EFFECT OF HOMOGENIZATION ON SELECTIVE NUTRITIONAL CHARACTERISTICS OF BUFFALLO MILK

BY

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I certify that the work reported in this thesis entitled "Effect of Homogenization on Selective Nutritional Characteristics of Buffalo Milk" was carried out by Mr. Ashok Kumar Mehta, as the requirement for the degree of DOCTOR OF PHILOSOPHY in the Faculty of Dairying, Animal Husbandry and Agriculture, Kurukshetra University, under my guidance and supervision.

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29/11/81
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fat remains in emulsified form which further increases the viscosity of milk, as a result of uniform dispersion of fat. Homogenization has been shown to alter the physical condition of milk proteins and in the process makes it more readily coagulable either by heat or acid treatment. Such a change together with the one produced by the reduction of fat globules, gives homogenized milk characteristics of a soft curd milk. Furthermore, this brings about other reactions such as interaction between lipid and proteins of milk, the nutritional consequence of which, is far from clear.

Pasteurized homogenized milk is a commercial product in several countries in the world, though in India it is being followed only in a few commercial milk plants. The general acceptance of homogenized milk due to its superiority over normal milk in respect of palatability, homogeneity, stability and digestibility, has implied the product to be wholesome. Furthermore, the treatment renders the milk so homogeneous in respect of its calorie and fat soluble vitamins that when consumed, these nutrients are proportionately available.

Although pasteurization of milk is known to affect the stability of most of water soluble vitamins very mildly, the relationship between oxidative destruction of vitamin C and stability of vitamin B_{12} and folic acid during sterilization of milk in the presence of oxygen (Ford, 1967), further raises

doubts about the stability of these vitamins during homogenization and pasteurization, when heating in the presence of oxygen, invariably occurs.

Controversy over the use of homogenized milk in view of its implied association with the development of atherosclerosis (Oster, 1971) has mooted a question about the desirability of its consumption, though studies carried out so far, have not provided any unequivocal evidence supporting such association.

The information accrued so far mainly explores the effect of homogenization treatment on cow milk. Little is known about the effect of such treatment on milk from other species. In India, buffalo milk, which constitutes about 63 percent of the total milk production, (FAO, 1974) has its own place in dairy industry. About 95 percent of the total milk handled by the organized dairy sector is comprised of buffalo milk and it is utilized both for supplying fluid milk and manufacture of milk products (Sindhu, 1979). Furthermore, buffalo milk being rich in fat, is often mixed with milk of other species to achieve desired fat and SNF level. The lowered curd tension of such mixed milks is implied to improve the digestibility, and in turn, their nutritive value. Buffalo milk has been used in the recent times more frequently in the preparation of infant food in which homogenization of milk assumes

significance in imparting soft curd characteristics to reconstituted milk.

It could thus be evident from the aforesaid that assumption of the feasibility of utilization of homogenized milk from nutritional stand point rests mainly on its palatability, curd tension and information available from limited in vitro digestibility studies, rather than on the biological availability and utilization of nutrients in the body. The knowledge about such parameters would be more meaningful, since apart from chemical and physical characteristics of the food, the physiological status of the subject equally influences the nutritive value of food.

The present study is therefore undertaken to ascertain the effects of homogenization of milk on certain nutritional characteristics on the following lines viz, to investigate

i) the effect of homogenization on the curd tension of milk from various species namely buffalo, cow and goat, as well as mixed milk having different proportion of buffalo and cow milk,

ii) the effect of homogenization on the protein and fat quality in terms of their utilization by the body using albino rats as the experimental model,

iii) the biological availability of fat soluble vitamin, vitamin A, from homogenized milk using albino rats as experimental animals,

iv) the stability of certain water soluble vitamins during homogenization and subsequent pasteurization.

CHAPTER - II

REVIEW OF LITERATURE

should be within 10 percent of the fat content in the rest of the portion of the well mixed milk, on maintaining the homogenized milk for 48 hours.

It is generally considered that homogenized milk has an edge over non-homogenized milk, in view of the altered physical as well as chemical characteristics of milk, further reflecting in better homogenity, palatability, digestibility and reduced curd tension. All these factors were subsequently implied to improve nutritive value of milk (Trout, 1948).

Palatability and acceptability

Palatability plays an important role in controlling the intake of food. Various factors such as appearance, texture, taste and flavour are known to influence the palatability of food (Babcock, 1939 and Trout, 1948). In respect of homogenized milk, Trout (1943) observed that homogenity and flavour retention over a prolonged period, mainly contribute towards its palatability. It was found that homogenized milk not only scored over non-homogenized milk in respect of distribution of fat but also regarding significant retardation of onset of oxidised flavour, otherwise witnessed in pasteurized milk. Similar observations were also made earlier by different workers (Thurston et al., 1936; Ross, 1937 and Larsen et al., 1941). Further probe

into oxidative reactions producing oxidized flavour showed the involvement of various factors such as phospholipids, oxygen and copper-protein complex. While surface of a fat globule was identified as the site for oxidation, decrease in number of vulnerable sites consequent to homogenization resulted in the retardation of oxidation reaction (Tarassuk and Koops, 1960).

Prucha and Tracy (1936) observed that the homogenization process imparted a pleasant and rich taste to the milk. Babcock (1939) attributed increase in the consumption of homogenized milk to its fresh flavour. Earlier, Trout et al. (1935) made a comparison between flavour of pasteurized milk and that of milk after homogenization, however, these workers failed to observe any improvement in the flavour as a result of homogenization. Doan (1943) found that consumer preference for homogenized milk over non-homogenized milk was influenced by the level of fat, when it was steadily raised from 2.5 to 5.0 percent. The highest preference was observed at 4.0 percent level. The consensus among the consumers was that the process made the milk richer, smoother and creamier. It was further observed that homogenization renders milk more opaque, more intensely white by being uniform as compared to non-homogenized milk. The uniformity and stability of colour was observed to contribute to its more attractive appearance, a factor affecting the acceptability

of the milk (Henderson, 1944; Stamberg and Theophilus, 1945). Such results of uniform consistency towards palatability have also been reported by Larsen et al. (1941). Sommer (1946) attributed such improvement in the colour characteristics to the increase in the number, as well as total surface area of fat globules responsible for reflecting and scattering the incident light. On the other hand, Wittig (1949) reported a higher viscosity, to be the major cause for the increased intensity of the colour of milk.

It would, thus, appear that homogenization increases the palatability and in turn the consumption by improving its appearance, flavour, and consistency resulting from uniform distribution of fat.

Effect of homogenization on fat and protein structure

Homogenization is often described as a mechanical process. Apart from the degree and duration of heating, mechanical factors such as stresses occurring while pumping, passing through the pipelines, valves, storing etc. involved during this process have been reported to profoundly affect fat globules and further produce physical as well as chemical changes.

Effect on fat particle size:

Fat globules exist as emulsion in milk, with sizes ranging between 0.1 and 10 microns with an average of

about 4 microns. Homogenization reduces the fat globule size to less than 2 microns, thereby increasing the fat surface area by 5 to 6 fold (Trout et al., 1935 and Jenness and Patton, 1959). Whereas Wittig (1949) observed that the average size of the individual fat globule in homogenized milk was about one micron, ~~much less that~~ values reported earlier.

The size of the fat globules in homogenized milk according to Walstra (1975) depends upon homogenization pressure, temperature, kind of valve and proper functioning of the homogenizer. Kazlauskaite and Vaitkus (1974) observed that homogenization of milk at different pressures ranging between 100 and 500 atm. at 60°C drastically reduced the fat globule size, further resulting in about 5 fold increase in fat surface area. Likewise, Stepanov and Kiseleva (1970) also found an increased disperson of fat with increase in temperature and homogenization pressure. Maximum disperson of fat was observed at 70 - 80°C corresponding to that of a homogenizer valve working at 100 to 120 atmosphere.

Ivanov et al. (1976) studied the specific efficiencies of homogenization at pressures ranging between 2.0 to 10 MPa and observed decreased efficiencies in reducing fat globule size with subsequent homogenization.

Homogenization and protein content in fat globule membrane:

In a freshly secreted milk, the lipoprotein complex of a fat globule membrane represents the major protein lipid interaction. Nordlund (1972) observed that the structure of fat globule membrane is comprised of two discrete lipoprotein layers differing in their structure and phospholipid content.

Quantitative estimates of the protein components in the lipoprotein complexes in non-homogenized milk were made by various workers. It was observed that values ranged between 0.3 to 0.9 g per 100 g of fat in fat globules (Palmer, 1944 and Roland, 1956). Much higher values ranging between 0.1 to 3.0 g were reported by Mulder and Menger (1958). The level of proteins was, however, markedly enhanced during homogenization. Tobias and Serf (1959) reported that the membrane protein to be around 2.3 g per 100 g of fat, almost four times higher than protein found in non-homogenized milk membrane and was commensurate with the increase in the intrafacial surface produced by homogenization.

Trout (1950) observed that during homogenization a film of proteinous material was adsorbed on the surface of the homogenized fat globules. The newely formed fat globules were thus resurfaced with fat globule membrane, entirely different from the one in the normal fat globules

in respect of protein characteristics. He further reported that the level of casein in newly formed membrane was about 25 percent as compared to nearly 2 percent in the original membrane. Similar observation was made earlier by Lundstedt (1936).

Nature of protein absorbed:

On the basis of the differences in the amino acids profile, Hare et al. (1952) considered the protein component of fat globule membrane from non-homogenized milk to be entirely different than other milk proteins such as casein, lactalbumin and lactoglobulin. Fox et al. (1960) studied the fat protein complex obtained on homogenization of milk. The complex particles were isolated from the sediment of the milk samples by centrifugation. It was observed that casein was the principle protein moiety of the fat protein complex. Similarly, Jackson and Brunner (1960) fractionated and separated protein from the fat globule membrane of homogenized milk and observed that it contained higher concentration of casein, on the other hand, relatively lesser quantities of serum proteins. This was further confirmed by Itoh and Nakanishi (1974), who observed that higher protein concentration in the fat globule membrane from raw homogenized milk was due to the presence of different fractions of casein as compared to those of raw non-homogenized milk.

Similar observation in respect of the adsorption of casein on enhanced fat surface, was made by Henstra and Schmidt (1970). By estimating the percent of casein micelles in total casein, Cerbulis (1969) reported that commercially homogenized and pasteurized milk had only 63 to 83 percent as compared to 93 percent in raw non-homogenized Holstein milk. The reduction was attributed to the adsorption of casein micelles on the fat surface. Other groups of workers, on the other hand, observed that proteins absorbed on the newely created surface were proteins from whey. Brunner et al. (1953a) observed differences in the amino acids profile of membrane proteins from homogenized and non-homogenized milk. There was an increase in the concentration of lysine and glutamic acid and a marked decrease in tryptophan, cystine and glycine concentration. The differences in amino acids composition further suggested that characteristics of fat globule membrane protein of milk was altered as a result of homogenization. From the electrophoretic and ultra-centrifugal characterization data, these workers identified membrane protein of homogenized milk as the plasma protein (Brunner et al., 1953b,c). Sasaki and Miyasawa (1959) in an electrophoretic study of milk proteins, observed that whey proteins were absorbed on the increased surface area of fat globule after homogenization. About 10 mg new membrane material mainly protein was adsorbed from

milk plasma on new surface following homogenization (Qwist, 1977).

Vaitkus et al. (1973), on the contrary, failed to observe any notable effect of homogenization on protein characteristics except that it lowered the proportion of high molecular weight protein component in the cream.

Factors affecting fat-protein complex formation:

The level of total solids in milk, particularly that of fat, and the pressure applied during homogenization appear to govern the lipid-protein complex formation. Stevens (1974) reported that with 30 to 40 percent of fat in the cream, the casein adsorption was found to increase on the fat surface by 90 percent as the pressure of homogenization was raised to 1500 psi. However, with 10 to 18 percent fat, casein adsorption was found to increase upto maximum pressure applied viz,3000 psi. Moreover, whey proteins and caseinate were not adsorbed with the same efficiency as that of casein micelles. Increase in the total solids upto 31 percent in milk and homogenization between 1000 to 8000 psi, a significant amount of fat binding to protein occured as the pressure was raised. In another experiment when calcium-ion concentration was increased by adding $CaCl_2.2H_2O$ and

homogenized at 800 psi, it was observed that as little as 2.5 mM calcium caused an increase in the amount of fat complexed, and at concentrations between 10 and 20 mM, of it was sufficient to complex 100 percent of the fat (Fox et al., 1960). Whereas Granikov et al. (1962) reported that increased fat-protein complex was mainly due to increased dispersion of protein particles during homogenization which was proportional to that of the dispersion of fat. The complex formation was considered to be due to adsorption forces of increased surface of the fat globules. Likewise, Henstra and Schmidt (1970) reported breakdown of casein particles into sub-units during homogenization which subsequently adhered to the surface of the fat globules, and thereby increasing the total casein concentration.

Thus apart from reducing fat globules into smaller size, homogenization was observed to sub-divide proteins into sub-units which were further absorbed on the increased area of fat globules, ultimately changing the composition of the fat globule membrane.

Homogenization and curd tension

Since long the curd tension has been the point of interest both to dairy technologists and nutritionists, in view of its implied association with digestibility. However, not much notice was given to this parameter

difference in breed and the type of coagulant used to clot the milk. Riddell et al. (1936) observed breed to be one of the important factors affecting the curd tension, whereas Hill (1923) and Rao et al. (1964) failed to find any difference in curd tension among various breeds of cow. Hill (1923) further stated that each cow has an individual milk curd character which may vary even to the extent of 10 fold.

As regards coagulant, using pepsin-HCl as coagulant instead of pepsin-$CaCl_2$ as suggested by Hill, Miller (1935) failed to observe any difference in curd tension of Holstein cow milk. However, a significant difference of 10 g was obtained with pepsin-HCl in comparison to pepsin-$CaCl_2$ method in the case of goat milk.

Apart from the factors mentioned earlier, the stage of lactation appears to have influence on the curd tension. Berry (1935) during a full lactational study, observed that colostrum had maximum curd tension, otherwise it was uniformly low in milk throughout the lactation. Riddell et al. (1936), however, observed a significant effect of lactation on curd tension, having low values upto 2 to 3 months of lactation, followed by tremendous increase during later months.

On an average curd tension for cow milk varied between 25 and 50 g (Babcock, 1939; Hadary and Sommer, 1939;

Kelly, 1941; Wolman, 1941; Rao et al., 1964; Jain et al., 1974 and Parsad et al., 1974), while for goat milk it varied between 40 to 55 g (Turner and Garrison, 1936-37; Rao et al., 1964 and Abou-Dawood and El-Sawaf, 1977).

Composition of milk:

Various milk constituents have been reported to have relationship with the curd tension. Riddell et al. (1936) showed a significant correlation of 0.76 between milk protein content and the curd tension. Weisberg et al. (1933) and Doan and Welch (1934) found that the proteinous ingredient in milk most closely associated with curd tension was casein. Jain et al. (1974) observed that curd tension increased with increase in solid-not-fat, total protein, casein and calcium contents of milk. High curd tension value for buffalo milk was attributed to its high calcium content by Rao et al. (1964). In the light of this, the low curd tension for milk with lower calcium content observed earlier by Weisberg et al. (1933) and Lundstedt (1936) could be well understood.

Changes in chemical composition of milk due to its dilution with water caused a reduction in curd tension and was found to be directly proportional to the degree of dilution (Doan and Welch, 1934 and Abou-Dawood and El-Sawaf, 1977).

Apart from total protein content, the physical state, of the protein has also been shown to influence the curd tension of milk. Flora and Doan (1938) reported a reduction in curd tension when milk was treated with trypsin. Similar reduction was observed after homogenization as well. Similar observations were made by Conquest et al. (1938) who noted a curd tension in a range of 20 to 30 g, when milk with curd tension value of 50 g, was digested with pancreatic juice.

Abdel-Salam et al. (1974) reported an increase in the curd tension on defatting buffalo milk. As the level of fat in raw milk with 6.5 to 7.0 percent was gradually reduced to 3 and 0 percent, the curd tension values changed from 55.7 to 64.5 g and 76.9 g, respectively. Conversely, when fat content of milk was increased from 0.02 to 5 percent, curd tension reduced from 66 to 45 g in non-homogenized milk (Kelly, 1941). This value further decreased to 16 g on homogenization at 3000 psi pressure. It was observed that at least 1 percent fat level was necessary to affect reduction in curd tension on homogenization. This was evident from the observation of Anderson and Weckel (1951), who noted higher reduction in curd tension on homogenization of milk with fat level of 1.5 and 1.0 percent in comparison to that with only 0.5 percent fat.

Effect of heat treatment:

Milk is often subjected to heat treatment of one kind or the other, in order to ensure safety for consumption. These include pasteurization either by holding or HTST method, UHT or sterilization. Such treatments have been reported to reduce the curd tension of milk, resulting due to conformational changes in the milk proteins. Haller et al. (1941) showed that pasteurization of milk by holding method lowered the curd tension by 48.4 percent, whereas pasteurization by flash method had relatively very small effect. Parsad et al. (1974), on the other hand, failed to observe any marked change in the curd tension due to pasteurization either by holding or HTST procedure. The respective decreases were 23 and 28 percent. Similarly, Rao et al. (1964) and Jain et al. (1974) could not find any reduction in curd tension on pasteurization either by holding or HTST method.

Boiling milk too, was found to have an equally pronounced effect on curd tension, similar to pasteurization. Lundstedt (1936) reported about 30 percent reduction in curd tension on boiling milk which was identical with that observed after pasteurization in other studies. Such effect of boiling further varied with the species. In goat milk a reduction of about 45 percent was observed on boiling, whereas it was 37 percent in the case of

Holstein cow milk (Miller, 1935). Rao et al. (1964), on the other hand, found a higher degree of reduction in the curd tension of both buffalo (79.5%) and cow milk (78%) as a result of boiling. Similar observations in respect of species about reduction of curd tension on boiling, were made by Jain et al. (1974). However, sterilization further lowered the curd tension i.e. 85.4 and 86.8 percent respectively . It would thus appear that the reduction in curd tension is more pronounced as the degree of heat treatment increases.

Effect of homogenization and pasteurization:

Homogenization was observed to be the most efficient treatment among all in reducing the curd tension, perhaps without affecting its nutritive value. Various workers have reported that homogenization of milk generally reduced the curd tension by 50 to 60 percent (Theophilus et al., 1934; Doan, 1938; Babcock, 1939; Kelly, 1941 and Parsad et al., 1974).

The effect of homogenization on curd tension depends on several factors such as pressure, temperature adopted during homogenization, fat content as well as initial curd tension of the milk.

Doan (1938) found that the maximum reduction in curd tension was observed when the initial curd tension

of the milk was 123 g as compared to one having curd tension of 56 and 34 g, when the milk was homogenized at 4000 psi. The respective values on homogenization were 40, 17 and 12 g. It was further seen that there was no difference in curd tension value when milk was homogenized either at single or double stage homogenization. Similar observations were also made by Chambers (1935) and Parsad et al. (1974).

As regards the influence of pressure and temperature during homogenization, both exhibited pronounced effect in reducing the curd tension. Theophilus and coworkers (1934) showed that as the homogenization pressure was increased from 500 to 2000 psi, the curd tension decreased progressively. Berry (1936) observed that soft curd milk could be obtained on homogenizing the milk at pressures ranging between 3000 and 5000 psi. Similar observations were made by Lundstedt (1936); Tracy (1936) and Kelly (1941) who noted a decrease in curd tension as the homogenization pressure was increased. However, several studies suggested that homogenization pressures beyond 2000 to 2500 psi had little effect in further reducing the curd tension (Caulfield and Martin, 1934; Doan,1938; Tracy,1938,41 and Babcock,1942). At pressure 2500 psi, hard curd milk resulted in medium soft curd, while medium soft milk resulted in soft curd.

The heat treatment of milk before and during homogenization seems to excrecise considerable influence on the curd tension. Doan (1938) observed that a greater effect was evident when the milk was processed at high temperature, as a result of cumulative action of heat and the homogenization, but at a still higher temperature above (82°C), homogenization produced very little, rather practically no additional reduction in the curd tension beyond what was accomplished by heat alone. Kelly (1941) also reported that as the temperature of homogenization was raised from 49 to 82°C the curd tension was reduced by about 50 percent. Babcock (1939) recorded a curd tension of 16.7 g as compared to the initial value of 50 g for milk which was pasteurized by holding method and homogenized at 2500 psi, whereas Berry (1936) demonstrated that milk heated at lower temperature (49°C) needed higher pressure of homogenization to record a similar lowering of curd tension. Similar observation was made by Lundstedt (1936). Kelly (1941) further observed that pasteurization of milk after homogenization, however, had almost similar effect in reducing the curd tension as compared to the reduction effect of pasteurization on non-homogenized raw milk. Prasad and coworkers (1974) were not able to demonstrate any such difference in the curd tension of milk pasteurized either by holding or HTST method before

or after the homogenization. The overall reduction due to heat treatment was 11.5 and 11.7 g, respectively.

Thus, in order to have a maximum effect of homogenization at a given pressure, it is essential to heat the milk at moderate but proper temperature as the milk, heated at very high temperature, homogenization produced little or no additional reduction in curd tension beyond what was accomplished by heat alone.

Curd tension and digestibility

Irrespective of various factors responsible to influence curd tension, this characteristic is generally considered in the light of digestibility of milk. Espe and Dye (1932) studied different types of soft curd milks produced either by dilution, boiling or sodium citrate addition and observed that for milk having higher curd tension, the digestion period was longer by 30 to 65 percent. Doan and Welch (1934) concluded that soft curd was broken considerably faster than hard curd, when the rapidity was assessed from the rate of development of non-protein nitrogen and visible disappearance of curd in the process of disintegeration. Kelly (1939) further confirmed during *in vitro* studies, that soft curd milk was digested more rapidly than hard curd milk. During an *in vitro* study, Turner (1945) found that when digestion

coefficient of milk from various species was calculated on the basis of conversion of acid insoluble to acid soluble protein, the digestibility coefficient was fairly high for human milk having low curd tension as compared with milk from cow, goat or mare. Further, *in vitro* experiments conducted by Doan (1938) and Conquest et al. (1938) on cow milk showed that enzymic treatment was found to be more effective in enhancing the digestibility than pasteurization or boiling. Likewise, Ilgner and Thurau (1951,52) demonstrated higher protein digestibility for homogenized cow milk during *in vitro* experiment than the non-homogenized one. It was observed that peptic and tryptic degradation of milk proteins was rapid and complete in the case of homogenized milk and was comparable with human milk (Ewerbeck and Jaeger, 1954).

Babcock (1939) observed that soft curd formation during homogenization provided larger surface area to combine with the digestive enzymes, thereby making milk more rapidly digestible rather than non-homogenized milk. It was seen that within first 15 minutes of digestion, boiled and homogenized milk showed 76.5 and 56.5 percent higher degradation than raw milk. However, when considered at the end of 5 hours period, apparently there was no difference between the boiled and homogenized milk. Similar observations were made by Flora and Doan (1938) and Kelly (1939).

