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Metabolism of Glucose by Sertoli Cells in Culture

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ABSTRACT

Overall rates of glucose utilization at physiological concentrations (5.5 mM) by primary cultures of rat Sertoli cells have been estimated to be 445 nmol/mg cell protein/h when determined by rates of incorporation of 5-[^{3}H]-glucose into [^{3}H]-H_2O, and 486 nmol/mg protein/h when determined by rates of incorporation of U-[^{14}C]-glucose into labeled products (CO_2, anions, lipids, and glycogen). Of total U-[^{14}C]-glucose utilized, 2.9% was converted to [^{14}C]-CO_2; 95.8% was converted to [^{14}C]-anions, most of which could be accounted for as lactate; and the remainder was incorporated into lipids and glycogen. About half the [^{14}C]-CO_2 formed from labeled glucose was calculated to be derived from oxidation via the pentose phosphate pathway. In the presence of the artificial electron acceptor phenazine methosulfate, the rate of conversion of 1-[^{14}C]-glucose to [^{14}C]-CO_2 was increased over 10-fold, whereas the incorporation of 6-[^{14}C]-glucose into [^{14}C]-CO_2 was not changed. Under these conditions, over 33% of total glucose utilized by Sertoli cells could be accounted for by the pentose phosphate pathway. However, this potential was not utilized in the absence of the artificial electron acceptor. Neither the overall rates of glucose utilization nor the metabolic fate of glucose was appreciably influenced by treatment of cells with follicle stimulating hormone, insulin, or dibutyryl cyclic AMP under any conditions investigated. The possible physiological importance of the high rate of lactate production by Sertoli cells was considered in relation to the role of lactate as a substrate for germinal cells in the adluminal compartment of the seminiferous tubule.

INTRODUCTION

Rates of glucose utilization and metabolism by testis preparations have been extensively investigated (Free, 1970; Setchell, 1978). However, only limited information is available on carbohydrate metabolism by isolated preparations of testicular cells, with the exception of spermatozoa (Voglmayer et al. 1966, 1967) and spermatids (Nakamura et al., 1978). Contributions of spermatogonia, spermatocytes, and somatic cells to overall rates of glucose metabolism by whole testicular or seminiferous tubule preparations have been largely deduced or inferred. For example, stimulation of incorporation of labeled amino acids into protein by glucose is greater in testes from mature rats than in preparations from sexually immature animals (Means and Hall, 1968), from which it has been concluded that advanced germinal cells (spermatids) probably utilize glucose at higher rates than the other types of testicular cells do and that spermatids may require glucose as an energy source. Other data obtained on testes from hypophysectomized or cryptorchid rats are consistent with these interpretations (Free, 1970).

Nakamura et al. (1981) have reported that lactate and pyruvate enhance the incorporation of labeled leucine into protein by spermatids by nearly tenfold, whereas the stimulation by glucose is only twofold. Similarly Jutte et al. (1980) have shown that addition of lactate but not glucose resulted in enhanced rates of respiration, protein synthesis, and RNA synthesis by isolated pachytene spermatocytes or round spermatids. Further, lactate was more effective than glucose in preventing degeneration of germinal cells in culture (Jutte et al., 1980).

Levels of glucose in seminiferous tubular fluid are very low (Voglmayr et al., 1966). In several mammalian species, the ratios of glucose concentrations in tubular fluid to those in plasma are less than 0.02, while the concentration of glucose in ram testicular lymph is ∿75% of that in plasma (Setchell, 1970; Setchell and

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Waites, 1975). These data indicate that glucose is readily available to cells in the basal compartment of the seminiferous tubule, but not to germinal cells in the adluminal compartment. Nonmetabolizable sugars, such as 3-0-methylglucose, readily penetrate the seminiferous tubule barrier to enter the adluminal compartment (Middelton, 1973; Middelton and Setchell, 1972). This suggests that low levels of glucose in tubular fluid do not result from an inability of sugar to penetrate the testis barrier, but result instead from the metabolism of glucose to various products by one or more cell types in the seminiferous tubule. Lactate is present in rete testis fluid at a concentration about half that in plasma (Setchell and Waites, 1975). The combined data are compatible with the possibility that advanced germinal cells in the adluminal compartment may be forced to use a substrate other than glucose as an energy source and that lactate is one such potential substrate.

We have previously demonstrated that Sertoli cells possess the enzymes required for the biosynthesis of myoinositol from glucose and that Sertoli cells in culture in a chemically defined inositol-free medium secrete inositol (Robinson and Fritz, 1979). In this communication, we present data showing the overall rates of utilization of glucose by Sertoli cells in culture under defined conditions and the percentages converted to lactate, CO_2, lipids, glycogen, and nonidentified anions. The very high rates of production of lactate by Sertoli cells to be documented may be of physiological importance in supplying substrate to germinal cells in the adluminal compartment.

MATERIALS AND METHODS

Cell Preparation and Culture Conditions

Sertoli cells, prepared from testes of 20-day-old Wistar rats by procedures previously described (Dorrington et al., 1975; Louis and Fritz, 1977), using mechanical agitation for the final step to obtain small aggregates, were cultured in serum-free modified Eagle's minimum essential medium (MEM). The cells were plated in Falcon petri dishes (60 × 15 mm) for tritiated glucose studies, or in Falcon tissue culture flasks with canted necks (64 × 38 mm) of 25 cm^2 growth area for [^{14}C]-glucose studies. Approximately 3–6 × 10^6 cells were plated per flask, equivalent to 0.2–0.4 mg cell protein per vessel, and corresponding to 17–33 μg DNA. Cells were cultured for 48 h at 32°C in a gas phase of 95% air:5% CO_2, with a change in fresh MEM after the initial 24 h. After the second 24 h of culture, experiments were performed on cells incubated under conditions described below to determine rates of glucose metabolism.

