420	365	1.0110	2.0000	330	0.9144	1.9609
Tryptophan	93	90	0.8737	1.9410	90	0.8737	1.9410	90	0.8737	1.9410
EAAI $\frac{a}{b}$			$\leq 20.7278/11$ = 1.8843 antilog =76.60			$\leq 20.8118/11$ = 1.8910 antilog =77.80			$\leq 20.7672/11$ = 1.8879 antilog =77.20	

* Oser (1959)
** Ratio above one is taken as 2.

Table 17 Contd.

Amino acid	Lactodex			Amul roller dried***			Glaxo***		
	Amino acid in infant food (mg/g)	Egg ratio	Log of egg ratio	Amino acid in infant food (mg/g)	Egg ratio	Log of egg ratio	Amino acid in infant food (mg/g)	Egg ratio	Log of egg ratio
	a			a			a		
Lysine	430	1.0666	2.0000	466	1.0355	2.0000	410	0.9111	1.9595
Histidine	202	1.5419	2.0000	175	1.3350	2.0000	152	1.1603	2.0000
Arginine	312	0.7800	1.8921	292	0.7300	1.8633	281	0.7026	1.8463
Threonine	277	0.9052	1.9566	361	1.1797	2.0000	317	1.0359	2.0000
Valine	291	0.6331	1.8048	222	0.4868	1.6866	206	0.4517	1.6542
Methionine	120	0.5911	1.7716	127	0.6479	1.8109	146	0.7448	1.8716
Isoleucine	265	0.5300	1.7243	248	0.4960	1.6955	203	0.4060	1.6085
Leucine	612	1.0643	2.0000	248	0.4313	1.6345	591	1.0278	2.0000
Tyrosine	236	1.0128	1.9445	208	0.8927	1.8890	218	0.9110	1.9101
Phenylalanine	350	0.9695	1.9863	237	0.6565	1.8169	268	0.7423	1.8704
Tryptophan	83	0.8050	1.9058	90	0.8737	1.9140	90	0.8737	1.9410
EAAI $\frac{a}{b}$			$\leq$20.9860/11 = 1.9078 antilog =80.9			$\leq$20.3107/11 = 1.8464 antilog =70.0			$\leq$20.6616/11 = 1.8784 antilog =75.6

*** Roller dried products.

These values when compared with the reported values for cow milk 88, casein 88, human milk 87,were lower by 12 to 19 percent (Oser, 1959).

Biological Evaluation of Protein Quality

Data presented in the earlier section on casein to whey protein ratio and the curd tension did not point out any difference in the digestibilities of proteins in various preparations. While the amino acid composition failed to show any difference, the biological availability of the limiting amino acid methionine determined microbiologically, showed higher losses of the amino acid in roller dried preparation in comparison to spray dried ones. It is possible that this may further reflect in their biological values. Further detailed biological evaluation was, therefore, carried out and data obtained are presented below.

Biological Value (BV)

The nitrogen balance study was conducted using conventional method of Mitchell (1923) using growing albino rats and data are given in Table 18. It was observed that the N-intakes were similar in all groups and ranged between 313.0 ±54 mg to 448 ±38 mg. The fecal and urinary excretion of nitrogen after making adjustment for endogenous losses, were found to vary between 41.00 ±41 mg and 118.00 ±25.46 mg in feces and between 15.00±0.44 mg and 16.00±0.54 mg in the urine. This further reflected in B V which ranged

Table 18 : Biological value (BV), true digestibility (TD) and nitrogen retention of some Indian infant foods.

	Product	Nitrogen intake (mg/7 days)	$F_n - F_e$ (mg/7 days)	$U_n - U_e$ (mg/7 days)	Biological value %	True digestibility %	Retention %
	Lactogen	313 ±54	34 ±5	16 ±0.51	94 ±2.40	88 ±5.24	83 ±2.70
	Amul spray	362 ±47	41 ±4	16 ±0.54	94 ±1.69	83 ±2.84	83 ±1.80
	Vijaya spray	448 ±38	64 ±12	15 ±0.60	95 ±0.70	86 ±3.13	82 ±0.80
	Lactodex	430 ±66	109 ±18	16 ±0.44	94 ±0.42	74 ±6.15	70 ±0.60
*	Amul roller dried	443 ±13	118 ±25	15 ±0.44	95 ±0.18	73 ±5.14	70 ±0.20
*	Glaxo	333 ±22	44 ±8	16 ±0.44	94 ±2.16	87 ±4.48	81 ±2.20

* Roller dried products.

Mean of six observations ± S.E.

Table 19 : Analysis of variance for true digestibility.

Source	d.f.	SS	MSS	F
Repitions	5	1085.80	217.36	2.026^{NS}
Treatment	5	759.80	153.96	1.435^{NS}
Error	25	2581.02	107.24	
Total	35	4537.63	129.6	

NS Not significant.
SE 5.97
CD 15.88

Table 20 : Analysis of variance for biological value.

Source	d.f.	SS	MSS	F
Repitions	5	34.13	16.82	1.75^{NS}
Treatment	5	41.47	8.29	3.63^{NS}
Error	25	240.02	9.60	
Total	35	365.53	10.443	

NS Not significant.
SE 1.788
CD 4.756

between 94.00 ±2.40 for Lactogen and 95.00±0.70 % in the case of Vijaya spray. The differences in B V were not significant.

Although the biological value was very close, the true digestibility varied in the case of Lactodex and Amul roller dried and ranged between 73.0 ±5.14 % for Amul roller dried and 88.0 ±5.24 %. The true digestibility though showed some difference, it was not statistically significant. It could be said, as mentioned earlier, that these infant foods showed low curd tension values, the digestibility was not effected and showed similarity. There was no difference between roller dried and spray dried products implying no difference in protein digestibilities and substantiated by biological evaluation.

In another experiment, the nitrogen deposited in the carcass of animals maintained on test diets, was determined and the net protein utilization was calculated. It was found that the same trend was evident (Table 21). The values for N-intake ranged between 1343.0 ±54.0 mg and 1554.0 ±53.0 mg, while retention of nitrogen in caracass was found between 312±68 and 442±58 mg. The differences were non-significant. The net protein utilisation value calculated was found to be between 71.0 ±3.13 % in the case of Lactodex and 78.0 ±1.76 % in the case of Amul spray.

It was evident from these data that the nutritional quality of proteins was similar irrespective of differences

Table 21 : Net protein utilization (NPU) of some Indian infant foods.

Product	Body nitrogen on protein diet. (mg/7 days)	Body nitrogen on protein free diet. (mg/7 days)	Nitrogen in protein free diet. (mg/7 days)	Nitrogen in protein diet. (mg/7 days)	Nitrogen retained. (mg/7 days)	N.P.U. %
Lactogen	1846 ±59	758 ±34	168 ±11	1517 ±139	329 ±61	72 ±0.10
Amul spray	1898 ±56	919 ±21	162 ±11	1452 ±49	446 ±47	78 ±1.76
Vijaya spray	1922 ±77	961 ±49	175 ±8	1479 ±48	442 ±58	76 ±1.76
Lactodex	1866 ±101	906 ±52	158 ±12	1554 ±53	312 ±68	71
* Amul roller dried	1868 ±95	940 ±46	164 ±4	1457 ±55	411 ±64	74 ±1.49
* Glaxo	1754 ±60	904 ±34	157 ±12	1343 ±54	411 ±54	74 ±2.44

* Roller dried products.

Mean of six observations ± S.E.

Table 22 : Analysis of variance for net protein utilisation.

Source	d.f.	SS	MSS	F
Repititions	5	118.99	23.79	1.044^{NS}
Treatment	5	248.33	49.66	2.17^{NS}
Error	25	569.66	22.78	
Total	35	937.0	26.77	

NS Not significant.

SE 2.756

CD 7.33

in the type of milk powders used for their preparations. Similar findings were reported by Singh (1983) who found 83 % net protein utilization (NPU) for whey protein based infant food manufactured at NDRI.

The biological values in this study were in agreement with the biological value of 91.85 % for Indec and 91.80 % for Amul spray reported by Bindra and Deodhar (1980). True digestibility reported for Indec was 85 % and 88 % for Amul spray which were comparable to the values found in the present study. Though Indec was cow milk based preparation, values found were similar to buffalo milk preparations.

As regards the biological values in the present study, Tomarelli and Bernhart (1962) reported somewhat lower biological value of 79.4 % for Oster milk and for humanised infant food 84.9 %. Although the values for humanised milk was higher, both values were lower than the values found in the present study.

As regards retention of nitrogen, it was found to range between 69.9 % and 83.82 %. These were higher than the values reported on human subjects. It could be seen that the retention even in the case of human milk was far below 70 %. It is possible that undeveloped enzyme system in an infant may not be able to digest proteins and utilise it effectively. In this context, Swaminathan (1977) reported that cow milk had a buffering ability towards acidity in the infant stomach which resulted in poor digestion of

protein. Harkova (1969) reported earlier that there was poor adaptation of gastric enzymes in the young human infant to protein other than human milk. Possibly similar condition did not arise in the case of experimental young rats and would explain somewhat lower retention of nitrogen in infants.

Barness et al. (1957) conducted balance studies on less than one month old infants by giving human milk and cow milk and found average retention for human milk 56.14 % and cow milk 54.42 %. On the other hand, Widdowson (1965) found in a study on one month old infants by giving human milk and humanised formulae S_{26}, SMA that the average retention was 57, 48.1 and 43.3 % respectively. Likewise, Singh (1983) found in a study on Indian infants of one month age by giving human milk, modified humanised infant food, developed at NDRI and infant foods, Lactogen and Amul spray, average retentions 68, 67 and 47 % respectively.

Fat

Data on fat content in different infant food preparations presented earlier (Table 1) signified the extent to which the infant calorie requirements are met by this constituent. However, the quality of fat,particularly its ability to provide essential fatty acids did not reflect in the same. Likewise, whether these fats differed in their nature, further influencing their absorption by the body, was not evident from such data. Determination of the extent of unsaturation in these fats, their absorption as well as content of essential fatty acids were made and data are presented in this section.

In order to ascertain whether infant foods under study contained only milk fat or also included vegetable fats, the degree of unsaturation was determined by estimating iodine value, as vegetable fats (except coconut oil) are known to exhibit relatively higher degree of unsaturation in comparison to milk fat. Data on the same are given in Table 23. It was observed that iodine values for different preparations varied within a narrow range between 27.0 $\pm$ 0.05 to 30.16 $\pm$ 0.05. When compared with cow milk or buffalo milk fat, there was close similarity among these values. However, in comparison to iodine value for human milk fat, these values were markedly low. It was apparent from the data that the fat component in these preparations

Table 23 : Iodine value of the Indian infant foods.

Product	Lacto-gen	Amul spray	Vijaya spray	Lacto-dex	Amul roller dried*	Glaxo	Human milk**	Cow milk**	Buffalo milk***
Iodine value	30.16 ±0.05	27.25 ±0.07	27.00 ±0.05	29.58 ±0.03	27.58 ±0.03	27.35 ±0.05	60	33-60	30-33

* Roller dried products

** Macy and Kelly (1961)

*** Arumughan (1978)

Mean of six observations ± S.E.

Table 24 : Analysis of variance for Iodine value

Source	d.f.	SS	MSS	F
Batch	1	0.756	0.756	2.161 NS
Treatment	5	72.582	14.51	51.57*
Batch error	5	2.308	0.46	1.318
Sampling error	36	12.595	0.349	

NS	Not significant
*	Significant at 5 % level.
SE	0.036
CD	0.073

was from milk. Further, it should be remembered that iodine value indicates, the degree of unsaturation in the given fat. This does not necessarily indicate the content of poly-unsaturated fatty acids (PUFA). As fatty acid like oleic acid (18 : 1) although unsaturated, is not dietary essential, yet feflects in the overall iodine value for the fat. It is known that milk fat contains fairly high level (about 18 %) of oleic acid.

Further experiments were done on essential fatty acid contents by determining these chemically.

Linoleic acid, Linolenic acid and Arachiodonic acid content.

The information on essential fatty acid content in infant food formulae becomes essential since these would be their only dietary source for infants. From data on the fatty acid composition of human milk (Widdowson, 1973), it was evident that sizable amount of linoleic acid was available to breast fed infants. Data on linoleic, linolenic, arachidonic acid contents are presented in Table 25.

It was seen that linoleic acid content varied between 1.09 $\pm$ 0.015g/100g fat in the case of Glaxo preparation to 2.47 $\pm$ 0.024 g/100 g in the case of Vijaya spray. Such kind of variation could be understood in the light of observation that milk obtained from buffalo, cow or mixed milks were used during these preparations.

Table 25 : Essential fatty acid composition of Indian infant foods.

Product	Linoleic acid	Linolenic acid	Arachidonic acid
	(g / 100 g)		
Lactogen	1.120 ±0.039	0.600 ±0.028	0.200 ±0.016
Amul spray	2.400 ±0.046	0.034 ±0.001	0.170 ±0.001
Vijaya spray	2.470 ±0.024	0.034 ±0.001	0.174 ±0.015
Lactodex	1.140 ±0.012	0.490 ±0.001	0.140 ±0.002
* Amul roller dried	2.320 ±0.079	0.034 ±0.001	0.170 ±0.001
* Glaxo	1.090 ±0.015	0.610 ±0.001	0.149 ±0.001

* Roller dried infant foods.

Mean of six observations ±S.E.

Table 26 : Analysis of variance for Linoleic acid.

Source	d.f.	SS	MSS	F
Batch	1	0.0028	0.0028	0.625^{NS}
Treatment	5	15.375	3.075	684.82*
Batch error	5	0.0212	0.0042	0.9473
Sampling error	24	0.1077	0.0044	

NS Not significant.
* Significant at 1 % level.
SE 0.0386
CD 0.0796

Table 27 : Analysis of variance ofor Linolenic acid.

Source	d.f.	SS	MSS	F
Batch	1	0.000354	0.0003547	1.855^{NS}
Treatment	5	2.639	0.5279	2761.33*
Batch error	5	0.002561	0.000512	2.678
Sampling error	24	0.00458	0.0001912	

NS Not significant.
* Significant at 1 % level.
SE 0.00797
CD 0.01645

Table 28 : Analysis of variance for Arachidonic acid.

Source	d.f.	SS	MSS	F
Batch	1	0.000103	0.0001033	0.01696^{NS}
Treatment	5	0.0831	0.0166	2.730^{NS}
Batch error	5	0.000625	0.000125	0.02054
Sampling error	24	0.14616	0.00609	

NS Not significant.

SE 0.0075

CD 0.0154

While Vijaya spray and Amul preparations were based from buffalo milk, Lactogen, Lactodex and Glaxo were based on cow milk. The differences in essential fatty acid composition of milk fats from these species, apparently reflected in the essential fatty acid composition.

It was evident from these observations that these preparations did not contain vegetable oils rich in linoleic acid.

As regards linolenic acid content, values ranged between 0.034 ± 0.001/100 g, 0.61 ±0.008/100 g milk fat. ($P<0.05$ Table 27) Again, preparations based on buffalo milk show distinctly low linolenic acid contents as compared to those based on cow milk. These values were decisively lower than 1.8 g/100 g fat for human milk, reported by Hanna et al. (1970).

Arachidonic acid content varied in the close range between 0.14 ±0.002 g/100 g for Lactodex and 0.20 ±0.016g/100g fat for Lactogen. A comparison with human milk fat showed that this essential fatty acid too, was markedly less in preparations studied than the reported value of 0.4/100 g fat in the case of human milk (Hanna et al., 1970). Apparently, these are the reflection of arachidonic acid contents in cow and buffalo milk fats.

Little is known about the fatty acid composition of infant food formula prevalent in this country. Data reported elsewhere in this regard on preparations popular

in European countries, in which fat was derived solely from cow milk showed the linolenic acid content to be identical to that as observed in the present study (Widdowson, 1973).

Several attempts have been made in recent years to simulate infant food formulae with human milk fat in this context, by way of incorporating vegetable fats rich in linoleic acid content which reflected in markedly higher values for linoleic acid contents.

In a study on several preparations popular in European countries, Widdowson (1973) reported 13 to 40 % linoleic acid in preparations, namely Humana, Pelargon, Eledon, Similar etc. Similar high values were reported in respect of linolenic acid and arachidonic acid which ranged between 1 to 2 % for linolenic acid and 0.2 % for arachidonic acid.

It was observed by Widdowson (1973) that a fat blend containing nearly half the amount as linoleic acid had no adverse effect and it was well absorbed by both term and pre-term infants.

It would appear that fatty acid contents in these humanised preparations showed close similarity to human milk fat, even when expressed on the basis of the calorie density. The desirability of such enrichment with vegetable oils appear doubtful in the light of reports made by Cuthbertson (1976) and Naismith et al. (1978) who observed

that the level of linoleic acid providing 1 % of total calories, was adequate to fulfill the nutritional needs of the infants.

Naismith et al. (1978) in their studies on term infants given modified cow milk formula with 0.55 % of total calories in the form of linoleic acid, did not observe either deficiency state or clinical signs of essential fatty acid deficiency. Similar observations were made earlier by Cutherbertson (1976) who found that preparations providing 0.6 % of total calories as linoleic acid were adequate to fulfill essential fatty acid requirements.

In the present study, it was seen that the total calories from three essential fatty acids varied between 0.664 % of the total calories in the case of Lactodex to 0.994 % in the case of Vijaya spray. It may be inferred in view of the above study that if 0.55 % of the total calories were provided by essential fatty acids in the infant foods under study, there was no possibility of onset of essential fatty acid deficiency. It should be noted that no reports of essential fatty acid deficiency among infants either in India or in foreign countries fed on unfortified formulae, have been made so far. Further, linoleic acid has been shown to be converted to linolenic acid and arachidonic acid. From the above, it would appear that no fortification of essential fatty acids, from vegetable fats in the case of present infant foods appear warranted. Such fortification would not only pose problem of storage of such infant foods high polyunsaturated fatty acids, but these may lead to rancid fats during storage more readily.

