THE METABOLISM OF GLUCOSE, FRUCTOSE, ACETATE, LACTATE AND PYRUVATE BY RAM, BULL, DOG AND RABBIT SPERMATOZOA

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Summary. Ram and bull spermatozoa oxidize acetate faster than or similarly to glucose, while dog and rabbit spermatozoa oxidize glucose faster than acetate; rabbit spermatozoa, in particular, have hardly any capacity to oxidize acetate.

Glucose and fructose are oxidized at about the same rate by the spermatozoa of all four species, each sugar giving rise to similar amounts of lactate.

Lactate is readily oxidized by spermatozoa of all four species; with rabbit and dog spermatozoa the rate of lactate oxidation greatly exceeds that of glucose, fructose, pyruvate or acetate.

Pyruvate is oxidized by ram and bull spermatozoa faster than acetate. With dog and rabbit spermatozoa, the recovery of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ -pyruvate is 2 to 4 times that from $[2-^{14}\text{C}]$ or $[3-^{14}\text{C}]$ pyruvate; this may be due to a dismutation of pyruvate.

INTRODUCTION

Fructose is the predominant sugar found in the semen of most species, e.g. the ram, bull and rabbit; both fructose and glucose are readily metabolized to lactic acid by mammalian spermatozoa even in species whose semen is devoid of reducing sugar, e.g. dog (see Mann, 1964). Under aerobic conditions, lactic acid is further oxidized to carbon dioxide via pyruvate and the Krebs cycle. Semen also contains some acetic and pyruvic acid (Flipse & Potter, 1955; Flerchinger, Erb, Mikota & Ehlers, 1956; Rhodes & Williams-Ashman, 1960; Marden, 1961; Scott, White & Annison, 1961; Ramsey, Lodge, Graves & Salisbury, 1963). While neither acetate nor pyruvate are likely to be of great significance as energy sources for ejaculated spermatozoa, studies of their oxidation are of interest in relation to the oxidative metabolism of other substrates.

Scott, White & Annison (1962b) found that ram and bull spermatozoa oxidize acetate in preference to glucose, but with dog spermatozoa the reverse appeared to be true. This suggested a different pattern of acetate and glucose metabolism in the spermatozoa of ruminants and non-ruminants. In this

connection, it is of interest that the blood of the former contains less glucose but more acetate than that of non-ruminants (Annison & Lewis, 1959), and that the acetate turnover is considerably higher in ruminants than in non-ruminants (Annison & Lindsay, 1961).

This study reports further work on the rates of oxidation of glucose, fructose, acetate, lactate and pyruvate by ram, bull, dog and rabbit spermatozoa.

MATERIALS AND METHODS

Semen

Ram semen was collected by electrical stimulation with a bipolar rectal probe (Blackshaw, 1954). Bull and rabbit semen were collected by means of an artificial vagina (White, 1955). Dog semen was collected by massage. Only samples of good initial motility were used; since the spermatozoa of all four species are susceptible to cold shock (White & Wales, 1960), precautions were taken to avoid a sudden drop in temperature during the collection and processing of semen.

Suspensions of washed spermatozoa

Ram and bull semen were diluted 1 : 5 and dog and rabbit semen 1 : 3 with calcium-free Krebs-Ringer phosphate (Umbreit, Burris & Stauffer, 1959). After centrifuging at 2000 rev/min (about 400 *g*) for 10 min, the supernatant was removed by means of a Pasteur pipette and the spermatozoa resuspended in fresh diluent and washed twice by the procedure of White (1953). Spermatozoal counts were made with a haemocytometer.

