REFERENCES

- Gomori, K. (1943). *J. biol. Chem.* **148**, 139.
 Harris, W. D. & Popat, P. (1954). *J. Amer. Oil Chem. Soc.* **31**, 124.
 Hers, H. G. (1957). *Le Métabolisme du Fructose*, p. 95. Brussels: Éditions Arscia.
 Lohman, K. (1933). *Biochem. Z.* **262**, 137.
 McKrasch, L. C., Davidson, W. P. & McGilvery, R. W. (1956). *J. biol. Chem.* **222**, 179.
 McKrasch, L. C. & McGilvery, R. W. (1956). *J. biol. Chem.* **221**, 909.
 Newsholme, E. A. & Randle, P. J. (1961). *Biochem. J.* **80**, 655.
 Newsholme, E. A., Randle, P. J. & Manchester, K. L. (1962). *Nature, Lond.*, **193**, 270.
 Pogel, B. M. & McGilvery, R. W. (1952). *J. biol. Chem.* **197**, 293.
 Regen, D. M., Davis, W. W. & Morgan, H. E. (1961). *Fed. Proc.* **20**, 83.
 Truman, D. E. S. & Korner, A. (1962). *Biochem. J.* (in the Press).
 Weber, G. & Cantero, A. (1959). *Cancer Res.* **19**, 763.
 Williamson, J. R. & Krebs, H. A. (1961). *Biochem. J.* **80**, 540.

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Oxidation of Short-Chain Fatty Acids (C_1 – C_8) by Ram, Bull, Dog and Fowl Spermatozoa

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Mammalian spermatozoa can utilize a glycolysable carbohydrate, such as the fructose usually present in seminal plasma, under both aerobic and anaerobic conditions, but in its absence motility is maintained by the oxidation of intracellular reserves (Mann, 1954). Hartree & Mann (1959) demonstrated that fatty acids are liberated when washed ram spermatozoa are incubated under aerobic conditions, and the low R.Q. (0.71) suggests that fatty acids participate in aerobic endogenous metabolism. Information on the oxidation of fatty acids by spermatozoa, however, is scanty and confusing. Lardy & Phillips (1944; 1945) found that acetate stimulated the oxygen uptake of bull epididymal spermatozoa, but with washed ejaculated spermatozoa the effect was consistently obtained only in the presence of 2,4-dinitrophenol. Similar results were obtained by Humphrey & Mann (1948) with washed ejaculated ram spermatozoa with acetate, propionate and butyrate as substrates. Flipse (1960) found that acetate increased the oxygen uptake of washed bull spermatozoa appreciably in the absence of 2,4-dinitrophenol, but no stimulation was obtained with butyrate and higher fatty acids. Turner (1960) showed that human spermatozoa can oxidize $[1-^{14}C]$ acetate.

In the present study the oxidation of short-chain fatty acids (C_1 – C_8) by ram, bull, fowl and dog spermatozoa has been investigated with ^{14}C -labelled substrates. The interrelation of acetate

and propionate oxidation by ram and dog spermatozoa has also been investigated.

MATERIALS AND METHODS

Preparation of suspensions of washed spermatozoa. Semen was collected by the techniques described by Scott, White & Annison (1961). Ram and bull semen was placed in a water bath at 37° immediately after collection and slowly cooled to room temperature to avoid cold shock. Ram and bull spermatozoa were washed twice with calcium-free Krebs–Ringer phosphate or bicarbonate buffer (Krebs, 1933) by the procedure of White (1953); the final concentration in the suspensions was 4×10^8 – 8×10^8 spermatozoa/ml. The diluent for fowl semen was a modified Krebs–Ringer phosphate with no added fructose (Wales & White, 1960). Fowl semen was diluted 1:4 and centrifuged at 300g for 10 min.; the supernatant was removed and the spermatozoa were resuspended at a concentration of about 8×10^8 /ml. The sperm-rich fraction of dog semen was diluted 1:4 with calcium-free Krebs–Ringer phosphate and the spermatozoa were washed by the procedure outlined for fowl spermatozoa; the final cell concentration of the suspension was 10^8 /ml. The spermatozoa were resuspended in the diluent used for washing and spermatozoal counts were made with a haemocytometer (White, 1953).

Incubation of spermatozoa with ^{14}C -labelled fatty acids. Suspensions of spermatozoa (2 ml.) were incubated at 37° with the sodium salts of the fatty acids (1 ml.) in Warburg flasks containing CO_2 -free 20% (w/v) NaOH (0.2 ml.) in the centre well. Oxygen uptakes were measured with air as gas phase, and a shaking rate of 120 strokes/min., over a period of 2–3 hr. At the end of the incubation the contents

of the centre well were removed quantitatively by washing with 3 ml. of CO₂-free water. A portion (2 ml.) of the flask contents was deproteinized with 25% (w/v) metaphosphoric acid (1 ml.) and steam-distilled in a Markham still to recover unchanged fatty acids, and to check the possible production of fatty acids. After titration with CO₂-free 0.01N-NaOH the distillates were concentrated and analysed by gas-liquid chromatography with the procedures described by Scott *et al.* (1961).

