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THE ROLE OF GLUCOSE, PYRUVATE AND LACTATE IN ATP PRODUCTION BY RAT SPERMATOCYTES AND SPERMATIDS

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The ATP content of pachytene spermatocytes and round spermatids, isolated from rat testes, was not maintained during incubation of the germ cells in the presence of glucose. Glucose was metabolized via glycolysis at a considerable rate, but the rate of oxidation of the resulting endogenous pyruvate in the mitochondria was too low to support fully ATP production. Exogenous pyruvate (0.25 mM) or exogenous L-lactate (3–6 mM), however, were effective energy substrates. The lactate dehydrogenase reaction in isolated germ cells favoured the rapid conversion of pyruvate to lactate, at the expense of reducing equivalents from mitochondrial NADH. Hence, to support ATP production by the germ cells via mitochondrial metabolism of endogenous pyruvate, a relatively high concentration of exogenous lactate may be essential. In the spermatogenic microenvironment in vivo, such high concentrations of lactate could result from the net production of lactate by Sertoli cells. The mitochondria of the isolated germ cells produced ATP probably at a close to maximal rate, and spermatogenesis therefore may be extremely sensitive to compounds which interfere with mitochondrial energy metabolism and respiratory control.

Introduction

Spermatocytes and round spermatids represent two successive stages in spermatogenesis. Four spermatids arise from one spermatocyte via meiotic divisions after the spermatocytes have completed the prophase of meiosis. The development of germ cells in the germinal epithelium is supported by Sertoli cells [1].

The energy requirements of isolated rat pachytene spermatocytes and round spermatids are not fully supported by glucose. It has been found that exogenous pyruvate and lactate exert an immediate effect on energy-dependent synthetic processes (RNA and protein synthesis) in these isolated germ cells [2,3]. Sertoli cells, on the other hand, metabolize glucose at a high rate via glycolysis and release pyruvate and lactate in vitro [4,5]. Moreover, the rate of conversion of glucose to pyruvate and lactate by Sertoli cells is enhanced by follitropin (FSH), a pituitary hormone which plays an important role in the hormonal control of spermatogenesis [3,6]. These observations have led us to propose that one aspect of the interaction between Sertoli cells and germ cells might be the exchange of pyruvate and lactate [5,7]. In other words, the metabolism of energy-yielding substrates by the different cell types in the germinal epithelium could be one of the most critical factors in germ cell development. To obtain a better in-

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Abbreviation: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

sight in germ cell energy metabolism, we have investigated the metabolism of carbohydrates and the production of ATP by isolated pachytene spermatocytes and round spermatids from rat testes. From the results, we have concluded that the rate of oxidation of endogenous pyruvate (produced via glycolysis) in the mitochondria was too low to fully support ATP production. Moreover, exogenous pyruvate was converted to lactate, at the expense of reducing equivalents from mitochondrial NADH. To support a high rate of ATP production via mitochondrial metabolism of pyruvate by the developing germ cells in vivo, a relatively high concentration of exogenous lactate may be essential.

Materials and Methods

Materials. The following radioactively labelled compounds were purchased from Amersham International, Amersham, U.K.: D-[1-^{14}C]glucose (50–60 Ci/mol), D-[6-^{14}C]glucose (50–60 Ci/mol), D-[5-^{3}H]glucose (10–20 Ci/mmol), [U-^{14}C]pyruvate (15–20 Ci/mol) and L-[U-^{14}C]lactate (161 Ci/mol).

The following radioactively labelled compounds were purchased from New England Nuclear, Boston, MA, U.S.A.: [1-^{14}C]pyruvate (5–20 Ci/mol) and [2-^{14}C]pyruvate (15–20 Ci/mol).

The pyruvic acid standard solution (0.04 mg/ml), the L-(+)-lactic acid standard solution (0.40 mg/ml), carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) and bovine serum albumin fraction V were from Sigma, St. Louis, MI, U.S.A.

Lactate dehydrogenase from pig heart, NAD$^+$-free acid grade I, NADH disodium salt grade I and ATP disodium salt were purchased from Boehringer Mannheim, Mannheim, F.R.G. The luciferin/luciferase mixture (Lumit PM) was from Lumac, Meise, Belgium.

All other chemicals were obtained from commercial sources and were of the highest purity available.

