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Metabolism of Glucose, Lactate, and Acetate by Testicular and Ejaculated Spermatozoa of the Ram

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Both testicular and ejaculated spermatozoa of the ram oxidized glucose, fructose, lactate, and acetate, and their metabolism was stimulated by the inclusion of potassium plus magnesium ions in the incubation medium.

There were differences in the metabolism of testicular and ejaculated spermatozoa. Testicular spermatozoa showed a proportionately greater oxidation of endogenous substrate in the presence of added glucose or acetate. During incubation for 4 hr, testicular cells formed more radioactive carbon dioxide from acetate-1-^{14}C than from acetate-2-^{14}C, whereas ejaculated spermatozoa oxidized both carbon atoms of acetate at a similar rate.

Acetate was always accumulated by ejaculated spermatozoa but only in trace amounts by testicular cells, except by one anomalous sample.

It is suggested that these results were due to the occurrence in the testicular cells of endogenous substrate which was readily oxidized in competition with added hexose or acetate, and to a greater synthetic capacity of testicular spermatozoa.

Voglmayr, Scott, Setchell, and Waites (1967) found that only about half of the radioactive glucose utilized by ram testicular spermatozoa could be accounted for as lactate, amino acids, and carbon dioxide. As ejaculated ram spermatozoa accumulate intracellular acetate from fructose (Wales and Humphries, 1969; O'Shea, 1970) the possibility that testicular cells also accumulate acetate was investigated. In addition, the capacity of these spermatozoa to oxidize lactate and acetate was examined.

MATERIALS AND METHODS

Testicular spermatozoa were obtained as described by Voglmayr *et al.* (1967) and ejaculated semen was collected after electrical stimulation (Blackshaw, 1954).

The basic diluents used were calcium-free Krebs-Ringer buffered to pH 7.2 with phosphate (Umbreit, Burris and Stauffer, 1959), and a phosphate-saline diluent (pH 7.2) consisting of 20 mM monosodium and disodium phosphate buffer and 127 mM sodium chloride. Both contained 30 mg penicillin and 50 mg streptomycin per 100 ml. The washed cells (Voglmayr *et al.*, 1967) were incubated at 37° in Warburg flasks of 5-ml volume with air as the gas phase. The final volume of the reaction mixture was 1 ml containing about 2×10^8 spermatozoa.

Radioactive compounds were obtained from the Radiochemical Centre, Amersham, Buckinghamshire, and their purity was checked before use. In the first two experiments labeled substrates were added to the incubation medium to yield a final concentration of 5 μmoles of glucose and 500 nCi of D-glucose-U-^{14}C, or 4 μmoles of L-lactate and 500 nCi of DL-lactate-1-^{14}C, or 5 μmoles of sodium acetate and 300 nCi of either acetate-1-^{14}C or acetate-2-^{14}C. For the final experiment the flasks contained 20 μmoles of fructose and 1 μCi of D-fructose-U-^{14}C. Oxidation of substrate was determined from the assay of $^{14}CO_2$ trapped in 0.05 ml 20% (w/v) KOH in the center well.

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After incubation, the cells were separated from the medium by density-gradient centrifugation (O'Shea, 1970), extracted with 2.5% (w/v) perchloric acid, and the carboxylic acids separated by partition chromatography on silicic acid with hexane–butanol solvents (O'Shea and Wales, 1968). Assay of radioactivity was carried out by liquid-scintillation techniques (Patterson and Greene, 1965). Where possible, the radioactive peaks from the silicic acid columns were recovered for further identification by paper chromatography and steam distillation. Most of the peaks obtained from testicular spermatozoa and tentatively identified as acetic acid by their position of elution from the silicic acid column were too small for further identification.

In the first experiment the effects of the various substrates on oxygen consumption were ranked using Duncan's multiple-range test (Duncan, 1955). The significance of the results shown in Table 4 was assessed by analysis of variance after conversion of the data to logarithms, and that of other results by paired *t* tests.

RESULTS

Accumulation of Metabolites from Glucose, Lactate and Acetate

The metabolism of testicular and ejaculated spermatozoa was examined in the presence of glucose, lactate, or acetate. In this experiment the cells were washed with, and incubated for 2 hr in, calcium-free Krebs-Ringer phosphate and the mean results are given in Tables 1, 2, and 3.

The oxygen uptake of both types of spermatozoa was greater with lactate or acetate than with glucose, and both produced more carbon dioxide from carbon atom one than from carbon atom two of acetate (Table 1). The oxygen consumption and carbon dioxide production of the ejaculated spermatozoa were greater. However, oxidation of added glucose or acetate accounted for a smaller percentage of the oxygen uptake by testicular than by ejaculated cells.