Hull (1938), on the other hand, observed superiority of heat treatment but not of homogenization on proteolytic digestibility of milk. It was observed that the pH at which curd was formed and size of the curd particles were interrelated with digestibility. Doan and Flora (1939) further reported that particle size was the better indicator of digestibility than curd tension, as the digestibility of natural pasteurized milk was only roughly proportional to curd tension. Though, homogenization reduces the curd tension and decreases the particle size, it has no effect on digestibility. Similarly, Doan and Dizikes (1942), too, were unable to demonstrate any correlation between digestibility and curd tension for different types of milk. It was observed that homogenized milk was inferior to acidified, superheated, evaporated and boiled milk.

In vivo experiment

Doan and Welch (1934), while conducting studies on human subjects as well as on calves and rats, observed that in most cases of human subjects, milk of low curd tension formed smaller curd mass. During animal studies, it was found to be assimilated faster in the intestine than hard curd milk. On distribution of fat in skim milk by viscolization, Espe and Cannon (1935) demonstrated that the milk with 6 percent fat left the calf stomach faster

than skimmed milk due to the difference in the texture of curd formed. Mortenson et al. (1935) confirmed that the boiled milk was digested faster and left the calf stomach earlier than raw milk. The difference in their behavior was ascribed to the coagulation time which was 1 to 10 minutes in the case of boiled milk, and 8 to 15 minutes in the case of raw milk.

Adam and Czech (1955) observed that infants fed homogenized milk required less amount of gastric juice for complete digestion. Further, Czech (1957) showed that when fed to fasted children for two successive days, homogenized milk left stomach more rapidly than non-homogenized one, with the mean difference of 80 minutes. In nine out of ten children, the amount of gastric juice secreted was much less when homogenized milk was given than when non-homogenized milk was given.

On the basis of nitrogen-balance study on rats Petrilli and Agnese (1960) were able to show that though digestion was almost similar viz, 89.3 percent with homogenized as well as non-homogenized milk, absorption was higher i.e. 79.4 percent in the case of homogenized milk. Likewise, the feed efficiency too, was better for homogenized milk.

Elias (1932), on the other hand, failed to observe any superiority of soft curd milk over hard curd milk

either in respect of gain in body weight or stomach emptying period in infants. The curd formed in the stomach was, however, softer than that formed from unboiled certified milk and the curd particles were larger as well as tougher than those obtained when evaporated or breast milk was given. Similarly, Hadary et al. (1943) failed to observe any faster stomach or colonic emptying in children fed soft curd milk produced by treating with barium salt. After examining the stomach content of infants fed raw and boiled milk, Ogilvie and Peden (1934) concluded that there was no difference between both types of milk in respect of gastric digestion. Assessment of the toughness and texture of curd formed in stomach of babies fed soft and hard curd formulae done by Wolman (1941) failed to show any significant difference. A mean curve drawn for growth, too, was found to be identical with all types of milk. Similarly, Ebel (1953) found almost similar time by which the homogenized and non-homogenized milk left the stomach of infants and children.

It could thus be seen from the evidence available so far that undoubtedly homogenization carried out at appropriate temperature and pressure does lower the curd tension. However, additional <u>in vivo</u> studies, are warranted to substantiate observations made during <u>in vitro</u> studies.

Biological evaluation of milk proteins from different species

As seen in the earlier section, in vitro studies conducted so far failed to demonstrate a definite relationship between curd tension of milk and its digestibility. Chemical constituents of milk, the fat content, in particular, was seen to affect the curd tension of milk. Since, buffalo milk differed markedly in this regard from milk of other species, it could possibly behave in different manner as regards its digestion. Rao (1956) found PER values for buffalo, cow and goat milk to be 2.4, 2.6 and 2.5, respectively, showing that proteins from different types of milk were identical in promoting growth. Chandrasekhara et al. (1959) observed that when infants were fed formulae prepared from buffalo milk, the growth rate was comparable to that for normal.

Nitrogen balance studies:

In a comparative study on utilization of milk proteins from various species, Rao (1956) noted almost identical values when milk protein from buffalo, cow and goat was fed to albino rats at 10 percent level, the B.V. was 91.9, 88.8 and 94.4, respectively.

Though homogenization is one of the essential steps in the preparation of number of milk products, little is known whether it has any effect on utilization of milk. Using albino rats as experimental animals, Henry et al(1942) reported a little higher value of digestibility coefficients

for sweetened condensed milk (98.8) than raw (94.2) and evaporated milk (93.7). However biological values were identical, which could be seen to be 84.1, 85.6 and 84.1 for condensed, raw and evaporated milk, respectively.

Therefore it would be seen that irrespective of variation in chemical composition, buffalo milk is utilized equally good as cow milk and even heat treatment did not alter its absorption and utilization.

Homogenized milk and fat utilization:

Milk fat is known to be digested almost completely with normal quantity of lipase secreted. The transport of lipid material in the body is favoured if it is present in dispersed state in biological fluid and its association with proteins. As seen earlier, during homogenization fat is maintained in a fine dispersion due to reduced size and uniform distribution of fat globules throughout the milk. This may help in enhanced utilization of milk fat. It would therefore be of great interest to know how efficiently the modified milk fat will be utilized in the body.

Effect of particle size:

Marriott and Schoenthal (1929) hypothesized that during homogenization process, before the preparation of evaporated milk, fat globules are brokendown into fine

particles. This results in the rapid digestion of homogenized fat due to larger surface area exposed for lipase action. Subsequent studies by Stejskal and Neuburger (1934) showed that size of fat globules influenced its utilization by adult human subjects. It was observed that the amount of fat excreted in faeces was only one third of that present in faeces from subjects fed non-homogenized milk. However, such absorption of fat was attributed to the type of the curd formed. Ilgner and Thurau (1951) observed, during in vitro study, a greater breakdown of fat from homogenized milk than from normal milk. It was further seen that pancreatic digestion of butter fat was almost double in the case of homogenized milk in comparison with normal milk, used in the preparation of vitamin D enriched milk. Ewerbeck and Jaeger (1954) demonstrated the superiority of homogenized milk over boiled or raw milk, in respect of breakdown of fat and liberation of fatty acids. Though, Agnese (1959) did not find any difference in the digestion coefficient of fat in homogenized and non-homogenized milk, there were significant differences with respect to absorption coefficients, and could be seen from the values 71.8 and 42.3 percent for homogenized and non-homogenized milk, respectively.

Contrary to the above findings during their studies on infant, Holt et al. (1933) failed to observe any

beneficial effect of feeding fat of reduced particle size on fat absorption. Likewise, Nevens and Shaw (1933) could not find any difference in the digestibility of fat when rats were fed either fresh whole milk, dried milk or evaporated milk. Further, Sager (1952) observed that it was emulsification in the digestive tract, affecting the optimum surface activity that enhanced the fat absorption rather than homogenization, since the particle size was similar in all the three types of milk viz. non-homogenized, homogenized cow and human milk. Secondly, homogenized milk also had lost its ability to form a creamy layer, suggesting that physical assimilation of cow milk to human milk was not affected by homogenization. It would thus appear doubtful whether homogenization produced any beneficial effect on fat utilization. The hypothesis formulated by Ewerbeck and Jeuger (1954) was later refuted as he could not find any difference in the quantity of fatty acid production through lipase action in homogenized as well as in non-homogenized milk.

Considering faecal excretion of fat in infants as a measure of fat metabolism, Fomon et al. (1970) observed that infants fed homogenized cow milk excreted maximum fat as compared to those fed either human milk or infant formulae.

Vitamins in milk

Milk is recognized as a good source of vitamin A and riboflavin, while it is the sole source of vitamin B_{12} in the vegetarian diet. However, it is found to be a poor source for both ascorbic and folic acid. Potential of milk in supplying both macro and micro nutrients makes its inclusion necessary in the diet. In view of this, an allowance of 200 ml of milk in a balanced diet is often recommended (Srilakshmi et al., 1970), which would meet about 25 percent requirements for riboflavin, 10 to 15 percent for each of vitamin B_6 and B_{12}, about 5 to 10 percent for thiamine and 1 to 2 percent of folic acid (NRC, 1974). The capacity of milk to meet the nutritional requirements could become still more limited due to deleterious effects of various processing treatments on the retention of vitamins in milk.

Fat soluble vitamin:

The content of vitamin A in milk has been reported to range between 100 to 225 I.U. per 100 g and is known to depend on various factors such as the level of feeding (Anantharamiah et al.,1950), seasons (Badr, 1954; Sampath et al., 1955 and Sirry and EL-Said Saleh, 1962); being highest during the pasture season and lowest during dry feeding of the animals.

Though the values reported so far were for natural milk, lactation has been shown to influence the level of vitamin in milk (Narayanan and Anantakrishnan, 1959; Elmoty and El-Malla, 1967). It was observed that vitamin A in colostrum of cow and buffalo decreases as the lactation advanced. Vitamin A in buffalo colostrum was lower than that in the cow colostrum.

Effect of processing treatments:

Several studies reported so far indicated that heat treatments such as pasteurization, either by holding or HTST methods, sterilization, drying or evaporation of milk caused little or no loss of vitamin A (Krauss et al., 1933; Gillam et al., 1938; Henry et al., 1939; Mattick et al., 1945-46 and Ford et al., 1969). However, Davidov et al. (1962) reported that the process of pasteurization and evaporation involved in the preparation of condensed milk brought about a 20 percent reduction in vitamin A. In the other study, Wagner (1957) reported that pasteurization lowered both vitamin A as well as carotene content by over 20 percent, whereas sterilization destroyed between 30 to 100 percent of the vitamin. The extent of destruction was much less, in milk sterilized by flash method at 145°C for 3 to 4 seconds (Rossihina et al.,1969).

Availability of vitamin A:

As stated earlier, it would appear that fat and to certain extent proteins undergo some interaction during

homogenization treatment. It was further evident that during such treatment, even subsequent pasteurization did not significantly affect the vitamin content. Although studies conducted showed that the larger fat globules are broken down still to smaller ones which are reported to be utilized in a manner different than the original one, little is known in respect of the utilization of fat soluble nutrients in milk, particularly vitamin A for which milk has been recognized to be a good source.

Earlier studies have established the role of vitamin A in growth and its association with vision in preventing xerosis, membrane structure, and deformity of bones, etc. Thus deficiency of vitamin A would not only affect the growth and vitamin A status of the individual, but also cause damage to other membrane in eyes leading to conditions such as xerophthalmia, keratomalacia and in extreme cases, extruction of lens.

Vitamin A and growth:

As early in 1934, Coward demonstrated that with vitamin A deficient animals, the growth response on vitamin A supplementation was a true measure of the vitamin activity. Several workers reported that though the growth of rats could be restored with the intake at a low level but for proper growth and storage, a higher amount of vitamin was required. With a dose equivalent

to 10 to 20 percent of the daily requirement when given to vitamin A deficient rats, resulted in gain in body weight. The growth observed under such condition was only about 70 percent of the normal growth (Braude et al., 1941; Lewis et al., 1942; Sherman and Campbell, 1945; Paul and Paul, 1946; Brown and Sturtevant, 1949 and Moore, 1957). Thus in order to achieve the normal growth and subsequent adequate storage, and further alleviate the deficiency symptoms in the eyes, a higher dose ranging between 20 to 50 I.U. was recommended by several workers (Lewis et al., 1942; Paul and Paul, 1946; Sherman and Trupp, 1949; Brown and Sturtevant, 1949 and Moore, 1957).

Hepatic vitamin A content:

Vitamin A is remarkable for its preference to liver as its site of storage. Over 80 percent of the total deposits of vitamin A in the body was reported to be present in the liver (Moore, 1957). This apart, relatively smaller reserves were reported to be present in lungs as well as in kidneys (Davies and Moore, 1934; Moore and Sharman, 1950 and Moore et al., 1951). This storage of vitamin A in liver depends on the amount of the vitamin ingested and has been reported to increase with an increase in the level of the dietary vitamin A (McCoord and Luce-Clausen, 1934 and Davies and Moore, 1948).

Furthermore, it was shown that a minimum dose of 10 to 50 IU/day/rat is needed for the storage of vitamin A in the liver of rats. However, below the intake of 10 IU/day, no storage could be observed (Baumann et al., 1934; Lewis et al., 1942; Little et al., 1943 and Vedrova et al., 1970).

As regards the vitamin level in the blood, studies conducted failed to show any correlation between the blood serum level and vitamin A deficiency, until reserves were completely depleted (McCoord and Luce-Clausen, 1934 and Ple-Galke, 1947). The plasma level was shown to be independent at higher level of vitamin A intake, however, a minimum quantity of 25 to 50 IU daily was required to restore serum vitamin A level to that of normal level of 80 IU vitamin A (Josephs, 1942; Lewis et al., 1942; Moore and Sharman, 1950; Moore, 1957 and Bring et al., 1965).

Availability of vitamin A from the dietary items:

Green vegetables contain sizeable amounts of carotene having provitamin A activity. On the other hand, food items of animal origin contain chiefly vitamin A. A study conducted on young men maintained on vitamin A deficient diet for 2 years and later given β-carotene or vegetables as a source of vitamin (Hume and Krebs, 1949), showed that feeding vegetables gave better response than feeding equivalent amount of β-carotene in oil. It

It was further observed that an intake 4 mg of β-carotene per day was essential for young adults. Its absorption depended largely on fineness of division of the vegetables and was about 50 percent when finely divided spinach and carrots were provided. Bhat (1973) also reported that vitamin A was retained better in liver when rats were fed vegetables than when β-carotene in oil was given.

On the other hand, Chari (1967) observed that synthetic vitamin A acetate was absorbed better than β-carotene supplemented through vegetables. When depleted rats fed green vegetables as a source of β-carotene or vitamin A acetate at the rate of 150 to 200 µg/100 g diet for the period of six weeks. Similarly, Murthy (1973) reported an increase in serum level of vitamin A (8.1 µg/100 ml) in young women given 425 µg of retinol for 20 days in comparison to those given 1980 µg of β-carotene from carrots (3.0 µg/100 ml).

In a comparative study on utilization of β-carotene, from different vegetables, Rajalakshmi et al. (1975) observed that availability of β-carotene from vegetables was better in the case of vitamin A deficient rats than normal rats. The magnitude of absorption was attributed to the extent of depletion of their reserves.

Availability of vitamin A from milk:

It has been well established that vitamin A is better absorbed in the body from an aqueous medium than when it is provided in fatty carrier, particle size apparently being the most important factor in this regard. Studies carried out on infants indicated that vitamin A in milk was absorbed almost equally well when the vitamin was given in the form of an aqueous dispersion, as vitamin was relatively poorly absorbed when administered through liquified butter fat (Lewis et al., 1950). Further, Lewis and Cohlan (1950) reported a large variation in serum and liver vitamin A levels in rats when vitamin A was supplemented in aqueous and oily preparation, respectively. Morales et al. (1950) showed that when infants were given skimmed milk homogenized with butter fat having fish liver oil as a source of vitamin A with a fat globule size having a maximum diameter of 2 μ, retained 63 percent of vitamin A, as against 43 percent when fat was supplied by gavage in six separate doses. The increased absorption was attributed to the reduced fat globule particle size. Although a minimum level of dietary fat has been suggested to be essential for adequate vitamin A absorption, studies with rats indicated that added vitamin A was absorbed better from fortified non-fat dry-milk than from a starch diet containing 5 percent fat

(Rasmussen et al., 1964). This was in conformity with the earlier observation of Vavich et al. (1955), that milk as such contains certain constituents which helped in increased storage of vitamin A in liver. Vitamin A deficient rats when given carotene (56 µg) emulsified in fresh non-fat milk and homogenized, had an average of 63.5 µg vitamin A per liver as against those received the supplement emulsified in water i.e. 42.3 µg/liver. However, at lower intake of 32 µg, such differences were not visible. Berger and coworkers (1966) further reported a better absorption of vitamin A from a casein complex in comparison when it was supplemented in oily solution. The recovery in liver was about 60 percent in the case of casein complex while it was only 40 percent from the oil solution. Similarly, Figueira et al. (1969) also observed better utilization of vitamin A by infants from vitamin A fortified non-fat dry milk.

Water soluble vitamins

Ascorbic acid in milk:

Ascorbic acid (vitamin C) is usually present in its reduced form in milk (Kon and Watson, 1936; Hand, 1943). However, it undergoes gradually oxidation to dehydroascorbic acid (Lechner and Kiermeier, 1969). Thus, depending on conditions and temperature during storage, market milk often contains vitamin C in reduced as well as oxidised form.

By and large, buffalo milk has been reported to have higher content of the vitamin C than cow milk. This was evident from the range of 18 to 39 mg/litre for buffalo milk reported by Kothavalla and Gill, (1943); Varma and Paul, (1947); Iordanov and Boev, (1956) and Barakat and Adel-Wahab, (1961) and the range of 14 to 20 mg for cow milk reported by Varma and Paul, (1947); Ford et al., (1959); EL-Rafey, (1962); Dluzewska and Bilinska, (1966) and Burton et al., (1970). Ewe and goat milk were, however, reported to have ascorbic acid content identical to those in buffalo milk (Iordanov and Boev, 1956 and Barakat and Adel-Wahab, 1961).

Losses during processing treatments:

Although milk is not considered as a potential source of vitamin C in term of meeting the human dietary requirement, its destruction during various processing treatments assumes special significance in the light of interaction with other nutrients, ultimately altering their levels. Losses of vitamin C occur at many stages during processing and also during storage and distribution of milk. It was as early as 1936, when Sharp reported gradual decrease in the vitamin C content of milk from the stage it was received from the milch animal and processed at dairy plant, until it was transported and delivered. Similar observation was made by Lechner and Kiermeier (1969), who studied vitamin C at different

stages of processing and packaging until it was delivered. An overall decrease observed was, however, not striking.

Pasteurization of milk has been shown to reduce vitamin C concentration in milk to a varying degree. Kothavalla and Gill (1943), observed a reduction of 24 percent and 17.5 percent in vitamin C content of milk after pasteurization either by holding or flash method, respectively. Similarly, Holmes et al. (1943) found that when milk was cooled and stored for 10 hours before pasteurization, vitamin C was reduced from an average of 19.7 to 15.9 mg per litre. A comparison of milk pasteurized by holding method, with that pasteurized by HTST method, showed that vitamin C was found to be more stable at higher temperature. Holmes et al. (1945) and Dluzewska and Bilinska (1966) on the other hand, found no loss of vitamin C in milk pasteurized by HTST method. However, flash method showed only 6 percent reduction. Furthermore, it was observed that all the dehydroascorbic acid was found to be degraded during the preheating period in the course of pasteurization (Woessner et al., 1940 and Hand, 1943). Thus, Ford et al. (1969) and Burton et al. (1970) observed that after pasteurization, milk contained no dehydroascorbic acid or at the most only a small amount formed at the end of the process. However, Lechner and Kiermeier (1969) reported that about 70 percent

of the total dehydroascorbic acid was lost between the period when milk was received and processed at dairy plant.

It was observed by Dluzewska and Bilinska (1966) that as the temperature for flash sterilization was raised from 85° to 145°C for 3 or 4 sec, the destruction of vitamin C progressively increased from 6 to 44 percent. Ford et al. (1969) found about 20 percent destruction of vitamin C content in milk during UHT processing. However, Burton et al. (1970) observed a smaller reduction of only 8 percent by both direct and indirect UHT processing.

Adoption of temperatures between 40 and 120°C considerably lower than UHT or sterilization, however, during longer duration upto 20 or 30 minutes was reported to cause still longer destruction i.e. upto 50 percent (Ford et al.,1957,59).

Effect of homogenization and pasteurization:

Bell and Sanders (1945) studied the vitamin C losses in milk fortified with 25 mg/quart after pasteurization and homogenization, found on an average loss of about 13 percent. Kyla-Siurola and Antila (1972) failed to observe any losses of vitamin C either during pasteurization alone or in homogenized milk and subsequently pasteurized. Further, they reported that about half of the original vitamin C content was destroyed in twice pasteurized and homogenized where milk was stored between the treatment

for 3 days at 4°C. Data, however, did not indicate the stage at which the activity was chiefly lost. Sterilization and homogenization too, did not show any effect on lowering the vitamin C content. However, Woessner et al. (1939) observed a 50 percent reduction in vitamin C content in pasteurized and homogenized milk.

B-group vitamins:

Levels of B-group vitamins in milk have been observed to vary considerably among species. This was evident from the wide range between 0.45 and 0.81 µg/ml in buffalo milk for thiamine (Rao and Basu, 1951; Boman, 1953; EL-Rafey, 1962 and Paolis and Gregory, 1963). Cow milk was also found to have similar values. Interestingly sheep milk was found to be richest in thiamine content with the average value of 0.97 µg/ml (Rao and Basu, 1951 and EL-Rafey, 1962).

As regards riboflavin content, the levels in buffalo milk varied between 1.02 to 2.48 µg/ml (Rao and Basu, 1951; Boman, 1953; EL-Rafey, 1962; Sirry and EL-Said Saleh, 1962 and Paolis and Gregory, 1963). Cow and sheep milk was also found to be identical in riboflavin content as of buffalo milk (Rao and Basu, 1951; Sirry and EL-Said Saleh, 1962), however, goat milk showed distinctly lower value (Rao and Basu, 1951).

Nicotinic acid content in milk from different species such as cow, sheep and goat were found to be

almost similar (Rao and Basu, 1951 and Paolis and Gregory, 1963), however, significantly higher concentrations between 1.71 and 2.60 µg/ml were observed by Boman (1953) and EL-Rafey (1962) in buffalo milk.

Pantothenic acid and folic acid concentrations in buffalo milk, too, did not vary much, with the average values ranging between 1.50 to 2.02 µg/ml and 5.50 µg/ml, respectively, in different studies (Boman, 1953; EL-Rafey, 1962 and Paolis and Gregory, 1963). Cow milk also did not differ from buffalo milk (Boman, 1953 and Burton et al., 1970).

As regards vitamin B_6, buffalo milk showed an average had a concentration of 0.325 µg/ml (Boman, 1953; and EL-Rafey, 1962), however, Paolis and Gregory (1963) found still lower concentration of 0.25 µg/ml. Cow milk showed somewhat lower levels ranging between 0.1 to 0.22 µg/ml with regards to vitamin B_6 (Chapman et al., 1957; Ford et al., 1959; and Dluzewska and Bilinska, 1966).

As regards vitamin B_{12} content, EL-Rafey (1962) and Paolis and Gregory (1963) reported buffalo milk to contain 4.0 to 4.32 mµg/ml. However, in the earlier study, Sreenivasamurthy et al. (1953) found a lower range of 2.8 to 4.0 mµg/ml for vitamin B_{12}. Zahriev and Kaloianov (1965) on the other hand, reported a little higher mean value of vitamin B_{12} in buffalo, cow and ewe milk

as 5.77, 6.14 and 9.09 mμg/ml, respectively.