Isolation and incubation of spermatocytes and spermatids. Cell suspensions enriched in either middle and late pachytene spermatocytes, or round spermatids (steps 1–7) were isolated from 32–35-day-old rats (Wistar, substrain RI-Amsterdam). The isolation procedure, and the cellular composition of the two cell suspensions have been described in detail previously [8].

The separation of spermatocytes and spermatids by sedimentation at unit gravity was carried out at room temperature in Dulbecco's phosphate-buffered saline [9] supplemented with 5.6 mM glucose, 1 mM sodium pyruvate and 3 mM sodium L-lactate. The cells were collected from the sedimentation column via an Isco type 6 optical unit connected to an Isco model UA-5 absorbance monitor (Isco Instrumentation Specialties, Lincoln, NE, U.S.A.). The distribution of the cells in the gradient was detected by measuring the light transmission at a wavelength of 340 nm, and the fractions containing spermatocytes or spermatids were pooled. The cells were washed once in phosphate-buffered saline and subsequently washed twice in the medium which was used for the incubations (centrifugations for 5 min at $150 \times g$).

In most experiments, the incubation medium was Eagle's minimum essential medium supplemented with 0.4% (w/v) bovine serum albumin. The energy substrates sodium pyruvate and sodium L-lactate were added as detailed for the individual experiments. Where indicated, glucose was omitted from the Eagle's minimum essential medium, or the cells were incubated in phosphate-buffered saline. The standard incubation temperature was 32°C, but where indicated, temperatures of 37 and 40°C were used. The phosphate-buffered saline was equilibrated with air, and the Eagle's minimum essential medium with 5% CO_2 in air.

Estimation of $^{14}CO_2$ and $^{3}H_2O$ formation. To estimate the rates of incorporation of ^{3}H- or ^{14}C-labelled compounds into $^{3}H_2O$ or $^{14}CO_2$, the spermatocytes ($(1.2–1.6) \cdot 10^6$ cells) and the spermatids ($(3–6) \cdot 10^6$ cells) were incubated in plastic tissue culture flasks (25 cm^2 surface area) in 2 ml of medium. The cells were preincubated for 30 min at 32°C, followed by incubation in the presence of labelled compounds in closed flasks. The amounts of radioactivity added were as follows: 1 μCi [^{14}C]glucose, 2 μCi [^{3}H]glucose, 0.2 μCi [U-^{14}C]pyruvate or [U-^{14}C]lactate, or 0.05 μCi of [^{14}C]pyruvate labelled at carbon-1 or carbon-2. Non-labelled substrate was added to obtain the substrate concentrations given in Results.

At the end of the incubations with ^3H- or ^{14}C-labelled substrates, the amounts of ^3H_2O or $^{14}CO_2$ formed were estimated essentially as described by Robinson and Fritz [4]. All incubations with radioactively labelled substrates were carried out also in the absence of cells, and the data were corrected for these blanks. The production of $^{14}CO_2$ or ^3H_2o was expressed as nmol of substrate converted, calculated as follows: (dpm in ^3H_2O or $^{14}CO_2$/dpm per ml medium) × (nmol substrate/ml medium). The contribution of the pentose phosphate pathway to glucose metabolism was calculated from the rates of ^3H_2O and $^{14}CO_2$ formation as described by Ashcroft et al. [10] by the equation of Katz and Wood [11].

Measurement of cellular ATP content. The spermatocytes ((0.10–0.15) · 10^6 cells) and the spermatids ((0.2–0.3) · 10^6 cells) were incubated for different time intervals in 250 μl Eagle's minimum essential medium, in plastic tubes with an internal diameter of 1 cm. The incubations were terminated at 0°C by addition of 20 μl of 50% perchloric acid, and the tubes were centrifuged for 5 min at 3000 × *g*. Subsequently, 200 μl of the supernatant was transferred to another tube and neutralized by addition of 40 μl of a solution of 0.5 M Tris and 6 M KOH. The precipitate of $KClO_4$ was removed by centrifugation, and the neutralized supernatants were used for the ATP assay. ATP was estimated by the bioluminescent firefly luciferin-luciferase reaction at room temperature, using the LKB-Wallac Luminometer 1250 (LKB Produkter AB, Bromma, Sweden) by measuring the light output of the following mixture: 0.5 ml Tris-EDTA buffer solution (0.1 M Tris, 1.7 mM EDTA, 5 mM magnesium acetate, adjusted to pH 7.75 with acetic acid), 25 μl of the luciferin/luciferase reagent and 10 μl of the neutralized sample. For each sample, the light output was estimated also after addition of an internal ATP standard (0.05 nmol).

