CHAPTER V

DISCUSSION

The lower sialic acid content of micellar casein compared to acid casein (Table 1 and 2) is presumably due to the association of calcium and inorganic phosphate ions with micellar casein (1,4,20,23) which on acidification dissociate (Ref.1, Tables 15 and 20). Acid casein is then likely to have more sialic acid per unit weight. Micellar casein particles have also the capacity to bind water (1,3) and more precisely κ-casein contains a strongly hydrophilic glycomacropetide (29) and thereby will cause a lowering of the sialic acid content of micellar casein than in acid casein. The present results also indicate a lower sialic acid content in buffalo casein preparations compared to that of cow irrespective of the physical status of casein i.e., micellar or acid (Table 1) as observed by Gupta and Ganguli (164). One might also attribute the lower content of sialic acid in whole casein from buffalo milk to the lower proportion of α-casein in whole casein of buffalo milk than cow samples as indicated earlier by Ganguli and Bhalewar (355). But considering the concentration of sialic acid in κ-casein (Table 4), it is quite evident that the buffalo casein molecule has low sialic acid per unit weight of the κ-casein fraction. The sialic acid content measured as N-acetyl neuraminic acid (NANA) was found to differ in different caseins prepared from milk of three breeds of cow (Table 2) as compared to published value on foreign breeds (163). Our results on acid and micellar caseins concur well
with the reported value on sialic acid from casein of Indian cattle by Gupta and Ganguli (154). Tharparkar milk caseins always exhibited higher level of sialic acid in both micellar and acid types than the other two breeds (Table 3). This sort of differential distribution of sialic acid in whole caseins in different breeds indicates towards, their differences in the micelle make up.

Neuraminic acid is chiefly important for the development of the gangliosides during the first stages after birth (386). Hence its presence in various milks, is of value to infant nutrition. From this stand point, therefore, the nutritional superiority of cow milk over buffalo (Table 1) and of Tharparkar milk over that of Red Sindhi and Sahiwal breeds (Table 2) can be claimed because of their higher sialic acid contents.

It would be of interest to note the correlation between hexose (Table 1) and sialic acid (Table 2) contents in caseins of different breeds of cow irrespective of the type of casein. One would expect such a trend in the light of the proportions of these two carbohydrates in k-casein (30).

The observations on the sialic acid in casein particles of different size (Table 3) can be explained in the light of findings by Amibaldi (3, 7, 39) and Yoshida et al. (39). These authors have shown that fractions settling at higher speeds (10,000–40,000 r.p.m.) mainly contained &lt;fraction (37), a fraction which is rich in sialic acid. This view is supported by the present findings, however, contamination of other fractions with
α-casein is also apparent from the presence of sialic acid in fractions collected at low speed (Table 3). Taking sialic acid as an index of k-casein content in whole casein (101) it can be stated that small micelles contain higher amount of k- fraction of casein. This view is further strengthened by our observations of starch gel electrophoretic pattern of the casein fractions obtained by differential ultracentrifugation (Chapter IV page 143) where small micelle fraction show relatively higher concentration of k-casein band irrespective of the source of casein micelle i.e. cow or buffalo. It may be further said that since the relative concentration of k-casein on electrophoresis in larger micelles is not as low as sialic acid content, it may be speculated that perhaps k-casein in larger micelles is of poorer sialic acid quality (166, 84).

A lower sialic acid content in k-casein isolated from whole micellar casein compared to the same from whole acid casein (Table 4) could be partially due to its source (it has been shown earlier that micellar casein contain lower sialic acid than acid casein (Table 1 and 2) due to varied reasons) and partially due to the presence of larger number of contaminants in micellar k-caseins as compared to acid k-casein as observed on starch gel electrophoresis (Fig. 11 and 12) in both cow and buffalo samples. The sialic acid concentration for k-caseins isolated from whole acid casein source was found to agree fairly well with the published data (186, 316, 387). A much higher concentration of sialic acid in k-casein fraction compared to its whole casein source(Table 4) is in confirmation of the earlier reports.
(151,3,296) that k-casein is the repository of sialic acid in whole casein. The results further signify that k-casein constitutes about 14% of the whole casein, since the approx. ratio of sialic acid in whole and k-casein comes to about 1:7, considering sialic acid as an index (151) of k-casein content. In a recent model proposed for the structure of casein micelle, Rose (48) has also shown a similar percentage of k-casein in whole casein. A lower whole casein: k-casein (1:4) ratio of sialic acid in case of Tharparkar (Table 4) suggests that k-casein content may be higher (about 28%) in case of Tharparkar casein compared to other two breeds of cow. Results recorded in Table 4 further indicate that k-casein of buffalo milk is of inferior quality as compared to that of cow milk as far as sialic acid is concerned.

No significant difference in the sialic acid content of caseins (micellar and acid) with different genetic k-casein variants (Table 5), suggests that sialic content is not dependent upon the k-casein variant in the milk.

No appreciable difference in the sialic acid content of casein micelle from dialysed and undialysed milk (Table 6) removes the suspicion that there could be some adhering lactose with micellar casein which may interfere with the sialic acid value (173). It may be concluded therefore that adhering lactose, if any, is washed out in the process of dehydrating the casein micelle (378,184) by acetone and ether.

Reduction in the sialic acid content of casein
micelle obtained from heated milk (Table 7) can be explained in the light of findings of Koning et al. (162). These authors have revealed that during heating of casein part of the neuraminic acid (NANA) is split off as free and as NPN-bound NANA. Release or destruction of sialic acid from casein due to heat treatment has been shown by other workers as well (213, 316, 178). Another probable reason could be the transfer of some casein micelle components (sialic acid containing fraction i.e. k-casein possibly) from sedimentable to soluble (in serum) state since such a transfer (though protein fraction was not characterized) has been observed (Table 32). The reduction in the intensity of k-casein band on electrophoresis of heat treated milk caseins (Chapter IV page 146 and fig. 13) further supports this possibility. Little or no change in the sialic acid content of heat treated milk acid casein (Table 7) is further in agreement with the above view, since the released sialic acid containing casein components precipitated along with acid casein on acidification of milk at pH 4.6 and hence the sialic acid content was not altered appreciably. The role of increase in content of calcium and phosphate of the casein micelle due to heat treatment (Tables 19 and 23) in diluting the sialic acid content of casein micelle in heat treated milk can also not be overlooked.

Differential rate of sialic acid reduction due to heat in cow and buffalo indicates their differential protection capability against heat-influenced sialic acid
destruction in the caseins of these two species. The
Buffalo casein micelle seems to be superior in this
regard (Table 7).