Analytical Determinations

Rates of total glucose utilization. Total glucose utilization by monolayers of Sertoli cells was estimated by determination of the rates of formation of tritiated water from glucose labeled with [^{3}H] either at carbon 2 (Hammerstedt, 1975) or at carbon 5 (Ashcroft et al., 1972). Incubations were carried out in 2 ml medium containing 5-[^{3}H]- or 2-[^{3}H]-glucose at concentrations specified in Table 1. Cells were incubated at 32°C at time intervals indicated, after which the medium was transferred into tubes containing 200 μl of 0.5 M HCl and 100 μl of 0.01% merthiolate solution. The cells were washed with Hanks' solution, the wash (0.1 ml) was added to the acidified incubation medium, and the total solution was brought to a known volume. Aliquots were allowed to equilibrate with 500 μl distilled water at 55°C for 48 h in glass counting vials (Ashcroft et al., 1972). The radioactivity in the water was determined by standard methods with a Nuclear Chicago model (ISOCAP/300) scintillation spectrometer, using Aquasol (10 ml) as scintillation fluid, and corrected for quenching. Blank samples consisted of flasks which contained no Sertoli cells, or which contained Sertoli cells acidified prior to addition of labeled substrate. Amounts of [^{3}H]-H_2O chemically formed from 5-[^{3}H]- or 2-[^{3}H]-glucose were also determined at zero time when intact cells were present. We calculated the final conversion rates of [^{3}H]-H_2O formation from the corrected dpm after subtracting these blanks. Total rates of utilization of glucose were determined in Sertoli cells maintained under basal conditions, and also in cells cultured or incubated in the presence of hormones [follicle stimulating hormone (FSH) or insulin], or dibutyryl cyclic AMP (dbcAMP), as indicated in the tables in Results.

Rates of Conversion of U-[^{14}C]-Glucose to [^{14}C]-CO_2

Specified concentrations of uniformly labeled [^{14}C]-glucose were added to Falcon tissue culture flasks in which Sertoli cells had been previously cultured for 48 h in serum-free MEM. Flasks containing these cells and MEM without bicarbonate were sealed with serum stoppers, fitted with suspended plastic center wells, and were flushed with oxygen and incubated at 32°C. At the end of time intervals indicated in the Tables, 50 μl hyamine hydroxide (0.5 M solution in methanol) were injected into center wells, followed by injection of 200 μl of 1 M HCl into the incubation medium to terminate the reaction and to release carbon dioxide from solution. Labeled carbon dioxide generated was trapped by the hyamine hydroxide during a subsequent incubation of sealed flasks for 60 min. Center wells containing hyamine hydroxide and labeled CO_2 were then transferred into plastic scintillation vials containing 10 ml of the counting cocktail (see Materials below for composition). The amounts of [^{14}C] were determined with an ISOCAP/300 scintillation counter. Counts were corrected to disintegrations per minute, from which the percentage conversion rates were calculated.

Rates of Conversion of U-[^{14}C]-Glucose to Anions

Aliquots of the medium and cell washes were

passed through minicolumns consisting of Pasteur pipettes (10.5 × 0.6 cm) containing washed Dowex 1-X8 (mesh 20–50) to a column length of 7.5 cm. The columns were washed several times with water until eluates were free of [^{14}C] activity, after which 11 ml 1 N HCl were added to the columns. This removed all [^{14}C]-anions bound to the resin. Aliquots of eluates were counted in Aquasol. From the corrected dpm, the conversion rates of [^{14}C]-glucose to [^{14}C]-anions were calculated. No detectable labeled cationic products were obtained when the chromatography was carried out using aliquots of medium on Dowex 50W-X8 columns and eluting with 1 N sodium hydroxide.

Conversion of U-[^{14}C]-Glucose to Lactate

Aliquots of incubation medium and cell washes, or aliquots from anion fractions obtained after elution from Dowex 1-X8 ion-exchange column chromatography, were applied to silica gel H thin layer chromatography plates, and the plates were developed in a solvent system consisting of diethyl ether and formic acid (7:1) saturated with water (Ting and Dugger, 1965), with authentic lactic acid as the reference compound (R_f 0.69). The regions in the silica gel plate corresponding to the lactic acid R_f area were scraped and transferred to glass vials; the free lactic was extracted by adding 1 ml water to gel and heating at 32°C for 30 min. The radioactivity was determined in Aquasol, and the corrected conversion rates were calculated as before.