Fat Absorption

Just as the quality of dietary fats, it is equally essential to understand how far these fats are utilised in the body. Knowledge on the fat absorption, thus becomes of interest in this context. Fat absorption, depends on the nature of the fat, especially its fatty acid composition and the positional distribution of fatty acids in the triglyceride molecules.

The fat absorption was determined according to the procedure described by Tomarelli et al. (1968) by conducting balance studies on albino rats of 80 to 90 g in weight. The data on these are presented in Table 29. The fat intake in all cases, except Lactodex, was about 5 g per day, while the fecal fat excretion after correcting the indigenous fat excretion, was about 0.47 to 0.52 g per day. In the case of Lactodex, however, the intake was 1.76 $\pm$0.29g/day and excretion was 0.32 $\pm$0.05 g/day. This was apparently a reflection of low fat contents in these preparations. Further, fat absorption was about 90 % in all cases except Lactodex which had 81.8 %.

Relatively few studies have been reported on fat absorption on Indian infant food formulae. In a study on preparations based on cow and buffalo milk, Bindra and Deodhar (1980) observed 92 % absorption in the case of Amul and Indec preparations based on buffalo and cow milk

Table 29 : Fat absorption for some Indian infant foods.

Product	Total fatty acid intake.	Total fatty acid excreted.	Total fatty acid in endogenous feces.	Net amount of fatty acid in feces corrected for endogenous excretion.	Fat absorption (% of intake).
	g / 100 g day				
Lactogen	5.14 ±0.85	0.56 ±0.09	0.09 ±0.01	0.47 ±0.07	90.85 ±0.60
Amul spray	5.00 ±0.85	0.55 ±0.09	0.07 ±0.01	0.48 ±0.08	90.40 ±0.36
Vijaya spray	5.23 ±0.85	0.57 ±0.09	0.04 ±0.01	0.52 ±0.08	91.96 ±0.68
Lactodex	1.76 ±0.29	0.36 ±0.06	0.04 ±0.06	0.32 ±0.05	81.81 ±0.49
Amul roller dried	5.62 ±0.90	0.60 ±0.09	0.09 ±0.01	0.51 ±0.08	90.92 ±0.80
Glaxo	5.90 ±0.95	0.58 ±0.09	0.06 ±0.01	0.52 ±0.88	91.18 ±0.38

* Roller dried products.

Mean of six observations.

Table 30 : Analysis of variance for fat absorption.

Source	d.f.	SS	MSS	F
Repititions	5	7.55	1.511	7.343^{NS}
Treatment	5	345.22	69.04	33.552*
Error	25	51.44	2.057	
Total	35	404.22	11.22	

NS Not significant
* Significant at 1 % level.
SE 8.28
CD 16.83

respectively. These values were in close agreement with the range observed in the present study (90.4 to 91.96 %).

In a study conducted on 4 week old infants, Singh (1983) observed relatively lower absorption of fat for commercial Indian formula. It was considered that such low absorption was possibly due to incompletely developed pancreatic lipase activity in such infants. Species difference affecting this parameter can also not be overruled. In the same study, Singh (1983) observed better absorption of vegetable oil fortified infant formula though less than human milk fat. In a study on rats, both young (40 - 50 g) and old (70 - 80 g), Raina (1980) found that fats with high linoleic acid were better absorbed. Similar observations were made by Widdowson (1965) on human infants, who found an absorption of 93 % for human milk, 91 % for S_{26} and 87 % for SMA.

Hanna et al. (1970) found that vegetable oil fortified infant formulae were quite well absorbed viz. 84 to 86 % as compared to 88.6 % for human milk, though the workers observed that it is difficult to imitate the fatty acid distribution of human milk fat and match the absorption to human milk fat.

Carbohydrates

The caloric contribution by carbohydrates towards total calories in human milk is markedly higher in comparison to cow or buffalo milk. In order to make up this deficiency, while formulating infant food formulae, use of carbohydrates of various types such as lactose, sucrose, glucose, dextrimaltose as well as corn and tapioca starches, either as such or in their modified form have been reported to be used as diluents (Anderson et al., 1972, 1974). While the use of lactose appears to be logical in this regard, economical constraints prohibit such use. In the case of infant food formula based on buffalo milk, Swaminathan and Parpia (1968) adopted sucrose in place of lactose, as this was less expensive as well as it is sweeter than lactose. While adopting any of these carbohydrates, other side effects, if any, too need tobe taken into account, besides their overall effect on utilisation of other nutrients. Thus, the use of starch is highly undesirable because of underdeveloped digestive system in the early stage of life. On the other hand, the use of excessive amounts of sucrose may lead to the development of dental caries (Keyes, 1969) and accelerated lipogenesis leading to subsequent obesity in the later life (Brooke, 1980).

While making a choice for infant food formula, among many orient countries as well as in India, the degree of prevalence of lactose intolerance in early stage of life,

also demands attention. In the light of this, a study was further extended to see the kind of carbohydrates used in the preparations under study(Table 31).

This was done by differentiating them chemically into reducing and nonreducing carbohydrates. None of these six preparations studied showed the presence of starch in them as evident from negative iodine test. Total reducing sugar content was determined according to the method of (Hane, 1980). These were found to range between 28.0 g in Amul preparations to 39.63 g in Lactodex. and the difference was significant(P 0.05) (Table 33).

Determination of lactose by the method specific for lactose (Nickerson et al. 1958), using methylamine hydrochloride, demonstrated that except Lactodex, in all other preparations lactose accounted for the total reducing sugars. The residual reducing sugars were found to range between traces of 2.23 g % (Vijaya spray) and could be considered as experimental errors. An attempt to determine glucose by using glucose specific enzymatic method of Slein (1963) failed to show the presence of even traces of glucose. In the case of Lactodex, lactose content accounted for about 63 % of total reducing sugars. This preparation was found to contain maltodextrin. In the light of this information, it was found that the remaining 37 % of reducing sugars may be present as reducing breakdown products made up of mono-, di- and tri reducing saccharides accompanying maltodextrin (Junk and Pancost, 1973). The glucose content in this preparation was found only 0.44 %. The non-

Table 31 : Carbohydrate composition of Indian infant foods.

Product	Total carbohydrate	Lactose	Glucose	Other reducing sugars	Sucrose	Malto-dextrose
	g/100 g					
Lactogen	52.18 ±0.26	28.12 ±0.36	Nil	1.88 ±0.01	22.18 ±0.10	-
Amul spray	52.18 ±0.26	27.62 ±0.29	Nil	0.12 ±0.02	24.68 ±0.11	-
Vijaya spray	52.18 ±0.26	27.77 ±0.31	Nil	2.23 ±0.01	22.18 ±0.12	-
Lactogex	71.87 ±0.37	22.59 ±0.52	0.44 ±0.001	13.03 ±0.22	1.56	34.25
* Amul roller dried	52.03 ±0.28	28.00 ±0.31	Nil	Nil	24.53 ±0.12	-
* Glaxo	52.18 ±0.26	28.00 ±0.31	Nil	2.01 ±0.07	22.18 ±0.13	-

* Roller dried products.

Mean of six observations.

Table 32 : Analysis of variance for Lactose.

Source	d.f.	SS	MSS	F
Batch	1	0.905	0.905	1.4587^{NS}
Treatment	5	189.23	37.846	61.003*
Batch error	5	2.067	0.4134	0.66
Sampling error	36	22.33	0.62	

NS Not significant
* Significant at 1 % level
SE 0.393
CD 0.8006

Table 33 : Analysis of variance for reducing sugar.

Source	d.f.	SS	MSS	F
Batch	1	0.01	0.01	0.01^{NS}
Treatment	5	352.60	70.5	38.68*
Batch error	5	0	0	0
Sampling error	36	65.61	1.82	

NS Not significant.
* Significant at 1 % level.
SE 0.674
CD 1.37

Table 34 : Analysis variance for total carbohydrate.

Source	d.f.	SS	MSS	F
Batch	1	0.15	0.15	0.25^{NS}
Treatment	5	2543.29	608.65	880.79*
Batch error	5	0.32	0.664	0.110
Sampling error	36	20.79	0.57	

NS Not significant
* Significant at 1 % level
SE 0.379
CD 0.772

reducing carbohydrate content in all preparations accounted for about 42 % of the total carbohydrates.

Regarding non-reducing sugars, all preparations except Lactodex and Glaxo declared the inclusion of sucrose. This was confirmed by modified procedure of Hanes (1980) as well as conformatory tests with resorsinol. Further check was also made by determining sucrose content by method of Pantulu *et al.* (1982). It was found that even Glaxo contained 22.18 ±0.13 g per 100 g sucrose in it. Other preparations except Lactodex had sucrose varying between 22.18 ±0.10 to 24.68 ±0.11 g per 100 g. Lactodex was virtually devoid of sucrose since the contents were less than 1 %. The maltodextrin content in Lactodex (taking into account 13.03 ±0.22 as breakdown products accompanying maltodextrin) was 34.25 g per 100 g. The maltodextrin content were determined according to Southgate(1969) by treating the sample devoid of reducing sugars, with taka-diastase and measuring reducing saccharides.

Though no detailed studies were conducted in other Indian preparations, the total carbohydrate content found by Udani *et al.* (1970) in Lever baby food was 48 g/100 g and those reported by Misra (1959) for Amul spray were 50 g/100 g, compatible with the values obtained in the present study.

While considering the carbohydrate component of the infant food formula, attention should alsobe given to its physiological significance, particularly in controlling the neurochemical mechanisms. Recent studies by Chiel and Wurtman (1981) demonstrated that as the ratio of carbohydrate

to proteins in the diet of rats markedly influenced the uptake of tryptophan and tyrosine in the brain tissue and consequently the levels of neurotransmittan such as seratonine, and citecholamine increased it. Ratios calculated from the data presented in Table 31 showed that except Lactodex, all other infant food formulae showed it to be approximately 2.4. However, in the case of Lactodex, it was almost double, viz. 4.9. Besides this ratio, Crane and Lachance (1983) demonstrated the effect of the chemical nature of carbohydrate to influence the activity and levels of monoamines in the brain tissue of rat pups. Earlier observations by Crook (1974) and Rapp (1978) showed that administration of sucrose to hyperactive children displayed symptoms of hyperactivity following a challenge test with sucrose.

In more detail experiments, Wurtman and Fernstrom (1975) showed that ingestion a single high carbohydrate meal providing dextrose, sucrose or dextin elevated brain serotonin levels. Likewise, Saller and Chiod (1980) showed that interavenous administration even of glucose, suppressed the firing of depamine containing neurons of the substantia nigra of rats. It would appear from these studies that the kind of carbohydrate to be used to fill the calorie gap in the formula or to improve the palate, need to be carefully tested and chosen. Although the use of sucrose or maltodextrin appeared to be more economical than the use of Lactose. The consumption of such formula by the babies over a prolonged period could very likely influence their behavioural patterns. Additional studies are warranted in this area.

Minerals in Infant Food Formulae

Like macronutrients discussed earlier, it is imperative to have knowledge on the micronutrients, especially mineral elements in the infant formulae. It is essential not simply because of the known established nutritional role of several major and trace elements, but also certain physiological constraints observed in the early stage. New born infants have relatively poor capacity to excrete excess salts in comparison to adults. Not only glomerular filtration rate is lower in early infancy, there is a specific inability of renal tubules to selectively reject sodium. Apart from this, the ability to regulate the concentration of plasma calcium, is not fully developed in the first few weeks of like. Thus, if due attention is not given during artificial feeding, hypocalcemic condition could be a distinct possibility (Gurr, 1981). Special attention is, therefore, warranted when the starting material is either cow's milk or buffalo's milk. Since these are high in protein as well as sodium content in comparison to human milk. Besides this, use of sodium phosphate, sodium citrate, sodium bicarbonate either as additive to lower the curd tension or as a neutralizer (Swaminathan and Parpia, 1968), further enhances the sodium content in the milk powder. In view of the aforesaid, data obtained on certain major and trace minerals are presented in this section.

Data on total ash content presented earlier showed considerable variation among different infant foods, with values ranging between 2.64 ±0.13 % in Lactodex to 4.26 ± 0.14 % in the case of Vijaya spray. Such values hardly elaborate about variations, if any, in their constituent elements. Besides their origin in the milk and other ingredients of the formulae, certain elements especially trace ones, are also known to be present as contaminants. In view of this, these infant foods were further analysed for certain major elements, namely,calcium, phosphorus, sodium,potassium, magnesium and chloride, as well as certain trace elements. Calcium, magnesium and phosphorus content in different preparations are given in Table 35.

Calcium

As regards calcium, it was observed that the values ranged between 0.85 ±0.03g/100 g for Lactodex and 0.89 ± 0.01 g/100 g for Lactogen, Amul preparations, Glaxo and Vijaya spray and the difference was not significant(P > 0.05).

While calcium content in all preparations, except Lactodex, could be reasonably accounted by the quantity present in the milk used, this was not so in the case of Lactodex. Apparently, this preparation was fortified with calcium salt although it was not evident on the container.

Table 35 : Calcium, phosphorus and magnesium contents in Indian infant foods.

Product	Calcium (g/100g)	Phosphorus (g/100g)	Magnesium (mg/100g)
Lactogen	0.89 ±0.01	0.57 ±0.12	87.50 ±0.20
Amul spray	0.89 ±0.01	0.61 ±0.04	126.00 ±0.17
Vijaya spray	0.88 ±0.01	0.62 ±0.04	97.00 ±0.18
Lactodex	0.85 ±0.03	0.38 ±0.04	58.00 ±0.17
* Amul roller dried	0.89 ±0.01	0.63 ±0.04	126.00 ±0.18
* Glaxo	0.89 ±0.01	0.63 ±0.04	87.00 ±0.18

* Roller dried products.

Mean of six observations ± S.E.

Table 36 : Analysis of variance in Calcium.

Source	d.f.	SS	MSS	F
Batch	1	0.006211	0.006211	5.699*
Treatment	5	0.001822	0.0003644	0.3343^{NS}
Batch error	5	0.001673	0.003346	0.3070
Sampling error	36	0.03923	0.00108	

NS Not significant.
* Significant at 5 % level.
SE 0.0165
CD 0.0335

Table 37 : Analysis of variance for Phosphorus.

Source	d.f.	SS	MSS	F
Batch	1	0.01	0.01	0.01^{NS}
Treatment	5	0.3953	0.0790	314.119*
Batch error	5	0.001094	0.0002188	0.8692
Sampling error	36	0.009063	0.000251	

NS Not significant.
* Significant at 1 % level.
SE 0.00793
CD 0.01612

Table 38 : Analysis of variance in Magnesium.

Source	d.f.	SS	MSS	F
Batch	1	1.95	1.95	0.0521 NS
Treatment	5	20238.46	4047.69	108.25*
Batch error	5	33.51	6.702	0.1792
Sampling error	36	1346.21	37.39	

NS Not significant
* Significant at 1 % level.
SE 3.057
CD 6.21

The preparations, Lactodex, Lactogen and Vijaya spray did not conform to I.S.I. specifications though the values are within the same range. The range of calcium observed in this study was comparable to 0.77 g/100 g in a cow milk based formula 'Indec', and 0.89g/100g in a buffalo milk based formula 'Amul Spray', reported by Bindra and Deodhar (1980). Similar values were reported in another study by Chaudhry and Kansal (1980) who found 0.82 g/100g in Amul spray, 0.80g/100g in Sapan, 0.78 g/100g in Lever Baby Food and 0.83g/100g in Indec. On the other hand, when compared with the values reported by Widdowson (1965) for different European preparations, namely 0.316 g/100 g in S_{26} and 0.468 g/100 g in SMA, those observed in the present study were considerably higher. It should be, however, noted that these preparations were for humanised milk and made using whey. Obviously, calcium was understandably lower.

Values observed in the present study were also somewhat lower than the range between 0.71g/100g and 0.95g/100g in several preparations of popular brands in U.S.A. reported by Fomon (1974).

Despite comparable levels on dry weight basis, when reconstituted as directed on containers, it was found that the amount ranged between 0.1g/100 ml in the case of Lactogen to 0.146g/100g in the case of Amul preparations (Appendix IV). These differences were mainly due to variations in prescribed dilutions. Whereas liquid formula for Lactogen contained 11.2 % powder, Amul preparations

were found to be three to six times higher than recommended, about 14 % powder in the liquid formula.

Phosphorus

Values for phosphorus in various formulae presented in Table 35, was found to range between 0.38 ±0.04 g/100 g in Lactodex and 0.63 ±0.04 g/100 g in the case of Glaxo and Amul roller dried. Except for Lactodex and Lactogen, all other values fall in the range of 0.61 g to 0.63 g/100 g. On the basis of quantity of milk used, it was evident that phosphorus contents could be accounted. Lactodex had significantly less phosphorus than other preparations ($p < 0.01$)(Table 37).

A comparison of the present data on phosphorus with those obtained by Bindra and Deodhar (1980) showed the content in the Amul spray similar (0.66 g/100 g). Calcium in 'Indec' was much low (0.52 g/100 g). Similar values were reported by Chaudhry and Kansal (1980) who obtained 0.68 g/100 g for Amul spray, 0.67 g/100 g for Sapan, 0.69g/100 g for Lever spray and 0.56 g/100 g for Indec.

When compared with values reported by Widdowson (1965) for some European preparations, it was observed that values obtained in the present study were much higher than 0.23 g/100 g for S_{26} and 0.35 g/100 g for SMA. Again, these preparations were of humanized milk, in which demineralised whey was used to make these preparations to resemble human milk. On the other hand, when compared with

phosphorus in several American preparations reported by Fomon (1974), values in the present study were somewhat lower. When reconstituted to make liquid formula as directed on containers, it was found that the amount of phosphorus ranged between 50 mg/100 ml in the case of Lactodex and 110 mg/100 ml in the case of Glaxo (Appendix IV) and were 4 to 8 fold higher than that observed in human milk.