An increase in the sialic acid content of casein
micelle (Table 8) due to chilling of milk was recorded. This
may be due to the clogging out of β-casein (non-sialic acid
casein moiety) from the casein micelle as observed by
Sullivan et al. (192). Rege and Colvin (18) further showed
that such a released β-casein component does not reenter
the micelles even on bringing the milk to normal temperature.
It seems that release of k-casein from the casein micelle
on chilling of milk as reported (281, 282) is not effective
at the extent of chilling applied (13 and 24 hours chilling
at 6°C) under the present experimental conditions. Had it
(k-casein) been liberated at this extent of chilling, a
decrease in the sialic acid content would have been expected,
which is increasing on the contrary (Table 31).

A decrease in micellar casein content and an increase
in soluble casein content (Table 30) on chilling of milk
further supports the above hypothesis that perhaps β-casein
has been extracted from the casein micelle due to chilling
and has thus enriched the soluble casein content. An
increased intensity of k-band (Fig. 14) in the electrophoretic
pattern of chilled milk casein micelle confirms that k-casein
is not liberated on chilling rather its concentration has
been increased due to decrease in other casein components.
Gradual decrease of γ-casein band (again a non-sialic
casein component) on electrophoresis (Fig. 14) of chilled
milk suggests that it could be so that \( \gamma \)-casein is also released on chilling and hence serves as an other cause for the increase in sialic acid content of casein micelle on chilling of milk. The removal of calcium and phosphate (Table 19 and 24) from casein micelle on chilling would also increase the sialic acid per unit weight. A higher rate of increase in sialic acid content on chilling in buffalo casein micelle compared to that of cow (Table 8) suggests that buffalo milk is more susceptible to cold compared to cow milk, which again is worth noting difference in the milk of the two species.

Caseins (micellar and acid) obtained from colostrum showed a very high content of sialic acid than normal milk (Fig. 2) and such a content gradually decreases with postpartum period till it becomes constant (by 5th day) and attains normal milk casein level (Fig. 3). Such a phenomenon has been reported by many other workers as well (167-173), but all these workers analysed colostrum and milk as such and not their caseins for such sialic acid analysis. The electrophoretic pattern of normal and colostrum casein (Fig. 18) indicates that higher sialic acid in colostrum is not due to difference in \( \kappa \)-casein content since no appreciable difference in \( \kappa \)-band is apparent in such samples. Greater attachment of sialic acid as glycosaminoepitope by mammmary in the \( \kappa \)-casein fraction of colostrum milk, however, could be speculated as a possible explanation. Such a phenomenon could be expected due to greater need of sialic acid for the development of gangliosides during the first stages
after birth (386). Like cow casein (Table 1), the cow
dialysed milk and cow blood serum (Table 9) also show a
higher concentration of sialic acid than corresponding
buffalo samples. Even the free sialic acid content in
the blood serum of two species show the similar differences.
The observed values (Table 9) on cow milk and blood serum
agrees fairly well with the same reported by Kiermeir and
Freisfeld (173) and Kinsella (388) on foreign breeds.

A much higher level of sialic acid in blood serum
compared to dialysed milk (Table 9) and similar distribution
differences in cow and buffalo may give a clue on the origin
of sialic acid in milk or k-casein molecule. It might give
the idea that perhaps sialic acid is of blood origin and
that mammary gland is involved in attaching the same with
k-casein molecule. Jordan et al. (389), however, has
shown the presence of enzymes associated with sialic acid
synthesis in rat mammary gland, though little is known
about similar possibility in ruminant mammary tissue.

The presence of the remarkably high concentration of
hexose in micellar casein compared to acid casein (Table 10
and 11) offers scope for speculation. The possibility
of increase in hexose content due to the adhering lactose
on casein micelle can probably be ruled out as the dialysis
of milk could not cause any change in the hexose content
of casein micelle (Table 10). The possibility of calcium
and phosphate ions in contributing towards the high value
in the method used, however, cannot be ruled out. Another
possibility could be that perhaps some of the hexose is
loosely attached to the casein micelle and the same is released on acidification of milk and hence acid caseins show a lower value.

The hexose contents of acid casein from cow milk, however resembled with that of the reported values on foreign breeds by Reynolds et al. (147). The strikingly low hexose content in buffalo casein samples is another noteworthy difference between the casein samples of cow and buffalo. Differential distribution of hexose in the milk of three breeds of cow further directs towards differences in their micelle make-up.

A lower nitrogen (and therefore protein) content (Table 12 and 13) in micellar caseins compared to acid caseins is obviously due to calcium and phosphate ions bound to it which get dissociated on acidification (in acid casein) to a great extent (Table 15 and 20). Similarly, a higher nitrogen content in cow casein micelle compared to buffalo casein micelle can be attributed to its comparatively lower calcium and phosphate contents. Amongst the breeds, Sahiwal casein micelle (Table 12) was found to show lower nitrogen content again because of its higher calcium and phosphate level (Table 16 and 21) compared to other two breeds studied.

Analysis for nitrogen (or protein) level of casein micelle fractions obtained by differential ultracentrifugation (Table 14) suggests that protein content is dependent upon particle size and is inversely proportional to it because the calcium and phosphate levels exhibit direct proportionality to particle size (Table 17 and 22).
It appears from the analysis of caseins for their calcium content that (Table 18) calcium is present only in micellar form and suggest therefore that this ion gets dissociated from the casein micelle on acidification of milk. The lower heat stability and faster clot formation by rennet (296, 382) in buffalo milk can be explained, possible because of its higher calcium content (Table 18). Higher calcium content of milk has been correlated with its lower heat stability by various authors (390 - 394). The difficulties felt in cheese preparation from buffalo milk (296, 382, 398) may also be attributed to its higher calcium content. The results further indicate that differences in the calcium level of cow and buffalo milk are true for their colloidal calcium content as well since both are higher in buffalo milk (Ref.382 and Table 18). Similar inferences can also be drawn about Sahiwal casein micelle which shows a higher content than the same from other two breeds (Table 18). The direct relationship of calcium content casein micelle with its particle size (Table 17) suggests that variation in the micelle size may be due to variations in calcium content in addition to other reasons. This also explains the larger casein micelle size in buffalo milk compared to that of cow (Table 34). An increase in the calcium content of casein micelle on heating (Table 18) and a decrease on cooling (Table 19) further suggests that structure of casein micelle is highly susceptible to changes in temperature.
Observations in this regard are in agreement with those of foreign workers on the milk of foreign breeds for effect of heating (204, 207, 202, 210) and chilling (245) both. It seems that buffalo casein micelle is less susceptible to such a temperature change as the percent increase or decrease on heating in calcium level and cooling was observed to be lesser than the same in cow milk (Table 18 and 19).

It appears that like calcium, the level of phosphate ions also decrease in the casein micelle on acidification of milk (Table 20 and 21). A higher phosphate level in buffalo casein micelle compared to that of cow and in Sahival compared to that of other two breeds further directs towards the differences in the milk of two species and different breeds of cow.

The role of phosphate in determining the particle size can be emphasized due to its greater association with larger particles (Table 22) irrespective of the source of casein micelle (i.e., cow or buffalo).

