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MONOSACCHARIDES AND MONOSACCHARIDE DERIVATIVES IN HUMAN SEMINAL PLASMA

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SUMMARY

Gas chromatography—mass spectrometry with an on-line data system was used to identify monosaccharides and monosaccharide derivatives in human seminal plasma. The carbohydrates were converted into the methoxime—trimethylsilyl derivatives before separation in open tubular glass capillary columns coated with SE-30. Twenty-one different compounds were detected in the seminal fluid, of which twelve have not been recognized before. Seventeen of the monosaccharides have previously been identified in urine. Similar patterns of sugars were found both in fertile and infertile individuals, including one with azoospermia. The compounds identified are, with the possible exception of D-ribose, present as free monosaccharides at the time of ejaculation, and they do not seem to be preformed by spermatozoa.

INTRODUCTION

Mann and Rottenberg [1] identified 6 monosaccharides in human seminal plasma, and later 2-acetamido-2-deoxy-hexoses [2] have also been found. It has been suggested [1, 2] that some of the sugars arise from the spermatozoa or by enzymic reactions in the seminal fluid.

Preliminary investigations [3] made it clear that several sugars in addition to those already described were present in human seminal plasma. Open tubular columns for gas chromatography attached to a mass spectrometer and an on-line data system have increased our analytical possibilities, and formed the basis for the present investigation.

MATERIALS AND METHODS

Chemicals

Reference sugars tested were: L-arabinitol, D-arabinose, erythritol, galactitol,

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D-glucitol, D-mannitol, L-rhamnose, D-ribose and L-sorbose from Nutritional Biochemicals (Cleveland, Ohio, U.S.A.), 2-acetamido-2-deoxy-D-galactose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-mannose, L-fucose and D-glycero-D-galacto-heptitol from Sigma (St. Louis, Mo., U.S.A.), L-arabinose, D-galactose and ribitol from Difco (Detroit, Mich., U.S.A.), D-fructose, D-glucose and glycerol from E. Merck (Darmstadt, G.F.R.), D-arabinitol and xylitol from Koch-Light (Colnbrook, Great Britain), D-mannose from Hopkins and Williams (London, Great Britain), D-xylose from BDH (London, Great Britain), threitol was a gift from Dr. Wold, Institute of Pharmacy, University of Oslo. L-Fucitol was made by reduction of L-fucose with sodium borohydride.

Methoxyamine—hydrochloride and N, O-bistrimethylsilyl-trifluoroacetamide (BSTFA) were obtained from Supelco (Bellefonte, Pa., U.S.A.).

Instrumentation

Gas chromatography—mass spectrometry (GC—MS) was performed on a Varian Model 112 combined gas chromatograph—mass spectrometer (Varian-MAT, Bremen, G.F.R.) to which an on-line data system (Spectrosystem 100, Varian-MAT), with dual discs and magnetic tape unit was attached. The gas chromatograph was a Varian Model 1400 (Varian Aerograph, Walnut Creek, Calif., U.S.A.) and the glass capillary column (LKB, Stockholm, Sweden) was 25 m × 0.28 mm I.D., and wall-coated with SE-30. The ion source of the GC detector was operated at 24 eV, and that for MS was operated at 70 eV. Injection port temperature was 250°. Samples were usually injected at an oven temperature of 80°. After elution of the solvent front the temperature control was set at 150° and the temperature programmed at 2° per minute up to 200°. Inlet pressure of helium carrier gas was 0.25 kp/cm^2, which gave a 10 cm/sec linear velocity of carrier gas. The split ratio of the injector was 1:20. The mass spectrometer scan time was 2 sec from start to reset.

A Fractovap Linea 2101 AC (Carlo Erba, Milan, Italy) gas chromatograph with a flame ionization detector and equipped with the same glass capillary column as described above, was also used. The chromatograph was operated at an injector—detector block temperature of 250°, and with the same column temperature conditions as for the GC—MS instrument. The split ratio was 1:20.

Ultrafiltration was done with a Millipore [illegible]edford, Mass., U.S.A.) PSAC filter 13 mm in diameter. The ultrafiltration [illegible] was operated with a magnetic stirrer, and at 3.5 kp/cm^2 pressure exerted from a nitrogen flask.

Samples

Samples analysed were obtained through masturbation. They were received from the outpatient infertility section of the Department of Gynecology and Obstetrics. A pilot study was undertaken on 13 samples. Of these, 6 were from men without findings indicating pathology, neither in medical history nor upon routine clinical examination of semen. Of the samples, 4 showed low motility of spermatozoa, and 3 showed more pronounced pathology, including one with azoospermia.

