The results presented in Table 1 reveal that buffalo milk possess significantly higher amounts of total nitrogen. This was in conformity with the results reported by Ismail et al (18) and Tandon and Ganguli (20). There was very little difference between the non-protein nitrogen content of buffalo and cow milk. On heat treatments, buffalo and cow milk non-protein nitrogen was not observed to be significantly different from that of raw milk samples. The results of Venkatappaih and Basu (17) and Ismail et al (18) are in agreement with the results reported in Table 1 for non-protein nitrogen, even in the case of UHT treatment Lyster et al (19) could not observe any change in the non-protein nitrogen content in the case of cow milk, Tandon and Ganguli (20) reported marginal increase in the non-protein nitrogen, when milk was subjected to graded heat sterilization. This may be due to high heat treatment given to milk during sterilization.

Non-casein nitrogen was higher in raw buffalo milk than that of cow milk. This is again in agreement with the findings of Ismail et al (18) and Tandon and Ganguli (20). On heat treatments the non-casein nitrogen decreased considerably. Reduction in the non-casein nitrogen was greater in the case of cow milk whey, the percentage of non-casein protein nitrogen was calculated
in terms of the non-casein nitrogen content. This decrease in the non-casein nitrogen content could be attributed to the fact that as a result of the heat treatments, the serum proteins get aggregated or structural changes take place, resulting in the co-precipitation of these serum proteins along with casein, when the pH heat treated milk is brought down to 4.6.

Heat treatment contributed to an increase in the casein nitrogen. Here again the casein nitrogen increased to greater amounts in cow milk than buffalo milk. Total albumin decreased in both buffalo and cow milk as a result of heat treatments. Total albumin of buffalo milk decreased the maximum, 84.3 per cent decrease at 95°C and 85 per cent at 100°C. Whereas the values for cow milk total albumin the decrease in total albumin nitrogen was 78.4 and 83.5 per cent. In agreement with the findings of Ismail et al. (18) and Tandon and Ganguli (20) it was observed that cow milk contained higher amount of β-lactoglobulin nitrogen. Considerable decrease in the β-lactoglobulin nitrogen resulted as a result of heat treatments. Between buffalo and cow milk β-lactoglobulin 88.7 per cent and 86 per cent of the β-lactoglobulin nitrogen got reduced. As reported by Gupta et al. (20b) buffalo milk contained lesser amount of proteose-peptone nitrogen. On heat treatment the proteose-peptone nitrogen showed decrease at both heating conditions in the case of buffalo milk. At the same time though heat treatment at
95°C decreased the proteose-peptone nitrogen. Heat treatment at 100°C for 10 minutes caused an increase in that fraction of milk proteins. The decrease in the proteose-peptone nitrogen content was in agreement with the reports of Lyster et al (19), Tandon and Ganguli (20) and Gupta et al (20b). However, Ismail et al (18) reported considerable increase in the proteose-peptone nitrogen when milk was heated to sterilizing temperatures.

Between buffalo and cow milk, residual albumin underwent greater decrease in cow milk than in buffalo milk. Globulin nitrogen was lower in cow milk. Globulin nitrogen in buffalo milk appeared to more resistant to heat treatment than cow milk.

Studies on the effect of various heat treatments on the non-protein nitrogen content of milk (Table 12A and 12B) did not show any significant difference in the non-protein nitrogen content of milk. It is now clear that heating milk for 10 minutes at lower temperatures are not sufficient to induce any increase in the non-protein nitrogen contents whereas sterilization conditions could bring about the increase in the non-protein nitrogen content.

The data relating to the rennet clotting time of buffalo milk is of great importance, since it differs from that of bovine milk. As reported earlier (25, 26) buffalo milk is found to clot faster than cow milk by the addition of rennet. This phenomenon could be
explained when the difference in the casein make up of the bovine casein, and ionic calcium which is very much required for the formation of the coagulum. Therefore it is noteworthy that these facts are to be given due consideration while discussing the difference in the clotting time of the milk of both species.

Heat treatments above 50°C, progressively increased the rennet clotting time of milk of both the species. However, there remained appreciable difference in the clotting of buffalo and cow milk when heated to 100°C/10 min. Heat treatments could induce a number of changes in the milk whereby the clotting time could be prolonged. Heat treatments could influence the mineral balance, whereby the rennet clotting time could be altered.