It has been reported by Ziegler and Fomon (1971) that infants fed formulae with phosphorus content higher than human milk, excrete large part of phorphorus in urine. This may contribute to high solute load.

Magnesium

Data on magnesium in various formulae are presented in Table 35 which showed the variation ranging between 58 ±0.17 mg/100 g in case of Lactodex and 126 ±0.17 mg/100 g in case of Amul preparations. These differences were statistically significant ($P<0.01$). Further, contents for Glaxo, Lactogen and Vijaya (spray) were in the same range.

When compared with the values reported by Chaudhry and Kansal (1980) i.e. 78 mg/100 g in Amul spray, 84 mg/100 g in Sapan, 72 mg/100 g in Lever spray and 87.5 mg/100g in Indec, values observed in the present study were in the similar range.

When compared with the values obtained by Widdowson (1965) for European humanized milk preparation S_{26}, 0.003 g/

100 g SMA, 0.049 g/100 g, the values in the present study are slightly higher. However, the values reported by Fomon (1974) for several preparations of popular brands in United States, ranged from 80 to 138 mg/100 g magnesium, were identical to those found in the present study.

On reconstitution as directed on containers, magnesium in liquid formula was found to vary between 9.84 mg/100 ml in the case of Lactogen and 21.0 mg/100 ml in the case of Amul preparations (Appendix IV). These levels were 2 to 3 times higher than magnesium in the human milk. Further comparison with the recommended international standards showed that on calorie basis, all formulae showed the magnesium content to be above 14 mg/100 KCal. This was much higher than the minimum of 6 mg/100 KCal recommended by Codex Alimentarius Commission (Appendix II).

Nutritional Adequacy

As mentioned earlier, the daily intake of calcium from reconstituted milks was found to be in the range of 0.36 g/day in the case of Lactogen and 0.78 g/day in the case of Glaxo. These,when compared with the value of 0.36 g/day recommended by American Nutrition Advisory Committee (Appendix III),were found to be higher.

Nutritional adequacy of these three elements, especially calcium, cannot be assured merely from the daily

intake of these elements. Several dietary factors have been recently found to influence the calcium absorption. These include Ca : P and Ca : Protein ratios, nature of the dietary carbohydrates, fatty acid composition and intake of dietary fat, besides vitamin D status of infants.

The Ca : P ratio in the formulae examined, and presented in Table 39 showed a variation between 1.41 for both the Amul products and Vijaya spray to 2.23 for Lactodex. In this context, Oppe and Redstone (1958) reported optimum calcium absorption in infants, when Ca:P ratio in the formula was about 2, which was similar to human milk. An identical ratio was also found to support optimal mineralization of bones too (ESPAGAN, Committee Report, 1977, Codex Alimentarius Commission, 1976), on the other hand, has prescribed that this ratio should not be less than 1.2 and exceed 2.0. In the light of these reports, ratios found in this study were in the desired range, with the exception of Lactodex. (Illustration III)

As regards Calcium to Protein ratio, in different formulae the ratio was maximum for Lactodex (0.059) in comparison to other formulae (Table 36) which varied in the close range between 0.040 to 0.044. Although De Portela et al. (1983) observed in their studies on infants, an increase in the excretion of calcium as the protein intake increased, two milk formulae examined by them did not show significantly higher calcium absorption in infants receiving formula with Ca/P ratio 2.34 and Ca : protein ratio 0.056,

Table 39 : Calcium : Phosphorus, Calcium : Protein, Nitrogen : Phosphorus ratios of some Indian infant foods.

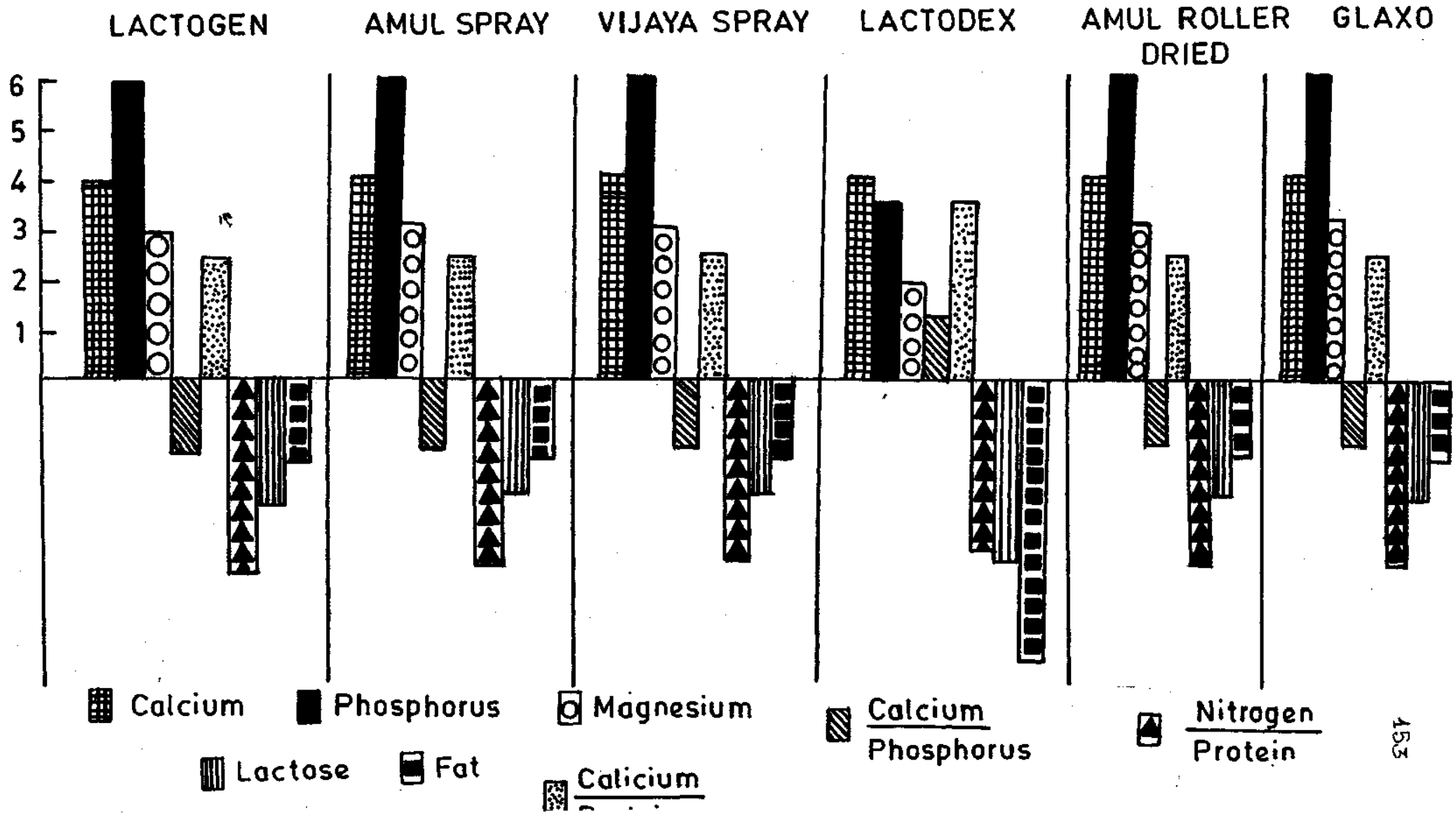
Product	Ca/P	Ca/Protein	Nitrogen/Phosphorus
Lactogen	1.56	0.041	5.89
Amul spray	1.46	0.041	5.50
Vijaya spray	1.41	0.040	5.41
Lactodex	2.23	0.059	6.05
* Amul roller dried	1.41	0.041	5.33
* Glaxo	1.41	0.041	5.33
Human milk	2.00	0.017	20.71

* Roller dried products.

Mean of six observations ±S.E.

ILLUSTRATION III

A COMPARISON OF THE INDIAN INFANT FOODS WITH NUTERIENTS IN HUMAN MILK



in comparison to other formula with Ca/P ratio 1.37 and Ca/Protein ratio 0.036.

The nature of carbohydrates in the formula does influence the calcium absorption in infants (Ziegler and Fomon, 1983; Wasserman and Comer, 1959) demonstrated that absorption of calcium by rats was not enhanced by the administration of sugars, namely glucose, fructose, galactalose and sucrose, which are rapidly absorbed from the intestinal tract. However, lactose and cellibiose that are absorbed more slowly, did enhance calcium absorption. Data presented earlier (Table 31) showed that sucrose was present in all preparations (except Lactodex), besides lactose which accounted over half of total carbohydrates. It is possible that maltodextrin used in Lactodex, would be absorbed more slowly than sucrose. This could influence calcium absorption from these formulae. The beneficial effect of lactose over sucrose as well as other carbohydrate sources was reported earlier by McCaulay and Watson (1967) in a study in which calcium absorption was decissively better from cow's milk in comparison to a formula with sucrose in place of lactose. In an another study, Ziegler and Fomon (1983) showed markedly higher absorption of calcium from formula with all the carbohydrate in the form of lactose, in comparison to formula in which lactose was replaced by corn starch hydrolysate and sucrose. However, this difference in the nature of carbohydrate did not affect phosphorus and magnesium absorption. Although absorption

studies on these minerals were not conducted in the present investigation, it is possible that Lactodex with lactose and maltodextrin as carbohydrates may show better absorption of calcium in comparison to other preparations consisting of slowly absorbable sucrose.

Another dietary factor known to affect the calcium absorption is fat. Shaw (1976) observed higher absorption of fat as well as calcium from human milk in comparison to either full cream cow's milk or humanised infant formula. A similar observation was made earlier by Widdowson (1965) and McCaulay and Watson (1957). In the present study, all infant foods contained milk fat as the only source of fat, and formula was not humanized in this respect. Again, the fat content were also fairly identical, barring Lactodex. Keeping in mind the inherent difference in the positional distribution of fatty acids between human and cow's milk fats, the similarity in calcium absorption is not anticipated between these preparations and human milk.

In the light of N/P ratio of 15 in soft tissues it was considered necessary to determine this ratio for the formulae examined (Table 39).

The ratio varied between 5.33 for Amul roller dried as well as Glaxo preparations and 6.05 for Lactodex. These ratios were markedly lower than the value of 20.7 for human milk. It should, however, be mentioned that absorption of phosphorus is also partly regulated by calcium absorption. A comparison of the ratios in the

present study with those computed from the data by Widdowson (1973) for several infant food formulations in European countries showed considerable similarity among them. It should be remembered that only certain amount of phosphorus is assimilated in soft tissues in proteins, while over 75 % of the body phosphorus is located in bones.

As regards anticipated daily magnesium intake from these infant foods, it was in the range of 39.32 mg/day in the case of Lactodex and 94.5 mg/day in the case of Amul preparations and were well within the recommended allowance of 50 mg/day by American Nutrition Advisory Committee(1980) (Appendix III). Except Lactogen and Lactodex, all other preparations fulfilled the requirement. The higher magnesium content ranging from 7.7 mg/100 ml in Lactodex to 8 mg/100 ml in Amul preparations in comparison to human milk, seem justifiable in the light of observations by Widdowson (1965), McCaulay and Watson (1957) who showed lower absorption of magnesium from formula milks than human milk.

Besides the group of macrominerals in the formulae discussed earlier, the other group of macro-nutrients comprised of sodium, potassium and chlorideis equally important in the light of its contribution to the renal solute load and further influence on the infant's water balance. Infants have a relatively poor capacity to excrete excess salts in comparison to adults. This is because of lower glomerular filtration rates as well as the inability of immature renal tubules selectively to reject sodium. Excessive sodium in the infant food may thus seriously affect the water balance in the body responsible for the clinical problems such as dehydration and condition of hypernatramia. The excessive sodium intake along higher calorie density of the diet in the early life may also lead to hypertension later (Dahl,1972).

In view of the practices often adopted in the dairy industry such as the use of sodium bicarbonate, hydroxides as neutralisers for milk, to retain its utility at milk plants for milk processing and also the addition of sodium phosphates, citrates, etc. to lower the curd tension during the manufacture of infant foods (Swaminathan and Parpia, 1968), it is highly essential to have knowledge of the contents of sodium and other macrominerals in this group. Data on these minerals are given in this section.

Sodium, Potassium and Chloride

Data on sodium, potassium and chloride content in various formulae under study are presented in Table 40. It was observed that sodium in these preparations ranged between 292.5 ±1.2 mg/100 g in Lactodex and 490.0 ±0.5 mg/100 g in Amul spray. Though Lactogen, Lactodex and Glaxo preparations did not conform to any uniform specifications in this regard, contents were relatively higher in these formulae in comparison to Vijaya spray and Lactogen. Even these four formulae showed significant differences ($P<0.01$) among them. On the basis of the quantity of milk used in their preparation, sodium content should not exceed 360 mg/100 g. However, as mentioned earlier, in order to lower curd tension the addition of sodium bicarbonate, phosphate and citrate, is often practised (Swaminathan and Parpia, 1968), which possibly reflected in the high sodium content of infant foods. Besides this, sodium bicarbonate is also used as neutraliser for milk before it is further processed in milk plants. This could also result in high sodium content in the resultant preparations.

The wide range for sodium observed in the present study was still within the broad variations reported in several other preparations in different countries. In a detailed comparative study on 30 dried milk preparations, popular in over seven European countries, Widdowson *et al.* observed sodium to range from 145 mg/100 g powder in Humana 2,

Table 40 : Sodium, potassium and chloride contents in some popular Indian infant foods.

Product	Sodium	Potassium	Chloride
	mg / 100 g		
Lactogen	455.00 ±1.40	825.00 ±5.40	498.63 ±7.10
Amul spray	490.00 ±0.50	834.00 ±3.80	513.04 ±7.20
Vijaya spray	361.25 ±0.66	832.50 ±3.60	508.49 ±5.10
Lactodex	292.50 ±1.20	402.50 ±5.40	356.80 ±9.10
* Amul roller dried	472.50 ±1.70	840.00 ±3.80	514.40 ±7.10
* Glaxo	471.25 ±1.50	878.00 ±5.40	519.67 ±10.10

* Roller dried products.

Mean of six observations ± S.E.

Table 41 : Analysis of variance for Sodium.

Source	d.f.	SS	MSS	F
Batch	1	8.8	8.8	0.0384^{NS}
Treatment	5	249050.5	49810.1	217.35*
Batch error	5	1816.2	363.24	1.585
Sampling error	36	8250.0	229.166	

NS Not significant.
* Significant at 1 % level.
SE 7.5691
CD 15.388

Table 42 : Analysis of variance for Potassium.

Source	d.f.	SS	MSS	F
Batch	1	0.000029	0.000029	0.1167^{NS}
Treatment	5	1.16908	0.23381	941.66*
Batch error	5	0.00108	0.000217	0.8747
Sampling error	36	0.008942	0.0002483	

NS Not significant.
* Significant at 1 % level.
SE 0.007
CD 16.01

Table 43 : Analysis of variance for Chloride.

Source	d.f.	SS	MSS	F
Batch	1	1074.169	1074.169	1.674^{NS}
Treatment	5	160640.50	32128.1	50.09*
Batch	5	161.86	32.37	0.05
Sampling error	36	23089.87	641.385	

NS Not significant.
* Significant at 5 % level.
SE 12.662
CD 25.74

a humanised formula in Switzerland to 458 mg/100 g in Ostermilk No.2. Shaw et al. (1973) found 198 mg/100 g in SMA whereas many other preparations ranged between 250 mg and 440 mg/100 g. Fomon (1974) reported sodium content between 280 and 420 mg/100 g in some popular infant foods in the United States.

Notwithstanding such parallel among infant foods from different sources, when contents were expressed on the basis of calorie contents of these infant foods, values were found to be alarmingly higher than the maximum limit of 60 mg/100 K Cal recommended by Codex Alimentarius Commission (Appendix II). The range observed varied from 73.7 mg/100 KCal in the case of Lactodex to 106 mg/100 K Cal in the case of Amul spray (Appendix V). Thus infants deriving their energy needs solely from the infant foods, receive enormously higher intake of sodium. This could have dangerous possible consequences of getting these infants predisposed to development of hypertension in adult life, as hinted by Ziegler and Fomon (1971).

When reconstituted as directed on the containers, the sodium content ranged between 39 mg/100 ml in Lactodex and 83 mg/100 ml in the case of Glaxo (Appendix IV). These values were much higher than the values observed in human milk (14 mg/100 ml). Apparently there is a strong case to reason more dilution atleast in the case of most preparations in order to attain atleast the upper limit of safety for the feeding of infants. This reasoning seems to be

further justified when a comparison was made with British Recommendations of 15 - 35 mg/100 ml sodium in formula milk. However, it may be equally important to note that the reports of incidence of hypernatraemia are scarce in this country. It may be necessary in this context to take into account sizable dermal excretion of sodium in the sweat, in tropical countries, the reassessment of the maximum limits, thus appears necessary.

Potassium

Data on potassium content in various formulae under study are presented in Table 40. It was observed that these contents ranged between 402 mg $\pm$5.4/100 g in the case of Lactodex and 878 mg $\pm$5.4/100 g in the case of Glaxo. Except Lactodex, for all the other preparations, potassium was in the close range of variations though these preparations did not conform any particular specifications. The statistical analysis of the data (Table 40) showed that potassium content was significantly lower ($P < 0.01$) in Lactodex and significantly higher($P < 0.01$) in Glaxo formulae as compared to other ones.

On the basis of the quantity of milk used, these preparations should contain not more than 700 mg/ 100 g. Possibly, excess of potassium in each of these food formulae could be the result of contamination.