While the total amount of substrate carbon accumulating intracellularly was similar in testicular and ejaculated spermatozoa, there were differences between the two types of spermatozoa in the metabolites making up this intracellular carbon (Table 2). Some 30 to 40% of the substrate carbon in the ejaculated cells was acetate. Little acetate was detectable in testicular spermatozoa, but other unidentified carboxylic acids formed a slightly larger proportion of their intracellular carbon pool.

The incubation medium with both cell types, when chromatographed on silicic acid, gave radioactive peaks coinciding with authentic formate and pyruvate (Table 3). These were too small for further identification. Most of the acetate formed from glucose by the ejaculated spermatozoa accumulated intracellularly (Table 3).

Effect of Time of Incubation on the Metabolism of Acetate

Spermatozoa were incubated for 2 or 4 hr with acetate, labeled specifically in the C_1 or C_2 position, to determine if the time of incubation altered the relative rate of oxidation of these two carbon atoms. The results showed that the time of incubation had a different effect on the oxidation of acetate by the two types of spermatozoa (Table 4). Thus, testicular cells oxidized more acetate-1-^{14}C than acetate-2-^{14}C, and this difference was greater at 4 hr than at 2 hr ($p < .05$). As in the first experiment, ejaculated spermatozoa oxidized more acetate-1-^{14}C than acetate-2-^{14}C during a 2-hr incubation ($p < .05$), but after 4-hr incubation the amounts of $^{14}CO_2$ formed from the two carbon atoms of acetate were not significantly different.

Effect of Potassium and Magnesium on the Metabolism of Testicular Spermatozoa

As only traces of acetate were accumulated by testicular spermatozoa in Krebs-Ringer phosphate, they were incubated under conditions known to increase acetate accumulation by ejaculated cells. Testicular spermatozoa (mean 1.2×10^8 cells/flask), washed in the phosphate–saline diluent, were incubated for 3 hr with 20 μmoles of fructose in the presence or absence of 1 mM KCl and 2 mM $MgCl_2$.

TABLE 1

METABOLISM OF GLUCOSE, LACTATE, OR ACETATE BY TESTICULAR (T) AND EJACULATED (E) RAM SPERMATOZOA[a]

Added substrate	Oxygen uptake[b]				Carbon dioxide from labeled carbon atom[b]		Accumulation of substrate carbon[b]			
	Total (μmoles/10^8 cells)		Percentage from oxidation of added substrate		(μmoles/10^8 cells)		In spermatozoal plug (μg atoms/10^{10} cells)		In PCA extract of plug (μg atoms/10^{10} cells)	
	T	E	T	E	T	E	T	E	T	E
A Glucose-U-^{14}C	1.25 ± 0.05	2.81 ± 0.24	52.5 ± 2.1	81.6 ± 2.7	0.67 ± 0.04	2.28 ± 0.24	41 ± 2[c]	48 ± 4[c]	34 ± 1[c]	46 ± 3[c]
B Lactate-1-^{14}C	1.49 ± 0.07***	3.41 ± 0.34***			0.32 ± 0.02	0.95 ± 0.08	1.0 ± 0.1	0.4 ± 0.2		
C Acetate-1-^{14}C	1.49 ± 0.07***	3.46 ± 0.37***	44.1 ± 1.1***	65.6 ± 1.7***	0.41 ± 0.02	1.21 ± 0.11	25 ± 1	25 ± 4		
D Acetate-2-^{14}C	1.47 ± 0.06***	3.59 ± 0.40***			0.27 ± 0.02**	1.09 ± 0.14**	46 ± 2**	37 ± 4*	41 ± 1	36 ± 4
No. of replicates	6	6	5	6	5	6	3	3	3	3

[a] Values are the means ± SE of the means over the 2-hr experimental period.

[b] * Significantly different from (C) $p < .05$; ** significantly different from (C) $p < .01$; *** significantly different from (A) $p < .01$.

[c] Four replicates.

TABLE 2

THE INTRACELLULAR METABOLITES ACCUMULATED FROM GLUCOSE-U-^{14}C OR ACETATE-2-^{14}C BY TESTICULAR (T) AND EJACULATED (E) RAM SPERMATOZOA[a]

	Acetate		Lactate		Peak 6 (Fraction 50)		Peaks 7 + 8 (Fractions 60–75)	
Substrate	T	E	T	E	T	E	T	E
Glucose-U-^{14}C	0.10 ± 0.04	38 ± 7	0.4 ± 0.1	1.4 ± 0.3	0	0	10–15	2–3
Acetate-2-^{14}C	0.2 ± 0.1	31 ± 11	[b]	[b]	[b]	[b]	10–20	4–9

[a] Values are the means ± SE of the means for four (glucose) or three (acetate) replicates expressed as percentages of the total substrate carbon accumulated intracellularly.

