Effects of osmolality, bicarbonate and buffer on the metabolism and motility of testicular, epididymal and ejaculated spermatozoa of boars

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Summary. Spermatozoa were collected from the rete testis of conscious boars, from the cauda epididymidis by retro-flushing, and by ejaculation. Testicular spermatozoa showed no progressive motility, and that of ejaculated was greater than that of epididymal spermatozoa. Glycolysis and respiration of testicular spermatozoa, while lower than that of the more mature cells, were only slightly affected by the incubation conditions. Epididymal spermatozoa converted 83% of the glucose they utilized to CO₂ or lactate, but testicular cells converted only 35% to these metabolites. Synthesis of lipid was greatest by testicular spermatozoa. With the more mature cells hyperosmolar conditions depressed CO₂ production, but increased lactate production, and these changes were greater for ejaculated than for epididymal spermatozoa. Glycolysis plus respiration of these cells was related to their motility. These results were interpreted as showing increasing motility, glycolysis and respiration with maturation, but also decreased synthetic capacity and increased sensitivity to the environment.

Introduction

That boar spermatozoa undergo changes as they pass through the epididymis has been inferred for a considerable time (Lasley & Bogart, 1944) but it was only the introduction of cannulation techniques (Voglmayr, Waites & Setchell, 1966) that enabled tissue-free testicular spermatozoa to be obtained. Changes in metabolism during sperm maturation in some species have recently been reviewed (Voglmayr, 1975). By adaptation of the techniques used in the ram it is possible to collect boar spermatozoa at various stages of maturation. Because the natural fluids in which these spermatozoa are suspended are different (Mann, 1975), a comparison was made of the metabolism of washed cells. The osmotic pressures of the native suspending fluids also differ (Levine & Marsh, 1971), and since osmotic pressure affects ejaculated spermatozoa (Blackshaw & Emmens, 1951; Stevermer, First & Hoekstra, 1964), this was included as one of the main variables in a comparison of the metabolism of boar spermatozoa at various stages of maturation.

Materials and Methods

Sperm samples. Large White boars aged 1–2 years were used. Ejaculated spermatozoa were from the 'sperm rich' fraction of ejaculates collected manually with a 'dummy sow' and filtered through gauze. The seminal plasma was removed after centrifugation at 900 g for 10 min. The

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spermatozoa were then washed twice at 25°C with the phosphate-buffered washing diluent (PBW: see below). Epididymal spermatozoa were obtained from the cauda epididymidis within 30 min of slaughter or castration of the animal. The tubule of the cauda epididymidis was flushed by injection of paraffin oil into the vas deferens and the spermatozoa were collected from a freed loop of the tubule. The cell suspension collected (about 4 ml) was diluted in 50 ml PBW at 25°C and washed twice at 25°C by centrifugation at 900 g for 10 min.

Testicular spermatozoa were obtained in rete testis fluid collected from conscious boars. The extratesticular rete was cannulated as described previously for the ram (Voglmayr *et al.*, 1966; Voglmayr, Scott, Setchell & Waites, 1967). The rete testis fluid was drawn caudally into a vessel, cooled to 4°C as described for the ram (Edwards, Dacheux & Waites, 1976). The boars were kept in a normal pen and the catheters remained patent for up to 8 days, with a fluid flow rate of about 2.5–3 ml/h and 150×10^6 spermatozoa/ml. Samples were removed after collection for 12 h. In the present study some testicular spermatozoa were obtained from animals cannulated and maintained under anaesthesia for several hours before slaughter. Spermatozoa were separated from the testicular fluid by centrifugation and washed once with PBW.

Diluents. All diluents contained 5 mM-KCl, 2 mM-MgCl₂, 1 mM-CaCl₂, sodium benzyl penicillin (500 000 i.u./l) and streptomycin sulphate (0.5 g/l). Spermatozoa were washed and resuspended in the phosphate-buffered washing diluent (PBW) which also contained 1 mM-Na₂HPO₄, adjusted to pH 7.4 with NaH₂PO₄ and to 310 mosmol/kg H₂O (with a Fiske Osmometer) with NaCl (about 145 mM). Spermatozoa were not in PBW for longer than 1 h.