Effect of different processing treatments:

Losses of B-group vitamins in milk during various processing treatments, accompanied increased shelf life. During the preparation of various by-products, such losses have been shown to be influenced by the duration and severity of heat treatment. Houston et al. (1940) observed that commercial pasteurization of milk caused 20 and 10 percent reduction in free and total thiamine content, respectively, whereas sterilization resulted over 53 percent loss of total thiamine as compared to 26 to 45 percent of free thiamine. Riboflavin has been reported to be heat stable and was not affected during commercial sterilization. Holmes et al. (1943,45) observed that HTST pasteurization reduced thiamine content by about 10 percent, while it did not show any effect on riboflavin content. Values before and after pasteurization were 1.50 and 1.48 mg/litre. Clemow (1951) reported, marginal losses between 4.9 to 9.5 percent of riboflavin during pasteurization.

As regards vitamin B_6, Debrit (1952) studied losses during pasteurization at 63°C for 30 minutes or at 72°C for 15 seconds with immediate cooling or sterilization

at 115°C in an autoclave for 20 minutes. While pasteurization did not result any significant loss of vitamin, sterilization caused only marginal destruction of 4.7 to 6 percent.

Ford (1957) and Chapman et al. (1957) observed that pasteurization, sterilization, UHT treatment and UHT treatment with subsequent sterilization of milk, did not cause any appreciable reduction in riboflavin, pantothenic acid, nicotinic acid, vitamin B_6 and biotin content as evident from negligible losses ranging between 0 and 2.6 percent. Losses in respect of thiamine were, however, higher and ranged between 10 and 50 percent depending upon the severity of heat treatment used in sterilization of milk.

Dluzewska and Bilinska (1966) found that flash pasteurization has no influence on riboflavin and pantothenic acid content, however, vitamin B_6 and B_{12} contents decreased by 7 and 10 percent, respectively. Losses in respect of all vitamins were much less during HTST pasteurization as compared to flash pasteurization. In an another study Kruglova and Gulko (1966) reported that thiamine losses during pasteurization by holding method and sterilization (115°C for 15 minutes) amounted to 30 and 50 percent, respectively, however, pasteurization at 95°C had negligible effect. Riboflavin and pantothenic acid

were found to be stable against these heat treatments. Vitamin B_{12} was found to be stable to heat at pasteurization temperature, but at sterilization temperatures the vitamin activity was reduced by 80 percent. Flash sterilization of milk at 85°C, 125°C, 135°C and 145°C for 3 or 4 sec showed negligible destruction of thiamine and riboflavin. On other hand, loss of vitamin B_{12} increased with the rise in temperature (Rossihina et al., 1969). Similarly, Burton et al. (1970) failed to observe any losses of thiamine during UHT sterilization of milk. Losses for folic acid were found to be about 10 percent in an indirect and about 4 percent in the direct UHT method, As regards vitamin B_6, losses were 10 and 6 percent, respectively. Reduction in vitamin B_{12} content was higher in direct (13 percent) in comparison to indirect method i.e. 4 percent.

Using microbiological method Karlin (1969) estimated the levels of folic acid, vitamin B_6 and B_{12} in milk subjected to various heat treatments, namely, (i) pasteurization at 91 to 92°C for 2 seconds, (ii) boiling for 2 to 5 minutes, (iii) homogenization and sterilization in bottle at 119-120°C for 13 minutes and (iv) homogenization and sterilization at 140°C for 3 to 4 seconds. The mean values for total folate and free folate activities were 68 and 44.8 µg/litre in untreated milk. Losses of total and free folic acid under these treatments were (i) 12 and 23, (ii) 17 and 71, (iii) 39 and 90, (iv) 8 and 92 percent, losses in

vitamin B_6 and B_{12} were (i) 3 and 8, (ii) 3 and 31, (iii) 16 and 73 and (iv) 0 and 100 percent, respectively. It was evident that the degree of destruction of vitamins increased with the extent of heat applied. It was not, however, clear from these observations, whether losses of vitamins were due to homogenization alone i.e. in group iv. In an another study, Karlin et al. (1969) found that by heating the milk at 62°C for 3 to 4 seconds most of the vitamins were retained without any significant loss. The mean losses expressed as percent were 2.6 for thiamine, 7.8 for riboflavin, 0.5 for nicotinic acid, 0.6 for pantothenic acid, 1.0 for vitamin B_6, 10.5 for vitamin B_{12}, 4.7 for biotin and 7.5 for folacin, respectively.

Ghitis and Candanosa (1966) found that where boiling of milk for 5 seconds caused a severe reduction of folic acid from 55 µg to 30 µg/litre, pasteurization at 50°C had little effect in respect of folic acid. Earlier, Knaut (1955) studied the effect of boiling on thiamine and riboflavin content in milk. After boiling for 3 minutes, the mean losses in percent for thiamine and riboflavin were 7.43 and 2.6, respectively.

Theophilus and Stamberg (1945) failed to observe any effect of pasteurization, homogenization or storage

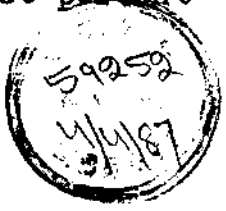
for 24 hours at 0°C on riboflavin content. Similarly Hassinen et al. (1954) did not observe any loss for vitamin B_6 in milk after pasteurization and homogenization. S_T-Pierre et al. (1963), on the other hand, observed a significant loss of vitamin B_{12} when homogenized milk was pasteurized at 62 to 65°C for 30 minutes than at 73°C for 15 seconds.

Relationship between ascorbic acid, folic acid and vitamin B_{12} during heat processing:

A study of factors which influence the thermal destruction of vitamin B_{12} in milk indicated that such process was essentially an oxidative in nature and was related directly/indirectly with the oxidative degradation of ascorbic acid affected by the presence of dissolved oxygen (Kon and Watson, 1936 and Rossenberg et al., 1956). This was further, confirmed by Ford (1957), who observed that aeration of milk before in-bottle sterilization greatly increased the losses of both vitamin B_{12} and ascorbic acid, whereas prior thorough aeration by flushing with nitrogen, reduced such destruction. The efficiency of such deaeration in stabilizing vitamin B_{12} to range between 0 and 50 percent. Ford (1967) further reported that removal of oxygen by nitrogen flushing and sterilization of milk either at 110°C for 20 minutes or at 120°C for 30 minutes, did not affect vitamin B_{12} and ascorbic acid activity. The mean concentration of vitamin B_{12} in deaerated milk was decreased from 4.2 to 3.8 µg/litre

and respective reduction in ascorbic acid was from 30 to 29 µg/ml.

Conditions during storage too, seem to be equally important in the retention of these vitamins. During direct heat processing and subsequent storage for longer periods, losses of ascorbic acid and folic acid were markedly influenced by residual oxygen level in the milk (Ford et al., 1968). It was observed that during processing about 20 percent of folic acid as well as ascorbic acid were destroyed. The remaining ascorbic acid disappeared in the next two weeks of storage, while folic acid level fell to zero in one case, while it was found to be stable in another case. The residual vitamin B_6 and vitamin B_{12} decreased by 40 percent after 90 days of storage. Such losses further increased over 50 to 60 percent during 180 days of storage. In another study, milk from three different plants was stored at 60 to 65°C for 0, 2, 7 and 14 days. Folic acid was found to be completely destroyed from all milk samples by 14 days of storage and ascorbic acid, vitamin B_6 and B_{12} also decreased by 20 percent. In the earlier study, Ford (1967) reported that if dissolved oxygen was excluded from milk, better protection against destruction was obtained. In the presence of oxygen in milk about 50 percent of the folate activity was lost on heating, while upto 80 percent



was further lost on exposure to sunlight. Burton et al. (1967) also reported relatively lesser losses of folic acid occured in the presence of ascorbic acid.

When the milk was heated at high temperature (UHT) in the presence of air or when oxygen was removed partially or totally, the ascorbic acid losses were about 20 percent. During storage in the absence of oxygen, no further destruction occured. However, at a concentration of 1 ppm oxygen, vitamin was completely lost in 14 days. About 20 percent of the folic acid was destroyed during processing and further losses on storage were dependent on the residual oxygen content of the milk. However, in the absence of oxygen, folic acid was found to be stable (Ford et al., 1969 and Burton et al., 1970).

Though, most of water soluble vitamins except ascorbic acid and thiamine are stable at pasteurization temperatures, these are more susceptible to destruction to exposure at higher temperature and prolonged storage in the presence of dissolved oxygen.

CHAPTER - III

MATERIALS AND METHODS

MATERIALS AND METHODS

Collection of milk samples

Buffalo, cow and goat milk samples were collected from the herd of respective species from cattle section of National Dairy Research Institute, Karnal at about 6.00 A.M. Keeping in mind the experimental design, the milk was divided into three lots and given following treatments.

Lot I : Buffalo milk (whole) was pasteurized by holding method (63°C for 30 minutes).

Lot II : Buffalo milk was standardised to a fat level of 4.5 percent by mixing it with skimmed buffalo milk, followed by homogenization as described in the later section. The homogenized milk was pasteurized by holding method.

Lot III : Buffalo milk (whole) was homogenized and pasteurized by holding method.

Homogenization of milk

Before homogenization milk was heated to 60°C to inactivate the enzyme, lipase. The heated milk was passed through a Gaulin piston type homogenizer at a standard pressure of 2500 psi in a double stage. The pressures in first and second stages were 2000 and 500 psi, respectively.

Determination of homogenization efficiency

The fat content in raw and homogenized milk was estimated according to Gerber method. On homogenization 50 ml portion of the homogenized milk was centrifuged at 1500 rpm for 30 minutes. An aliquot of 11 ml was removed from the bottom section using 11 ml pipette and fat content was estimated as mentioned above.

Homogenization efficiency was calculated according to the procedure described by Ridgway (1957) as described below:

$$\text{Homogenization efficiency} = \frac{F_1}{F_2} \times 100$$

F_1 = the fat content of milk in bottom 11 ml of the centrifuge tube

F_2 = the initial fat content of milk before centrifugation.

Pasteurization of milk

Whole milk or homogenized milk was pasteurized either according to Holding method or by high temperature short time (HTST) method as required in different experiments.

Pasteurization by holding method:

Fresh or homogenized milk taken in a clean container was placed in a water bath maintained at 64 to 65°C to attain temperature of 63°C at which the milk was maintained for 30 minutes with intermittent stirring to ensure the uniform temperature.

Pasteurization by HTST method:

Fresh or homogenized milk in a clean container was placed in water bath maintained at 74 to 75°C. As the milk attained a temperature of 71 to 72°C, was maintained for 15 seconds.

On cooling to room temperature, the homogenized and pasteurized milk samples were filled in cleaned and dried plastic bottles and kept in refrigerator at 5°C until used in feeding experiments.

Evaluation of protein quality

Keeping in view the limitations of various procedures adopted to assess the protein quality the experiments were conducted to study growth promoting ability as well as nitrogen balance, on giving test milk samples.

Modified protein efficiency ratio determination

Male weanling albino rats weighing between 35 to 40 g were obtained from small animal house of the institute. These were maintained on a protein-free diet (Table A) for a period of 10 days. During this period they lost about 15 to 20 percent of their body weights. At this stage the animals were weighed and divided into three groups in randomized fashion. Each group consisted of 8 animals and had identical body weights. These were maintained on the following dietary regimes for another 10 days.

Group I : Buffalo milk (whole) pasteurized.

Group II : Buffalo milk (standardized) homogenized and pasteurized.

Group III : Buffalo milk (whole) homogenized and pasteurized (pasteurization was done using holding method in this experiment).

Animals were maintained individually in anodised aluminium cages, with free access to water, test milk samples were given ad libitum.

Milk was offered three time a day, i.e. in the morning at 6.00 A.M., 12 noon and 7.00 P.M. and residual milk was recorded, to calculate the daily intake. After completion of the experimental period the animals were weighed to record the gain in their body weight. The modified protein efficiency ratio (PER_D) was calculated according to Venkatrao et al. (1964).

$$PER_D = \frac{\text{Gain in body weight(g) during protein repletion period of 10 days}}{\text{Protein intake (g) during the period of 10 days}}$$

Nitrogen estimation:

The nitrogen content in test milk samples was determined according to microkjeldhal method described in AOAC (1970) and the protein content was calculated by multiplying the nitrogen content by the factor 6.38.

Nitrogen balance studies

Determination of biological value and digestibility coefficients:

Male weanling albino rats weighing between 40 to 45 g were obtained from the small animal house of National Dairy Research Institute, Karnal. These were divided into three groups of eight animals each in randomized fashion so that the average body weights (g) for all groups, were identical. Rats were housed individually in metabolic cages and were fed ad lib. protein-free diet (Table A) for the period of 10 days. During this period animals had free assess to water. Daily collections of urine and faeces were made. Urine was collected in bottles containing a few ml of 10 percent H_2SO_4, while faeces were collected daily and dried in an oven maintained at 100 $\pm$ 1°C. Samples of urine and faeces were pooled and stored appropriately in the refrigerator until taken for analysis for nitrogen content. On the eleventh day animals were switched over to feeding on test milk samples for a period of 10 days as given below:

Group I : Buffalo milk (whole) pasteurized.

Group II : Buffalo milk (standardized), homogenized and pasteurized.

Group III : Buffalo milk (whole) homogenized and pasteurized.

The milk was offered three times a day as described earlier. The daily intake of milk was recorded individually by measuring the residual leftover milk. The daily collection of urine and faeces was done as described earlier and nitrogen content in these samples was determined.

Calculations:

Biological value and digestibility coefficients were calculated according to Mitchell (1924).

Digestibility coefficient (D.C.) =

$$= \frac{\text{Nitrogen digested}}{\text{Nitrogen intake}} \times 100$$

$$= \frac{I_n - (F_n - F_{en})}{I_n} \times 100$$

$$\text{Biological value} = \frac{I_n - (F_n - F_{en}) - (U_n - U_{en})}{I_n - (F_n - F_{en})} \times 100$$

Where

I_n = Nitrogen intake on test protein diet

F_n = Faecal nitrogen, on test protein diet.

F_{en} = Endogenous faecal nitrogen on protein-free diet.

U_n = Urinary nitrogen, on test protein diet.

U_{en} = Endogenous urinary nitrogen on protein-free diet.

TABLE A

Composition of protein-free diet

Ingredient	Percent added
Starch (maize)	73
Sucrose	9
Refined Groundnut oil	8
Cellulose	5
Salt mix*	4
Vitamin mix**	1
Total	100

* Composition of salt mix (AOAC, 1970)

Weigh 70 g of NaCl and grind 0.5 g. KI with a portion of NaCl. Similarly grind together remainer of the NaCl with 194.5 g KH_2PO_4, 58.5 g $MgSO_4$ (anhydrous), 140.5 g $CaCO_3$, 13.5 g $FeSO_4.7H_2O$, 2 g $MnSO_4$, 0.2744 g $ZnSO_4.7H_2O$, 0.2380 g $CuSO_4.5H_2O$ and 0.0115 g $CoCl_2$, finally adding the NaCl-KI mixture. Reduce entire mixture to fine powder.

** Composition of vitamin mixture (AOAC, 1970)

Weigh 50 mg thiamine hydrochloride, 400 mg riboflavin, 50 mg pyridoxine hydrochloride, 400 mg nicotinic acid, 400 mg *Ca-pantothenate*, 20 mg folic acid, 4 mg biotin, 0.3 mg vitamin B_{12}, 10 mg inositol, 10 mg para-amino-benzoic acid and 99.6 g of starch to mix these well.

Fat soluble vitamins/100 g diet:

Vitamin A	2,000 IU
Vitamin D	200 IU
Vitamin E	10 IU
Choline chloride	200 mg

Determination of net protein utilization (NPU):

Net protein utilization was determined according to the method of Miller and Bender (1955).

Young male albino rats weighing between 50 to 60 g were taken for this study. These were grouped according to randomized design into four groups of eight animals each. The average weight for each group was identical. These were subjected to the following dietary treatments for the period of 10 days.

Group I : Protein-free diet

Group II : Buffalo milk (whole) pasteurized

Group III : Buffalo milk (standardized), homogenized and pasteurized.

Group IV : Buffalo milk (whole) homogenized and pasteurized.

Each rat was housed individually. The milk was offered three times daily and the intake of milk recorded as described earlier.

At the end of the experimental period, animals were sacrificed and the carcass was dried in an oven maintained

at 95°C ± 1°C till it attained a constant weight. These were then uniformly powdered. Nitrogen content in the carcass as well as test milk samples was determined according to microkjeldahl procedure described earlier. The net protein utilization value was calculated as given below:

$$\text{NPU (\%)} = \frac{\text{Body-N content in test protein group} - \text{body-N content in protein-free group}}{\text{Nitrogen intake}} \times 100$$

Fat absorption

The absorption of milk fat from different test milk samples was determined according to the procedure of Tomarelli et al. (1968).

Young male albino rats weighing between 80 to 100 g were taken in this experiment. They were maintained on a fat-free diet (Table B) for the period of three days, for acclimatisation. These rats were then divided into four groups of eight animals each, according to randomized design. The average body weight (g) for all groups were identical. These were kept on the following dietary treatment for the period of three days followed by fat-free diet for another 3 days.

Group I : Fat-free diet (control)
The control group was kept on fat-free diet for the period of six days.

Group II : Buffalo milk (whole) pasteurized

Group III : Buffalo milk (standardized), homogenized and pasteurized.

Group IV : Buffalo milk (whole) homogenized and pasteurized.

The test milk samples were offered three times every day and the daily intake was recorded. During this period faeces in all the four groups were collected daily. These were weighed and preserved in 95 percent ethanol, until taken for total fatty acid analysis according to Van de Kamer et al. (1949).

The absorption of total fatty acids was calculated from the ratio of the amount in faeces (corrected for endogenous excretion) to the amount ingested.

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Composition of salt mixture and vitamin mixture is similar to that given under Table A (AOAC, 1970).

In addition, fat soluble vitamins were also added at the rate of 20,000 IU of vitamin A, 2,000 IU of vitamin D and 100 IU of vitamin E per kg of the diet.

Determination of curd tension

Six different trials were conducted to determine the curd tension of milk from different species, namely, buffalo, cow and goat. As regards mixed milk samples were made using whole milk from buffalo and cow in the ratios of 90:10, 50:50 and 10:90. All these samples were homogenized and pasteurized as described earlier.

The curd tension in milk was determined by adopting the procedure of Chandrasekhara et al. (1957).

Fifty ml milk samples to be tested were taken in 100 ml beakers and warmed to 37°C. To this 2 ml of 0.1 percent rennet solution (Hansen's) was added rapidly into the beaker and the milk was thoroughly mixed using the knife. The beaker was then placed in a thermostatically controlled water bath at 37°C and allowed to stand for a period of 3 hours. The pan was loaded with lead shots till the curd tension knife cuts its way through the curd. The weight of the lead shots expressed in grams was taken as a measure of curd tension.

Determination of total solids

Total solid content in test milk samples before homogenization was estimated gravemetrically according

to AOAC (1970). Milk samples of known weight were dried to a constant weight in a moisture dish in an oven maintained at 95° ± 1°C. Total solids content was calculated as

$$\text{Total solid (\%)} = \frac{\text{Weight of dried milk}}{\text{Weight of sample taken}} \times 100$$

Biological availability of vitamin A

Experimental animals:

Weanling male rats of 24 to 27 days old weighing between 35 and 48 g were made deficient in vitamin A by maintaining on vitamin A deficient diet (Table C) till the cessation of growth was evident and characteristic symptoms of vitamin A deficiency were shown by the rats as illustrated in (Illustration I). At this stage, the gain in body weight during the period of one week was not more than one gram. Some of the animals exhibited loss in body weight. Body weight of rats was recorded on every 3rd day of the experimental period. Vitamin A deficient animals were divided into four groups in randomized fashion with six animals in each group and were given the following dietary treatment.

Group	I	:	Control
Group	II	:	Buffalo milk (whole) pasteurized, (20 ml).
Group	III	:	Buffalo milk (standardized), homogenized and pasteurized milk (30 ml).
Group	IV	:	Buffalo milk (whole) homogenized and pasteurized (20 ml).

Twenty five IU of vitamin A was provided to the animals in group II, III and IV, from test milks. The animals had free assess to vitamin A deficient diet as well as water during the treatment. Animals were given these supplements till they regained the normal growth rate of 2 g per day. At this stage, animals were sacrificed, blood and liver were analysed for vitamin A content.

TABLE C

Composition of vitamin A deficient diet

Ingredient	(g)
Casein (vitamin free)	180
Starch (maize)	650
Groundnut oil (vitamin A free)	50
Yeast dried powder	80
Salt mix*	40
Total	1000

To every kg of diet was added vitamin D 2000 IU, vitamin E 500 IU and vitamin K 50 mg, respectively.

* Salt mix was of the same composition used in earlier experiments (Table A).

Estimation of vitamin A in blood serum :

Vitamin A in blood serum was estimated according to Yudkin (1941). To 1 to 2 ml of blood serum taken in a stoppered centrifuge tube were added equal volumes of 95 percent ethanol with constant stirring. This was followed by the addition of double the volume of diethyl ether. Tubes were stoppered and shaken vigorously for 10 minutes. Later these were centrifuged at 2000 rpm to separate the aqueous and etherial layers. The etherial layer was collected and a suitable aliquot was evaporated in a water bath maintained at 35°C and the residual ether was eliminated in the stream of CO_2. The residue was taken in 1 ml of choloroform in a cuvette. The colour was developed according to Carr and Price (1926) by adding 9 ml of 20 percent antimony trichloride with a drop of acetic anhydride to remove moisture and the colour density was measured at 620 mμ using Spectronic-20. The vitamin concentration was calculated from the standard curve obtained.

Vitamin A in liver:

Vitamin A from liver was extracted by direct extraction method proposed by Hinds et al. (1968).

One g of liver tissue was ground well with anhydrous sodium sulphate taken in the ratio of 1 : 6 in a pestle mortor. The ground sample was extracted with 100 ml of diethyl ether in a stoppered flask under inert atmosphe-

(nitrogen gas) for 24 hours at 0°C. Following extraction, the diethyl ether was decanted, the residue was reextracted with 50 ml of diethyl ether and shaken gently for 15 to 20 minutes on a mechanical shaker. Both the etherial layers were pooled and an aliquot of 75 ml of the etherial extract was passed through column containing anhydrous sodium sulphate. The ether was evaporated, dissolved in chloroform and volume was made to 10 ml. The vitamin A was determined as described earlier.

Determination of vitamin A in milk:

Vitamin A in milk was estimated according to the method proposed by Kochen (1944). One hundred ml of test milk sample was saponified with alcoholic KOH for 10 minutes. On cooling, it was extracted with diethyl ether. Subsequent steps were similar to those followed for vitamin A determination in serum, as described earlier.

Estimation of protein in liver:

The protein content in liver homogenate was estimated according to Richlerich (1969) using biuret reagent.

Determination of water soluble vitamins

Certain water soluble vitamins were estimated in untreated, pasteurized milk, homogenized, homogenized and pasteurized by holding and HTST method according to the standard procedures.

Ascorbic acid:

Preparation of sample: 15 ml of 6 percent TCA was added in a dropwise fashion to 5 ml of milk with intermittent mixing. After keeping for 5 minutes it was centrifuged at 6000 rpm for 10 minutes and supernatant collected. It was mixed with about 0.5 g of acid washed activated charcoal and shaken well and later filtered after 30 minutes through whatman filter paper No.1. The filtrate was taken for further estimation.