Incubation experiments

Suspensions of ram and bull spermatozoa (2 ml) were incubated with 1 ml of substrate in 15 ml Warburg flasks containing CO₂-free 20% (w/v) KOH (0.2 ml) in the centre well. Suspensions of dog and rabbit spermatozoa (0.5 ml) were incubated with 0.5 ml of substrate in 6 ml Warburg flasks containing CO₂-free 20% (w/v) KOH (0.05 ml) in the centre well; the smaller flasks were used to increase the sensitivity of recording the oxygen uptake. The amounts of substrate/flask were (μ moles): pyruvate, 36; acetate, 54; glucose, 18; fructose, 18; DL lactate, 36. The manometers were filled with air, and shaken at 114 strokes/min, over a period of 3 hr at 37° C. At the end of that period the contents of the centre well were removed quantitatively by washing with 3 ml of CO₂-free water. A portion (1 to 2 ml) of the contents from the main part of the flask was deproteinized with 0.3 N-Ba(OH)₂ (0.5 ml) and 5% (w/v) ZnSO₄·7H₂O (0.5 ml) and in the filtrate lactate estimated by the enzymatic method of Barker & Britton (1957).

The ¹⁴CO₂ absorbed in the centre well and removed from there by washing, was precipitated as Ba¹⁴CO₃ in the presence of 5% ammonium chloride. The Ba¹⁴CO₃ was collected by filtration on Whatman No. 542 paper as described by Annison & White (1961), and assayed for radio-activity with an end-window Geiger-Müller tube. The counts were corrected for self-absorption by the method of Hendler (1959).

Radio-active chemicals

These were obtained from the Radiochemical Centre, Amersham, Bucks. Labelled pyruvate was stored in acid solution (Von Korff, 1964) and the radio-activity of each substrate checked by counting in a liquid scintillator (Nuclear Chicago).

Statistical analyses

Where necessary the oxygen uptake data have been subjected to standard analyses of variance with isolation of sums of squares attributable to differences between treatments and differences between ejaculates. The treatment-ejaculate interaction mean square has been used as error term.

In Table 3, the significance of differences in oxygen uptake between substrate and control has been assessed by a *t*-test using the interaction mean square from the analysis of variance (not published) as the standard error and the degrees of freedom associated with it.

RESULTS

Rates of glucose and acetate metabolism

The results of experiments in which [U-¹⁴C]glucose (where U indicates uniform labelling) and [1-¹⁴C]acetate were used, separately or in combination, are given in Table 1.

When used separately, both glucose and acetate stimulated the oxygen uptake of ram and bull spermatozoa, but the amounts of oxygen taken up and of labelled carbon dioxide liberated were greater with acetate. When glucose and acetate were used together, their effects were not additive; an analysis of variance of the oxygen uptake data (Table 2) showed a highly significant interaction between the substrates. When the two substrates were used together acetate constituted 68 to 78% of the total C oxidized (calculated as μ atoms) and glucose only 22 to 32%.

With dog and rabbit spermatozoa glucose was more effective than acetate in stimulating oxygen uptake; in fact, acetate did not increase significantly the oxygen consumption of rabbit spermatozoa above the control. The higher O₂ uptake in the presence of glucose was accompanied by higher yields of labelled carbon dioxide. When glucose and acetate were used together, glucose constituted 65 to 90% of the total C oxidized (calculated as μ atoms) and acetate only 10 to 35%.

With all four species there was considerable accumulation of lactate when glucose was the substrate. Rabbit spermatozoa produced the greatest amounts of lactate (per 10⁸ cells) and their ability to oxidize glucose and acetate is clearly much less than that of ram, bull or dog spermatozoa.

Further comparison between the rates of glucose, fructose, lactate, pyruvate and acetate metabolism

Table 3 gives the results of experiments in which the metabolism of [U-¹⁴C]-glucose, [U-¹⁴C]fructose, DL[1-¹⁴C]lactate, [1-¹⁴C]pyruvate, [3-¹⁴C]pyruvate and [1-¹⁴C]acetate was compared using ram, bull, dog and rabbit spermatozoa.