Samples from 3 healthy donors were ejaculated into 80% ethanol to stop

enzymic action and action of spermatozoa. For comparison, the same three donors also produced samples which were left to liquefy for 30 min before separation of spermatozoa and plasma.

Methods

Semen will clot immediately after ejaculation, and then it liquefies spontaneously during the next 30 min. Seminal plasma was separated from spermatozoa through centrifugation, 30—60 min after ejaculation. A 0.5-ml volume of seminal plasma was ultrafiltered, and then passed through a mixed-bed ion-exchange resin (Dowex 2 (CO_3^{2-}) and Dowex 50 (H^+), both X8 and 100—200 mesh). The eluate was lyophilized and dissolved in 1 ml of pyridin. About 2 mg of methoxyamine—HCl was added and the mixture heated at 80° for 2 h. After addition of 100 μl of BSTFA the heating was continued for 15 min [4].

RESULTS

A gas chromatogram of the neutral fraction of pooled human seminal plasma is shown in Fig. 1. In all, 25 of the peaks have been identified, they represent 21 monosaccharides or monosaccharide derivatives.

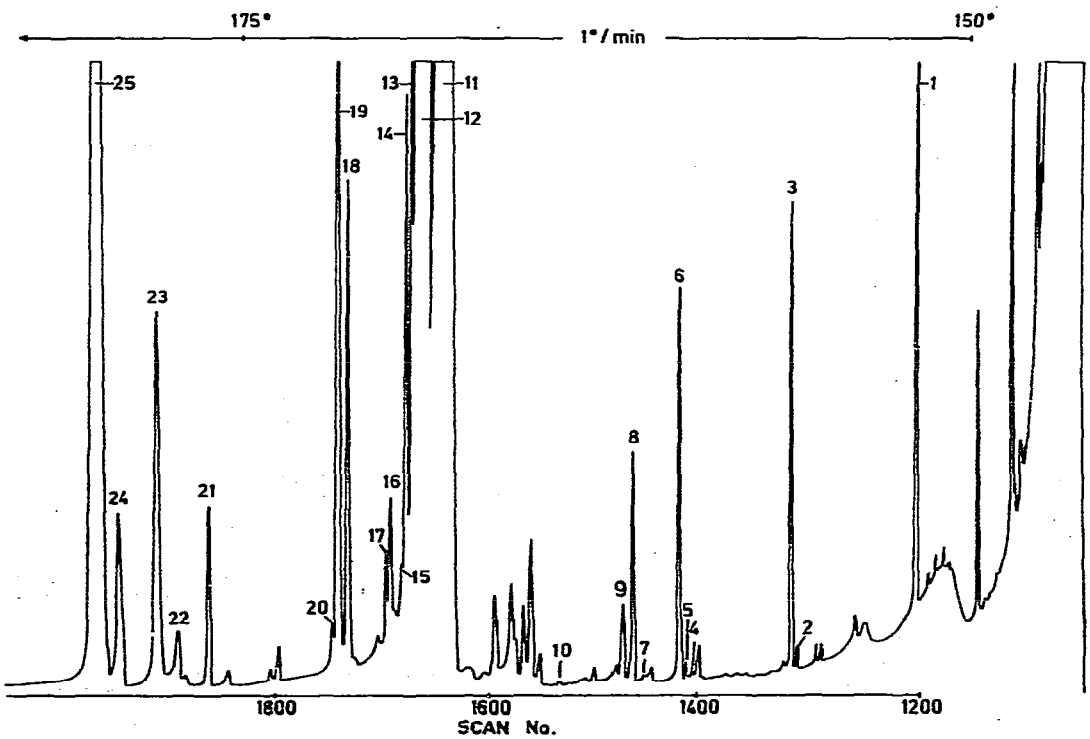

Fig. 1. Gas chromatogram of the neutral fraction of pooled human seminal plasma. A 25 m long, open-tubular, glass capillary column coated with SE-30 was used. The oven temperature at injection was 80°. After elution of the solvent front, the temperature control was set at 150° and the temperature was programmed at 1°per min to 200°. The numbering of the peaks is the same as in Table I, which also shows the identities of the compounds.

TABLE I

MONOSACCHARIDES AND ALDITOLS IDENTIFIED IN HUMAN SEMINAL PLASMA

The numbering of the peaks is the same as in Fig. 1. Retention times (SE-30 glass capillary column) are given relative to the first peak of fucose, and have been taken from a GC run with a temperature programming of 2°/min from injection at 120°.