Phosphate seems to behave similarly as calcium on heating or cooling of milk (Table 23 and 24) for similar seasons. Here again the observations are in agreement with the same reported by other authors (282).

The higher opacity in case of buffalo micellar casein (Table 25) may be attributed to the higher initial content of Ca++ in buffalo milk (282) or buffalo casein micelle (Table 15) which can contribute to opacity through its binding in the micellar particles. Similar explanation can be given for higher opacity in case of Sahival (cow) milk micellar casein compared to that of Tharparkar.
breed micellar casein. It may be said that the differences in opacity obtained by different buffers viz. maleate, citrate and phosphate (Table 26) might be due to their natural property. It has been observed that at lower pH opacity was more than the same at higher pH and more specifically with citrate buffer (Table 26) which could be due to partial precipitation of casein nearing its isoelectric point.

Increase in opacity in micellar casein by increasing buffer levels leads to the conclusion that opacity is more dependent on buffer constituents. The opacity has also been found to increase with time (Fig. 4 - 6). Similar conclusion can also be drawn from the observations indicating the difference in opacity due to storage period (Fig. 6 a-c). A sudden rise in opacity after 24 hours in case of Tris buffer (Fig. 6 a-c) indicates that perhaps by this period the micelles become quite unstable in that solution. Where in phosphate buffer, micelles remained more stable as compared to tris.

Opacity of casein micelle in maleate buffer (Table 27 and 28) further confirms the earlier observations that buffalo casein micelle is more opaque than sow and amongst the three breeds of sow Sahival showed highest opacity. A higher opacity in buffalo casein micelle (Table 27) may also be possible factor for the low solubility of whole and skim milk powder from buffalo milk compared to that of cow milk (396), irrespective of the process employed i.e. roller or spray drying (396). Dependence of opacity on casein concentration (Table 27) also supports the similar view. Since buffalo milk is
known to have higher content of casein (Ref. 286, 383, 383 and Table 37). Lower solubility of milk powder in buffalo can also be predicted from our observations that buffalo micellar caseins took much longer time than cow for dissolution for a similar concentration of casein both the cases. On the basis of these results, therefore, lower solubility of milk powder from Sahival milk compared to that of other two breeds can be predicted since opacity is also higher in this case (Table 28).

A positive correlation between Ca content (Table 17) and opacity (Table 29) of the different casein micelle fractions obtained by successive ultracentrifugation emphasizes the role of calcium in determining the opacity of casein micelle and hence solubility of milk powder from the milk of different species of breeds. It also strengthens the view expressed earlier that the higher opacity in buffalo casein micelle may be attributed to its higher calcium content and likewise for Sahival casein micelle. Higher opacity in all buffalo fractions compared to corresponding cow fractions further stresses the differences in the casein molecule of the two species.

A dramatic increase in the opacity of casein micelle on addition of calcium in the system (Table 30) is expected due to the destabilization of the calcium sensitive $\alpha$-casein fraction of the micelle (64, 1, 9). This view is further confirmed by the finding (Fig. 7) that opacity further increased with time in the presence of calcium chloride at a very sharp rate in the beginning and ultimately
even led to precipitation in some cases. The observations (Table 30 and Fig.7), therefore, leave no room for doubt that \( \alpha \)-casein slowly precipitate on addition of calcium in the Alkali-buffer system. Similar increase on addition of calcium ions was also observed by Yoshida (218) in their studies on colloidal aspects of casein micelle. Yoshida concluded that casein micelle aggregated and was destabilised in a centrifugal field on addition of calcium ion. His (218) explanation is in accordance with the aggregation studies of Zittle and Pepper (397). The present investigation supports the view of Zittle and Pepper (397) that the principal functions of the calcium in the casein aggregation is through binding to the casein to reduce the net charge, in order to make it comparable to isoelectric point. Differential rate of aggregation (or destabilization of \( \alpha \)-casein) in different breeds of cow or in buffalo compared to cow (Fig.7) may be due to differential calcium (Table 18 and 16) and casein contents (Table 37 and Ref. 296) since it has been proved by Zittle and Pepper (397) that calcium : casein ratio is a factor which influences the rate of aggregation.

An increase in the opacity observed on addition of phosphate (Table 30) may be explained due to formation of insoluble calcium phosphate on such addition (306). A higher opacity in phosphate buffer system compared to maleate buffer (Table 28) also suggests that addition
of phosphate is likely to cause an increase in the opacity.

Reduction in the opacity on addition of sodium citrate (Table 30) indicates that sodium citrate has a solubilising effect on calcium caseinate, which is quite natural because of the great affinity of citrate to the calcium ion (309). The present observations (Table 30) regarding the effect of citrate on opacity of casein micelle gets support from the finding of Sommer and Hart (400) who has shown the solubilizing effect of citrate on calcium citrate precipitates. A decrease in the calcium concentration through binding by citrate, together with the effect of sodium ion (309) is sufficient to explain lower or almost negligible opacity observed on addition of citrate in the alkali-buffer system.

An increase in the opacity (Tables 31 and 32) of casein micelle solution (whole micellar casein or caseins of different particle sizes) indicates that heating is capable to induce some change in colloidal properties or structure of the casein micelle. It may be emphasized that such an increase was irrespective of the species, breed or particle size (Table 31 and 32). Similar observations were also made by Yoshida (317) on ew casein micelle. The alteration in the opacity of casein micelle due to heat and its direct proportionality with extent of heat (Table 31 and 32) may be of importance for understanding the effects of heat on milk. Such a
change is obviously due to aggregation of casein micelle particles and their slow destabilization and hence coagulation as observed in some cases (Table 31) by heat. An irregular pattern in the increase of opacity by heat in different casein micelle samples is explainable in the light of findings by Zittle et al. (397, 398) and Dyachenko and Vlodavets (401). The authors have concluded that calcium, phosphate and citrate etc. (constituents of casein micelle) and the concentration of these ions have a big role to play in such a heat induced coagulation of casein.

It is interesting to note that the opacity of the casein micelle obtained from heated milk did not differ from/same of unheated milk (Table 33) except when the heat treatment was too severe (i.e., sterilization) where the opacity show an increase. The higher opacity in sterilized milk casein micelle compared to that of raw milk (Table 33) indicates the lower solubility of the former. The comparison of the direct influence of heat (Table 31 and 33) on casein micelle and indirect influence through heating milk as such (Table 33) suggests that treatments given normally to milké like pasteurisation or boiling) does not greatly influence the colloidal properties of casein micelle.

A lower opacity in chilled and dialysed milk (Table 33) casein micelle compared to that of normal milk may be attributed to the loss of calcium on cooling of milk (Table 19) as such or during dialysis as the case may be,
since the presence of calcium has been proved to be a stronger factor in contributing towards micellar opacity (217, 48, 3).

Differential pattern of micellar casein distribution (obtained at different speeds) in cow and buffalo milk can be explained on the basis of differential particle sizes (Table 34). Settling of major portion of buffalo micellar casein at low speed indicates that buffalo micellar casein particles are larger in size than that of cow.

