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Glucose and Acetate Metabolism by Ram, Bull, Dog and Fowl Spermatozoa

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The utilization of carbohydrate by ram and bull spermatozoa has been examined in some detail (Mann, 1945; Mann & Lutwak-Mann, 1948; Mann, 1954; Flipse, 1955) and the break-down of glucose and fructose to lactate by the Embden-Meyerhof pathway has been demonstrated. In contrast with ram and bull semen, which contains high concentrations of fructose, dog and fowl semen is characterized by an almost complete absence of reducing sugar and little is known about the carbohydrate metabolism of the spermatozoa. Lorenz (1958) reported that fructose can be formed by fowl spermatozoa from glucose added to the semen or to washed sperm suspensions, but this observation was not confirmed by van Tienhoven (1960).

Flipse (1956) reported evidence of pentose-shunt activity in some samples of ejaculated bull spermatozoa, but this was not confirmed by Salisbury & Nakabayashi (1957). The possible existence of this alternative pathway of carbohydrate metabolism in ram, bull, dog and fowl spermatozoa has been investigated in the present study with specifically

labelled glucose. The glycolytic activities of the spermatozoa of the four species have also been compared.

Observations on the oxidation of acetate by ram, bull, dog and fowl spermatozoa have been reported by Scott, White & Annison (1962), and there is evidence that the endogenous substrate of spermatozoa is a long-chain carbon compound which would presumably be oxidized as C_2 units (Hartree & Mann, 1959). The interrelation of glucose and acetate utilization by spermatozoa is the main subject of the present investigation, and the use of ^{14}C -labelled substrates has allowed the marked effects of added substrates on endogenous oxidation to be assessed for the first time.

The possible role of the tricarboxylic acid cycle in synthetic reactions in spermatozoa has been investigated by comparing the yields of labelled carbon dioxide from the oxidation of $[1-^{14}C]$ acetate and $[2-^{14}C]$ acetate (Weinman, Strisower & Chaikoff, 1957). Only in fowl spermatozoa was evidence obtained of the entry of unlabelled intermediates into the tricarboxylic acid cycle.

MATERIALS AND METHODS

The techniques described by Scott *et al.* (1962) were used for the preparation of spermatozoal suspensions, manometric measurements and assays of radioactivity. After incubation, 2 ml. of the flask contents was deproteinized with 0.3N-Ba(OH)₂ (1 ml.) and 5% (w/v) ZnSO₄·7H₂O (1 ml.) and the filtrates were used for glucose, fructose and lactate analyses.

Analytical methods. Glucose was determined by the glucose-oxidase method (Huggett & Nixon, 1956), and fructose by the procedure of Mann (1948), with heating for 20 min. (White, 1959). For lactate, the procedure of Barker & Summerson (1941) was used.

Radioactive chemicals. These were obtained from The Radiochemical Centre, Amersham, Bucks.

Statistical analysis. The oxygen uptake data of Table 1 have been subjected to analysis of variance as outlined by Scott *et al.* (1962).

RESULTS

Glucose and acetate interrelation. Tables 1 and 2 give the results of experiments in which [1-¹⁴C]-acetate and D-[U-¹⁴C]glucose (where U indicates uniform labelling) were incubated, separately and in combination, with ram, bull, dog and fowl spermatozoa.

Both glucose and acetate stimulated the oxygen uptake of ram and bull spermatozoa with liberation of labelled carbon dioxide, and their effects on oxidative metabolism were competitive, not