The intensity of the colour developed due to osazone was measured at 540 mμ in Klett Summerson photoelectric colorimeter. Total ascorbic acid in milk samples was extracted from the standard curve.

Total ascorbic acid in different milks was estimated as dehydroascorbic acid according to the procedure of Roe and Kuether (1943).

Determination of B-group vitamins

Milk samples were defatted according to the method of Rose Gottlieb (1959).

Estimation of thiamine:

Preparation of samples for assay: The vitamin from the defatted material was liberated enzymatically. Twenty ml of milk sample was heated with 25 ml of 0.1 N H_2SO_4 for 30 minutes in a boiling water bath. On cooling, the pH was adjusted to 4.5 with 2.5 M sodium acetate. Twenty

milligram each of papain (Biochemicals Unit, V.P.Chest Inst. Delhi) and takadiastase (Park-Davis) were added. This was incubated for 24 hours at 37°C under a thin layer of toluene. At the end of incubation the enzymes were inactivated by heating the reaction mixture for 30 minutes in a boiling water bath and the mixture was later filtered through whatman filter paper No.1. The filtrate was saved for further determination.

Total thiamine content of milk samples was determined according to the method of Fitzgerald and Hughes (1949) described by Barton-Wright (1952) using Lactobacillus fermenti - 36 as the test organism.

Determination of riboflavin

Preparation of sample:

Extraction and hydrolysis: Defatted milk samples were hydrolysed with 30 ml of 0.1 N HCl and 5 ml of 2.5 M sodium acetate by autoclaving at 15 lbs pressure for 15 minutes. On cooling, the pH was adjusted to 4.5 with 0.1 N NaOH and filtered through whatman filter paper No.1. The filtrate was again adjusted to pH 6.8 and made to aliquot, and was subsequently used in the determination.

Total riboflavin in milk was determined microbiologically using Lactobacillus casei -ATCC-7469 as the test organism according to Snell and Strong (1939) as described in Methods of vitamin assay by Freed (1966).

Determination of niacin

Preparation of sample: To 10 ml of defatted milk was added 50 ml of 1 N H_2SO_4 in 250 ml conical flask. The mixture was autoclaved at 15 lbs pressure for 30 minutes. After autoclaving, the samples were cooled and pH was adjusted to 6.8 with 1 N NaOH and the volume was made. The solution was filtered through Whatman filter paper No.1 and filtrate was used for the estimation.

The basal medium for nicotinic acid estimation was obtained from Hindustan Dehydrated Media and used at 7.3 percent concentration.

Niacin in milk was determined microbiologically using Lactobacillus plantrum ATCC 8014 according to the method of Snell and Wright (1941) described in methods of vitamin assay by Freed (1966).

Determination of pantothenic acid

Liberation of bound pantothenic acid: An aliquot of 1.5 ml of defatted milk was incubated at 37°C for 4 hours with 0.2 ml of phosphatase, 0.05 ml of chicken liver extract and 0.05 ml of 0.1 M $NaHCO_3$. The volume was made to 5.0 ml with water, of this 2 ml was pipetted into 50 ml cylinder, pH adjusted to 4.5 and volume made to 40 ml with water. This was filtered through Whatman filter paper No.40. The filtrate was used for further estimation.

Chicken liver extract: Liver from freshly killed one month old chicken was quickely chilled to approximately to 4°C, minced and homogenized with 20 volumes of cold acetone in a waring blender for 1 minute. The precipitate was washed with ether and dried in a vacuum desiccator. The resultant powder was stored in a deep freezer at -4°C.

The powder was extracted with 10 times its weight with cold, freshly prepared 0.02 M $NaHCO_3$ and was centrifuged. The supernatant was subsequently used as chicken liver extract.

Basal medium for pantothenic acid:

Dehydrated basal medium for pantothenic acid assay was obtained from Hindustan Dehydrated Media.

Pantothenic acid was estimated microbiologically using Lactobacillus plantrum ATCC 8014 as the test organism according to Hoag et al. (1945) as described in methods of vitamin assay by Freed (1966).

Determination of folic acid

Preparation of sample using conjugase: To 5 ml of defatted milk, 5 ml of 0.2 M sodium acetate buffer was added. This was heated at 100°C for 5 minutes in a water bath. On cooling to room temperature, 20 mg of dry chicken pancreas powder was added, incubated at 37°C for 24 hours under a thin layer of toluene. After inactivating the enzyme by heating to boil for 5 minute, the mixture

was cooled and the pH adjusted to 6.6 to 6.8 with 1 N NaOH solution. This was suitably diluted to give a final solution having a concentration of 0.2 mμg/ml of folic acid. The solution was filtered through Whatman filter paper No.40.

Chicken pancreas extract: The pancreas from freshly killed chicken was ground in a blender with five volumes of chilled acetone. This was filtered, removed and washed with acetone and subsequently air dried and stored in deep freezer.

Folic acid content in milk was determined microbiologically using Streptococcus faecalis ATCC 8043-R as the test organism according to the method Teply and Elvehjem (1945) as described by Freed (1966), using basal medium obtained from Hindustan Dehydrated Media was used at 7.5 percent level.

Determination of total vitamin B_6

Preparation of sample: To 10 ml of defatted milk sample 180 ml of water containing 1 ml of 10 N HCl was added and autoclaved at 15 lbs for 4 hours. On cooling, the pH was adjusted to 4.5 with 15 percent NaOH. The solution was diluted to give an approximate concentration of 0.01 μg vitamin B_6/ml. This was filtered and later used for microbiological assay. The basal medium for vitamin B_6 obtained from Hindustan Dehydrated Media, was used at 7.3 percent concentration.

Vitamin B_6 content was estimated microbiologically using Saccharomyces carlsberensis 4288 as the test organism according to the procedure of Atkin et al. (1943) as described in methods of vitamin assay by Freed (1966).

Determination of vitamin B_{12}

Extraction of sample: Vitamin B_{12} from milk was liberated according to Gregory (1954).

To 1 ml of milk sample, 1 ml of 0.1 M sodium acetate buffer was added, warmed to 60°C and 50 mg of papain (Biochemicals Unit) and one drop of sodium cyanide was added. It was incubated for 1 hour and then steamed for 10 minutes to inactivate the enzyme. The solution was diluted, filtered to obtain a clear solution and pH adjusted to 6.0.

The basal medium obtained from Hindustan Dehydrated Media was used at 8.5 percent concentration.

Vitamin B_{12} was determined microbiologically using Lactobacillus leichmannii ATCC 7830 according to Skeggs et al. (1950) as described in methods of vitamin assays by Freed (1966).

Statistical analysis

The statistical analysis of the experimental data was carried out according to the Snedecor and Cochran (1967).

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CHAPTER - IV

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RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

EFFECT OF HOMOGENIZATION AND PASTEURIZATION ON CURD TENSION OF MILKS FROM DIFFERENT SPECIES

An effecient homogenization of milk has been recognised to lower the curd tension which inturn, is considered as an index of better digestibility. However, certain gross chemical constituents of milk are known to influence the curd tension. Furthermore, homogenization is seldom adopted without subsequent heat treatment such as pasteurization, sterilization etc. Keeping in mind the differences in curd tension of different milks, it becomes necessary to ascertain whether the fat component has any role in determining the curd tension of milk samples. Experiments were therefore conducted to study the homogenization efficiency for milks from different species as well as the effect of homogenization and pasteurization on the curd tension. Keeping in mind the overall significance of total solids content in respect of curd tension, these too have been determined and data are presented in this section.

Homogenization efficiency

Although the homogenization treatment could be described as the one in which large fat globules are split into smaller ones, the process, is somewhat complex

with many facets yet not clearly understood. During homogenization, fat globules get evenly dispersed in the milk, no cream line formed on standing. In other words, fat globules form a stable emulsion.

Data on the fat content of milk before and after homogenization and homogenization efficiency for milk from different species as well as mixed milk, are presented in Table 1. It was seen that despite of wide variation in the fat content in different milks samples used in this study, the homogenization efficiency varied within a narrow range of 88.68 and 90.62 percent. The maximum value observed in the case of buffalo milk was, however, not significantly higher than the minimum value for mixed milk (buffalo : cow 10 : 90). It was thus apparent from the statistical analysis (Table 1A) that all milks could be homogenized with same degree of efficiency, irrespective of the level of fat. Values observed in the present study compared well with those reported by Ridgway (1957), who reported maximum efficiency of 87.5 percent for milk homogenized at the pressure 2500 psi with subsequent centrifugation at 1100 rpm for 30 minutes. These values correlated well as well with those of USPH index of 1.4 and burrette method value of 96.6.

Curd tension

As mentioned earlier, apart from homogenization, the extent of heat treatment, as well as difference in the

TABLE - 1

Homogenization Efficiency for Milk from Different species and Mixed Milk

Milk	Average fat content (%) Before homogenization	After homogenization	Average efficiency (%)
Buffalo	7.78 ±0.35	7.05 ±0.34	90.62 ± 1.15
Cow	3.36 ±0.13	3.00 ±0.10	89.29 ± 1.26
Goat	3.79 ±0.12	3.40 ±0.09	89.71 ± 1.09
Mixed (Buffalo:Cow)			
90 : 10	6.98 ±0.42	6.25 ±0.34	89.54 ± 1.89
50 : 50	5.23 ±0.35	4.72 ±0.35	90.25 ± 1.02
10 : 90	3.80 ±0.16	3.37 ±0.17	88.68 ± 1.31

Mean of six observations with ± S.E.

TABLE - 1A

Analysis of variance for homogenization efficiency

Source	d.f.	S.S.	M.S.S.	'F' value
Between milk	5	14.41	2.882	1.515 N.S.
Between replicate	5	1.87	0.374	0.197 N.S.
Error	25	38.05	1.902	

N.S. = Non Significant

TABLE - 2

Effect of homogenization and pasteurization on curd tension of different types of milk

Treatment	Milks					
	Goat	Buffalo	Cow	B : C 90:10	B : C 50:50	B : C 10:90
Untreated	21.4 ± 0.07	42.8 ± 6.69	19.7 ± 0.25	37.8 ± 4.38	28.9 ± 1.49	20.3 ± 1.21
Pasteurized	18.4 ± 0.23	39.8 ± 4.57	16.7 ± 0.92	33.5 ± 1.91	25.7 ± 0.79	17.8 ± 1.18
Homogenized	12.0 ± 0.84	16.6 ± 2.32	9. 6 ± 0.32	16.1 ± 1.13	13.1 ± 0.76	13.2 ± 0.35
Homogenized and Pasteurized	6.4 ± 0.91	10.1 ± 1.04	6.1 ± 0.69	8.7 ± 1.03	8.8 ± 1.01	7.9 ± 0.82

Values are expressed as gram

Mean of six observations with ± S.E.

B:C Proportion of buffalo(B) and cow(c) milk.

TABLE - 3

Analysis of variance - curd tension of different types of milk

Source of variation	d.f.	S.S.	M.S.S.	'F' value
Between treatment	3	10137.07	3379.02	499.27**
Between replicates	5	189.93	37.98	5.61**
Types of milk	5	4013.05	802.61	118.59**
Interaction	15	1900.59	126.71	18.77**
Error	115	778.30	6.76	-

** Significant at 1% level.

CD at 5% - Treatment - 3.6055

Replicates - 2.4939

Types of milk - 2.4939

to influence the curd tension. A mixed milk sample with predominance of buffalo milk (90 parts buffalo milk and 10 parts of cow milk) showed the curd tension of 37.8 g whereas that having only 10 parts of buffalo milk showed a low curd tension of 20.3 g.

The values observed for curd tension of buffalo milk in the present study compared well with those reported earlier (Chandrasekhara et al., 1957 and Jain et al., 1974). These were, however, found to be considerably lower than the range of 55 to 56 g observed by Abdel-Salam et al. (1974) and Abou-Dawood and EL-Sawaf (1977). The differences observed could be due to the difference in the breed of buffaloes, because the type of the coagulant used in most of these studies was similar.

The higher curd tension for buffalo milk as compared to other species could be attributed to its chemical composition. This could be well conceived in the light of an established correlation between curd tension and the chemical composition particularly its total solids, total protein, casein and calcium content (Weisberg et al., 1933; Doan and Welch, 1934; Riddell et al., 1936; Jain et al., 1974 and Abou-Dawood and EL-Sawaf, 1977). In the present study total solids (Table 5) were also highest in buffalo milk. Interestingly, these values were almost similar for cow and goat milk.

The curd tension values viz. 19.7 and 21.4 g observed for cow and goat milk, respectively, were markedly lower than the values ranging between 25 to 56 for cow milk and 40 to 55 for goat milk reported by various workers (Kelly, 1941; Turner and Garrison, 1936-37; Rao et al.,1964; Jain et al., 1974 and Abou-Dawood and EL-Sawaf, 1977). Such large variation could either be due to difference in the breed of milch animals or due to difference in the type of coagulant used in these studies. Such a possibility would seem plausible in the light of the observation by different workers who showed significantly different curd tension values when pepsin-$CaCl_2$ and pepsin-HCl were used as coagulant (Miller, 1935 and Kelly, 1939).

The low curd tension in both cow and goat milk as compared to buffalo milk may be due to the species differences which reflected in lower total solids, total protein, casein and calcium content (Doan and Welch, 1934 and Abou-Dawood and EL-Sawaf, 1977).

As regards mixed milks, higher curd tension for the mixed sample with higher proportion of buffalo milk as compared to those with lesser quantities could be attributed to differences in chemical composition contributed proportionately by buffalo and cow milk. The statistical analysis of the data given in Table 3 showed that different types of milk differed significantly

($P < 0.01$) in respect of curd tension; with buffalo milk showing distinct superiority over other types of milk.

Effect of pasteurization

As regards the effect of pasteurization, it was further seen that pasteurization of milk at 62°C for 30 minutes reduced the curd tension only moderately, as evident from 7.0 to 15.0 percent reduction in different milk samples in comparison with that for raw milk samples (Table 4). The reduction was somewhat higher in the case of goat and cow milk than buffalo milk. In general, the decreases observed were significant ($P < 0.01$).

These results are in close agreement with those reported by Abou-Dawood and EL-Sawaf (1977) who found relatively lesser reduction in curd tension in buffalo and ewe milk as compared to cow and goat milk when pasteurized at 62°C for 30 minutes. This could be partially attributed to the better heat stability of buffalo milk than other milks as reported by Weisberg et al. (1933) and Abou-Dawood et al. (1976).

The results observed as regards to reduction in curd tension in the present study were in agreement in respect of lowering the curd tension on pasteurization with those reported earlier (Mortenson et al., 1935; Berry, 1936; Chandrasekhara et al., 1957; Rao et al., 1964 and Jain et al., 1974).

TABLE - 4

Percent reduction in curd tension of different types of milk due to homogenization and pasteurization

Treatment	Milks					
	Goat	Buffalo	Cow	B : C 90:10	B : C 50:50	B : C 10:90
Untreated	-	-	-	-	-	-
Pasteurized	14.1	7.0	15.0	11.3	10.9	12.1
Homogenized	44.0	61.0	51.0	57.4	54.6	34.6
Homogenized and Pasteurized	70.1	76.3	68.8	68.8	69.3	60.7
Pasteurization alone after homogenization	26.0	15.2	17.8	19.3	14.7	26.1

B: C Proportion of buffalo (B) and cow(C) milk.

The decrease in curd tension due to heat treatment could be due to (i) lower calcium ion concentration and increase in the electrostatic charges carried by the casein micelles as reported by Richardson and Palmer (1929), (ii) denaturation of whey proteins, rendering these insoluble, (iii) decrease in the degree of hydration of casein and (iv) preparation as the degree of heat treatment increases (Rao et al., 1964).

Effect of homogenization

On homogenization the maximum reduction of 61.0 percent in the curd tension was observed in the case of buffalo milk, and minimum was found for goat milk i.e. 44.0 percent. The greater reduction in the case of buffalo milk was in conformity with the observation made earlier by Doan (1938) who reported that reduction was maximum in the case of milk with initial high curd tension. The percent reduction observed in the case of cow and goat milk were identical with the values observed earlier by different workers (Theophilus et al., 1934; Doan, 1938 and Kelly, 1941). The maximum softening effect of homogenization in buffalo milk could be partly attributed to the higher fat level which absorbed higher quantity of protein due to its increased surface area after homogenization. Similar observations were made earlier by Doan (1938); Kelly (1941) and Webb et al. (1978), who noted greater reduction in curd tension in milk with higher fat level in comparison with

a lower fat content. The increased number of fat globules during homogenization would serve as the point of weakness in the coagulum (Sommer, 1946).

Effect of homogenization and pasteurization

The combined effect of homogenization and the subsequent pasteurization was found to be yet more pronounced on the curd tension. Such effect was maximum in the case of buffalo milk i.e. 76.3 percent followed by goat 70.1 percent and cow milk 68.8 percent. Mixed milks too showed similar effects. Under identical conditions Parsad et al. (1974) observed a similar reduction in the curd tension by 74 percent in the case of buffalo milk after homogenization and pasteurization.

The effect of pasteurization in reducing curd tension was greater after homogenization than before. It is possible that homogenization alters stability of milk proteins towards various treatments. The different behaviour of homogenized milk in this regard, could as well be partly attributed to the changes in the heat stability of milk proteins during such treatment. Such a possibility would appear fairly distinct in view of various observations made in different studies, indicating that homogenization lowers the heat stability of milk proteins and so also

at higher pressures (Sommer, 1934; Doan, 1938; Alfonsus, 1949; Hollender and Weckel, 1941 and Carr and Trout, 1942). The adsorption of plasma proteins on the fat globule surface seems to be a major contributing factor. The denaturation of heat labile proteins predisposes the entire plasma system to protein - protein and protein-lipid interaction and the aggregation of protein coated fat globules into larger ones is thus promoted. Heating of such destabilised proteins could result in the product with a lower curd tension.

Relationship between different milk constituents and curd tension

Data on total solid, fat and SNF content in milk from different species and mixed milk as well as curd tension, are presented in Table 5 and 6. Understably, the buffalo milk had significantly higher level of total solids (17.28 percent), than goat (11.78 percent) and cow milk viz. 11.43 percent, respectively. The higher total solid content in buffalo milk is primarily the reflection of its higher fat content. Values obtained for all the three species are in close agreement with those values reported by several workers (Asker et al., 1957; Dastur, 1958, ; Paul and Malhan, 1962; Ghosh and Anantakrishnan, 1964,65 and Juma and Alsafar, 1970).

TABLE - 5

Average composition of different types of milk and their curd tension

Milk	Fat (%)	S.N.F. (%)	Total solids (%)	Curd tension (g)
Buffalo	7.78 ±0.35	9.50 ±0.20	17.28 ± 0.46	42.8 ± 6.69
Cow	3.36 ±0.13	8.07 ±0.15	11.43 ± 0.27	19.7 ± 0.25
Goat	3.79 ±0.12	7.99 ±0.08	11.78 ± 0.10	21.4 ± 0.07
B:C 90:10	6.98 ±0.42	9.27 ±0.10	16.25 ± 0.53	37.8 ± 4.38
B : C 50:50	5.23 ±0.35	8.71 ±0.17	13.94 ± 0.50	28.9 ± 1.49
B : C 10:90	3.80 ±0.16	8.26 ±0.10	12.06 ± 0.25	20.3 ± 1.21

Mean of six observations with ± S.E.

B:C Proportion of buffalo (B) and cow(C) milk

TABLE - 6

Analysis of variance table for total solids

Source	d.f.	S.S.	M.S.S.	'F' value
Between treatments	1	0.8844	0.8844	2.0459
Between replicates	5	36.0388	7.2077	16.6737**
Types of milk	5	363.8747	72.7749	16.8350**
Interaction (types of milk and treatment)	5	0.1516	0.0303	0.0702
Error	55	23.7756	4.3228	
Total	71	424.7251		

** Significant at 1 percent level

CD at 5 percent - replicate - 4.691.

Total solids as well as fat content in three types of mixed milk increased as the proportion of buffalo milk increases i.e. highest in milk having B:C, 90:10 followed by 50:50 and 10:90.

Though total solids, fat, protein, ash etc. are known to influence the curd tension of milk, the most important one among these is the casein. In the present study a correlation coefficients were observed between curd tension and total solid ($r = 0.82$) and curd tension and SNF ($r = 0.71$). These observations are in close agreement to those made earlier by Jain et al., (1974) and Abou-Dawood and EL-Sawaf (1977), who reported that curd tension increased as SNF, total casein and total solids increases in the milk. However, no such correlation was observed between fat and curd tension in different types of milk.

It was seen from the results presented in this section that, irrespective of marked variations in their total solid and fat contents, milks from different species as well as mixed milks could be homogenized equally efficiently. This, further produced a distinct reduction in the curd tension of milk. Although heat treatment such as pasteurization does lower the curd tension, the homogenized milk showed greater reduction than non-homogenized one on pasteurization. It was further seen that both total

solids and SNF content of milk were highly correlated with the curd tension. The extent to which this would influence the utilization of milk nutrients by the body, however, can not be ascertained from these observations.

BIOLOGICAL EVALUATION OF PROTEIN FROM HOMOGENIZED MILK

Although homogenization of milk does not appreciably alter the distribution of nitrogen among various nitrogenous constituents such as nonprotein, noncasein, casein, globulin, albumin and proteose fractions it enhances the absorption of casein on fat globules, eventually altering the amino acid profile of the fat globule membrane proteins (Brunner et al.,1953c). In the light of the observation that homogenization drastically reduced the curd tension of milk, as evident from the data presented in the earlier section, the influence of homogenization on the utilization of proteins would appear possible. Though, it is largely assumed that a decrease in the curd tension of milk reflects in improved digestibility, relatively very few studies have been reported so far to substantiate such assumption. As regards pasteurization, data available did not show any conspicuous deleterious effect on the nutritive value of milk proteins, except certain degree of denaturation of whey proteins (Henry et al., 1937 and Henry, 1957). Little is, however, known about changes in the nutritive value

if any, when pasteurization is performed after homogenization, in view of the protein-lipid interactions mentioned earlier. In the light of the aforesaid, experiments were conducted to find the utilization of proteins of homogenized milk by determining (a) the growth promoting ability as well as (b) nitrogen balance when experimental animals were given test milk samples.

Modified protein efficiency ratio (PER_D)

Under defined conditions, the growth rate of animals is related to the dietary protein quality and therefore considered as an index of protein quality. Furthermore, the evaluation of protein quality on the basis of growth is based upon the assumption that the optimum of a given protein is determined not merely by the absolute quantity furnished but rather by its quality (Osborne et al., 1919). Later, Cannon and coworkers (1944) observed that animals made protein sensitive by maintaining on protein-free diet respond more effectively to differences in protein qualities than a normal ones, since growth and nitrogen retention are higher under these conditions.

