

## Differences in Carbohydrate Content between Caseins from Cow's and Human Milk

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Important biochemical differences between human and cow's caseins have been described, *e. g.* in elementary composition, physico-chemical properties and digestibility<sup>1</sup>. During preparation of human casein our attention was directed to the rather mucinous consistence of this protein in contrast to the bovine preparations. No investigation of carbohydrates in human casein has so far appeared in the literature. The present authors have therefore compared the content of hexose, hexosamine and sialic acid in human and cow's caseins.

The material was obtained from fresh, unpasteurized cow's milk and pooled frozen human milk. Bovine casein was prepared merely by acidifying the skimmed milk to pH 4.6 and treated according to conventional methods. Human casein was precipitated at pH 4.6 after diluting skimmed milk several times and further treated as the bovine casein. In no case reducing sugar was present in the casein preparations.

The caseins were investigated for hexoses by the orcinol method<sup>2</sup>, hexosamine by Morgan-Elson's method<sup>3</sup> and sialic acid by Bial's reaction<sup>4</sup>.

	% Hexose	% Hexos- amine	% Sialic Acid
Cow's casein	0.23	0.19	0.45
Human »	1.98	1.32	0.75

The variance of the results between different samples from the same species was small. Thus, the carbohydrate content of human casein was found to be much higher than that of bovine casein. This difference was still more pronounced when the human casein was prepared from undiluted milk after dialysing against distilled water for several days.

The carbohydrate content of caseins from certain other species (*e. g.* sheep, goat and whale) and its correlation to hydrolysis with proteolytic enzymes will be discussed.

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## Isolation of Sialic Acid from Brain Gangliosides

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It was from gangliosides that neuraminic acid was first isolated (Klenk)<sup>1</sup>. Using the same procedure he also isolated the acid from submaxillary mucin<sup>2</sup>. Blix, however, had more than 10 years earlier obtained from the same mucin an acid which was later named sialic acid<sup>3</sup>. The relation between the two acids was supposed to be that neuraminic acid was a deacetylated methoxy-derivative of sialic acid<sup>4</sup>. Thus, it seemed quite possible that sialic acid would also be the naturally occurring acid in gangliosides, and this the author has demonstrated to be the case.

Gangliosides were prepared by the cellulose column technique<sup>5</sup>. Hydrolysis was performed with 0.1 N H<sub>2</sub>SO<sub>4</sub> for one hour at 90° C. The hydrolysate was poured into a cellophane bag and dialysed against distilled water changed twice a day. The dialysates were pooled, neutralised with barium hydroxide, and after filtration passed over Amberlite IR 120 (H) to remove excess Ba<sup>++</sup>. The sialic acid was fixed on a Dowex 2 column (formate form) and eluted with increasing concentration of formic acid. Sialic acid was indicated in the eluates with Bial's reagents. Free sialic acid appeared when the normality of the formic acid in the eluate was 0.4—0.5. The fractions containing sialic acid were put together and evaporated *in vacuo*. Crystallization occurred in some instances already during evaporation.

The isolated substance, like sialic acids from mucin and serum (the latter isolated in the same way by the author), gave positive direct Ehrlich reaction (neuraminic acid: neg.). Ninhydrin reaction was negative (neuraminic acid: pos.).

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