Table 1a. [1-¹⁴C]Acetate and D-[U-¹⁴C]glucose metabolism by spermatozoa

Spermatozoa were incubated in Warburg flasks at 37° in air for 2 hr. (ram and bull) or for 4 hr. (dog and fowl). Ram and bull spermatozoa were washed twice; dog and fowl spermatozoa were unwashed. The amounts of substrate in each flask were: ram and bull: acetate, 36–54 μ moles (25 μ mc); glucose, 12–18 μ moles (25 μ mc); dog: acetate, 18–36 μ moles (25 μ mc); glucose, 6–12 μ moles (25 μ mc); fowl: acetate, 18 μ moles (25 μ mc); glucose, 6 μ moles (25 μ mc). The numbers of spermatozoa ($\times 10^8$)/flask were: ram, 5.7–16.3; bull, 8.2–16.4; dog, 1.7–2.9; fowl, 15.6–25.4. The acetate and glucose oxidized have been calculated from ¹⁴CO₂ recovered. Oxygen uptake (observed) is expressed as μ l.; acetate, glucose and lactate as μ moles. All values are calculated/10⁸ spermatozoa, and represent the means of three ejaculates. The total volume of solution in each flask was 3 ml. (sperm suspension, 2 ml.; substrate, 1 ml.).

Substrate	Ram				Bull			
	Oxygen uptake	Acetate oxidized	Glucose oxidized	Lactate produced	Oxygen uptake	Acetate oxidized	Glucose oxidized	Lactate produced
Control	16.9	—	—	—	8.0	—	—	—
[1- ¹⁴ C]Acetate	70.4	2.35	—	—	28.6	0.53	—	—
D-[U- ¹⁴ C]Glucose	53.6	—	0.43	1.70	26.0	—	0.15	1.42
D-[U- ¹⁴ C]Glucose + acetate	49.5	—	0.19	1.89	23.7	—	0.07	1.31
[1- ¹⁴ C]Acetate + glucose	50.6	1.45	—	1.84	25.3	0.39	—	1.37

Substrate	Dog				Fowl				
	Oxygen uptake	Acetate oxidized	Glucose oxidized	Lactate produced	Oxygen uptake	Acetate oxidized	Glucose oxidized	Fructose produced	Lactate produced
Control	45.7	—	—	—	8.2	—	—	—	0.19
[1- ¹⁴ C]Acetate	67.7	1.04	—	—	9.4	0.04	—	—	0.29
D-[U- ¹⁴ C]Glucose	81.7	—	0.49	5.66	10.8	—	0.02	0.21	0.42
D-[U- ¹⁴ C]Glucose + acetate	71.3	—	0.39	5.09	11.5	—	0.02	0.19	0.46
[1- ¹⁴ C]Acetate + glucose	70.3	0.33	—	5.06	11.7	0.05	—	0.19	0.43

Table 1b. Analysis of variance of the oxygen-uptake data

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

	D.F.	Variance ratio			
		Ram	Bull	Dog	Fowl
Between treatments:	3				
Effect of acetate alone	1	75.6†	58.3†	4.8	3.1
Effect of glucose alone	1	35.5†	44.2†	12.3†	24.1†
Sum of individual against combined effects	1	3.7	1.0	0.4	3.4
Between ejaculates	2	14.2†	6.0*	6.9*	23.9†
Residual	6†	<i>5695</i>	<i>110</i>	<i>1520</i>	<i>72</i>

* $P < 0.05$.

† $P < 0.01$.

‡ Degrees of freedom (D.F.) reduced to 5 for the fowl because of a missing value inserted.

additive. A 30% suppression of acetate oxidation occurred in the presence of glucose, but glucose oxidation was depressed by more than 50% in the presence of acetate. Acetate was as effective as glucose in stimulating the oxygen uptake of the spermatozoa of both ruminants and with glucose as substrate there was considerable accumulation of lactate.

Glucose was more effective than acetate in stimulating the oxygen uptake of dog spermatozoa, and again the effects of the two substrates were not additive. Glucose oxidation was decreased by 20% in the presence of acetate, but glucose suppressed acetate oxidation by about two-thirds, in marked contrast with the effects observed with ruminant spermatozoa. Lactate was the main end-product of glucose metabolism by dog spermatozoa.

The overall metabolic rate of fowl spermatozoa was lower than those of the other species when activities were expressed in terms of spermatozoal

numbers. Oxygen uptake was only slightly increased by glucose and acetate, the effect being greater with the former. The oxygen-uptake data and recovery of labelled carbon dioxide indicated that the stimulatory effects of glucose and acetate were additive. Fructose as well as lactate was formed from glucose.

