STUDIES ON THE EFFECTS OF SUGARS ON WASHED HUMAN SPERM MOTILITY

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The motility of ejaculated human sperm which was washed thoroughly by Ficoll gradient centrifugation to remove seminal plasma was investigated in the presence of various concentrations of sugars. Washed human sperm showed high motility in an artificial medium containing glucose and fructose, which were major sugar components of semen. However, mannitol and 3-*O*-methyl-D-glucose had no effect on sperm motility. Dose-response curves of sperm motility in the additions of glucose or fructose were bell-shaped. The maximum sperm motility was found in the medium with 1 mM glucose or 15 mM fructose. When, however, both 1 mM glucose and 15 mM fructose were added to the medium, sperm motility was higher than that with an individual sugar. Although phlorizin decreased glucose activated sperm motility, it has no effect in case fructose was the activator. The results suggest that the effect of glucose on human sperm motility is due to a different mechanism from that of fructose.

Keywords — sperm; sperm motility; glucose; fructose; 3-*O*-methyl-D-glucose; phlorizin

INTRODUCTION

Seminal plasma consists of the secretions from seminal vesicle, prostate gland and bulbourethral gland, and it contains proteins, lipids, sugars, inorganic and organic salts and physiologically active substances.¹⁾ Most of those compositions are similar to those of blood plasma. However, the composition of sugars in seminal plasma with higher fructose and low glucose contents differs from that of blood plasma. Sperm motility is related to fertility and sugar is an important element for sperm motility. Fructose in seminal plasma (approx. 15mM)²⁾ has been thought to be a main energy source.³⁾ Whereas little attention has been paid to the physiological significance of glucose because of its low concentration in seminal plasma (approx. 0.5 mM).⁴⁾ Some investigators have studied the effects of sugars on sperm motility, but in most cases, native semen or poorly washed sperm was used.

In this communication, we used highly

washed sperm obtained by a density gradient centrifugation with Ficoll to examine the effects of sugars on sperm motility in the artificial medium.

MATERIALS AND METHODS

Human Semen — Freshly ejaculated human semen was kindly provided from Department of Obstetrics and Gynecology, School of Medicine, Keio University (Tokyo). The ejaculates were obtained after four days abstinence and only normal semen, in which sperm density was higher than 4×10^7 sperm/ml with >60% motility was employed in all experiments.

Chemicals — Ficoll ($M_r \approx 4 \times 10^5$), *N*-2-hydroxyethylpiperazine *N'*-2-ethanesulfonic acid (HEPES) and 3-*O*-methyl- α -D-glucose were purchased from Nakarai Chemicals Co. (Tokyo). D-Glucose, D-fructose and mannitol were the products of Kanto Chemicals Co. (Tokyo). Phlorizin was from Sigma Chem. Co. (St. Louis, Mo., U.S.A.). F-kit Glucose/Fructose

(Boehringer Mannheim Co.) as described by Bergmeyer⁵⁾ was used to determine the concentrations of glucose and fructose.

Preparation of Washed Human Sperm — Sperm were washed by the centrifugation with Ficoll as described previously.⁶⁾ Semen was diluted with an equal volume of medium (20 mM HEPES, 138 mM NaCl, 2 mM NaHPO₄, 4 mM MgCl₂, 2.5 mM KOH, 7.5 mM NaOH, pH 7.4 at 22°C) and 1 ml of the diluted semen was layered on 5 ml of the medium containing 7.5% (w/v) Ficoll, and centrifuged at $40 \times g$ for 5 min and then at $250 \times g$ for 10 min. Supernatant was removed and the sperm pellet was suspended into the above medium. Sperm suspension was adjusted to $5-10 \times 10^7$ sperm/ml.

Measurement of Sperm Motility — Sperm motility was examined on the Thoma's hemocytometer according to the method of Ishii *et al.*⁷⁾ with minor modification. Sperm suspension was dropped on a hemocytometer and the glass was placed on the stage of a microscope (magnitude; 10×40) which was kept at 22°C on thermal insulation plate. The number of sperm passed through a 0.25 mm line on the glass in one minute was counted. For good reproducibility every measurement was repeated six times by using two hemocytometers. Motility index was obtained by the following equation: Motility index = Number of sperm passed a 0.25 mm line $\times 10^8$ / Number of sperm per milliliter.

RESULTS

Ficoll centrifugation method gave highly washed human sperm suspension which contained only 0.01 mM glucose (<0.1%), 0.3 mM fructose (<3%) and 2% of protein of seminal plasma. Such suspension was incubated in the medium containing sugars for 1 h, when sperm showed maximum motility in 1 mM glucose or 15 mM fructose containing medium. Both glucose and fructose increased sperm motility, but at higher concentrations motility decreased considerably (Fig. 1). Relatively low concentration of glucose (1 mM) showed a maximum effect on sperm motility. Whereas ten to twenty times

higher concentrations of fructose was required to be effective. The maximum effect of fructose was recognized in 15 mM, however, it was only 50% effective of 1 mM glucose. When both 1 mM glucose and 15 mM fructose were added together in the medium, sperm motion was most active and was about 120% of that with glucose only after 1 h incubation and about 140% of that with fructose only.

Mannitol was added at 20 mM concentration to test the effect of osmotic pressure to sperm

FIG. 1. Sperm Motility in the Various Concentrations of Glucose (A) or Fructose (B) after 1 h Incubation

motility. The sperm motilities in the presence and in the absence of 1 mM glucose did not change by the addition of mannitol. Furthermore, sperm motility in the presence of 1 mM 3-*O*-methyl glucose instead of glucose was

exactly the same as that of control. This result indicates that the sperm motility is dependent on the energy produced by glucose metabolism.