Conversion of U-[^{14}C]-Glucose to Lipids

After terminating the incubation with U-[^{14}C]-glucose, cells were washed twice with 1 ml Hanks' balanced salt solution. Cells, completely solubilized in 1% sodium dodecyl sulfate containing 1 mM disodium ethylenediamine tetraacetate, were subjected to sonication with a Bronwill Biosonik III equipped with a needle probe and run at the maximum permissible power input for 20 sec. Microscopic examination of plates after this treatment revealed the absence of any residual cellular material. An aliquot of the solubilized cells was treated with 20 volumes of chloroform and methanol (2:1) to extract lipids according to Folch et al. (1957). The subsequent two extractions consisted of using 20 volumes of chloroform:methanol:HCl (200:100:1, v/v/v) followed by washing the combined extracts with 0.9% NaCl solution using 5 times the volume of the solubilized cells. The upper phase was washed once with synthetic lower phase consisting of chloroform:methanol:0.58% NaCl (344:56:4, v/v/v), using 10 times the volume of the solubilized cells. The combined lower organic phases were neutralized with ammonia vapor from filter paper strips dipped in ammonium hydroxide. The organic layers were then evaporated in a thin stream of nitrogen at 32°C. The residue was dissolved in hexane, counted in the same scintillation fluid that was used for [^{14}C]-CO_2 measurements (see Materials, below), and corrected conversion rates were calculated.

Conversion of U-[^{14}C]-Glucose to Glycogen

Glycogen was isolated from the solubilized cells by the procedure of Chambost et al. (1972). Aliquots of solubilized cells (0.5 ml) were refluxed at 100°C for 30 min with 30% potassium hydroxide solution (1 ml). To the cooled solutions four drops of saturated solution of potassium chloride were added, followed by 2.6 ml methanol. The solutions were allowed to stand for 15 min and centrifuged; the pellet was dissolved in 1 ml hot water. Precipitation and solution steps were repeated twice. The precipitated glycogen was dissolved in water and counted in Aquasol from which the corrected conversion rates were calculated.

Determination of Pentose Phosphate Shunt Activity

Pentose-phosphate shunt activity was estimated by the methods of Katz and Wood (1963) by comparing rates of conversion of 1-[^{14}C]-glucose and 6-[^{14}C]-glucose to [^{14}C]-CO_2, and correlating these values with the total glucose utilized determined by the formation of [^{3}H]-H_2O from 5-[^{3}H]-glucose. The stimulation by phenazine methosulfate (100 μM) of this pathway in Sertoli cells was also measured.

Materials

The following isotopes were purchased from New England Nuclear, Boston MA: U[^{14}C]-D-glucose (310 mCi/mmole); 1-[^{14}C]-D-glucose (0.25 mCi/1.8 mmole); and 6-[^{14}C]-D-glucose (0.05 mCi/1.8 mmole), all initially dissolved in ethanol:water (9:1). The following isotopes were purchased from Amersham, Oakville, Ontario: 2-[^{3}H]-D-glucose (13.5 Ci/mmole) and 5-[^{3}H]-glucose (10 Ci/mmole), both initially dissolved in water.

Hyamine hydroxide (trade name for diisobutylcresoxyethoxylethyl dimethylbenzylammonium hydroxide), 1 M solution in methanol, was purchased from Amersham/Searle; Dowex 1-X8 (20–50 mesh) from J. T. Baker Chemical Co.; Silica gel H from E. Merck, Darmstadt, West Germany; dbcAMP from Sigma; phenazine methosulfate (PMS) from Eastman; and Aquasol from New England Nuclear. Plastic center wells for trapping [^{14}C]-CO_2 were purchased from Kontes.

Ovine follicle stimulating hormone (FSH) was obtained from the USPHS (NIH-FSH-S13) and from Dr. M. Sairam, Clinical Research Institute, Montreal, (S-1528C2). Insulin was a gift from Dr. C. C. Yip, Banting and Best Dept. of Medical Research, Toronto.

The scintillation cocktail used for the measurement of [^{14}C]-CO_2 is a solution of 0.4% PPO (2,5-diphenyloxazole) and 0.01% POPOP (1,4-bis-2-[5-phenyloxazolyl]-benzene) in a toluene and methanol (4:1, v/v).

RESULTS

Overall Rates of Utilization of [^{3}H]-Glucose by Sertoli Cells Prepared from Testes of 20-Day-Old Rats

At concentrations of glucose in the medium approximating those in plasma (5.5 mM), rates of utilization of tritiated glucose were estimated to be 459 nmole/mg protein/h from 5-[^{3}H]-

TABLE 1. Overall rates of utilization of [^{3}H]-glucose (5.5 mM) by Sertoli cells.[a]

Site of tritium label on glucose	Incubation time (min.)	[^{3}H]-H_2O formed from [^{3}H]-glucose[b] (nmol/mg cell protein)
5-[^{3}H]-D-glucose	30	248 ± 4.7
	45	372 ± 5.3
	60	459 ± 4.2
2-[^{3}H]-D-glucose	15	94 ± 3.2
	30	192 ± 6.2
	60	388 ± 4.1

[a]Data are expressed as mean ± SEM for triplicate determinations in four to five separate experiments.

[b][^{3}H]-H_2O formed from labeled glucose was determined as described in Materials and Methods.

D-glucose and 388 nmole/mg protein/h from 2-[^{3}H]-D-glucose (Table 1). Rates from both substrates were linear during the initial hour of incubation. For reasons given in the Discussion, we believe the higher figure provides a more reliable estimate. Rates of glucose utilization at four lower concentrations of 5-[^{3}H]-glucose were also determined (Fig. 1). Overall rates of glucose utilization by Sertoli cells were not detectable altered by prior treatment of cells with high concentrations of FSH, insulin, or dbcAMP under conditions investigated (Table 2).

