STUDIES OF THE METABOLISM OF HUMAN SPERMATOZOA

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Summary. The metabolism of human spermatozoa has been studied using concentrated suspensions of washed cells incubated with radioactive substrates in small Warburg flasks, enabling the oxygen uptake to be measured accurately.

Oxidative metabolism was greater at pH 7.0 than at pH 8.5. Phosphate ions (20 mM) depressed oxidative metabolism at pH 7.0 but increased it at pH 8.5. Fructolysis was stimulated by phosphate ions irrespective of the pH. Phosphate ions decreased the oxidation of lactate and acetate by about the same absolute amount which suggests that it may restrict the entry of acetyl CoA into the Krebs cycle.

Potassium ions (15 mM) increased both fructolysis and oxidative metabolism, and bicarbonate increased the oxygen uptake.

Glucose and fructose were oxidized more readily than acetate, glycerol or sorbitol, but only the polyols significantly increased the oxygen uptake.

INTRODUCTION

While there is abundant literature on the metabolic behaviour of the spermatozoa of several mammalian species, work on human spermatozoa is rather sparse. This has been mainly due to the low density of spermatozoa in the human ejaculate which makes it difficult to make an accurate estimate of metabolic activity.

Ross, Miller & Kurzrok (1941) and MacLeod (1941) have shown that the oxygen consumption of human semen is predominantly associated with the seminal plasma and some workers have doubted the existence of true respiratory activity in human spermatozoa (MacLeod, 1943, 1956; MacLeod & Freund, 1958). On the other hand, MacLeod (1943) and Mann (1951) have shown that human spermatozoa possess components of the cytochrome oxidase system and the latter author suggests that their metabolic pattern is essentially similar to that of other mammalian spermatozoa. The work of Turner (1960) and Nevo (1966) indicates the existence of an actively functioning oxidative system in human spermatozoa and Rothschild (1960) suggests that the use of phosphate buffer in manometric experiments has contributed to the belief that human spermatozoa do not respire.

We have recently been able to obtain large volumes of freshly ejaculated human semen and by concentrating the spermatozoa and using ^{14}C labelled substrates in small manometer flasks, we have been able to measure their oxygen uptake with confidence and to extend knowledge of the metabolic behaviour of the human sperm cell.

MATERIALS AND METHODS

Semen

Semen was supplied by fourteen normal students and the time from collection to the start of the experiments was always less than 2 hr. Bull semen was collected with an artificial vagina.

Diluents

Experiment 2. (1) pH 7.0; no phosphate: 0.123 M-NaCl, 0.005 M-KCl, 0.001 M-MgSO₄ · 7H₂O, 0.205 M-tris [tris-(hydroxymethyl)-amino-methane], 0.023 M-HCl.

(2) pH 7.0; phosphate: 0.092 M-NaCl, 0.005 M-KCl, 0.001 M-MgSO₄ · 7H₂O, 0.025 M-tris, 0.023 M-HCl, 0.012 M-Na₂HPO₄ · 12H₂O, 0.008 M-NaH₂PO₄ · 2H₂O.

(3) pH 8.5; no phosphate: 0.123 M-NaCl, 0.005 M-KCl, 0.001 M-MgSO₄ · 7H₂O, 0.037 M-tris, 0.011 M-KCl.

(4) pH 8.5; phosphate: 0.092 M-NaCl, 0.005 M-KCl, 0.001 M-MgSO₄ · 7H₂O, 0.037 M-tris, 0.011 M-HCl, 0.020 M-NaH₂PO₄.

Where necessary, the buffers were adjusted to the respective pH with NaOH.

Experiment 3. Diluents (1) and (2) as above.

Experiment 4. 0.124 M-NaCl and 0.016 M-sodium phosphate (Umbreit, Burris & Stauffer, 1959) to which 0.015 M-KCl was added in place of the equivalent amount of NaCl.

Experiments 5 and 6. Ca-free Krebs-Ringer phosphate buffer (Umbreit, Burris & Stauffer, 1959).

All diluents contained penicillin and streptomycin sulphate (0.5 mg of each /ml of diluent).

Radio-active chemicals

These were obtained from the Radiochemical Centre, Amersham, England, and were added separately to the diluents to give the desired concentration and radio-activity. The radio-activity of the substrates was checked in a liquid scintillation counter (Nuclear Chicago).

Preparation of spermatozoal suspensions

Ejaculates were pooled and the spermatozoa washed by diluting the semen with 3 volumes of diluent and centrifuging for 10 min at 400 g. After aspirating off all the supernatant, the spermatozoa were washed again and diluted to the appropriate volume with the diluent used for washing.