Measurement of the rates of pyruvate and lactate consumption or production. The spermatocytes (0.5 · 10^6 cells) and the spermatids (1 · 10^6 cells) were incubated for different time intervals in 2 ml Eagle's minimum essential medium in plastic Petri dishes with a diameter of 4 cm. Glucose and/or pyruvate and/or lactate were included in the medium as indicated in Results. At the end of the incubation, the amounts of lactate and pyruvate in the spent incubation medium were estimated using enzymatic methods, as described by Hohorst [12] and Czok and Lamprecht [13].

Results

ATP content of isolated germ cells

The ATP content of isolated pachytene spermatocytes and round spermatids was increased when the glucose-containing incubation medium was supplemented with lactate (Table I). In particular for the round spermatids, it was observed that the ATP content in the absence of lactate was almost as low as the ATP content which was obtained when the NADH-Q reductase complex of the electron-transport chain was inhibited by rotenone (Table I). Furthermore, the results in Table I show that the spermatocytes and spermatids had exactly the same ATP content after incubation in the presence of lactate for 60 min at 32, 37 or 40°C.

The effect of lactate on ATP production was compared to that of pyruvate, by measuring the ATP content of round spermatids after incubation in glucose-containing medium, supplemented with different concentrations of lactate or pyruvate. The results (Fig. 1) indicate that in comparison to lactate a much lower concentration of exogenous

TABLE I

EFFECTS OF LACTATE AND INCUBATION TEMPERATURE ON THE ATP CONTENT OF ISOLATED SPERMATOCYTES AND SPERMATIDS

The ATP content was estimated after incubation for 60 min at 32, 37 or 40°C. The incubation medium was Eagle's minimum essential medium, supplemented with 6 mM sodium L-lactate. Rotenone (15 μM) was added 30 min after the start of the incubations. The results represent the means ± S.D. of triplicate incubations.

Incubation conditions	*T* (°C)	Spermatocytes ($1 \cdot 10^6$)	Spermatids ($4 \cdot 10^6$)
Glucose	32	6.8 ± 0.5	2.0 ± 0.0
Glucose and lactate	32	14.6 ± 1.0	15.4 ± 0.7
Glucose and lactate	37	14.8 ± 1.2	15.1 ± 1.0
Glucose and lactate	40	14.7 ± 0.1	15.2 ± 0.8
Glucose, lactate and rotenone	32	0.7 ± 0.1	0.3 ± 0.0

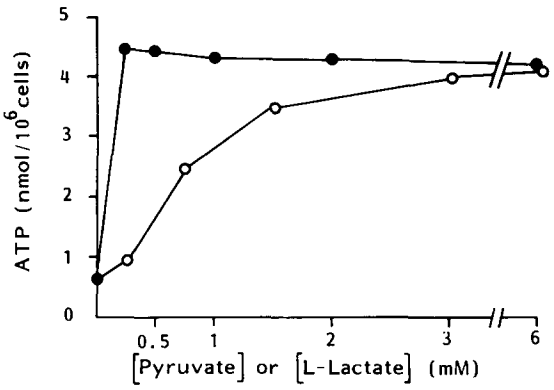

Fig. 1. Effect of substrate concentrations (sodium pyruvate or sodium L-lactate) on the ATP content of spermatids. The ATP content was estimated after incubation for 90 min in Eagle's minimum essential medium (5.6 mM glucose) supplemented with different concentrations of pyruvate (●) or L-lactate (○). The results represent the means of duplicate incubations (the duplicate values differed by maximally 0.5 nmol/10^6 cells).