When comparing buffers, incubation diluents containing 40- or 60-mM phosphate buffer (sodium salts), or 60 mM-Tris ((hydroxymethyl) aminomethane)-HCl buffer (Tris buffer), or 60 mM-N-Tris ((hydroxymethyl) methyl 2-aminoethane sulphonic acid)-HCl buffer (Tes buffer), and 10 mM-glucose were used. To study the effect of osmotic pressure 60 mM-Tris-HCl and 40 mM-phosphate-buffered incubation diluents containing 10 mM-glucose and with and without 12 mM-sodium bicarbonate were used. The osmotic pressure was varied by altering the amount of NaCl to obtain diluents with 150, 190, 250, 310, 370 or 430 mosmol/kg (Tris) and 190, 250, 310 or 430 mosmol/kg (phosphate). The pH and osmotic pressure of diluents containing sodium bicarbonate were adjusted after addition of the bicarbonate.

Incubation system. Spermatozoa suspended in 0.5 ml PBW were incubated at 37°C with gentle shaking for 3 h in 10-ml Erlenmeyer flasks containing 0.5 ml of the appropriate incubation diluent and 1 µCi [U-¹⁴C]glucose (sp. act. 316 mCi/mmol: Radiochemical Centre, Amersham, England). The final concentration of spermatozoa in the flasks was about 1×10^8 /ml. Buffer concentrations during incubation were 30 mM-Tris, 30 mM-Tes, and 20 mM- or 30 mM-phosphate. The osmotic pressures referred to in the text are the final values obtained during incubation in the flasks.

Carbon dioxide production. The flasks were fitted with air-tight stoppers and plastic hanging wells. CO₂ production was estimated as described previously (Edwards *et al.*, 1976). At 10 min before the end of incubation 0.2 ml hyamine hydroxide (1.0 M in methanol) was injected into the hanging wells. The CO₂ dissolved in the buffer was liberated by the injection of 0.2 ml 1 M-H₂SO₄ into the flasks at the end of the incubation. The hanging vials were removed 1 h later, wiped, and placed in scintillation vials containing 10 ml scintillation mixture (toluene:triton, 2:1 v/v; PPO 4 g and POPOP 0.1 g/l toluene). Radioactivity in the trapped CO₂ was estimated after the chemiluminescence had disappeared (at least 24 h). Spermatozoa incubated under the same conditions but with the H₂SO₄ added at the start of incubation gave background values for each treatment.

Incorporation of glucose into lipids. When the flasks were opened the incubation medium was centrifuged and the supernatants removed. After washing in 2 ml PBW containing 50 mM-glucose and 50 mM-lactate the spermatozoa were extracted three times with chloroform:methanol (2:1 v/v). The lipid extract was washed once with 0.9% (w/v) KCl and four times with methanol:water (1:1 v/v). The washing was judged to be satisfactory because there

was no radioactivity in the aqueous phase at the last wash. The extracted lipid was dried under N_2 and dissolved in scintillation fluid for estimation of the radioactivity present in the lipids.

Lactate production. The supernatants from the incubations were deproteinized with 0.2 ml 3.6 M-perchloric acid, neutralized with 5 N-KOH, and lactate was estimated enzymically. Glucose levels were measured enzymically on the same supernatants from the epididymal spermatozoa. With the epididymal cells the [^{14}C]lactate formed from [^{14}C]glucose was measured after separation by paper chromatography with butanol:acetic acid:water (4:1:5 by vol.) as the eluant. This was more difficult with the testicular cells because of the greater diversity of labelled products formed, but [^{14}C]glucose and [^{14}C]lactate were isolated from some replicates by paper chromatography.

Motility of spermatozoa. The percentage of motile spermatozoa and the progressive motility of the cells were assessed after the 3 h incubation (Paquignon, Dacheux & Courot, 1977) by using aliquots of sperm suspension which were not treated with acid.

Statistical analyses. For a preliminary experiment mean values were compared with Duncan's multiple range test (Duncan, 1955). Analyses of variance were used on the data from the other experiments. Partitioning of treatment sums of squares was carried out with respect to the effects of osmotic pressure and its interactions by using matrices of orthogonal polynomial coefficients (Fisher & Yates, 1963). The terminology 'L' and 'Q', taken from Cochran & Cox (1957), indicates the linear regression (L) and the deviation from linearity or quadratic curvature (Q). Only the relevant portions of the analyses of variance are given in the tables or text, and some data, although examined by analysis of variance, are presented as mean $\pm$ standard error (s.e.m.).