Incubation of seminal plasma and spermatozoa

Cell-free seminal plasma (1 ml), as in Exp. 1, or suspensions of washed spermatozoa (0.5 ml) plus 0.5 ml of substrate, as in subsequent experiments,

were incubated at 37° C in 6 ml Warburg flasks containing CO₂-free 20% (w/v) KOH in the centre well. The manometers were filled with air and shaken at 114 strokes/min over a period of 3 hr to measure oxygen uptake. The oxidation of substrate was determined from the assay of ¹⁴CO₂ trapped in the KOH in the centre well. The results are expressed as μ atoms of C oxidized and can be converted to μ moles of substrate oxidized by dividing by the number of C atoms in the molecule. Sperm respiration in the presence of CO₂ and bicarbonate (Exp. 6) was studied by using single-side-arm Warburg flasks and the method of Pardee (1949) as modified by Krebs (1951). Appropriate amounts of Krebs' 'CO₂-buffer' (4 M-2,2'-iminodiethanol with 0.1% thiourea) were added to the side-arm to give a 2% CO₂ atmosphere above the fluid after equilibration. The flasks were then gassed for 2 to 3 min with 5% CO₂-95% O₂ and allowed to equilibrate for 10 min with stop-cocks open. The stop-cocks were then closed and a further 15 min allowed for the atmosphere above the fluid in the flask to reach the proper CO₂ concentration. Flasks having no CO₂ in the atmosphere had KOH in the sidearm and were closed the entire 25 min period so that all CO₂ would be removed. For experiments requiring anaerobic conditions, single-side-arm Warburg flasks containing 20% (w/v) KOH in the centre well and yellow phosphorus in the side-arm were gassed for 10 min with nitrogen.

A portion (1 ml) of spermatozoal suspensions before incubation and of the flask contents after incubation were deproteinized with 0.3 N Ba(OH)₂ (0.5 ml) and 5% (w/v) ZnSO₄ · 7H₂O (0.5 ml) and glucose, fructose and lactate estimated in the filtrates. Counts of spermatozoa were made in duplicate and all values expressed on a 10⁸ cells basis over the experimental period. The final spermatozoal concentrations were 1.5 to 3.0 × 10⁸/ml.

Analytical methods

Lactate was determined by the enzymic method of Barker & Britton (1957) and glucose by glucose oxidase (Huggett & Nixon, 1956). Fructose was estimated by the method of Mann (1948) with an incubation period of 20 min for colour development (White, 1959). The amount of substrate utilized was calculated from the amounts in the flask at the start and end of experiments.

Assay of radio-activity

The ¹⁴CO₂ absorbed in the centre well, and removed from there by washing, was precipitated as Ba¹⁴CO₃ in the presence of 5% ammonium chloride. The Ba¹⁴CO₃ was collected by filtration on Whatman No. 542 paper as described by Annison & White (1961) and assayed for radio-activity with an end-window Geiger-Müller tube. The counts were corrected for self-absorption by the method of Hendler (1959).

Statistical analyses

The statistical significance of the results has been assessed by analyses of variance and the residual mean square is given in italics at the base of the variance ratio columns in the tables.

In some experiments the significance of difference between treatments has

been assessed by a *t*-test using the interaction mean square from the analysis of variance to determine standard error (S.E.) of differences between means as presented in these tables.

Where comparisons to be made between the oxidation of labelled substrates could not be decided in advance of performing the experiment (Table 4), the multiple-range test (Duncan, 1955) was used to compare treatment means.

RESULTS

Experiment 1. Oxygen uptake of seminal plasma

In view of early reports that the oxygen consumption of human seminal plasma is characteristically high (Ross, Miller & Kurzrok, 1941; MacLeod, 1941), comparisons were made with bull seminal plasma. Text-fig. 1 shows that

TEXT-FIG. 1. The oxygen uptake of human (○) and bull (●) seminal plasma incubated at 37° C for 3 hr. Values represent the means of six ejaculates for each species.

the oxygen uptake of human seminal plasma (9.2 μl/ml/hr) is more than twice that of the bull (4.6 μl/ml/hr).

Experiment 2. Effect of pH and phosphate on the metabolism of fructose

The pH of human semen obtained from four donors within 3 to 4 min of ejaculation was measured in a pH meter immediately and at regular intervals for 8 hr when the semen was left standing in open tubes (15 mm internal diameter). The initial pH was 7.5 ± 0.25 and remained constant over the 8-hr period at room temperature. Since, however, human spermatozoa show maximum motility at about pH 8.5 (Blackshaw & Emmens, 1951), the rate of metabolism at this pH was compared with that at pH 7.0. The effect of 20 mM-phosphate was also investigated in this experiment.