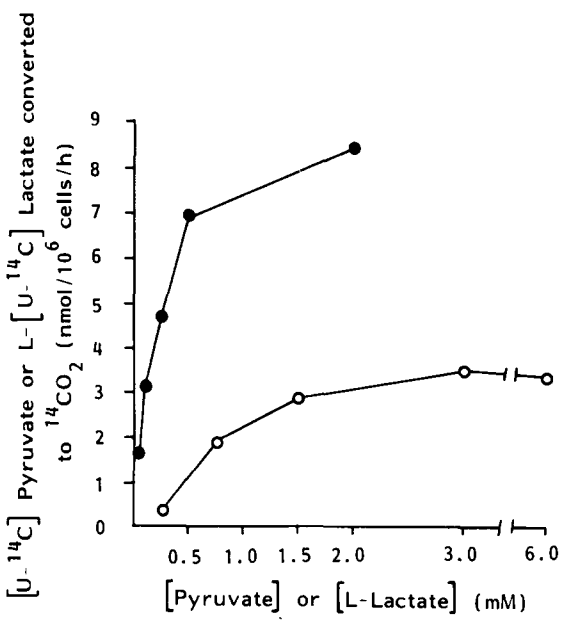

Fig. 2. Rates of conversion of [U-^{14}C]pyruvate or L-[U-^{14}C]lactate to $^{14}CO_2$ at different substrate concentrations. The amount of $^{14}CO_2$ liberated was estimated after incubation of spermatids for 2 h in Eagle's minimum essential medium (5.6 mM glucose) supplemented with different concentrations of [U-^{14}C]pyruvate (●) or L-[U-^{14}C]lactate (○). The results represent the means of duplicate incubations (the duplicate values differed by maximally 0.6 nmol/10^6 cells per h).

pyruvate could maintain the ATP content of the isolated cells.

Rates of mitochondrial oxidation of pyruvate and lactate

The rates of mitochondrial oxidation of L-[U-^{14}C]lactate and [U-^{14}C]pyruvate by round spermatids at different substrate concentrations are shown in Fig. 2. The rate of incorporation of ^{14}C from L-[U-^{14}C]lactate into $^{14}CO_2$ at 3–6 mM sodium L-lactate was much lower than that of [U-^{14}C]pyruvate at 2 mM sodium pyruvate. At these substrate concentrations, however, the same cellular ATP content was obtained (Fig. 1).

The rate of $^{14}CO_2$ production from [U-^{14}C]-pyruvate, at a substrate concentration of 2 mM, was 8.4 nmol/10^6 spermatids per h (Fig. 2). For the same cell preparation, the rates of $^{14}CO_2$ production from [1-^{14}C]pyruvate and [2-^{14}C]pyruvate were 14.2 and 5.4 nmol/10^6 spermatids per h, respectively. This can be explained, because carbon-2 (and carbon-3) of pyruvate, but not carbon-1, are incorporated into pools of citric acid cycle intermediates and subsequently into CO_2 after one or more complete turns of the citric acid cycle. Moreover, carbon-2 (and carbon-3) of pyruvate could be incorporated into acetyl-CoA and subsequently into lipids.

Reduction of pyruvate to lactate

During incubation of spermatocytes and spermatids in medium containing 1 mM pyruvate, pyruvate was converted to lactate, and lactate was released into the incubation medium (Table II). This metabolism of exogenous pyruvate resulted in a ratio lactate produced/pyruvate consumed in the range of 0.55–0.65. In other words, from 10 mol pyruvate consumed approx. 6 mol was converted to lactate and not more than 4 mol was oxidized in the mitochondria. The rate of reduction of exogenous pyruvate to lactate was not significantly altered after addition of glucose (Table II). Apparently, NADH formed from NAD^+ by mitochondrial oxidation of pyruvate was reoxidized not only via the electron-transport chain, but also at the conversion of other pyruvate molecules to lactate. This observation could explain the results presented in Fig. 2, where it was shown that the rate of [U-^{14}C]pyruvate oxidation

TABLE II

PYRUVATE CONSUMPTION AND LACTATE PRODUCTION BY SPERMATOCYTES AND SPERMATIDS

The amounts of pyruvate and lactate in the spent incubation media were estimated after 20 h of incubation of the cells in Eagle's minimum essential medium, supplemented with 2 mM sodium pyruvate, either in the absence or in the presence of 5.6 mM D-glucose. The results represent the means ± S.D. of quadruplicate incubations and are expressed as nmol/10^6 cells per 60 min.

