BRIEF COMMUNICATION

THE METABOLISM OF CARBOHYDRATES AND OTHER
COMPOUNDS BY EPIDIDYMAL-LIKE BOVINE
SPERMATOOZOA

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Summary. The ability of bovine spermatozoa to metabolize carbohydrates and other compounds oxidatively has been studied, using spermatozoa free of the components of the seminal fluids. Only glucose, fructose, mannose and galactose of the hexoses were significantly oxidized by the spermatozoa.

An uptake of substrates from the seminal plasma and their subsequent utilization by the spermatozoa interferes with any attempt to assess the ability of ejaculated spermatozoa to oxidize other substrates. Any critical examination of substrate utilization therefore requires the use of epididymal spermatozoa. Recent investigations in our laboratory have resulted in the collection of exogenous substrate-free ejaculated sperm cells (Salisbury & Graves, 1963) which are immobilized immediately after collection and which have the substrates of the seminal plasma partly removed by washing. These cells exhibit metabolic characteristics similar to epididymal spermatozoa and therefore have been termed 'epididymal-like cells' (ELC).

This paper reports the results of experiments designed to measure the ability of these seminal plasma-free mammalian spermatozoa to oxidize many carbohydrates, amino acids and short-chain fatty acids. The influence of CO₂ on spermatozoan respiration in the presence of these substrates was investigated also.

The sperm samples (ELC) used in this study were collected as previously described (Salisbury & Graves, 1963) and resuspended, after washing, to a concentration of 1 to 2×10^9 cells/ml in 0.9% NaCl. The oxygen consumption was measured manometrically at 37° C in 17 ml single side-arm flasks with air as the gas phase. The effect of CO₂ on respiration under a gas phase of 20% O₂, 5% CO₂ and 75% N₂, was studied in 17 ml single side-arm Dixon-Keilin flasks.

Since maximum respiration of bull spermatozoa during short-time aerobic incubation occurs in saline (Bishop & Salisbury, 1955), this was used as the suspending medium and each substrate was added at a concentration of 0.027 M (pH 7.0). The cell suspension in the side-arm (0.2 ml) was tipped into the main body of the flask containing the 0.8 ml of incubation medium after a

10-min equilibration period. The endogenous respiration (in the presence of saline) and the respiration in 0.027 M fructose (500 mg/100 ml, the approximate level in seminal plasma) were used as controls. Aliquots, taken initially and after incubation, were analysed for fructose, lactic acid, phosphate and pyruvate as previously reported (Salisbury & Graves, 1963).

Only the 4-hr values are given, with the respiration data given as the percentage of oxygen uptake above the endogenous controls. Analysis of variance was used to test for significant differences in the oxygen uptake data.

Of the ninety-six carbohydrates, amino acid, fatty acid and nucleic acid compounds investigated in this study, only the substrates shown in Table 1

TABLE 1
SUBSTRATES WHICH SIGNIFICANTLY ($P < 0.01$) STIMULATED
OXYGEN UPTAKE OF ELC DURING 4 hr AT 37°C, ALONG
WITH THE % O₂ UPTAKE ABOVE THE ENDOGENOUS LEVEL,
THE FINAL % MOTILE AND THE FINAL LACTATE PRODUCTION
VALUES

Substrate	% O ₂ uptake above endogenous level*	% motile	Lactate ($\mu\text{g}/10^8$)
Maltose	309.1	45.0	111.0
Pyruvate	301.5	12.7	135.2
Glycerol	258.4	16.2	0.0
Lactate	232.9	15.7	-178.2
Mannose	206.3	17.8	77.3
Sorbitol	190.7	14.2	0.0
Glucose	189.5	22.2	66.7
Fructose	187.6	22.5	47.2
Acetate (K)	181.1	7.7	0.0
Acetate (Na)	150.3	10.0	0.0
Fructose-6-phosphate	133.0	8.0	0.0
Glyceraldehyde-3-phosphate	107.8	1.1	35.1
Fructose-1,6-phosphate	101.0	0.0	17.1
Glycerol-phosphate	100.0	13.0	10.3
Glucose-1-phosphate	96.9	17.5	0.0
Oxaloacetate	80.7	13.3	0.0
Glucose-6-phosphate	78.4	7.0	0.0
Ribose-5-phosphate	58.8	2.2	0.0
Galactose	51.4	21.1	0.0
Glyceraldehyde	44.3	0.0	0.0
Glycogen	33.7	0.0	0.0
Oxalosuccinate	28.6	11.4	0.0

* Saline = 12.1 $\mu\text{l}/10^8$ cells/4 hr.

significantly ($P < 0.01$) stimulated the oxygen uptake. Five per cent CO₂ in the presence of these same compounds significantly ($P < 0.01$) stimulated cellular respiratory activity only in the presence of glucose, fructose, mannose, pyruvate, isocitrate, α -ketoglutarate and oxaloacetate over the respiration in the absence of the 5% CO₂. Chemicals previously shown to affect cell permeability, *trans*-1,2-cyclopentane dicarboxylic acid (Seaman & Houlihan, 1950) and dimethylsulphoxide (Lovelock & Bishop, 1959), had little effect on respiration in the presence of any of the compounds.

The results show that only a few compounds can serve as sources of energy for mammalian spermatozoan motility as measured by increased respiratory

activity and maintenance of motility. The significant increase in oxygen uptake of the spermatozoa incubated in the presence of the phosphorylated compounds, oxaloacetate, galactose, glyceraldehyde, glycogen and oxalosuccinate is undoubtedly due to the characteristic low endogenous respiration of ELC incubated in the absence of substrate (saline), in which small stimulatory effects due to metabolizable substrates are readily observed. Glycogen, for example, caused no detectable stimulation to respiration of either whole semen (Redenz, 1933) or washed spermatozoa (Mann, 1946), both of which have high endogenous respiration rates due to the uptake by the spermatozoa of substrates from the seminal plasma. Redenz (1933) using whole semen was also unable to detect a utilization of the phosphorylated compounds.

The stimulatory effect of low levels of CO₂ has previously been reported to occur in spermatozoa by Salisbury & Kinney (1957) and Jones & Salisbury (1962).

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