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Glycolysis by Washed Suspensions of Human Spermatozoa

Effect of Substrate, Substrate Concentration, and Changes in Medium Composition on the Rate of Glycolysis

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The rate of formation of lactic acid from glucose by motile human spermatozoa suspended in plasma-free medium is constant over a 30-fold change in substrate concentration (.001-.03 M) while lactic acid formation from fructose increases less than 2-fold over the same concentration range. The additional observation that glucose-6-phosphate enter cells without prior dephosphorylation and is metabolized to lactic acid at the same maximum rate as fructose and glucose indicates that glycolysis in human sperm is ordinarily not substrate limited. These results also indicate that the fructose levels ordinarily present in semen are sufficient to sustain a high rate of glycolysis even when considerably diluted such as may occur in the female reproductive tract. A transport barrier to sugar influx also does not limit glycolysis since the initial rate of uptake of radioactive glucose into cells continues to increase over a concentration range in which the rate of lactic acid formation is constant. Further, from the observation that the activity of hexokinase in sperm homogenates exceeds the rate of glucose utilization by intact cells, it is possible to conclude that the catalytic capacity of intracellular hexokinase does not limit glycolysis. The sum of these observations indicates that a major rate limiting step (or steps) functions beyond hexokinase. In other experiments aerobic glycolysis was found to be only slightly stimulated by dinitrophenol. Inorganic phosphate also stimulates glycolysis but the magnitude of this effect and the concentration required to produce it are small. The significance of these observations are discussed in terms of the small Pasteur effect exhibited by these cells. A sensitive fluorometric method for determining lactic acid in washed sperm suspensions is also described.

Energy metabolism in human spermatozoa is predominantly a glycolytic process although recent isotope experiments have also established the presence of a small oxidative metabolism (Terner, 1960; Peterson and Freund, 1968; Murdoch and White, 1968). Comparatively few studies on the glycolytic process and its control have been reported due to the low cell numbers in average human ejaculates and the consequent difficulties encountered in measuring small amounts of metabolic end products. We have initiated a study of this metabolism in plasma-free medium using the sensitive fluorometric technique to measure lactic acid production and glycolytic enzyme activities. In this paper we examine the effects of changes in substrate, substrate concentration, and medium composition on the overall rate of glycolysis. Our results lead us to conclude that glycolysis is not ordinarily limited by substrate concentration nor by the rate of substrate transport into the cell. We further conclude that a rate limiting step (or steps) functions beyond the hexokinase step.

MATERIALS AND METHODS

Semen specimens obtained from several donors were pooled before use. The work up of these specimens and the procedure used for preparing plasma-free cell suspensions have been previously described (Peterson and Freund, 1968). The salt medium used in

these studies had the following composition: 0.113 M sodium chloride; 0.020 M tris(hydroxymethylamino) methane (Chloride salt); 0.0125 M monopotassium phosphate; 0.0025 M dipotassium phosphate; 0.003 M magnesium cloride, and 0.0004 M disodium EDTA. The pH was 7.4. This medium provided adequate buffering capacity and was sufficiently low in phosphate concentration to maintain good sperm motility (av 40–50%).

In experiments in which the effects of inorganic phosphate concentration on glycolysis were studied the following changes in medium composition were made: Potassium phosphate was replaced by 0.015 M potassium chloride, sodium phosphate buffer (pH 7.4) was added, and sufficient sodium chloride removed to provide a medium isoosmolar with our standard medium.

Substrates were added to the salts medium at concentrations dictated by the aims of a particular experiment.

In most instances experiments were carried out by adding 1 ml of the washed sperm suspension (0.1–3.0 $\times 10^8$ cells) to stoppered 10-ml Erlenmeyer flasks. Substrates and other compounds dissolved in buffer were then added to give final volumes of 1.5–3.0 ml. Incubations were carried out with shaking in a Warburg bath at 37 C. Warburg flasks (6-ml capacity) attached to horizontal capillary differential syringe manometers (Peterson, Freund, and Gilmont, 1967) were used in several experiments carried out under anaerobic conditions. In these experiments 1–2 ml of the sperm suspension were added to the flask's main compartment and substrate was added to the side arm. A stream of oxygen-free nitrogen was passed through the flask for 10 min before closing the gas vents. Five minutes were allowed for equilibration before substrate was tipped into the main compartment.