Time course of glucose metabolism. Table 3 shows that ram, bull, dog and fowl spermatozoa metabolized glucose at a linear rate over 2-3 hr. under aerobic conditions. The disappearance of glucose was accompanied by the production of roughly equivalent amounts of lactate, except with fowl spermatozoa where a considerable amount of fructose also accumulated. No fructose was detected when ram, bull or dog spermatozoa were incubated with glucose under the conditions indicated in Table 3.

Existence of pentose phosphate-shunt pathway. The possible existence of this alternative aerobic path-

Table 2. *Progressive oxygen uptake of spermatozoa metabolizing acetate and glucose*

For details of procedure see Table 1. All values (μL) are expressed/ 10^8 spermatozoa and are the means of three ejaculates.

Substrate	Min. ...	Ram			Bull		
		30	60	120	30	60	120
Control		6.6	10.5	16.9	4.9	6.5	8.0
[1- ^{14}C]Acetate		17.2	35.3	70.4	10.2	17.2	28.6
D-[U- ^{14}C]Glucose		14.0	28.3	53.6	9.0	15.4	26.0
[1- ^{14}C]Acetate + [U- ^{14}C]glucose		13.3	26.3	50.6	7.8	14.1	25.3

Substrate	Min. ...	Dog					Fowl			
		30	60	120	180	240	30	60	120	210
Control		10.3	17.2	29.8	39.1	45.7	1.8	3.1	5.5	8.2
[1- ^{14}C]Acetate		14.7	26.2	44.8	59.2	67.7	1.9	3.4	6.1	9.4
D-[U- ^{14}C]Glucose		13.8	25.6	50.6	71.3	81.7	2.0	3.9	7.4	10.8
[1- ^{14}C]Acetate + [U- ^{14}C]glucose		13.1	23.1	45.6	59.2	70.3	2.1	4.0	7.7	11.7

Table 3. *Rate of D-[U- ^{14}C]glucose metabolism by spermatozoa*

Ram and bull spermatozoa were washed twice; dog and fowl spermatozoa were unwashed. Suspensions were incubated in Warburg flasks at 37° in air with 18 μmoles (25 μmc) of glucose/flask. The number of spermatozoa ($\times 10^8$)/flask were: ram, 17.5; bull, 9.3; dog, 4.3; fowl, 8.3. Oxygen uptake is expressed as μL ; glucose, lactate, carbon dioxide and fructose as μmoles . All values are calculated/ 10^8 spermatozoa and represent the means of duplicates.

Species	Time (min.)	Oxygen uptake	Glucose utilized	Lactate produced	$^{14}\text{CO}_2$ recovered	Fructose formed
Ram	30	3.8	0.23	0.46	0.06	0
	60	9.8	0.42	0.79	0.30	0
	120	21.0	0.63	1.47	0.72	0
Bull	30	1.9	0.60	0.82	0.06	0
	60	6.5	0.73	1.25	0.18	0
	120	13.1	1.26	2.69	0.42	0
Fowl	60	3.7	0.41	0.17	0.04	0.30
	120	6.5	0.67	0.28	0.06	0.73
	180	9.7	0.78	0.32	0.10	0.85
Dog	60	11.3	1.40	2.15	0.36	0
	120	23.4	2.09	3.40	0.72	0
	180	36.3	2.35	4.60	1.20	0

Table 4. ^{14}C -Labelled carbon dioxide ratios after incubating spermatozoa with D-[6- ^{14}C]glucose and with D-[1- ^{14}C]glucose

Spermatozoa were incubated at 37° in air. The amount of glucose was 18 μmoles (25 μmc)/flask. Values are expressed as the ratio ($^{14}\text{CO}_2$ from D-[6- ^{14}C]glucose)/($^{14}\text{CO}_2$ from D-[1- ^{14}C]glucose). Results are shown as means $\pm$ S.E. where necessary.