When compared with other studies, the contents, observed in this study were found to be within the broad range for several European preparations (438 - 1182 mg) by

Widdowson (1965). Imamva et al. (1969) also reported 903 mg/100 g in Japanese preparations. Similar higher values were reported by Fomon (1974) in popular infant formulae in the United States (1080 mg/100 g).

When reconstituted based on the instructions by the manufacturers, the content in liquid formula ranged between 55 mg/100 ml for Lactodex and 155 mg/100 ml in the case of Glaxo. These were markedly higher than potassium content in human milk (55 mg/100 ml), except Lactodex. Likewise, when expressed on the basis of calorie contents, potassium content ranged from 101.4 mg/100 K Cal in Lactodex to 191.2 mg/100 K Cal in Glaxo formula (Appendix V). Still these values were within the minimum-maximum range of 80 to 200 mg/100 K Cal recommended by Codex Alimentarius Commission (Appendix II).

Chloride

As regard chloride content in various formulae (Table 40), it was observed that these ranged between 356.80 ±9.1 mg/100 g in Lactodex and 519.67 ±10.1 mg/100 g in Glaxo preparation. Except Lactodex, all the other preparations showed chloride content in a small range of values, though Lactogen, Amul preparations, Vijaya spray and Glaxo did not conform any uniform specifications. It was significantly less in Lactodex ($P < 0.05$) (Table 43).

On the basis of the amount of milk used in these preparations, the content should not exceed 520 mg/

100 g. When compared with the values of 630 mg/100 g (Imamva et al.,1969) and 480 mg/100 g (Fomon, 1974), the values in the present study appear to be in the similar range. When compared with the recommended limits of 50-150 mg/100 K Cal, by Codex Alimentarius Commission(Appendix II), all preparations were found tobe within these limits.

On reconstituting into liquid formula as directed by the manufacturers, it was found that the amount ranged between 55 mg/100 ml in the case of Lactogen and 91 mg/100 ml in the case of Glaxo. These were 2 to 4 times higher than the content of 22 mg/100 ml in human milk, but in the broad range of 40 - 80 mg/100 ml recommended by Department of Health and Social Security of U.K. (1980).

Nutritional Adequacy

Though excessive intake of Na, K and Cl could have serious consequences, appropriate intakes of these elements are indeed essential in view of their role in maintaining water balance in the body. The daily intake of sodium from reconstituted milks was found to vary between 198.90 mg/day in the case of Lactodex and 420 mg/day in the case of Glaxo. The recommended allowance by American Nutrition Committee (1980) (Appendix III) were 115 - 350 mg/day. The values found in the present study were found to be in this range.

Similarly the daily intake of potassium from the reconstituted milks was found to vary between 280 mg/day

In the case of Lactodex and 785 mg/day in the case of Glaxo, which was well within the allowances recommended by American Nutrition Advisory Committee (350 - 925 mg/day).

The daily intake of chloride from reconstituted milks was found to be between 239 mg/day in the case of Lactodex and 462 mg/day in the case of Glaxo. The recommended value of American Nutrition Advisory Committee were 275 mg to 700 mg/day. The values in the present study were found in this range.

Renal Solute Load

The renal solute load depends primarily on the non-metabolizable dietary components, especially electrolytes ingested in excess over body needs, as well as certain metabolic end products. In most infant formulae, a high percentage of solutes is comprised of disaccharides such as lactose, sucrose and maltose and low molecular weight polysaccharides, namely, dextrdmaltose. These are normally metabolized yielding only a few solutes for renal excretion. On the other hand, urea, the metabolic end product of proteins, markedly contributes to the renal solute load.

It was seen from the data on various major elements in infant foods that, in terms of their contents these are sufficient to meet the nutritional requirements. However, in respect of certain elements, especially sodium, the content appear to be alarmingly high and could produce deletarious effects. Being non-metabolizable dietary components, many

of these major elements when ingested in excess over body needs, contribute to the renal solute load. This latter aspect deserves serious consideration in view of the under-developed nature of infant kidneys, their poor capacity to excrete excess salt, lower glomerular filtrate rates, and inability of renal tubules to selectively reject sodium. Administration of high solute loads, therefore, could neither be considered desirable nor safe. In view of this, renal solute load for all the preparations were computed according to Ziegler and Fomon (1971).

The solute load of infant foods under study were computed by taking into account recommended dilution and are given in Table 44. It could be seen that Lactodex had the lowest solute load of 124 m Osmol followed by Lactogen 200.27, Vijaya spray 184.50, both Amul preparations 226 m Osmol and Glaxo 253.50 m Osmol. Clearly, all preparations showed distinctly higher solute loads than 79 m Osmol reported for human milk. The higher osmolar content in these preparations appear tobe comparable to 260 m Osmol/ lit reported for cow milk (Samadi et al., 1983) and could possibly be due to the high concentrations of the feed recommended on the containers. Further scrutiny of the data showed that protein contributed maximally to the renal solute load.

As pointed out earlier, all these preparations had protein contents much higher (about two times) than those recommended allowance of 10.5 g/day by Indian Council of Medical Research. If these preparations restricted protein

Table 44 : Solute load of some Indian infant foods under study (0 - 1 month).

Product		Sodium (mg/lit)	Potassium (mg/lit)	Chloride (mg/lit)	Protein (g/lit)	Total osmolar load
Lactogen	Nutrient content	655.00	1180.00	717.00	30.90	200.87
	Osmolar Conc.(m Osmol)	28.47	29.50	19.30	123.00	
Amul spray	Nutrient content	602.00	1388.80	855.00	35.84	
	Osmolar Conc.(m Osmol)	26.20	35.58	24.40	140.00	226.18
Vijaya spray	Nutrient content	480.00	1100.00	677.00	29.13	
	Osmolar Conc.(m Osmol)	20.88	28.10	19.00	116.52	184.50
Lactodex	Nutrient content	390.00	550.00	470.00	20.00	
	Osmolar Conc.(m Osmol)	16.90	14.10	13.42	80.00	124.00
* Amul roller dried	Nutrient content	602.00	1388.80	855.00	35.84	
	Osmolar Conc.(m Osmol)	26.2	35.58	24.40	140.00	226.48
*Glaxo	Nutrient content	831.60	1550.90	917.00	38.08	
	Osmolar Conc.(m Osmol)	36.16	39.66	25.56	152.12	253.50
Human milk	Nutrient content	161.00	508.30	389.95	12.00	
	Osmolar Conc.(m Osmol)	7.00	13.00	11.00	48.00	79.00
Cow milk	Nutrient content	575.00	1365.00	1026.60	33.00	
	Osmolar Conc.(m Osmol)	25.00	35.00	29.00	132.00	221.00

* Roller dried products.

level in the light of this, the solute load would appreciably come down from 250 to 150 m Osmol per day. It is evident that all the preparations incorporated high level of protein apparently with a view that increased protein may favour better growth. On the contrary, it is possible that it could exert adverse effect on infant kidneys in the aforesaid manner.

The osmolar load of sodium in these milks was 13.84 % of overall solute load as compared to 8.86 % in the case of human milk. Similarly the osmolar load of potassium in these milks was about 15.4 % as compared to 16.4 % in human milk. As regards chloride, it was 10.7 % as compared to 13.9 % in the case of human milk.

It could be seen that the contribution of sodium to the net osmolar load is more than the other two elements As has been discussed elsewhere, that these preparations ar often fortified with sodium salts to reduce curd tension. Such extra addition of sodium could be refrained in order to attain a low solute milk.

When viewed on the basis of sodium/protein ratio, it was seen that all the preparations exceed the ratio of 0.013 relevant for human milk.

A comparison of the solute load for infant foods with some foreign preparations are given inTable 45. These were found to contain more solute load. These foreign infant foods were of two kinds viz., humanised and non-humanised. The humanised milk preparations such as SMA,

Table 45 : Solute load of some foreign infant foods.

Product		Sodium (mg/lit)	Potassium (mg/lit)	Chloride (mg/lit)	Protein (g/lit)	Total osmolar load
*Nutramigen	Nutrient content	322.00	1053.00	850.80	22.00	
	Osmolar Conc.(m Osmol)	14.00	27.00	24.00	88.00	153.00
*Lofenalac	Nutrient content	483.00	1053.00	815.30	22.00	
	Osmolar Conc.(m Osmol)	21.00	27.00	23.00	88.00	159.00
*Portagen	Nutrient content	414.00	1053.00	638.10	23.00	
	Osmolar Conc.(m Osmol)	18.0	27.0	18.0	92.0	155.00
*Pregestimil	Nutrient content	322.00	936.00	815.35	22.00	
	Osmolar conc.(m Osmol)	14.00	24.00	23.00	88.00	149.00
**SMA	Nutrient content	230.00	819.00	638.10	22.00	
	Osmolar Conc.(m Osmol)	10.00	21.00	18.00	88.00	137.00
**Similac	Nutrient content	391.00	1092.00	850.80	24.00	
	Osmolar Conc.(m Osmol)	17.00	28.00	24.00	92.00	161.00
Whole cow milk	Nutrient content	575.00	1365.00	1026.60	33.0	
	Osmolar Conc.(m Osmol)	29.00	25.00	35.00	132.00	221.00
Present study	Nutrient content	390-831	550-1550	470-917	20-38	
	Osmolar Conc.(m Osmol)	17-36	14-39	13-25	80-152	124-254

* Fomon (1974).
** Widdowson (1965).

S_{26} had lower sodium, potassium and chloride. The ratio of sodium/protein too almost neared to that for human milk. Inspite the ratio, the total solute load was still high in these preparations and the protein content was more than the recommended allowance of 13.2 g/day. The protein in these infant foods was 22 g/100 g which indicated that humanisation in respect of protein quantity was not done.

Keeping in mind the immature nature of infant kidneys and the possible osmolar load, it would have to clear, when liquid formulae made from these preparations given during first four months of lives. Data on water load and osmolar load were calculated and given in Table 46. As mentioned before, dilutions recommended by manufacturers were taken into consideration while computing these values. It was found that in all cases except Lactodex the osmolar load decisively exceeded the load for human milk and was more similar to cow milk. Assuming that the extra renal losses of fluid by the infants remains at about 400 ml/day, as suggested by Fomon (1974) and with the intakes of the magnitude of 700 ml of these liquid formulae, the infant could be in negative water balance.

It would thus be evident from the aforesaid that this condition could be overcome by either increasing the water intake by infant or by decreasing the concentration of liquid formulae. Because of the limited capacity of child to consume higher volumes of liquid, it would be rather desirable to lower the concentration of liquid formula so as to come and closer to human milk.

Table 46 : Water and Osmolar load of some Indian infant foods.

Month	Lactogen			Amul spray			Vijaya spray		
	Milk powder (g)	Qnty. of water (ml)	Osmolar Conc. (m Osmol)	Milk powder (g)	Qnty. of water (ml)	Osmolar Conc. (m Osmol)	Milk power (g)	Qnty. of water (ml)	Osmolar Conc. (m Osmol)
Ist	95	690	194	120	720	234	105	720	197
2nd	115	700	227	150	750	282	105	750	196
3rd	135	850	219	180	950	266	125	900	194
4th	135	850	219	210	1050	282	150	1050	193
	Lactodex			Amul roller dried*			Glaxo*		
Ist	72	510	126	120	720	234	105	600	248
2nd	108	575	144	150	750	282	125	750	235
3rd	180	1125	144	180	950	266	150	850	249
4th	180	1125	144	210	1050	282	175	1000	248

* Roller dried products.

Microminerals

Trace elements assume important position in the nutrition of the living body by way of their involvement in various biochemical functions. Certain elements namely, iron, zinc, copper and manganese were selected because of their role in infant nutrition and the amount present in milk which is the chief diet of the infant. Thus, while ensuring adequacy of macro-nutrients, these micro nutrients in infant nutrition cannot be over looked. Although these preparations are based on milk and could be considered as a good source of certain macro nutrients, little is understood about this class of nutrients. Data on trace element contents in milk reported from several laboratories indicated that human milk, cow milk and buffalo milk are poor sources of trace elements (Macy and Kelly, 1961; Dang et al., 1982).

Iron

From the data on iron content in various formulae analysed (Table 47), it was found to range between 4.0 ± 0.001 mg/100 g in the case of Lactodex and 5.75 ±0.08 mg/100 g in the case of Vijaya Spray, Amul Spray and Glaxo. The statistical analysis of the data showed that Lactodex had iron content significantly lower than other preparations ($P < 0.01$) (Table 48). Differences in contents among other preparations were non-significant. When considered on the basis of iron content in the milk viz. 0.2mg/100 g,

Microminerals

Trace elements assume important position in the nutrition of the living body by way of their involvement in various biochemical functions. Certain elements namel iron, zinc, copper and manganese were selected because of their role in infant nutrition and the amount present in milk which is the chief diet of the infant. Thus, while ensuring adequacy of macro-nutrients, these micro nutrient in infant nutrition cannot be over looked. Although thes preparations are based on milk and could be considered as a good source of certain macro nutrients, little is understood about this class of nutrients. Data on trace element contents in milk reported from several laboratories indicated that human milk, cow milk and buffalo milk are poor sources of trace elements (Macy and Kelly, 1961; Dang et al., 1982).

Iron

From the data on iron content in various formulae analysed (Table 47), it was found to range between 4.0 ± 0.001 mg/100 g in the case of Lactodex and 5.75 ±0.08 mg/100 g in the case of Vijaya Spray, Amul Spray and Glaxo.

Table 47 : Content of certain trace elements in Indian infant foods.

Product	Iron	Copper	Zinc	Manganese
	mg/100 g			
Lactogen	5.500 ±0.070	0.370 ±0.004	3.38 ±0.021	0.025 ±0.003
Amul spray	5.750 ±0.080	1.000 ±0.001	3.41 ±0.022	0.025 ±0.0029
Vijaya spray	5.750 ±0.070	0.370 ±0.001	3.38 ±0.021	0.026 ±0.003
Lactodex	4.000 ±0.001	0.250 ±0.001	1.88 ±0.070	0.020 ±0.0001
* Amul roller dried	5.500 ±0.070	1.000 ±0.001	3.39 ±0.021	0.025 ±0.0029
* Glaxo	5.750 ±0.080	0.375 ±0.001	3.41 ±0.022	0.028 ±0.003

* Roller dried products.

Table 48 : Analysis of variance for iron.

Source	d.f.	S.S.	M.S.S.	F
Batch	1	0.0117	0.011	0.130^{NS}
Treatment	5	188.87	37.77	420.44*
Batch error	5	0.1212	0.024	0.269
Sampling error	36	3.23	0.089	

NS Not significant
* Significant at 1 % level
SE 0.149
CD 0.303

Table 49 : Analysis of variance for copper.

Source	d.f.	S.S.	M.S.S.	F
Batch	1	0.01	0.01	0.01^{NS}
Treatment	5	4.68	0.92	4.60*
Batch error	5	0.10	0.02	10.00
Sampling error	36	0.002	0.002	

NS Not significant
* Significant at 1 % level
SE 0.0223
CD 0.04533

Table 50 : Analysis of variance for zinc.

Source	d.f.	S.S.	M.S.S.	F
Batch	1	0.063	0.063	0.71^{NS}
Treatment	5	11.75	2.35	26.70*
Batch error	5	8.66	1.71	19.43
Sampling error	36	3.17	0.088	

NS Not significant
* Significant at 5 % level
SE 0.0148
CD 0.361

Table 51 : Analysis of variance for manganese.

Source	d.f.	S.S.	M.S.S.	F
Batch	1	0.0000625	0.0000625	0.1329^{NS}
Treatment	5	0.01079	0.0021	4.46*
Batch error	5	0.000001	0.0000002	0.0004
Sampling error	36	0.017	0.00047	

NS Not significant
* Significant at 1 % level
SE 0.0013
CD 0.0027

the values obtained are considerably higher. It was observed from the nutrition labels for all the formulae were fortified with ferrous sulfate. It should also be kept in mind while considering the advantages of iron fortification of such preparations that added ferrous compounds could as well saturate lactoferrin present in these infant formulae and in turn, reducing their ability to exert bacteriostatic effect, as reported by Weingberg (1978).

The possibility of some iron contamination also cannot be ruled out. Its nutritional significance,however, seems to be remote since such iron has been reported to be stored in the form of siderophilin in the liver(Underwood, 1956).

A comparison of the present data with values reported by Imamwa et al. (1969) for popular infant food formulae in Japan (4.84 mg/100 g), Gregorio et al (1983) for Itablian preparations namely Dodelac, Eulac, Preaptamil, Premifiori and Similac, 0.24 mg to 2.1 mg/100 g, Kirkpatrick et al. (1983) for Canadan preparation 2.0 mg to 3.0 mg/100 g, the values obtained in the present study are similar to the values obtained by Imamwa et al. (1969) but higher than the values obtained by Gregorio et al. (1983) and Kirpatrick et al. (1983), which was possibly due to lower level of fortifications in these infant foods.

On reconstitution of liquid formula according to the manufacturers, it was found that the iron content ranged between 0.53 mg/100 ml in the case of Lactodex to 0.86 mg/100 ml in case of Glaxo (Appendix IV). These, when compared with the values reported by Fomon (1974) **viz.** 1.27 mg/100 ml in Nutramigen, Lofenalac, Portagen and Pregestimil, the values are on the lower side as the American preparations were fortified at a higher level.

Nutritional Adequacy

When the iron intake from different infant foods were calculated on the basis of prescribed intakes of infant food, the level of iron available varied between 2.703 mg/day in the case of Lactodex and 4.51 mg/day in the case of Glaxo. These values were found much lower than 10 mg/day prescribed by American Nutrition Advisory Committee (1980) (Appendix III) when expressed on the basis of 100 K Cal. These infant foods contained 1.01 mg/100 K Cal in the case of Lactodex and 1.25 mg/100 K Cal in rest of the preparations. These contents were within the limits of Codex Alimentarius (Appendix II).