Lactic Acid Assay. Lactic acid was estimated enzymatically by measuring the amount of NADH formed when aliquots of cell suspensions were incubated in the presence of lactic dehydrogenase, NAD, and a pyruvate trapping agent. In some early experiments, the increase in optical density at 340 mμ that occurs when NAD is reduced, formed the basis of the assay. Fluorescence measurements were employed in later experiments, however, since they were found to be simpler, more sensitive, and less expensive. The fluorometric procedure we have adopted is a modification of that developed by Loomis (1961) for the determination of serum lactic acid. Duplicate aliquots (0.1 ml) of sperm suspensions were added to fluorometric tubes (1.3 cm ID × 10 cm, matched, pyrex) which were placed in boiling water for 60 sec to stop metabolism. Deproteinization was not necessary because of the small cell concentrations. A 0.6-ml volume glycylglycine-semicarbazide buffer (0.1 M, pH 10, Sigma) containing 1.0 mg NAD and 0.25 mg lactic dehydrogenase (beef heart, Sigma, Type 111) were added to the tubes which were then incubated at 37 C for 90 min. After the incubation period, 4.4 ml pyrophosphate buffer (0.1 M, pH 10, containing 0.35 mg/ml EDTA) were added to all tubes. The fluorescence of samples and standards was read at room temperature in a Turner Model 110 fluorometer. Filters were chosen to provide excitatory light at 375 mμ and to pass emitted light at 470 mμ. The fluorescence of standards was linear over the tested range, 10^{-6} to 10^{-5} M (0.005–0.05 μmoles lactic acid per tube).

In the experiments to be described, single runs were made with each treatment and the average of duplicate determinations on a given sample are reported. This average was reproducible to within 2.3% using the fluorometric assay and to within 1.3% using the spectrophotometric assay.

Glucose and Protein Assays. Glucose was estimated enzymatically with glucose oxidase (Worthington Biochemicals). Protein was determined by the method of Lowry *et al.* (1951).

Homogenate Preparation. Sperm homogenates were prepared by centrifuging (760*g*, 3×) and washing pooled semen three times with 0.25 M sucrose. The packed cells (1.5 ml) were chilled in ice and then added to an ice-jacketed stainless-steel micro cup of a VirTis "23" homogenizer equipped with micro blades. Acid-washed glass beads (0.6 ml, av diam. 100 μ, B. Braum Co., Melsungen, Germany) were added to the suspension which was homogenized at full speed for 100 sec.

The homogenate (which consisted mainly of separated sperm heads, midpieces and tails, and some apparently intact cells) was decanted into a glass centrifuge tube. An additional 0.5 ml of cold sucrose medium was used to rinse the micro cup and this washing was added to the decanted homogenate. Most of the remaining glass beads were removed by a 60-sec spin at minimum speed in a clinical centrifuge.

Hexokinase Assay. Hexokinase activity in sperm homogenates was assayed fluorometrically by following the rate of NADPH formation from NADP in the presence of substrate and glucose-6-phosphate dehydrogenase. Assay tubes for glucose contained: Tris chloride (0.02 M, pH 7.4); potassium phosphate (0.015 M, pH 7.4); magnesium chloride (0.005 M); EDTA (0.0001 M); NADP (0.000012 M); glucose-6-phosphate dehydrogenase (10 units); glucose (0.0005–0.001 M), and 0.05 ml sucrose homogenate (0.22 mg protein). The total volume was 4.55 ml. Assay conditions with fructose as substrate (0.002–0.02 M) were the same except that the sucrose homogenate contained 0.27 mg protein and tubes contained 0.01

mg phosphohexoseisomerase. Prewarmed tubes were placed in a constant temperature sample chamber (37 C) of the Model 110 Turner fluorometer where reactions were initiated by the addition of ATP (0.001 M). Fluorescence changes were measured at half-minute intervals for 10 min and rates were calculated from the linear portion of the resulting curves. All measurements were corrected for the small fluorescence changes occurring in controls (homogenate omitted).

