Changes in the Metabolism of Guinea Pig Sperm from Different Segments of the Epididymis (37508)

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The epididymis is the part of the male reproductive tract in which the final steps in sperm maturation and development occur. Most studies of sperm maturation in the epididymis have been concerned with morphological changes that accompany changes in fertilizing capacity. Blandau and Rumery (1) showed that the percentage of rat epididymal sperm capable of fertilizing ova increased from 8% in the caput to nearly 90% in the cauda. In the rabbit, it was observed that fertilizing capacity increased dramatically when sperm passed through a particular segment of the epididymis (2). Fawcett and Hollenberg (3) observed similar changes in acrosome development as sperm passed through the second to fourth segment of the guinea pig epididymis. In view of these findings, it was of interest to determine whether such developmental and morphological changes are accompanied by changes in the metabolic behavior of epididymal sperm.

The purpose of this study, therefore, was to determine the basic patterns of energy metabolism in guinea pig epididymal sperm and to characterize the changes in metabolic behavior associated with sperm passage through various segments of the epididymis.

Methods and Materials. Sperm suspensions. Adult guinea pigs (Carworth, New City, NY) weighing approximately 1 kg were used in all the experiments. The animals were anesthetized (Nembutal, 40 mg/kg) and exsanguinated by partial decapitation. The epididymides were isolated at room temperature in a Tris based salts medium (pH 7.4) (4) containing 1 m*M* glucose.

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The epididymis was separated into 3 segments, caput, corpus and cauda according to macroscopic appearance. Each segment was minced into small pieces to release most of the sperm. The sperm suspension was filtered through a stainless steel filter (100 μm pore size), washed once in the incubating solution, centrifuged at 300*g* for 10 min and resuspended in the buffer. Sperm from the vas deferens were collected by flushing the vasa with the buffer solution. The sperm were washed once, centrifuged and resuspended in the buffer. During these procedures, the medium contained 1 m*M* glucose unless otherwise specified.

Sperm suspensions were counted in a hemocytometer in quadruplicate and the motility was estimated. Smears were taken of each fraction and stained according to Casarett (5). Caput sperm were quite fragile and were easily disrupted and special care in centrifuging and washing was necessary to avoid excessive damage. Microscopic examinations of the smears from all segments revealed no contamination by other cells.

Metabolic experiments. Experiments were performed in stoppered 25 ml Erlenmyer flasks. Incubations were carried out with shaking in a thermostated Dubnoff bath at 37°. For anaerobic experiments, flasks stoppered with rubber septa were flushed with pure nitrogen (99.999%) for 10 min to remove oxygen and to achieve thermal equilibrium. Anaerobic conditions were maintained by slow flushing with nitrogen throughout the experiment. Small portions of the suspensions were taken at various times to determine motility.

Aliquots of the sperm suspensions were removed from the flasks at zero time and after

an incubation interval, they were deproteinized immediately by boiling for 10 min, centrifuged, and the clear solution was then frozen and stored until assay.

Lactic acid and glucose were determined fluorometrically in a Turner Model 111 as previously described (6).

Respiration experiments. Oxygen uptake was measured polarographically using the Beckman Model 160 oxygen analyzer. The electrode was calibrated by estimating the amount of oxygen consumed by glucose oxidase (Glucostat, Worthington Biochemicals) in the presence of a known amount of glucose. Homovanilic acid 1 mg/ml was added to consume peroxide and to drive the reaction to completion (4).

Experiments were carried out by adding 2 ml of sperm suspension to an oxygen chamber and the changes in oxygen tension were recorded with continuous stirring at 37°. Substrates were added with a microsyringe (Hamilton) through a hole in the Teflon sleeve.

All materials used in the experiments were analytically pure and were purchased from Sigma, Baker or Boehringer Mannheim.

Results. Preparation of sperm suspension. The epididymis was divided into three segments according to macroscopic appearance by a method similar to that described by Glover and Nicander (7). The caput was identified as the brownish-pink area of the epididymis characterized by the fat pad and the first appearance of small tubules, the corpus as the area of small narrow tubules and the cauda as the area of large tubules on the distal apex of the epididymis, attached to the gubernaculum.