Rates of Conversion of [^{14}C]-Glucose to [^{14}C]-CO_2

At a glucose concentration of 5.5 mM, 459 nmole were utilized/mg protein/h of which 14.2 nmole (3.1%) were converted to [^{14}C]-CO_2 (Table 2). However, when total glucose utilization was reduced by lowering glucose concentrations, the relative amount of glucose oxidized to CO_2 was increased. For example, at a glucose concentration of 0.1 mM, 10% of glucose utilized was incorporated into CO_2 (Table 3). The concentration of glucose at which the maximum utilization rate is reached is greater than 3 mM (Fig. 1), whereas the concentration at which the maximal rate of conversion of glucose to CO_2 occurs is no more than 2 mM (Fig. 2).

To evaluate levels of glucose oxidized via the pentose phosphate pathway, we determined the amounts of 1-[^{14}C]-glucose and 6-[^{14}C]-glu-

TABLE 2. Rates of conversion of U-[^{14}C]-glucose (5.5 mM) and 5-[^{3}H]-glucose (5.5 mM) to metabolites by Sertoli cells.[a]

	Incorporation of U-[^{14}C]-glucose into					Incorporation of 5[^{3}H]-glucose into [^{3}H]-H_2O
	CO_2	Total anions	Lipids	Glycogen	Sum of products	
	(nmol glucose utilized/mg cell protein/h)					(nmol/mg cell protein/h)
Control	14.2 ± 0.7	474 ± 8	4.5 ± 0.3	2.3 ± 0.1	496	459 ± 4.2
Insulin[b]	13.6 ± 0.2	462 ± 11	6.4 ± 0.1	2.1 ± 0.06	484	436 ± 6.2
oFSH[c]	13.9 ± 1.3	457 ± 12	5.4 ± 0.6	2.1 ± 0.02	478	449 ± 14.9
dbcAMP[d]	13.0 ± 0.3	469 ± 12	5.6 ± 0.2	2.1 ± 0.02	489	436 ± 6.2

[a]Cells, cultured for 48 h in the presence or absence of indicated levels of hormones, were incubated for 60 min in MEM containing labeled glucose, and products were isolated as described in Materials and Methods. Values are means ± SEM for triplicate determinations in four separate experiments.

[b]10 mU/ml.

[c]Ovine FSH, 300 ng/ml, Sairam's S-1528C2.

[d]0.1 mM dibutyryl 3',5' cyclic AMP.

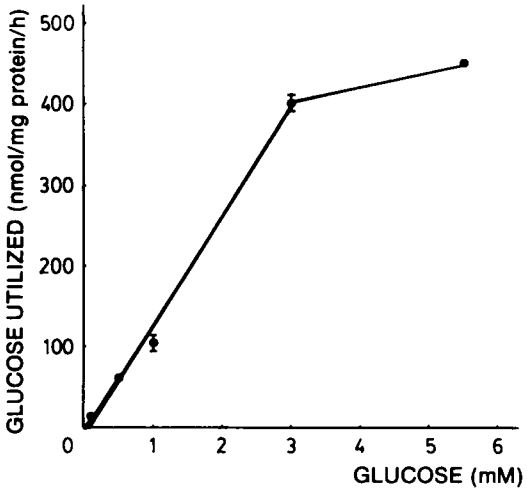


FIG. 1. Rates of total utilization of 5-[^{3}H]-glucose by Sertoli cells in culture at varying concentrations of glucose. The [^{3}H]-H_2O formed was measured as described in Materials and Methods. The rates are expressed as glucose utilized in nmol/mg cell protein/h (mean ± SEM for triplicate determinations for each point). Results are from a single experiment, representative of five different experiments.

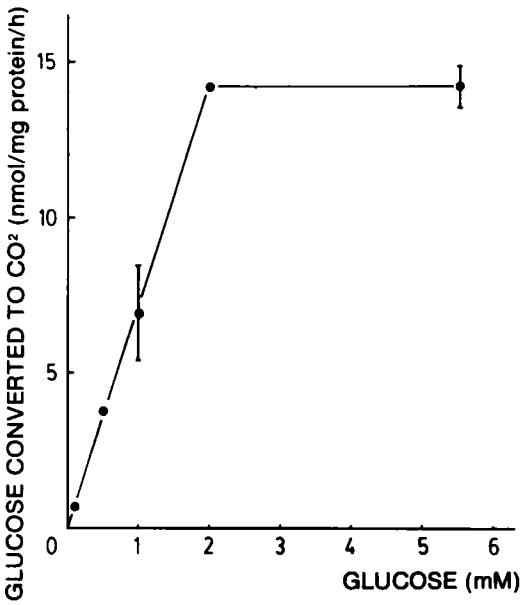


FIG. 2. Rates of conversion of U-[^{14}C]-glucose to [^{14}C]-CO_2 by Sertoli cells in culture at varying concentrations of glucose. The [^{14}C]-CO_2 was measured by trapping it in hyamine hydroxide as described in Materials and Methods. The rates are expressed as glucose utilized in nmol/mg cell protein/h (mean ± SEM of triplicate determinations for each point). Results are from a single experiment, representative of four separate experiments.

cose converted to [^{14}C]-CO_2 by Sertoli cells. Employing the equation of Katz and Wood (1963), we estimated that at glucose concentrations between 0.5 and 5.5 mM, ∿1.6–2.8% of the total glucose utilized was oxidized via the pentose phosphate shunt (Table 3). This accounts for about 50% of total glucose conversion to [^{14}C]-CO_2 at these glucose concentrations. At lower glucose concentrations (0.1 mM), the amounts of glucose oxidized via the pentose shunt tended to be relatively larger (Table 3).