	Pyruvate consumed	Lactate produced
Spermatocytes		
without glucose	169 ± 3	91 ± 6
5.6 mM glucose	169 ± 3	93 ± 2
Spermatids		
without glucose	52 ± 1	33 ± 1
5.6 mM glucose	48 ± 1	30 ± 1

was much greater than that of L-[U-^{14}C]lactate.

Furthermore, it was observed that the pyruvate pool from the incubation medium (1 mM at the start of the incubation, no lactate added) could be exhausted by spermatocytes, until the pyruvate concentration was below the detection limit (0.01 mM). At that point, the concentration of lactate in the spent incubation medium was 0.53 ± 0.01 mM (± S.D., eight incubations). No pyruvate was detected in the spent incubation medium of spermatocytes and spermatids (detection limit 0.01 mM) after incubation of the germ cells for 20 h in medium supplemented with 6 mM L-lactate.

The pyruvate dehydrogenase complex can attain its maximal activity

The lactate dehydrogenase reaction and the electron-transport chain may compete for the reducing equivalents from NADH. Hence, we investigated the effects of an inhibitor of the electron-transport chain (rotenone) and uncoupling agents which cause a loss of respiratory control (2,4-dinitrophenol and FCCP) on the metabolism of pyruvate by germ cells. These experiments were performed only with spermatocytes during short-term incubations. Spermatids could not be used for such experiments, because damage was observed in spermatids (phase-contrast microscopy) shortly after addition of compounds which inhibit ATP synthesis (or during short-term incubation in the absence of pyruvate and lactate).

Inhibition of the NADH-Q reductase complex of the electron-transport chain by rotenone resulted in a ratio lactate produced/pyruvate consumed of 0.76 (Table III). A ratio of 0.8 is the maximal ratio that could be expected, when 4 mol NADH is produced from complete mitochondrial oxidation of 1 mol pyruvate and all mitochondrial NADH is subsequently oxidized via reduction of 4 mol pyruvate to 4 mol lactate (a total amount of 5 mol pyruvate is consumed to produce 4 mol lactate). The uncoupling agents 2,4-dinitrophenol and FCCP caused inhibition of the reduction of pyruvate to lactate (ratio b/a in Table III). Moreover, the loss of respiratory control induced by FCCP resulted in an increased rate of oxygen consumption by spermatocytes (Fig. 3). These results indicate that the rate of reduction of pyruvate

TABLE III

EFFECTS OF ROTENONE, 2,4-DINITROPHENOL AND FCCP ON PYRUVATE CONSUMPTION AND LACTATE PRODUCTION BY SPERMATOCYTES

The amounts of pyruvate and lactate in the spent incubation media were estimated after 150 min of incubation. The incubation medium (Eagle's minimum essential medium) did not contain glucose, but was supplemented with 1 mM sodium pyruvate. Rotenone (15 μM), 2,4-dinitrophenol (50 μM) or FCCP (0.5 μM) were added at the start of the incubations. The results represent the means ± S.D. of triplicate incubations.

Additions	Pyruvate consumed (a) (nmol/10^6 cells per 60 min)	Lactate produced (b) (nmol/10^6 cells per 60 min)	$a - b$	Ratio b/a
None	205 ± 21	130 ± 17	75	0.63
Rotenone	89 ± 6	68 ± 10	21	0.76
Dinitrophenol	102 ± 6	27 ± 6	75	0.26
FCCP	94 ± 8	16 ± 3	78	0.17

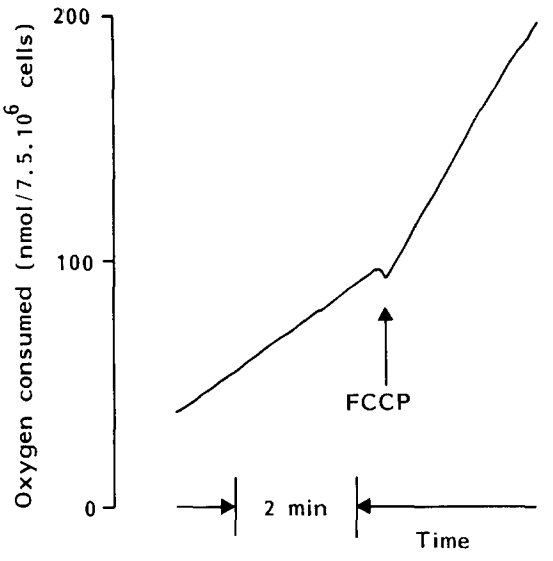

Fig. 3. Rate of oxygen consumption by spermatocytes before and after addition of 0.5 μM FCCP. The spermatocytes ($7.5 \cdot 10^6$) were incubated at 32°C in 2 ml phosphate-buffered saline supplemented with 1 mM sodium pyruvate.

to lactate (involving oxidation of NADH) decreases when the rate of oxidation of NADH via the electron-transport chain is increased.