Species	No. of washings	No. of ejaculates	$^{14}\text{CO}_2$ ratio			
			30 min.	60 min.	120 min.	180 min.
Ram	2	4	—	—	1.14 $\pm$ 0.04	—
	2	1	1.30	1.24	1.08	—
Bull	2	4	—	—	1.11 $\pm$ 0.05	—
	2	1	0.97	0.97	1.22	—
	0	1	—	—	—	0.95
Dog	1	4	—	—	—	1.18 $\pm$ 0.05
	0	1	—	0.95	1.06	1.17
Fowl	1	4	—	—	—	1.00 $\pm$ 0.06
	0	1	—	0.57	0.52	0.68

Table 5. Oxidation of [1- ^{14}C]acetate and [2- ^{14}C]acetate by spermatozoa

Ram and bull spermatozoa were washed twice; dog and fowl spermatozoa were washed once. Suspensions were incubated in Warburg flasks at 37° in air. The amount of acetate/flask was 54 μmoles (25 μmc). Specific activities (sp.act.) are expressed as $\mu\text{mc}/\text{mg.}$ of barium carbonate after incubation. Results are shown as means $\pm$ S.E.

Species	No. of ejaculates	Sp.act. of [1- ^{14}C]acetate	Sp.act. of [2- ^{14}C]acetate	Sp.act. of [1- ^{14}C]acetate Sp.act. of [2- ^{14}C]acetate
Ram	3	0.86	0.88	0.99 $\pm$ 0.03
Bull	4	0.55	0.50	1.10 $\pm$ 0.07
Dog	3	0.59	0.71	0.83 $\pm$ 0.12
Fowl	3	0.18	0.08	2.25 $\pm$ 0.13

way of glucose metabolism was examined by comparing the yields of labelled carbon dioxide obtained by incubating spermatozoa with D-[1- ^{14}C]glucose and with D-[6- ^{14}C]glucose. When washed spermatozoa were examined after incubating for 2–3 hr., ratios of labelled carbon dioxide from D-[6- ^{14}C]glucose to that from D-[1- ^{14}C]glucose were about one (Table 4), indicating the absence of pathways for the direct aerobic oxidation of glucose. As a further check on the existence of the pentose cycle, spermatozoa of each species were incubated with 1- ^{14}C - and 6- ^{14}C -labelled glucose and ratios of labelled carbon dioxide measured at various time intervals (Table 4).

No evidence of shunt activity was obtained when twice-washed ram and bull spermatozoa were incubated for 30, 60 and 120 min., but the ratio of 1.30 for ram spermatozoa after 30 min. was unexpected.

The ratios of labelled carbon dioxide of unwashed dog and fowl spermatozoa were measured after 60, 120 and 180 min. With dog spermatozoa, ratios of one were obtained, but unwashed fowl spermatozoa gave a much lower value (0.68). This finding suggested that with fowl spermatozoa the lactate normally present in the semen might cause preferential oxidation of C-1 of glucose and experiments were undertaken to check this possibility.

Six fowl ejaculates were pooled; half was diluted 1:4 and the ratio of labelled carbon dioxide was measured after incubating for 3 hr.; the second half was washed once, diluted 1:4 and the ratio measured in the presence of added lactate (5 mg./ml.). The ratios obtained were 0.74 and 0.55 respectively, both values being the means of duplicates.

Oxidation of [1- ^{14}C]acetate and [2- ^{14}C]acetate. Spermatozoa were incubated with carboxyl- and methyl-labelled acetate and the ratio of labelled carbon dioxide from [1- ^{14}C]acetate to that from [2- ^{14}C]acetate was determined (Table 5). Ratios of about one were obtained for ram, bull and dog spermatozoa, indicating that there was no entry of unlabelled endogenous substrate into the tri-carboxylic acid cycle (Weinman *et al.* 1957). With fowl spermatozoa, however, the ratio of 2.25 indicated substantial influx of unlabelled tri-carboxylic acid-cycle intermediates.