When phenazine methosulphate (PMS) (100 μM) was added during incubation, the rate of conversion of 1-[^{14}C]-glucose to [^{14}C]-CO_2 was increased by over 10-fold (Table 3). In the presence of PMS at a glucose concentration of 5.5 mM, over 33% of total glucose utilized could be accounted for by oxidation via the pentose phosphate pathway (Table 3).

Rates of Conversion of U-[^{14}C]-Glucose to Anions and Lactate

The conversion of labeled glucose to anionic compounds accounted for most of the total glucose utilized. During the initial 30 min of incubation, [^{14}C]-lactate formation represented over 90% of total labeled anions produced (Fig. 3). By 60 min, ∿350 nmole U-[^{14}C]-glucose had been converted to lactate, corresponding to the formation of 700 nmoles of labeled lactate per milligram Sertoli cell protein, and accounting for ∿77% of the [^{14}C]-products measured.

Rates of Conversion of U-[^{14}C]-Glucose to Glycogen and Lipids

Relatively small amounts of labeled glucose were incorporated into cell glycogen or lipid (Table 2). These rates were not greatly influenced by prior treatment of cells with insulin, FSH, or dbcAMP, although treatment with insulin did appear to increase lipid formation slightly ($P<0.05$) (Table 2).

Summary of Rates of Conversion of Labeled Glucose to Various Products

A balance sheet, summarizing rates of incorporation of U-[^{14}C]-glucose into labeled CO_2, anions, glycogen, and lipids, is presented in Table 2. The total amounts of glucose

TABLE 3. Calculation of rates of glucose metabolized via the pentose phosphate pathway by Sertoli cells.[a]

Glucose concentration (mM)	PMS (100 μM)	U-[^{14}C]-Glucose to [^{14}C]-CO_2 (nmol/mg cell protein/ 60 min) 1	1-[^{14}C]-Glucose to [^{14}C]-CO_2 (nAtom carbon #1/ mg cell protein/ 60 min) 2	6-[^{14}C]-Glucose to [^{14}C]-CO_2 (nAtom carbon #6/ mg cell protein/ 60 min) 3	5-[^{3}H]-Glucose to [^{3}H]-H_2O (nmol/mg cell protein/60 min) 4	Percentage of total glucose metabolized by pentose phosphate pathway[b] 5	Glucose metabolized via pentose phosphate pathway[c] (nmol/mg cell protein) 60 min 6	Percentage of total CO_2 from glucose via pentose phosphate pathway 7
0.1	–	1.0 ± 0.04	2.98 ± 0.24	0.3 ± 0.02	10.8 ± 0.6	10.2	1.1	100
0.5	–	4.64 ± 0.05	6.86 ± 0.24	0.37 ± 0.1	79.6 ± 9.1	2.8	2.23	49.1
1.0	–	9.0 ± 1.0	10.2 ± 0.3	1.1 ± 0.2	148 ± 7.5	2.2	3.26	36.1
5.5	–	14.2 ± 0.7	24.2 ± 0.5	3.08 ± 0.33	455 ± 3.7	1.6	7.31	51.5
5.5	+	84.7 ± 7.5	271.4 ± 6.2	4.62 ± 0.11	446 ± 3.1	33.7	150.5	>100

[a]Conditions were the same as those described in Tables 1 and 2. Each value is the mean ± SEM for triplicate determinations of three different preparations.

[b]The percentage of total glucose utilized which was metabolized by the pentose phosphate pathway was calculated by the equation of Katz and Wood (1963) using the observed rates of glucose utilized (column 4) and rates of conversion of 1-[^{14}C]-glucose and 6-[^{14}C]-glucose to [^{14}C]-CO_2 (columns 2 and 3). The equation is

$$\frac{G_{CO_2(1)} - G_{CO_2(6)}}{1 - G_{CO_2(6)}} = \frac{3PC}{1+2PC}$$

where G represents the specific yield and PC represents pentose cycle pathway.

[c]The rate of glucose conversion of CO_2 via the pentose pathway was calculated by multiplying the fraction of total glucose utilized (column 5) by the total amounts of glucose utilized (column 4).

[d]The percentage of CO_2 from glucose contributed by the pentose phosphate pathway was calculated by dividing the values of column 6 (nmol glucose converted to CO_2 via pentose phosphate pathway) by values in column 1 (nmol U-[^{14}C]-glucose converted to CO_2, representing the total glucose oxidation to CO_2).

converted to these products correspond reasonably well with overall rates of utilization of glucose, estimated by determining the incorporation of 5-[^{3}H]-glucose into [^{3}H]-H_2O (Table 2) (see Discussion).

Absence of Demonstrable Effects of Hormones on Carbohydrate Metabolism by Sertoli Cells

At physiological concentrations of glucose (5.5 mM), neither FSH nor dbcAMP had any discernible influences on overall rates of glucose utilization or on the rates of incorporation of labeled glucose into any of the products measured (Table 2). Similarly, hormones added did not alter the rates of oxidation of glucose via the pentose phosphate shunt pathway (Table 4 and unpublished observations). Addition of insulin resulted in a small but statistically significant increase in the incorporation of labeled glucose into lipids (Table 2). However, insulin did not alter the rates of incorporation of glucose into other products, nor did it influence the overall rate of glucose utilization (Table 2). We examined possible effects of FSH and insulin on sugar metabolism at lower concentrations of glucose and during shorter periods of incubation (Table 4). At a glucose concentration of 0.5 mM, neither FSH nor insulin altered the rates of conversion of 1-[^{14}C]-glucose to [^{14}C]-CO_2 (Table 4). When PMS was added, the rates of formation of [^{14}C]-CO_2 from 1[^{14}C]-glucose were increased by nearly a factor of 10, but these rates were independent of the presence of FSH or insulin (Table 4).