The total number of cells and the *in vitro* motility of sperm from various epididymal segments are shown in Table I. Sperm content was found to be low in the caput and vas segments and high in the corpus and cauda. Because of the small number of sperm in the caput, it was necessary in most cases to sacrifice 3 or 4 animals and to pool the sperm.

Striking differences were found in the motility of sperm taken from various segments. Sperm motility was poor in the caput, higher in the corpus and highest in the cauda.

TABLE I. Total Sperm Content and Sperm Motility from the Segments of the Epididymis.

Segment	No. of estimations	Sperm content (millions)	Motility (%)
Caput	13	10 ± 1[a]	<10
Corpus	15	87 ± 7	28 ± 4
Cauda	15	99 ± 8	47 ± 3
Vas deferens	10	28 ± 4	41 ± 4

[a] Mean ± SEM.

Rouleaux formation (aggregation of sperm into large bundles with their heads stacked vertically) described by Freund (8) in ejaculated semen and by Fawcett and Hollenberg (3) was found in all sperm preparations from the corpus, cauda and vas deferens. Caput sperm did not form rouleaux.

Glucose metabolism and respiration. Table II summarizes the results of experiments in which the rates of aerobic and anaerobic glycolysis and the rates of sperm respiration from the segments of the epididymis were determined. Observations were made over a 2-hr period since initial experiments had shown that glucose utilization and lactic acid production were linear with time for at least 3 hr of incubation. As Table II shows, there was a large increase in glucose utilization and lactic acid production under aerobic conditions, when sperm passed from the corpus to the cauda segment; both glycolytic parameters, however, declined again in vasa sperm. These changes in the rate of glycolysis are also reflected in changes of the kinetics of glucose utilization by sperm taken from the epididymal segments (Table III). K_m and V_{max} values were calculated using the integrated form of the Michaelis–Menten expression. Both K_m and V_{max} showed increases from caput to cauda with the largest difference occurring between corpus and cauda sperm. Both constants, however, were lower in vasa sperm.

The oxidative nature of epididymal sperm metabolism is evident from the observation that only about 60% of the glucose utilized (Table II) in caput and corpus sperm was

TABLE II. Aerobic and Anaerobic Glycolysis in Sperm from the Segments of the Epididymis.[a]

Segment	Parameter:	Aerobic				Anaerobic	
		ZO_2	ZL	ZG	CZG	ZL	ZG
Caput		58.50[b]	0.420	0.400	0.515	0.280[d]	0.194
		±4.11	±0.035	±0.106	±0.058	±0.098	±0.087
		(6)	(4)	(4)	(4)	(4)	(4)
Corpus		42.00[c]	0.383	0.384	0.465	0.324	0.293
		±4.94	±0.098	±0.040	±0.069	±0.094	±0.070
		(6)	(6)	(6)	(6)	(6)	(6)
Cauda		44.93[c]	1.084[e]	0.814	0.836	0.396[d]	0.329
		±7.42	±0.306	±0.289	±0.242	±0.080	±0.084
		(6)	(6)	(6)	(6)	(6)	(6)
Vas deferens		35.55[c]	5.594	0.508	0.539	0.260	0.264
		±2.63	±0.192	±0.098	±0.094	±0.094	±0.058
		(6)	(6)	(6)	(6)	(6)	(6)

[a] Numbers in parentheses are number of estimations. ZO_2 = μl $O_2/10^8$ sperm/hr; ZL, ZG = μmoles of lactic acid produced or μmoles of glucose utilized/10^8 sperm/hr; CZG = calculated value for glucose utilized based on ZO_2 and ZL values.

[b] Mean ± SEM.

[c] Significantly lower than the caput ZO_2 ($p < 0.05$) by t test.

[d] Significantly lower than the aerobic ZL ($p < 0.05$) by t test.

[e] Significantly higher than the caput ZL ($p < 0.05$) by t test.

converted into the lactic acid that appeared in the medium. A large portion of the glucose taken up was oxidized as reflected in the very high ZO_2 values observed. Calculations of material balance (CZG) showed that virtually all the glucose taken up by sperm could be accounted for in terms of the lactic acid formed and the oxygen consumed. The direction of the magnitude of the respiratory response in the various segments was opposite of that observed when glycolysis was measured. Respiration was highest in caput sperm, declined in the other segments, and was lowest in sperm taken from the vas deferens.