TABLE 1
METABOLISM OF ACETATE AND GLUCOSE BY SPERMATOOZOA

Species	Substrate	Progressive oxygen uptake (μ l)			Acetate oxidized (μ moles)	Glucose oxidized (μ moles)	Lactate accumulating (μ moles)
		1 hr	2 hr	3 hr			
Ram (4)	Control	12.1	19.3	23.9	—	—	—
	[1- 14 C]Acetate	29.5	57.1	83.6	1.19	—	—
	[U- 14 C]Glucose	26.6	49.7	63.7	—	0.25	3.02
	[U- 14 C]Glucose + acetate	25.8	47.1	64.7	—	0.07	3.08
	[1- 14 C]Acetate + glucose	25.8	47.3	64.3	0.76	—	3.85
Bull (6)	Control	7.4	9.4	10.9	—	—	—
	[1- 14 C]Acetate	25.8	46.6	62.3	0.71	—	—
	[U- 14 C]Glucose	17.0	30.9	41.7	—	0.11	1.79
	[U- 14 C]Glucose + acetate	18.9	35.3	50.3	—	0.06	1.96
	[1- 14 C]Acetate + glucose	14.5	27.7	43.3	0.38	—	1.77
Dog (3)	Control	4.1	5.9	9.8	—	—	—
	[1- 14 C]Acetate	9.1	16.4	23.8	0.25	—	—
	[U- 14 C]Glucose	17.3	31.9	45.6	—	0.12	3.58
	[U- 14 C]Glucose + acetate	17.5	31.0	42.0	—	0.11	3.63
	[1- 14 C]Acetate + glucose	16.3	29.9	39.7	0.18	—	3.38
Rabbit (4)	Control	3.2	4.1	4.5	—	—	—
	[1- 14 C]Acetate	3.0	4.3	5.0	0.01	—	—
	[U- 14 C]Glucose	6.9	10.1	12.5	—	0.04	4.80
	[U- 14 C]Glucose + acetate	5.4	9.2	11.2	—	0.03	5.29
	[1- 14 C]Acetate + glucose	5.7	9.2	11.2	0.01	—	5.02

Values represent means for the number of ejaculates shown in parentheses. 50 μ mc of substrate were added to each flask. The numbers of spermatozoa ($\times 10^8$)/flask were: ram, 3.1 to 12.5; bull, 3.9 to 12.7; dog, 0.8 to 2.7; rabbit, 3.1 to 5.1. All values are calculated/10⁸ spermatozoa. The $^{14}\text{CO}_2$ data have been used to calculate the amount of acetate and glucose oxidized.

TABLE 2
ANALYSIS OF VARIANCE OF THE 3 HR OXYGEN UPTAKE DATA IN TABLE 1

Source of variation	Ram		Bull		Dog		Rabbit	
	d.f.	Variance ratios	d.f.	Variance ratios	d.f.	Variance ratios	d.f.	Variance ratios
Between treatments	3		3		3		3	
Effect of acetate	1	23.5**	1	46.3**	1	3.7	1	0.1
Effect of glucose	1	2.7	1	3.6	1	104.0**	1	22.4**
Interaction	1	21.9**	1	31.0**	1	13.0*	1	0.4
Between ejaculates	3	5.9*	5	3.3*	2	3.7	3	2.4
Residual	9	156	15	104	6	20	9	9

The residual mean square is given in italics at the base of the variance ratio columns.

* $P < 0.5$; ** $P < 0.01$.

Ram and bull. All labelled substrates stimulated the oxygen uptake of ram and bull spermatozoa and produced labelled carbon dioxide. Lactate, pyruvate and acetate stimulated oxygen uptake to about the same extent, and their effect was greater than that of glucose or fructose. The $^{14}\text{CO}_2$ data confirm that ram and bull spermatozoa oxidize pyruvate and lactate faster than glucose and