In the present study growth promoting ability of proteins from different preparations of homogenized and non-homogenized buffalo milk, was ascertained using protein depleted weanling male albino rats as the experimental animals and the results are presented in Table 7. It was

TABLE - 7

Modified protein efficiency ratio (PER_D) in rats fed different test milk samples

Dietary treatment	No.of animals	Body wt. (g)			Total milk intake (ml)	Total protein intake (g)	$PER_{(D)}$ Value
		Initial	After 10 days on protein-free diet	Gain on test diet after 10 days			
Group I	8	37.4 ± 0.59	30.8 ± 0.61	29.1 ± 2.18	142.9 ± 6.82	6.73 ± 0.15	4.31 ± 0.25
Group II	8	36.6 ± 0.54	30.1 ± 1.16	35.1 ± 1.79	249.6 ±10.49	11.75 ± 0.52	2.98 ± 0.32
Group III	8	36.8 ± 0.52	30.1 ± 0.68	37.9 ± 2.45	226.3 ± 9.18	10.66 ± 0.43	3.53 ± 0.11

Mean of eight observations ± S.E.

Group I - Buffalo milk (whole) pasteurized
Group II - Buffalo milk (Standardized) homogenized and pasteurized
Group III - Buffalo milk (whole) homogenized and pasteurized

TABLE - 8

Analysis of variance - PER_D

Source of variation	d.f.	S.S.	M.S.S.	'F' value
Between treatment	2	7.11	3.56	15.021**
Between replicate	7	1.40	0.20	0.843
Error	14	3.32	0.237	

** Significant at 1 percent level

TABLE - 9

Analysis of variance - milk intake

Source of variation	d.f.	S.S.	M.S.S.	'F' value
Between treatment	2	5077.14	2538.57	4.3658*
Between replicate	7	5351.90	764.55	1.3148
Error	14	8140.60	581.47	

* Significant at 5 percent level

seen from the data that the group I receiving non-homogenized pasteurized whole milk showed higher average PER_D value of 4.31 in comparison with those receiving homogenized milk either whole (Group III) or standardized (Group II). The differences observed were found to be statistically significant ($P < 0.01$., Table 8). This is interesting particularly because the total milk intake for the group I and consequently the protein intake, was much lower than other two groups. The higher consumption of milk in groups II and III could be a reflection of improved palatability of milk due to homogenization (Babcock, 1939 and Trout, 1943).

Though the protein efficiency ratio determined by rat depletion-repletion method, would depend on the extent to which the protein reserves are depleted during protein-free dietary regime, the differences observed could not be attributed to this, as it was observed that animals in all groups, were protein depleted identically as evident from 18 to 20 percent decrease in body weights.

The ratios obtained for group I and III were higher than the conventional protein efficiency ratio reported earlier for buffalo milk by Rao (1956) and Daniel et al. (1968), who observed the value to range between 2.6 to 3.3. The modified PER value for group II was however, lower

than the reported one. The higher value observed in the present study was similar to that reported by Deodhar and Srivastava (1977) for cow milk. The higher value for PER observed could be due to the higher retention of nitrogen since protein depleted animals were used. This could be well conceived in the light of the observation of Venkatroa et al. (1964), who reported a higher PER_D value of 4.16, when protein depleted rats were fed skim milk based diet. The significantly low PER_D value obtained for the groups II and III, receiving homogenized milk in comparison with group I could be attributed to any of the following possibilities, viz;

(a) the difference in protein intakes in different groups,

(b) a change in the protein quality as a result of homogenization,

(c) quicker passage through the alimentary canal, thereby incomplete digestion of proteins from homogenized milk and

(d) catabolism of excess protein after meeting body needs.

As pointed out earlier, it could be seen from Table 7, the total protein intake in the group I receiving non-homogenized milk was significantly less ($P < 0.01$, Table 9) than other two groups receiving homogenized milk. The lower value for PER_D for group III could be partly explained on the basis of the observation of Allison (1964) that the

relationship between the gain in body weight and protein intake is linear only at lower levels of protein intake. Further, as reported earlier by Fixsen et al. (1934), the protein efficiency ratio decreases as the level of dietary protein level progressively increases. This is particularly true in the case of good quality proteins (Venkatrao et al., 1964). At the higher level, when the protein intake exceeds the need for the growth, the excess quantity would be channeled to catabolic degradation.

The possibility of changes in protein quality resulting during homogenization and subsequent pasteurization treatments, reflecting in the differences in PER_D values between non-homogenized and homogenized milks, can not be easily ruled out. However, as pointed out by Venkatrao et al. (1964) the evaluation of protein quality on the basis of gain in body weight, has several limitations. The increase in body weight could as well be due to increase in body fat and/or body water contents and not merely due to nitrogen deposition in the carcass, as assumed.

Apart from this, a possibility can not be ruled out that homogenized milk is digested more easily but less completely as reported by Adam and Czech (1955). It is possible that the period for emptying of stomach could be

shorter in the case of homogenized milk as compared to that required for non-homogenized milk as reported by several workers (Doan and Welch, 1934; Flora and Doan, 1938; Babcock, 1939 and Adam and Czech, 1955).

Observations made while assessing the growth promoting ability of different test milk samples, however, do not permit to conclude in respect of digestion or metabolic breakdown of proteins. Studies were therefore carried out on the nitrogen balance by maintaining experimental rats on test milk samples.

Furthermore, just as the growth response, nitrogen balance too, has been shown to depend on the quality as well as quantity of the dietary protein (MacLaughlan, 1972) when other experimental conditions are normal. The major limitation of protein quality evaluation by PER method mentioned above, could be overcome by conducting nitrogen balance studies. In view of this, the biological value, digestibility coefficient, net protein utilization values as well as test protein from experimental milk samples, were determined, taking into consideration the intake and excretion of nitrogen, as proposed by Mitchell (1924) and data are given in the next section.

Biological value and digestibility coefficient

Data on protein intake as well as faecal and urinary nitrogen excreted during different dietary regimes and

the digestibility coefficient and biological values computed from the same are presented in Table 10,11 and 12. It could be seen from the results that the average milk consumption was almost double in both groups fed homogenized milk in comparison with the control group receiving non-homogenized milk. Further computation of the values given for nitrogen intake and the faecal nitrogen excreted, showed that there was relatively lesser excretion of faecal nitrogen in the group I as compared to groups II and III receiving homogenized milk. The magnitude of faecal excretion as well as the differences between these values were markedly significant ($P/0.01$) (Table 13). This observation supports the hypothesis that proteins from homogenized milks are digested in shorter durations but relatively less completely as compared to non-homogenized milk as suggested by Doan and Welch (1934); Doan and Flora (1939); Babcock (1939) and Adem and Czech (1955).

Similar scrutiny of the data on urinary excretion suggested that about 6.8 percent of the total nitrogen intake was excreted by animals given non-homogenized milk. On the other hand, 21.7 percent (Group II) and 11.2 percent (Group III) of the dietary nitrogen were excreted when the animals were maintained on homogenized milk (Table 14). Somewhat higher excretion of urinary nitrogen observed in group III as compared to group I suggests that considerable amount of protein nitrogen in group III was excreted as a result

TABLE - 10

Biological value, digestibility coefficient of different test milks

Groups	Total milk intake in 10 days (ml)	Total-N intake in 10 days (mg)	Faecal-N excretion in 10 days on the test diet (mg)	Endogenous Faecal-N excretion (mg)	Total-N excreted in faeces $F_n - F_{en}$ (mg)	Urinary-N excretion in 10 days on test diet(mg)	Endogenous urinary-N excretion (mg)	Total-N excretion in urine $U_n - U_{en}$ (mg)	Digestibility coefficient (%)	Biological value (%)
Group I	177.2 ± 24.95	1121.25	81.58	43.08	38.50 ± 3.96	165.06	89.27	75.79 ± 0.62	96.56 ± 0.71	93.00 ± 0.68
Group II	370.5 ± 16.16	2344.53	216.63	50.83	165.80 ±11.28	593.25	86.27	506.98 ±2.50	92.93 ±0.90	76.73 ± 3.65
Group III	359.4 ± 11.64	2274.13	173.53	47.64	125.89 ± 8.13	343.16	91.49	252.17 ±26.70	94.46 ± 0.32	88.26 ± 1.09

Mean of 8 observations with ± S.E.

Group I Buffalo milk (whole) pasteurized

Group II Buffalo milk (Standardized) homogenized and pasteurized

Group III Buffalo milk (whole) homogenized and pasteurized

TABLE - 11

Analysis of variance for biological value

Source of variation	d.f.	S.S.	M.S.S.	'F' value
Between treatment	2	1049.20	524.60	27.11**
Between replicate	7	24.48	3.21	0.165
Error	14	271.02	19.35	

** Significant at 1 percent level

CD at 5 percent - 4.717

TABLE - 12

Analysis of variance - milk intake for biological value

Sourceof variation	d.f.	S.S.	M.S.S.	'F' value
Between treatment	2	188195.3	94297.6	27.556**
Between replicate	7	3730.7	532.9	0.1558NS
Error	14	44462.2	3420.2	

** Significant at 1 percent level

NS Non significant

CD at 5 percent - 62.72

TABLE - 13

Analysis of variance - total faecal-N excretion ($F_n - F_{en}$)

Source of variation	d.f.	S.S.	M.S.S.	'F' value
Between treatment	2	67813.6	33906.8	55.444**
Between replicate	7	3389.1	4841.6	7.931**
Error	14	8546.1	610.4	

** Significant at 1 percent level

CD at 5 percent - 26.49

TABLE - 14

Analysis of variance - total urinary-N excretion ($U_n - U_{en}$)

Source of variation	d.f.	S.S.	M.S.S.	'F' value
Between treatment	2	739965.6	369982.8	13.999**
Between replicate	7	15369.9	2195.1	0.167
Error	14	183216.3	13086.9	

** Significant at 1 percent level

CD at 5 percent - 122.69

of higher catabolic degradation. Significantly ($P<0.01$, Table 14) larger excretion of nitrogen by animals fed standardized homogenized milk (Group II) as compared to group III receiving homogenized milk could be attributed to distinctly lower ratio of nitrogen to total dietary calories in group II. It is possible that this factor might have adversely influenced the protein efficiency ratios for animals given homogenized milks.

It was seen from the digestibility coefficient for milks calculated from these values, that it did not change significantly after homogenization. This was evident from nonsignificant differences in different groups (Table 10). The trend observed in this study was similar to that one reported earlier by several workers, who reported that though homogenization reduced the curd tension as well as particle size of the curd, it had no effect on its digestibility (Doan and Flora, 1939; Doan and Dizikes, 1942 and Petrilli and Agnese, 1960). On the other hand, Doan and Welch (1934); Kelly (1939) and Ilgner and Thurau (1951,52) found during <u>in vitro</u> studies, soft curd milk to be digested better and completely than hard curd milk. The values for digestibility coefficient observed in the present study were somewhat higher than 89.3 percent observed for homogenized cow milk by Petrilli and Agnese (1960).

However, these were, by and large, in close agreement with those reported for cow milk (Henry et al., 1939; Henry et al., 1942 and Henry and Toothill, 1962). The relatively higher value for pasteurized non-homogenized milk receiving group could be attributed to the lower intake of nitrogen together with lower faecal excretion as compared to the group receiving homogenized milk, in which case the intake was more than double while excretion was also about 3 to 4 times higher than the values found in control group. This may have lowered the digestibility coefficients.

As regards biological value (93.0 percent) it was higher for the group receiving whole milk pasteurized than other groups. The statistical analysis Table 11 of the data showed that pasteurized milk had significantly higher ($P < 0.01$) biological value in comparison with other two groups which, inturn, differed significantly among themselves.

The biological values observed in present study for the control group I and group III were higher than those reported by Petrilli and Agnese (1960) for homogenized cow milk i.e. 79.4 percent. However, the value 79.4 percent is very close to the value observed for milk having almost identical fat level. Similarly the biological value for group I, namely, buffalo milk

pasteurized was very close to that value of 91.0 reported for buffalo milk (Rao, 1956). In another study, Mitra and Mittra (1942) reported a much lower value of 66.7 percent for buffalo milk. Such kind of variations in the biological value could be explained in view of the fact that biological value for the same sample could be different, depending upon the degree to which the body protein reserves are lowered during protein-free dietary regime. In the present study, all the groups lost about 18 to 20 percent of their body weight, suggesting the protein depletion was identical in all groups. The low biological value in both groups administered homogenized milk as compared to control group, seems to be due to large quantity of nitrogen excreted in urine, which in turn differed significantly ($P < 0.01$) from the quantity excreted by the rats receiving pasteurized milk. The higher excretion of urinary-N in group II, receiving standardised homogenized milk in comparison with other groups could be due to higher amino acid oxidation, and was probably influenced by higher nitrogen/calorie ratio in standardised buffalo milk than whole buffalo milk as mentioned earlier. Thus with lower levels of calorie in the diet, the protein was preferentially utilized to provide energy, with the consequent loss of nitrogen in the urine.

The differences in biological values in the present study could also be explained on the basis of observations of Forbes et al. (1958), who showed that the biological value of proteins decreases with the increase in the intake of proteins. Similarly Barns et al. (1946), reported earlier that percentage of protein retained falls, with increase in nitrogen intake. Likewise, Mitchell (1924) attributed a low biological value to higher protein intake and observed that such decrease was probably due to lowered utilization of nitrogen for growth than for maintenance. This was probably due to increased use of protein fed at higher level for fulfilling the energy demand of the body.

Net protein utilization (NPU)

Apart from the nitrogen balance studies reported above from which NPU could as well be computed, the nitrogen deposited in the carcass of animals, maintained on test milk diets was determined according to Miller and Bender (1955) and data are given in Table 15. As observed in the previous feeding experiments, the milk consumption was significantly higher in groups receiving homogenized milk in comparison with that receiving pasteurized non-homogenized milk. It was further seen from the data presented as well as the statistical analysis of the data on N-retained in the body that the groups receiving homogenized milk (group III and IV)

TABLE - 15

Net protein utilization values for different test milk samples

Groups	Body wt. (g)			Total milk intake in 10 days (ml)	N-intake through milk in 10 days (mg)	Body-N (mg)	N-retained (mg)	NPU (%)
	Initial (g)	After 10 days on respective diet (g)	Gain (g)					
Group I	55.94	47.70	-8.24	-	-	1138.77	-	-
Group II	55.94	74.44	18.50	236.5 ± 8.11	1340.0	2209.82	1071.50 ± 47.20	79.96 ± 1.67
Group III	55.90	88.70	32.80	384.3 ±12.96	2184.47	2410.08	1271.31 ± 75.04	58.20 ± 2.35
Group IV	55.72	91.00	35.28	366.4 ±11.70	2082.83	2583.70	1445.00 ± 72.80	69.38 ± 2.55

Mean of 8 observations with ± Standard error

Group I - Protein-free diet

Group II - Buffalo milk (whole) pasteurized

Group III - Buffalo milk (standardized), homogenized, pasteurized

Group IV - Buffalo milk (whole), homogenized, pasteurized.

TABLE - 16

Analysis of variance - net protein utilization (NPU)

Source of variation	d.f.	S.S.	M.S.S.	'F' value
Between treatment	2	1821.30	910.65	19.363**
Between replicate	7	163.14	26.16	0.556
Error	14	644.44	47.03	

** Significant at 1 percent level

CD at 5 percent - 7.355

TABLE - 17

Analysis of variance - N-retained in body

Source of variation	d.f.	S.S.	M.S.S.	'F' value
Between treatment	2	542533.0	271278.0	8.758**
Between replicate	7	304249.0	43464.7	1.4039
Error	14	433603.0	30971.6	

** Significant at 1 percent level

CD at 5 percent level - 188.74

retained significantly higher quantity of nitrogen in the carcass as compared to a group receiving pasteurized non-homogenized milk. It was interesting to note that the nitrogen retention was significantly higher in group IV than in group III which excreted largest amount of nitrogen in the urine (as seen earlier in Table 14). However, when judged on the basis of N-consumed in different groups, while calculating NPU value, it was observed that the NPU value was highest for pasteurized non-homogenized group. The average NPU values were 79.96, 58.20 and 69.38 for whole milk pasteurized (group II), standardised homogenized milk (group III) and homogenized pasteurized milk (group IV), respectively. It was seen from the statistical analysis (Table 17) of the data that group II had significantly higher value ($P<0.01$) than group III and IV, while, group III had the lowest value. Very few studies have been reported on the determination of NPU value by carcass analysis for buffalo milk. A comparison of the values showed that the values observed in the present study for group II compared well with those reported for cow milk i.e. 75 to 81 (Miller and Bender, 1955; Henry and Toothill, 1962; Myna et al., 1964; Parthasarahty et al., 1964 and Venkatrao et al., 1964).

The low NPU values for homogenized milk could be partly explained on the basis of observation by Barns et al. (1946) that percentage of protein retained in the carcass falls with increasing consumption of protein in the diet. Similarly Togle and Donoso (1967) observed that when protein consumption was low the free amino acids that enter the liver pool, are primarily utilized for tissue protein synthesis and may have a lesser chance of being channelled into the urea cycle. It was further observed that the NPU values for different milk samples obtained in this experiment showed identical trend observed in respect of biological value, determined earlier.

It would appear from the data obtained on the utilization of protein from homogenized and non-homogenized milk, that although homogenization did reduce the curd tension, it did not show any salutary effect on the protein quality. There was no change in the digestibility of proteins, as contemplated earlier. On the other hand, the growth promoting ability, (PER_D), the biological value as well as net protein utilization values were significantly lower for homogenized milk. The extent of such decreases was more pronounced when the ratio of nitrogen to calorie was reduced as a result

of standardisation of milk. Though such possibilities have been discussed before, the influence of altered lipid-protein complex on the nutritional value of proteins can not be ruled out.

Apart from the reasons given above for the decreased modified protein efficiency ratio, biological value as well as net protein utilization value the possibility of diminished protein quality as a result of homogenization and subsequent pasteurization can not be ignored in view of the observation that homogenization as well as heat treatments above 65°C increase the total free SH moieties in treated milk samples (Rotkiewicz and Kisza, 1971).

The simultaneous action of the enzyme sulfahydryl oxidase resulting in the formation of disulphide bonds (Janolino and Swaisgood, 1975,78) within the protein molecules, could as well result in the decreased biological availability of S-amino acids from milk proteins. The limitation of milk proteins in respect of S-containing essential amino acids is thus likely to be further compounded due to such a treatment. This, however, will have to be further probed before arriving at a final conclusion.

Fat absorption

One of the basic tenets of pharmacology states that the particle size determines the rate of absorption of

a given compound in the body and its micronization into particles further facilitates its entry into the blood stream from the intestinal lumen. In the case of milk fat, fat globules size markedly varies among different species, ranging from 2.9 μ in the case of goat to about 4.0 to 4.5 μ in cow and buffalo milk. Among various treatments used to micronize the milk fat globules, homogenization has been demonstrated to be most efficient one, which increases total fat surface area by 4 to 6 fold. This would further facilitate the absorption of fat, apart from providing increased surface area for lipase action.

In order to assess the effect of homogenization on the absorption of fat, a study was carried out using young male albino rats according to Tomarelli et al. (1968). Data obtained are given in Table 18. It was observed that rats given standardised and homogenized (group III) and whole homogenized milk (group IV) consumed greater quantity of milk as compared to rats fed pasteurized milk (group II), which further reflected in the varying amounts of fatty acid intake in different groups.

The mean fat absorption values for groups II, III and IV were 78.3, 78.2 and 88.8, respectively. The statistical analysis of the data (Table 20) showed that fat absorption was significantly ($P/0.01$) higher in

TABLE - 18

Effect of homogenization treatment on milk fat absorption in young male rats

Groups	Total milk intake in 3 days (ml)	Total fatty acids intake (g)	Total fatty acids excreted in faeces (g)	Net amount of fatty acids in faeces corrected for endogenous excretion (g)	Fat absorption (%)
Group I	-	-	0.135	-	-
Group II	80.3 ± 4.94	6.18 ±0.37	1.474 ±0.13	1.339	78.3 ± 2.08
Group III	134.1 ± 0.17	6.04 ± 0.17	1.466 ± 0.07	1.331	78.2 ± 1.24
Group IV	139.7 ± 0.46	10.75 ± 0.35	1.339 ±0.07	1.204	88.8 ± 0.63

Mean of 8 observations with ± S.E.

Group I - Control
Group II - Buffalo milk(whole) pasteurized
Group III - Buffalo milk (standardized) homogenized and Pasteurized
Group IV - Buffalo milk (whole) homogenized and pasteurized

TABLE - 19

Analysis of variance - milk intake, during fat absorption experiment

Source of variation	d.f.	S.S.	M.S.S.	'F' value
Between treatment	2	17243.4	8621.7	58.12**
Between	7	1333.1	191.2	1.28
				114

TABLE - 19

Analysis of variance - milk intake, during fat absorption experiment

Source of variation	d.f.	S.S.	M.S.S.	'F' value
Between treatment	2	17243.4	8621.7	58.12**
Between	7	1333.1	191.2	1.28
				114

TABLE - 19

Analysis of variance - milk intake, during fat absorption experiment

Source of variation	d.f.	S.S.	M.S.S.	'F' value
Between treatment	2	17243.4	8621.7	58.12**
Between	7	1333.1	191.2	1.28
				114

TABLE - 19

Analysis of variance - milk intake, during fat absorption experiment

group IV in comparison with groups II and III. However, fat absorption in groups receiving pasteurized non-homogenized milk and standardised homogenized milk (group II and III) was similar. It was seen that the intake of fatty acids was also similar in these groups, so was the levels excreted in the faeces. Apparently there was higher abosorption of fatty acids in group IV, which could be attributed to lower excretion of fatty acids through faeces as compared to other two groups despite of higher intake. Thus, although the net values for faecal fatty acids were not significantly different in different groups, when looked in the light of the total amount of fatty acids ingested it was observed that hardly about 11 percent of the total fatty acids ingested were excreted in the faeces by rats in group IV, in contrast to about 22 percent in groups II and III. The increased absorption in the group IV receiving homogenized milk, when compared with group I could be ascribed to the reduction of fat globule size during homogenization, Since the level of milk fat in both the cases was identical. Inspite of higher intake of fat in group IV, there was no increase in the faecal excretion of fatty acids.

Similar increased absorption of fat from homogenized milk was reported earlier by Stejskal and Neuburger (1934) among human subjects. It was observed that the amount of

fat in the faeces was only one third of that was found when non-homogenized milk was given. It would thus appear that less fat was excreted by subjects for homogenized milk than rather non-homogenized. However, these workers attributed this to the kind of curd formed.

A lower degree of fat absorption in group III as compared to group IV could be partly explained on the basis of observation of Zoula et al. (1966), who reported that infants fed partially skimmed milk absorbed minimum fat. The ratios for protein to energy, in the present study was distinctly lower in group III than that in group IV due to possibly a change in ratio of fat to protein in such preparation. On the other hand, Fomon et al. (1970) observed higher fat excretion among infants fed homogenized cow milk in comparison to those fed human milk. This was over-come when sucrose was added in the homogenized milk, which suggests that if milk of low fat is to be homogenized, it must have same energy value to that of the control group. Standardised homogenized milk, clearly had lower calorific content than homogenized whole milk.