The higher content of micellar casein in buffalo milk could also be attributed to the higher calcium level (252) in this milk since such ion contributes significant impact on the micellar structure (3, 73). The absence of soluble casein in buffalo milk (Table 35) might as well explain its differential behaviour towards rennet action (252, 296) compared to cow milk.

The lower soluble casein content and the higher MC/SC ratio (Table 36 A) in the milk of Sahiwal breed compared to other two breeds gives a clue on the particle size of the casein and indicates that Sahiwal milk has a larger micelle size compared to other two breeds tested. Statistical analysis (Table 36 B) further enlightens that the particle size of casein micelle of Tharparkar and Red Sindhi is of the same order. Our data on the composition of protein contents (Table 37) in buffalo and cow milk agree fairly with the reported values on these Indian species of animals (293, 402 - 404).
The micelle size has a great significance in relation to the physico-chemical and biochemical phenomena. A large micelle size indicates towards lower density (308), faster electrophoretic mobility (124), lower sialic acid residue (Table 30), higher sialic acid release (Table 44), higher rate of turbidity formation by rennin (Fig.36), higher calcium (Table 17) and higher phosphate (Table 22) content of the casein particles.

The low speed needed in sedimenting casein micelle in case of buffalo milk (56,000 x g, 30 min.) compared to that of cow (105,000 x g, 30 min.), (Fig.2) further indicates towards the larger particle size of buffalo casein micelle (Fig.8). Such basic difference in the casein micelle of buffalo and cow milk further fortifies the findings of Ganguli (296) on the compositional differences of caseins of these two species.

An increase in the sedimentable protein content or decrease in soluble protein content (Table 39) on heating the milk at various temperatures gives scope for speculation that perhaps soluble casein particles aggregated to form larger particles due to heat. Hence such aggregates settled along with the sedimented casein micelle. Another reason could be the co-settling of whey proteins denatured by heat. The possibility of such a change in the sedimentable and soluble protein distribution pattern due to heat-induced changes in colloidal calcium (Table 18) and phosphate (Table 25) level can also not be ruled out (75). A higher soluble protein content on sterilization compared
to boiling of milk (Table 38) gives the impression that on sterilization some proteinous components of casein micelle have been transferred to soluble state. Loss of such a temperature-sensitive casein from the micelle is the cause of additional serum protein (73) content in case of sterilization compared to boiling where it may not have happened. This contention is further confirmed by the soluble protein content of resuspended and recentrifuged casein micelle suspension in milk dialysate (Table 38). The soluble protein content in such a suspension was not affected by pasteurization or boiling but was increased by sterilization indicating therefore that some heat-sensitive casein micelle component has been released on sterilization of milk and transformed into soluble state.

The decrease in micellar casein content and an increase in soluble casein content recorded on chilling of milk in both cow and buffalo samples (Table 39) can be explained in the light of findings by Rose (73). According to Rose (73), the loss of either calcium phosphate or of temperature-sensitive casein from the micelle releases additional casein to the serum; loss of both has a more than additive effect. The loss of colloidal calcium and phosphate in the casein micelle on chilling of milk has been observed experimentally (Table 19) and (Table 24) and that of temperature-sensitive casein (mostly /β-casein) is evidenced from the literature (73, 192, 16, 202, 203, 288).

Differences in the rate of transfer of protein of
casein from sedimentable to soluble state on heating (Table 38) and cooling (Table 39) of milk in cow and buffalo further enlighten on focus the difference in casein micelle structure of the two milks. These observations (Table 38 and 39) further suggest that heating or cooling of milk greatly alters the casein micelle structure.

The slower mobilities and poorer resolution of the casein components in micellar form compared to the acid casein samples (Fig. 9 and 10) appear to be a distinct property associated with the micellar structure of the molecule. It appears from the results presented (Fig. 9 and 10) that casein in its micellar state is less ionizable than its acid form. This may be due to the fact that casein in its micellar state exists as calcium caseinate phosphate complex (1, 9), whereas on acidification it looses both calcium and phosphate ions (Ref.1, 44, 60) and (Table 15 and 20) and therefore, on acidification it gains net charge due to the removal of these ions from the micelle (1). Suppression of the charges with the calcium and phosphate radicals in micellar casein may cause ultimately a lower mobility and poorer resolution of the protein components in the same electric field compared to the acid casein components. Slower mobility of micellar casein compared to its acid counterpart on starch gel (Fig. 9 and 10) is in agreement of the similar observations made by Sabarwal and Ganguli (124) using paper strip electrophoresis.

Slower rate of migration of buffalo casein components compared to that of cow have been reported by many
workers (96, 288, 289, 361) by resolving acid casein by paper electrophoresis. Such differential behaviour was even recorded in micellar caseins from these two species on paper electrophoresis by Sabarwal and Ganguli (124). But starch gel electrophoresis, however (Fig. 9 and 10) did not show any appreciable difference in the rate of migration or it showed a very slight decrease (Fig. 14) compared to cow samples. The difference in the two species, however, were observed in some minor fractions and k-fractions. At this juncture, it is not possible to characterize the minor bands (unidentified bands) detected in caseins (Fig. 9 - 18). Several positively charged proteins zones were also detected by Neelin (143) in bovine casein. Our buffalo and cow caseins behaved similarly in this regard. Differences in the k-casein variants in cow and buffalo caseins (Fig. 9 and 10) is yet another very prominent difference in casein micelle make-up of the two species. Such a difference was also observed by Ganguli and Majumder (121) in acid casein samples. Our observations are also in agreement with the results of Ganguli and Majumder (121).

A considerably lower concentration of K_A band compared to K_B band in case of buffalo appears to be a probable factor responsible for the difficulties experienced during the preparation of milk products from buffalo milk (362), since the influence of k-casein variant on the processing pattern of some milk products has been reported recently by Schmidt (240). The above contention
is further fortified by the interesting observations of Ganguli and Majumder (131) that casein samples (sealed in filter paper) of both cow and buffalo on storage at room temperature (15-25°C) in a dessicator slowly lose k-casein B. Whereas under the same conditions of storage, k-casein A does not undergo any change. The electrophoretic pattern of caseins of different particle sizes (obtained by differential ultracentrifugation) unequivocally demonstrates that all micellar caseins are mixture of \( \alpha_s \), \( \beta \), \( \gamma \) and k-casein. Dependence of migration rate on the particle size observed on paper electrophoresis (124) was not observed on starch gel. The heterogeneity of k-casein molecule on starch gel (Fig.11 and 13) has also been reported by other workers. The observance of a sharp k-zone also occurs well with the findings of others (115, 111, 112), on k-casein isolated from acid casein source. It is for the first time, however, that the electrophoretic pattern of micellar k-casein (Fig. 11 and 13) has been revealed. Larger number of fast moving components and other bands in micellar k-casein compared to acid k-casein may be regarded as presence of larger number of contaminants in the former. At this juncture, however, it could not be possible to characterize such contaminants and other positively charged components. The difference observed in k-casein patterns of cow and buffalo further enlighten the earlier observations on the differential make-up of casein micelle of these two species.
Better resolution of casein micelle from dialysed milk compared to that of undialysed milk could be due to some compositional differences in the casein micelle caused during dialysis. It has been shown earlier that casein micelle loses its calcium and phosphate (Table 19 and 26) to a certain extent on cooling of milk. Therefore, since dialysis of milk was carried out at refrigeration temperature (4°C) some loss of these ions is expected which could be a probable factor in better resolution of the dialysed milk's casein micelle since such a change would make the casein micelle more ionizable (1, 124).