TABLE 3
METABOLISM OF GLUCOSE, FRUCTOSE, LACTATE, PYRUVATE AND ACETATE BY SPERMATOZOA

Species	Substrate	Progressive oxygen uptake (μl)			Substrate yielding $^{14}\text{CO}_2$ (μmoles)	Lactate accumulating (μmoles)
		1 hr	2 hr	3 hr		
Ram (4)	Control	12.8	18.5	22.4	—	—
	[U- ^{14}C]Glucose	30.8	50.6	67.9**	0.39	1.64
	[U- ^{14}C]Fructose	30.6	50.2	64.9**	0.41	1.75
	[1- ^{14}C]Lactate	35.7	66.5	94.5**	0.98	—
	[1- ^{14}C]Pyruvate	37.7	70.2	99.2**	1.35	0.84
	[3- ^{14}C]Pyruvate	37.5	70.4	97.4**	1.16	1.44
	[1- ^{14}C]Acetate	35.4	66.9	96.0**	1.01	—
Bull (4)	Control	9.0	11.8	13.0	—	—
	[U- ^{14}C]Glucose	16.8	29.0	36.0**	0.20	2.33
	[U- ^{14}C]Fructose	18.0	31.3	41.3**	0.29	2.67
	[1- ^{14}C]Lactate	21.3	39.5	56.0**	0.64	—
	[1- ^{14}C]Pyruvate	24.5	45.4	63.5**	0.94	1.22
	[3- ^{14}C]Pyruvate	23.5	42.3	63.5**	1.02	2.26
	[1- ^{14}C]Acetate	20.5	36.3	48.8**	0.56	—
Dog (3)	Control	6.5	10.8	14.5	—	—
	[U- ^{14}C]Glucose	19.5	30.5	38.2**	0.22	1.65
	[U- ^{14}C]Fructose	18.1	26.3	32.4**	0.25	1.52
	[1- ^{14}C]Lactate	24.8	46.7	70.9**	0.70	—
	[1- ^{14}C]Pyruvate	6.6	13.2	20.5	0.79	—
	[3- ^{14}C]Pyruvate	6.9	14.2	21.7	0.22	—
	[1- ^{14}C]Acetate	10.7	18.4	26.4*	0.55	—
Rabbit (4)	Control	1.8	2.6	3.0	—	—
	[U- ^{14}C]Glucose	5.1	7.5	7.8*	0.06	1.67
	[U- ^{14}C]Fructose	5.5	8.1	8.9**	0.08	1.55
	[1- ^{14}C]Lactate	8.1	12.9	15.7**	0.20	—
	[1- ^{14}C]Pyruvate	4.4	6.5	7.5*	0.41	0.34
	[3- ^{14}C]Pyruvate	4.6	6.7	7.6*	0.14	0.22
	[1- ^{14}C]Acetate	2.3	3.4	3.7	0.12	—

Values represent the means for the number of ejaculates shown in parentheses. 50 μmc of substrate were added to each flask containing ram and bull spermatozoal suspensions; 500 μmc of substrate were added to each flask containing dog and rabbit spermatozoal suspensions. The numbers of spermatozoa ($\times 10^8$)/flask were: ram, 5.8 to 14.2; bull, 5.4 to 9.6; dog, 2.0 to 3.1; rabbit, 2.8 to 4.0. All values are calculated/ 10^8 spermatozoa.

* Significantly different from the control, $P < 0.05$.

** Significantly different from the control, $P < 0.01$.

fructose; however, in this experiment the $^{14}\text{CO}_2$ production from acetate was no greater than from the sugars. Both the oxygen uptake and the yield of $^{14}\text{CO}_2$ were similar when [1- ^{14}C] and [3- ^{14}C]pyruvate had been used as substrate. Lactate accumulated when pyruvate, glucose or fructose were incubated with the spermatozoa.

Dog. Lactate was by far the most effective substrate in stimulating the oxygen uptake of dog spermatozoa. Glucose and fructose were only about half as effective, and acetate and pyruvate even less so. The yields of $^{14}\text{CO}_2$ from $[3\text{-}^{14}\text{C}]$ -pyruvate and from the other substrates also indicate ready oxidation of lactate with the oxidative rates of fructose, glucose, acetate and pyruvate in descending order when calculated in terms of μatoms of C. In contrast to the results found with the spermatozoa of ruminants the recovery of $^{14}\text{CO}_2$ from $[1\text{-}^{14}\text{C}]$ pyruvate was more than three times that from $[3\text{-}^{14}\text{C}]$ pyruvate. Furthermore, the accumulation of lactate due to the metabolism of pyruvate by dog spermatozoa was much smaller than that produced by the metabolism of fructose or glucose.