Results

Ejaculated spermatozoa

Comparison of buffers. In a preliminary experiment 30 mM-phosphate, 20 mM-phosphate, 20 mM-phosphate + 6 mM-bicarbonate, and 30 mM-Tris at pH 7.4 were compared for 4 ejaculates. After incubation for 3 h there were no differences in the lactate production, percentage of motile spermatozoa and progressive sperm motility. Production of CO_2 was 1.06, 1.26, 2.16, and 2.12 $\mu\text{mol}/10^8$ spermatozoa in 3 h with 30 mM-phosphate, 20 mM-phosphate, 20 mM-phosphate + 6 mM-bicarbonate, and 30 mM-Tris respectively. Examination of the data with Duncan's multiple range test showed that CO_2 production with 20 mM-phosphate + 6 mM-bicarbonate or with Tris was greater ($P < 0.05$) than that with 20 mM- or 30 mM-phosphate.

In a second experiment 30 mM-Tris and 30 mM-Tes at pH 7.4 with and without addition of 6 mM-sodium bicarbonate were compared for 6 ejaculates. Again there were no differences in the percentage of motile spermatozoa and in their progressive motility; CO_2 production was 1.81, 2.44, 0.81, and 1.94 $\mu\text{mol}/10^8$ cells in 3 h with Tris, Tris + bicarbonate, Tes, and Tes + bicarbonate respectively. The effects of buffer and bicarbonate addition were both significant ($P < 0.001$). Lactate production was decreased from 0.57 to 0.47 $\mu\text{mol}/10^8$ cells over 3 h by addition of bicarbonate to Tris buffer but was increased from 0.55 to 0.59 $\mu\text{mol}/10^8$ cells over 3 h by addition of bicarbonate to Tes buffer (buffer $\times$ bicarbonate interaction: $P < 0.05$).

Because of these results, Tris was subsequently used as the main buffer in factorial experiments which could be statistically examined with analyses of variance; phosphate was used to a lesser extent.

Effect of pH of buffer. This was studied for 4 ejaculates with Tris-HCl buffer at pH 7.2, 7.4 and 7.6, and with final osmotic pressures of 280, 310, 340 and 370 mosmol. There were no interactions between pH and osmotic pressure; CO_2 production and sperm motility were not altered by change in pH. Lactate production ($\mu\text{mol}/10^8$ cells over 3 h) increased linearly ($P < 0.001$) as the pH of the medium was raised (mean $\pm$ s.e.m. 0.43 ± 0.05 at pH 7.2; 0.55 ± 0.05 at pH 7.4;

Table 1. Effect of osmotic pressure and 6 mM-bicarbonate on the motility of boar spermatozoa after incubation and on their production of CO₂ and lactate (measured enzymically) during incubation, which was for 3 h at 37°C in Tris-HCl buffer ($n = 4$, except for CO₂ production by testicular spermatozoa where $n = 6$)

Sperm type	Bicarbonate	Osmotic pressure (mosmol)						s.e.m.*	d.f.	
		230	250	280	310	340	370			
% Spermatozoa motile										
Ejaculated	—	—	—	55.5	55.0	55.9	53.4	1.215‡	33	
Ejaculated	—	57.1	60.0	55.0	54.1	—	—	0.794‡	12	
Epididymal	mean†	48.1	48.8	50.0	51.3	53.1	50.6	0.151‡	33	
Progressive motility										
Ejaculated	—	—	—	3.58	3.62	3.48	3.00	0.073	33	
Ejaculated	—	3.50	3.70	3.62	3.54	—	—	0.046	12	
Epididymal	mean†	3.14	3.08	3.23	3.15	3.23	3.20	0.147	33	
CO ₂ production (μmol/10 ⁸ cells/3 h)										
Ejaculated	—	—	—	2.28	1.80	1.19	0.62	0.160	33	
Ejaculated	—	2.17	2.68	2.45	2.10	—	—	0.167	12	
Epididymal	—	1.98	2.37	2.37	2.19	2.03	1.71	0.179	15	
Epididymal	+	1.73	2.59	2.72	2.70	2.64	2.29			
Testicular	mean†	0.70	0.77	0.85	0.80	0.82	0.73	0.064	24	
Lactate accumulated (μmol/10 ⁸ cells/3 h)										
Ejaculated	—	—	—	0.39	0.57	0.62	0.54	0.059	33	
Ejaculated	—	0.12	0.15	0.24	0.45	—	—	0.039	12	
Epididymal	—	0.37	0.44	0.48	0.54	0.56	0.55	0.034	14	
Epididymal	+	0.47	0.58	0.60	0.60	0.57	0.54			
Testicular	mean†	0.28	0.25	0.24	0.25	0.25	0.26	0.041	14	