Measurement of Cellular Uptake of Radioactive Glucose. Uptake of radioactive glucose into sperm cells was measured by the technique of rapid Millipore filtration (Britten, Roberts, and French, 1955). Details have been described in a previous report (Peterson and Freund, 1968).

Materials. Reagents were reagent grade or *Grade* A and were purchased from Calbiochem and the Sigma Chemical Company. Purified enzymes were purchased from Sigma. [U—^{14}C] glucose was purchased from Calbiochem.

RESULTS AND INTERPRETATION

Table 1 summarizes experiments in which a number of carbohydrates and glycolytic intermediates were tested as substrates for glycolysis by washed sperm suspensions. In each experiment glucose served as a control substrate and the results are presented as percentages of this rate. Considering the non-phosphorylated hexoses, the pattern of utilization is not unlike that reported for other species (Mann, 1945). Glucose, fructose, and mannose are rapidly metabolized while galactose is metabolized more slowly. An interesting finding was that glucose-6-phosphate is converted to lactic acid as rapidly as either glucose or fructose. Since sperm homogenates lack glucose-6-phosphatase activity the phosphorylated compound apparently crosses the cell membrane intact. If, however, the pH of the suspending medium is raised, glucose-6-phosphate metabolism is retarded and at pH 8.1 it is converted to lactate at less than one-third the rate at pH 7.4 (1.467 vs. 0.450 μmoles/hr/10^8 sperm). Glucose, on the other hand, is metabolized at about the same rate at both pH values. This probably reflects an increase in negative charge on both membrane and phosphorylated substrate under alkaline conditions which would decrease the ability of substrate to penetrate the membrane. We will return to the significance of glucose-6-phosphate metabolism below.

TABLE 1

RELATIVE RATES OF LACTIC ACID FORMATION FROM VARIOUS SUBSTRATES BY WASHED SPERM SUSPENSIONS

Assay conditions were, as follows: Exp. no. 1, 0.51 × 10^8 sperm in 2.1-ml medium, 180-min incubation; exp. no. 2, 1.3 × 10^8 sperm in 1.3-ml medium, 150-min incubation; exp. no. 3, 1.3 × 10^8 sperm in 2.5-ml medium, 90-min incubation; exp. no. 4, 0.5 × 10^8 sperm in 1.4-ml medium, 90-min incubation. All substrates were present at 0.01 M final concentration.

Exp. no.	Substrate	Lactic acid formed (μmoles/hr/10^8 sperm)	% of Control
1	Glucose	1.018	(100)
	Fructose	1.018	100.0
	Mannose	0.860	84.5
	Galactose	0.336	33.0
2	Glucose	1.298	(100)
	Glucose-6-phosphate	1.269	97.8
	Fructose-1,6,-diphosphate	0.616	47.4
	DL-Glyceraldehyde-3-phosphate	0.694	53.5
	Glycerol	0.002	0.2
3	Glucose	1.264	(100)
	Glucose + pyruvate	1.539	121.8
	Pyruvate	1.025	81.1
	Pyruvate + (DNP) (10^{-4} M)	0.547	43.3
4	Glucose	1.004	(100)
	α-Glycerophosphate	0.000	0.0

Other phosphorylated intermediates are more slowly metabolized; in this case penetration and/or cofactor requirements may limit the rate. Lactic acid is not formed in measurable quantities when either glycerol or α-glycerophosphate is added to cell suspensions. Thus, human sperm differ in this respect from ram and bull sperm which readily convert glycerol (presumably via phosphorylation and oxidation) to lactic acid (White, Blackshaw, and Emmens, 1954; Mann and White, 1956, 1957).

The rapid conversion of pyruvic acid to

TABLE 2

EFFECT OF SUBSTRATE CONCENTRATION ON THE RATE OF LACTIC ACID FORMATION FROM GLUCOSE AND FRUCTOSE

Assay conditions were, as follows: Exp. no. 1, 1.08×10^8 sperm in 2.5-ml medium, 60-min incubation; exp. no. 2, 0.70×10^8 sperm in 3.0-ml medium, 210-min incubation.