Rabbit. In general, the ability of rabbit spermatozoa to oxidize the various substrates tried is much smaller than that of ram, bull and dog spermatozoa. However, by increasing the specific activity of the labelled substrate ten-fold, that is, to 500 μmc , it has been possible to obtain distinct data. Acetate failed to increase the oxygen uptake significantly.

TABLE 4
METABOLISM OF SPECIFICALLY LABELLED PYRUVATE AND LACTATE BY SPERMATOZOA

Species	Substrate	Oxygen uptake (μl)	Lactate utilized (μmoles)	Lactate accumulating (μmoles)	Radioactivity in respiratory $^{14}\text{CO}_2$ (μmc)
Ram	$[1\text{-}^{14}\text{C}]$ Pyruvate	62.5		4.42	1.37
	$[2\text{-}^{14}\text{C}]$ Pyruvate	64.6		4.41	1.00
	$[3\text{-}^{14}\text{C}]$ Pyruvate	64.9		4.53	1.68
	$[1\text{-}^{14}\text{C}]$ Lactate	62.6	2.03		1.67
	$[2\text{-}^{14}\text{C}]$ Lactate	63.8	1.73		1.41
Dog	$[1\text{-}^{14}\text{C}]$ Pyruvate	27.3		0.26	0.87
	$[2\text{-}^{14}\text{C}]$ Pyruvate	26.9		0.22	0.22
	$[3\text{-}^{14}\text{C}]$ Pyruvate	24.6		0.20	0.36
	$[1\text{-}^{14}\text{C}]$ Lactate	70.1	1.66		1.82
	$[2\text{-}^{14}\text{C}]$ Lactate	71.4	1.72		1.25

Values represent the means of three ejaculates for each species. Fifty μmc of substrate were added to each flask. The numbers of spermatozoa ($\times 10^8$)/flask were: ram, 2.7 to 6.7; dog, 1.3 to 2.6. All values are calculated/ 10^8 spermatozoa/3 hr.

Pyruvate was oxidized more rapidly. As with dog spermatozoa, the recovery of $^{14}\text{CO}_2$ from $[1\text{-}^{14}\text{C}]$ pyruvate was approximately three times that from $[3\text{-}^{14}\text{C}]$ pyruvate. In contrast to findings with dog spermatozoa, however, there was some accumulation of lactate when pyruvate had been used as substrate.

Differences in the metabolism of pyruvate and lactate labelled with radio-active carbon in various positions

In view of the different yields of $^{14}\text{CO}_2$ arising from the metabolism of $[1\text{-}^{14}\text{C}]$ and $[3\text{-}^{14}\text{C}]$ pyruvate by dog and rabbit spermatozoa, further studies were conducted with $[1\text{-}^{14}\text{C}]$ pyruvate, $[2\text{-}^{14}\text{C}]$ pyruvate, $[3\text{-}^{14}\text{C}]$ pyruvate, $[1\text{-}^{14}\text{C}]$ lactate and $[2\text{-}^{14}\text{C}]$ lactate using ram and dog spermatozoa (Table 4).

Ram. As in the previous experiment, no significant difference was observed between the oxygen uptake of ram spermatozoa in the presence of lactate and pyruvate. Moreover, the yields of $^{14}\text{CO}_2$ from the specifically labelled substrates

were similar except that less $^{14}\text{CO}_2$ was produced from $[2\text{-}^{14}\text{C}]\text{pyruvate}$ than from either $[1\text{-}^{14}\text{C}]$ or $[3\text{-}^{14}\text{C}]\text{pyruvate}$.

Dog. Lactate was more effective than pyruvate in stimulating the oxygen uptake and production of labelled CO_2 by dog spermatozoa. With pyruvate as substrate the $^{14}\text{CO}_2$ recovered from the metabolism of the $[1\text{-}^{14}\text{C}]$ compound was 2 to 4 times that from either $[2\text{-}^{14}\text{C}]$ or $[3\text{-}^{14}\text{C}]$ compound.