The rate of incorporation of [1-^{14}C]pyruvate into $^{14}CO_2$ represents oxidative decarboxylation of pyruvate via the mitochondrial pyruvate dehydrogenase complex. The results of this direct estimate of the rate of decarboxylation (Table IV) were similar to the results of the indirect estimate, calculated from the overall rate of pyruvate consumption and the rate of lactate production ($a - b$ in Table III). The results presented in Tables III and IV demonstrate that the activity of the pyruvate dehydrogenase complex was not enhanced in the presence of 2,4-dinitrophenol or FCCP. Hence, during incubation in the presence of pyruvate, the pyruvate dehydrogenase complex in the isolated germ cells probably has attained its maximal activty. This maximal activity was observed in the absence as well as in the presence of glucose (Table IV). Arsenite, an inhibitor of the pyruvate dehydrogenase complex, caused strong inhibition of $^{14}CO_2$ production from [1-^{14}C]pyruvate (Table IV).

TABLE IV

CONVERSION OF [1-^{14}C]PYRUVATE TO $^{14}CO_2$ BY SPERMATOCYTES AND SPERMATIDS

The amount of substrate converted to $^{14}CO_2$ was estimated after incubation of the isolated cells for 2 h in phosphate-buffered saline containing 1 mM sodium [^{14}C]pyruvate, in the presence or absence of 5.6 mM glucose. 2,4-Dinitrophenol (50 μM) or sodium meta-arsenite (1 mM) were added at the start of the incubation. The results represent means ± S.D. of 1–4 different cell preparations (duplicate or triplicate determinations for each cell preparation).

Additions	[1-^{14}C]pyruvate converted to $^{14}CO_2$ (nmol/60 min)	
	Spermatocytes ($1 \cdot 10^6$)	Spermatids ($4 \cdot 10^6$)
None	68 ± 13	61 ± 10
Glucose	69 ± 13	60 ± 13
Dinitrophenol	74 ± 20	
Arsenite	4	

Metabolism of glucose

Glucose had no effect on the oxidation of pyruvate and the conversion of pyruvate to lactate, and glucose failed to maintain the ATP content of the isolated germ cells. Apparently, glucose has no important role in the energy metabolism of the spermatogenic cells. If glucose metabolism by isolated germ cells is estimated in the absence of pyruvate and lactate, inaccurate data may be obtained (because under these conditions, the cellular ATP content is very low). However, we have tried to estimate the rate of glucose metabolism during short-term incubations (2 h) by spermatocytes (but not by spermatids, for reasons described above).

The overall rate of glucose utilization by spermatocytes (formation of ^3H_2O from [^{3}H]glucose) was significant, and in addition carbon-1 and carbon-6 of [^{14}C]glucose were incorporated into $^{14}CO_2$ at a low rate (Table V). Overall utilization of glucose at a rate of 10.9 nmol/10^6 spermatocytes per h (Table V) could result in formation of maximally 20–22 nmol pyruvate/10^6 cells per h, whereas oxidation of carbon-6 of glucose at a rate of 2.7 nmol/10^6 spermatocytes per h (Table V) equals oxidation of carbon-3 of pyruvate at a rate of maximally 5.4 nmol/10^6 cells per h. The results would indicate, that only one out of four pyruvate molecules originating from glucose was completely

TABLE V

CONVERSION OF [^{3}H]GLUCOSE OR [^{14}C]GLUCOSE TO ^3H_2O OR $^{14}CO_2$ BY SPERMATOCYTES AND SPERMATIDS

The amounts of glucose converted to ^3H_2O or $^{14}CO_2$ were estimated after incubation of the isolated cells for 2 h in phosphate-buffered saline containing [^{3}H]glucose or [^{14}C]glucose (5.6 mM), in the presence or absence of 1 mM sodium pyruvate. The results (nmol/10^6 or $4 \cdot 10^6$ cells per 60 min) represent two (single values), three or four (means ± S.D.) different cell preparations. The values for each cell preparation were determined in duplicate or triplicate incubations, and the number of cell preparations used is indicated between brackets.