In addition to the shift in the mineral equilibrium of the milk salts, there occurs, interaction between casein components and β-lactoglobulin. Such an interaction could considerably be of very much importance as far as the coagulation of milk is concerned. It has been very clearly shown by Kannan and Jenness (29) that the interaction between β-lactoglobulin and caseinate micelles might make the coagulability more critically dependent on the concentration of colloidal phosphate. It is noteworthy that, it is not only the composition of the caseins micelle is important but also, the constitution of the surrounding medium is of important as far as the rennet clotting time of milk. Further a
a higher content of $\beta$-lactoglobulin might also contribute to the prolongation of rennet clotting time after heat treatments.

The impact of the surrounding medium in relation the clotting time on heat treatment could well be studied from Tables 14A and 14B. When buffalo casein micelle was dispersed in cow ultracentrifugal whey, and vice versa, there was considerable difference in the clotting time of both the systems from that of the original milk from which these systems were devised. The clotting time of buffalo micelles were prolonged even in the unheated condition in the new surrounding. Since the serum of both buffalo and cow milk differ in terms of soluble casein, $\beta$-lactoglobulin and the calcium ion content it is understandable that these facts might have contributed to the longer periods of clotting time. By interchanging the micelles, it was observed that, the rennin clotting time of cow casein micelles were very much decreased than when they were in the original milk. However, this interchanging of the casein in the milk serum resulted in rennin clotting time falling in between that of the original milk samples from which these were derived.

Ultracentrifugal sedimentation was used as one of the criteria to study the heat induced changes of milk proteins. Although ultracentrifugal sedimentation of milk proteins at a particular force of gravity may not yield any valid information regarding the sedimentation
behaviour of milk proteins, use had been made on the differential ultracentrifugation, studies. Sabarwal and Ganguli (44) have clearly established the difference in the ultracentrifugal sedimentation of buffalo and cow milk. Their observation that buffalo milk contains much less soluble nitrogen is confirmed by the findings in these studies. A critical analysis of the Table 15 reveals the fact that at 11,739 x g unheated buffalo milk contained less supernatant nitrogen than cow milk. On heat treatment at both the heating conditions, in the case of buffalo milk about 5 per cent decrease in the supernatant was observed. Whereas at identical conditions the decrease in the supernatant nitrogen was 2.2 per cent and 4.2 per cent at 95°C and 100°C for 10 minutes in the case of cow milk. When centrifugal force used for sedimentation was increased, the supernatant nitrogen in unheated milk decreased. In the case of heat treated milk, also the supernatant nitrogen was decreased. The difference between buffalo and cow ultracentrifugal whey obtained at 46,956 x g was much less than at 11,739 x g. In this case also the reduction in the supernatants were 5.5, and 7 per cent of the total ultracentrifugal whey in case of buffalo milk heated at 95°C and 100°C for 10 minutes. At the same time, the reduction in the case of cow milk was only 4.8 and 5.7 per cent. It follows from the fact that heat treatment of milk at 95°C and 100°C for 10 minutes did not alter the reduction in the
supernatant nitrogen of buffalo and cow milk at these two forces of sedimentation. The reduction in the supernatant nitrogen between the heat treatments was not appreciably different. This means that heat treatments at 100°C could appreciably increase the molecular weights of the serum proteins so that these proteins could also be centrifuged down with casein.

Centrifugal force of 105,000 x g was sufficient to settle down all the colloidal proteins of buffalo and cow milk. These difference, between cow and buffalo supernatants was evidenced by the fact that buffalo milk contained less amount of soluble casein than cow milk. The supernatant nitrogen of the heat treated milk samples at 95°C/10 min. showed considerable difference from that obtained from unheated milk. In the case of buffalo milk the decrease in the supernatant nitrogen was about 17 per cent, while that of cow milk supernatant, the reduction was only 7 per cent. Heating at 100°C caused a further decrease in the non-sedimentable nitrogen. It was reported (40) that heating cow milk to 242°F for 2 minutes removed about 52 per cent of the whey protein was.

The findings reported in Table 15 are in agreement with that of Sullivan et al. (47), that the supernatant nitrogen in milk decreased immediately after heating. Nakai et al. (48) also reported that the non-sedimentable nitrogen and non-casein nitrogen decreased, as a result of heat treatment of cow skim milk. While these reports
showed that heat treatment of milk resulted in a decrease of the non-sedimentable nitrogen. Morr (51) did not observe any particle size distribution in heat treated milk from that of unheated skim milk. This difference may be attributed to the fact that on storing the heat treated milk before centrifuging may lead to a number of changes whereby the effect of heat treatment on the non-sedimentable nitrogen of milk is not observable.