* Standard error of the difference between means calculated from the error mean square of the analysis of variance.

† Mean of values obtained with and without bicarbonate.

‡ On data transformed to the arcsin $\sqrt{\text{percentage}}$.

0.61 $\pm$ 0.06 at pH 7.6). There was a small but significant ($P < 0.05$) linear increase in the mean percentage of motile spermatozoa with increasing pH (53.5% at pH 7.2; 55.2% at pH 7.4; and 56.3% at pH 7.6).

Effect of osmotic pressure. The effect of osmotic pressure during incubation was studied in two experiments, the first associated with pH effects (see above) and an osmotic pressure range between 280 and 370 mosmol, and the second at pH 7.4 between 230 and 310 mosmol.

At the end of the 3-h incubation the percentage of spermatozoa which were motile (Table 1) was greatest at 250 mosmol ($P < 0.01$ for L; $P < 0.05$ for Q) and decreased ($P < 0.05$ for L) between 280 and 370 mosmol. Progressive motility was also maximal at 250 mosmol ($P < 0.001$ for Q), and decreased further above 310 mosmol ($P < 0.001$ for L and Q). The production of CO₂ was maximal ($P < 0.01$ for Q) around 250 mosmol, and decreased linearly ($P < 0.05$) between 280 and 370 mosmol (Table 1). Lactate accumulation was greatest ($P < 0.001$ for Q) at 310–340 mosmol, and was higher at 370 than at 280 mosmol ($P < 0.001$ for L). In the second experiment lactate accumulation increased linearly ($P < 0.001$) between 220 and 310 mosmol, although there was a small but significant ($P < 0.01$) quadratic effect.

Epididymal spermatozoa

Spermatozoa from 4 boars were incubated over a range of osmotic pressures in Tris-HCl or phosphate buffers at pH 7.4.

Table 2. Effect of osmotic pressure and 6 mM-bicarbonate on the metabolism of washed boar spermatozoa incubated for 3 h at 37°C in Tris-HCl buffer with glucose as substrate (values are for 10⁸ spermatozoa/3 h)

		Osmotic pressure (mosmol)						s.e.m.*	d.f.	n
Bicarbonate		230	250	280	310	340	370			
Epididymal spermatozoa										
Glucose utilized (μmol)	mean†	0.60	0.79	0.84	0.81	0.76	0.75	0.103	15	4
Glucose converted to lipid (nmol)	—	1.38	1.51	1.92	2.27	2.58	2.70	0.097	15	4
	+	1.49	1.51	1.73	1.97	1.99	2.02		15	4
% Glucose utilized found as CO ₂	—	53	55	49	41	41	37	0.046‡	15	4
	+	43	45	47	51	50	47		15	4
Testicular spermatozoa										
Glucose utilized (μmol)	—	0.87	0.86	0.82	0.72	0.73	0.78	0.163	7	3
Glucose converted to lipid (nmol)	mean†	2.58	2.74	2.88	2.84	3.00	2.77	0.100	24	6
Glucose converted to lactate (μmol)	—	0.106	0.098	0.103	0.114	0.129	0.145	0.013	11	2
	+	0.122	0.112	0.106	0.112	0.108	0.132		11	2
Glucose converted to CO ₂ + lactate (μmol)	—	0.227	0.223	0.230	0.234	0.241	0.276	0.018	9	3
	mean†	0.208	0.197	0.203	0.214	0.214	0.256	0.017	11	2

* Standard error of the difference between means calculated from the error mean square of the analysis of variance.