Table 1 shows that the higher pH significantly reduced the oxidative metabolism of human spermatozoa but increased fructolysis. At pH 7.0, phosphate

decreased oxidative metabolism whereas at pH 8.5 an increase was observed in the presence of phosphate. Phosphate ions, however, increased fructolysis irrespective of the pH of the medium.

Experiment 3. Effect of phosphate on the metabolism of acetate and lactate

To ascertain whether phosphate depressed the oxidation of fructose by inhibiting the oxidation of its breakdown products, the metabolism of lactate and acetate was studied in the presence and absence of 20 mM-phosphate (Table 2).

TABLE 1

EFFECTS OF pH AND PHOSPHATE IONS (20 mM) ON THE METABOLISM OF D-[U-¹⁴C]FRUCTOSE BY WASHED HUMAN SPERMATOZOA

Phosphate (mM)	pH	Oxygen uptake (μl)	Fructose oxidized (μatoms C)	Fructose utilized (μmoles)	Lactate produced (μmoles)
0	7.0	16.3	0.420	0.75	1.34
	8.5	6.9	0.090	0.78	1.45
20	7.0	11.9	0.318	0.93	1.84
	8.5	8.9	0.120	1.16	2.26

SUMMARY OF THE ANALYSES OF VARIANCE

Source of variation	d.f.	Variance ratios			
		Oxygen uptake	Fructose oxidized	Fructose utilized	Lactate produced
Effect of phosphate	1	1.15	1.44	14.60**	43.04**
Effect of pH	1	32.76**	77.18**	3.19	7.15*
Interaction	1	8.45*	4.82	1.78	2.28
Between replicates	2	23.64**	8.69*	7.20*	1.18
Residual	6	3.56	0.0027	0.0163	0.0302

* $P < 0.05$; ** $P < 0.01$.

Values represent the means of three replicates and are calculated/10⁸ spermatozoa/3 hr. 4.5 μmoles, 200 μmc of labelled fructose were added to each flask.

Lactate was more effective than acetate in increasing the oxygen uptake of human spermatozoa and was oxidized at a much faster rate. Phosphate depressed the oxidation of both substrates as judged by the sensitive oxygen uptake and ¹⁴C data although it was not possible to detect any depression of lactate utilization by chemical estimation.

Experiment 4. Effect of potassium on the aerobic and anaerobic metabolism of fructose

Table 3 shows the effect of potassium (15 mM) on the aerobic and anaerobic metabolism of fructose by human spermatozoa.

Potassium ions stimulated both oxidative metabolism and fructolysis, the greatest effects on fructose utilization and lactate production being seen under aerobic conditions.

TABLE 2
EFFECT OF PHOSPHATE IONS (20 mM) ON THE METABOLISM OF [1-¹⁴C]
ACETATE AND [1-¹⁴C]LACTATE BY WASHED HUMAN SPERMATOZOA

Phosphate (mM)	Substrate	Oxygen uptake (μl)	Substrate oxidized (μatoms C)	Lactate utilized (μmoles)
0	[1- ¹⁴ C]Acetate	20.7	0.182	—
	[1- ¹⁴ C]Lactate	26.7	0.581	0.16
20	[1- ¹⁴ C]Acetate	17.1	0.138	—
	[1- ¹⁴ C]Lactate	21.6	0.488	0.17

SUMMARY OF THE ANALYSES OF VARIANCE

Source of variation	d.f.	Variance ratios	
		Oxygen uptake	Substrate oxidized
Effect of phosphate	1	7.48*	10.23*
Effect of substrate	1	10.92**	306.10**
Interaction	1	0.20	1.31
Between replicates	3	6.04*	4.80*
Residual	9	10.04	0.002

* $P < 0.05$; ** $P < 0.01$.

Values represent the means of four replicates and are calculated/10⁸ spermatozoa/3 hr. The amounts of substrate added to each flask were: acetate, 27 μmoles, 200 μmc; lactate, 18 μmoles, 200 μmc.