Several reports available in India and other countries indicated incidence of iron deficiency among infants (subsisting on commercial formulae (Gopalan and Raghavan, 1961; Woodruff et al., 1977). Since lactoferrin was effected due to processing, iron fortification in limited amount seem justified (Qarterman, 1982).

Copper

Data on copper content in various formulae under study are presented in Table 47. It was observed that these contents ranged between 0.25 ±0.001 mg/100 g in the case of Lactodex and 1.0 ±0.001 mg/100 g in the case of Amul spray as well as Amul roller dried preparations. The statistical analysis of the data showed that Lactodex had copper content significantly lower than other preparations ($P < 0.01$) (Table 49). Differences in the content among other preparations were, non-significant.

In the case of Vijaya Spray and Lactogen, values obtained were identical viz. 0.37 ±0.001 mg/100 g, whereas that in respect of Glaxo preparation was in between these two extremes. It was interesting to note that despite having identical ISI specifications, copper content was distinctly different in Vijaya spray and Amul preparations. It was observed that possibly in the case of latter, the formula was fortified with copper salt to make up the deficiency of milk in providing this nutrient to the extent upto 0.15 mg though it was not declared on the containers. It was further noted that except Lactogen, none other preparation declared such fortification on the container, though such a fortification was implied from the difference between the copper contents in the milk and those actually observed on analysis. Higher levels due to contamination arising from utensils during processing of these formulae do not appear feasible.

A comparison of data on Indian preparations reported by Dang et al. (1982) with our observations showed that the values for Lactogen and Amul preparations were in conformity with those observed in the present study. Small difference among other preparations could be accounted towards variation in the sampling as a result of batch to batch variation. Observations made by Dang et al. (1982) were based on a smaller number of samples.

In the present study, average values reported were a mean of twelve replicates derived from 4 batches.

When compared with values reported for popular infant food formulae in Japan containing 0.08 mg/100 g(Imamwa et al. 1969), Italian preparations namely Dodelac, Eulac, Preaptimil, Primifiori and Similac, containing 0.01 mg to 0.35 mg/100 g (Gregorio et al., 1983), Swedish preparations containing 0.04 to 0.44 mg/100 g(Soderhjelm, 1959), values obtained in the present study were considerably higher. This could be due to the fact that most of these were not fortified with respect to this trace element. The values reported by Kirkpatrick et al. (1980) for Canadian infant foods ranged from 0.43 mg to 0.79 mg/100 g and were quite similar to the values obtained in the present study.

On reconstituting liquid formula according to directions, it was found that the level of copper ranged between 0.33 mg/100 ml in case of Lactodex and 0.144 mg/100 ml in the case of Amul preparations (Appendix IV). These were comparable with those reported by Collip et al. (1983) who found

the copper content to vary between 0.06 mg to 0.170 mg/100 ml in several popular infant formulae in United States.

A comparison with the copper content showed that these levels were much higher than (0.02 to 0.05 mg/100 ml) present in human milk.

Nutritional Adequacy

When the copper intake from different infant formulae were computed on the basis of prescribed allowances of infant food, the level of copper available was found to vary between 0.168 mg/day in the case of Lactodex and 0.756 mg/day in the case of Amul preparation. It was observed that except Glaxo and Amul preparations, none other fulfil the nutritional requirements of 0.5 to 0.7 mg/day as defined by American Nutrition Advisory Committee (1980; Appendix III). These infant foods contained 0.08 mg/100 K Cal in the case of Lactogen and Vijaya spray to 0.218 in the case of Amul preparations which was within the limits of Codex Alimentarius (Appendix II). On the other hand, when the intakes from these preparations were viewed in the light of Codex Alimentarius recommendations (Appendix II), provided entire calorie requirements were met by respective preparations, the copper requirements could be satisfied. Although no report has been made so far on the incidence of copper deficiency among Indian subjects,especially among infants subsisting on commercial formulations, several reports in other countries showed existance of copper deficiency among infants given infant formulae (Lonnerdal et al., 1981). This was ascribed to the binding of added copper salts to high

molecular weight proteins, impeding copper absorption.

According to Widdowson (1973), breast fed infants who received 0.62 ug/ml copper showed no deficiency of copper. In the present study, the copper content in the liquid formula ranged from 0.4 ug/ml to 1.66 ug/ml. Apparently, Lactogen, Vijaya spray and Lactodex fail to meet this criterion. On the other hand, Cardano and Graham (1966) found that infants subsisting entirely on formula showed copper deficiency.

Zinc

Data on zinc content in various formulae under study are presented in Table 47. It was observed that the contents ranged between 1.88 ±0.021 mg/100 g in the case of Lactodex and 3.41 ±0.022 mg/100 g for Amul spray and Glaxo. The statistical analysis of the data showed that content in Lactodex was significantly lower than other preparations (Table 50) ($P < 0.05$). Differences in the content among other preparations were non-significant. Except for Lactodex, these values varied in a close range, though only Amul preparations and Vijaya spray conformed ISI specifications. It was implied that these formulae were fortified with zinc salt to make up the deficiency of milk powder which has approximately 2 mg/100 g of zinc mineral.

It was further noted that except for Lactogen which was fortified with zinc sulfate, to provide 3.5 mg/100 g of

zinc, none other preparations declared such fortification in the nutrition table, though such fortification was apparent from the difference between the zinc content in milk and those actually found on analysis.

A comparison with data reported elsewhere in this regard, it was found that there was similarity in values reported by Dang *et al.* (1982) viz. 3.41 mg/100 g for Amul, 3.4 mg/100 g for Glaxo, 3.9 mg/100 g for Lactogen and those found in the present study.

When compared with the values (0.33 mg/100 g to 4.55 mg/100 g) reported by Gregorio *et al.* (1983) for Italian preparations, Dodelac, Eulac, Preaptimil, Primifiori and Similac, the values in the present study were found similar in range. The values obtained by Kirkpatrick *et al.* (1983) for Canadian preparations, 1.64 mg and 4.94 mg/100 g were found to be higher than those found in the present study.

On reconstituting as directed on the container, it was found that zinc content ranged between 0.19 mg/100 ml for Lactodex and 0.48/100 ml in Amul preparation (Appendix IV). The values reported by Collip *et al.* (1983) for several liquid infant formulae ranged between 0.4 mg/100 ml to 1.2 mg/100 ml and were highly comparable with those found in the present study. Collip *et al.* (1983) had reported that the formulae were fortified with this mineral. These levels were markedly higher than those reported for human milk (0.11 mg/100 ml) (Fomon, 1974).

Nutritional Adequacy

When the zinc intake from different formulae were compared on the basis of prescribed allowances of 3 mg/day (American Nutrition Advisory Committee, 1982; Appendix III), the level of zinc available from these formulae varied from 0.96 mg/day in the case of Lactodex to 2.49 mg/day in the case of Amul preparations and were somewhat less. When expressed on the basis of calorie density, the zinc content in these infant foods was between 0.46 mg/100 K Cal in the case of Lactodex and 0.86 in the case of Amul preparations which was within the limits of Codex Alimentarius (Appendix II). Though no information is available on the incidence of zinc deficiency among Indian subjects specially among infants subsisting on commercial formulations, several reports in other countries showed existance of zinc deficiency among infants, given infant formulae (Lonnerdal et al., 1981; Hambridge et al., 1979; John and Evans, 1978). This was implied due to the binding of zinc to high molecular weight proteins impeding zinc absorption.

As pointed out by John and Evans (1978) that the zinc deficiency condition is remote in infants receiving breast milk, whereas it is fairly common among infants subsisting on infant food formulae unfortified in respect of zinc. Lonnerdal et al. (1981) showed the superiority of human milk over infant formulae in terms of bio-availability of zinc. Thus, in order to overcome the limitation of poor zinc bio-availability from artificial formulae, extra allowance has to be made by fortifying these preparations.

The quantities ranging between 0.96 mg/day from Lactodex to 2.49 mg/day available from Amul preparations would apparently meet about 32 to 83 percent of the recommended allowance for this trace element.

It should, however, be kept in view that biological availability of zinc is dependent on the simultaneous presence of other elements, particularly calcium and copper. The amount available from different preparations, therefore, have to be viewed in the light of contents of other minerals.

Manganese

Data on manganese content in various formulae under study are presented in Table 47. It was observed that these contents ranged between 0.020 ±0.0001 mg/100 g in Lactodex and 0.028 ±0.003 mg/100 g in the case of Glaxo. Except for Lactodex, the rest of the preparations were in close range. The statistical analysis of the data showed that Lactodexhad manganese content significantly lower than other preparations ($P<0.01$) (Table 51). Differences in the content among other preparations were non-significant. Though Lactogen, Glaxo and Lactodex did not conform to ISI specifications. (It would appear on the basis of milk powder used in these preparations that the manganese content should be 0.02 mg/100 g and it could be said that these were fortified with manganse salt).

A comparison of data obtained by Indian workers (Dang et al., 1982) showed that Amul contained 0.0458 mg/100 g, Glaxo contained 0.0465 mg/100 g and Lactogen contained 0.0357 mg/100 g which were higher than the findings in the present study.

When compared with values reported by Gregorio et al. (1983) for Italian preparations, Dodelac, Eulac, Preaptamil, Primifiori and Similac ranging from 0.03 mg to 0.199 mg/100 g, the values in the present study were lower. When compared with Kirkpatrick et al.(1983) for Canadian preparation, 0.084 mg/100 g to 0.113 mg/100 g, the values in the present study were lower which could be due to higher fortification, as reported by the worker.

In reconstituted preparations, it was found that the contents ranged between 0.002 mg/100 ml in the case of Lactodex and 0.003 mg/100 ml in all other preparations (Appendix IV) and when these were compared with findings of Collip et al. (1983) who found values for different brands to range between 0.02 to 0.01 mg/100 ml. These values in the present study were lower when compared with human milk (0.001 mg/100 ml) the values in the present study were higher.

Nutritional Adequacy

When quantities of manganese available from different infant formulae were computed on the basis of prescribed intakes of infant food, these were found to vary between

0.01 mg/day in the case of Lactodex and 0.018 mg/day in the case of Glaxo which were much below the recommended value of 0.5 to 0.7 mg/day by American Nutrition Advisory Committee (1980) (Appendix III). The manganese content was between 0.005/100 K Cal in the case of Lactodex and 0.0036/100 K Cal in the case of Lactogen which was within the limits of Codex Alimentarius (Appendix II).

It was observed that none of these preparations fulfilled the nutritional requirements. Although no information is available on the incidence of manganese deficiency among Indian as well as foreign subjects, a lone report by Collip et al. (1983) about higher manganese content in hairs in mentally disabled infants. This was attributed to high level of fortification of infant formulae in the range of 0.2 mg/100 ml to 0.1 mg/100 ml.

The recommended allowance between 0.5 and 0.7 mg/day by the American Nutrition Advisory Committee (Appendix III) was found to be too high as the fortification done in the light of this in several preparations such as Similac, Isomil, ProSobec and SMA, resulted in manganese intake in the range of 0.2 to 1.0 mg/day. It was observed that intake even at the level of 0.2 mg/day would raise the manganese content of hairs. This raises the question about the precise manganese requirement of infants and on the desirability of fortification of infant food formulae in this respect.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSION

This study was undertaken to assess the nutritional characteristics of certain popular milk based infant food formulae prevalent in the country. This was found necessary in view of the influence of manufacturing processes on the nutritional value of milk. Six milk based infant food formulae popular in different parts of the country were chosen for this investigation. These were prepared either by spray drying or roller drying process and under different managemental and manufacturing conditions. For every formula two independent replicates from each of three different batches were taken in this study. Of these six formulae, two were prepared by roller drying process, while the rest were manufactured using spray drying technique. Products included Lactogen (Delhi), Amul spray and Amul roller dried (Anand, Gujarat State), Glaxo (Bombay, Maharashtra State), Lactodex (Madras, Tamil Nadu State) and Vijaya spray (Hyderabad, Andhra Pradesh State).

These were evaluated in respect of their (i)proximate composition, (ii) protein quality by chemical as well as biological tests, (iii) nature of carbohydrates used in these preparations, (iv) fat and certain fat derived nutrients as well as its absorption, and (v) macro and micro minerals. Data obtained was critically evaluated in view of the recent norms stipulated by the Codex Alimentarius Commission and

the adequacy of nutrients discussed in the light of established recommended dietary allowances for infants.

a) Data on proximate composition expressed per 100 g showed protein to range from 14.39 g ±1.90 (Lactodex) to 21.86 g ±0.09 (Vijaya spray), fat between 5.76 g ±0.09 (Lactodex) and 18.50 g ±0.16 (Lactogen and both Amul preparations), total carbohydrates between 52.03 g ±0.28 (all preparations except Lactodex) and 71.87 g ±0.37 (Lactodex), total ash between 2.46 g ±0.13 (Lactodex) and 4.26 g ±0.14 (Vijaya spray), moisture between 2.33 g ±0.12 (Lactodex) and 2.41 g ±0.12 (Vijaya spray) and vitamin A between 1959 IU ±37 (Lactodex) and 2365 IU (Amul spray). In Lactodex the differences were significantly lower for all nutrients except carbohydrate which was significantly higher ($P < 0.01$) in comparison to other formulae. Differences in case of other preparations were non-significant. When data were viewed in the light of minimum and maximum limits stated by Codex Alimentarius Commission, it was found that whereas fat content was within the range in all preparations except Lactodex, protein content exceeded the upper limit. In the case of Lactodex, fat content was below, while protein content barely met the lower limit. Total carbohydrate content in all the preparations was much above the minimum level of 35 % specified by I.S.I. The daily

protein intake varied from 10.2 g (Lactodex) to 19.5 g (Glaxo). Except Lactodex, all other preparations exceeded values recommended by American Nutrition Advisory Committee, as well as Nutrition Expert Group of Indian Council of Medical Research (I.C.M.R.). As regards energy intakes from these infant foods, these varied from 306 K Cal/day (Lactodex) to 540 K Cal/day(Glaxo). These were within the prescribed limits given by Nutrition Advisory Board of Indian Council of Medical Research (I.C.M.R.). As regards vitamin A, these provided within the limit of 500 I.U./100 K Cal. The Carbohydrate content in liquid reconstituted formulae ranged between 6.91 g (Vijaya spray) and 7.80 g(Glaxo) per 100 ml which was close to the value of 7 g/100 ml in human milk.

b) The nutritive value of proteins was assessed by determining casein/whey ratio, curd tension, amino acid profile as well as with biological evaluation studies on rats. The casein content varied between 11.0 g $\pm$ 0.06 (Lactodex) and 16.70 g $\pm$0.001 (Lactogen). The difference was significant ($P/0.05$). On the other hand, whey protein ranged between 3.39 g $\pm$0.06 (Lactodex) and 5.34 g $\pm$0.12(Amul roller dried). Again differences were significant ($P/ 0.05$). The ratio however, appeared much closer, ranging from 3.04 $\pm$ 0.04 (Amul roller dried) to 3.47 $\pm$0.028(Lactogen).

These ratios, resembled the values for cow and buffalo milk, rather than human milk.

c) As regards curd tension for different formulae, values varied between 2 for Lactodex and 6 in case of Amul spray and Vijaya spray. These were markedly lower than the reported values for cow and buffalo milk, but higher than zero, value reported for human milk.

d) Amino acid profile on milk protein in these preparations was determined chromatographically by amino acid analyser. The values were expressed per 100 g protein. As regards the essential amino acid content, the values varied as follows : lysine 6.56 g (Glaxo) to 8.72 g (Vijaya spray), histidine 2.36 g (Vijaya spray) to 3.24 g (Lactodex), arginine (3.0 g (Amul spray) to 5.0 g (Lactodex), threonine 4.44 g (Lactodex) to 5.78 g (Amul roller dried), valine 3.0 g (Lactogen) to 4.66 g (Lactodex), methionine 2.02 g (Lactogen) to 3.20 g (Vijaya spray) to 4.24 g (Lactodex), tyrosine 3.34 (Amul roller dried) to 4.80 g (Vijaya spray), phenylalanine 3.8 g (Amul roller dried) to 5.84 g (Amul spray) and tryptophan 1.33 g (Lactodex) to 1.45 g (rest of the preparations.

e) Essential amino acid indices (EAAI) calculated from these data showed the values as follows : Lactogen 77.6, Amul spray 77.8, Vijaya spray 77.2, Lactodex 80.9, Amul roller dried 70.0, and Glaxo 75.6. The

values for roller dried (72.5) were slightly lower than spray dried (78.1). Also these values were lower than those reported for cow milk (88.0) and human milk(87.0).

f) Available methionine was determined microbiologically by using Spreptococcus zymogenes MCD 592. It was found that the values ranged between 1.46 g ±0.11/100 g protein (Amul roller dried) and 1.89 g ±0.001 (Amul spray). In comparison to total methionine content, availability varied between 66.4 % in the case of Amul roller dried and 81.20 %, in the case of Lactodex. Roller dried preparations showed about 9 % lower available methionine level than spray dried ones.

g) When daily essential amino acid intakes were computed and compared with FAO reference pattern, these were found to be four to five folds higher.

h) During biological evaluation for protein quality, nitrogen balance studies were conducted on rats. It was found that the true digestibility for the preparations was as follows : Amul roller dried 73.0 ±5.14 %, Lactodex 74.0 ±6.15 %, Amul spray 83.0 ±2.84 %, Vijaya spray 86.0 ±3.13 %, Glaxo 87.0 ±4.48 % and Lactogen 88.0 ±5.24 %. The difference in these values was non-significant. The values for roller dried were somewhat lower than spray dried ones. However, the differences were nonsignificant. Biological values

were found to be 94 ±2.40 % (Lactogen), 94 ±1.69%(Amul spray), 95 ±0.70%(Vijaya spray), 94 ±0.42%(Lactodex), 95 ±0.18% (Amul roller dried) and 94 ±2.16 % (Glaxo). The nitrogen deposited in the caracasses was estimated to determine NPU. for different proteins. NPU values were 72 ±0.10 % (Lactogen), 78 ±1.76 % (Amul spray), 76 ±1.76 % (Vijaya spray), 71 ±0.001 (Lactodex), 74 ± 1.49 % (Amul roller dried) and 74 ±2.44 %(Glaxo). Small differences observed were nonsignificant.

i) The quality of fat was determined by finding iodine value, content of essential fatty acids and fat absorption. The iodine value varied in the narrow range between 27.0 ±0.05 (Vijaya spray) and 30.16 ±0.05 (Lactogen). The difference was statistically significant ($P < 0.05$). These values were similar to cow and buffalo milk fat, but much lower than human milk fat. This suggested that the vegetable fat rich in polyunsaturated fatty acids was not used in any of these formulae.

j) As regards essential fatty acids, linoleic acid content expressed per 100 g fat, varied between 1.09 g ±0.015 (Glaxo) and 2.47 ±0.024 (Vijaya spray), linolenic acid between 0.03 g ±0.001 (both Amul preparations) to 0.61 g ±0.001 (Glaxo), arachidonic acid between 0.14 g ±0.002 (Lactodex) and 0.20 g ±0.016 (Lactogen).