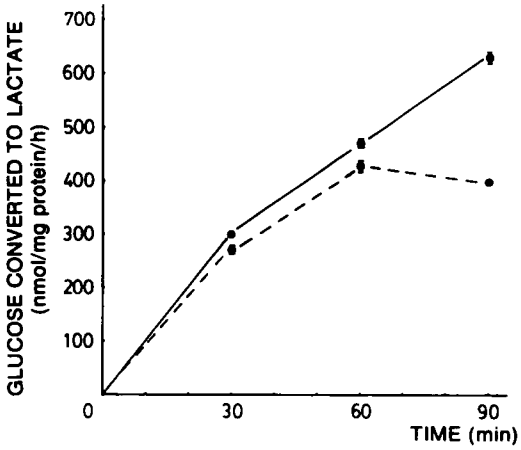


FIG. 3. Conversion of U-[^{14}C]-glucose to labeled anions and labeled lactate at different time intervals. The labeled anions (——) were estimated by ion-exchange column chromatography (Dowex 1- X 8) as described in Materials and Methods, and the labeled lactate (----) was estimated by thin layer chromatography (see Materials and Methods). The amounts of both labeled anions and labeled lactate are expressed as glucose utilized in nmol/mg cell protein (mean ± SEM of triplicate determinations for each point). Results are from a single experiment, representative of three different experiments for lactate analyses, and four experiments for total anion determinations.

DISCUSSION

Rates of Lactate Formation from U-[^{14}C]-Glucose

The incorporation of U-[^{14}C]-glucose into lactate accounted for most of the glucose utilized by Sertoli cells (Fig. 3). During the first

TABLE 4. Absence of effects of insulin and FSH on the incorporation of 1-[^{14}C]-glucose into [^{14}C]-CO_2 by Sertoli cells.[a]

Conditions	Incorporation of 1-[^{14}C]-Glucose (0.5 mM) into [^{14}C]-CO_2 (nAtom carbon #1/mg protein/10 min)	
	PMS added (100 μM)	
	−	+
Control	3.4 ± 0.05	28.2 ± 0.7
FSH (300 ng/ml Sairam S1528C2)	3.4 ± 0.08	24.3 ± 0.28
Insulin (10 mU/ml)	3.2 ± 0.06	28.1 ± 0.47

[a]Data are expressed as mean ± SEM for triplicate determinations of three different preparations. PMS represents phenazine methosulfate (100 μM) added at the beginning of incubation. Cells treated with FSH had been cultured in its presence for 48 h prior to incubation. Insulin was added only at the beginning of incubation with labeled glucose.

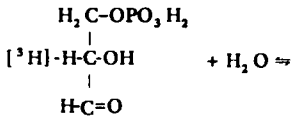
hour of incubation, label incorporated into lactate accounted for over 75% of the [^{14}C] present in anions formed from U-[^{14}C]-glucose, corresponding to the production of ~700 nmol lactate/mg protein/h. If this is representative of what occurs in vivo, the lactate released by Sertoli cells would be potentially available as a substrate for adjacent germinal cells, and could therefore be of considerable physiological importance.

Rat follicles are reported to have high rates of lactate production, most evident in preovulatory follicles stimulated by luteinizing hormone (Nilsson, 1974; Tsafriri et al., 1976). It has been appreciated since the studies of Biggers et al. (1967) that meiotic maturation of oocytes is supported by pyruvate and oxaloacetate more than by glucose. This has recently been extended by studies of Ahrén et al. (1978), who measured the respiration of isolated oocytes, and reported an increase elicited by pyruvate, oxaloacetate, or lactate but not by glucose. Lactate and other metabolites produced from glucose by granulosa cells surrounding the oocyte are likely to be of importance in providing substrates necessary for oocyte metabolism and maturation.

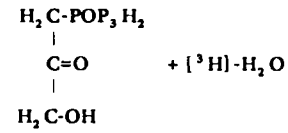
By analogy, a similar situation may prevail among the cells in the seminiferous tubule. As indicated in the Introduction, several recent observations demonstrate that lactate is a better energy source than glucose for spermatids and pachytene spermatocytes (Nakamura et al., 1981; Jutte et al., 1980). The low concentrations of glucose in tubular fluid make it essential for germinal cells in the adluminal compartment to have access to alternate substrates as sources of energy. Perhaps, part of the role of Sertoli cells as nutrient or "nurse" cells consists of providing lactate. If so, this simple metabolic function constitutes a portion of the important cellular interactions required between gonadal somatic cells and germinal cells for spermatogenesis to proceed normally (for review see Fritz, 1978).