TABLE 3
EFFECT OF POTASSIUM IONS (15 mM) ON THE AEROBIC AND ANAEROBIC METABOLISM OF D-[U-¹⁴C]FRUCTOSE BY WASHED HUMAN SPERMATOZOA

Potassium (mM)	Gas phase	Oxygen uptake (μl)	Fructose oxidized (μatoms C)	Fructose utilized (μmoles)	Lactate produced (μmoles)
0	Aerobic	15.8	0.299	0.98	1.64
	Anaerobic	—	—	0.90	1.79
15	Aerobic	19.6	0.423	1.10	2.23
	Anaerobic	—	—	1.06	1.96

SUMMARY OF THE ANALYSES OF VARIANCE

Source of variation	d.f.	Variance ratios			
		Oxygen uptake	Fructose oxidized	Fructose utilized	Lactate produced
Effect of potassium	1	8.52	34.25**	19.95**	28.89**
Effect of gas phase	1	—	—	3.75	0.65
Interaction	1	—	—	0.35	8.68*
Between replicates	3	3.57	1.49	6.20*	6.62*
Residual	3	3.39	0.0009	0.004†	0.0196†

* $P < 0.05$; ** $P < 0.01$; †9 degrees of freedom.

Values represent the means of four replicates and are calculated/10⁸ spermatozoa/3 hr. 9 μmoles, 200 μmc of labelled fructose were added to each flask.

Experiment 5. Comparison of rate of oxidation of sugars, polyols and acetate

The rate of metabolism of labelled glucose, fructose, glycerol, sorbitol and acetate by human spermatozoa is shown in Table 4.

Glucose and fructose were oxidized at about the same rate and gave rise to about equal amounts of lactate. Glycerol, sorbitol and acetate were oxidized less readily but only the two polyols produced a statistically significant increase

TABLE 4

METABOLISM OF GLUCOSE, FRUCTOSE, GLYCEROL, SORBITOL AND ACETATE BY WASHED HUMAN SPERMATOZOA

Substrate	Progressive oxygen uptake (μl)			Substrate oxidized ($\mu\text{atoms C}$)	Lactate produced (μmoles)
	1 hr	2 hr	3 hr		
Control	4.9	9.3	11.9	—	—
D-[U- ^{14}C]glucose	5.3	10.4	13.0	0.215	1.89
D-[U- ^{14}C]fructose	4.8	9.0	12.0	0.231	2.02
[1- ^{14}C]glycerol	5.7	11.0	14.2*	0.107	Trace
[U- ^{14}C]sorbitol	5.4	10.7	14.1*	0.131	Trace
[1- ^{14}C]acetate	4.8	9.7	12.6	0.113	—
	S.E. = 0.82				

* Significantly different from control, $P < 0.05$.

Values represent the means of four replicates and are calculated/10⁸ spermatozoa. The amount of substrate added to each flask was: D-[U- ^{14}C]glucose, 9 μmoles , 500 μmc ; D-[U- ^{14}C]fructose, 9 μmoles , 500 μmc ; [1- ^{14}C]glycerol, 18 μmoles , 500 μmc ; [U- ^{14}C]sorbitol, 9 μmoles , 500 μmc ; [1- ^{14}C]acetate, 27 μmoles , 500 μmc .

For the substrate oxidized values, the multiple range test gave the following ranking: glucose = fructose > glycerol = sorbitol = acetate ($P < 0.05$).

TABLE 5

EFFECT OF BICARBONATE ON THE METABOLISM OF GLUCOSE BY WASHED HUMAN SPERMATOZOA

Bicarbonate (mm)	Progressive oxygen uptake (μl)			Glucose utilized (μmoles)	Lactate produced (μmoles)
	1 hr	2 hr	3 hr		
0	4.2	8.3	10.7	1.13	2.11
6.00	5.0	8.7	12.0**	1.20	2.30
	S.E. = 0.13			S.E. = 0.037	S.E. = 0.91

** Highly significantly different from 0 mm bicarbonate. $P < 0.01$.

Values represent the means of three replicates and are calculated/10⁸ spermatozoa. 9 μmoles of glucose were added to each flask.

in oxygen uptake above that of the controls. Trace amounts of lactate could be detected when glycerol or sorbitol were used as substrate but none was present with acetate.

Experiment 6. Effect of bicarbonate on the metabolism of glucose

Sodium bicarbonate (6.00 mm) slightly increased the oxygen uptake of human spermatozoa but had no significant effect on glycolytic activity (Table 5).

DISCUSSION

Our estimate of the pH of freshly ejaculated human semen using a glass electrode agrees with previous published data (Schuermann & Doepfmer, 1960; Mann, 1964; Raboch & Škachova, 1965). We did not, however, find any evidence of a rise in pH on standing, as apparently sometimes occurs with ejaculates of low sperm concentration (see White & MacLeod, 1963). pH papers have, in our experience, proved unreliable and may give values up to 1.0 pH units above that of the glass electrode. The finding that fructolysis proceeds at a faster rate at pH 8.5 than at pH 7.0 may explain the observation of Blackshaw & Emmens (1951) that human spermatozoa show maximum motility under alkaline conditions.