Poorer resolution of casein micelle on starch gel on heating of milk suggests aggregation of the casein particles which presented difficulty in separation. Increase in the colloidal calcium and phosphate (Table 10 and 23) on heating of milk could make the casein micelle less ionizable (1, 124) and hence a poorer resolution is expected. Destruction of k-casein band on heating can as well explain the reduction of sialic acid content of heated milk's casein micelle (Table 7) and changes in the sedimentable and soluble protein contents on ultracentrifugation (Table 30).

The effect of k- and \(\bar{\kappa}\) casein bands in case of sterilised milk acid casein (Fig.13) may be a reason for differential gel filtration pattern (Fig.16 D' and 17 D') in this case. Results on heated milk casein micelle regarding their electrophoretic separation support the
view that heating is capable of inducing molecular changes in the casein micelle.

Observations of chilled milk samples on starch gel (Fig. 14) gives the idea that even cooling can alter the structure of casein micelle. An increase in the sialic acid content of casein micelle from chilled milk (Table 8) can be explained on the basis of these results (Fig. 14). These observations (Fig. 14) further project the difference between micellar structure of the caseins from cow and buffalo milk.

It is known that presence of dissociating agents like urea, and 2-mercaptoethanol in the starch gel medium (120, 121) helps in the effective separation of protein components. The present results indicate that further addition of these agents (i.e. beyond normal concentration given by El-Hegazy (120) and Ganguli and Majumder (123)) do not further help in the better resolution of the casein moeity.

Better resolution of &s-component in colostrum casein (Fig. 15) as evidenced by an extra fastest moving band signifies the difference in colostrum casein to normal milk caseins. The significance of no appreciable difference in the k-casein fraction in the pattern has been discussed earlier (Chapter V, Page 187).

Further studies are however, need to find a satisfactory explanation for such an electrophoretic change.

Results on gel filtration pattern of micellar and acid caseins (Fig. 16 and 17) elicit the differences in the molecular size in the two types of caseins. Molecular
size of micellar caseins seems to be much higher compared to acid casein since the former filters out as a single peak against at least two clear peaks observed in case of latter (Fig. 16 and 17). It seems from the results, therefore, that micellar casein is excluded from the Sephadex G-100 gel bed because its molecular weight has been reported to be in order of millions (107, 1, 3) and hence filters out as a single peak. Faster filtration rate of buffalo micellar casein on Sephadex G-100 (Fig. 17 A) compared to cow micellar casein (Fig. 16 A) suggests that molecular weight of buffalo casein micelle is higher than that of cow. Similarly in case of acid casein higher molecular weight of components (or some component) of 1st peak seems to be higher in case of buffalo (as it filters out faster) compared to that of cow (Fig. 16 A and 18 A). The gel filtration pattern of acid caseins obtained are quite similar to those obtained by Nakanishi and Itoh (213). Yaguchi and Tarasuk (103) were, however, able to get at least three protein peaks on Sephadex gel filtration of acid caseins. Less number of peaks (only two) observed in our samples against the higher number of peaks (three) observed by Yaguchi and Tarasuk (103) could be attributed to lower gel height (43 cm) compared to the gel height used by these foreign workers. The results of these authors indicated that $\kappa$-casein is present only in first peak, since it consists of $\alpha$- $\delta$ and $\beta$-casein against $\alpha$- $\delta$ and $\alpha$-casein consisting the 2nd and 3rd peaks, respectively. Second peak in cow and buffalo filters out in the same
fraction (tube) but the difference was observed in the first peak (the only peak having k-casein). In the light of findings by Nakamichi and Itoh (213) and Yaguchi and Tarassuk (162, 104), it may be safely stated that difference in molecular size may be in k-casein fraction since first peak is a mixture of k-, \( \alpha \) s and \( \beta \) - and second peak has no k-casein fraction but only \( \alpha \) s and \( \beta \)-casein, which do not differ in cow and buffalo since second peak (\( \alpha \) s and \( \beta \)-casein peak) comes in the same tube (34th) as revealed by Fig.16 A" and 17 A".

Separation of heat treated milk's casein micelle (particularly in case of sterilized milk’s casein micelle) into two peaks against one in case of raw milk’s micellar casein (Fig.16 and 17) suggests that heating of milk can cause the molecular degradation of the casein micelle. No difference in the gel filtration pattern of heat treated milk (except that of sterilized milk) acid casein compared to that of raw milk, suggests that the difference in heating in the corresponding micellar fractions could be due to their colloidal calcium and phosphate content (Table 18 and 23). The increase in first peak and decrease in the second peak in case of sterilized milk’s acid casein (Fig.16 D" and 17 D") gives the idea that \( \alpha \) s and \( \beta \)-(or one of them) components exhibited heat induced aggregation and hence filtered out faster along with the first peak(213,227).

Differential gel filtration pattern of casein micelle obtained from chilled and unchilled milk (Fig.18) further suggests that like heating, even the cooling of milk can cause molecular changes in the casein micelle. Such a change on cooling may be explained on the basis of changes
on cooling may be explained on the basis of changes occurring in the casein micelle on cooling of milk such as release of $\beta$-casein (182, 19) and a decrease in the calcium and phosphate content (Table 19 and 24) of the casein micelle. The cold dissociation of the casein micelle may be suggested on the basis of these results. It seems that the dialysis of milk does not affect the molecular size of casein micelle as apparent from no change in the gel filtration pattern.

Studies with dissociating agents suggest further that the molecular size of the casein micelle or its micellar integrity is not challenged by its modification with dissociating agents like urea, potassium oxalate and 2-mercaptoethanol (in the levels tried) as revealed by no change in the gel filtration pattern before and after modification.