Exp. no.	Substrate concentration (Molar)	Glucose (μmoles lactic acid/hr/10^8 sperm)	Fructose (μmoles lactic acid/hr/10^8 sperm)
1	0.001	0.967	0.549
	0.005	0.947	0.867
	0.030	0.960	0.920
2	0.010	1.049	1.037
	0.030	1.001	0.972

lactate by sperm suspensions indicates the presence of an active pathway for the regeneration of the reduced pyridine nucleotide needed for continuous pyruvate reduction. This pathway may involve a pyruvate dismutation such as has been found in bull sperm by Terner (1959). Analogous to the reaction in bull sperm, pyruvate reduction in human sperm is inhibited by dinitrophenol.

Effect of Substrate Concentration on the Glycolytic Rate. Table 2 shows the results of experiments which tested the effects of varying the concentration of glucose and fructose on the glycolytic rate. The table shows that lactic acid formation from glucose was essentially constant over a 30-fold increase in glucose concentration (0.001–0.03 M) and that fructose metabolism increased less than two-fold over the same concentration range. In other experiments, lactic acid formation from fructose was essentially constant at concentrations above 0.01 M. In human ejaculates the average fructose concentration approximates 300 mg percent (0.016 M) and the sperm concentration is about 10^8 cells/ml (MacLeod and Freund, 1958). Since the average rate of fructose utilization is only about 0.9 μmoles/hr/10^8 sperm (MacLeod and Freund, 1958), these fructose levels are more than adequate to sustain the maximum fructolytic rate for many hours. It is, therefore, unlikely that fructolysis in semen is ordinarily limited by substrate concentration. In the presence of glucose, substrate limitation would not occur unless the glucose concentration fell below .001 M.

It has been previously observed that when both glucose and fructose are present in human semen, glucose is preferentially utilized (Freund and MacLeod, 1958). This observation was explained by assuming a higher affinity of sperm hexokinase for glucose. Since this could also explain the smaller rate of lactic acid formation from fructose at low substrate levels (0.001 M), we decided to test the explanation directly by examining the kinetics of hexokinase activity in sperm homogenates. The results of these experiments are shown in Fig. 1 where the hexokinase rate is plotted at various substrate concentrations by the Lineweaver-Burk method. The much higher affinity of glucose for hexokinase as compared to fructose is reflected in the

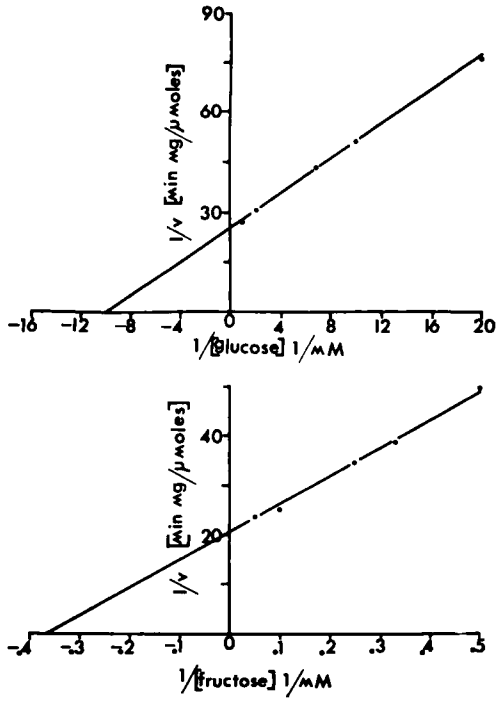

FIG. 1. Kinetics of glucose and fructose phosphorylation by hexokinase in sperm homogenates Assay conditions are described in Methods. Velocity units: μmoles hexose phosphate/min/mg protein.

K_m values estimated from these plots which were found to be 1.02×10^{-4} M and 2.82×10^{-3} M for glucose and fructose, respectively. These values are not too different from those recently reported for bull sperm by Rikmenspoel and Caputo (1968) (0.60×10^{-4} M for glucose and 1.04×10^{-3} M for fructose). However, in the work with bull sperm, calculations were based on the measurement of the rates of fructose and glucose utilization by intact sperm cells over 15-min intervals. The validity of this method is questionable since it assumes that hexokinase limits substrate utilization (transport barriers are absent) and that the rates observed are equivalent to initial rates.

