SUMMARY

1. Of the different types of whey fed for collection of abomasal juice, 3/4th diluted whey prepared from boiled skim milk was found to be the best in terms of total rennet activity (764 rennin units) and specific activity ($32 \times 10^{-2}$), present in the abomasal juice. Rennet isolated from above type of abomasal juice had greater solubility than from other types of abomasal juice. Fortification of whey with certain additives like glutamic acid (0.1%), aspartic acid (0.1%), cysteine (0.1%) and sodium chloride (2%) had no beneficial effect on the total rennin secretion and its specific activity in abomasal juice.

2. A retention period of 15-25 minutes of whey in the abomasum gave maximum yield of rennin. The volume of whey to be fed to calf for good rennet activity as well as yield, was determined to be 1.5 litres.

3. Calves upto the age of 3½ months donated abomasal juice with better coagulation (rennet) activity. A typical data on a calf ranged from 420 to 1080 rennin units with an average of 750 units per collection.

4. Average of 50 collections of abomasal juice from a calf yielded 36743 rennin units. Isolation of rennet from above abomasal juice resulted in a
preparation containing 8044 rennin units thus giving 23% recovery of enzyme. Such preparation was sufficient to coagulate 4400 to 4500 litres of milk at 37°C in half an hour.

8. Powder rennet was successfully prepared from liquid fistulated calf rennet by both lyophilisation and vacuum drying processes. Powder rennet preparations had higher protein and low salt as compared to Hansen rennet powder although both rennets had similar coagulation activity per unit weight. Furthermore fistulated calf rennet was more mobile electrophoretically than Hansen rennet. The salt and protein contents of lyophilised fistulated calf rennet powder were slightly different from vacuum dried powder of same type.

6. Attempts to crystalline rennin from fistulated calf rennet was not successful. However, a 10-fold purified product was obtained.

7. Proteolytic activities attributed due to pepsin and trypsin were observed to be associated with abomasal juice of fistulated calves, which, however, did not exhibit regular trends.

8. Of the different solvent systems used for storage studies of fistulated calf rennet, 0.03M sodium lactate and sucrose (10%) containing thymol (0.5%) were found to be more efficient for preserving rennet activity at room temperature (20 to 30°C) for
2 months. While in refrigerator (4 to 10°C) rennet was stable in 0.03M sodium lactate with or without 0.8% thymol up to 240 days storage with only 20% loss in its activity. Sucrose (10%) with thymol (0.8%) was also another good preservative for rennet activity during storage in refrigerator.

9. Heat denaturation studies revealed lesser susceptibility of fistulated calf rennet (inactivation of enzyme preparation at 70°C in half an hour) compared to Hansen rennet (inactivation at 60°C in half an hour).

10. Optimum activity (stability) of fistulated calf rennet solution was observed at pH 5.5. pH value above or below 5.5 caused activity losses. Hansen rennet behaved in same manner.

11. Paper, starch gel and polyacrylamide gel disc electrophoretic studies of fistulated calf rennet revealed the presence of a single protein band at pH 8.6 in veronal buffer indicating the apparent electrophoretic homogeneity of the product. Hansen rennet, on the other hand, resolved into a single but trailed protein band with slower electrophoretic mobility than fistulated calf rennet. However, on polyacrylamide gel, Hansen rennet resolved into two protein bands as against one of fistulated calf rennet, but with slower mobilities.
Rennets from fistulated buffalo calves and goat kids manifested similar electrophoretic pattern on paper and polyacrylamide gel as of fistulated calf rennet. On starch gel fistulated buffalo calf rennet showed lower mobility compared to fistulated calf and goat kid rennets.

12. Molecular sieving of rennets from fistulated cow and buffalo calves and goat kids on Sephadex G-100 resulted in its separation into two protein peaks compared to three protein peaks of Hansen rennet, thus signifying the molecular heterogeneity of these enzyme preparations.

13. The percentage release of sialic acid from a particular type of casein by different rennets (from fistulated cow, buffalo calves and goat kids and Hansen rennets) was insignificantly influenced by the types of rennet. But casein from milk of different species of animals caused varied percentage release of sialic acid. The casein-agar diffusion studies further substantiated the above mentioned observation.

14. Of the various chemicals added to fistulated calf rennet, it was found that addition of BaCl₂, MgCl₂, FeCl₃, MnCl₂ and KCl stimulated rennet activity, whereas CuCl₂, NiCl₂, NiSO₄, CoSO₄, FeSO₄ and ZnSO₄ inhibited, while addition of NaCl, NH₄Cl and (NH₄)₂SO₄
were without effect on the rennet activity.

15. Incorporation of BaCl$_2$, MgCl$_2$ and FeCl$_3$ in standard skim milk substrate resulted in reduction of its coagulation time by fistulated calf rennet. Whereas incorporation of NiCl$_2$, CuCl$_2$ and ZnSO$_4$ caused an increase in coagulation time while KCl, NaCl, NH$_4$Cl and (NH$_4$)$_2$SO$_4$ had no effect.

16. Fistulated calf rennet lost minimum activity due to photooxidation at pH 8.5. Change of pH from this value caused loss in rennet activity.

17. Dansylation studies showed that fistulated calf and Hansen rennets were modified by dansyl chloride above pH 5.0 only. Hansen rennet was observed to be more prone to dansylation than fistulated calf rennet.

18. Kinetic studies in relation to development of turbidity in k-casein by rennet action (fistulated calf rennet and Hansen rennet) revealed that optimum pH to obtain maximum turbidity development in maleate buffer was 6.5. Optimum concentration of cow and buffalo k-caseins were 0.4% and 0.5%, respectively. The ideal volume of rennet was 0.4 ml (50 mg/ml) with 5 ml of substrate in a fixed time.

19. Immunological studies on rennets provided a useful tool for differentiating fistulated cow calf
rennet from fistulated buffalo calf and goat kid rennets. Such techniques also highlighted the immunological homogeneity of fistulated calf rennet preparation and heterogeneity of Hansen rennet.

20. The presence of rennin as prorennin in the abomasal juice obtained from a typical fistulated calf (age up to 85 days) fed with 3/4th diluted whey prepared from boiled skim milk was in the range of 6 to 12% with an average of 9%. The percentage of prorennin in the abomasal juice was dependent on the kind of whey fed to fistulated calves. Highest amount (17%) of prorennin was obtained with rennet whey feeding. Presence of additives like glutamic acid (0.1%) and aspartic acid (0.1%) in whey reduced the prorennin content in abomasal juice while sodium chloride (2%) and cysteine (0.1%) abolished its presence.

21. There was variation in the recovery of prorennin from the abomasal juice of same calf.

22. Cysteine (0.1%) and sodium chloride (2%) when added to prorennin solution at pH 4.5, stimulated its conversion to rennin unlike glutamic and aspartic acids (0.1%) which inhibited the conversion. But at pH 2.0, with due exception of sodium chloride (2%), the remaining additives stimulated prorennin activation.
23. Paper electrophoresis of prorennin in veronal buffer pH 8.6 and molecular sieving on Sephadex G-100 revealed the presence of one protein band and one protein peak, respectively, thereby indicating the homogeneity of prorennin.