Cell type	Substrates added	Glucose converted to ^3H_2O or $^{14}CO_2$			Glucose metabolized via the pentose phosphate pathway
		[5-^{3}H]glucose	[1-^{14}C]glucose	[6-^{14}C]glucose	
Spermatocytes ($1 \cdot 10^6$)	glucose	10.9 ± 1.0(4)	2.8 ± 0.6(3)	2.7 ± 0.5(3)	0.1 ± 0.0(3)
Spermatocytes ($1 \cdot 10^6$)	glucose + pyruvate	2.6 ± 0.2(3)	1.6; 1.7(2)	0.1; 0.1(2)	0.8; 1.0(2)
Spermatids ($4 \cdot 10^6$)	glucose + pyruvate	8.9 ± 1.3(3)	0.5; 0.8(2)	0.1; 0.2(2)	0.2; 0.2(2)

oxidized via the citric acid cycle. However, a somewhat larger amount of endogenous pyruvate may have been decarboxylated via the pyruvate dehydrogenase complex (as described above with respect to the metabolism of exogenous [^{14}C]pyruvate). In addition, pyruvate originating from glucose may have been converted to lactate.

After addition of exogenous pyruvate (1 mM), the overall rate of glucose utilization (^3H_2O from [5-^{3}H]glucose) by spermatocytes was decreased 4-fold (Table V). This inhibitory effect of pyruvate may result from inhibition of regulatory enzymes of the glycolytic pathway when the cellular ATP content is high. In spermatids, glucose was metabolized via glycolysis at a considerable rate in the presence of exogenous pyruvate (Table V).

In the absence of exogenous pyruvate, the rate of oxidation of [1-^{14}C]glucose by spermatocytes was equal to the rate of oxidation of [6-^{14}C]glucose, and virtually no glucose was oxidized via the pentose phosphate pathway (Table V). After addition of exogenous pyruvate, the rate of incorporation of [6-^{14}C]glucose into $^{14}CO_2$ by spermatocytes and spermatids was very low, because the exogenous non-labelled pyruvate caused dilution of the endogenous [3-^{14}C]pyruvate (which is produced from [1-^{14}C]- and [6-^{14}C]glucose). Production of $^{14}CO_2$ from [1-^{14}C]glucose, in contrast, was observed also in the presence of exogenous pyruvate, in particular for spermatocytes. The results in Table V indicate that under these incubation conditions, a significant amount of glucose was metabolized by spermatocytes via the pentose phosphate pathway.

Discussion

Several observations on pyruvate and lactate metabolism by round spermatids from rats, as reported earlier by Mita and Hall [14] are in disagreement with the present results. The most significant differences concern the observations of Mita and Hall [14] that pyruvate could not support ATP synthesis, and that the ATP content of spermatids incubated in the presence of lactate was approx. 1 nmol/10^6 cells (as compared to approx. 4 nmol/10^6 spermatids in the present experiments). This disagreement might be explained, because Mita and Hall [14] isolated the spermatids at 4°C and incubated the cells at 37°C, whereas it has been reported that rat spermatids which were isolated at 4°C and subsequently incubated at 37°C had lost respiratory control and had a low ATP content [15]. In the present experiments, the cell separation procedure was carried out at room temperature (20–24°C), and the spermatocytes and spermatids maintained a high ATP content at 32, 37 and 40°C. In rats, there is a body-testis temperature gradient of about 3.5°C [16], and in all other experiments the isolated germ cells were incubated at 32°C.

Glucose cannot fully support the energy requirements of the isolated germ cells. Hence, the rate of metabolism of glucose via glycolysis could be too low. Nakamura et al. [17] have suggested that glycolysis in round spermatids from rats is inhibited at the glyceraldehyde-3-phosphate dehydrogenase reaction, as a result of inhibition of this enzyme by AMP and ADP. Nevertheless, lactate

had accumulated in these spermatids (final concentration 36 nmol/10^6 cells) during incubation of the cells for 1 h in the presence of 10 mM glucose [17]. The present results show, that glucose was metabolized via glycolysis at a considerable rate by spermatocytes and spermatids (in the absence or presence of exogenous pyruvate, respectively), but the resulting endogenous pyruvate was not oxidized completely. In isolated spermatogenic cells, the transport of reducing equivalents from cytosolic NADH into the mitochondrial matrix via a shuttle system may occur at a rate which is too low to maintain a low NADH/NAD^+ ratio in the cytosol. This might result in endogenous pyruvate (from glucose) being converted to lactate in the cytosol, rather than being oxidized in the mitochondria.