Incorporation of labelled acetate by spermatozoa. After incubation with labelled acetate the spermatozoa were killed by adding 0.1N-hydrochloric acid, and the flask contents were transferred to a centrifuge tube. The contents were twice washed with unlabelled acetate (54 $\mu\text{moles}/\text{ml.}$) to remove any labelled acetate adsorbed on the surface of the spermatozoa. The precipitated spermatozoa were

Table 6. *Percentage contribution of exogenous substrates (acetate and glucose) to the total oxidation of spermatozoa*

Ram and bull spermatozoa (washed twice) and dog and fowl spermatozoa (washed once) were incubated at 37° in air with [^{14}C]acetate (54 μmoles , 25 μmc), and with D-[^{14}C]glucose (18 μmoles , 25 μmc). Values are means $\pm$ s.e. of several ejaculates (numbers in parentheses) for each species.

Species	Acetate	Glucose
Ram	91.5 $\pm$ 2.38 (6)	61.5 $\pm$ 4.50 (2)
Bull	57.8 $\pm$ 3.43 (8)	54.3 $\pm$ 6.42 (4)
Dog	59.7 $\pm$ 12.75 (4)	75.5 $\pm$ 11.05 (4)
Fowl	14.0 $\pm$ 3.58 (6)	22.3 $\pm$ 4.18 (3)

washed, and then twice extracted with 5 vol. of chloroform-methanol (2:1, v/v); the lipid fractions were pooled for radioactive assay with a Packard Tri-Carb Scintillation Spectrometer. A sample (one-fifth vol.) was placed in a scintillation vial and dried down under nitrogen. The scintillation mixture (10 ml.) consisted of 2,5-diphenyloxazole (3 g./l.) and 1,4-di-(5-phenyloxazol-2-yl)benzene (0.1 g./l.) in toluene. There was no incorporation of acetate into lipid components of ram, bull, dog and fowl spermatozoa.

Relative oxidation of exogenous and endogenous substrates. The relative contribution of exogenous and endogenous substrates to the total oxidation was assessed by comparing the specific activities of liberated labelled carbon dioxide with those of the original substrates. The ratio (100 $\times$ specific activity of labelled carbon dioxide)/(specific activity of substrate carbon) gives the percentage contribution of exogenous substrate; values for ram, bull, dog and fowl spermatozoa metabolizing acetate and glucose are shown in Table 6.

The contribution of exogenous substrate was greater than the endogenous for all species except the fowl. The endogenous contributions were least for ram spermatozoa metabolizing acetate (8.5%) and dog spermatozoa metabolizing glucose (24.5%).

DISCUSSION

The observation that acetate and glucose increase the oxygen uptake of spermatozoa, which with ruminant species must first be washed to remove plasma fructose, confirms previous work (Lardy & Phillips, 1944; Mann, 1948; Mann & Lutwak-Mann, 1948; Mann, 1954; van Tienhoven, 1960; Scott *et al.* 1962). In addition, however, the present study reveals marked differences in the metabolic patterns of ruminant and non-ruminant spermatozoa when incubated with a combination of the two substrates. Ram and bull spermatozoa oxidize acetate in preference to glucose, but with dog spermatozoa the reverse is true and glucose

exerts a 'sparing effect' on acetate oxidation. A similar ruminant-non-ruminant difference in glucose and acetate utilization has been observed with sheep- and rat-mammary-gland slices (Balmain, Folley & Glascock, 1954).

Many conflicting reports have appeared on the interrelation of glucose and fatty acid oxidation in other tissues. Pennington & Pfander (1957) reported that propionate and butyrate lowered the uptake of glucose by rumen epithelium and that fatty acid metabolism was increased by glucose. In the rat diaphragm, glucose and acetate compete with each other and the evidence suggests that glucose is metabolized preferentially (Parnes & Wertheimer, 1950; Wertheimer & Ben-Tor, 1951). In other rat tissues the catabolism of one substrate is probably not greatly influenced by the other (Allen, Friedmann & Weinhouse, 1955). The different patterns of acetate and glucose metabolism in ruminant and non-ruminant spermatozoa reflect the relative availability of these substrates to the tissues of each species. The blood of ruminants contains less glucose but more acetate (Annison & Lewis, 1959), and, although glucose turnover is not markedly lower in ruminants than in non-ruminants (Annison & White, 1961), acetate turnover is considerably higher (Annison & Lindsay, 1961). The preferential oxidation of acetate by ruminant spermatozoa indicates their greater ability to activate acetate in the presence of pyruvate, which is an intermediate in glucose metabolism and an alternative precursor of acetyl-CoA. CoA availability may well limit the rate of overall oxidation in ram and bull spermatozoa.

