THE CARBOHYDRATES OF BUFFALO AND GOAT SEMEN*

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Available studies on the carbohydrates of buffalo and goat semen are limited to estimations of fructose in whole semen. In order to have a complete picture of the 'free' and 'bound' carbohydrates present, the seminal plasma of these species was subjected to 'extensive dialysis' as described by Mann & Rottenberg (1966). The results obtained are reported in this communication.

Semen was collected by artificial vagina and the plasma was separated from the spermatozoa in a clinical centrifuge. Pooled seminal plasma (5 to 10 ml) was dialysed against distilled water with five changes for 24 to 48 hr at 5° C. The two fractions, dialysable and non-dialysable, were taken to the original volume of the seminal plasma at 40° C under vacuum. The free sugars in the dialysable fraction were estimated after deproteinization with $\text{Ba}(\text{OH})_2$ and ZnSO_4 followed by deionization. The bound sugars in both the dialysable and non-dialysable fractions were hydrolysed with 1 N- H_2SO_4 for 6 hr and the hydrolysed samples were deionized by passing through a column of Amberlite IR-120 (H^+) and Dowex AG-(Cl^-) as described by Mann & Rottenberg (1966). Orcinolreactive compounds were estimated spectrophotometrically (Vasseur, 1948). Fructose was determined by the resorcinol method (Mann, 1946) and glucose by glucose oxidase and horse radish peroxidase (Hugget & Nixon, 1957). For sialic acid estimation, the samples were hydrolysed with 0.1 N- H_2SO_4 at 80° C for 1 hr and the amount was estimated by the thiobarbituric acid assay method of Warren (1959). The hexoseamines were determined in the hydrolysed samples (1 N-HCl for 12 hr at 100 to 105° C) by the procedure of Cessi & Piliego (1960). The amount of total nitrogen was estimated by the micro-Kjeldahl technique.

The sugars present in the seminal fractions were separated by paper chromatography on Whatman paper No. 1, using the solvent systems, propan-1-ol : ethylacetate : water (7 : 1 : 2, by vol.) (Glegg & Eidinger, 1954) and ethylacetate : pyridine : water (8 : 2 : 1, by vol.). These were detected on paper by spraying with AgNO_3 -NaOH (Trevelyan, Proctor & Harrison, 1950) and identified by co-chromatography with known samples.

The 'free' sugars in the semen of both the species consisted chiefly of fructose with small amounts of inositol (buffalo) and glucose (goat). This is in accord with the observations on human and bovine semen (Mann & Rottenberg, 1966; Baronos, 1971b) but differs from those on boar and stallion semen (Baronos, 1971a) where inositol occurs in preference to fructose. Another

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Table 1. Carbohydrates of the seminal plasma of buffalo and goat

<i>Species</i>	<i>Fraction</i>	<i>Orcinol- reactive material</i>	<i>Fructose</i>	<i>Glucose</i>	<i>Hexoseamine</i>	<i>Sialic acid</i>	<i>Nitrogen</i>
Buffalo	Dialysable non-hydrolysed	1100 to 1200(4)	800 to 1000(4)	1.0 to 1.3(2)	7.2 to 9.90 (4)	Trace	21 to 42(2)
	Dialysable hydrolysed	—	—	2.4 to 3.7(3)	16.0 to 18.9 (4)	27.2 to 37.20(4)	—
	Non-dialysable hydrolysed	130 to 250 (4)	0.00	1.3 to 5.20(3)	78 to 122.8(4)	30.5 to 90.0(4)	126 to 168(2)
Goat	Dialysable non-hydrolysed	600 to 700(3)	450 to 456(3)	8.8(1)	10.8 to 11.80(3)	Trace	31.5(1)
	Dialysable hydrolysed	—	—	—	28.0 to 50.0(2)	27.0 to 31.0(3)	—
	Non-dialysable hydrolysed	100 to 200(3)	0.00	4.81(1)	65.4 to 70.0(3)	23.0 to 28.0(3)	55.9(1)

Values expressed in mg/100 ml. Numbers in parentheses indicate the number of samples estimated.

interesting feature is the occurrence of fructose at the highest concentration (800 to 1000 mg/ml) in buffalo semen which also accounts for its high carbohydrate content. A part of the 'bound' carbohydrate fraction in the seminal plasma of both species occurred in the dialysable fraction. The sugars released on hydrolysis were sialic acid, hexoseamines, mannose, galactose, glucose, xylose, fucose and ribose. Here, they differ from the carbohydrate composition of human and bovine seminal plasma (Mann & Rottenberg, 1966; Baronos, 1971b) where sialic acid and xylose were not found in the dialysed fraction and the 'bound' carbohydrates were mainly localized in the non-dialysed fraction. For buffalo and goat semen, the non-dialysed fraction was resolved by paper chromatography into four compounds; one of these was not identified while the remaining three had the same chromatographic mobility as the authentic mannose, galactose and fucose. These were eluted from the paper and their subsequent estimation by the orcinol method showed them to be in the ratio of 4 : 3 : 2 for buffalo and 10:5:9 for goat. The detailed composition of both the species is given in Table 1.

The sugars released on hydrolysis from both dialysed and non-dialysed fractions could be present as a component of a polysaccharide or in 'bound' form with protein. No evidence for the presence of polysaccharide of any sugar (mannose, glucose and galactose) was obtained when they were prepared by the procedure of alkaline hydrolysis and ethanol precipitation (Pflüger, 1903; Somogyi, 1934). Similar evidence has been obtained for human (Mann & Rottenberg, 1966) and bovine (Baronos, 1971b) seminal plasma. Galactose constituted the major 'bound' carbohydrate of bovine, boar and stallion semen (Baronos, 1971a, b) but in both the buffalo and goat seminal plasma, mannose was present at higher concentrations than galactose. It would seem, therefore, that 'bound' carbohydrates (mannose, galactose, fucose) are present as glycoproteins with minor differences in composition for the different species. The seminal vesicles were shown to be the source of fructose and 'bound' carbohydrates containing galactose in bovine seminal plasma (Baronos, 1971b).

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