DISCUSSION

The observation that acetate and glucose increase the oxygen uptake of washed ram, bull and dog spermatozoa, confirms previous work (Lardy & Phillips, 1944; Mann & Lutwak-Mann, 1948; Humphrey & Mann, 1949; Scott *et al.*, 1962a, b). The present study and also Turner's (1960) work on human spermatozoa support the suggestion (Scott, White & Annison, 1962b) that the pattern of glucose and acetate metabolism differs in the spermatozoa of non-ruminant and ruminant species. Thus it would appear that while dog, rabbit, fowl and human spermatozoa oxidize glucose at a greater rate than acetate, ram and bull spermatozoa oxidize acetate either more readily than glucose or at a similar rate.

The lower oxidation rate of glucose by ram and bull spermatozoa is unlikely to be due to a slower formation of CoA from pyruvate than from acetate since, in both species, ram and bull spermatozoa oxidize pyruvate more readily than either acetate or glucose. A high rate of pyruvate oxidation by bull spermatozoa has been previously reported by Melrose & Turner (1953) and ready utilization of pyruvate compared with other substrates is also characteristic of spermatozoa of man (Turner, 1960), certain fish (Turner & Korsh, 1963) and the rabbit (present study).

Our work also confirms in, and extends to, other species (bull, dog and rabbit) the observation originally made by Mann & Lutwak-Mann (1948) that ram spermatozoa utilize glucose at approximately the same rate as fructose, the natural sugar of semen. The breakdown of both sugars is probably initiated by the same hexokinase-catalysed reaction and leads to the accumulation of similar quantities of lactic acid. Some of the lactic acid produced from sugar undergoes further oxidation. In some species, e.g. the dog and rabbit, the rate of lactate oxidation exceeds that of pyruvate.

The greater recovery of $^{14}\text{CO}_2$ from $[1\text{-}^{14}\text{C}]\text{pyruvate}$ than from $[3\text{-}^{14}\text{C}]\text{pyruvate}$ metabolized by dog and rabbit spermatozoa suggests the presence of a mechanism whereby the carboxyl group is removed selectively, possibly by a dismutation reaction of the type envisaged by Melrose & Turner (1953) and Turner (1959). It might be noted in this connection that the rate of oxidation of acetate in dog and rabbit spermatozoa is relatively lower than in ram and bull spermatozoa where the yields of $^{14}\text{CO}_2$ from C_1 and C_3 labelled pyruvate are more similar. Since lactate is most probably oxidized via pyruvate it is not surprising that the yields of $^{14}\text{CO}_2$ from the $[1\text{-}^{14}\text{C}]\text{lactate}$ are also greater than from $[2\text{-}^{14}\text{C}]\text{lactate}$ in dog spermatozoa.

The significance of the rather lower yield of $^{14}\text{CO}_2$ from $[2\text{-}^{14}\text{C}]\text{pyruvate}$ than from $[3\text{-}^{14}\text{C}]\text{pyruvate}$ metabolized by ram spermatozoa is not obvious.

The experiments do, however, support Scott, White & Annison's (1962b) finding that the amount of $^{14}\text{CO}_2$ produced from the carboxyl-C atom of acetate is no greater than from the methyl-C atom.