Studies on heat-denaturation pattern of whey proteins in the milk system with or without casein micelles (Table 40) clearly suggest that the casein micelle exerts the protective influence on whey proteins against heat denaturation and thus is able to stabilize them partially (Table 41, Fig.19). Differences in the extent of stabilization of whey proteins by casein micelle of cow and buffalo milk may be attributed to the differences in the composition of the casein micelle from two milks (296, 352). This contention, however, still remains to be examined.
The greater release of sialic acid as sialeopeptide from micellar casein than the corresponding acid caseins by rennet irrespective of breed and species of the animal. (Table 42 and 43) immediately gives an impression that casein in its native state appears to be more susceptible to rennet action probably because of the presence of more calcium and phosphate ions (Tables 18, 30 and 31) imbeded in its structure. The stimulation of the secondary phase of rennet action by calcium has been established through many reports (3, 290, 320, 164), present results reveal that possibly it stimulate the primary phase as well (This resulting in greater sialic acid release in micellar casein).

Such a contention is sponsored by the turbidimetric studies on the release of 125 TCA-soluble material by action of rennet on milk by Banerjee and Ganguli (406) where these authors recorded a stimulation by calcium. A greater release of sialic acid from cow acid casein was observed compared to corresponding buffalo acid caseins but the micellar casein from these two species show similar rates of sialic acid release (Table 42). The not difference between the rates of sialic acid release in micellar and acid casein is wider in case of buffalo compared to cow casein (Table 42). These results also indicate that calcium content of micellar caseins helped the buffalo samples to reach upto cow levels in micellar caseins. The caseins from different breeds of cow did not exhibit any difference in their susceptibility towards rennet as far as primary phase (glycotopeptide release) is concerned (Table 43).
In the light of higher content of calcium and phosphate in buffalo casein micelle over that of cow (Tables 18 and 20) and Sahiwal micelle over that of other breeds (Table 16 and 21) one would expect a higher rate of sialic acid release in these two cases compared to that of others. But the present investigation (showing similar rates) gives the idea that perhaps calcium stimulates the rennet action up to a certain level beyond which there is no effect. It may be suggested therefore that the minimum level of calcium was present in the casein micelle of all cases under study and hence no appreciable difference in glycopeptide release in different caseins was observed. Considering the absolute amount of sialic acid released, such difference between cow and buffalo casein samples, becomes more prominent since buffalo casein has less sialic acid than cow casein (Table 1). Gupta and Ganguli (1944) has also observed similar results on release of sialic acid from cow and buffalo acid caseins. Results on the sialic acid release using successive micellar casein fractions of different particle size (Table 44) indicates that although the larger micellar particle is poor in sialic acid (Table 3) maximum release of the sugar moiety was accomplished by rennet with this fraction. The trend of sialic acid release from k-casein fraction of micellar and acid caseins (of cow and buffalo milk) was similar to corresponding whole caseins except that buffalo micellar k-casein did not show any appreciable release as shown by its whole casein micelle source (Table 49). This may be due to the dissociation of calcium and phosphate in the
process of preparing k-casein from whole micellar casein during acidification (378). Results on sialopeptide release of k-casein from different breeds of cow (Table 48) further show that the breeds do not differ in their susceptibility towards primary phase of rennet action. It may be emphasized that k-casein in both forms exhibited lesser sialic acid release in case of buffalo compared to cow (Table 46). Ganguli and Bhatera (355) have reported that the α-fraction of cow casein and buffalo casein is 80% and 40% respectively and since the α-fraction of casein contains k-fraction and hence is involved in rennin action (3). One would reasonably expect a difference in the percent release of sialic acid firstly due to the difference in the composition of whole casein and secondly due to higher content of sialic acid in whole (Table 1) k-casein (Table 4) and successive micellar casein fractions (Table 3) in cow milk than in buffalo milk. The preferential release of sialic acid from cow micellar and acid k-caseins over buffalo k-caseins by rennet (Table 45) has revealed that the difficulties encountered in the preparation of cheese from buffalo milk (332,335,407) can probably be attributed to the differences in the release of glycopeptide (184).

No appreciable difference in the sialopeptide release from dialysed (Table 46), heat treated (Table 47) and cold treated (Table 48) milk reveals that the site of rennet action is not influenced by these treatments to milk. So far as primary phase is concerned, Pyne (237) has also
reported a similar phenomenon on heating of milk, though such a treatment (heat) may delay the clot formation by rennin (1). This report on the rennet susceptibility of casein micelle fractions of different particle sizes, dialysed, heated and cold milk casein micelle (as measured by sialopeptide release) is first of its kind.

For the first time, it was observed that colostrum casein show lesser sialic acid release than milk casein, the sialic acid release gradually increases till it attains a constant level after 5 days of parturition (Fig. 21). These results are just the reverse of sialic acid content of micellar and acid casein from post-partum milk (Fig. 2). The higher release in micellar caseins is maintained in all such cases compared to corresponding acid casein (Fig. 21). The result suggests two possibilities for such a phenomenon i.e. either sialic acid content in colostrum caseins is too high to be released by the used concentration of rennin or there may be some enzyme (rennin) inhibitory substances present in colostrum casein. However, these possibilities are subject to experimental verification.

It appears that the addition of dissociating agents like 2-mercaptoethanol, urea and potassium oxalate neither inhibited nor stimulated the primary phase of the enzyme action, at least in the concentrations tried (Table 49) irrespective of the source of casein micelle (cow or buffalo). Addition of various milk constituents salts also failed to change the course of enzyme action (Table 50). However, a slight increase in sialic acid release of acid caseins in
presence of extra calcium (and phosphate as well to some extent) confirms the role of these ions in boosting the rennet action. No appreciable change in sialopeptide release in micellar casein (Table 50) on addition of these ions again support the view that perhaps more calcium content than required (which is already imbeded in the casein micelle) does not help the action of rennet.

Studies on turbidity with different buffers reveal (Fig.22-24) that the optimum pH for rennet action with phosphate, citrate and maleate buffers is 5.7, 6.0 and 6.5, respectively. Since micellar casein in milk exists as calcium-caseinate phosphate complex (1-4) maleate buffer (pH 6.5) can be considered most suitable as it is non-interfering with the constituents of micellar casein. Tris although a non-interfering buffer could not be used for turbidimetric studies because of its higher pH range. No increase in turbidity with citrate buffer above pH 5.0 (Fig.22 c, 23 a and 24 a) could be due to removal of calcium by citrate (either citric acid or sodium citrate).

Higher rate of turbidity formation in all experiments with buffalo casein as compared to cow provides additional support that buffalo milk clots faster than cow milk (296, 352). The formation of turbidity is mainly dependent upon the secondary phase (3) in rennet action involving Ca++ ion concentration of the medium (1,3). One probably can explain the faster formation of turbidity in buffalo micellar casein due to the presence of more calcium in it (Table 15). Since Sahiwal casein is showing faster turbidity than
Tharparkar, it may be due to the higher calcium content of former (Table 10). Increase in turbidity (Fig.25 and 26) is compatible with increase in rennet level (104). The subsequent decrease in turbidity with rennet (Fig.26) in case of buffalo can be explained on the basis of faster precipitation rate of casein.