Peak No.	Compound	Relative ret. time	Ten most abundant *m/z* fragments									
1	Glycerol	0.28	73	147	205	103	117	218	74	45	148	133
2	Threitol	0.55	73	217	147	103	205	117	189	218	204	191
3	Erythritol	0.56	73	147	217	103	205	117	204	189	191	116
4	Xylose	0.84	73	103	217	307	147	189	218	160	191	117
5	Arabinose	0.86	73	103	217	147	307	189	104	133	74	160
6	Ribose	0.90	73	103	217	147	307	189	74	104	160	218
7	Xylitol	0.98	73	103	217	205	147	307	189	148	218	117
8	Fucose, 1st peak	1.00	117	73	160	118	147	277	129	75	161	219
8	Arabinitol	1.01	pure spectrum not obtained									
9	Ribitol	1.02	pure spectrum not obtained									
9	Fucose, 2nd peak	1.03	pure spectrum not obtained									
10	Fucitol	1.18	73	117	147	217	205	219	129	103	319	133
11	Fructose, 1st peak	1.38	73	103	217	307	147	74	104	308	133	218
12	Fructose, 2nd peak	1.41	73	103	217	307	147	74	308	218	104	75
12	Mannose, 1st peak	1.42	pure spectrum not obtained									
13	Galactose, 1st peak	1.43	73	205	147	319	103	217	160	320	117	74
14	Glucose, 1st peak	1.44	73	205	319	147	160	103	217	320	157	117
15	Mannose, 2nd peak	1.45	73	103	147	205	319	217	160	74	117	129
16	Galactose, 2nd peak	1.46	73	205	147	319	103	217	160	320	129	117
17	Glucose, 2nd peak	1.47	73	147	205	103	319	160	217	74	117	157
18	Mannitol	1.54	73	205	319	147	103	217	320	117	157	206
19	Glucitol	1.55	73	205	319	147	103	217	320	307	117	206
20	Galactitol	1.56	73	217	103	147	205	319	307	218	117	74
21	Scyllo-inositol	1.77	73	217	318	305	147	191	204	319	306	103
22	2-Acetamido-2-deoxy-glucose, 1st peak	1.80	73	147	205	129	202	87	103	75	173	319
23	2-Acetamido-2-deoxy-glucose, 2nd peak	1.82	73	147	205	129	202	87	319	103	173	75
24	2-Acetamido-2-deoxy-galactose	1.87	73	147	205	129	87	319	202	103	173	75
25	Myo-inositol	1.90	73	217	305	147	191	318	204	306	218	265

Table I gives relative retention times and the 10 most abundant *m/z* fragments for the compounds identified. Identification is based on both mass spectra and retention times. Mass spectra have been compared to mass spectra of authentic sugars, and to mass spectra described in the literature. Retention times have been compared by adding authentic sugars to samples of seminal fluid.

For scyllo-inositol the retention time has not been verified. The mass spectrum, however, identifies peak 21 of Fig. 1 to be an inositol. Furthermore, the spectrum indicates that it is scyllo-inositol, since no other inositol has such a low relative abundance of *m/z* 265 [5].

Due to overlapping of neighbouring GC peaks, no pure mass spectrum could be obtained for some of the compounds in Table I. MS may, in spite of this, give data which are of importance for the identification. This is shown in Fig. 2. As can be seen from the traced fragments, the GC peaks clearly consist of two compounds each. However, no reliable order of relative ion abundance can be given.

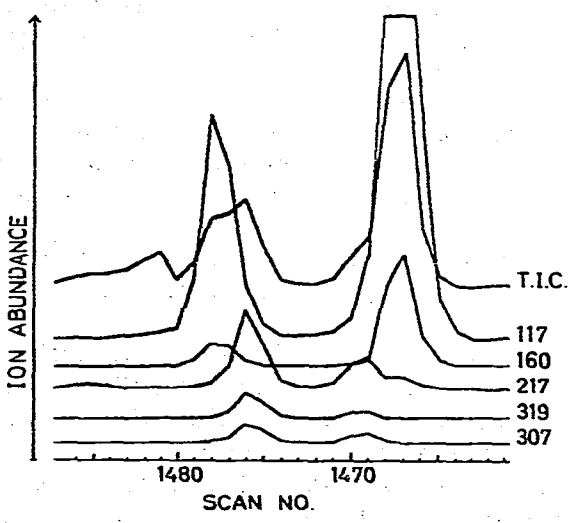

Fig. 2. Characteristic mass spectral fragments traced through peaks 8 and 9 of Fig. 1. At least two compounds are shown to be present in each peak. T.I.C. is total ion current.