Since heat treatments of milk brings about changes in the serum proteins of milk, it is of great interest to know how far these changes result from heat treatment. Tables 16A and 16B show the impact of heat treatment on the serum proteins of buffalo and cow milk. One of the methods of studying such changes is the determination of the non-casein protein nitrogen after heat treatment. It is very clear that increase in temperature of heating resulted in the progressive decrease in the non-casein protein nitrogen. Further such a study revealed the fact that the whey proteins of buffalo milk are more vulnerable to heat treatment than cow milk whey proteins.

Another criteria used to assess the heat induced changes on the milk proteins is to determine the particle size distribution. Buffalo and cow milk when subjected to gel filtration on Sephadex G-100 resolved into three protein peaks, corresponding to casein, and the whey proteins. Although qualitatively there was no difference between buffalo and cow milk gel filtration patterns,
there existed quantitative difference as far as peak areas were concerned. The Sephadex gel filtration patterns of unheated buffalo and cow milk were (Fig. 2) in agreement with that reported by Majumder and Ganguli (64). Since the effluents were monitored at 253.7 nm, the gel filtration patterns showed in addition to the three protein bands of raw milk one more band. This component of milk, which has very high absorption of UV at 253.7 nm was a dialysable constituent (Peak IV).

Heating at 70°C for 10 minutes caused a change in the filtration patterns. The peaks due to the whey proteins were transformed into a single peak of small area. At the same time, at the position of the casein peak, a broad peak also appeared. This sort of gel filtration patterns suggest that heating milk at 70°C for 10 minutes induces certain changes on milk proteins in terms of molecular weight. Heat treatment at 80°C for 10 minutes showed, that the changes were not complete. But the whey protein peaks merged as one peak, which was still reduced. Filtration pattern at 90°C for 10 minutes showed that the molecular particle size and thereby the molecular weight, transformation was almost complete. Gel filtration patterns of heat treated skim milk at 95°C and 100°C for 10 minutes revealed that, the proteins resolved into a major and minor band. The minor band can be attributed to the whey proteins which did not undergo any molecular transformations either in terms of aggregation.
or interactions with the major protein-casein. The shifts in the peak volume and finally, merging with the casein peak is a clear indication that the molecular weight of these whey proteins which are approximately 18,000 in the unheated condition increases to about 10 times as a result of heat treatment. The results obtained with heat treated milk are in agreement with that reported by Mörr et al. (64) for bovine, heat treated skim milk. Further the results indicated that these changes were rapid in the case of buffalo milk than bovine milk. This indicates that buffalo milk proteins are more vulnerable to heat treatment than cow milk.

Differential ultracentrifugal whey from heat treated skim milk (Fig. 3, 4 and 5) presents certain interesting aspects. Supernatants obtained at 105,000 x g and 46,936 x g filtration patterns on Sephadex G-100 was almost identical. The protein peak corresponding to the position of casein was very similar to that of casein from unheated milk sample. This was also true in the case of supernatants obtained at 11,739 x g except that the peak corresponding to the whey proteins was larger in area. These findings point out that in heated milk there remained protein components, whose molecular weight (sieving properties) was very near to that of casein.

The question whether casein micelles when dispersed in distilled water or buffer (phosphate pH 6.8) on heat
treatments at 95°C and 100°C for 10 minutes give rise to any components has been investigated. An examination of the results presented in Table 19 shows that when nitrogen fractionation of the casein micellar dispersions are carried out (10), the nitrogen content of the various fractions are negligible. Whatever minor nitrogen fractions present may be from the milk serum which could not be completely removed from the sediment. Further, none of the protein fractions showed increase as a result of heat treatments. Hence it is established that when micellar casein dispersions were subjected to heat treatments at 95°C and 100°C for 10 minutes, no degradation of the casein micelles took place.

No significant difference in the sialic acid release by remains could be observed as a result of heat treatment of the casein micelle solution. This was true in the case of casein micelles settling at 105,000 x g, 48,956 x g and 11,739 x g (Tables 20A to 22B). These results indicate that heat treatments did not effect the sialic acid moiety. Further in the absence of other reactive groups, heat treatments did not cause any structural changes on casein micelles, whereby the release of sialic acid could be facilitated or hampered. The effect of heat treatment on acid casein was also the same as far as acid casein from both the species in relation to heat treatments.
From Table 244, it is clear that heat treatment of $\alpha$-casein obtained from buffalo milk released around 4 per cent more of sialic acid than the unheated sample. This may imply that heat treatments may have caused disaggregation of the $\alpha$-casein.