In spermatocytes and spermatids utilizing exogenous pyruvate, the lactate dehydrogenase reaction and the electron-transport chain were competing for reducing equivalents from NADH, so that cytosolic NAD^+ was reduced at the expense of mitochondrial NADH. The shuttle system by which reducing equivalents from mitochondrial NADH are carried into the cytosol in the germ cells is not known. The malate-aspartate shuttle tends to operate unidirectionally to oxidize cytosolic NADH [18], but might be used also to reduce cytosolic NAD^+. In this respect, it could be of interest that spermatocytes and spermatids contain an isoenzyme of lactate dehydrogenase which is composed of germ cell-specific C subunits (LDH-C_4 or LDH-X) [19,20]. The suggestion that LDH-C_4 is located in the mitochondrial matrix [21] has been adopted by many authors. Intramitochondrial localization of LDH-C_4 would allow the NADH formed from pyruvate oxidation to be used for the reduction of other pyruvate molecules to lactate in the mitochondrial matrix [22]. Moreover, direct intramitochondrial oxidation of exogenous lactate via LDH-C_4 [23] would make a shuttle system for reoxidation of cytosolic NADH redundant. The observations regarding the localization of LDH-C_4 in the mitochondrial matrix, however, are still controversial and it is certain that most of the LDH-C_4 is not associated with the mitochondrial matrix [24].

The present results indicate that the lactate dehydrogenase reaction in the isolated germ cells favours the rapid conversion of endogenous or exogenous pyruvate to lactate. A high concentration of exogenous lactate apparently is required for spermatocytes and spermatids to use endogenous pyruvate as the predominant energy-yielding substrate. A possible supply of lactate from the circulation may not be sufficient to fully support ATP synthesis by the germ cells, since the concentration of lactate in rat blood is approx. 1 mM [25], whereas a lactate concentration of 3–6 mM was required to maintain the ATP content of isolated germ cells. Hence, net production of lactate via glycolysis by Sertoli cells could be essential to raise the concentration of lactate in the spermatogenic microenvironment. Therefore, the present results lend further support to the hypothesis that stimulatory effects of hormones (follitropin, insulin) on the rate of glycolysis by Sertoli cells are a prerequisite to maintain a large population of developing germ cells [3,26].

The rate of $^{14}CO_2$ formation from [1-^{14}C]pyruvate (60–70 nmol/10^6 spermatocytes or $4 \cdot 10^6$ spermatids, which gives an estimate of the maximal activity of the pyruvate dehydrogenase complex) was approx. 4-times lower in spermatids than in spermatocytes. One spermatocyte gives rise to four spermatids via the meiotic divisions, and the results would indicate that there is no increase of the number of pyruvate dehydrogenase complexes towards or after completion of the meiotic divisions. The ATP content of the isolated cells (approx. 15 nmol/10^6 spermatocytes or $4 \cdot 10^6$ spermatids) was proportional to the maximal activity of the pyruvate dehydrogenase complex. In other words, spermatocytes and spermatids may produce ATP in the mitochondria at a close to maximal rate. In this respect, it is of interest that spermatogenesis in vivo is impaired after inhibition of the citric acid cycle. Fluoroacetate, which is converted to fluorocitrate and then inhibits the enzyme aconitase, can induce regression of the germinal epithelium at a sublethal dose [27]. Furthermore, it has been observed that gossypol, a compound which exerts strong detrimental effects on developing germ cells as well as spermatozoa and which is currently being tested as a male contraceptive, can act as an uncoupling agent on oxidative phosphorylation in different cell types [28]. The more or less specific effects of fluoro-

acetate and gossypol on spermatogenesis in vivo may be related to a high sensitivity of spermatogenic cell types, as compared to other cell types, to compounds which interfere with mitochondrial energy metabolism and respiratory control.

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