Lactogen, Lactodex and Glaxo had significantly lower linoleic acid than rest of other three preparations ($P<0.05$). Amul spray, Amul roller dried and Vijaya spray had significantly less linolenic acid than rest of the three preparations($P<0.05$). The difference in arachidonic acid was, however, nonsignificant. The linoleic acid contributed between 43 mg/100 K Cal and 98 mg/1 K Cal in these preparations, which was far below 300 mg/100 K Cal recommended by Codex Alimentarius Commission.

k) The fat absorption was determined by conducting balance studies on rats. The fat absorption was 90.85 $\pm$0.60 % (Lactogen), 90.40 $\pm$0.36 % (Amul spray), 91.96 $\pm$0.68 % (Vijaya spray), 81.81 $\pm$0.49 %(Lactodex), 90.92 $\pm$0.80 % (Amul roller dried) and 91.18 $\pm$0.38 % (Glaxo). Fat absorption was identical in all formulae except Lactodex which showed significantly lower value ($P<0.05$).

l) The carbohydrates were further analysed to find various sugars used in these infant foods. Lactose was inherent in all preparations, which originated from milk. In most preparations except Lactodex, sucrose was used. In Lactodex, however, maltodextrin was used. None of the preparations contained either starch or glucose. The lactose content expressed per 100 g varied between 22.59 g $\pm$0.52 (Lactodex) and 28.12 g $\pm$0.36 (Lactogen). Lactodex had significantly less lactose ($P<0.05$). There was no difference in the lactose content of rest

of the preparations. Sucrose varied between 22.18 g ±0.13 and 24.68 g ±0.11. The differenceswere non-significant. Maltodextrin was 34.85 g in Lactodex.

m) As regards mineral content, certain major minerals namely calcium phosphorous, magnesium, sodium, potassium and chloride and trace elements namely iron, copper, zinc and manganese were determined. The calcium content expressed per 100 g, ranged between 0.85 g ±0.03 (Lactodex) and 0.89 g ±0.001 (Lactogen and Amul preparations), phosphorous content ranged between 0.38 g ±0.04 (Lactodex) and 0.63 g ±0.04 (Amul roller dried and Glaxo), magnesium content ranged between 58.0 mg ± 0.17 (Lactodex) and 126.0 g ±0.17 (Amul preparations). In the case of calcium, the differences were nonsignificant. In the case of phosphorous and magnesium, Lactodex contained significantly lower quantities of these minerals($P<0.05$). There was no difference between the rest of the preparations. The calcium to phosphorous ratios ranged between 1.4 and 2.34 and were within the prescribed range of 1.2 and 2.0 of Codex Alimentarius Commission.

n) The sodium content expressed per 100 g, varied between 292.50 mg ±1.20 (Lactodex) and 490.0 mg ±0.50 (Amul spray). Lactodex had significantly less sodium ($P<0.01$). There was no difference between the rest of the preparations.

The potassium content was between 402.50 ±5.40(Lactodex) and 878.0 mg ±5.40 (Glaxo). Chloride content was between 356.80 mg ±9.10 (Lactodex) and 519.67 mg ±10.10 (Glaxo). Lactodex had significantly less of these two minerals ($P < 0.05$). There was no significant difference in the content of these minerals in rest of the preparations. When compared with the values of Codex Alimentarius Commission, it was found that sodium, potassium and chloride content when expressed per 100 K Cal, these values were 40, 60 and 20 % more than Codex values, except Lactodex which was within the range.

From the osmolar load computed on the basis of protein and mineral content in a litre of liquid reconstituted formula, it was found that values varied between 124 m Osmol (Lactodex) and 254 m Osmol (Glaxo). These values were two to three times higher than 79 m Osmol reported for human milk and were closer to cow milk in most preparations, except Lactodex.

o) As regards trace minerals, iron, copper, zinc and manganese were analysed. Iron content expressed per 100 g ranged between 4.0 ±0.001 (Lactodex) and 5.75 mg ±0.08 (Glaxo, Amul spray and Vijaya spray), copper content ranged between 0.25 mg ±0.001 (Lactodex) and 1.0 mg ±0.001 (Amul preparations), zinc content varied between 1.88 mg ±0.07 (Lactodex) and 3.41 mg ±0.022 (Glaxo and Amul spray), manganese content was between 0.02 mg ±0.001 (Lactodex) and 0.028 mg ±0.003(Glaxo).

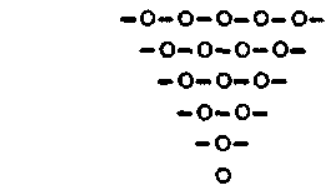
Lactodex had significantly lower iron, copper, zinc and manganese content than rest of the preparations ($P<0.05$). There were significant differences in the rest other preparations except in the case of Copper for which Amul preparations had significantly higher content of copper ($P<0.05$). These values were about 15 % higher than the values prescribed by Codex Alimentarius Commission except for manganese which was within the range.

CONCLUSIONS

The nutritional evaluation of various infant formulae made in the course of this investigation demonstrated that the manufacturing or managemental practices do not affect the quality of important nutrients such as protein and fat. Although the level of available methionine was somewhat reduced in roller dried preparations in comparison to spray dried ones, this did not apparently affect the protein quality. The amount of methionine available was still sufficient to meet body requirements. The protein content in all these preparations, barring a few, warrants a serious reconsideration.

The redundant supply of protein in the formulae which was about two fold higher than actual protein requirement, may not only result in metabolic wastage of this precious nutrient, but it would substantially contribute to

the Osmolar load which needs to be carefully controlled, in view of premature infant kidneys. Likewise, the use of various sodium salts as neutralisers, stabilisers or curd tension lowering agents should be curbed. It was found that sodium content in all preparations grossly exceeded the maximum limit set by Codex Alimentarius Commission. As regards fat content, though it is adequate to fulfil the recommendation of Codex Alimentarius Commission, it was found to be awfully inadequate to fulfil essential fatty acid requirements of infants. Modification of existing formulae would go a long way not only in ensuring more nutritious and wholesome product but also may work out to be more economically feasible proposition.



REFERENCES

Abou Dawood,A.E. and El. Sawaf S.	1977	Egypt.J.Dairy Sci.5, 129.
Ainscough, E.W., Brodie, A.M. and Plowman, J.E.	1980	Am.J.Clin.Nutr. 33,1314-1315.
American Oil Chemists Society (AOCS)	1959	Cd. No.7.78 Published by 35, East Wacker Drive,Chicago 1, Illinois.
Ames, B.N.	1966	In "Methods of Enzymology", Vol.VIII, 115.
American Nutrition Advisory Committee	1980	United States Congress, Infant Formula Act (1980). Washington D. Room Public Law 96-359. United States Capital Health Documents. Cited. Am.J.Clin.Nutr. 1982,35(2),381.
Andrews, N.	1969	Biochem. J. 111, 14. Cited. Am.J.Clin.Nutr.33, 1861.
Anderson, T.A., Fomon, S.J. and Filer, L.J.	1972	J. Lab.Clin.Med. 79, 31.
Anderson, T.A., Meennise, G. and Fomon, S.J.	1974	In "Infant Nutrition". Ed. Fomon,S.J. Pat Sander Company, Philadelphia. Lond. Toronto.
Arvidson, U.B., Draper, W.L. and Walker, A.R.P.	1954	Trans. Roy. Soc. Med. 48,315.
Association of Official Analytical Chemists (AOAC).	1980	Cd.No.2.049. Pub. Benjamin, F. Franklin Station,Washington,D.C.
Association of Official Analytical Chemists (AOAC).	1980	Cd.No.31.040. Pub.Benjamin. Franklin Station,Washington,D.C.
Bartlop,D. and Oppe,T.E.	1970	Lancet ii, 1333-35.
Barness, L., Baker, D., Guilbert, P., Torres, F.E. and Gyorgy, P.	1957	J. Pediat. 51 (1), 29.

Belavady, B. and Gopalan,C.	1956	J. Trop. Paediat. 2, 89.
Belavady, B. and Gopalan, C.	1959	J. Med. Res. 47, 225.
Belavady, B.	1960	Ind. J. Med. Res. 48, 654.
Belavady, B. and Gopalan, C.	1962	Bull. WHO 26, 203.
Bender and Miller	1955	Brit. J. Nutr. 9, 382.
Bingham, E.C. and Zittle, C.A.	1964	Arch. Biochem. Biophys. 106,235. Cited. Am.J.Clin.Nutr.33, 1861.
Bindra, H. and Deodhar, A.D.	1980	J.Dairy Sci. 33, 508.
Bodwell, C.E.	1967	In "Evaluation of Proteins for Humans". Ed.Bodwell,C.E. Pub. AVI.
Bown,K.H., Black, R.E., Robertson,A.D. and Akhter,N.A.	1982	Am. J. Clin. Nutr. 35, 745-756.
British Standards Institute Bulletin (BSI)	1963	Cd. No. 1741 British Standards House 2, Park St., London.
Brooke, O.G.	1980	Brit.J.Nutr., 44, 13-23.
Carbo, S.	1959	Lattante. 30, 625-659. Cited. Nutr.Abst.Rev.Abst.4317.
Cardano,A. and Graham, G.G.	1966	Pediat. 38, 596-604.
Carr, F.H. and Price, E.A.	1926	Ed.No.2052(1968) ISI Bull. Pub. Manak Bhawan,NewDelhi.
Caruso, P. and Ciliberto, R.	1970	Minnerva Pediat. 22 (4) Suppl. 23 - 25. Cited. Nutr.Abst.Rev.40 Abst.7828.
Chandrashekhara,M.R., Sreeniwasmurthy,V., Swaminathan, M., Bhatia,D.S. and Subrahmanyan.	1957	Fd. Sc. Mysore, 6, 228-232.

Chaudhury,S. and Kansal,V.K.	1980	M.Sc.Thesis "Bio-availability of calcium,magnesium, phosphorous from milk and milk products and weaning foods", NDRI, Karnal.
Chandra, R.K.	1978	Nutr. Rev. 36, 265.
Cheftel, J.C.	1979	In "Nutritional Safety Aspects of Food Processing". Ed.Tannenbaum, S.R. Pub.Marcel Bekker INC. NY. Basel.
Chiel, H.J. and Wirtman,R.J.	1981	Science, 213, 676.
Chiodot, L.A. and Saller, C.F.	1980	Science, 210, 1269.
Cockran, W.G. and Cox, G.M.	1977	In "Experimental Designs". Ed. Cockran, W.G. and Cox,G.M. Pub.Asia Publishing House.
Codex Alimentarius Commission	1976	CAC/RS 72/74. FAO/WHO Bull. Pub. Rome.
Collip, A.J., Chen, S.Y. and Maitinsky, S.	1983	Ann. Nutr. Metab. 27, 488-494.
Cosin,R.J. and Smith,K.T.	1979	Nutr.Rev. 37(4) 97-103.
Crane, S.C.and Lachance, P.A.	1983	Nutr. Report Inter.28(5),991-997.
Crook, L.	1974	Pediat. 54, 656.
Crosse,V.M., Wallis, P.G., Low, A. and Henly, A.V.	1960	Nutr. 14, 65-74.
Cuthbertson,W.F.J.	1976	Am.J.Clin.Nutr.29, 559-568.
Dahl,L.K.	1972	Am.J.Clin.Nutr. 25, 231.
Dallman,P.R., Simes, M.A. and Stekel,A.	1980	Am.J.Clin.Nutr. 35, 86.
Dang,H.S., Jaiswal, D.C., Dacosta, H. and Samasundaram, S.	1982	J.Radio Anal. Chem.70(2),163-174.

Daniel, V.A., Desai, B.L.M?, Rao,S.V., Swaminathan, M. and Parpia,H.H.B.	1968	J.Fd.Sci. 33(3), 331-34.
Davies, D.T. and White, C.D.	1962	J.Dairy Res. 29, 285.
Dean,F.J. and Wetsch, R.C.	1934	"Penn Agri. Expt.Station, Bull. 312, 35. In "Homogenised Milk". Ed.Trout,G.M. 1950. Pub. Michigan State College Press, East Lansing.
Dellany,R.A.M.	1976	J.Soc.Dairy Tech.,29, 91.
Deodhar,A.D.and Ramakrishnan,C.V.	1960	J.Trop.Pediat, 6(2), 44-47.
Deodhar,A.D., Rajalakshmi, R. and Ramakrishnan,C.V.	1964	Acta. Pediat. 53,42-48. Cited. Am.J.Clin.Nutr.33, 1861.
De Portella, M.L., Zeni, S., Piazza, N., Garcia, N., Garcia, H. and Rio, M.E.	1983	Nutr.Reports Inter. 28(5),1091.
De Vittali,H.P.	1965	Lattante. 36, 537-544. Cited. Nutr.Abst.Rev.36,Abst.2959.
Edelman, C.M.Jr., Barnet, H.L.and Tronpkov, V.	1960	J.Clin.Invest. 39, 1062.
Eid, E.E.	1970	Brit.Med.J. 2, 74.
SPAGAN Committee Report.	1977	Kinderarztliehe Prascis, 47(1), 11-26. Cited. Acta Paediatrica Scandinanica,4977 Suppl.262.
Farquhar,J.D.	1963	Am.J.Dischild,106,201-206.
Fomon, S.J.	1960	Pediat. 26(1),51-61.
Fomon, S.J.and Owen,G.M.	1962b	Pediat. 29, 1005.
Fomon, S.J., Owen,G.H., Jensen, R.L. and Thomas,L.N.	1963	Am.J.Clin.Nutr. 12, 346-357.

Fomon, S.J. and Filer.	1967	In "Amino Acid Metabolism and Genetic Variation". Ed. Nyan,W.L. Pub.McGraw Hill.p391, NY.
Fomon, S.J.	1974	In "Infant Nutrition". Ed.Fomon, S.J., Pub.Sander Co.Philapelphaa Lond. Toronto.
Fomon, S.J.and Ziegler,E.E.	1971	Am.J.Clin.Nutr.23(10),1293-1313.
Food Agriculture Organisation(FAO)	1965	WHO Tech.Report Ser. 301, 35.
Ford,J.E.	1964	Brit.J.Nutr. 18,449.
Forsum, E. and Hambreus,L.	1972	Nutr. Metab. 14, 48.
Forsum, E.and Lonnerdal, B.	1980	Am. J. Clin. Nutr. 33, 1809-13.
Friedman, C.P., Jack,E.L.and Smith, L.M.	1965	WHO Tech. Report Ser.301, 35.
Ganguli, N.C.	1964	J.Dairy Sci. 47, 13-18.
Ganguli, N.C.	1979	World Animal Rev. 30, 2-40.
Gaull, G.E., Rassin, D.K., Raiha, N.C.R. and Heinoven, K.	1977	J. Pediat. 90, 348.
Ghosh, S.	1977	In "The Feeding and Care of Infants and Young Children", Pub.Voluntary Health Association of India.
Goalwin, A. and Pomerange,J.	1962	Arch.Pediat. 79, 58-62.
Gopalan, C.and Raghavan, K.V.	1961	In "Nutrition Atlas", Pub. NIN, Hyderabad.
Gopalan, C., Ramashastri, V. and Balasubramanian,C.	1981	In "Nutritive Value of Indian Food." Pub. NIN, Hyderabad.
Gregario, P., Sircusano, C., and Toscano,G.	1983	Igiene Moderna, 77(6),801-810. Cited. Nutr.Abst.Rev. 53, Abst. 1240.

Gunther, M., and Stanier,J.E.	1952	Brit. J. Nutr. 6, 215.
Gurr, M.	1981	J. Dairy Res. 48, 519.
Gyorgy, P.	1955	J. Bact. 69, 483.
Gyorgy, P.	1962	Science, 137,338-340.
Hall, B.	1975	Lancet, 1, 779-781.
Hall, B. and Muller, D.P.R.	1980	In "Human Milk - Its Biologic and Social Value". Ed.Frier,S., Eidelman,A.I. Pub.Excerpta Medica Amsterdom, Oxford, Princeton.
Hamosh, M.	1979	Pediat. Res. 13, 615-622.
Hambridge,K.M., Walravens, P.A., Casex, C.E., Bown, R.H. and Bender, C.	1979	J.Pediat, 94(4), 607-608.
Hanesen, H.E., Haggard, M.E., Boelsche, A.N., Adam, D.J.D., and Wiese, H.F.	1958	J. Nutr. 66, 565-576.
Hanna, F.M., Navarrette,D.A. and Hso,F.A.	1970	Pediatrics, 45, 216.
Hanes P.	1980	In "Sugar Analysis", Ed.Bown,C.A. and Zertain,F.W. Pub.John Wiley & Sons,N.Y.
Harkova, R.M.	1969	Vop.Pitan. 28(1), 43-49. Cited. Nutr.Abst.Rev.38, Abst. 3328.
Harvey,D.R., Cooper, L.V.,and Stevens,J.F.	1970	Arch.Dis.Child. 45, 506-509.
Harvel, O.and Oliver Crona,T.	1974	Biochem. Biophys. Acta., 369, 234.
Have,V.D. and Mulder, H.	1957	Neth. Milk Dairy J. 11, 128.