$K$-casein in solution on heat treatments, did not show significant changes in the release of sialic acid (Table 25A and 25B). This would mean that the methionine-phenylalaine peptide bond susceptible to rennin cleavage was not marked by heat treatment and thereby did not alter the rate of release of sialopeptide by rennin.

Observations of Zittle (71) on the viscosity of acid casein solution, heat treated in presence of calcium chloride, led to the conclusion that the increase in viscosity was due to the aggregation of casein.

Buffalo micellar casein obtained at 105,000 x g and dispersed in maleate buffer pH 6.8 on heat treatment in presence of added calcium chloride showed turbidity. This was in agreement with the results reported by Zittle (72) for acid casein. From an examination of the Fig. 6, it is evident that the turbidity increased as the calcium chloride concentration and temperature increased. The formation turbidity with increase in calcium chloride solution could be treated as an interaction or association of the protein with calcium chloride, new species of calcium casein aggregates being formed. It can also be concluded that heating micellar
casein in presence of calcium chloride favours the interaction of casein micelles and calcium resulting in the casein aggregates. From Fig. 6, it is evident that maleate buffer of pH 6.8, the aggregation of micellar casein in presence of calcium chloride followed the first order reaction kinetics. It is also noteworthy that, micellar casein, in addition to the calcium existing with it, could attach or interact with large amounts of calcium, and that this phenomenon requires energy of activation. Between buffalo and cow, casein micelles aggregated more than the cow casein micelles.

On the other hand, it was observed that, heating casein micelles solution up to 90°C and addition of calcium chloride resulted in turbidity which was not different from that obtained from that to which calcium chloride was added and heat treated. However, such turbid solution at high temperature showed reversal of the phenomenon, as the temperature of the solution was lowered. Although this is in agreement with the findings of Zittle (72) for acid casein, the reversal phenomenon in the case of micellar casein was not complete, even smaller amounts of calcium chloride solutions were added.

The reversal of aggregation could take place, when all the calcium chloride bound to the casein molecules were dissociated and removed. This incomplete reversal phenomenon would mean that, the calcium chloride casein boundage is quite strong, and that by cooling it could not
be dissociated.

From Fig. 7, 8 and 9, it can be observed that pH of the buffer solution did not affect heat induced aggregation of acid casein from buffalo and cow milk, and its reversal phenomenon. Aggregation of acid casein and its reversal phenomenon was in conformity with the results reported by Zittle (72). Comparing the turbidity (optical density at 660 nm) and temperature of heating, curve of acid casein and micellar casein, it was observed that there was no linearity in the turbidity formation in the case of acid casein. This may also lead to the conclusion that the casein aggregation in the case of acid casein did not follow the first order reaction kinetics. However, the maximum turbidity attained at 90°C for the same amount of calcium chloride did not differ in the case of buffalo and cow for acid and micellar casein. It may also be found that the phenomenon of reversal of aggregation was not same in micellar and acid casein samples. In the case of acid casein, the reversal was complete at 25°C. It may inferred from this phenomenon that calcium chloride which was associated with the protein at high temperatures, could no longer remain attached with it at lower temperatures. This shift in the attachment of calcium to the protein may be attributed as a criteria for the reversal phenomenon. It is also evident that the pH of the solution in the
range of 6.4 - 6.8 had no effect on the heat induced aggregation and its reversal phenomenon.

The heat induced aggregation of \( \alpha_s \)-casein, showed some interesting features. There was slight difference in the rate of aggregation of \( \alpha_s \)-casein at low pH (6.4) from that of pH 6.6 or pH 6.8 maleate buffer. A critical examination of the Fig. 10, 11 and 12, will reveal that pH had some effect in the aggregation when higher amounts of calcium chloride solutions were used, the reversal phenomenon was not demonstrated. Rate of aggregation and disaggregation at pH 6.8 is of great importance. Since \( \alpha_s \)-casein is sensitive to calcium ions, at higher concentrations of precipitation of the protein took place. The work of Krescheck et al. (73) pointed to the fact that the complex of \( \alpha \)-casein dissociated at elevated temperatures, which was followed by aggregation.