Additional information can be taken from the plot in Fig. 1 if the units for V_{max} are converted to μmoles per 10^8 sperm per hour. Rates were estimated in these units by determining the protein content of sucrose washed intact cell suspensions of known sperm count and then comparing these values with the protein content of homogenates. The results of such analyses, in which duplicate determinations of sperm concentration and protein content were made on nine different pooled and washed specimens, gave an average value of 3.33 ± 0.59 mg protein per 10^8 sperm. In terms of hexokinase activity this conversion factor gives V_{max} values of 7.69 and 9.75 μmoles of sugar phosphorylated per hour per 10^8 sperm for glucose and fructose, respectively. By comparing these values with the data in Table 1, it can be seen that the maximum activity of hexokinase in the homogenate exceeds by more than 10-fold the rate of lactic acid formation in intact cells (1 mole of sugar $\cong$ 2 moles lactic acid). This result means that the *amount* (catalytic capacity) of intracellular hexokinase is in excess and is not a rate limiting factor in glycolysis.

The presence of hexokinase in excess suggested the possibility that glucose metabolism in intact cells was being limited by the presence of a transport barrier to glucose uptake. Evidence against this possibility came

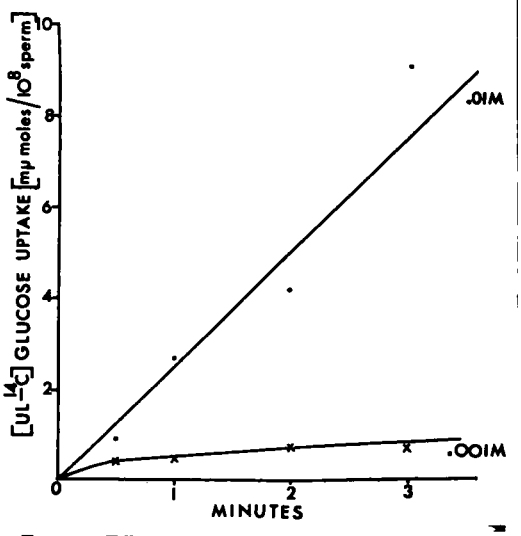

FIG. 2. Effect of substrate concentration on the initial rate of [U—^{14}C] glucose uptake by washed sperm suspensions. Uptake for each time interval was determined by adding 0.4 ml [U—^{14}C] glucose (0.02 M, 55,540 dpm/μmoles or 0.002 M, 1,076,580 dpm/μmoles) in standard medium at 37 C to a 0.4-ml aliquot (0.17×10^8 cells) of a prewarmed washed sperm suspension. At the indicated times the entire suspension was filtered through a 3-μ Millipore filter mounted on an ice-cold, stainless-steel filtration apparatus. Sperm collected on the filters were washed three times with 3-ml portions of ice-cold medium before the filters were dried and counted.

from the results of the experiment shown in Fig. 2, in which the initial rate of [U—^{14}C] glucose uptake into sperm cells at 0.001 M and 0.01 M glucose concentrations is plotted. It can be seen that the uptake of glucose into the cell continued to increase when the substrate concentration was raised 10-fold although the glycolytic rate is the same at both concentrations. This result excludes the rate of transport as a limiting step in glycolysis.

It also appears that a rate limiting step (or steps) functions beyond the hexokinase step. This follows from the observation that glucose and glucose-6-phosphate, which is not acted upon by hexokinase, are converted to lactate at essentially the same rate over a large concentration range and from the evidence mentioned suggesting that glucose-6-phosphate penetrates the cell without being dephosphorylated.

TABLE 3

EFFECT OF INORGANIC PHOSPHATE CONCENTRATION AND ADENINE NUCLEOTIDES ON LACTIC ACID FORMATION

Assay conditions were, as follows: Exp. no. 1, 0.16×10^8 sperm in 1.0-ml medium, 120-min incubation. The medium changes required to maintain iso-osmolarity in this experiment are described in Methods; exp. no. 2, 0.3×10^8 sperm in 1.30-ml medium, 150-min incubation. Glucose concentration in both experiments was 0.01 M.