From studies on effect of storage period (Fig.27) it appears that phosphate engulfs all calcium of micellar casein gradually and thus inhibits turbidity formation which is not apparent with Tris buffer. A sudden rise in turbidity in case of Tris at the end of 12 hours, leads to the concept that perhaps prolonged content of casein micelle in that buffer destabilizes the system and thereby ultimately causes precipitation of the casein.

It is suggested that this turbidimetric method can become a good tool to evaluate the complexity of structure of casein micelle. However, the source of casein (cow, buffalo or any other milk animal), nature and pH of the solvent (buffer) and the storage period of the substrate (the micellar casein solution) should be always kept in mind in adopting the described turbidimetric method of rennet assay.

The superiority of buffalo and Sahiwal casein micelle over that of cow and other two breeds (Tharparkar and Red Sindhi) regarding rate of turbidity formation is further established through studies with maleate buffer pH 6.8 (Fig.26). The rate of enzyme (rennet action was also observed to be dependent on substrate (casein) concentration
by the cheese technologist. Although milk coagulation
times have been used as an index of rennin activity (318),
a turbidimetric procedure would seem more reliable as the
developed turbidity can be measured optically compared to
the visual clotting time procedure. The results on the
turbidity formation with whole micellar casein from buffalo
milk, however, cannot be correlated with its sialic acid
release if one wants to compare such results with cow milk
casein (because turbidity development is much faster in
buffalo whereas the extent of sialic acid release is more
or less the same in casein micelle of the two species). On
the basis of these results it may be pointed out that a
true picture on the action of rennet on casein can be
elicited only on the basis of the sialic acid release from
casein and not on the turbidity formation of milk clotting
property of rennet. The importance of turbidity production
as a measure of secondary phase of rennet action in a cheese
vat, however, cannot be ignored.

A much higher rate of turbidity production in k-casein
without addition of calcium, establishes that the rennet
acts upon k-casein fraction only (3). Such turbidity
development was observed in both micellar and acid k-casein,
whereas Gupta and Ganguli (164) had shown earlier that acid
caseins failed to produce turbidity when acted upon by
rennet. It is obvious that turbidity production in k-casein
(Fig.31) is due to gradual insolubilization of para-k-
casein due to release of glycopeptide from the k-casein
moiety through rennet action (3). The observations of
Gupta and Ganguli (164) on whole acid caseins can, therefore, be explained due to a much less content of k-casein in whole casein (2, 48, 161) and since enzyme action is dependent upon substrate (k-casein) concentration (164, 319, 320) a low or apparently no-turbidity development in acid caseins is expected. One might wonder therefore on the rennet dependent turbidity production in whole micellar casein where the k-casein content should be even less because of lesser salic acid content compared to acid casein. But obviously the presence of bound calcium in micellar casein and its absence in acid casein (Table 18) due to dissociation on acidification (1, 46, 67) can very well explain the differential turbidimetric behaviour of whole micellar and acid casein towards rennet since the calcium ion is known to be associated with faster rennet action (1, 3).

A much lower rate of turbidity production in buffalo k-casein (Fig. 31) immediately gives the impression that perhaps the rennet acts at a very slow rate on buffalo casein, but the faster formation of clot rules out such impression. On the basis of the present investigation, it can be added that the higher calcium of buffalo milk (352) stimulates the secondary phase of rennet action but infact the rennet action is slower in buffalo milk. This sort of a confusing phenomenon in buffalo milk is the most probable factor responsible for the difficulties encountered in the preparation of cheese from buffalo milk (395, 407). Since there is a decrease in the turbidity development by rennet
action on micellar casein obtained from dialysed milk (dialysis was carried out in cold, Fig. 32) and chilled milk (Fig. 33), it appears likely that some alteration in the micellar structure has happened, during the chilling process. This probably can happen through the phenomena explained below. If the micelle is deprived of its calcium through the chilling process (Table 19) there could be a reduction in the rate of rennet dependent turbidity development due to calcium deficiency in the micelle. There can be as well a possibility of a loss in some of the protein fractions (Table 39) comprising the casein micelle which might be essential for turbidity development. A marking of the site of rennet action in the casein micelle induced through chilling procedure can also be speculated as a possible cause for the observed alteration in the rate of turbidity development by rennet.

It appears that dissociating agents like urea and 2-mercaptoethanol stimulate the rennet action (as evidenced by higher rate of turbidity production) on the casein micelle (Fig. 34) probably through cleavage of S-S (disulphide) linkage in the casein micelle. However, potassium oxalate appeared to be ineffective in this regard (Fig. 34). Such a influence of dissociating agents is however limited to secondary phase of rennet action only since sialic acid release which is the measure of primary phase (5) is not affected (Table 40).

A dramatic increase caused in the rate of turbidity development (even leading to precipitation in some cases)
by the addition of calcium in the casein micelle solution (Fig. 35) confirms the earlier speculation that calcium has a great role to play in the stimulation of rennet action irrespective of breed of species of the animal. The importance of calcium in rennet dependent turbidimetric studies has also been emphasized by Ashworth (319, 320). Addition of citrate on the other hand completely inhibited the turbidity formation by rennet in the casein (Fig. 35). This may be due to the binding of calcium by citrate (399) and hence due to non-availability of calcium, the formation of insoluble calcium para-caseinate may be inhibited. Since the release of sialic acid is not affected by the presence of citrate in the system (Table 50) the observations on turbidity gives scope to predict that primary phase of the rennet action can be separated from the secondary phase by way of blocking the latter with the help of citrate. Since these two phases of rennet are known to start simultaneously (3) the separation of primary phase through citrate (as proposed above) would facilitate a better study of this phase since it is known to be the main enzymatic phase of the rennet action (3). The inhibition of turbidity by citrate (Fig. 35) explains the formation of no turbidity production in the casein micelle solution when citrate buffer of pH above 5.0 was used earlier (Fig. 22 c, 23 c and 24 c) and hence the earlier hypothesis stands verified.

The role of phosphate, however, in the rennet action could not be understood clearly since it showed both increase and decrease in the rate of turbidity production in some
samples (Fig. 35). It can be speculated from these observations that perhaps phosphate stimulates the rennet action up to certain concentration beyond which it may cause inhibitory action. In the light of this interpretation, therefore, it may be stated that the original phosphate content imbeded in the casein micelle may be variable from sample to sample and hence may cause such a variable effect. This hypothesis, however, needs to be tested.

The above discussion suggests the importance of ionic constituents of casein micelle in determining the rate of turbidity development by rennet in different breeds of cow and buffalo. The variations observed can hence be safely attributed to the variations in the ionic constituents of the casein micelle from the milk of these different species or breeds of animal. It also justifies the use of maleate butter for turbidimetric studies since none of its components corresponds with the constituents of casein micelle.

The addition of various milk constituents salts (Table 51) failed to cause an alteration in the rate of turbidity development by rennet irrespective of the source of casein micelle (i.e. cow or buffalo). These results suggests that only the constituents of casein micelle (and not other milk constituents) interfere with the enzyme (rennin) action.