When k-casein in maleate buffer 6.8 was used, for the aggregation studies, (Fig.13) it was revealed that, aggregation of the protein to a limited extent took place only at higher concentrations of calcium chloride and higher temperatures. Although k-casein is not sensitive to calcium ions, the low turbidity formed may be due to the presence of \( s \) in preparation as contaminant.

At pH 6.8, the turbidity formation at low calcium chloride concentration was not pronounced in the case of
\( \beta \)-casein (Fig. 14). However, at higher calcium chloride concentrations and temperatures, aggregation of \( \beta \)-casein took place. This rate of aggregation or disaggregation did not follow the first order reaction kinetics. This may be due to the fact that at elevated temperatures, (90°C) dissociation and further aggregation did not take place unlike \( \alpha \)-casein.

Studies on the rennet action on the casein micelles of buffalo and cow revealed that the rennet action measured in terms of the turbidity formation was different with micelles of different particle size. It also revealed the difference between unheated buffalo and cow casein micelles obtained at different gravitational forces. From Fig. 15 it is clear that, the casein micelles obtained at different gravitational forces differed considerably. Thus casein micelles obtained at 11,739 x g formed higher rate of turbidity. Next in the order was casein micelles obtained at 46,945 x g and the least from the casein micelles obtained at 105,000 x g. One probable explanation for this can be the presence of more calcium in micelles settling at low gravity.

Between buffalo and cow the difference in the turbidity formation can also be attributed to the presence of greater amounts of calcium in the buffalo casein micelle. Heat treatments at 95°C and 100°C for 10 minutes did not cause any increase in the turbidity and thereby higher rennet action. This may be due to the fact that
these heat treatments could not mask the peptide bond susceptible to cleavage by rennin, and thereby did not affect the rennet action.

Slight higher turbidity in all heated samples can be attributed to the fact that micellar casein solutions on heat treatment became turbid.

Paper strip electrophoresis of micellar casein obtained at 105,000 x g and heat treated showed difference (Table 26) in the relative proportions of the different fractions. Buffalo and cow unheated micellar casein showed difference. While buffalo micellar casein possessed (Fig. 16 and 17) relatively higher β-casein, in cow micellar casein α-casein fraction was predominant. Heat treatments caused changes in the α-casein fraction. This may be in agreement with the results reported by Krescheck et al (73) that α-casein dissociated at elevated temperatures. Starch gel electrophoretic patterns, (Fig. 18) did not show in great detail any changes as a result of heat treatment. It may be inferred from that heat treatments did not show any drastic changes.

Acid casein samples from buffalo and cow milk showed on paper strip electrophoresis, relatively higher proportions of α- in the case of cow and β- in the case of buffalo. From Fig. 19 and 20 it is evident that heat treatments caused slight alteration in the electrophoretic patterns. Starch gel electrophoresis (Fig. 21) could not reveal any difference in the case of
unheated and heat treated acid casein samples. Results from these experiments clearly indicate, that heat treatments at 95°C and 100°C for 10 minutes could not induce any change on casein. Alternatively, casein samples are very resistant to changes at these heating conditions.

In similar manner, studies on fractionated caseins, \( \alpha_s \) and \( \beta \), and \( k \)-casein, on paper strip and starch gel electrophoresis did not reveal any change in the electrophoretic pattern. This again indicates that casein fractions are also very resistant to heat induced changes, when heating conditions used were only 95°C and 100°C for 10 minutes.

Another possible change that could occur as a result of heat treatment is the change in molecular weight. Buffalo and cow acid casein and micellar casein samples, dissolved in phosphate buffer pH 6.4, 6.6 and 6.8 on heat treatment at 95°C and 100°C for 10 minutes resolved only into a single peak, thereby indicating that heat treatments could not induce any change in the molecular weight. This is expected since the conditions employed for the elution was not sufficient even to fractionate the whole casein on Sephadex G-100. Since all the chromatographic resolutions, carried out, were at room temperatures, the samples were in the polymeric state. As a result, changes in molecular weight if any could not be observed.
The action of dissociating agent like 2-mercaptoethanol was studied to gain information as to how heat treatment brings about changes on acid, micellar and k-casein samples. Figs. 22, 23, 24, 25, 26, 27, 28, 29 and 30 reveal the molecular sieving properties of micellar casein, acid casein and k-casein subjected to heat treatments and the effect of dissociating agent thereafter. Although all the unheated samples resolved into two fractions on treatment with 2-mercaptoethanol, the heat treatments and dissociating agent did not reveal any qualitative or quantitative change in the gel filtration patterns on Sephadex G-100.