Seminal fluid samples from 13 men of infertile couples were examined with gas chromatography. In some of these chromatograms the peaks for D-xylose, arabinitol, arabinose, ribitol and L-fucitol could not be detected. This might be due to the somewhat lower sensitivity of the flame ionization detector of the gas chromatograph used for these analyses, as compared to the electron impact detector of the GC–MS combination instrument. In all the samples examined, the other peaks identified in Fig. 1 were present with about the same relative heights. This was also true for the sample of the azoospermic individual. The concentrations of the sugars were, however, somewhat lower in this sample than in the others.

In order to avoid possible changes of the seminal plasma after ejaculation,

samples from three healthy donors were ejaculated directly into 80% ethanol. D-ribose was absent in two of the samples, but present in the third one. D-ribose was present in samples from the same three donors after the samples had been left to liquefy for 30 min before separation of spermatozoa from seminal plasma. The ribose-containing sample, which had been treated with ethanol, contained only small amounts of fructose. This was also true when a sample from the same donor was left to liquefy. The other compounds identified in the pooled plasma were present also in the 3 samples ejaculated into ethanol, and the GC peaks were of similar relative heights as those of the pooled sample.

DISCUSSION

A variety of derivatives have been used for the GC analysis of sugars, most of them are unsuitable when ketoses are present. The methoxime—TMS derivatives can be applied also on ketoses. This is probably the main advantage of the present method compared to others. The methoxime—TMS derivatives give one GC peak for alditols, and two peaks for most aldoses and ketoses, due to isomerism arising during methoxime formation. Without prior reaction of the carbonyl function, most other derivatization procedures, like trimethylsilylation, methylation or acetylation, will give 2—4 peaks for each sugar. Reduction of the carbonyl group will give one peak for each monosaccharide, but will not distinguish between e.g. D-glucose and D-glucitol. Aldononitriles give just one peak for each aldose and alditol, but cannot be applied to the analysis of ketoses. The oxime—TMS derivatives can be used, but they are rather unstable [6].

The methoxime—TMS derivatives are stable for months when kept at —20° in PTFE-capped vials. When destroyed by hydrolysis, the samples can easily be taken to dryness and rederivatized.

For identification of monosaccharides, mass spectra alone will not suffice. As can be seen from Table I, mass spectra of optical isomers are quite similar. Mass spectrometry is, however, well suited to the identification of classes of sugars. The literature contains mass spectra of methoxime—TMS derivatives of various sugars [4] and of 2-acetamido-2-deoxyaldoses [7], and mass spectra of TMS derivatives of inositols [5]. For identification it is, in addition, necessary to determine gas chromatographic retention times. Even then a complete identification of some of the monosaccharides is at present not possible.

Only a few of the 21 compounds identified in the present paper have previously been recognised as constituents of seminal fluid. Mann [1] showed that 6 sugars were present: D-fructose, D-glucose, D-ribose, L-fucose, D-glucitol and myo-inositol. 2-Acetamido-2-deoxy-hexoses were shown to be present in seminal fluid of many mammals, including man, by Rodger and White [2]. It was, however, not possible for them to distinguish between the different isomers. High concentrations of these sugars are found in the seminal fluid of marsupials, where they dominate as much as does D-fructose in the seminal fluid of most eutherians. Scylio-inositol is also present in seminal fluid of different mammals [8], but has not been described in the semen of man before. This sugar is found in the same body fluids and tissues as myo-inositol, and is thought to be a part of myo-inositol metabolism [9].

It has been suggested [1, 2] that some of the sugars previously found in seminal fluid, are the result of enzymic or spermatozoic action occurring during liquefaction. The findings in the samples ejaculated into ethanol do not support this hypothesis. The results from the azoospermic sample make it, in addition, unlikely that the monosaccharides are formed by spermatozoa before ejaculation. The volume of this sample was 6 ml, which excludes retrograde ejaculation as the cause of the azoospermia. Most likely all the sugars found are present as free monosaccharides at the time of ejaculation, with the possible exception of D-ribose.

The concentrations of D-fructose and myo-inositol are known to vary within wide ranges in human seminal plasma, with means of about 12.5 mmole/l and 2.3 mmole/l, respectively. Semiquantitative calculations for the remaining 19 compounds identified give concentrations ranging from less than 5 μmole/l for xylitol to about 2 mmole/l for glycerol.

Although most of the 21 compounds have not been identified in human seminal plasma before, 17 of them are known to occur in human urine [10]. This suggests that these compounds may be part of the general carbohydrate metabolism in man.

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