Estimation of Overall Rates of Glucose Utilized by Sertoli Cells

The total amount of U-[^{14}C]-glucose incorporated into labeled CO_2, anions, lipids, and glycogen was estimated to be an average of 486 nmol/mg protein/h (Table 2). This is ~8.5% greater than the rate of incorporation of 5-[^{3}H]-glucose into tritiated water (445 nmol/mg protein/h), estimated from the average of values obtained for cells incubated under four different sets of conditions (Table 2). The discrepancy could be accounted for by an incomplete equilibration with cellular water of tritium on carbon 2 of D-glyceraldehyde-3-phosphate, which originated from the tritium on carbon 5-[^{3}H]-glucose, in the triosephosphate isomerase reaction:



D-glyceraldehyde-3-phosphate



Dihydroxyacetone phosphate

If D-glyceraldehyde-3-phosphate were converted to diphosphoglyceric acid prior to the completion of equilibration in the triosephosphate isomerase reaction, this would contribute to the apparently lower rate of glucose utilization of 5-[^{3}H]-glucose than of U-[^{14}C]-glucose.

On the other hand, the rate of glucose utilization from 2-[^{3}H]-glucose was estimated to be only 388 nmol/mg protein/h (Table 1). It is possible that the lower rates in this case result from an incomplete equilibration of the phosphohexose isomerase reaction:

$$H_2O + 2\text{-}[^3H]\text{-glucose-6-phosphate} \rightleftharpoons$$

$$\text{fructose-6-phosphate} + [^3H]\text{-}H_2O$$

If this occurred, some of the nonequilibrated labeled glucose-6-phosphate could be converted to other intermediates which need not lose [^{3}H] from the 2 position, such as 6-phosphogluconic acid in the pentose phosphate pathway; glucose-1-phosphate and UDPG in the glycogen synthetic pathway; or myoinositol-1-phosphate in the myoinositol biosynthetic pathway. The quantitative importance of each, relative to the total rate of glucose utilization, seems minor, and it is therefore not possible to provide an explanation for the lower rate of total glucose utilization measured with this technique. It is probable that there is an enzy-

matic discrimination against [^{3}H] (Hammerstedt, 1975).

We are impressed with the close approximation of values obtained by using U-[^{14}C]-glucose and 5-[^{3}H]-glucose as substrates, and therefore are of the opinion that measurements with these substrates provide more reliable estimates of overall rates of glucose metabolism through the combined glycolytic and pentose cycle pathways.

Absence of Detectable Influences of FSH, Insulin, and dbcAMP on Glucose Metabolism

Under all conditions examined, neither FSH, insulin, nor dbcAMP elicited any change in overall rates of glucose utilization (Table 2). We employed concentrations of these reagents which have been found under similar conditions to influence other metabolic aspects of Sertoli cells in culture. For example, both FSH and dbcAMP increase the rates of formation of androgen binding protein, plasminogen activator, estrogens from testosterone, sulfo-proteins, and myoinositol (for review see Fritz, 1978). We also determined if expression of the considerable potential capacity for oxidation of glucose via the pentose phosphate pathway could be influenced by insulin or FSH. The addition of the artificial electron acceptor PMS greatly increased pentose phosphate pathway activity (Table 3), evident by the increased rate of oxidation of 1-[^{14}C]-glucose to [^{14}C]-CO_2 (Table 4). In the presence or absence of PMS, neither FSH nor insulin altered rates of [^{14}C]-CO_2 formation at all concentrations of 1-[^{14}C]-glucose employed (0.1–5.5 mM). Data from only two sets of conditions have been shown (Tables 3, 4). In other experiments we have also measured possible effects of insulin on initial rates of uptake by Sertoli cells of the nonmetabolizable sugar 3-0-methylglucose. Equilibration occurred rapidly into total cell water in the presence or absence of insulin, and no influence of insulin on rates could be detected (data not shown). From combined observations we conclude that modulation of various Sertoli cell functions by FSH and insulin is probably not directly associated with effects on glucose metabolism.

We have recently examined the possible influences of 5-thio-D-glucose on carbohydrate metabolism by Sertoli cells. This agent, which prevents spermatogenesis when given to rats or mice (Zysk et al., 1975; Homm et al., 1977; Burton and Wells, 1977), has been postulated to act via inhibition of glucose metabolism and protein synthesis by spermatids (Nakamura and Hall, 1976, 1977). We have observed that 5-thio-D-glucose inhibits glucose utilization and lactate formation by Sertoli cells (Robinson and Fritz, submitted). It is possible that chemical agents which specifically inhibit glucose utilization by Sertoli cells could elicit a failure of spermatogenesis by blocking the formation of lactate required by germinal cells for their development. This possibility is being investigated.

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REFERENCES

Ahrén, K., Dekel, N., Hamberger, L., Hillensjo, T., Hultborn, R., Magnusson, C. and A. Tsafriri. (1978). Metabolic and morphological changes produced by gonadotrophins and cyclic AMP in the oocyte and cumulus oophorus of preovulatory rat follicle. Ann. Biol. Anim. Biochim. Biophys. 18, 409–417.

Ashcroft, S.J.H., Weerasinghe, L.C.C., Bassett, J. M. and Randle, P. J. (1972). The pentose cycle and insulin release in mouse pancreatic islets. Biochem. J. 126, 525–532.

Biggers, J. D., Whittingham, D. G. and Donahue, R. P. (1967). The pattern of energy metabolism in the mouse oocyte and zygote. Proc. Nat. Acad. Sci. USA 58, 560–567.

Burton, L. E. and Wells, W. W. (1977). Studies on the effect of 5-thio-D-glucose and 2-deoxy-D-glucose on myoinositol metabolism. Arch. Biochem. Biophys. 181, 384–392.

Chambost, J. P., Favard, A. and Caltaneo, J. (1972). Synthèse de novo de glycogene par un mutant d'*Escherichia coli* de pourvu d'activités glucose-phosphate isomerase et D-glucose-6-phosphate des hydrogénase. Carbohydr. Res. 24, 379–391.