Exp. no.	Addition	Concentration (Molar)	Lactic acid formed (μmoles/hr/10^8 sperm)
1	Phosphate	0	0.839
	Phosphate	0.001	0.895
	Phosphate	0.005	1.012
	Phosphate	0.015	1.012
	Phosphate	0.040	1.169
2	Control (standard medium)	—	1.243
	Adenosine	0.003	1.308
	Adenosine-5'-phosphate	0.003	1.330
	Adenosine-5'-diphosphate	0.003	1.330
	Adenosine-5'-triphosphate	0.003	1.252

Effect of Medium Changes and Cofactor Additions on Glycolysis. Both inorganic phosphate (P_i) and adenine nucleotides have been implicated in the control of glycolysis in other cells (Racker, 1965) and, in a recent report, Murdoch and White (1968) have shown that in the absence of inorganic phosphate human sperm metabolize glucose at a lower rate. Table 3 shows results from experiments in which the effect of increasing P_i concentrations and the presence of adenine nucleotides on the rate of lactic acid production by sperm were tested. Stimulation by adenine nucleotides and adenosine, if any, was only marginal, while inorganic phosphate caused a 28% increase in the glycolytic rate. However, most of this increase occurred after the addition of a relatively small amount of inorganic phosphate (0.005 M). In other experiments in which the P_i concentration was as high as 0.080 M, the glycolytic rate was never more than 10% above the rate observed at the 0.005 M level. As discussed in the next section, the relatively low P_i concentration needed to maximize the glycolytic rate could be explained by the absence of significant barriers to phosphate entry and also to a limited competition for this ion by respiratory processes in the cell. The latter possibility receives support from the data shown in Table 4 which compares the rates of aerobic and anaerobic glycolysis by sperm suspensions in the presence and absence of 10^{-4} M dinitrophenol. The difference between the anaerobic and aerobic rates of glucose utilization and lactate formation is small and confirms similar observations recently reported by Murdoch and White (1968). Consistent with this small difference is that dinitrophenol, an uncoupler of oxidative phosphorylation, which might be expected to release the respiratory inhibition of glycolysis, causes only a slight stimulation of the aerobic rate of glycolysis.

Finally, in other experiments, we found that the glycolytic rate was not stimulated by a number of changes in medium composition. Bicarbonate addition (0.001–0.005 M) had no effect on the rate. In media in which sodium was completely replaced by potassium and vice-versa, glycolysis was inhibited

TABLE 4

EFFECT OF DINITROPHENOL (DNP) ON GLUCOSE UTILIZATION AND LACTIC ACID FORMATION BY WASHED SPERM SUSPENSIONS

Flasks contained 0.85×10^8 sperm in 1.3-ml medium, 80-min incubation. Glucose concentration 10^{-3} M, dinitrophenol (DNP) 10^{-4} M.

Gas phase	Addition	Glucose utilized	Lactic acid formed
		(μmoles/hr/10^8 sperm)	
Air	Glucose	0.565	1.177
	Glucose + DNP (10^{-4} M)	0.617	1.213
Nitrogen	Glucose	0.649	1.205
	Glucose + DNP (10^{-4} M)	0.617	1.171

by 25% but it was not possible to stimulate glycolysis above the rate occurring in the standard medium by any manipulation of the ratio of these ions. The rate of lactic acid production was increased by 22% after the addition of seminal plasma but this may have been due to the presence of pyruvate in the plasma which, as shown in Table 1, adds to the total lactate produced by cells without stimulating the overall glycolytic process.