The response of fowl spermatozoa to exogenous acetate and glucose is lower, and it is difficult to study the interrelations. The overall metabolic activity, per unit cell, is lower than with other species, and the pattern of carbohydrate metabolism is different. Morphologically fowl spermatozoa are dissimilar to mammalian spermatozoa, and the enzyme complement could be much less and thus account for a lower metabolic rate. A possible explanation of the non-competitive nature of acetate and glucose oxidation is that CoA is not limiting in fowl spermatozoa. The formation of fructose from glucose is an intriguing problem; our results eliminate the pentose-shunt pathway. The process is not reversible so that a sorbitol dehydrogenase similar to that found in ram spermatozoa (King & Mann, 1959) and other tissues (Blakely, 1951; McCorkindale & Edson, 1954; Williams-Ashman & Banks, 1954; Hers, 1955) is probably not involved.

Ram and bull spermatozoa readily metabolize fructose, which is normally present in the semen of these species (Mann, 1954), and it is an important source of energy after ejaculation. In

contrast fowl semen has negligible amounts of reducing sugar, but does contain about 30 mg. of lactate/100 ml. The present results indicate that fowl spermatozoa metabolize lactate and hence it would be available as substrate.

The high glycolytic activity of ram and bull spermatozoa is well recognized (Mann, 1954), and an almost quantitative breakdown of glucose to lactate by dog spermatozoa is also shown in the present studies. Ram and bull spermatozoa metabolize fructose at a linear rate under anaerobic conditions (Mann, 1948), and our results indicate that this also occurs aerobically with glucose as substrate. The accumulation of lactate when these spermatozoa are incubated under aerobic conditions indicates that glycolytic activity considerably exceeds the oxidative capacity of the tricarboxylic acid cycle. Concurrent metabolism of endogenous substrate would also contribute intermediates to the tricarboxylic acid cycle.

We have not been able to confirm the finding of Flipse (1956) that some samples of bull spermatozoa possess pentose-shunt activity. Glock & McLean (1955) have shown a good correlation between pentose-shunt activity and NADP concentrations in tissues; the absence of NADP in bull spermatozoa (Salisbury & Nakabayashi, 1957) therefore suggests that there is no direct oxidation of glucose. Wu, McKenzie, Fang & Butts (1959), with specifically labelled glucose, have also been unable to demonstrate the pentose shunt in bull epididymal spermatozoa, although there was evidence for this pathway in testicular spermatozoa. The mode of glucose dissimilation by spermatozoa at different sites in the male reproductive tract might reflect changes in the ratio of NADP to NAD in the spermatozoa.

A possible explanation of the preferential oxidation of C-6 of glucose during the early stages of the incubation of glucose with ram spermatozoa is that the experimental conditions favoured the accumulation of dihydroxyacetone phosphate. The effect of lactate in lowering the ratio of labelled carbon dioxide when fowl spermatozoa is incubated with [$1-^{14}\text{C}$]glucose and with [$6-^{14}\text{C}$]glucose is difficult to explain in the absence of pentose-shunt activity. If lactic acid inhibited the major pathway from glyceraldehyde 3-phosphate to 3-phospho-D-glycerate and accelerated the pathway via D-glyceraldehyde there would be preferential liberation of C-1 as carbon dioxide. The latter pathway has, however, not as yet been demonstrated in spermatozoa.