† Mean of the values obtained with and without bicarbonate.

‡ On data transformed to angle = arcsin √percentage for the analysis of variance.

Effect of bicarbonate and osmotic pressure with 30 mM-Tris-HCl buffer. The osmotic pressure of the fluid from the cauda epididymidis (the supernatant after centrifuging) was about 335 ± 10 mosmol. For nearly all the variables measured there were large interactions between addition of bicarbonate and different boars and when applicable this has been allowed for in the statistical analyses. The peak value for percentage of motile spermatozoa was at 250 mosmol in the presence of bicarbonate but was at 340 mosmol in its absence (bicarbonate $\times$ osmotic pressure interaction: $P < 0.05$ for $L \times Q$). There was also an overall linear increase in percentage motile spermatozoa with rising osmotic pressure ($P < 0.05$). Bicarbonate and osmotic pressure had no significant effect on progressive motility (Table 1). Addition of bicarbonate resulted in the pH of the medium at the end of incubation (pH 7.37) being higher ($P < 0.001$) than in its absence (pH 7.11).

The production of CO₂ by epididymal spermatozoa (Table 1) was affected by the presence of bicarbonate. Therefore, although there was an overall quadratic effect of osmotic pressure ($P < 0.05$), peak CO₂ production occurred at 250–280 mosmol in the absence of bicarbonate and at 250–340 mosmol in its presence (bicarbonate $\times$ osmotic pressure interaction: $P < 0.05$ for $L \times Q$). Addition of bicarbonate at low osmotic pressures had no effect but increased CO₂ production at higher osmotic pressures (i.e. a significant interaction of osmotic pressure and addition of bicarbonate: $P < 0.001$ for $L \times L$).

Lactate accumulation, whether measured enzymically (Table 1) or as [¹⁴C]lactate from precursor [¹⁴C]glucose, showed a quadratic effect of osmotic pressure ($P < 0.001$). As with CO₂ production, there was a linear $\times$ linear interaction of the effects of osmotic pressure and bicarbonate addition ($P < 0.01$) but addition of bicarbonate had a stimulatory action on lactate accumulation below 310 mosmol.

Lipid synthesis from glucose (Table 2) increased with rising osmotic pressure but the rate of increase was less when bicarbonate was included in the medium (osmotic pressure $\times$ bicarbonate

interaction: $P < 0.001$ for $L \times L$). Glucose utilization by epididymal spermatozoa was markedly affected by osmotic pressure, with a maximum at 280 mosmol ($P < 0.05$ for Q). Because of the lack of effect of addition of bicarbonate on glucose utilization, CO_2 production as a percentage of glucose utilized was virtually unchanged in the presence of bicarbonate, but fell with increasing osmotic pressure in the absence of bicarbonate (Table 2: osmotic pressure $\times$ bicarbonate interaction: $P < 0.001$ for $L \times L$). The proportion of glucose utilized which accumulated as lactate (36%) and as lactate + CO_2 (83%) was constant under all conditions of incubation.

Table 3. Effect of buffer, 6 mM-bicarbonate, and osmotic pressure on the metabolism of glucose by washed spermatozoa from the cauda epididymidis of the boar (means for 4 boars, for 10^8 spermatozoa/3 h)

Buffer	Bicarbonate	Osmotic pressure (mosmol)	CO_2 formed (μmol)	Lactate accumulated (μmol)	Glucose utilized (μmol)	% glucose utilized found as CO_2	% glucose utilized found as lactate	Glucose converted to lipid (nmol)
Tris	—	250	2.37	0.44	0.68	55	36	1.51
		280	2.37	0.48	0.75	49	36	—
		310	2.19	0.54	0.79	41	35	2.27
		370	1.71	0.55	0.72	37	38	2.70
	+	250	2.59	0.58	0.89	45	33	1.51
		280	2.72	0.60	0.92	47	32	—
		310	2.70	0.60	0.83	51	39	1.97
		370	2.29	0.54	0.77	47	37	2.02
Phosphate	—	250	2.46	0.40	0.71	46	34	1.93
		280	2.01	0.44	0.70	44	32	—
		310	1.88	0.50	0.67	43	41	2.31
		370	1.14	0.57	0.57	26	53	2.63
	+	250	3.09	0.49	0.91	53	28	2.10
		280	2.65	0.56	0.77	57	37	—
		310	2.67	0.54	0.84	49	34	2.14
		370	1.67	0.54	0.70	33	40	2.22