DISCUSSION AND CONCLUSIONS

The results of the experiments reported here permit an evaluation of the importance of several potentially limiting mechanisms in glycolysis by human sperm. With regard to the significance of substrate concentration, it was shown that the fructose level ordinarily present in fresh semen is sufficient to support glycolysis at its maximum rate. Further, since the rate of fructose utilization is small, an average of 6–7 hours would be required for metabolism to deplete initial fructose levels to limiting values. This clearly is important when considering sperm survival in the female reproductive tract. There is considerable evidence that in most species sperm and seminal plasma are transported together from the site of deposition to the site of fertilization (Mann, Polge, and Rowson, 1956). Transport is rapid, occurring within a matter of minutes under the force of continuous uterine contractions. Undoubtedly, this mechanism is involved in sperm transport in the human although it has yet to be directly demonstrated. In the absence of dilution by uterine fluids, we have pointed out that the nutritional environment provided by the seminal plasma is adequate to maintain the energy status of the sperm cell for an extended period of time. However, we should add that even if semen were diluted 16-fold by the uterine fluid the fructolytic rate would decrease by less than 2-fold and probably still would be sufficient to meet the prolonged energy demands of the sperm cell. In more extreme circumstances, the ability of sperm to utilize very low concentrations of glucose which may be present in uterine fluid and their ability to metabolize endogenous lipids may assume more important roles in maintaining the energy status of the sperm cell.

The decrease in fructose metabolism that occurs below 0.01 M substrate levels can be attributed to the low affinity of this substrate for sperm hexokinase. This is concluded from the experiments which directly measured the substrate concentration dependence of hexokinase activity in sperm homogenates and from experiments which showed that transport barriers do not limit sugar uptake. The catalytic capacity of sperm hexokinase, however, does not limit glycolysis since the activity of this enzyme in sperm homogenates exceeds the rate of glucose utilization by intact cells. Since exogenous glucose is not restricted in its access to the enzyme *in situ*, hexokinase activity may be lowered by some form of feedback control such as is known to occur in other cells. Supplemental control mechanisms, however, must also operate at other sites in the glycolytic chain in order to account for the observation that the glycolytic rate is not changed when hexokinase is bypassed by using glucose-6-phosphate as substrate.

An important feature of human sperm metabolism is the absence of an appreciable Pasteur effect. This is evident from the results in Table 4 from which a 2.3% Pasteur effect can be calculated. It is also supported by the observation that dinitrophenol, an agent known to release the inhibitory effects of oxidative metabolism on glycolysis, causes only a slight stimulation of the aerobic rate of glycolysis. The absence of a significant Pasteur effect in these cells also provides an explanation for the relatively small stimulation of glycolysis by inorganic phosphate. Wu and Racker (1959) and Racker (1965) have observed that in cells in which phosphate control of glycolysis is important, transport barriers control the flow of this ion from the external medium and perhaps also from competing respiratory compartments. These

workers showed that the uptake of inorganic phosphate was severely restricted in those cells having a pronounced Pasteur effect but was most rapid in cells in which the Pasteur effect was absent. In view of the relatively small concentration of inorganic phosphate needed to optimally stimulate aerobic glycolysis in human sperm and the small magnitude of this stimulation, it would appear that the transport barriers to P_i entry and the competition for this ion by respiratory processes are small.

The factors responsible for the high aerobic glycolytic rate in human sperm remain to be resolved. Although human sperm respire at low rates (Terner, 1960; Peterson and Freund, 1968) it is not likely that a high aerobic glycolysis results from a lower capacity of these cells for oxidative metabolism. If it is assumed that respiration is tightly coupled to ATP formation, an O_2 uptake as small as 2 $\mu l/10^8$ sperm/hr would still be sufficient to produce ATP at a level comparable to that developed by glycolysis and would probably lead to significant competition by both processes for common intermediates.

An interesting alternative explanation is provided by the earlier studies of Lardy (1949) on bull sperm metabolism. This investigator has shown that bull epididymal sperm have a lower aerobic glycolysis and exhibit a more pronounced Pasteur effect than do ejaculated sperm. In order to account for this change, it was suggested that the ejaculatory process releases some type of metabolic regulator that, in effect, uncouples oxidative phosphorylation, releasing the competitive effects of oxidation on glycolysis. Morton (1965) has further suggested that the metabolic regulator acts by diverting high energy respiratory intermediates into transport mechanisms that become active once sperm are ejaculated. This interesting hypothesis may explain the small Pasteur effect observed in ejaculated human sperm but awaits the availability of human epididymal sperm for further testing.

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