THE BOUND CARBOHYDRATE OF BOVINE SEMINAL PLASMA

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Summary. Galactose has been identified as the main bound carbohydrate of bovine seminal plasma. It was detected chromatographically and determined enzymatically (by galactose dehydrogenase) in acid-hydrolysed material. Bound galactose was shown to occur mainly in the non-dialysable portion of bovine seminal plasma in amounts varying from 30 to 150 mg/100 ml seminal plasma. It occurs in a protein-bound form, and is secreted by the seminal vesicles.

INTRODUCTION

Bull seminal plasma, in common with that of other mammals, contains a considerable amount of carbohydrate which occurs partly free and partly bound. The free carbohydrate is present mainly in the form of fructose and originates in the seminal vesicles and ampullae (Mann, 1946, 1964). The bound carbohydrate occurs in the form of polysaccharides (Mann & Rottenberg, 1966).

The main purpose of the present study was to identify the sugars which compose the bound carbohydrate of the bovine seminal plasma. Evidence is presented to show that the predominant sugar occurring in the bound form is galactose and that bound galactose originates in the seminal vesicle, the same organ which secretes fructose.

MATERIALS AND METHODS

Collection of semen and of the seminal-vesicle secretion

Semen from adult bulls was collected by an artificial vagina (Walton, 1945), and the seminal plasma separated from the spermatozoa by centrifugation. Most experiments were carried out with pooled seminal plasma but, on two occasions, instead of pooled material individual semen samples were used; the electrical stimulation method (Lutwak-Mann & Rowson, 1953; Davies, Mann & Rowson, 1957) was also used in order to collect semen fractions from an adult bull and to obtain an ejaculate from a 6-month-old calf. The seminal-vesicle secretion was obtained at autopsy.

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Separation of free and bound carbohydrate

Seminal plasma or vesicular fluid was dialysed at 3 to 5° C against glass-distilled water, which was changed five times at 12-hr intervals. The non-dialysable portion was concentrated *in vacuo* at 40 to 50° C to the original volume of the seminal plasma. The dialysable portion was concentrated *in vacuo* to half the original volume of the seminal plasma. For the analysis of neutral sugars, samples of both the dialysable and non-dialysable portions, each containing about 0.5 to 0.8 mg orcinol-reactive carbohydrate/ml were hydrolysed with 1 N-H₂SO₄ in sealed tubes for 6 hr at 105 to 110° C. For the liberation of hexosamines, samples were hydrolysed with 1 N-HCl for 12 hr at 100 to 105° C. Sialic acid was liberated by hydrolysis for 1 hr in 0.1 N-H₂SO₄ at 80° C.

Glycogen, together with any 'galactogen' (i.e. polysaccharide composed solely of galactose) was separated by the procedure of alkaline hydrolysis and ethanol precipitation (Pflüger, 1903; Somogyi, 1934). The final precipitate was dissolved in 2 ml water and hydrolysed with 1 N-H₂SO₄ before enzymatic determination of glucose and galactose.

Preparation of material for paper chromatography

Before chromatography, ions were removed by means of an ion-exchange-resin column containing Dowex AG-1 (OAc⁻) and Amberlite IR-120 (H⁺) (Mann & Rottenberg, 1966). Following deionization, the combined effluents were concentrated in a rotary evaporator at 40 to 50° C to near dryness. The residue was dissolved with a small amount of water and an aliquot of the solution, corresponding to about 100 µg orcinol-reactive carbohydrate, was applied to the paper. Ethyl acetate:pyridine:water (8:2:1 by vol) was used as the solvent system, and the spots produced by sugars, sugar alcohols and other silver-reducing substances were detected by the method of Trevelyan, Proctor & Harrison (1950).

Glucose, galactose, fructose and total carbohydrate

Glucose was estimated by the glucose oxidase-tolidine method (Middleton & Griffiths, 1957). Galactose was estimated by galactose dehydrogenase supplied by C. F. Boehringer & Soehne GMBH. Fructose was estimated according to the colorimetric method of Roe (1934) as adapted to semen by Mann (1946). Total carbohydrate was measured by the orcinol method (Vasseur, 1948). All these estimations were carried out on deionized material.

Hexosamine and sialic acid

Hexosamine was determined spectrophotometrically according to Cessi & Piliego (1960). Sialic acid, free and bound (liberated by hydrolysis), was determined according to Warren (1954).

Nitrogen

Total nitrogen was determined by the micro-Kjeldahl procedure using Markham's distillation apparatus (King & Wootton, 1956).

RESULTS

Preliminary paper chromatographic analysis carried out on samples of non-fractionated bovine seminal plasma which had been subjected to acid hydrolysis revealed the presence of a surprisingly large amount of galactose varying from 30 to 150 mg/100 ml. However, no galactose was found in the protein-free extracts prepared from seminal plasma by means of $\text{Ba}(\text{OH})_2$ and ZnSO_4 . The possibility that galactose may have arisen as an artifact from galactosamine or from fructose was immediately excluded by employing identical procedures of hydrolysis and deionization to solutions of pure galactosamine, glucosamine and fructose.