The greater fat absorption in the case of homogenized milk preparations are in conformity with observations made by several other workers who ascribed the reduction of fat particle size to be responsible for enhanced

fat absorption (Ilgner and Thurau, 1951,52 and Ewerback and Jaeger, 1954). On the other hand, Nevens and Shaw (1933); Holt et al. (1933) and Sager (1952), however, failed to observe any significant increased absorption of fat due to reduction in fat particle size.

Fat absorption values obtained in the present study are higher than those reported by Agnese (1959), who observed a value of 71.8 percent for homogenized milk and 42.3 percent in non-homogenized milk. Absorption to similar extent was observed by Morales et al. (1950), who observed only 40 percent when normal fat was given, while on administration of homogenized milk fat during which fat globules were reduced to a diameter of lesser than 2 μ, the absorption was enhanced to 71.5 percent.

Data obtained in this study thus clearly demonstrated that the homogenization treatment distinctly improved the process of fat absorption.

BIOLOGICAL AVAILABILITY OF VITAMIN A FROM HOMOGENIZED MILK

It was seen in the earlier section that the utilization of fat was markedly improved when the milk was subjected to homogenization treatment. Though several studies reported so far, amply demonstrated that fat serves as a carrier for various fat soluble nutrients, little is known whether the distribution of these fat soluble

nutrients in lipid phase undergoes any change during homogenization and whether their biological availability is, in any way, altered in this process. Keeping in mind the nutritional importance of milk in meeting the body requirements for vitamin A, a study was conducted to find its utilization from the homogenized milk, in comparison with the non-homogenized one, using vitamin A deficient albino rats as experimental model, to whom different test milk samples were given as the only source of vitamin A. The degree of utilization was determined by recording gains in body weight as well as levels of the vitamin A in serum and in liver of rats given different dietary treatments. Results are presented in this section.

Vitamin A in homogenized milk

It was seen from the contents of vitamin A in milk before and after homogenization (125 IU and 123 IU, respectively) that there was virtually no change in the same. This is to be expected since vitamin A is fairly stable during heating at 60°C (the temperature adopted for preheating the milk before homogenization). However, such data do not indicate if there is any change in the biological availability of vitamin A. In the present study vitamin A deficient male rats were

preferred over normal rats, to ascertain biological availability, with the view that animals sensitized in this manner, would respond better than normal ones, to the dose of vitamin A. Further, although it was observed earlier by (Lewis et al., 1942; Paul and Paul, 1946 and Brown and Stutevant, 1949) that the intake 2 to 4 IU of vitamin A was adequate to support the growth, the dose of 25 IU of vitamin A was chosen in this study, in the light of its capacity to restore not only growth rate but also vitamin reserves in the body.

Data on the gain in body weight as well levels of vitamin A in blood serum and liver from rats in different experimental groups are presented in Table 21.

Body weights

It was seen from the data given in Table 21 that during vitamin A deficient dietary regime, rats chosen at weanling stages gained about 110 g body weight during the period of 8 to 9 weeks. It could be further seen from (Illustration 7A) that the growth rate (about 2 g/day) was normal upto 40 days, on vitamin A deficient diet. Thereafter the growth retarded and in the later stage i.e. after 56 days, the weights tend to decline. At the end of this period, apart from cessation of growth animals showed characteristic syndroms of vitamin A deficiency viz. appearance of xerophthalmia, paralysis

TABLE - 21

Effect of vitamin A supplement from homogenized milk on growth and vitamin A activity in blood and liver of rats

Diet	Body wt. (g)				Total wt. of liver (g)	Vitamin A IU/liver	Vitamin A IU/g liver	Blood serum vitamin A IU/100 ml
	Initial	At the time of milk feeding	On feeding test milk samples	Gain				
Group I	38.7 ± 1.89	146.3 ± 9.45	-	-	4.42 ±0.31	Traces	Traces	Traces
Group II	37.5 ± 0.93	148.2 ± 10.10	275.5 ± 11.40	127.3 ± 5.31	8.54 ±0.41	200.9 ± 9.81	23.5 ± 2.25	43.1 ± 3.18
Group III	38.7 ±1.94	145.7 ±14 .20	259.7 ± 12.86	114.1 ± 9.65	8.65 ±0.66	223.1 ± 12.11	25.8 ± 1.20	36.1 ± 2.51
Group IV	38.6 ± 1.58	150.6 ± 5.81	262.2 ± 8.55	111.6 ± 5.71	8.02 ±0.15	224.1 ± 9.59	28.0 ± 1.26	42.0 ± 4.20

Mean of six observations with ± S. E.

Group I - Control

Group II - Buffalo milk (whole) pasteurized

Group III - Buffalo milk (standardized) homogenized and pasteurized

Group IV - Buffalo milk(whole) homogenized and pasteurized

TABLE - 22

Analysis of variance - weight gain in rats

Source of variation	d.f.	S.S.	M.S.S.	'F' value
Between treatment	2	874.78	437.39	1.815^{NS}
Between replicate	5	2251.60	450.31	1.869^{NS}
Error	10	2409.90	241.99	

NS - Non significant

TABLE - 23

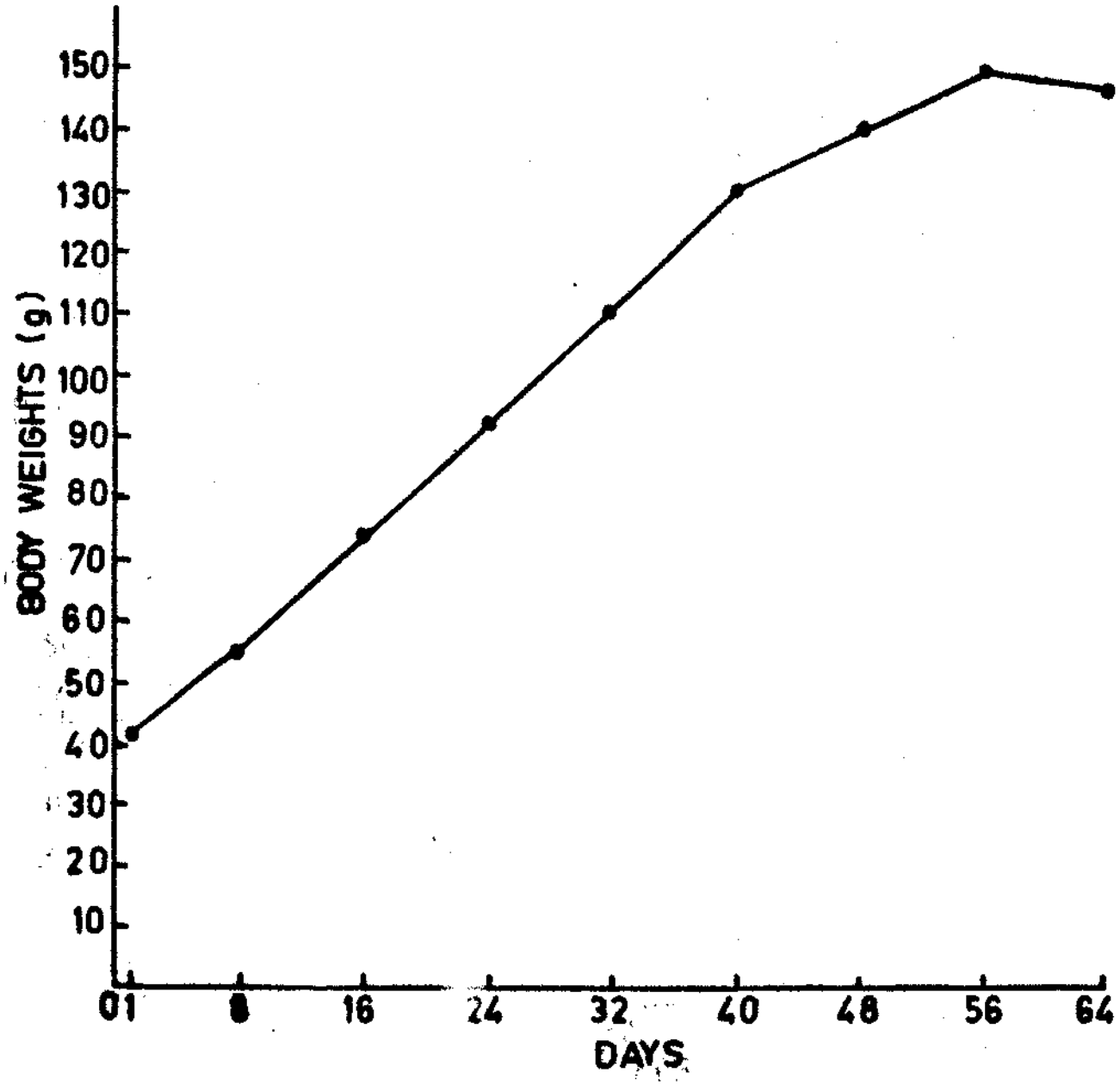
Analysis of variance - growth and vitamin A in blood and liver of rats

Source of variation	d.f.	Vitamin A IU/100 ml Blood serum			Vitamin A IU/g liver			Liver vitamin A (IU)		
		S.S.	M.S.S.	'F'	S.S.	M.S.S.	'F'	S.S.	M.S.S.	'F'
Between treatment	3	7418.8	2472.9	75.39**	3064.4	1021.5	79.81**	72.7	24.23	28.28**
Between replicate	5	538.9	107.8	3.28**	50.8	10.1	0.78	8.8	1.76	2.053
Error	15	492.0	32.8		192.6			12.9	0.860	

** Significant at 1 percent level

CD at 5 percent - Serum vitamin A - 7.046
Vitamin/g liver - 4.401
Vitamin A/liver -12.13

FIG. A. GROWTH CURVE - RATS FED VITAMIN A DEFICIENT DIET



of hind limbs etc. (Illustration C). In the present study the initial body weights of experimental animals were about 38 g whereas Underwood (1979) used rats with somewhat higher body weights. Such differences in the initial weights would have pronounced influence on the vitamin A reserves in the body.

On feeding test milk samples as the sole source of vitamin A animals gained an average of 127.3, 114.1 and 111.6 g in body weight when buffalo milk (whole) pasteurized (group II), standardised and homogenized milk (group III) and buffalo milk (whole) homogenized (group IV), respectively, were given. The statistical analysis (Table 22) did not show any difference in body weight gain to be significant during the experimental period. However, it would appear from the illustration B that during the early stage after the commencement of milk feeding the gain in body weight was higher in group IV as compared to group II, thereafter it showed a tendency for smaller gain. On an average rats gained 3.6, 3.3 and 3.2 g/day in group II, III and IV, respectively. Vitamin A deficient rats fed 25 IU/day of synthetic vitamin A (Lewis et al., 1942) found to gain about 131 g during an experimental period of six weeks with an average gain of 3.1 g/day. On the other hand, Rajalakshmi et al. (1975), failed to observe any significant difference in growth rate in vitamin A

FIG. B. GROWTH CURVES IN VITAMIN A DEFICIENT RATS FED VITAMIN A FROM DIFFERENT MILKS

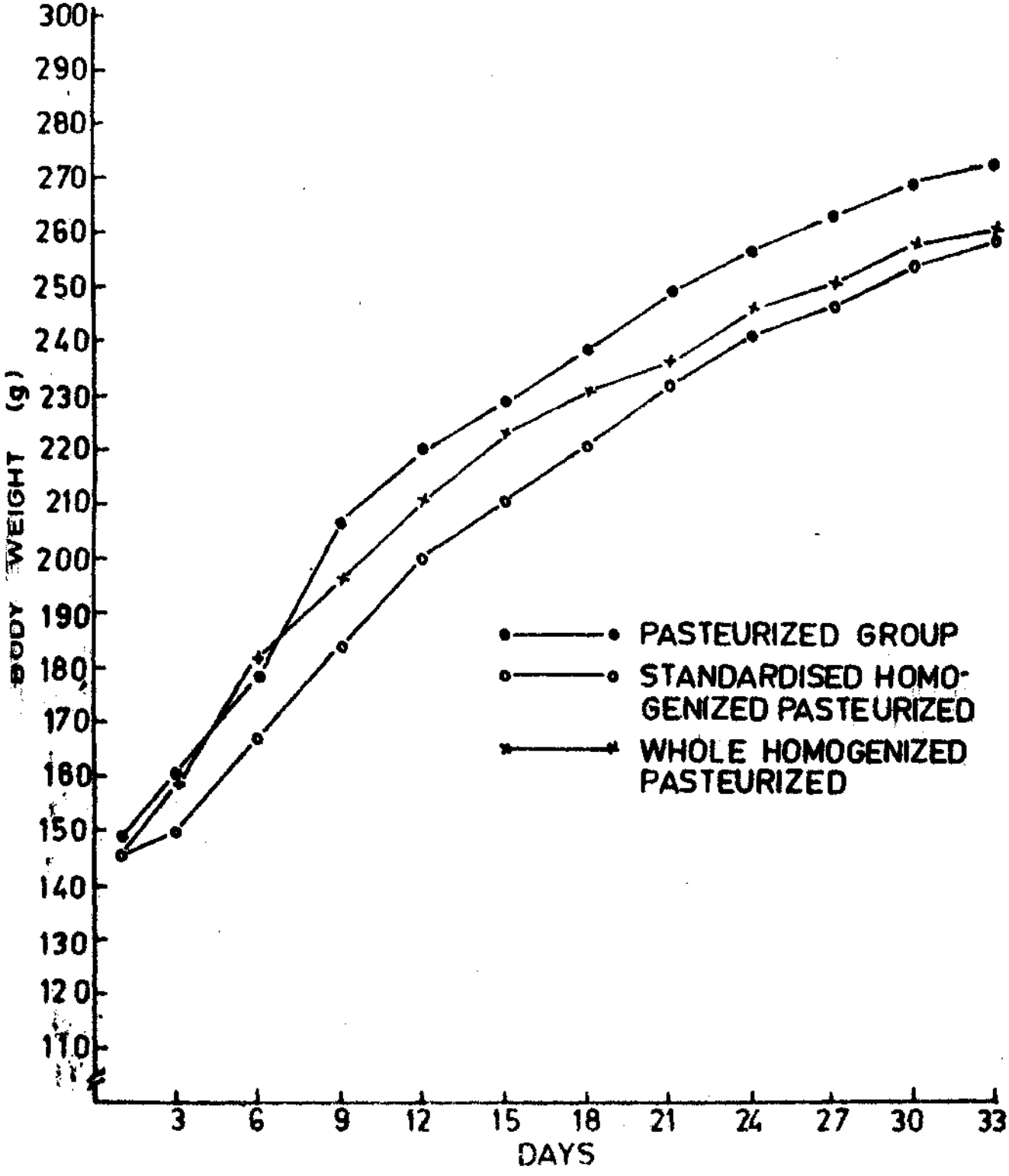


Illustration C. Rats showing vitamin A deficiencysyndroms.

deficient rats fed vitamin A either from vegetables, pure β-carotene or vitamin A acetates. The dosage adopted in these studies, were much longer.

It is possible that the amount of vitamin A required for growth is much less viz. 2 to 4 IU/day (Braude et al., 1941; Lewis et al., 1942; Paul and Paul, 1946; Brown and Stuntevant, 1949) than supplementation of vitamin A 25 IU done in this study. Thus the consumption of the vitamin dose at this level apparently had no effect on growth rate, irrespective of the form in which it is present in the diet.

Blood serum level vitamin A

It was seen from the data on serum vitamin A levels in different experimental groups, the levels were extremely low (traces) when animals were maintained on vitamin A deficient diet for a period of 8 to 9 weeks. The subsequent supplementation of vitamin A at the dosage of 25 IU/day, through test milk samples markedly enhanced the levels in blood serum and were 43.1, 36.1 and 42.0 IU/100 ml in groups receiving buffalo milk (whole) pasteurized(group II), standardized and homogenized (group III) and buffalo milk (whole) homogenized (group IV), respectively. Although supplementation through all test milk samples resulted in an increase in the blood vitamin A levels in groups II, III and IV, there were

no significant differences amongst themselves (Table 23). Such non-significant differences, suggest that the availability of vitamin A from milk was not affected due to homogenization. However, when these were examined on the basis of observations made by Lewis et al. (1942), who showed that the intake of vitamin A as low as 1 IU/ day was adequate to produce measurable increases in blood vitamin A level, it appeared that probably because of administration of a larger dose of 25 IU/day, the efficiency of different milk samples to produce effect on blood vitamin A level was subdued. Observations made in the present study were, however, in general agreement with those made in different studies in which animals were given more than 20 IU/day, which failed to show differences in the serum vitamin A level (Moore and Sharman, 1950).

It could be seen from the aforesaid that though a minimum amount of vitamin A is essential to produce a visible level of vitamin A in blood, however, it is not linear with the dose given or source of vitamin A in the diet.

Hepatic vitamin A levels

Data presented on vitamin A content in liver as well as weight of liver for rats from different experimental groups showed that there was significant difference ($P < 0.01$)

in regeneration of liver vitamin A when animals were given experimental milk samples. The statistical analysis of the data, however, failed to show any significant difference in the weight of liver when given milk either standardized homogenized or buffalo milk homogenized or buffalo milk pasteurized.

As regards hepatic contents of vitamin A, maintenance of rats on vitamin A deficient dietary regime till the visible vitamin A deficiency symptoms were evident as seen in the Illustration A, drastically depleted vitamin A stores of the liver with the result that there was practically no vitamin A content in the liver after animals were kept on deficient diet for a period of 8 to 9 weeks. On supplementing diets with test milk samples to provide 25 IU/day (the body requirement to support normal growth) it was seen that the livers from the group receiving buffalo milk (whole) pasteurized (group II) had 23.5 IU/g concentration whereas those receiving homogenized pasteurized milk showed the level of 28.0 IU/g of liver. The difference in hepatic levels of these two groups was found to be significant. The deposits of vitamin A computed, on the basis of the total liver weight further conformed the observation that vitamin A content in liver for groups receiving homogenized milk was significantly higher than those receiving non-homogenized milk as a source of vitamin A.

The extent of depletion-repletion of liver in respect of vitamin A witnessed in the present investigation was identical to those reported by several workers in different studies (Lewis et al., 1942; Glover et al., 1948; Chari, 1967 and Rajalakshmi et al., 1975). It would appear from the observations made in the present investigation that animals receiving homogenized milk had better reserves of vitamin A in the liver than the one receiving non-homogenized milk, indicating that vitamin A from homogenized milk was better utilized than the vitamin present in non-homogenized milk. This could be attributed to the reduce particle size of fat globule during homogenization and emulsification (Lewis et al., 1950). Similar observations were made earlier by Morales et al. (1950) who administered vitamin A either from butter fat or fish liver oil, either through homogenized or non-homogenized fat and showed that vitamin A was better absorbed from homogenized preparation in which the particle size of fat globule was markedly reduced, apart from better emulsification of vitamin A in liquid phase. Secondly, the contribution of certain unidentified factors present in milk, towards better absorption of vitamin A can not be ruled out in the light of observations made by Vavich et al. (1955) on experimental vitamin A deficient rats, and were provided vitamin A through different sources. It was shown that

incorporation of skimmed milk along with vitamin A resulted better absorption of vitamin A than when the polysorbic acid or aqueous solution of vitamin A were administered. It would be interesting to mention the possible involvement of vitamin A-casein complex in the better absorption of vitamin A was indicated by the observation of Berger et al. (1966) who showed that rats given vitamin A-casein complex showed better hepatic reserves of vitamin A than those receiving only vitamin A and casein. It is possible that the process of homogenization further brings about the complex formation between vitamin A and casein. Such a possibility appears feasible in view of the observation that the extent of absorption of casein in the lipid phase enhanced markedly as a result of homogenization, which could be seen from the finding that 25 percent casein component in fat globule membrane in the homogenized milk preparation in contrast to hardly 2 percent in the case of unhomogenized milk (Lundstedt, 1936).

STABILITY OF CERTAIN WATER SOLUBLE VITAMINS IN HOMOGENIZED MILK

The ability of milk to fulfill the body requirement for vitamins is generally, ascertained on the basis of their contents often stated in food tables. However, these values usually indicate levels occuring in the unprocessed product. Apart from being thermolabile,

quite a few of these vitamins exist in free as well as in bound form in milk, and have been shown to be susceptible for destruction during heat processing (Housten et al., 1940; Hassinen et al., 1954; Parkhim et al., 1965; Karlin, 1969 and Webb et al., 1978). Though pasteurization of raw milk has been shown to result losses of certain B-group vitamins ranging between negligible and moderate, very few studies have been carried out so far to find the effect of such treatment on vitamin levels in milk after homogenization Whether the altered distribution of milk proteins between fat globule membrane lipids and the aqueous phase, also changes the ratio of bound to free vitamins, ultimately affecting their stability during pasteurization, remains far from clear. Experiments were therefore conducted to study how far the levels of certain vitamins of B-group in the buffalo milk are affected during homogenization and subsequent pasteurization. Further, in the light of the influence of oxidative destruction of ascorbic acid on the stability of vitamin B_{12} and folic acid contents in milk, ascorbic acid content was also determined in the present study. Data obtained are presented in this section.

Effect of homogenization and pasteurization on thiamine, riboflavin, niacin, pantothenic acid and vitamin B_6 content

Data on the levels of different B-group vitamins in the untreated buffalo milk, as well as in

(i) pasteurized, (ii) homogenized and (iii) homogenized and pasteurized milk samples are given in Tables 24 and 27. It was observed that the fresh buffalo milk contained on an average of 51.6 µg of thiamine; 196.0 µg of riboflavin; 88.6 µg of niacin; 352.4 µg of pantothenic acid and 3.8 µg of vitamin B_6 when expressed on 100 ml basis.

The mean values observed for these vitamins in general, were in close agreement with those reported by several workers for buffalo milk (Rao and Basu, 1951; Boman, 1953; Contini and Damiano, 1959 and Sirry and EL-Said Saleh, 1962). However, slight variations in the contents were seen in comparison with those observed in the present study in respect of riboflavin, nicotinic acid, pantothenic acid and vitamin B_6 (Rao and Basu, 1951; Boman, 1953 and Paolis and Gregory, 1963). These variations could be well understood in the light of several factors, such as environmental; genetic, physiological, stage of lactation and nutrition etc, on the vitamin content. This apart, methodologies adopted for sampling, liberation of the vitamins in the free form and their determination, in addition to conditions during storage (especially in the case of riboflavin) markedly reflect on the absolute values reported in different studies.

TABLE - 24

Effect of homogenization and pasteurization on the stability of certain water soluble vitamins in buffalo milk

Vitamin/Treatments	Untreated	Pasteurized		Homogenized	Homogenized + Pasteurized	
		Holding Method	HTST Method		Holding Method	HTST Method
Thiamine	51.6 ± 2.03	49.1 ± 1.96	48.7 ± 2.11	48.0 ± 2.12	43.6 ± 2.22	44.0 ± 2.91
Riboflavin	196.0 ± 7.07	186.2 ± 6.54	190.8 ± 6.28	188.2 ± 6.77	176.2 ± 6.07	178.6 ± 6.92
Niacin	88.6 ± 4.15	82.4 ± 2.14	85.2 ± 3.88	82.6 ± 2.50	78.6 ± 2.61	79.7 ± 3.18
Pantothenic acid	352.4 ±13.60	332.4 ±14.10	334.5 ±15.60	320.6 ±15.20	309.8 ±15.01	313.9 ±13.60
Vitamin B_6	3.8 ± 0.13	3.6 ± 0.16	3.7 ± 0.15	3.6 ± 0.15	3.5 ± 0.14	3.5 ± 0.14

Values expressed as µg/100 ml

Mean of six observations ± S.E.