Henry, K.M. and Kon, S.K.	1953	Brit.J.Nutr. 1, 29.
Hendrick,X.G.V.	1962	Ann.Pediat. 198, 358. Cited. Am.J.Clin.Nutr.,33, 1861.
Hendrick,X.G.V.	1963	Pediat 31, 1019. Cited. Am.J.Clin.Nutr.,33, 1861.
Heinz, F., Welsch, H., Weichmann, B., and Shihlfanth,K.	1966	Klin Wockonscher 44,1189-1193. Cited. Nutr.Abst. Rev. 37, Abst. 3015.
Hendrick, X.G.V.	1971	Ann.Pediat 238, 371. Cited. Am.J.Clin.Nutr.,33, 1861.
Heyn, S.T., Miller, E.L., Carpenter,K.J., and Milner,C.K.	1965	Brit.J.Nutr. 19, 547.
Holman, R.T., Caster, W.O. and Wiese, H.F.	1964	Am.J.Clin.Nutr.,44, 70-75.
Holt,L.E. and Synderman, S.E.	1967	In "Amino Acid Metabolism and Genetic Variation", Ed. Nyan, W.L., Pub.McGraw Hill,NY.
Husband, J., Husband, P.and Mallinson, C.N.	1970	Lancet, 1, 290.
Huffman, S.L., Chowdhury,D.A., and Chakraborty,D.L.	1980	Am.J.Clin.Nutr.,33, 144-154.
Hytten, F.E.	1954	Brit. Med.J., 1, 176.
Immamwa,T., Naito, H. and Aoki, T.	1969	J.Jap.Soc.Fd.Nutr., 22(6), 361-366.
Indian Standards Institute Bulletin (ISI).	1958	Cd.No.1224, Part I, Pub.Manak Bhawan, New Delhi.
Indian Standards Institute Bulletin (ISI)	1966	Cd. No.3508, Pub.Manak Bhawan, New Delhi.

Kirkpatrick, D.C., Collins, B., Conacher, H.B.S., Mckenzie, A.D., Meranger, J.C., Lactoix,G.M.A., Dabenka, R. and Sauary, G.	1983	Cand. J. Fd. Tech. 11(3),154-161.
Klee,W.A. and Klee, C.B.	1970	Biochem. Biophys. Rescommun. 39, 833. Cited. Am.J.Clin.Nutr.,33,1861.
Kleinbaum, H.	1967	Ztscher Kinderheik,101,111-119. Citd. Am.J.Clin.Nutr.33,1861.
Kon,S.K. and Mawson,E.H.	1950	In "Human Milk,Wartime studies of certain vitamins and other constituents". Ed.Kon,S.K.,Meuson,E.H. M.R.C.Suppl.Rep. No.269, Pub.H.M.Stationery House,Lond.
Lohr, H., Stunn, J., and Wolf, H.	1965	Med.Ernährung, 6,7-9. Citd.Nutr.Abst.Rev.35,Abst.4325.
Lonnerdall,B., Forsum, E., and Hambreus, L.	1976	Am. J. Clin. Nutr.,29, 1127.
Lonnerdal, B., Keen, C.L., and Hurley, L.S.	1981	Ann. Rev. Nutr. 1, 149-174.
Macy, I.G. and Kelly, H.J.	1961	In "Milk. The Mammary Gland and and its Secretion." Ed.Kon,S.K.Cowie, H. Pub. Academic Press,N.Y. Lond.
Martin, M.T., Jacobs, F.A.,and Brushmiller,J.G.	1984	J.Nutr., 114, 869-879.
Matson,F.H.	1964	J.Biol.Chem.,239, 2772.
Mauron, J., Mottu, F., Bujard, E.,and Egli,R.H.	1955	Arch. Biochem. Biophys. 59, 433-451.
McCaulay,D.and Watson, K.	1957	Arch. Dis Child.,42, 485.
McDonald,F.J.	1966	Nature, 209, 1134.

Kirkpatrick, D.C., Collins, B., Conacher, H.B.S., Mckenzie, A.D., Meranger, J.C., Lactoix,G.M.A., Dabenka, R. and Sauary, G.	1983	Cand. J. Fd. Tech. 11(3),154-161.
Klee,W.A. and Klee, C.B.	1970	Biochem. Biophys. Rescommun. 39, 833. Cited. Am.J.Clin.Nutr.,33,1861.
Kleinbaum, H.	1967	Ztscher Kinderheik,101,111-119. Citd. Am.J.Clin.Nutr.33,1861.
Kon,S.K. and Mawson,E.H.	1950	In "Human Milk,Wartime studies of certain vitamins and other constituents". Ed.Kon,S.K.,Meuson,E.H. M.R.C.Suppl.Rep. No.269, Pub.H.M.Stationery House,Lond.
Lohr, H., Stunn, J., and Wolf, H.	1965	Med.Ernaerung, 6,7-9. Citd.Nutr.Abst.Rev.35,Abst.4325.
Lonnerdall,B., Forsum, E., and Hambreus, L.	1976	Am. J. Clin. Nutr.,29, 1127.
Lonnerdal, B., Keen, C.L., and Hurley, L.S.	1981	Ann. Rev. Nutr. 1, 149-174.
Macy, I.G. and Kelly, H.J.	1961	In "Milk. The Mammary Gland and and its Secretion." Ed.Kon,S.K.Cowie, H. Pub. Academic Press,N.Y. Lond.
Martin, M.T., Jacobs, F.A.,and Brushmiller,J.G.	1984	J.Nutr., 114, 869-879.
Matson,F.H.	1964	J.Biol.Chem.,239, 2772.
Mauron, J., Mottu, F., Bujard, E.,and Egli,R.H.	1955	Arch. Biochem. Biophys. 59, 433-451.
McCauley,D.and Watson, K.	1957	Arch. Dis Child.,42, 485.
McDonald,F.J.	1966	Nature, 209, 1134.

Mc Millan, J.A., Landaw, S.A. and Oski, F.A.	1976	Pediatrics (USA), 58(5),686-691.
Mehta, A.K.	1981	Ph.D. Thesis "Effect of Homogenisation on Selecting Nutritional Characteristics of Buffalo Milk", NDRI, Karnal.
Mellander, O., Vahlquist, B. and Mellbin,T.	1959	Acta Pediat. 48,Suppl.(116),108.
Miller,D.S. and Bender,A.E.	1955	Brit.J.Nutr., 9, 382.
Miller,G.A. and Lachance,P.A.	1975	In "Evaluation of Protein for Humans."p.149. Ed. Bodwell,C.E. Pub. A.V.I.
Misra,R.P.	1959	Ind. J.Pediat. 26, 290.
Mitchell,H.H.	1923-24	J.Biol.Chem., 58, 387. Cited by Venkata,Rao., Narayana Rao, Swaminathan, M. and Subrahmanyan, V., J.Nutr.Dietet.1,42, (1964).
Mukherjee,P.S.	1959	Ind.J.Pediat. 26, 467-476.
Nagasawa, T., Kiyosana, I., and Kuwahara,L.	1970	J.Dairy Sci., 53, 136.
Naismith, D.J., Deeprose, S.P., Supramanian, G., and Williams,M.J.H.	1978	Arch.Dis Child.,53(11),845-849.
Narayana,Rao M., Sreenivas, M., Swaminatha, M., Carpenter,K.J. and Morgan,C.B.	1963	J.Sc.Fd.Agri., 14, 544.
Natelson, S., Pennicall, R., Crawford, W.L., and Munsey,F.A.	1955	J. Dis Child., 89, 656-668.
Nickerson, T.A., Vujicacie,I.F. and Lin, A.Y.	1976	Dairy Sc. J., 59, 386.

Ogra,P.L., Fishant,M., and Theodore,C.	1980	In "Human Milk, its Biological and Social Value". Ed.Frier,S.,Edelman,A.I., Pub.Excerpta Medica,Amsterdom, Oxford,Princeton.
Oppe,T.E.and Redstone,D.	1968	Lancet 1, 1045-1048.
Oser,B.L.	1959	In "Protein and Amino Acid Nutrition". Ed. Albanese,A.A. Pub.Academic Press,N.Y.
Pantulu,P.C., Unnikrishnan,V., and Ramamurthy,MK.	1982	J.Fd.Sc.Tech., 19, 113.
Parpia,H.A.S.	1972	In "Proceedings of the First Asian Congress of Nutrition", Pub.NIN,Hyderabad.
Peter,F.E.	1953	Brit.J.Nutr., 7, 208.
Plenert,W., Gassman,B. and Heine, W.	1965	Deutsch.Gesundherwesen, 20, 1139-1143. Cited. Nutr.Abst.Rev.36, Abst.1036.
Pop, O., Hurgoin,V.and Harcu,A.	1977	Pediat Romania,26(2),177-181. Cited. Nutr.Abst.Rev. 48, Abst. 6024.
Qartermann,J.	1982	Proc.Nutr.Soc., 42, 45A.
Raiha,N.C.R., Heinman, K., Rassin, D.K.and Gaull,G.E.	1976	Pediatrics, 57(5),659-674.
Raina, R.	1980	Ph.D. Thesis "Some Metabolic Aspects of Utilization of Milk Fat from Cow Fed with Protected Fat", NDRI, Karnal.
Rajlakshmi,R.and Ramakrishnan,C.V.	1964	Acta Paediat., 52, 473-78.
Rajlakshmi,R., Ramakrishnan,C.V. and Deodhar,A.D.	1964	Acta Paediat., 54, 101.
Rajlakshmi, K. and Srikantia,S.G.	1976	Am.J.Clin.Nutr., 29, 664.
Rao,R.V., Chopra,V.C., Stephen,J. and Bhalerao, V.R.	1964	J.Fd.Sc.Tech., 1. 19.

Rapp,D.J.	1978	J.Learning Dis., 11, 56.
Rassin,D.K., Gaull,G.E., Raiha,N.C.R.and Heinoven,K.	1977	J.Pediat. 90, 356.
Rolls,B.A. and Porter,J.W.G.	1973	Proc.Nutr.Soc., 32, 13.
Rosero,O.R., McDonald,L.R., Martin,F.G., Conrad,J.H., and Ellis,G.L.	1983	Nutr.Reports, 28(5), 1199.
Rowlands,S.J.	1938	J.Dairy Res., 9, 42.
Rubini, L., Landucci, L. and Mazzeo,G.	1959	Lattante,30, 464-482. Cited.Nutr.Abst.Rev.,31, Abst.945.
Saller,C.F. and Chiodo,L.A.	1980	Science, 210, 1269.
Samadi,A.R., Wahed,M.A. and Islam, M.R.	1983	Nutr.Reports Inter. 28(5), 1101.
Sandell,S.	1959	In "Colorimetric Determination of Trace Elements." Pub.Inter Science,N.Y.
Schmidt,C.W.	1968	Med.Ernaharung, 9,124-125.
Schultz,D.L.and Ashworth,U.S.	1974	J.Dairy Sci., 57, 992.
Seiffert,E.	1969	Kinderharztl Prox.,36,183-90. Cited. Dairy Sci.Abst.31, Abst. 1356.
Shaw,J.C.L.	1973	In "Nutritional Problems in a Changing World", Ed.Hollingworth,D. and Russell, M., Pub. Applied Science.
Shaw,J.C.L., Jones,A. and Gunther,M.	1973	Brit.Med.J., 2, 12-15.
Shaw,J.C.L.	1976	Pediat. 57(1), 16-25.
Spies, J.H., and Chamber,D.C.	1949	Analyst Chem.,21, 1240.

Singh,M.N.	1983	Ph.D. Thesis "Studies on the Modification of Bovine Milk for Development of Infant Formulae", NDRI, Karnal.
Sklanunu, Zurukzoglu,S., Mametetzis,C. and Katriu,D.	1965	Hew Paediat Acta. 20, 193. Cited.Am.J.Clin.Nutr.33, 1861.
Slein,M.W.	1963	In "Methods of Enzymatic Analysis" Ed.Hans Ulrick Bergmeyer, Pub.Academic Press,N.Y.,Lond.
Snedecor,G.W.and Cochran,W.G.	1967	In "Statistical Methods", Pub.Iowa State University Press, Ames, USA.
Soderhjellam,L.	1959	Acta Soc.Med.Upsala, 64, 499-501. Cited. Nutr.Abst.Rec., 30, Abst. 4452.
Someswara,Rao, K. Swaminathan,M.C., Swarup,S. and Patwardhan,V.N.	1959	WHO Bull. 20, 603-639.
Southgate,D.A.T. and Barrett,M.	1966	Brit.J.Nutr., 20, 363.
Southgate,D.A.T.	1969	J.Sc.Fd.Agri., 20, 326.
Spies,J.R. and Chamber,D.C.	1949	Analyst Chem., 21, 1240.
Staemler,H.G.	1969	Med.Ernaharung, 10, 236-238.
Stampar,Plasaj B., Valjak,B., Louric, M., Barisic Seper, I. and Kelerbaccha,M.	1974	Harna-1. Isharana 15,11/12,509-22. Cited.Nutr.Abst.Rev.46, 3047.
Stastny,D., Vogel,R.S.and Picdano,M.F.	1984	Am.J.Clin.Nutr., 39, 872-878.
Stewart,R.H.E., Platon, and Kelly,V.J.	1958	J.Biol.Chem., 232, 777. Cited.Am.J.Clin.Nutr., 33, 1861.

Swaminathan,M.S. and Parpia,H.A.S.	1968	World Rev.of Nutr. and Dietet. 9, 206-226.
Swaminathan,M.	1977	In "Essentials of Food and Nutrition". Pub.Ganesh and Co.,Madras-17.
Taitz,L.S.	1971	Brit.Med.J., 1, 315.
Taitz, L.S.	1973	In "Nutritional Problems in a Changing World", Pub.Applied Science Publishers, Lond.
Taitz, L.S.	1979	Arch.Dis Child, 53(9), 697-700.
Tanaka, Y., Haltano, S., Nishi,Y.and Usin,T.	1980	J.Pediat, 96(2),255-57.
Tomarcelli,R.H., and Bernhart,F.N.	1962	J.Nutr., 78, 44.
Tomarelli,R.H., Meyer,B.J., Weaber,J.R.and Bernhart,F.N.	1968	J.Nutr., 95,583-590.
Tracy,V.V. and Harper,J.R.	1971	Brit.Med.J. 1, 16.
Underwood,E.J.	1956	In "Trace Elements". Pub.Academic Press,N.Y.
Udani,M., Shah,P.M., Ramdas,R., Shendarkar,S. and Parekh,V.C.	1970	Ped.Clin.Ind., 5, 123.
Van,De., Kramer,J.H., Hutnick,H., Tenbokeland, Weyers,H.A.	1949	J.Biol.Chem., 177, 347.
Vaughan,O.W. and Filer,L.J.	1960	J.Nutr., 71, 10.
Vaughan,L.A. Weber,C.W.and Kemberling,S.R.	1979	Am.J.Clin.Nutr.,32,2301-2305.

Venkatachalam,P.S.	1962	In "A Study of the Diet Nutritional and Health of the People of the Chimbu Area,New Guinea", Monograph No.4, Cited."Infant Nutrition in Sub-tropics and Tropics", Ed.Jelliffee,D.B.
Venkatachalam,P.S., Susheela,T.P. and Parvati,R.	1967	J.Trop.Pediat. 13, 70-76.
Vignetti,P. and Sbraccia,C.	1958	Lattante, 29, 172-184. Cited. Nutr.Abst.Rev. 27, Abst. 1118.
Walravens,P.A., and Hambridge,K.M.	1976	Am.J.Clin.Nutr.,29(10),1114-21.
Walravens,P.A.	1980	Clin.Chem., 26(2),185-189.
Wardlaw,H.S.H., and Dart,E.P.	1926	Aus.J.Expt.Biol.Med.Sci., 3, 129.
Wasserman, R.H. and Comer,C.L.	1959	Proc.Soc.Expt.Biol. Med., 101, 314.
Weinberg,E.D.	1978	Microbiological Reviews, 42(1), 45-66.
Widdowson,E.M.	1965	Lancet, 2, 1099.
Widdowson, E.M.	1973	In"Nutritional Problems in a Changing World." Ed.Hollingworth,D.,Russel,M. Pub.Applied Science,Lond.
Widdowson,E.M.	1974	Arch.Dis Child, 49, 867.
Wiese,H.F., Hansen,A.E.and Adam,D.J.D.	1958	J.Nutr.,66, 345.
Williams,M.L., Morrow,C.S.G., Sloan,S.E. and Barness,L.E.	1970	Am.J.Clin.Nutr.,23,1322-30.
Woodruff,C.W. Latham,C.,and Mc David,S.	1977	J.Pediat. 90(1),36-38.

Wurtman,D.and Fernstrom,T.	1975	In "Frontiers in Neurology and Neurosamine Research". Ed. Seeman P., Brown,G., Pub.University of Toronto Press.
Ziegler,E.E. and Fomon,S.J.	1971	J.Pediat, 78, 561.
Ziegler,E.E. and Fomon,S.J.	1983	J.Pediat Gastr.Nutr.,2,288-294.
Zoppi,G., Zamboni,G., Sitzere,M., Zellini,P. and Cancellieri,M.L.	1978	Pediat(USA), 62(6),1010-1018.