Dorrington, J. H., Roller, N. F. and Fritz, I. B. (1975). Effects of follicle-stimulating hormones on cultures of Sertoli cell preparations. Mol. Cell. Endocr. 3, 57–70.

Folch, J., Lees, M. and Sloane-Stanley, G. (1957). A simplified method for isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Free, M. J. (1970). Carbohydrate metabolism in the testis. In: The Testis. Vol. 2. (A. D. Johnson, W.

R. Gomes and N. L. Vandemark, eds.). Academic Press, New York. pp. 125–191.

Fritz, I. B. (1978). Sites of action of androgens and FSH on cells of the seminiferous tubule. In: Biochemical Actions of Hormones. Vol. 5. (G. Litwack, ed.). Academic Press, New York. pp. 249–281.

Hammerstedt, R. H. (1975). Tritium release from [2-^{3}H] D-glucose as a monitor of glucose consumption by bovine sperm. Biol. Reprod. 12, 545–551.

Homm, R. E., Rusticus, C. and Hahn, D. W. (1977). The antispermatogenic effects of 5-thio-D-glucose in male rats. Biol. Reprod. 17, 697–700.

Jutte, N.H.P.M., Koolen, L. M., Jansen, R., Grootegoed, J. A., Rommerts, F.F.G. and Van der Molen, H. J. (1980). Lactate essential for biochemical activities in isolated germinal cells. First European Workshop on Molecular and Cellular Endocrinology of the Testis, Geilo, Norway, April 8–11, 1980. Abstr.

Katz, J. and Wood, H. G. (1963). The use of $C^{14}O_2$ yields from glucose-1- and -6-C^{14} for the evaluation of the pathways of glucose metabolism. J. Biol. Chem. 238, 517–523.

Louis, B. G. and Fritz, I. B. (1977). Stimulation by androgens of the production of androgen binding protein by cultured Sertoli cells. Mol. Cell. Endocr. 7, 9–15.

Means, A. R. and Hall, P. F. (1968). Protein biosynthesis in the testis. I. Comparison between stimulation by FSH and glucose. Endocrinology 82, 597–602.

Middelton, A. (1973). Glucose Metabolism in Rat Seminiferous Tubules. Ph.D. Dissertation. University of Cambridge, England.

Middleton, A. and Setchell, B. P. (1972). The origin of inositol in rete testis fluid of the ram. J. Reprod. Fertil. 30, 473–475.

Nakamura, M. and Hall, P. F. (1976). Inhibition by 5-thio-D-glucopyranose of protein biosynthesis *in vitro* in spermatids from rat testis. Biochim. Biophys. Acta. 447, 474–483.

Nakamura, M. and Hall, P. F. (1977). Effect of 5-thio-D-glucose on protein synthesis *in vitro* by various types of cells from rat testes. J. Reprod. Fertil. 49, 395–397.

Nakamura, M., Hino, A. and Kato, J. (1981). Regulation of protein synthesis in spermatids from rat testes: Role of adenosine triphosphate (ATP). Am. J. Physiol. In Press.

Nakamura, M., Romrell, L. J. and Hall, P. F. (1978). The effects of temperature and glucose on protein biosynthesis by immature (round) spermatids from rat testes. J. Cell Biol. 79, 1–9.

Nilsson, L. (1974). Acute effects of gonadotrophins and prostaglandins on the metabolism of isolated ovarian follicle from PMSG-treated immature rats. Acta Endocrinol. 77, 540–558.

Robinson, R. and Fritz, I. B. (1979). Myoinositol biosynthesis by Sertoli cells, and levels of myoinositol biosynthetic enzymes in testis and epididymis. Can. J. Biochem. 57, 962–967.

Setchell, B. P. (1970). The secretion of fluid by the testis of rams and goats with some observations on the effect of age, cryptorchidism and hypophysectomy. J. Reprod. Fertil. 23, 79–85.

Setchell, B. P. (1978). Metabolism in the testis. In: The Mammalian Testis. Paul Elk, Publisher, London. pp. 285–311.

Setchell, B. P. and Waites, G.M.H. (1975). The blood-testis barrier. In: Handbook of Physiology. Sect. 7, Endocrinology. Vol. 5, Male Reproductive System. pp. 143–172.

Ting, I. P. and Dugger, W. H. (1965). Separation and detection of organic acids on silica gel. Anal. Biochem. 12, 561–578.

Tsafriri, A., Lieberman, M. E., Ahrén, K. and Lindner, H. R. (1976). Dissociation between LH-induced aerobic glycolysis and oocyte maturation in cultured Graafian follicles of the rat. Acta Endocrinol. 83, 151–157.

Voglmayr, J. K., Scott, T. W., Setchell, B. P. and Waites, G.M.H. (1967). Metabolism of testicular spermatozoa and characteristics of testicular fluid collected from conscious rams. J. Reprod. Fertil. 14, 87–99.

Voglmayr, J. K., Waites, G.M.H. and Setchell, B. P. (1966). Studies on spermatozoa and fluid collected directly from the testis of the conscious ram. Nature 210, 861–863.

Zysk, J. R., Bushway, A. A., Whistler, R. L. and Carlton, W. W. (1975). Temporary sterility produced in male mice by 5-thio-D-glucose. J. Reprod. Fertil. 54, 69–72.