Under anaerobic conditions, glycolysis and motility decreased. This decrease in metabolic activity was noted in sperm taken from all segments of the epididymis, but was particularly striking in sperm taken from the cauda and vas. Glucose utilization could not be entirely accounted for by the appearance of lactic acid in the medium and it is possible that lactate or pyruvate are diverted into other pathways (*i.e.*, acetate) under anaerobic conditions (9). The motility decreased by 20%.

Endogenous respiration was high and similar in magnitude to respiration in the presence of glucose in sperm from all segments

TABLE III. Kinetic Constants for Glucose Utilization by Sperm from the Segments of the Epididymis.[a]

Segment	Sperm conen (millions/ml)	Motility (%)	K_m/(mM)	V_{max} (μmoles/10^8 sperm/hr)
Caput	26.2	<10	0.36	0.23
Corpus	102.0	20	0.44	0.38
Cauda	98.4	50	1.00	1.07
Vas deferens	69.1	40	0.69	0.60

[a] Glucose consumption was followed for 3 hr. Samples were taken every 5 min for the first hour and every 15 min for the following 2 hr.

TABLE IV. Effect of Glucose and Lactic Acid on Endogenous Respiration in Sperm.[a]

Segment	Oxygen uptake		
	Endogenous	+ Glucose (1 m*M*)	+ Lactic acid (3 m*M*)
	ZO_2 (μl O_2/10^8 sperm/hr)		
Caput	77.8 (1)	82 (1)	94.5 (1)
Corpus	42.92 ± 4.53[b] (9)	51.37 ± 9.96 (3)	75.5 ± 13.50[c] (4)
Cauda	36.00 ± 3.79 (6)	42.20 ± 8.73 (4)	68.75 ± 4.75[c] (4)
Vas deferens	21.34 ± 2.35 (5)	33.55 ± 1.95[c] (2)	45.75 ± 10.75[c] (4)

[a] Numbers in parentheses are numbers of measurements.
[b] Mean ± SEM.
[c] Significantly different from endogenous ZO_2 ($p < 0.05$) by t test.

of the epididymis. Endogenous respiration in vas sperm, however, was significantly lower than glucose-supported respiration (Table IV). Respiration was increased only slightly when glucose was added to endogenously respiring cells but was markedly stimulated by the addition of lactic acid (Table IV). Addition of glucose after addition of lactic acid decreased respiration slightly while addition of lactic acid after glucose addition increased respiration significantly.

The addition of the uncoupling agent 2,4-dinitrophenol (DNP), was found to be markedly inhibitory to respiration in sperm from all segments (Table V).

TABLE V. The Effect of 2,4-Dinitrophenol on the Respiration of Epididymal and Vas Sperm.[a]

Segment	Oxygen uptake	
	Endogenous	+ 6×10^{-5} *M* DNP
	ZO_2 (μl O_2/10^8 sperm/hr)	
Caput	127 (1)	83 (1)
Corpus	41.40 ± 6.43[b] (4)	22.58 ± 4.19[c] (3)
Cauda	34.90 ± 5.28 (4)	16.06 ± 4.26[c] (3)
Vas deferens	36.45 ± 3.97 (4)	17.25 ± 4.18[c] (3)

[a] Numbers in parentheses are numbers of measurements.
[b] Mean ± SEM.
[c] Significantly different from endogenous ZO_2 ($p < 0.05$) by t test.

Discussion. The procedure adapted in this study for the isolation of guinea pig epididymal sperm has proven to be a simple and reliable method for obtaining metabolically active, motile spermatozoa from several segments of the guinea pig epididymis. Even caput sperm, known for their poor motility *in vitro* (10), were shown to be metabolically active.

Several findings have emerged from this work. First, the glycolytic apparatus of sperm appears to be relatively undeveloped or inactive in the caput and corpus segments of the epididymis, but rises to high levels of activity in the caudal segment. These changes are reflected in the changes of the kinetic parameters of glucose utilization. The increase in the K_m and V_{max} of glucose uptake may be due to changes in hexokinase or other enzyme activity during epididymal sperm transit such as that noted in the bull (11). The striking increase in glycolytic metabolism as sperm pass from the corpus to cauda segments may be related to the marked changes in fertilizing capacity that are known to occur in the same epididymal segments of other species. Cummins and Orgebin-Crist (12), for example, showed that rabbit spermatozoa isolated from the distal corpus epididymis were capable of fertilizing only half the number of ova fertilized by sperm taken from the ejaculate, but almost four times more than those fertilized by sperm isolated from the proximal corpus. As noted earlier, Blandau and Rumery (1) observed almost a 90% increase in fertilizing ability as sperm passed

from the caput to the cauda epididymis. The function of the cauda epididymis in inducing these changes remains obscure and this should be a fruitful area of future research.