[b] Trace in some replicates.

TABLE 3

THE ACCUMULATION OF ACETATE AND LACTATE FROM GLUCOSE-U-^{14}C BY TESTICULAR (T) AND EJACULATED (E) RAM SPERMATOZOA[a]

	Intracellular		Extracellular	
Metabolite	T	E	T	E
Acetate	0.04 ± 0.02	18.2 ± 4.4	0.05 ± 0.02	1.3 ± 0.8
Formate	0	0	0.32 ± 0.11	0.03 ± 0.03
Pyruvate	0	0	0.36 ± 0.16	0.03 ± 0.02
Lactate	0.10 ± 0.04	0.7 ± 0.2	55 ± 17	827 ± 64

[a] Values are the means ± SE of the means for four replicates expressed as μg atoms of substrate carbon/10^{10} cells over the 2-hr experimental period.

TABLE 4

EFFECT OF TIME OF INCUBATION ON THE METABOLISM OF ACETATE BY TESTICULAR (T) AND EJACULATED (E) RAM SPERMATOZOA[a]

Incubation period (hr)	Label	Oxygen uptake		Carbon dioxide from carbon atom labeled	
		T	E	T	E
2	Acetate-1-^{14}C	1.65 ± 0.15	2.25 ± 0.21	0.48 ± 0.06	0.84 ± 0.09
	Acetate-2-^{14}C	1.67 ± 0.16	2.11 ± 0.26	0.33 ± 0.05	0.69 ± 0.05
4	Acetate-1-^{14}C	3.42 ± 0.21	3.71 ± 0.45	1.12 ± 0.10	1.59 ± 0.15
	Acetate-2-^{14}C	3.43 ± 0.26	3.87 ± 0.47	0.86 ± 0.10	1.54 ± 0.16

[a] Values are the means ± SE of the means for four replicates expressed as μmoles/10^8 cells over the experimental period.

The addition of the ions increased the oxygen uptake, fructose oxidation, and extracellular accumulation of lactate (Table 5). As in the first experiment, only traces of a material, tentatively identified as acetate, were present within the cells. Extracellular acetate was accumulated in greater amounts by one sample, both with (16.2 μg atoms of substrate carbon per 10^{10} cells) and without (25.7 μg atoms of substrate carbon per 10^{10} cells) the addition of potassium and magnesium ions. This material was steam-volatile and ran with authentic acetate when chromatographed on paper using ethanol : ammonia (7.6 N) : water (8 : 1 : 1) as solvent. All the other parameters for this replicate were considerably greater than those for the other three replicates; namely, oxygen uptake 353 and 298 μmoles/10^{10} cells/3 hr, carbon dioxide formation from fructose 320 and 268 μmoles/10^{10} cells/3 hr, lactate accumulating in the medium 303 and 197 and intracellularly 0.6

TABLE 5
EFFECT OF ADDING POTASSIUM (1 mM) AND MAGNESIUM (2 mM) IONS ON THE METABOLISM OF WASHED TESTICULAR RAM SPERMATOZOA WITH FRUCTOSE AS SUBSTRATE[a]

Ions	Oxygen uptake (μmoles)	Carbon dioxide from fructose (μmoles)	Lactate accumulated from fructose (μg atoms of substrate carbon)		Acetate accumulated from fructose (μg atoms of substrate carbon)	
			Intracellular	Extracellular	Intracellular	Extracellular
—	208 ± 31	162 ± 35	0.3 ± 0.2	134 ± 43	Traces	7.3 ± 6.2
+[b]	246 ± 37**	189 ± 44*	0.3 ± 0.1	205 ± 54**	Traces	4.3 ± 4.0

[a] Values are the means ± SE of the means/10^{10} cells for four replicates over the experimental period (3 hr).
[b] * Significant effect $p < .05$; ** significant effect $p < .01$.

and 0.8 μg atoms of substrate carbon/10^{10} cells/3 hr, and percentage of oxygen uptake due to oxidation of fructose 90 and 90 with or without added ions. It was found that the spermatozoa collected for this replicate were obtained after a 48-hr period of restricted flow through the catheter in the rete testis.