Summary of the analyses of variance

Variance ratios							
Source of variation	d.f.	CO_2	Lactate accumulated	Glucose utilized	% glucose as CO_2 †	% glucose as lactate†	Lipid
A. Between buffers	1	7.46*	3.42	5.92*	3.07	0.72	13.25
B. Bicarbonate	1	72.30***	23.86***	24.67	9.11**	2.67	14.39
C. Osmotic pressure							
L	1	105.11	11.59	8.14**	33.78	11.39**	69.90
Q	1	14.09**	1.74	2.44	6.02*	0.68	2.04
D. Amongst boars	3	231.23***	135.30*	83.34*	62.72***	56.24***	38.13**
A $\times$ C							
L $\times$ L	1	22.07***	2.50	2.42	5.84*	3.58	8.19**
B $\times$ C							
L $\times$ L	1	0.90	10.55**	2.30	4.17	0.35	17.62***
B $\times$ D	3	1.02	14.81***	3.53*	0.14	1.59	1.16
C $\times$ D	9	3.01*	3.92**	2.04	0.30	0.80	2.69*
Higher-order boar interactions (error mean square)	26	0.0627	0.0027	0.0108	0.0059	0.0068	0.0452‡

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (using the higher-order interactions where appropriate).

† On data transformed to the arcsin $\sqrt{\text{percentage}}$.

‡ For 21 d.f.

Table 4. Effect of buffer, 6 mM-bicarbonate, and osmotic pressure on the metabolism of washed testicular spermatozoa of the boar (values are for 10^8 spermatozoa/3 h; $n = 5$ for CO_2 , 3 for lactate and 4 for lipid)

Buffer	Bicarbonate	Osmotic pressure (mosmol)	CO_2 formed (μmol)	Lactate accumulated (μmol)	Glucose converted to lipid (nmol)
Tris	—	250	0.90	0.26	3.11
		310	1.03	0.25	3.18
		370	0.87	0.24	3.02
	+	250	0.81	0.24	2.87
		310	0.90	0.27	3.09
		370	0.78	0.28	2.96
Phosphate	—	250	0.70	0.21	3.75
		310	1.06	0.29	4.49
		370	0.96	0.23	4.32
	+	250	0.72	0.22	3.65
		310	0.77	0.24	4.14
		370	0.69	0.21	3.31

Summary of the analyses of variance

Source of variation	CO_2		Lactate		Lipid	
	d.f.	Variance ratio	d.f.	Variance ratio	d.f.	Variance ratio
A. Between buffers	1	5.64	1	3.02	1	28.40***
B. Bicarbonate	1	27.23***	1	0.00	1	3.31
C. Osmotic pressure						
L	1	1.60	1	0.01	1	0.09
Q	1	22.53**	1	0.97	1	3.84
D. Amongst boars	4	116.55***	2	7.94**	3	56.72***
A $\times$ B	1	1.88	1	1.34	1	1.12
A $\times$ C	2	2.49	2	1.04	2	0.69
A $\times$ D	4	11.27***	2	7.48**	3	14.62***
B $\times$ C	2	3.58	2	0.19	2	0.45
B $\times$ D	4	3.88*	2	0.35	3	2.10
C $\times$ D	8	2.63*	4	0.53	6	0.91
A $\times$ B $\times$ C						
L $\times$ L $\times$ L	1	4.63*	1	0.05	1	0.14
L $\times$ L $\times$ Q	1	1.01	1	0.53	1	1.62
Higher-order interactions (error mean square)	25	0.0114	13	0.0031	16	0.2601

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (using the higher-order interaction where appropriate).

Comparison of Tris-HCl and phosphate buffers. There were no significant effects on the percentage of motile spermatozoa or on progressive sperm motility. The pH of the medium at the end of incubation was similar in Tris + bicarbonate (pH 7.37) and phosphate + bicarbonate (pH 7.31) but fell more ($P < 0.05$) in phosphate (pH 6.92) than in Tris (pH 7.08).