Chromatography was repeated using fractionated seminal plasma in the form of (i) dialysable non-hydrolysed, (ii) dialysable and acid-hydrolysed, and (iii) non-dialysable but acid-hydrolysed material. The dialysable non-hydrolysed material contained mainly fructose and also some inositol and sorbitol; this was in agreement with the earlier observations of Mann (1946, 1954), Hartree (1957) and King, Isherwood & Mann (1958). The same dialysable fraction after hydrolysis was also found to contain a small amount of galactose. On the other hand, the non-dialysable and acid-hydrolysed material was shown to contain a much larger amount of galactose, as well as a little mannose. The discovery that the bulk of the bound carbohydrate in bovine seminal plasma consists mainly of galactose led us to consider the possible sources of that sugar. One possibility was that galactose occurs in the seminal plasma bound to a protein. The other possibility was that it may be present in the form of a 'galactogen'-like polysaccharide. In order to explore both these possibilities, one aliquot of the non-dialysable portion of bull seminal plasma was hydrolysed with H_2SO_4 while another one was subjected to alkaline hydrolysis and ethanol precipitation (Pflüger, 1903; Somogyi, 1934). The acid hydrolysates prepared from two different specimens of bull seminal plasma contained 37 and 132 mg galactose/100 ml, respectively. After ethanol precipitation, the alkaline hydrolysates prepared from the same material contained neither glycogen nor galactogen. From this, it can be concluded that galactose occurs in seminal plasma in a protein-bound form and not as 'galactogen'.

In every sample of bull seminal plasma so far examined, including the electro-ejaculate of an immature bull calf, galactose was shown to be the principal bound sugar, though on no occasion was it possible to detect any free galactose. The results of analyses of the various neutral sugars, amino-sugars, sialic acid and nitrogen performed on fractionated seminal plasma and vesicular secretion are shown in Table 1. As can be seen, the non-dialysable and acid-hydrolysed portion of bovine seminal plasma contained, in addition to galactose, an appreciable amount of hexosamine and sialic acid. Table 1 also shows that bound galactose is in fact derived mainly from the seminal-vesicle secretion.

In order to investigate the origin of bound galactose, analyses were performed using, instead of semen, separate fractions of an electro-ejaculate collected from an adult bull. In the experiment, the results of which are recorded in Table 2, the combined volume of all the fractions was 16 ml. Of this volume, about 7 ml was contributed by the first three fractions and constituted

TABLE 1
CONCENTRATION OF FRUCTOSE, GLUCOSE, HEXOSAMINE, SIALIC ACID AND NITROGEN IN THE DIALYSABLE AND NON-DIALYSABLE PORTION OF BOVINE SEMINAL PLASMA AS WELL AS IN THE VESICULAR SECRETION COLLECTED DIRECTLY FROM THE SEMINAL VESICLES OF AN ADULT BULL

	Fructose	Galactose	Glucose	Hexosamine	Sialic acid	Nitrogen
Seminal plasma						
Dialysable non-hydrolysed portion	300 to 700 (4)	0 to 2.2 (5)	0 to 1.2 (3)	1.5 to 1.7 (2)	trace (2)	17.5 (1)
Dialysable acid-hydrolysed portion	245 to 570 (4)	2.9 to 13 (3)	0 to 4 (3)	11.0 to 13.6 (2)	0.1 to 0.3 (2)	
Non-dialysable acid-hydrolysed portion	0 (4)	30 to 150 (5)	0 to 1 (3)	120 to 240 (3)	7.9 to 12.6 (2)	364.0 (1)
Vesicular secretion						
Dialysable non-hydrolysed portion	1133 (1)	0.4 (1)	24 (1)			
Dialysable acid-hydrolysed portion	800 (1)	26.4 (1)	35 (1)			
Non-dialysable acid-hydrolysed portion	0 (1)	124 (1)	0 (1)			

Results are expressed in mg/100 ml. Number of samples in parentheses.

the so-called 'pre-sperm' fraction, that is, the secretion of the urethral glands. This fraction was sperm-free and contained neither fructose nor bound galactose but did contain an appreciable amount of hexosamine and sialic acid, as in the observations by Lutwak-Mann & Rowson (1953). Fractions 4 and 5 represented mainly the sperm-rich fraction. They contained little fructose and little bound galactose, hexosamine and sialic acid. The remaining two fractions (6 and 7), which constitute the 'post-sperm' fraction derived mainly from the seminal vesicle, had not only a high concentration of fructose but also of bound galactose.

TABLE 2

CONCENTRATION OF NITROGEN, HEXOSAMINE, SIALIC ACID, FRUCTOSE AND BOUND GALACTOSE IN SEVEN FRACTIONS OF BULL SEMEN, COLLECTED IN ORDER OF THEIR APPEARANCE DURING ELECTRO-EJACULATION

	Fraction of ejaculate						
	1st	2nd	3rd	4th	5th	6th	7th
Volume	1.8	2.4	2.6	1.5	1.8	3.3	2.6
Sperm density	—	—	0.26	1.042	1.728	1.600	0.728
Fructose	—	—	—	52.0	167.0	380.0	290.0
Galactose	—	—	—	18.0	59.0	66.0	61.0
Hexosamine	53.0	51.0	51.0	74.0	116.0	121.0	111.0
Sialic acid	15.0	10.0	9.8	—	23.3	44.5	29.0
Nitrogen	90.0	80.0	67.0	183.0	530.0	560.0	600.0

Results are expressed in mg/100 ml fraction; volume is expressed in ml; sperm density in 10^9 cells/fraction; other results in mg/100 ml.

DISCUSSION

It has been recognized for some time that mammalian seminal plasma is characterized by a high content of carbohydrate both in free and bound form. In the past, most investigations were directed towards the elucidation of the nature and function of the free carbohydrate. More recently, however, the presence of a small amount of bound galactose in human seminal plasma has been reported by Mann & Rottenberg (1966). The present study reveals that bovine seminal plasma is extraordinarily rich in bound galactose, so much so that bound galactose can almost be considered as yet another macro-constituent of semen. Most of the bound galactose is contained in the non-dialysable portion of bovine seminal plasma and appears to be present in those parts of the bovine ejaculate which are also rich in fructose. This observation together with the results of direct analysis of seminal-vesicle secretion demonstrates that free fructose and bound galactose are produced in the bull by the same accessory gland, namely, the seminal vesicle.

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