It is tempting to conclude on the basis of our observations that the modification of rennet by treating
with dissociating agents like 2-mercaptoethanol and urea
did not challenge the activity of rennin. But modification
of rennet by heat inactivated the enzyme completely. Since
such a heat inactivation is a phenomenon usual with the
enzymes. A higher rate of turbidity production in casein
micelles by the action of trypsin compared to that of rennin
(Fig.36) is natural because of the higher proteolytic
activity of the former since turbidity production is
essentially a slow degradation process (3). The results
(Fig.36) further suggests that trypsin is non-specific in
its action towards casein since it continued showing its
normal action even when added in rennetted casein or on
addition of rennin during its action. Formation of turbidity
by trypsin even in acid casein (Fig.37) without addition of
calcium unlike rennin (104) suggests that turbidity
formation in acid casein may be used as a tool to
differentiate between the two enzymes.

A higher rate of turbidity production in buffalo
micellar and acid caseins initially compared to that of
cow using trypsin (Fig.36 and 37) suggests a higher rate
of proteolysis of buffalo casein (compared to cow ones) in
the initial stages of protein degradation. But longer
time (18 to 20 hours) taken in buffalo casein for decrease
in turbidity and ultimate precipitation compared to the cow
samples (7 to 9 hours) suggests slowing down of the rate
of proteolysis in case of buffalo in the later stages, and
hence show a ultimate faster rate of proteolysis of cow
casein than buffalo caseins which is in agreement with the
observations of Ganguli et al. (359, 355, 371) who used various proteolytic enzymes. The development of turbidity by rennet in ultracentrifugal milk serum fractions collected at different speeds (Fig. 38) is obviously due to the presence of soluble casein in it (Table 35 and 36) which acted as a substrate for rennet action (3, 164). It is also known that with decrease in the substrate concentration (Fig. 38) the rate of turbidity development by rennet decreases (164). Thereby the decrease in the rate of turbidity in supernatant fractions obtained at higher speeds is quite expected because of the greater removal of casein micelle (Table 34).

A lower rate of turbidity production in buffalo supernatants fortifies the earlier findings (Fig. 8) that buffalo casein micelles settle at a much lower speed (50,000 x g) compared to cow casein micelles (105,000 x g). A constant rate in buffalo supernatants at higher speeds (Fig. 38 and 39) indicates complete absence of casein micelle and the slight turbidity production was due to small amount of soluble casein present in it (Table 38). A higher rate of turbidity increase in serum fractions obtained at 105,000 x g in cow milk compared to buffalo counterpart fractions confirms the earlier findings (Table 38), that cow milk has a much higher soluble casein content than buffalo milk. A linear relationship between turbidity values in different ultracentrifugal milk serum fractions (Fig. 39) further directs towards the direct dependence of turbidity production on substrate (casein) concentration (164).
A similar capacity of k-casein from micellar and acid casein source (Fig. 40 A to H) to stabilize \(\kappa\)-casein against calcium precipitation suggests that stabilizing power of k-casein is not associated with its sialic acid content since micellar and acid k-casein were observed to differ in their sialic acid contents (Table 4). Therefore, the contention of Marier et al. (181) of association of sialic acid content with stabilizing ability of k-casein does not seem to hold good. The present findings (Fig. 40 A to H) in this regard gains additional support from the observations by Joshi and Ganguli (82) that the presence of proteose-peptone, a sialic acid rich protein fraction (181) did not help in the stabilisation of \(\kappa\)-casein by k-casein against calcium precipitation. This is further supported by findings of Thompson and Pepper (318) that stabilizing ability of k-casein towards \(\kappa\)-casein was not destroyed even after its treatment with neuraminidase. Several other reports (84, 48) also verify the view that sialic acid has no association with the stabilisation capacity of k-casein. A greater ability of cow k-casein (micellar or acid) as revealed by Fig. 40 (A, C, E, G) to stabilize \(\kappa\)-casein compared to buffalo k-casein (Fig. 40 B, D, F, H) apart from indicating the differences in chemical make up of k-casein from the two species also gives the impression that the relative concentration of k-casein in buffalo is higher than cow taking \(\kappa/\kappa\) ratio as the possible index.

A much higher \(k/\kappa\) ratio (0.20 in case of cow and 0.233 in case of buffalo) was needed to solubilise
equivalent amount of $\alpha_s$-casein under the present conditions against a much lower ratio (0.10) suggested by Zittle (49). This may be explained in the light of observations by Joshi and Ganguli (52), Carrel and Thompson (19) and Thompson et al. (53) that stabilizing ability of $k$-casein is also dependent upon the genetic variants of $k$-casein and $\alpha_s$-casein respectively. The dehydration of the $k$-casein used, by acetone and ether, against the same through lyophilization by Zittle (49, 378) may also necessitate a higher $k/\alpha_s$ ratio to achieve similar solubilization of $\alpha_s$-casein. Such a variation due to breed and season also cannot be ruled out. Moreover, using $k/\alpha_s$ ratio (0.10) of Zittle (49) El-Nagawy (51) and Joshi and Ganguli (52) could achieve only about 82% and 30% stabilization of $\alpha_s$-casein respectively against 98% achieved by Zittle (49). The purity of $k$-casein and $\alpha_s$-casein could still be other factors causing variation. Therefore, the present deviation of $k/\alpha_s$ ratio from that of Zittle (49) may be due to varied reasons which is difficult to explain at present.

The results (Table 52) further indicate that these $\alpha_s$- $k$-casein stabilized systems were also susceptible to rennet like normal caseins.

Analysis of glycomacropeptide (GMP) released by action of rennet on micellar and acid $k$-casein (Table 53) suggests that the two forms of $k$-casein may differ in rate of GMP release by rennet (Table 42 and 43) but the released material (GMP) from the two forms of $k$-casein does not differ in its composition. The value for nitrogen content
(Table 55) observed in cow GMP concurred very well with those reported in literature (39, 34, 278, 337), but the values for sialic acid content were much less compared to those cited in literature (29, 34, 278, 333). This lower value of sialic acid in cow GMP could be attributed to the difference in the method used for isolation of GMP. The method employed was precipitation of GMP with the help of phospho-tungstic acid from 12% TCA soluble material obtained after rennet action (337) against preparation of GMP by lyophilization of the same 12% TCA soluble material used by other workers. Differences in the chemical composition of GMP from cow and buffalo (Table 53) further enlighten the differences in casein structure of the two species.

A lower sialic acid content in buffalo casein per unit weight (Table 1) may be explained on the basis of lower sialic acid in GMP attached to the k-casein molecule in this species compared to that of cow. A lower total yield of GMP in case of buffalo than cow from the equal amounts of k-casein further offers an explanation for the lower sialic acid content in buffalo casein (Table 1) since all the sialic acid of casein is known to be attached to the glycomacropeptide of the k-casein molecule (3, 34, 289).