-o-o-o-

APPENDIX

APPENDIX I

Composition of assay medium
(*S. zymogenes* NCDO 592)

Basal medium	
Glucose	12 g
K_2HPO_4	12 g
Citric acid	0.5 g
Sodium acetate trihydrate	0.5 g
Tween 80	1.0 ml
Solution of mineral salts	10 ml
Adenine	5.0 mg
Guanine	5.0 mg
Uracil	5.0 mg
Xanthine	5.0 mg
Thiamine	2.0 mg
Pyridoxal ethylacetal hydrochloride	2.0 mg
Riboflavin	2.0 mg
Nicotinic acid	2.0 mg
Calcium pantothenate	2.0 mg
P-amino benzoic acid	2.0 mg
Folic acid	0.2 mg
Biotin	10.0 ug
Ascorbic acid	0.5 g
Vitamin B_{12}	2.0 ug

pH was adjusted to 7.2 and water was added to make the volume 200 ml.

Contd...

Amino acid supplement

L-glutamic acid	1.0 g	L-cystine**	0.2 g
L-leucine	0.5 g	L-serine	0.2 g
L-isoleucine	0.5 g	L-tyrosine	0.2 g
L-valine	0.5 g	L-proline	0.2 g
L-lysine	0.5 g	L-histidine	0.2 g
L-alanine	0.5 g	L-phenylalanine	0.2 g
L-aspartic acid	0.5 g	L-threonine	0.2 g
L-arginine	0.2 g	L-tryptophan	0.2 g
Glycine	0.2 g		

pH was adjusted with N-KOH to 7.2 and water was added to make the volume 250 ml.

** First dissolved separately in 10 ml boiling water by addition of N-KOH.

Composition of solution of mineral salts (per litre)

$MgCl_2.6H_2O$	20 g	$CoCl_2.6H_2O$	0.25 g
$CaCl_2$	5 g	$CuSO_4.5H_2O$	0.25 g
$FeCl_3.6H_2O$	0.5 g	VSO_4	0.25 g
$ZnSO_4.7H_2O$	0.5 g	Na_2MoO_4	0.25 g
$MnSO_4.4H_2O$	0.25 g		

Dissolved in water with addition of $N\text{-}H_2SO_4$ to clear.

APPENDIX II

Codex Alimentarius Commission Recommendations.

	Values per 100 K Cal	
	Minimum	Maximum
Protein (g)	1.8	4.5
Fat (g)	3.3	6.0
Essential fatty acids(g)	300 mg	N.S.
Vitamin A(I.U.)	250	500
Vitamin D(I.U.)	40	80
Vitamin K(ug)	4.0	N.S.
Vitamin E(I.U.)	0.7	N.S.
Vitamin C(mg)	8.0	N.S.
Vitamin B_1(ug)	40.0	N.S.
Vitamin B_2(ug)	60.0	N.S.
Vitamin B_6(ug)	35.0	N.S.
Vitamin B_{12}(ug)	0.15	N.S.
Niacin (ug)	250	N.S.
Folic acid (ug)	4.0	N.S.
Pantothenic acid (ug)	300	N.S.
Biotin (ug)	1.5	N.S.
Choline (mg)	7.0	N.S.
Inositol (mg)	4.0	N.S.
Calcium (mg)	50	N.S.
Phosphorus (mg)	25	N.S.
Magnesium (mg)	6.0	N.S.
Iron (mg)	1.0	N.S.
Zinc (mg)	0.5	N.S.
Iodine (ug)	5.0	N.S.
Copper (ug)	60	N.S.
Manganese (ug)	5.0	N.S.
Sodium (mg)	20	60
Potassium (mg)	80	
Chloride	55.0	

APPENDIX III

American Nutrition Advisory Committee Recommendations (1980).

	0-6 months, values/day
Energy (KCal)	570-870
Protein (g)	13.2
Essential fatty acids (% KCal)	3
Vitamin A (I.U.)	1400
Vitamin D (I.U.)	400
Vitamin K (ug)	12
Vitamin E (I.U.)	4.5
Vitamin C (mg)	35
Vitamin B_1 (ug)	300
Vitamin B_2 (ug)	400
Vitamin B_6 (ug)	300
Vitamin B_{12} (ug)	0.5
Niacin (mg)	6
Folacin (ug)	30
Pantothemic acid (mg)	35
Biotin (ug)	--
Choline (mg)	--
Inositol (mg)	--
Calcium (mg)	360
Phosphorus (mg)	240
Magnesium (mg)	50
Iron (mg)	10

Contd..

Appendix III. Contd.. . .

Iodine (mg)	3.0
Copper (ug)	500 - 700
Manganese (ug)	500 - 700
Sodium (mg)	115 - 350
Potassium (mg)	350 - 925
Chloride (mg)	275 - 700
Floride (ug)	100 - 500
Chromium (ug)	10 - 40
Selenium (ug)	10 - 40
Molibdenum (ug)	30 - 60

-.

APPENDIX IV

Nutrient composition of 100 ml of liquid reconstituted Indian infant foods.

Nutrient	Product					
	Lacto-gen	Amul spray	Vijaya spray	Lacto-dex	Amul roller dried*	Glaxo*
Quantity of powder(g)	64.8	75.0	60.0	60.0	75.0	90.0
Quantity of water (ml)	450	525	510	510	525	600
Calories(KCal)	64.8	64.3	59.8	53.3	64.3	67.5
Protein (g)	3.09	3.07	2.85	2.00	3.07	3.25
Carbohydrate(g)	7.4	7.48	6.91	9.60	7.43	7.80
Fat (g)	2.59	2.57	2.39	0.80	2.57	2.70
Calcium (g)	0.128	0.127	0.117	0.113	0.127	0.133
Phosphorus (g)	0.082	0.087	0.082	0.050	0.090	0.094
Sodium (mg)	65.52	76.07	48.0	39.0	67.49	70.50
Potassium (mg)	118.8	119.26	110.00	55.0	120.12	131.70
Chloride(mg)	71.7	73.35	67.56	47.46	73.50	77.85
Iron (mg)	0.79	0.82	0.76	0.53	0.82	0.86
Copper (mg)	0.053	0.144	0.049	0.033	0.143	0.093
Zinc (mg)	0.42	0.48	0.35	0.19	0.48	0.43
Manganese(mg)	0.003	0.003	0.002	0.002	0.003	0.003
Magnesium (mg)	12.50	13.0	12.9	9.7	18.0	13.05
Vitamin A (IU)	239	394	297	284	394	394

* Roller dried products.

APPENDIX V

Nutrient composition per 100 KCal of Indian Infant foods.

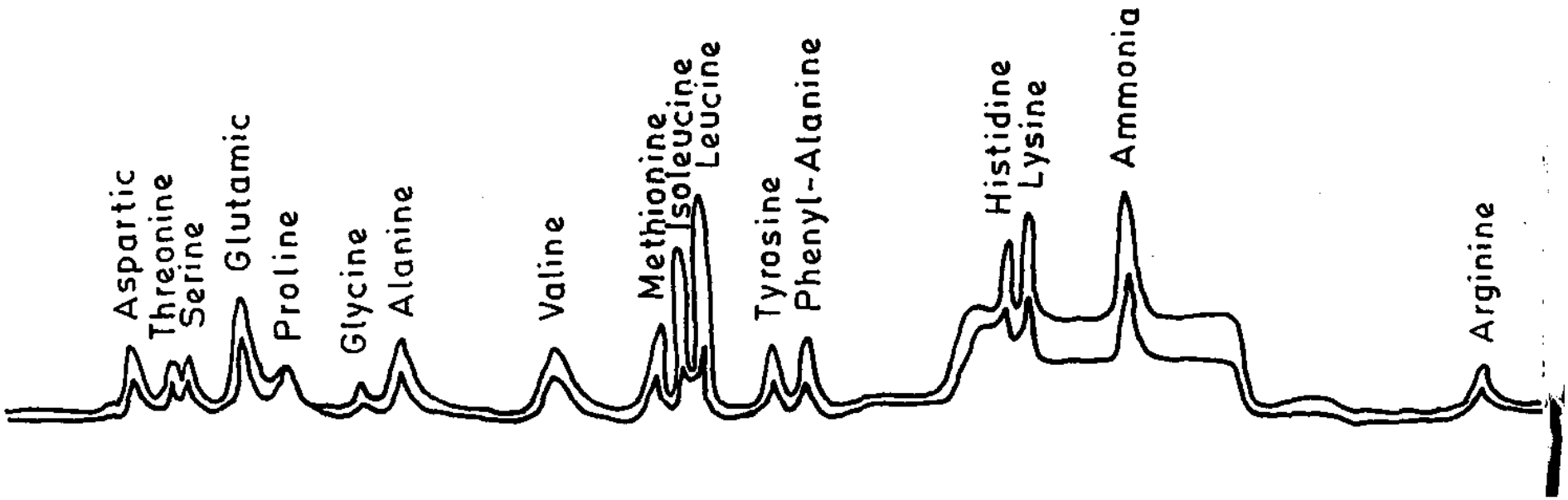
Nutrient	Product					
	Lacto-gen	Amul spray	Vijaya spray	Lacto-dex	Amul roller dried*	Glaxo*
Quantity of powder (g)	64.8	75.0	60.0	60.0	75.0	90.0
Quantity of water (ml)	450	525	510	510	525	600
Calories(KCal)	290.25	337.00	270.00	240.00	337.00	405.00
Protein(g)	4.42	4.68	4.76	1.78	4.68	4.70
Carbohydrate(g)	11.42	11.50	11.50	18.10	11.80	11.50
Fat (g)	4.04	4.00	4.00	1.50	4.00	4.00
Calcium (g)	0.19	0.19	0.18	0.21	0.19	0.19
Phosphorus(g)	0.124	0.134	0.136	0.094	0.134	0.135
Sodium (g)	0.099	0.078	0.078	0.073	0.078	0.102
Potassium (g)	0.180	0.181	0.180	0.103	0.181	0.191
Chloride (g)	0.108	0.111	0.110	0.089	0.111	0.113
Iron (mg)	1.20	1.25	1.25	1.01	1.25	1.25
Copper (mg)	0.08	0.218	0.08	0.063	0.218	0.136
Zinc (mg)	0.854	0.86	0.88	0.47	0.86	0.865
Manganese (mg)	0.0038	0.0038	0.003	0.003	0.0038	0.0038
Magnesium (mg)	19.05	27.5	21.16	14.50	27.50	19.06
Vitamin A(I.U.)	459	515	459	492	515	486

Appendix XI A

AMINOGRAM OF
LACTOGEN

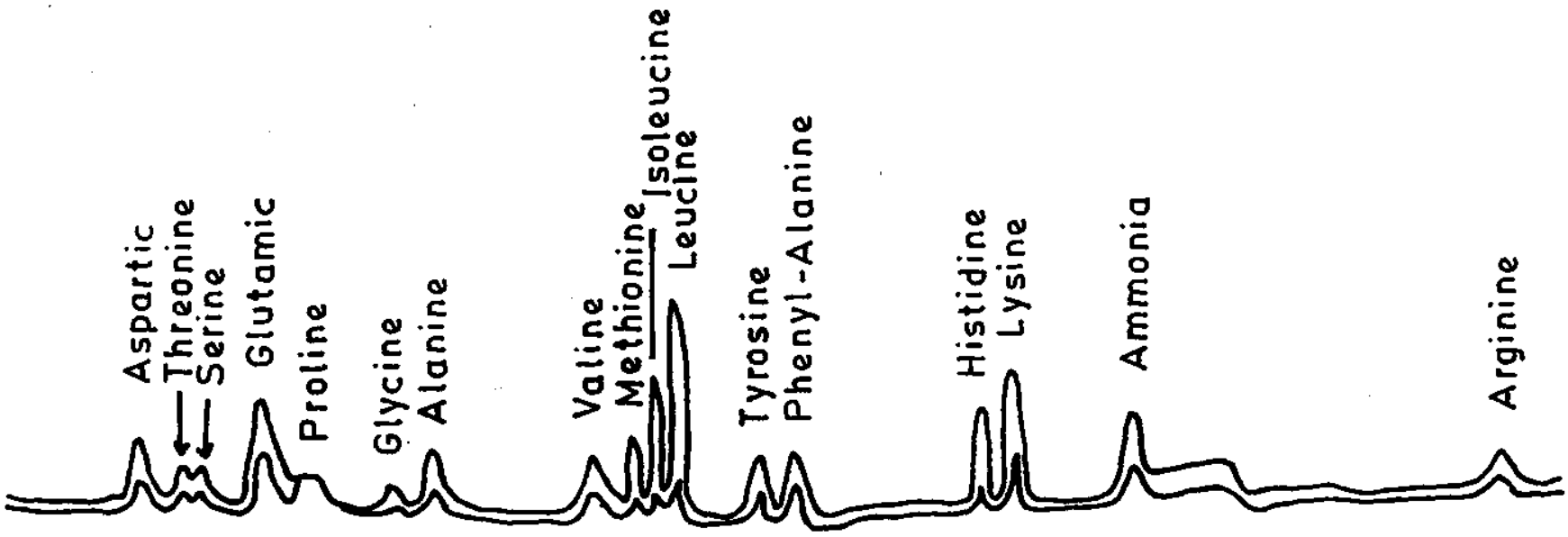
Appendix VI B

AMINOGRAM OF
AMUL SPRAY



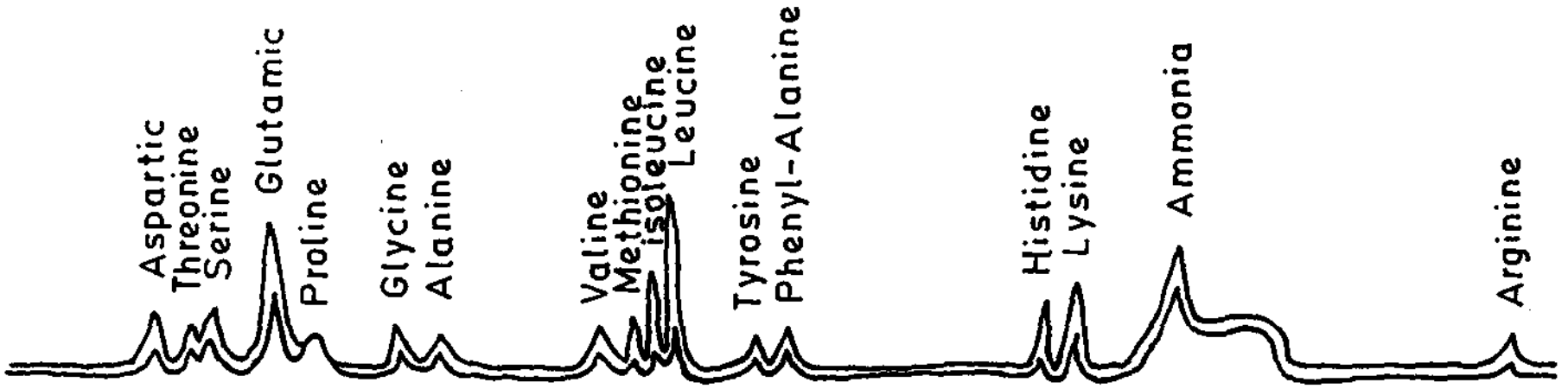
Appendix VI C

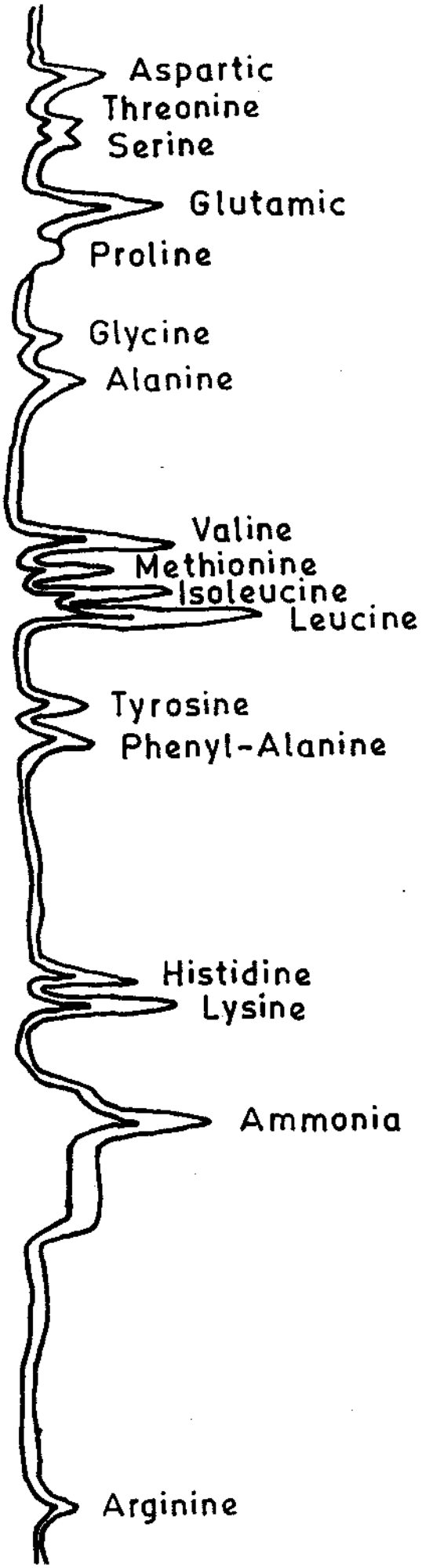
AMINOGRAM OF
VIJAYA SPRAY



Appendix VI D

AMINOGRAM OF
LACTODEX





AMINOGRAM OF
AMUL ROLLER DRIED

Appendix VI

AMINOGRAM OF
GLAXO