Weinman *et al.* (1957) reported that the tricarboxylic acid cycle in animal tissues has synthetic activity. The present investigations indicate that ram, dog and bull spermatozoa have negligible synthetic activity and possess only catalytic amounts of C_3 and C_4 intermediates. However, the

ratios obtained from the oxidation of carboxyl- and methyl-labelled acetate by washed fowl spermatozoa strongly suggest the influx of unlabelled endogenous substrate into the tricarboxylic acid cycle.

In general the relative contributions of acetate and glucose to the total oxidation (Table 6) reflect differences in the rate of oxidation of these substrates (Table 1). Ram spermatozoa oxidize acetate much more readily than glucose but the reverse is true for dog spermatozoa. The low rate of oxidation of both acetate and glucose (Table 1) by fowl spermatozoa is reflected in the low contribution of these substrates to overall oxidation (Table 6).

The use of isotopes has made possible an accurate assessment of the endogenous metabolism of spermatozoa in the presence of an exogenous substrate. Hitherto it has been assumed that the endogenous metabolism of spermatozoa is not changed by adding substrates and that the difference between oxygen uptake in the presence and absence of exogenous substrate is a true reflection of its oxidation. If an added substrate fails to increase the oxygen uptake of washed spermatozoa above the endogenous rate it is generally assumed not to be oxidized. The present study with isotopes, however, indicates that such a conclusion may not always be correct. Thus a comparison of Tables 1 and 6 suggests that in at least two instances, namely when ram spermatozoa metabolize acetate and dog spermatozoa metabolize glucose, the endogenous oxidation may be depressed. Exogenous acetate might be expected to compete favourably with the C_2 fragments derived from the endogenous phospholipid of ram spermatozoa (Hartree & Mann, 1959). On the other hand, glucose might be expected to be oxidized preferentially to the endogenous C_3 fragments in dog spermatozoa.

SUMMARY

1. Ram, bull, dog and fowl spermatozoa metabolize acetate and glucose, but there are differences between the species.
2. Ram and bull spermatozoa oxidize acetate in preference to glucose, but with dog spermatozoa the reverse is true. The preferential oxidation of acetate by ruminant spermatozoa indicates their greater ability to activate acetate in the presence of pyruvate, an alternative precursor of acetyl-CoA.
3. Ram, bull and dog spermatozoa break glucose down to lactic acid almost quantitatively under aerobic conditions, but with fowl spermatozoa there is an accumulation of fructose as well as lactate.

4. There was no evidence of pentose-shunt activity from the labelled-carbon dioxide ratios (1.00–1.18) obtained after incubating spermatozoa with D-[6-¹⁴C]glucose and with D-[1-¹⁴C]glucose.

5. The labelled-carbon dioxide ratios (0.83–1.10) obtained with [1-¹⁴C]acetate and with [2-¹⁴C]acetate as substrates indicate that ram, bull and dog spermatozoa have negligible synthetic activity and possess only catalytic amounts of C₃ or C₄ intermediates. The corresponding ratio (2.25) for fowl spermatozoa suggests an influx of unlabelled tricarboxylic acid-cycle intermediates.

6. With labelled acetate and glucose it has been possible to obtain an accurate estimate of the endogenous oxidation in spermatozoa in the presence of an exogenous substrate. The endogenous contribution was less than the exogenous for all species except the fowl.

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The Exchange of ¹³¹I-Labelled Lipid and ¹³¹I-Labelled Protein between Red Cells and Serum

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The exact nature of the red-cell surface has not yet been determined. Observations on the permeability characteristics (Overton, 1895) and on the birefringence of red cells (Schmitt, Bear & Ponder, 1936) have suggested that the surface is lipid in nature. Analysis of the lipid content of red cells has shown that there is sufficient lipid to form a bimolecular layer around the red cell (Gorter &

Grendel, 1925). Others have suggested that the red-cell surface is formed of a mosaic of lipid and the red-cell protein, stomatin, and several possible models have been suggested (Nathanson, 1904; Ponder, 1948; Parpart & Ballentine, 1952; Burgen, 1959). Electrophoresis of red cells carried out at varying pH values has suggested that the phosphoric acid groups of kephalin and related lipids