Earlier reports (66, 67 and 68) have indicated that heat treatments brings about the cleavage of the serine-phosphorus linkage. According to Belloc and Jenness (67), the liberation of phosphorus in the form of inorganic phosphorus, followed the first order reaction. Table 28 reveals data on the determination of the dephosphorization of casein and its fractions subjected to heat treatment. The greatest percent release was from -casein. The release of phosphorus soluble in 12 per cent TCA observed was much less than what has been reported in literature (66, 67, 68 and 69). This may be due to the fact that heat treatments employed were much more severe than what has been used in the present study.
Of the various proteins of milk the serum proteins are most vulnerable to heat treatment. Isolation of these proteins and heat treatment would furnish information as to how heat induces changes in these proteins. The results of the analysis of the distribution of nitrogen in the ultracentrifugal whey obtained at 105,000 x g are presented in Table 29. Total nitrogen in buffalo milk was higher than in cow milk UCW. Heat treatments did not cause any change in total nitrogen. This is expected since heating at the temperatures studied do not induce the formation of any volatile nitrogenous compounds. As in the case of whole milk samples buffalo milk contained more of non-protein nitrogen than cow milk. Heat treatments did not induce the formation of non-protein nitrogen substances.

Non-casein nitrogen content was higher in the case of buffalo UCW. Heat treatments considerably decreased the non-casein nitrogen. The maximum decrease was in the case of cow UCW.

Cow milk contained greater amount of casein (soluble) than buffalo UCW. This is in conformity with the results reported by Gupta and Ganguli (44). On heat treatments, the casein nitrogen estimated increased 6.5 - 6.6 times that of the unheated in the case of buffalo UCW. At the same time in the case of cow milk UCW, the increase was only 3 - 3.1 fold. As a result of heat treatments, the whey proteins were denatured. As the pH of the heat
treated whey was adjusted to 4.6 for the determination of casein nitrogen, the whey proteins also got precipitated with casein. Hence the many fold increase in the casein nitrogen is the result of the whey proteins getting precipitated along with casein. This may indicate that the whey proteins of buffalo UCW are more vulnerable to heat treatment than cow UCW proteins. Furthermore the higher content of soluble casein of cow UCW may exert a protective action on the denaturation of whey proteins. Such a phenomenon has already been reported by Sabarwal and Ganguli (105).

Total albumin nitrogen content of buffalo milk UCW was higher than cow UCW. Heat treatments reduced the total albumin nitrogen content of UCW.

$\beta$-lactoglobulin content of buffalo UCW was lower than cow UCW. As much as 90 per cent and 94 per cent of the UCW decreased as a result of heat treatment in the case of buffalo and cow UCW, respectively. The fact that, higher percent of $\beta$-lactoglobulin decrease occurred in cow UCW may be due to the fact cow UCW contained higher $\beta$-lactoglobulin nitrogen.

Results presented in Table 30 indicate that buffalo UCW contained higher residual albumin than cow UCW. The maximum decrease of residual albumin was in the case of buffalo UCW.

From Table 37 it is clear that the proteose-peptone nitrogen content was higher in the case of cow UCW.
Heat treatment at 95°C for 10 minutes decreased the proteose-peptone nitrogen to the maximum. Heat treatment at 100°C for 10 minutes did not cause as much decrease in proteose-peptone nitrogen as heating at 95°C for 10 minutes. This was in conformity with the findings of Gupta et al (20b) that proteose-peptone decrease to about 50 per cent when milk was heat sterilized at 120°C for 10 minutes.

Globulin nitrogen was lower in cow UCW than in buffalo UCW. Heat treatments completely decreased the globulin nitrogen in the case of cow UCW. But a small portion of the globulin nitrogen remained in heat treated buffalo UCW.