DISCUSSION

In the present studies the oxygen uptake of ejaculated spermatozoa was greater than that of testicular spermatozoa. Voglmayr *et al.* (1967) obtained similar results with ram testicular spermatozoa incubated in phosphate-free diluent, but they also showed that addition of phosphate to the incubation medium depressed the respiration of ejaculated spermatozoa and stimulated that of testicular spermatozoa. This could be due to the high level of phosphate ion in their diluent, as 80 mM phosphate has been shown to depress the metabolism of ejaculated ram spermatozoa (Mann and White, 1957; Murdoch and White, 1966a). Although concentrations of up to 60 mM phosphate stimulate the metabolism of ejaculated ram spermatozoa, the significant interactions between the effects of potassium and phosphate on the oxidative metabolism of epididymal ram spermatozoa and their absence with ejaculated spermatozoa (Wallace and Wales, 1964) indicate that also in this respect testicular spermatozoa may differ from ejaculated cells.

The lower oxygen uptake with glucose than with either lactate or acetate, in the presence of potassium and magnesium, confirms earlier results with ejaculated spermatozoa (Murdoch and White, 1966b; Wales and O'Shea, 1966). As a smaller percentage of the oxygen taken up by testicular spermatozoa is required for the oxidation of either fructose or acetate, these cells must oxidize a larger proportion of endogenous substrate than ejaculated spermatozoa, which, in the presence of hexoses, oxidize little endogenous material (Scott, White, and Annison, 1962; O'Shea and Wales, 1966).

Although the addition of potassium and magnesium increased the metabolism of testicular spermatozoa, the effect was not as great as with ejaculated ram spermatozoa of similar concentration (Wales and O'Shea, 1966; O'Shea, 1970). The present results also confirm that, under aerobic conditions, testicular spermatozoa accumulate less lactate than ejaculated cells from fructose as well as from glucose (Voglmayr, Waites, and Setchell, 1966; Murdoch and White, 1968). With both types of spermatozoa almost all the lactate formed accumulates in the incubation medium, as reported for ejaculated spermatozoa (Wales and Humphries, 1969; O'Shea, 1970).

With the exception of one abnormal sample, only trace amounts of acetate were accumulated from fructose or glucose by testicular spermatozoa, and, in contrast to

ejaculated cells, there was no increase in extracellular acetate when the cells were incubated in a medium deficient in potassium (O'Shea, 1970). In the anomalous replicate spermatozoa remained in the rete testis for up to 48 hr prior to their collection, and it seems likely that under these conditions the spermatozoa can undergo a certain degree of maturation without the mediation of the epididymis. With another ram, after blockage of the catheter for up to 35 hr, a large increase was observed in the oxidative metabolism of testicular spermatozoa which also exhibited rapid tail oscillation (Voglmayr and White, unpublished).

Scott, Voglmayr and Setchell (1967) have shown that when testicular and ejaculated ram spermatozoa were incubated with glucose-U-^{14}C, small amounts of labeled acetic and formic acids accumulated in the incubation medium. These authors also showed that testicular sperm medium contained more formate than acetate while the reverse was true for ejaculated spermatozoa; similar results were obtained in the present experiments. It is interesting that the incubation medium of the abnormal testicular sperm replicate contained only traces of material tentatively identified as formate but much more acetate, again resembling ejaculated more than testicular spermatozoa.

Testicular spermatozoa have a greater synthetic capacity than ejaculated spermatozoa, as demonstrated by the incorporation of glucose into phospholipid (Scott, Voglmayr, and Setchell, 1967) and amino acids (Setchell, Hinks, Voglmayr, and Scott, 1967). Their slightly greater oxidation of acetate-1-^{14}C than acetate-2-^{14}C could be due to entry of unlabeled endogenous substrate into the tricarboxylic acid cycle (Weinman, Strisower, and Chaikoff, 1957), as endogenous substrate does contribute to their metabolism. However, it is likely that the process is a dynamic one with both synthesis and breakdown of endogenous materials. That there is considerable pooling of acetate carbon in testicular spermatozoa is also consistent with the apparent acceleration in $^{14}CO_2$ production from labeled acetate with time of incubation. The present investigation has shown that this pool consists mainly of a compound or compounds that are not carboxylic acids. However, recent studies by Voglmayr and White (1970) have shown that freshly collected testicular spermatozoa accumulate an intracellular "reserve carbohydrate" from glucose.

Although ejaculated spermatozoa accumulate as much substrate carbon intracellularly as testicular cells, interchange with compounds other than through the glycolytic or citric acid cycle pathways must be minimal. Their differential oxidation of the carbon atoms of acetate at 2 hr suggests some pooling. A similar interaction between length of incubation time and labeling of carbon atom was observed by Scott *et al.* (1962) when ejaculated ram spermatozoa were incubated with glucose-6-^{14}C and glucose-1-^{14}C. These workers also suggested pooling of intermediates by the sperm cell.

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