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REFERENCES

- ANNISON, E. F. & LEWIS, D. (1959) *Metabolism in the rumen*. Methuen, London.
- ANNISON, E. F. & LINDSAY, D. B. (1961) Acetate utilization in the sheep. *Biochem. J.* **78**, 777.
- ANNISON, E. F. & WHITE, R. R. (1961) Glucose utilization in sheep. *Biochem. J.* **80**, 162.
- BARKER, J. & BRITTON, H. G. (1957) The enzymatic estimation of L(+) lactic acid. *J. Physiol., Lond.* **138**, 3p.
- BLACKSHAW, A. W. (1954) A bipolar rectal electrode for the electrical production of ejaculation in sheep. *Aust. vet. J.* **30**, 249.
- FLERCHINGER, F. H., ERB, R. E., MIKOTA, L. E. & EHLERS, M. H. (1956) Metabolism of bull semen. III. Relation of lactic acid and its accumulation during incubation with other semen quality measurements and non-returns. *J. Dairy Sci.* **39**, 1006.
- FLIPSE, R. J. & POTTER, F. E. (1955) Presence of volatile fatty acids in bovine semen. *Proc. Soc. exp. Biol. Med.* **89**, 432.
- HENDLER, R. W. (1959) Self-absorption correction for carbon-14. *Science, N.Y.* **130**, 772.
- HUMPHREY, G. F. & MANN, T. (1949) Studies on the metabolism of semen. 5. Citric acid in semen. *Biochem. J.* **44**, 97.
- LARDY, H. A. & PHILLIPS, P. H. (1944) Acetate utilization for maintenance of mobility of bull spermatozoa. *Nature, Lond.* **153**, 169.
- MANN, T. (1964) *The biochemistry of semen and of the male reproductive tract*. Methuen, London.
- MANN, T. & LUTWAK-MANN, C. (1948) Aerobic and anaerobic utilization of fructose by spermatozoa and seminal vesicles. *Biochem. J.* **43**, 266.
- MARDEN, W. G. (1961) Source of endogenous pyruvic acid in bovine seminal fluid and utilization. *J. Dairy Sci.* **44**, 1688.
- MELROSE, D. R. & TERNER, C. (1953) The metabolism of pyruvate in bull spermatozoa. *Biochem. J.* **53**, 296.
- RAMSEY, H. A., LODGE, J. R., GRAVES, C. N. & SALISBURY, G. W. (1963) Organic acids in fresh bovine semen. *J. Dairy Sci.* **41**, 1132.
- RHODES, J. B. & WILLIAMS-ASHMAN, H. G. (1960) Oxidizing enzymes in human seminal plasma. *Med. exp.* **3**, 123.
- SCOTT, T. W., WHITE, I. G. & ANNISON, E. F. (1961) Fatty acids in semen. *Biochem. J.* **78**, 740.
- SCOTT, T. W., WHITE, I. G. & ANNISON, E. F. (1962a) Oxidation of short-chain fatty acids ($\text{C}_1\text{-C}_8$) by ram, bull, dog and fowl spermatozoa. *Biochem. J.* **83**, 392.
- SCOTT, T. W., WHITE, I. G. & ANNISON, E. F. (1962b) Glucose and acetate metabolism by ram, bull, dog and fowl spermatozoa. *Biochem. J.* **83**, 398.
- TERNER, C. (1959) The effects of 2,4-dinitrophenol and p-nitrophenol on the aerobic and anaerobic metabolism of bull spermatozoa. *Biochim. biophys. Acta*, **36**, 479.
- TERNER, C. (1960) Oxidation of exogenous substrates by isolated human spermatozoa. *Am. J. Physiol.* **198**, 48.
- TERNER, C. & KORSH, G. (1963) The oxidative metabolism of pyruvate, acetate and glucose in isolated fish spermatozoa. *J. cell. comp. Physiol.* **62**, 243.
- UMBREIT, W. W., BURRIS, R. H. & STAUFFER, J. F. (1959) *Manometric Techniques*. Burgess Publishing Co., Minneapolis.
- VON KORFF, R. W. (1964) Pyruvate- C^{14} , purity and stability. *Ann. Biochem.* **8**, 171.
- WHITE, I. G. (1953) The effect of washing on the motility and metabolism of ram, bull and rabbit spermatozoa. *J. exp. Biol.* **30**, 200.
- WHITE, I. G. (1955) The collection of rabbit semen. *Aust. J. exp. Biol.* **33**, 367.
- WHITE, I. G. & WALES, R. G. (1960) The susceptibility of spermatozoa to cold shock. *Int. J. Fert.* **5**, 195.