The significant effects obtained with the two buffers are presented in Table 3. Carbon dioxide production from glucose was increased (mean 25%) by the addition of bicarbonate. Increasing osmotic pressure resulted in a greater depression of CO_2 production with the phosphate buffer (decrease of 50%) than with Tris buffer (decrease of 20%). Accumulation of lactate was increased by addition of bicarbonate at low osmotic pressure but was unaffected at high osmotic pressure. Lipid formation from glucose was greater in phosphate buffer than in Tris buffer at 250 mosmol, but the amounts formed were similar in the two buffers at 310 and 370 mosmol. Addition of bicarbonate had no effect on lipid synthesis at 250 mosmol but depressed lipid synthesis at 310 and 370 mosmol. Glucose utilization was greater with Tris than with phosphate

buffer. Bicarbonate enhanced glucose utilization but the increase varied amongst boars. As the osmotic pressure increased there was less utilization of glucose. The percentage of glucose utilized and found as CO_2 fell with high osmotic pressure, the decrease being greater with phosphate than with Tris buffer, while that found as lactate increased with increasing osmotic pressure because lactate formed from glucose remained constant. However, the proportion of glucose disappearing that was found as CO_2 + lactate was high (81%) and constant under all conditions.

Testicular spermatozoa

The rete testis fluid of the boar had an osmotic pressure of about 273 ± 11 mosmol ($n = 5$). These spermatozoa showed no progressive motility, and progressive motility was not initiated by any treatment during incubation at 37°C for 3 h at pH 7.4.

The results with Tris buffer are shown in Tables 1 and 2 and for the comparisons of Tris and phosphate buffers in Table 4.

Production of CO_2 was slightly depressed ($P < 0.01$ for Q) at high or low osmotic pressures (Table 1). A similar mean trend in the comparison of buffers (Table 4) was not statistically significant as it did not occur for all boars. Although total lactate accumulation (i.e. enzymic estimation) was not affected by osmotic pressure, addition of bicarbonate or by type of buffer, there were some small but statistically significant changes in the accumulation of [^{14}C]lactate from [^{14}C]glucose (Table 2). In the absence of bicarbonate, lactate formed from glucose increased with rising osmotic pressure (bicarbonate $\times$ osmotic pressure interaction: $P < 0.05$ for $\text{L} \times \text{L}$). Overall lactate production from glucose was least at 250–280 mosmol ($P < 0.05$ for Q). Glucose converted to lactate + CO_2 was least at 250 mosmol and then increased as the osmotic pressure rose ($P < 0.01$ for L and Q). Because of the small amount of glucose utilized the errors in the estimation of the amount of glucose utilized were large. Glucose utilized (0.72 ± 0.16 $\mu\text{mol}/10^8$ spermatozoa over 3 h), lactate formed from glucose as a percentage of glucose utilized ($20 \pm 2.4\%$), and lactate formed from glucose + CO_2 formed from glucose as a percentage of glucose utilized ($35 \pm 4.8\%$) were not altered by osmotic pressure changes or by addition of bicarbonate. No chromatography was carried out after incubation in phosphate buffer. Lipid production from glucose increased as the osmotic pressure was raised from 230 to 340 mosmol but then fell ($P < 0.01$ for L and Q).

Discussion

The changes in metabolism of boar spermatozoa as they mature are similar to those found in other species (see Voglmayr, 1975). Under optimal incubation conditions, production of CO_2 and lactate increased with maturation (Table 1). Conversely, synthetic capacity, as measured by incorporation of glucose into lipid, was greater in testicular than in epididymal spermatozoa (Table 2). As with other species, most of the glucose utilized by mature boar epididymal spermatozoa of boars was converted to CO_2 and lactate, but this conversion by boar testicular spermatozoa was lower than that by the testicular cells of other species (Voglmayr, 1975). During maturation boar spermatozoa become more sensitive to changes in incubation conditions, whether CO_2 and lactate production are expressed as absolute values or as percentages.