Sperm taken from the vas deferens invariably showed reduced oxidative and glycolytic rates which may be due to the loss of the protective environment of the epididymal duct.

The diminished glycolysis of epididymal sperm under anaerobic conditions is a very unusual feature of the metabolism of these cells, since abolition of respiration ordinarily enhances glycolysis in most tissues. This phenomenon may be related to the observation that dinitrophenol (ordinarily a respiratory stimulant) inhibits oxidation in these cells. Since DNP is also known to stimulate mitochondrial ATPase, it is possible that ATP generated in the mitochondria may be essential for the phosphorylation of hexose and the stimulation of glycolysis, and also for the activation of the fatty acid breakdown involved in endogenous respiration. Although this explanation is somewhat speculative, it receives additional support from the fact that a portion of cellular hexokinase is known to be bound to the mitochondrial membrane in several types of mammalian cells (13). If this binding is extensive in guinea pig sperm it could mean that mitochondrial ATP is needed to initiate glycolytic reactions and thus account for the decreased anaerobic glycolysis.

The rate of respiration observed in caput guinea pig epididymal sperm is the highest yet observed for any species of mammalian sperm. In contrast to the changes in glycolysis, respiration declined as sperm passed from the caput to cauda segments. Since endogenous sperm respiration showed a similar change, it would appear that endogenous reserves, presumably lipids, are present in high concentration in caput sperm. These lipids may serve as a source of energy for sperm during epididymal passage. This idea is supported by the fact that anaerobic glycolysis is low in all segments, which would increase sperm dependence on endogenous reserve nutrients. The depletion of other endogenous reserves, especially adenine nucleotides, may also play a role in activating the glycolytic apparatus (14, 15). This possibility is currently being tested.

The metabolic behavior of guinea pig epididymal sperm can be compared to that of ram epididymal sperm (16) and bull epididymal sperm (17–19). The metabolic rate of ram epididymal sperm (isolated mostly from the cauda segment) resembles that of caudal epididymal sperm from the guinea pig with slight differences in the rates of oxidation and glycolysis. Only a small Pasteur effect was observed in ram sperm. On the other hand, bull epididymal sperm show a large Pasteur effect and have a low rate of aerobic glycolysis which increases markedly when sperm are ejaculated (19). Endogenous respiration in the epididymal bull sperm is low but increases markedly upon ejaculation. Since these data and measurements of P/O ratios indicated a general uncoupling of oxidative phosphorylation during ejaculation in the bull, Lardy, Ghosh and Plaut (17) postulated the existence of a metabolic regulator, released at ejaculation, that induced the uncoupling and diverted mitochondrial energy into ion transport pathways. Recent reports by Hoskins, Stephens and Casilas (14) and Garbers *et al.* (20) suggest that adenylcyclase may be involved in these changes. Our observations indicate that significant metabolic changes also occur in sperm during epididymal transit, prior to ejaculation. Whether further changes are induced after ejaculation in this species is currently being investigated.

Summary. A method has been adapted for the isolation of metabolically active sperm from the caput, corpus and cauda epididymis and vas deferens of the guinea pig. The following changes in *in vitro* sperm metabolism associated with epididymal transit were observed:

1. A significant increase in the glycolytic rate (caput to cauda).

2. A marked decrease in glucose-supported respiration and endogenous respiration (caput to vas deferens).

3. A significant inhibition of sperm respiration by the uncoupling agent dinitrophenol in all segments of the epididymis.

4. A significant decrease in sperm anaerobic glycolysis in all segments of the epididymis.

These findings are discussed in terms of their correlation with known changes in sperm morphology and fertilizing capacity during epididymal transit and in terms of possible physiological mechanisms that ensure sperm survival during epididymal transit.

The authors gratefully acknowledge the technical assistance of Miss Cecil Miller and Miss Ollie Brown.

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Received May 7, 1973. P.S.E.B.M., 1973, Vol. 143.