Phlorizin, a competitive inhibitor of active transport of glucose⁸⁾ was tested for its effect on sperm motility (Fig. 2). Although 1 mM phlorizin was ineffective in the absence of glucose, glucose-evoked sperm motility was suppressed at least 50% by 1 mM phlorizin. However, phlorizin did not influence on the sperm motility induced by fructose.

DISCUSSION

It has been reported that the main sugar component in mammalian semen are glucose and fructose. Shimazaki *et al.*¹⁾ reported that the concentrations of glucose and fructose in normal semen were 0–5 mM (mean: 0.4 mM) and 5–30 mM (mean: 15 mM), respectively. Thus the elimination of those sugars in semen is essential to prepare exact sugar concentration in the medium used in our experiments. Kaneko *et al.*⁶⁾ have obtained highly washed intact human sperm with Ficoll density gradient centrifugation. Sperm suspension prepared by this method contained negligible amount of glucose and fructose and the method was useful enough to remove other low molecular substances in semen.

Sperm motility showed maximum activity in the medium added 0.5–1.0 mM glucose or 10–20 mM fructose. In higher concentrations of two sugars at 10 mM glucose and 40 mM fructose, however, sperm motions were very slow. These findings indicate that about 1 mM of glucose and 15 mM fructose corresponding to the concentrations in human semen are appropriate to sperm motility. Lowering of sperm motility by the addition of high concentrations of sugars was not simply due to the increase of osmotic pressure. Freund and MacLeod⁹⁾ have reported that the addition of 30 mM fructose in human semen at final concentration increased fructolysis and lactate production, but depressed the motility. The physiological role of high concentration of fructose remains unexplained.

FIG. 2. Inhibitory Effect of Phlorizin on Sperm Motility in the Presence of Glucose (A) or Fructose (B)

(A); Each medium contained 1 mM phlorizin (Δ), 1 mM glucose (●), and 1 mM glucose with 1 mM phlorizin (▲). Control did not contain any sugar (○).

(B); Each medium contained 1 mM phlorizin (Δ), 15 mM fructose (●), and 15 mM fructose with 1 mM phlorizin (▲). Control did not contain any sugar (○).

Peterson and Freund¹⁰⁾ have reported that hexokinase from human sperm has K_m values to fructose and glucose of 2.82 mM and 0.1 mM, respectively. Therefore, glucose is metabolized more readily than fructose in sperm. It has been reported that sperm predominantly utilizes glucose *in vitro* in the presence of both glucose and fructose.¹¹⁾ However, the motility in the medium with 1 mM glucose and 15 mM fructose was higher than in the individual case. Furthermore, phlorizin suppressed the glucose-evoked sperm motility, but not the fructose-evoked one. These results suggest that the systems utilizing glucose and fructose in sperm may be different or independent. Glander and Dettmer^{12,13)} studied the uptakes of ^{14}C -glucose and ^{14}C -fructose by human sperm and reported that the transport systems of these two sugars were different. Thus the difference between the effects of glucose and fructose in human sperm seems to be dependent upon the sugar transport mechanisms rather than metabolic pathways.

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REFERENCES

- 1) J. Shimazaki, T. Katayama, H. Aikawa and S. Yanagi: Biochemistry of seminal fluid, *Metabolism and Disease*, **16**, 471–480 (1979).
- 2) K. Shida, J. Shimazaki, N. Furuya and M. Taya: Some biochemical analysis of semen from male infertility patients, *Jap. J. Fert & Ster.*, **16**, 217–223 (1971).
- 3) T. Mann, "The Biochemistry of Semen and of the Male Reproductive Tract," Methuen Co., London, 1964.
- 4) R. Eliasson: Accurate determination of glucose in human semen, *J. Reprod. Fertil.*, **9**, 325–330 (1965).
- 5) H. U. Bergmeyer, "Methods of Enzymatic Analysis," 2nd ed., Academic Press, New York, 1974.
- 6) S. Kaneko, C. Moriwaki, H. Sato, T. Kobayashi and R. Iizuka: Development of a multiple exposure photography method for analysis of sperm motility and preparation of washed sperm with Ficoll density gradient, *Jap. J. Fert & Ster.*, **25**, 491–497 (1980).
- 7) N. Ishii, S. Mitsukawa and M. Shirai: Sperm motile efficiency, *Andrologia*, **9**, 55–62 (1977).
- 8) F. Alvarado: Hypothesis for the interaction of phlorizin and phloretin with membrane carriers for sugars, *Biochim. Biophys. Acta*, **135**, 483–495 (1967).
- 9) M. Freund and J. MacLeod: Effect of addition of fructose and glucose on the fructolysis and motility of human sperm, *J. Appl. Physiol.*, **13**, 506–509 (1958).
- 10) R. N. Peterson and M. Freund: Glycolysis by washed suspensions of human spermatozoa—Effect of substrate, substrate concentration and changes in medium composition on the rate of glycolysis, *Biol. Reprod.*, **1**, 238–246 (1969).
- 11) J. Masaki: Carbohydrate metabolism in sperm, *Protein, Nucleic acid and Enzyme*, **24**, 259–263 (1979).
- 12) H. J. Glander and D. Dettmer: Monosaccharide transport across membranes of human spermatozoa—Development of a radiochemical method of measuring monosaccharide uptake by spermatozoa, *Andrologia*, **10**, 69–73 (1978).
- 13) H. J. Glander and D. Dettmer: Monosaccharide transport across membranes of human spermatozoa—Basic properties of D-fructose and D-glucose uptake, *Andrologia*, **10**, 273–277 (1978).