Osmotic pressure changes were carried out by changing the amount of NaCl. With Tris buffer, at the two extremes of 230 and 370 mosmol, the NaCl added was about 70 and 140 mM respectively. The change in the Na:K ratio thus caused would not have affected the motility of the boar spermatozoa (Boender, 1976). Although chloride levels were also changed, it is

assumed that any effects seen were due to osmotic pressure *per se*, a view supported by the data of sperm motility at different osmotic pressures. Low osmotic pressures stimulated motility, as observed previously with stored boar spermatozoa (Steevermer *et al.*, 1964). The effects of osmotic pressure on motility were smaller with epididymal spermatozoa.

The effects of osmotic pressure on CO_2 and lactate production were greatest with ejaculated spermatozoa, and minimal with testicular cells. Glucose incorporation into lipid was more affected by osmotic pressure in epididymal than in testicular spermatozoa. This may be related to the increasing susceptibility to cold shock of spermatozoa as they mature (Lasley & Bogart, 1944; Voglmayr *et al.*, 1967).

Decreased CO_2 production with hyperosmolality supports the suggestion with osmotic pressure affects the morphology of the mitochondria, as it does the volume of the whole cell (Drevius, 1972). The increased lactate and lipid accumulation with hyperosmolar conditions may not be similarly affected because lipid synthesis occurs in the cytoplasm and glycolytic enzymes are concentrated in the tail (Mohri, Mohri & Ernster, 1965). Inhibition of oxidative metabolism in the presence of increased glycolysis could in itself increase lipid synthesis by accumulation of precursors. Also, reduced oxidative metabolism may have reduced degradation of lactate and lipid, thus giving an apparent increase in their formation. However, CO_2 and lactate production are both related to motility, and the osmolality at which the motility of ejaculated spermatozoa was maintained included the peaks for both CO_2 and lactate formation, i.e. as CO_2 production is decreased glycolysis increases to maintain the energy supply. However, the increased motility of ejaculated cells under hypo-osmolar conditions is consistent with oxidation rather than glycolysis supplying the energy for progressive motility of boar spermatozoa (Nevo, Polge & Frederick, 1970).

The effects on matured spermatozoa of the addition of sodium bicarbonate are likely to be partly due to the greater buffering capacity that it confers. This may apply particularly to the intracellular medium (Salisbury, VanDemark, Lodge & Cragle, 1960), and would be less important in testicular spermatozoa because of their lower lactate production. A direct action of bicarbonate on sperm metabolism cannot be ruled out because bicarbonate is involved in sperm metabolic pathways (Mounib & Eisan, 1968; O'Shea & Wales, 1970). The interactions of bicarbonate and osmotic pressure with respect to CO_2 and lactate production by epididymal cells are such as to decrease the detrimental effects of increasing and decreasing osmotic pressure respectively. The interaction of osmotic pressure and bicarbonate on lipid synthesis and progressive motility may also be related to their interactive effects on CO_2 production. Bicarbonate caused both increased glycolysis and respiration of ejaculated ram spermatozoa (Wales & Murdoch, 1971) but not in all circumstances.

The greater energy available to epididymal spermatozoa in Tris buffer may be related to the differential ionic permeability of membranes because Tris can penetrate cell membranes (Omachi, Macey & Waldeck, 1961) and may act as an intracellular buffer. The entry of phosphate into spermatozoa is controlled by the plasma membrane, and high levels of extracellular phosphate do not always result in increased cellular phosphate levels (Babcock, First & Lardy, 1975). Phosphate has also been reported to inhibit hexokinase in muscle (Leuck & Fromm, 1974). In other species Tris has been reported to be better than phosphate for maintaining sperm motility (Jones & Foote, 1972).

This study of conditions during incubation *in vitro* has shown that boar spermatozoa become more sensitive to their environment as they mature. It is tempting to infer that this may result from some form of membrane transformation. The diminution of lipid (Grogan, Mayer & Sikes, 1966; Johnson, Pursel & Gerrits, 1972) and the possible change in fatty acid composition (Voglmayr, 1975) during transit through the epididymis could be related to such a transformation. This decrease in resistance of spermatozoa to environmental changes with maturity parallels their decreased resistance to cold shock (Lasley & Bogart, 1944), which is also a membrane phenomenon.

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