The discussion on the distribution of nitrogen in unheated and heat treated ultracentrifugal whey indicates that the order of decreasing of the whey protein nitrogen are globulin nitrogen, residual albumin nitrogen, -lactoglobulin nitrogen, total albumins nitrogen proteose-peptone nitrogen. Although Larson and Rolleri (83) reported that proteose-peptone did not change as a result of heat treatment, the findings reported here, as well as that of Gupta et al (20b) led support that proteose-peptone also underwent reduction due to heat treatment. This difference can also be the outcome of the method adopted for the determination of this protein fraction. In the original Rowland (5) procedure, proteose-peptone was determined after heat treatment.
Milk sera isolated, acid whey, rennet whey and ultracentrifugal whey on heat treatment, revealed certain interesting aspects in relation to the, destabilization and denaturation of the serum proteins. Results presented in Fig. 30 for acid whey indicates that approximately 75 per cent of the proteins were denatured on heat treatment at 95°C and 100°C for 10 minutes. Between buffalo and cow, cow acid whey proteins underwent more denaturation than buffalo acid whey. On heating cow milk acid whey at different pH, Rowland (4) observed that at pH 4.75 - 4.85, as much as 70 per cent of the soluble nitrogen was rendered coagulable when boiled. Although in the present study, heat treatments were done after readjusting the pH to 6.8, the heat denaturable serum protein was very near to the earlier report (4). Kenkare et al (101), Morr and Josephson (102) and Guy et al (104) also reported the extensive denaturation of acid whey proteins as a result of various heat treatments. About 65 per cent of the acid whey proteins were destabilized as a result of heat treatment.

Denaturation and destabilization of rennet whey studied are shown in Fig. 31. Here as in the case of acid whey, about 50 per cent of the whey proteins got denatured when heated to 95°C and 100°C for 10 minutes. The destabilization of rennet whey at 95°C and 100°C for 10 minutes was only about 50 per cent of the total whey
proteins. Acid whey and rennet whey differed in respect of destabilization. This difference (higher percent of destabilization) between acid whey and rennet whey may stem from the fact that, in the case of acid whey, readjustment of the pH and introduction of cations and anions may alter the salt equilibria, whereby the serum proteins get destabilized as a result of heat treatments.

About 70 per cent of ultracentrifugal whey obtained at 105,000 x g was denatured as a result of heat treatment at 95°C and 100°C for 10 minutes. The rate of denaturation showed that buffalo UCW proteins were denatured more than cow UCW. Destabilization of these proteins were only up to 65 per cent (Fig. 32).

The fact that lower ultracentrifugal forces have comparatively more of micellar casein unsettled had been reported by Sabarwal and Ganguli (44). Ultracentrifugal whey obtained at 46,956 x g showed distinct difference in the denaturation and destabilization of the whey proteins. Only about 60 - 65 per cent serum proteins were denatured and 55 per cent destabilization. The overall lower denaturation and destabilization (Fig. 33) is evidenced by the fact, that more of micellar casein remained in the UCW, than the UCW obtained at 105,000 x g. Between buffalo and cow, it has been reported (44) that cow UCW contained more of micellar casein in the UCW than its buffalo counterpart. The presence of micellar casein protected
the whey proteins from undergoing extensive denaturation. Sabarwal and Ganguli (105) have reported such a phenomenon. Cow milk UCW proteins were destabilized and denatured to lesser extent than buffalo UCW proteins.

UCW obtained at 11,739 x g showed more distinct difference between buffalo and cow, UCW protein denaturation. In both cases denaturation was below 60 per cent and destabilization below 45 per cent. Here again it was the buffalo UCW proteins which underwent extensive denaturation.

Acid whey dialysed and heat treated presented an interesting picture (Fig. 35). Only 60 per cent of the buffalo acid whey was denatured and in the case of cow it was about 90 per cent. Similarly destabilization reached upto 80 per cent.

A number of investigators (88, 90, 99, 100, 101, 102) has studied the denaturation of milk serum using electrophoretic techniques. At room temperature paper strip electrophoresis resolved acid whey into two protein bands. Densitometer tracings (Fig. 36) indicated that heat treatments at 95°C and 100°C for 10 minutes completely denatured the whey proteins of both buffalo and cow. Polyacrylamide gel electrophoresis of buffalo acid whey revealed that unheated acid whey samples resolved into 4 protein bands. Heat treatment above 75°C for 10 minutes denatured the whey proteins completely. Undestabilized whey proteins subjected to electrophoresis on the same gel,
revealed that electrophoretic patterns up to 75°C were very similar to that of acid whey. Whereas at 80°C traces of β-lactoglobulin and α-lactalbumin remained unstabilized. Cow acid whey also behaved in similar manner.

Dialyzing and heat treatment (Fig. 38) of acid whey, denatured and destabilized the whey proteins even at 70°C.

The densitometer tracings (Fig. 39) of the paper strip electrophoresis of unheated and heat treated rennet whey showed two peaks corresponding to β-lactoglobulin and α-lactalbumin. Heat treatment at 95°C for 10 minutes flattered the densitometer tracings and the differentiation of the peaks were very little, and same protein fractions which did not migrate from the origin appeared. At 100°C/10 min. there existed only one peak, at the same time the proteins concentration at the origin increased.