TABLE - 25

Percent reduction in certain water soluble vitamin during homogenization and pasteurization in buffalo milk

Vitamin/treatments	Untreated	Pasteurized		Homo-genized	Homogenized + Pasteurized	
		Holding method	HTST method		Holding method	HTST method
Thiamine	-	4.8	5.6	6.9	15.5	14.7
Riboflavin	-	5.0	2.6	3.7	10.0	8.8
Niacin	-	6.9	3.7	6.7	11.2	9.9
Pantothenic acid	-	8.5	5.1	9.0	12.3	10.9
Vitamin B_6	-	3.9	3.1	4.9	7.8	8.0

TABLE - 26

Analysis of variance- water soluble vitamins

Source of variation	d.f.	Thiamine			Riboflavin			Nicotinic acid			Pantothenic acid			Vitamin B_6		
		S.S.	M.S.S.	'F'	S.S.	M.S.S.	F	S.S.	M.S.S.	'F'	S.S.	M.S.S.	'F'	S.S.	M.S.S.	'F'
Between treatment	5	301.52	60.3	31.08**	7201.2	1440.2	51.84**	395.80	79.16	7.86**	7599.0	1519.8	19.89**	0.40	0.08	40.00**
Between replicate	5	775.39	135.1	69.02**	1673.1	334.6	12.04**	1676.60	335.32	32.30**	36201.9	7240.4	94.79**	4.10	-0.82	410.00**
Error	25	48.61	1.9		694.1	27.78		251.8	10.07		1909.6	76.4		0.05		

** Significant at 1% level.

CD at 5% level

Thiamine	1.656
Riboflavin	6.262
Nicotinic acid	3.769
Pantothenic acid	10.382
Vitamin B_6	0.053

Effect of pasteurization:

It could be further seen from the data given in Table 24, 25 and 26, that there was slight but significant decrease ($P<0.01$) in thiamine content of milk on pasteurization either by holding (4.8 percent) or by HTST method (5.6 percent). Values, observed were, by and large, lower than the losses of 10 to 20 percent during pasteurization by holding method reported in different studies (Houston et al., 1940; Woessner et al., 1940; Kruglova and Gulko, 1966 and Rossihina et al., 1969). However, these were in agreement with those reported by Karlin et al. (1969).

Losses of thiamine during HTST pasteurization, observed in this study were identical to those reported by Holmes et al. (1945); Chapman et al. (1957) and Dluzewska and Bilinska (1966). Lower losses witnessed during HTST pasteurization could be attributed to the relatively milder heat treatment for the shorter duration, during which the vitamin is known to be stable.

Riboflavin, nicotinic acid and pantothenic acid are known to be fairly heat stable at neutral as well as acidic pH of the medium. These vitamins appeared to remain stable at temperatures meted during pasteurization. In the present study decreases were very small but significant ($P<0.01$) when the milk was pasteurized by

holding method. These losses were 5.0, 6.9 and 8.5 percent for riboflavin, nicotinic acid and pantothenic acid, respectively. It was further seen that the losses were much smaller during HTST treatment.

Losses in riboflavin, nicotinic and pantothenic acid contents were seen to exhibit a similar trend, being lower in HTST method (2.6, 3.7 and 5.1 percent) than the one observed during holding method of pasteurization. The statistical analysis of the data (Table 26) showed that the losses observed during holding methods were small but significant ($P < 0.01$).

The trend observed in the present study was identical to that reported earlier for riboflavin (Houston et al., 1940; Holmes et al., 1943,45; Ford, 1957; Chapman et al., 1957; Dluzewska and Bilinska, 1966 and Karlin et al., 1969), for nicotinic acid (Clemow, 1951 and Karlin et al., 1969) and in the case of pantothenic acid (Chapman et al., 1957).

Decreases in vitamin B_6 content during pasteurization by holding and HTST method, though small viz. 3.9 and 3.1 percent, were significant ($P < 0.01$). Similar observations were made in different studies (Debrit, 1952; Chapman et al., 1957 and Dluzewska and Bilinska, 1966).

Effect of homogenization:

As described in the earlier section, homogenization was performed by heating the milk to 60°C and then subsequent

treatment at 2500 psi in a double stage homogenizer. The changes in the level of vitamins would thus be the combined effect of initial heating followed by homogenization.

It was seen from Table 25 that the decreases in the B-group vitamins ranged between 3.7 percent in the case of riboflavin to 9.0 percent in the case of pantothenic acid. The magnitude of losses was of the same order observed during pasteurization by either method, and could be attributed to the heat treatment prior to homogenization (60°C) and to the increase in temperature by 4.5 to 9.3°C depending upon the pressure applied during homogenization. Relatively little has been reported about the losses of vitamins content in milk as a result of homogenization. However, Theophilus and Stamberg (1945) failed to observe any loss in riboflavin content after homogenization at 1500 psi pressure and subsequent pasteurization by holding method. The marginal differences in the losses in both studies could be due to different pressures employed. Similar observations of non-significant destruction for vitamin B_6 in milk after homogenization and pasteurization by holding method or sterilization during evaporation of milk have also been made by Debrit (1952); Hassinen et al. (1954) and Chapman et al. (1957).

Effect of pasteurization on homogenized milk:

When milk was pasteurized either by holding or HTST method after homogenization, it was observed that in respect of almost every vitamin, the degree of destruction was higher in comparison with that observed after pasteurization of raw milk. The overall reductions in thiamine content were 15.5 and 14.7 percent (Table 25) during holding and HTST methods, respectively, after homogenization. Bell and Sanders (1945) observed an identical loss of thiamine i.e. 12 percent, when cow milk fortified with thiamine and vitamin C was homogenized and pasteurized. Similarly Chapman et al. (1957) reported only 10 percent destruction of thiamine in homogenized and sterilized milk. While riboflavin content decreased by 10.0 and 8.8 percent in homogenized and pasteurized milk, in the present study Theophilus and Stamberg (1945) failed to observe any significant loss in riboflavin content in milk after homogenization at 1500 psi pressure and pasteurization by holding method. This could be attributed to the (i) differences in free and bound form of vitamin present and/or (ii) the efficiency of homogenization at lower pressure to liberate bound vitamin in the free form.

The reduction in nicotinic and pantothenic acid contents too, was higher in homogenized and pasteurized

milk, when compared with the losses when raw milk was pasteurized. These values are almost similar to those observed earlier by Chapman et al. (1957) who reported a loss of about 10 percent of these vitamins in homogenized and sterilized milk.

Vitamin B_6 losses were almost of equal order when homogenized milk was pasteurized by either method and were 7.8 and 8.0 percent, respectively. Chapman et al. (1957), on the other hand, failed to observe any significant differences in homogenized sterilized milk in comparison to untreated milk.

It was evident from the data presented in this section that pasteurization of buffalo milk resulted in mild destruction in respect of all the water soluble vitamins studied. The observations, were by and large, in conformity with the those made by earlier workers in respect of cow milk. The lowering of vitamin contents due to homogenization process too, was not any manner larger than that observed during pasteurization by either method. The destruction was however, greater, during subsequent pasteurization. Although the form of vitamin was not determined in this study, it has been reported that barring nicotinic acid, about 5 to 25 percent of these vitamins, exist in the bound form (Hartman and Dryden, 1965). It is possible that homogenization of milk

could lead to liberation of vitamins from protein binding, when adsorption of protein on fat globule membrane increases. These liberated vitamins could be susceptible for destruction during pasteurization.

Ascorbic acid, folic acid and vitamin B_{12} stability:

In the light of the reported interaction between oxidative destruction of ascorbic acid and contents of vitamin B_{12} and folic acid, these were determined in milk during different treatments and data presented in Tables 27, 28 and 29. The average content of ascorbic acid, folic acid and vitamin B_{12} were found to be 3.3 mg/100 ml, 5.8 µg/100 ml and 373 mµg/100 ml, respectively, in buffalo milk.

The value observed for total ascorbic acid in buffalo milk in the present study was slightly higher than those reported by other workers (Kothavalla and Gill, 1943; Varma and Paul, 1947; Iordanov and Boev, 1956 and EL-Rafey, 1962), which ranged between 2.57 and 2.97 mg/100 ml. However, yet larger variation between 1.95 to 4.0 mg/100 ml was reported by Barakat and Abdel-Wahab (1961) and EL-Rafey (1962). These reported variations in the values could be attributed to differences in methods employed in the estimation of ascorbic acid. Whereas Kothavalla and Gill (1943)and Varma and Paul (1947) determined the vitamin titrimetrically using 2 : 6 dichlorophenol indophenol,

TABLE - 27

Effect of homogenization and pasteurization on stability of ascorbic acid, folic acid and vitamin B_{12} in buffalo milk

Vitamin/Treatments	Untreated	Pasteurized		Homogenized	Homogenized + Pasteurized	
		Holding method	HTST method		Holding method	HTST method
Ascorbic acid mg/100 ml	3.3 ± 0.14	3.0 ± 0.15	3.1 ± 0.15	3.1 ± 0.12	2.7 ± 0.11	2.8 ± 0.18
Folic acid µg/100 ml	5.8 ± 0.25	5.2 ± 0.23	5.3 ± 0.24	5.0 ± 0.22	4.4 ± 0.28	4.6 ± 0.18
Vitamin B_{12} mµg/100ml	373.0 ±22.6	359.0 ±21.3	359.0 ±22.3	344.0 ±19.3	344.0 ±18.3	331.0 ±19.7

Mean of six observations with ± S.E.

TABLE - 28

Percent reduction in total content of ascorbic acid, folic acid and vitamin B_{12} during homogenization and pasteurization in buffalo milk

Vitamin/treatment	Raw	Pasteurized		Homogenized	Homogenized and pasteurized	
		Holding method	HTST method		Holding method	HTST method
Ascorbic acid	-	11.3	7.6	6.9	19.2	15.5
Folic acid	-	8.6	8.0	10.8	22.3	19.5
Vitamin B_{12}	-	3.8	3.8	8.0	10.5	11.3

TABLE - 29

Analysis of variance - Ascorbic acid, Folic acid and Vitamin B_{12}

Source of variation	d.f.	Ascorbic acid			Folic acid			Vitamin B_{12}		
		S.S.	M.S.S.	'F' value	S.S.	M.S.S.	'F' value	S.S.	M.S.S.	'F' value
Between treatment	5	1.59	0.138	32.83**	10.90	2.18	118.48**	8078.50	1615.7	20.44**
Between replicate	5	2.97	0.594	61.36**	10.72	2.14	116.30**	75518.30	15013.7	191.09**
Error	25	0.242	0.0097		0.46	0.0189		1975.90	79.04	

** Significant at 1% level

CD at 5% level = Ascorbic acid - 0.117
Folic acid - 0.161
Vitamin B_{12} -10.568

the method that accounts only for reduced form of ascorbic acid, rather than total ascorbic acid content. In the present study the total ascorbic acid content, inclusive of reduced and oxidized forms, was estimated using 2,4 dinitrophenylahydrazine according to the method described by Roe and Kuether (1943). This apart, the conditions and the period for which the milk is held during storage would equally reflect in the differences in various reported values.

As regards the content of folic acid, the value witnessed in the present investigation compared well with those (5.51 µg/100 ml) reported in earlier studies (Boman, 1953; and EL-Rafey, 1962). The mean concentration for vitamin B_{12} was somewhat lower than the wide range between (400 to 577 mµg/100 ml) reported by Sreenivasamurthy et al. (1953); EL-Rafey (1962); Paolis and Gregory (1963). However, Zahriev and Kaloianov (1965) observed much higher value. Such a large variation could be partly attributed to the genetic factors as well as the differences in the test organisms used for estimations e.g. Lactobacillus lactis Dorner MB 367 by Sreenivasamurthy et al. (1953), whereas in present study Lactobacillus leichmannii ATCC 7830 was used as the test organism.

Effect of pasteurization:

The effect of pasteurization of milk either by holding or HTST method on the ascorbic acid content could be further

seen from Tables 28 and 29. There was a decrease of 11.3 and 7.6 percent, respectively. Stastical analysis of the data (Table 29) showed that pasteurization by holding method produced more deleterious effect on ascorbic acid level than HTST treatment. Similarly, Dluzewska and Bilinska (1966) reported a reduction of similar magnitude in ascorbic acid on pasteurization of buffalo milk by HTST method, as observed in the present study. The extent of reduction obtained in the present study by either method was much less than the losses of about (20 percent) reported for buffalo milk by Kothavalla and Gill (1943) and for cow milk (Kon and Watson, 1935; Holmes et al., 1943 and Hand, 1943) during pasteurization by holding method. Here again, the losses reported by these workers were only in respect of reduced form of the vitamin and not for total vitamin as determined in the present study. The loss of only 6 percent was also comparable to that observed by Rossihina et al. (1969) during flash pasteurization (85 to 90°C for few seconds). Similar type of reduction was observed for cow milk by Burton et al. (1970). However, Kyla-Siurela and Antila (1972) failed to observe any significant loss in ascorbic acid content during HTST pasteurization when total vitamin content was estimated using 2,4 dinitrophenylhydrizine method.

At the stage of its extraction from the udder, milk is known to contain vitamin C in the reduced form. It should be mentioned that dehydroascorbic acid has been reported to contribute about 20 percent of the total vitamin content present in raw untreated cow milk (Burton et al., 1970). However, subsequently it slowly gets oxidized to dehydroascorbic acid and process is often accelerated by the factors such as metallic contacts, exposure to light, oxygen, rise in temperature etc. The amount of dehydroascorbic acid present in milk, would thus depend on several aforesaid factors.

The higher losses observed in the ascorbic acid content in pasteurized milk by holding method could primarily be due to losses of dehydroascorbic acid present in milk, since this form of ascorbic acid has been reported to be more heat labile than its reduced form (Hand, 1943; Ford et al., 1968,69; Lechner and Kiermeier, 1969 and Burton et al., 1970). All these studies were carried out on cow milk. The differences in the extent of losses in ascorbic acid during pasteurization reported in different studies would appear plausible in the light of the aforesaid. On the other hand, during pasteurization of milk by HTST method, ascorbic acid was more stable and could be partly ascribed to relatively very short duration

of heat treatment. Further, liberation of sulphydryl groups during heating at 73°C and by their virtue of being reducing agents, these could have resulted in lowered oxidation-reduction potential of milk and apparently protected ascorbic acid from oxidation during HTST pasteurization. Such phenomenon may not be effective in holding method since the optimum temperature for production of these sulphydryl compounds is reported to be 73°C (Burgwald and Josephson, 1947).

As regards folic acid and vitamin B_{12}, these were found to be decreased by 8.6 and 8.0, and 3.8 and 3.8 percent during pasteurization by holding and HTST method, respectively. The extent of reduction in folic acid levels was similar to that reported earlier by Karlin (1969) and Karlin et al. (1969), during flash pasteurization either at 92°C for 3 to 4 seconds or by heating the milk at 62°C for 3 to 4 seconds. Ghitis and Candanosa (1966) on the other hand, failed to observe any loss of folic acid on milk pasteurization at 50°C. The failure of these workers to observe any loss of folic acid, could be the relatively lower temperature adopted for pasteurization.

The reduction in folic acid content during pasteurization could be attributed to the destruction of free form of the vitamin, which is more heat labile than the bound form. It was observed that at higher temperature, viz.

boiling at 100°C for 2 to 3 minutes, total folacin activity was reduced by 40 percent, while free folacin was destroyed completely. Even at pasteurization temperatures loss of free form of the vitamin was double than the reduction in total activity (Karlin,1969).

A decrease in vitamin B_{12} contents during pasteurization using either method was found to be only very small (3.8 percent). The loss of vitamin B_{12} content observed in the present study was identical to that reported by several workers (Chapman et al.,1957; Ford et al., 1959; Kruglova and Gulko, 1966 and Burton et al., 1967). However, these losses were comparatively much lower than 7 to 10 percent reported by other workers (Dluzewska and Bilinska, 1966; Karlin, 1969 and Karlin et al., 1969).

The stability of vitamin B_{12} at lower temperature could be attributed to the fact that most of the vitamin is present in the bound form (cobalamin) and accounted for about 95 percent of the total vitamin B_{12} percent in milk (Parkhim et al., 1965).

Effect of homogenization:

As regards the effect of homogenization on the contents of these vitamins, it could be seen (Table 28) that the loss of ascorbic acid was 6.9 percent and was comparatively less than that seen during pasteurization.

This could possibly be due to heating of milk at relatively lower temperatures of 60°C before homogenization. Further increase of temperature by 4.4 to 9.2°C apparently did not result in additional destruction of vitamin C. On the other hand; the destruction of folic acid as well as vitamin B_{12} during homogenization was markedly higher, viz. 10.8 and 8.0 percent, respectively. It is possible that during homogenization treatment, increased adsorption of proteins on the fat globule membrane resulted in the liberation of these vitamins into free form, which were susceptible for thermal destruction. Apart from this, the influence of oxidative destruction of vitamin C on the degradation of folic acid and vitamin B_{12}, can not be ruled out.

Effect of homogenization and pasteurization:

The effect of pasteurization done after homogenization was almost similar to those observed on untreated milk for ascorbic acid and vitamin B_{12} content, however, it was somewhat higher for folic acid. Total losses in percent due to homogenization and pasteurization were almost double the extent noted due to pasteurization in the case of ascorbic acid, however, these were still higher in rest of the two vitamins.

The statistical analysis of data (Table 29) showed that pasteurization by either method significantly ($P < 0.01$) decreased vitamin C and folic acid in homogenized milk,

whereas in respect of vitamin B_{12}, it was only HTST treatment.

Losses of vitamin C observed in the present study were markedly lower than those reported by Kyla-Siurola and Antila (1972) who reported about 50 percent decrease in vitamin C content in milk pasteurized and homogenized, second time after a storage period of 3 days at 4°C, after pasteurization and homogenization. Higher losses in their study, could be due to oxidation of ascorbic acid by dissolved oxygen to its oxidised form during storage. Similar type of reduction was also observed when milk was pasteurized and homogenized (Woessner et al., 1939). However, this decrease could be attributed to methodology used for determination, the indophenol dye used in this study as would only estimate reduced form of vitamin C. On the other hand, Bell and Sanders (1945) reported a loss of 12 percent in vitamin C in milk (fortified with 25 mg/quart) after homogenization and pasteurization.

More destruction of folic acid and vitamin B_{12} content could also be attributed to the increased destruction of ascorbic acid. The losses observed in the case of folic acid were similar to those observed earlier in milk heated (UHT) in the presence of air

(Ford et al., 1969 and Burton et al., 1970). However, the losses observed in the present study were much lower in comparison with those found by Karlin (1969).

As regards vitamin B_{12}, losses observed in the present study were identical to those reported by Chapman et al. (1957) who recorded less than 10 percent reduction in vitamin B_{12} during sterilization of homogenized milk. However, Karlin (1969) observed a 73 percent decrease in vitamin B_{12} in homogenized milk followed by sterilization at 120°C for 13 minutes. Pasteurization by holding method lowered vitamin B_{12} significantly greater than those observed in HTST pasteurization. This observation was in close agreement of St-Pierre et al. (1963) who observed a significantly higher losses of vitamin B_{12} in homogenized milk, pasteurized by holding method in comparison to those observed in HTST method.

Nutritional consequences

Though milk exhibits wide range of water soluble as well as fat soluble vitamins in it, their contents in milk do not adequately fulfil the nutritional requirements for most of these vitamins. While contents of vitamins B_2 and vitamin A have been known to be adequate to meet the nutritional requirements to a large extent, it is not so in respect of other vitamins. Computation of indices

TABLE - 30

Effect of homogenization and pasteurization on index of nutritional quality (INQ)* of buffalo milk

Vitamin	INQ for untreated milk	INQ for homogenized and pasteurized milk
Ascorbic acid	1.10	0.91
Thiamine	0.80	0.70
Riboflavin	2.50	2.30
Nicotinic acid	0.10	0.09
Pantothenic acid	0.75	0.65
Folic acid	0.30	0.23
Vitamin B_6	0.04	0.04
Vitamin B_{12}	2.70	2.40

* $INQ = \frac{\% \text{ of nutrient allowance}}{\% \text{ of energy allowance}}$ (Lofgren and Speckmann, 1979)

of nutritional quality done by Lofgren and Speckmann (1979) showed that on the basis of nutrient density, whole milk could hardly be considered as a good source in respect of ascorbic acid, vitamin B_1 and nicotinic acid in the normal human diet. Further computation on similar basis suggested that milk do not appear to be important dietary source for folic acid, pantothenic acid and vitamin B_6 as well (Table 30). Even, taking into account the losses ranging between 10-11% in respect of vitamin B_2 and vitamin B_{12} during homogenization and subsequent pasteurization of buffalo milk, the dietary significance of milk in respect of these vitamins appears to be only marginally minimised, as a result of such treatment.

CHAPTER - V

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Though homogenization is practised in fluid milk processing to improve the palatability as well as in the manufacture of several dairy products, the effect of such treatment on nutritional characteristics of milk has been largely implied on the basis of improved palatability and change in curd tension. Keeping in view the paucity of systematic information on this aspect, the present study was undertaken to find the effect of homogenization on certain nutritional qualities of milk.

In this study, the milk was homogenized in a double stage Gaulin piston type homogenizer, at a standard pressure of 2500 psi. It was subsequently pasteurized either by holding method (63°C for 30 minutes) or HTST method at 72°C for 15 seconds according to the experimental requirements.