The high oxygen uptake of human seminal plasma compared to that of the bull may be accounted for by the presence of a spermine-diamine oxidase system in human seminal plasma (Zeller, 1941). At all events, spermine occurs in high concentrations in human semen (Mann, 1964) but bull semen contains only a trace. The seminal plasma in human semen consumes about twice as much oxygen as the spermatozoa (9 versus 5 $\mu\text{l/hr/ml}$ of semen). By contrast, the oxygen consumption of the seminal plasma in bull semen is less than a tenth that of spermatozoa (5 versus 70 $\mu\text{l/hr/ml}$ of semen—see Murdoch & White, 1966a). The oxygen uptake of the individual spermatozoon is, however, comparable for the two species, i.e. human, about 5 $\mu\text{l}/10^8$ spermatozoa/hr; bull, about 7 $\mu\text{l}/10^8$ cells/hr. Our results leave little doubt that human spermatozoa, like those of the bull and other mammals examined, have an actively functioning oxidative system since the oxygen uptake of the plasma-free spermatozoa has been accurately measured along with the radio-active $^{14}\text{CO}_2$ produced from labelled substrates.

Human spermatozoa resemble ram and bull in their response to phosphate and potassium (cf. Lardy & Phillips, 1943; White, 1953; Wallace & Wales, 1964; Lodge, Salisbury, Schmidt & Graves, 1963; Wales & Wallace, 1964) and the depressing effect of phosphate on oxygen uptake at pH 7.0 would, as Rothschild (1960) suggests, make respiration more difficult to measure. Since the oxidation of acetate is depressed to about the same extent as lactate, the rate-limiting action of phosphate may be concerned with the entry of acetyl CoA into the Krebs cycle and not merely with inhibition of lactic dehydrogenase (Rothschild, 1960). The stimulatory effect of phosphate on the breakdown of fructose to lactic acid is not unexpected in view of the large number of phosphate intermediates in the Embden-Meyerhof series of reactions. Since potassium stimulates anaerobic as well as aerobic fructolysis in human spermatozoa it must have a stimulatory effect on the Embden-Meyerhof pathway as well probably as Krebs-cycle reactions as also shown in the ram and bull (Dott & White, 1964; Wales & O'Shea, 1966; Murdoch & White, 1966b).

Human spermatozoa clearly oxidize glucose more readily than acetate (Terner, 1960; Minassian & Terner, 1966) and fall into the pattern of other non-ruminant spermatozoa (e.g. dog and rabbit) in this respect (Scott, White & Annison, 1962; Murdoch & White, 1966a). Human spermatozoa, like those of other mammalian species (Mann & White, 1956, 1957; White, 1957; O'Shea &

Wales, 1965), also have the ability to oxidize sorbitol which is present in semen and is metabolized via the glycolytic cycle after conversion to fructose (King & Mann, 1959; O'Shea & Wales, 1965). Only trace amounts of lactate were produced by human spermatozoa, however, when either sorbitol or glycerol was used as substrate. This suggests that the conversion of glycerol to phosphoglycerol and of sorbitol to fructose in human spermatozoa is slow and only just adequate to meet the demands of the Krebs cycle after the conversion products have entered the glycolytic pathway. Since fructose and glucose give rise to a considerable amount of lactate under aerobic conditions glycolysis must proceed at a greater rate than the Krebs cycle reactions in human spermatozoa. The results, however, dispel any doubt that human spermatozoa can oxidize lactate and glycerol (see White & MacLeod, 1963). Ram and bull spermatozoa are also able to oxidize glycerol (White, Blackshaw & Emmens, 1954; O'Dell, Flipse & Almquist, 1956; Mann & White, 1956, 1957; White, 1957) but it seems unlikely that the protective action of glycerol at low temperatures is dependent upon its metabolism by spermatozoa.

The stimulating effect of bicarbonate on the respiration of human spermatozoa confirms the work of Hamner & Williams (1964) although the magnitude of our stimulation was not as great. Bull, rooster and rabbit spermatozoa show a similar response to bicarbonate (Hamner & Williams, 1964; Murdoch & White, unpublished data) and the former authors suggest that the stimulation is due to an increase in the concentration of Krebs-cycle intermediates.

Since the addition of exogenous substrates such as glucose, fructose and acetate failed to increase the oxygen uptake of human spermatozoa above the control, these substances must cause a decrease in the oxidation of endogenous substrate in the spermatozoa, as has been observed in the ram and bull (Scott, White & Annison, 1962). The nature of the endogenous substrate in human spermatozoa is not known with any certainty but is most likely phospholipid.

It is concluded that, in general, the metabolic behaviour of human spermatozoa *in vitro* resembles that of other mammalian species although there may be some quantitative differences.

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