Polyacrylamide gel electrophoretic patterns showed (Fig. 40) that heat treatments up to 80°C did not cause any significant change in the electrophoretic patterns. Heat treatments above caused significant decrease in the whey protein patterns. Complete denaturation did not take place as a result of heat treatment. Undestabilised whey on gel electrophoresis also gave rise to same pattern.

In the case of rennet whey dialysed and heat treated, polyacrylamide gel electrophoresis (Fig. 41) showed the disappearance of the major bands of rennet whey, as a result of heat treatments above 65°C. This may be
attributed to the fact that during dialysis slight acidity may have developed.

Paper strip electrophoresis (Fig. 42) of ultracentrifugal whey obtained at 105,000 x g resolved in two fractions of serum proteins. Heat treatments at 95°C and 100°C for 10 minutes caused the merging of the two major bands and increase in the area of the point of application and the non-migration of the protein components.

Polyacrylamide gel electrophoresis (Fig. 43) revealed that heat treatments above 75°C caused extensive denaturation of serum fractions, as evidenced by the disappearance of the major bands as well as from the protein remaining in the origin.

On the basis of polyacrylamide gel electrophoresis (Fig. 46) and gel filtration pattern on Sephadex G-100, the addition of N-ethylmaleimide showed protective action on the denaturation and destabilization of individual serum proteins of acid whey. This phenomenon was reported by Norr and Josephson (102) and Choudry and Humbert (103).

Measurement of turbidity formed when rennet whey or ultracentrifugal whey was heated, (Fig. 47) at 660 nm was used, to assess, the heat denaturation or destabilization of whey proteins to a certain extent.

Results of molecular sieving experiments (Fig. 48) on the particle size distribution of acid whey showed major changes in the protein bands. As reported by Norr and
Josephson (102) for cow milk whey, heat treatments of
buffalo acid whey lead to the formation of serum protein
aggregates which when sieved, occupied the position of
casein. This would mean that heat treatments aggregated
the serum proteins to such an extent that their molecular
weight rose to that of casein. The same arguments would
hold good for rennet whey, and ultracentrifugal whey at
different forces of gravity for the whey protein
aggregation.

Heat denaturation of $\alpha$-lactalbumin was
temperature dependent (Table 39). On heating
$\alpha$-lactalbumin in distilled water formation of a turbidity
was observed. Turbidity formation was almost complete
when the solution attained temperature of 90°C (Fig. 54).

Polyacrylamide gel electrophoresis (Fig. 55)
showed that complete denaturation of $\alpha$-lactalbumin did
not take place as a result of heat treatments. This was
expected since $\alpha$-lactalbumin was the most thermo
resistant component among all the whey proteins.

Gel filtration (Fig. 56) on Sephadex G-100
indicated the step wise increase in the molecular weight
due to aggregation of the protein as temperature of
heating increased.

The heat denaturation of $\beta$-lactoglobulin was
time temperature dependent (Table 40). Polyacrylamide
gel electrophoresis revealed that complete denaturation of
this protein could not be effected. This may be due to
the fact that 10 minutes heating was insufficient at any
given temperature to completely denature a one per cent
β-lactoglobulin solution. Gel filtration pattern shifts
could be explained as the increase in molecular weights
as a result of heat treatments at various temperatures.
The gel filtration pattern clearly showed that heating
β-lactoglobulin to 95°C or 100°C for 10 minutes increased
eutered
the molecular size to that extent that they are also/at
the same volume as that of casein

The emergence of whole casein, and β-lactoglobulin, α-casein and β-lactoglobulin, k-casein and
β-lactoglobulin as a single peak (Fig. 59, 60 and 61) from
heat treated mixtures, on Sephadex gel filtration would
mean that an association or interaction of the protein
might have taken place as a result of heat treatment.

The migration of the β-lactoglobulin and k-casein
mixture after heat treatment as one zone in polyacrylamide
gel electrophoresis would suggest an interaction between
these two proteins, as reported in literature (126, 127,
128, 129, 130 and 135) for bovine k-casein and
β-lactoglobulin. The gel filtration pattern of heat
treated α-lactalbumin and β-lactoglobulin mixture (1:1)
showed the interaction between these proteins, since on
heat treatments these two proteins moved as a single unit
on gel filtration. Similarly, the migration of these two
proteins together on polyacrylamide as a single unit would suggest the formation of a complex between these two proteins.