Experiments on the effect of variation in total solids contents in milk on (i) the homogenization efficiency, (ii) curd tension and (iii) relationship between certain chemical constituents and curd tension, were carried out. Milk samples from different species viz. buffalo, cow and goat and mixture of buffalo and cow milk in different proportions were used in these experiments.

a) It was observed that the homogenization efficiency for different milk samples varied in the narrow range between 88.8 to 90.6 percent. It was maximum in the case of buffalo milk and minimum in the case of mixed milk having least proportion of buffalo milk. Differences observed were, however, non significant.

b) As regards curd tension, buffalo milk showed significantly higher ($P < 0.01$) curd tension (42.8 g) than goat milk (21.4 g) and cow (19.7 g). It was further seen that as the proportion of buffalo milk in the mixed milk samples decreased, there was a progressive decrease in the curd tension value.

c) Pasteurization by either holding or HTST treatment resulted 7 to 15 percent reduction in the curd tension. Decreases observed were, however, non significant.

d) As regards homogenization treatment, there was significant decrease ($P < 0.01$) in curd tension in all types of milk samples. The maximum reduction was witnessed in the case of buffalo milk whereas minimum was seen in the case of goat milk. It was further observed that predominance of buffalo milk in the mixed milk samples influenced the reduction in curd tension.

e) As regards the effect of pasteurization subsequent to homogenization of milk, it was seen that the reduction in

curd tension was significant in all cases and with pasteurization with either method. The overall reduction of 76.3 percent in curd tension was observed in buffalo milk as against i.e. 68.8 and 70.1 percent in cow and goat milk, respectively.

f) There was a definite positive relationship between the total solids content and the curd tension of milk ($r = 0.82$) and also between SNF and the curd tension ($r = 0.71$).

g) In a study on the effect of homogenization on the protein quality, the growth promoting ability assessed by determining modified protein efficiency ratio (PER_D) and the N-balance in experimental rats maintained on test milk samples, were determined. It was observed that PER_D was significantly higher ($P/0.01$) in the group of rats receiving non-homogenized buffalo milk than those receiving either standardised or whole homogenized milk.

h) It was observed in the N-balance studies that though there were no differences in the digestibility coefficients for test milk protein samples, the biological value was significantly higher ($P/0.01$) for the group receiving non-homogenized whole buffalo milk (93.0) than the group receiving either standardised homogenized (76.7) and in whole milk homogenized (88.2). It was further observed that there was significantly higher excretion of urinary

nitrogen in animals receiving homogenized milk than other group.

i) As regards the deposition of dietary-N in the body carcass, the group receiving non-homogenized milk showed distinct superiority (NPU = 79.9) over other groups fed homogenized milk (NPU = 58.2 and 69.3), respectively.

j) In Experiment on the effect of homogenization on the absorption of fat, it was observed that the group maintained on whole homogenized buffalo milk showed significantly higher fat absorption (88.8%) than the group receiving non-homogenized milk i.e. 78.3%.

k) Attempts to study the biological availability of vitamin A from buffalo milk homogenized as well from non-homogenized, were made using vitamin A deficient male rats. By providing vitamin A at a dose of 25 IU per day from these milk samples, it was observed that vitamin A deficiency symptoms disappeared by 30 to 33 days of feeding these milk samples as the source of vitamin A. This was evident from restoration of growth rate and serum vitamin A levels. However, it was observed that the groups receiving homogenized buffalo milk either standardized or whole milk had significantly higher ($P < 0.01$) levels of vitamin A in the liver than those receiving non-homogenized milk.

l) As regards the stability of certain water soluble vitamins such as, ascorbic acid, thiamine, riboflavin, nicotinic acid, pantothenic acid, folic acid, vitamin B_6 and B_{12} on homogenization and also after subsequent

pasteurization, It was observed that pasteurization of untreated milk by either method, lowered the levels of these vitamins by 2.6 to 11.3 percent.

m) Homogenization treatment of the untreated milk showed reduction in respect of these vitamins identical to pasteurization, which ranged between 3.7 to 10.8 percent.

n) Pasteurization of homogenized milk either by holding or HTST method reduced respective vitamins with equal degree. The overall reduction due to homogenization and pasteurization as regards to these vitamins ranged between 8 to 22 percent, being minimum in vitamin B_6 and maximum in folic acid.

CONCLUSION

Experiments conducted in the course of this investigation showed that at a standard pressure of 2,500 psi, milk from different species as well as mixed milk could be homogenized with equal degree of efficiency, irrespective of their fat content. Such treatment produced milk with soft curd characteristics.

As regards the utilization of nutrients it was observed that fat as well as fat soluble vitamin A from homogenized milk, were utilized much better than non-homogenized milk. Though homogenization and subsequent pasteurization of buffalo milk significantly lowered the modified protein efficiency ratio, biological value as

well as net protein utilization, the decreases witnessed in respect of these parameters, cannot be considered to be of that magnitude so as to suggest homogenization treatment undesirable. The improvement in the palatability of milk decisively increased the net protein intake in the groups receiving homogenized milk, resulting in maximum average gain in body weights. This could partially counter the limited adverse effect homogenization may have on the quality of milk proteins.

The extent of losses in B-group vitamins studied were higher when the milk was homogenized and subsequently pasteurized. However, milk is hardly considered as a good source of ascorbic acid as well as most of B-group vitamins except vitamin B_2 and vitamin B_{12}. An overall reduction in the levels of these vitamins due to homogenization and subsequent pasteurization thus do not appear to be that large, so as to warrant the exclusion of homogenized milk in the balanced diet.

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FIG.I. STANDARD CURVE FOR ASCORBIC ACID

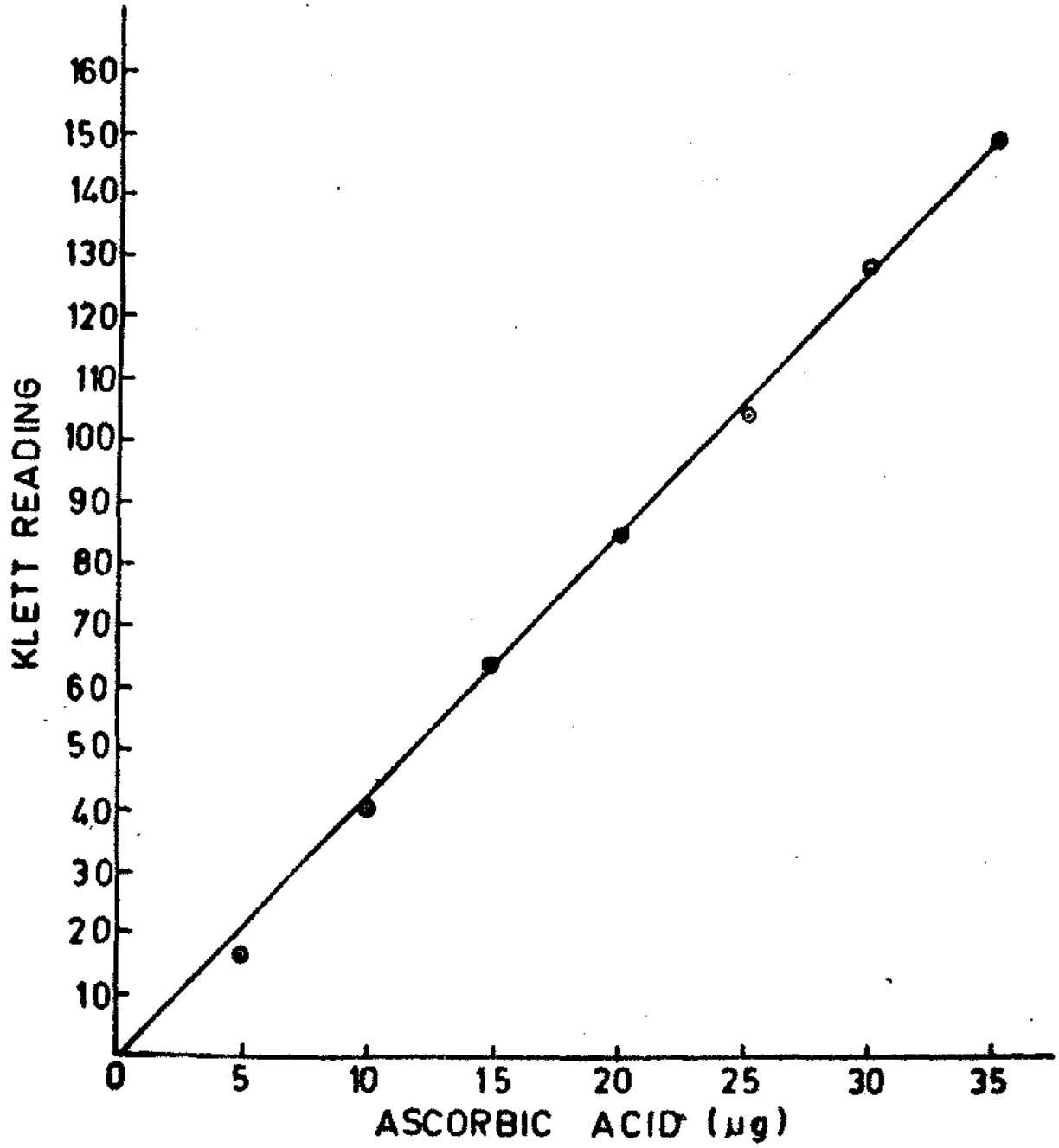

FIG-2. STANDARD CURVE FOR FOLIC ACID

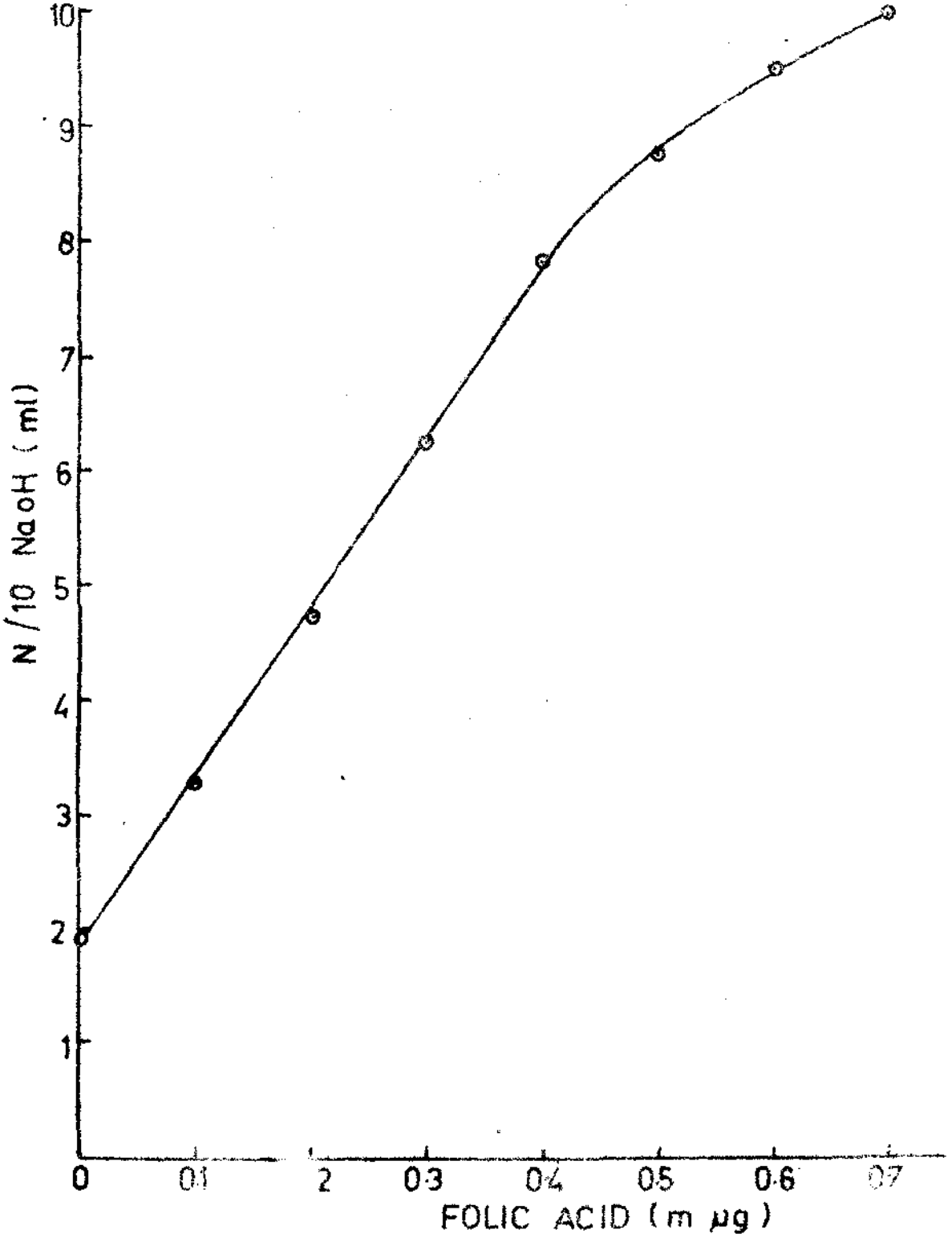

FIG. 3. STANDARD CURVE FOR RIBOFLAVIN

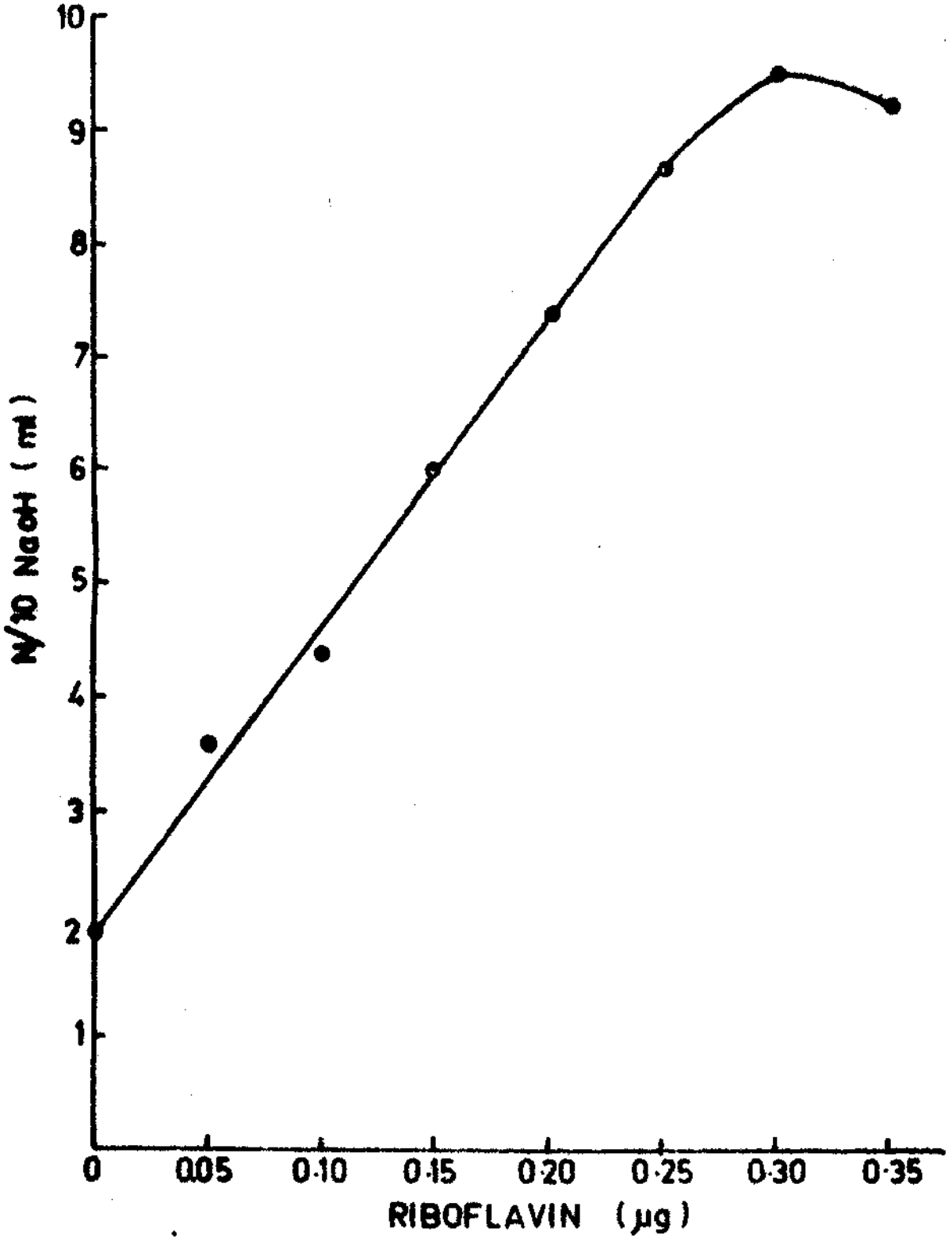

FIG. 4. STANDARD CURVE FOR NICOTINIC ACID

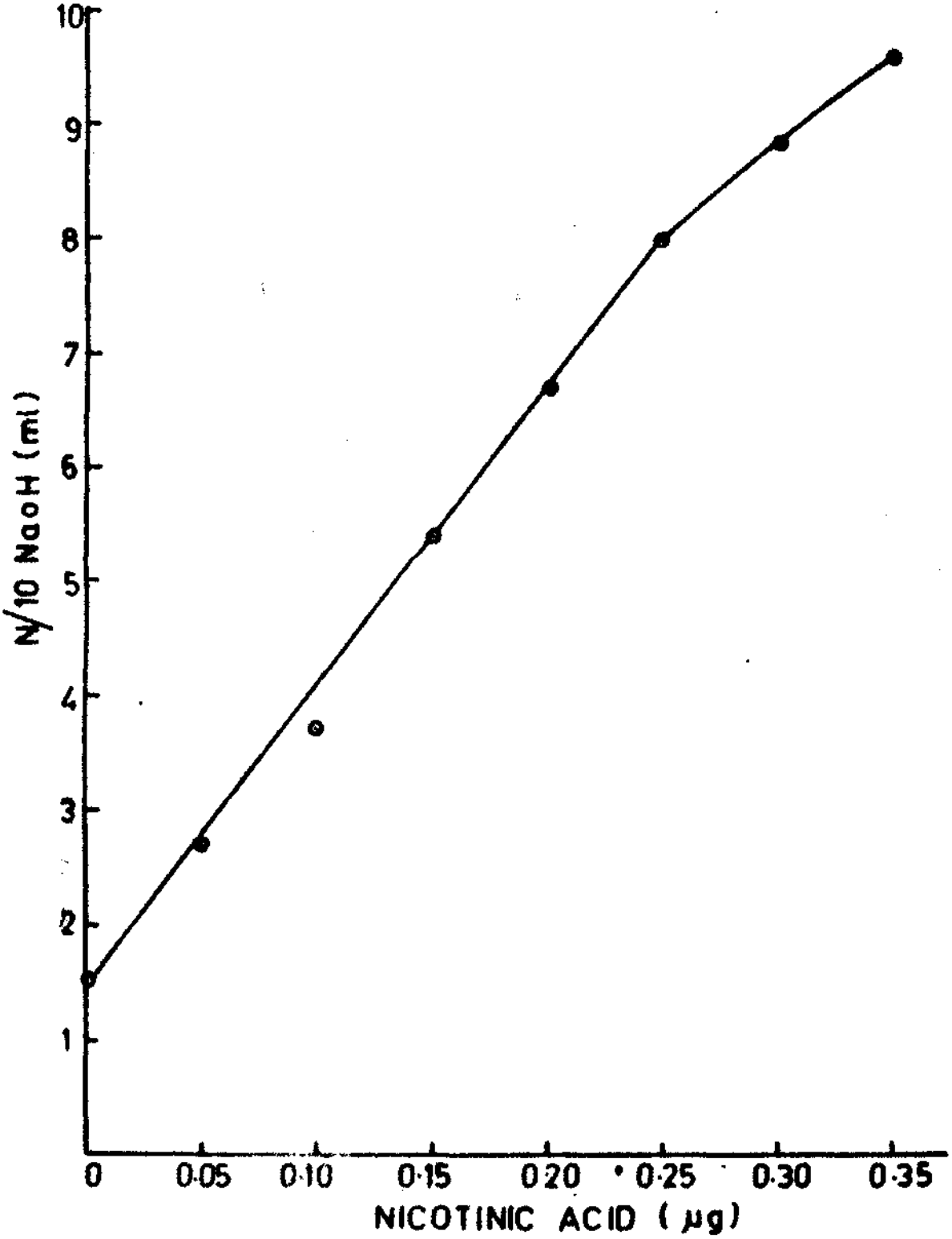

FIG.5. STANDARD CURVE FOR PANTOTHENIC ACID

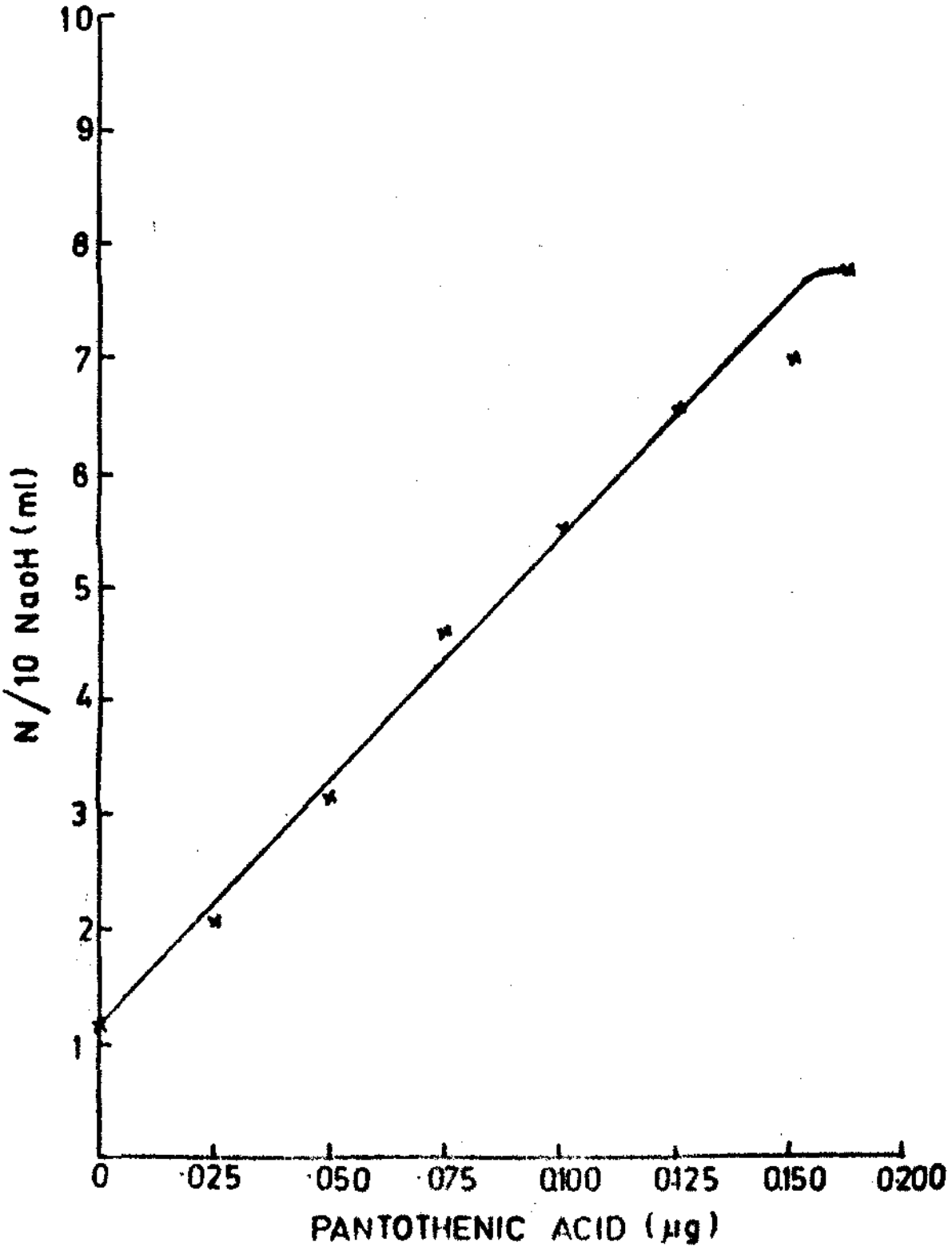

FIG. 6. STANDARD CURVE FOR VITAMIN A