communication (Wheelock & Penney, 1970). An integrated form of the Michaelis-Menten equation was used to calculate values for K_m and V from the results obtained. Values for milks from different cows varied from 6.70×10^{-6} to 5.42×10^{-5} M for K_m and from 7.01×10^{-11} to 4.52×10^{-10} mol of glycopeptide $\cdot$ s $^{-1} \cdot$ l $^{-1} \cdot$ μ g $^{-1}$ of rennin $\cdot$ l $^{-1}$ of milk for V .

There was a linear relationship between initial velocity of release of glycopeptides and enzyme concentration, which varied between milks. The proportionality constant was used as an estimate of specific activity and values obtained were between 0.60 and 1.15 mg of N $\cdot$ min $^{-1} \cdot$ l $^{-1} \cdot$ μ g $^{-1}$ of rennin $\cdot$ ml $^{-1}$ of milk.

Taken together these results suggest that there is a change in the affinity of the enzyme for the κ -casein substrate between different milk samples. This could be due to the ionic environment of the milk, the amount of κ -casein in the milk, which may determine the size of the casein micelles (Sullivan, Fitzpatrick & Stanton, 1959), or the composition of the κ -casein. Variation in κ -casein composition is largely due to differences in the carbohydrates attached to it (Mackinlay & Wake, 1965).

Recently Sinkinson & Wheelock (1970) have shown that the *N*-acetylneuraminic acid/*D*-galactose molar ratio in the glycopeptides increases as the glycopeptides are released by rennin action. This finding suggests that the glycopeptides with a lower proportion of *N*-acetylneuraminic acid are released at a faster rate than those with a high proportion. Therefore the kinetic parameters for the release of each glycopeptide may be partly determined by the carbohydrates that are attached to it. For an individual milk sample, the values of K_m and V would therefore be an average value, determined by the relative proportions of different κ -caseins present. As the proportions probably vary between milks, then there should be corresponding changes in K_m and V . The specific activity would be determined by the composition of the κ -casein that is initially attacked.

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Carbohydrates of Bovine κ -Casein Glycopeptides

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The carbohydrates *N*-acetylneuraminic acid, *D*-galactose and 2-acetamido-2-deoxy-*D*-galactose (Alais & Jollès, 1961; Wheelock & Sinkinson, 1969) are attached to the glycopeptides released from κ -casein by the action of rennin (EC 3.4.4.3). Quantitative variations in these carbohydrates are largely responsible for the heterogeneity of κ -casein (Mackinlay & Wake, 1967). For an individual milk sample the average value for the carbohydrates attached to all the glycopeptides may be obtained by estimating the increase in carbohydrates attached to glycopeptides soluble in the 12% (w/v)-trichloroacetic acid filtrate after rennin action on the milk. In a previous investigation (Sinkinson & Wheelock, 1970a) we obtained molar proportions 3:5:2 and 2:2:1 for *N*-acetylneuraminic acid:*D*-galactose:2-acetamido-2-deoxy-*D*-galactose in the κ -casein glycopeptides. Values of 1:1:1 (Jollès, Alais, Adam, Delfour & Jollès, 1964) and 1:2:1 (Kuwata, Niki & Arima, 1969) have also been reported. There is, however, little information on the carbohydrates attached to the individual glycopeptides that contribute to the average value.

The glycopeptides present in the trichloroacetic acid filtrate of milk after rennin action were separated by ion-exchange chromatography. Then 30–40 mg of glycopeptides was dissolved in 2.5 ml of buffer (49 mM-KH₂PO₄–1 mM-Na₂HPO₄–0.1 M-NaCl adjusted to pH 5.1). This solution was eluted on a DEAE-cellulose column with 600 ml of buffer by using a linear 0.1–0.2 M-NaCl concentration gradient. Five major fractions and one minor fraction were obtained, which were each analysed for carbohydrates by g.l.c. (Sinkinson & Wheelock, 1970b).

Each glycopeptide contained *D*-galactose and 2-acetamido-2-deoxy-*D*-galactose. The first glycopeptide eluted was the only one to contain *D*-mannose, but was devoid of *N*-acetylneuraminic acid. The second glycopeptide contained *N*-acetylneuraminic acid, but the proportion was less than 1 mol/mol of glycopeptide and may have been due to contamination by the glycopeptides eluted later. These all contained approx. 1 mol of *N*-acetylneuraminic acid, 2 mol of *D*-galactose and 1 mol of 2-acetamido-2-deoxy-*D*-galactose/mol of glycopeptide.

The results show that the carbohydrate composition of the greater proportion of the κ -casein glycopeptides is similar and therefore will largely

determine the average molar proportions of the carbohydrates attached to all the glycopeptides. The existence of a D-mannose-containing glycopeptide is in agreement with previous observations that small amounts of D-mannose were attached to the glycopeptides released by rennin action on whole milk (Sinkinson & Wheelock, 1970a).

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Carbohydrates of Bovine α -Lactalbumin Preparations

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Recently there has been renewed interest in the protein α -lactalbumin, because of the discovery of its role as a component of lactose synthetase (Brodbeck, Denton, Tanahashi & Ebner, 1967).

Crystalline α -lactalbumin prepared by conventional methods has been found to consist of the major protein, present in the greater proportion, and a smaller amount of a second component. This secondary protein has the same amino acid composition as the major protein and has been reported to contain 1% of hexosamine (Gordon, Aschaffenburg, Sen & Ghosh, 1968). Apart from this there have been no reports of carbohydrates being present in α -lactalbumin (Ebner & Brodbeck, 1968).

α -Lactalbumin was prepared by ammonium sulphate fractionation of the 3%-(w/v)-trichloroacetic acid precipitate of whole milk (Aschaffenburg, 1968) and by ammonium sulphate fractionation of whole milk followed by separation on Sephadex G-100. Crystalline preparations were also purchased from Koch-Light Laboratories Ltd. (Colnbrook, Bucks., U.K.) and from Nutritional Biochemicals Corp. (Cleveland, Ohio, U.S.A.).

Polyacrylamide-gel disc electrophoresis of these preparations showed only the main protein band

and a faster-moving band due to the minor component.

G.l.c. of the trimethylsilyl derivatives of the methyl glycosides released on methanolysis (Clamp, Dawson & Hough, 1967; Sinkinson & Wheelock, 1970) showed that all these preparations contained D-mannose, D-galactose, 2-acetamido-2-deoxy-D-galactose and 2-acetamido-2-deoxy-D-glucose. The total concentration of carbohydrates varied from 0.25 to 1.5%. Assuming that the carbohydrates are attached to the α -lactalbumin, then there is considerably less than 1 mol of each carbohydrate/mol of protein. If all the carbohydrates are associated together in one moiety then this moiety must be present in only a proportion of α -lactalbumin molecules. As hexosamine has already been detected in the minor component it is possible that all the carbohydrates are attached to it. This would provide an explanation for the difference in properties between the major protein and the minor component.

The presence of carbohydrates in α -lactalbumin may have an effect on its role in lactose synthesis.

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The Aggregation Properties of C-Phycocyanin

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Phycocyanin from several algal sources has been examined over a wide range of concentrations (0.005–4.0 mg/ml). Osmotic-pressure, sedimentation-velocity, gel-filtration, disc-electrophoresis and immunodiffusion measurements all indicate that phycocyanin is a highly aggregated protein (Scott & Berns, 1965; Berns, 1967, 1970; Kao & Berns, 1968). Large aggregates exist at both extremely low and high concentrations (Lee & Berns, 1968a,b). Measurements with an analytical ultracentrifuge, equipped with a monochromator and photoelectric