Assay of radioactivity. The ¹⁴CO₂ absorbed in the centre well was precipitated as barium carbonate and collected and assayed for radioactivity by the procedures described by Annison & White (1961).

Estimation of ketone bodies. The method of Reid (1960) was used. Suspensions of spermatozoa (2 ml.) were deproteinized with 0.15M-ZnSO₄ (1 ml.) and 0.3N-NaOH (1 ml.).

Estimation of lactic acid. The method of Barker & Summerson (1941) was used.

Labelled fatty acids. [1-¹⁴C]-Labelled fatty acids (C₁-C₈) were obtained from The Radiochemical Centre, Amersham, Bucks. The acids were diluted 1:10 with carrier (purified unlabelled acid) and the specific activity was determined before and after analyses by gas-liquid chromatography. No change in relative specific activity occurred, proving the purity of the labelled acids. Unlabelled acids were purified by distillation, and were homogeneous when examined by gas-liquid chromatography. The radioactivity of added substrates was determined after wet oxidation (Van Slyke & Folch, 1940).

Statistical analysis. Where necessary the oxygen-uptake data have been subjected to standard analysis 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. The standard error of the difference between a pair of treatment means is calculated, taking it as being equal to $\sqrt{(2s^2/r)}$, where s^2 is the interaction mean square and r the number of replications.

The significance of differences between pairs of treatment means has, in Table 1, then in effect been assessed by a t test with the standard error of difference of means and the degrees of freedom associated with s^2 , except in Expts. A and C for the ram in which one acid was tested in each experiment. The error mean squares for Expts. A and C of the ram were almost identical and hence for an F -test have been combined to give an overall estimate based on two degrees of freedom rather than the one available in each separate test.

Three missing values (two in Table 1 and one in Table 3) have been inserted by the method of Cochran & Cox (1950) and the degrees of freedom for error reduced accordingly.

RESULTS

Oxidation of fatty acids. The spermatozoa of all species showed some capacity to liberate labelled carbon dioxide from each of the acids examined (Table 1), and a significant increase in oxygen uptake often occurred (Table 2). Although for each species except fowl oxygen uptake was greatest in the presence of acetate, the pattern of activity of each species was different. These

differences are well exemplified by comparing the results for spermatozoa of the two ruminant species, ram and bull (Table 1). Ram spermatozoa oxidized most acids, but those containing an even number of carbon atoms were more readily attacked (Table 1). Octanoate stimulated oxygen uptake at a concentration of 3.6 μ moles/ml., but at a concentration of 9 μ moles/ml. the oxygen uptake was similar to that of the control, and at 18 μ moles/ml. some depression of oxygen uptake occurred. Bull spermatozoa oxidized each acid examined, as measured by labelled carbon dioxide production, although their activity relative to that of ram spermatozoa was low. Acetate stimulated the oxygen uptake by bull spermatozoa, but the other acids did not.

Dog and fowl spermatozoa oxidized propionate as effectively as acetate (Table 1), in marked contrast with the relative rates of oxidation of these substrates by ram and bull spermatozoa. Only fowl spermatozoa showed a major respiratory response to octanoate. With both dog and fowl spermatozoa recoveries of labelled carbon dioxide from acids of chain length C₅-C₈ were considerably in excess of the theoretical amount of acid oxidized as calculated from the oxygen uptake (Table 1). The incomplete oxidation of these acids was not due to the formation of ketone bodies or of acids of lower chain length.

[¹⁴C]Formate gave rise to labelled carbon dioxide when incubated with the spermatozoa of the four species investigated, although a significant stimulation of oxygen uptake was observed (Table 1) only with the fowl. Addition of methylene blue (1.3 μ -moles/ml.) increased production of labelled carbon dioxide from [¹⁴C]formate with ram spermatozoa, but the marked spermicidal effect of methylene blue may have vitiated these attempts to demonstrate formate-dehydrogenase activity.

Acetate and propionate metabolism by dog and ram spermatozoa. The interrelation of acetate and propionate oxidation was investigated with the combinations of labelled and unlabelled substrates shown in Table 3. The recoveries of labelled carbon dioxide show that propionate had no effect on the oxidation of acetate by dog spermatozoa, and little if any on that by ram spermatozoa. The oxidation of propionate (as measured by production of labelled carbon dioxide) by dog spermatozoa was halved in the presence of an equal concentration of acetate, but with ram spermatozoa was only slightly reduced.

Factorial analysis of the oxygen-uptake data, with the mean of the values for the two combinations of acetate and propionate on each occasion, confirms the significant effect of acetate for both species and of propionate for the dog. There was no conclusive evidence that propionate inhibited

the oxygen uptake of ram spermatozoa in the presence of acetate, as the effects were additive in this instance.

Effect of inhibitors. Fluoroacetate, a specific inhibitor of the tricarboxylic acid cycle (Peters, 1952), greatly depressed the oxygen uptake and production of labelled carbon dioxide from [1-¹⁴C]-acetate in experiments with ram spermatozoa (Table 4).

The possibility of a non-specific spermicidal action of fluoroacetate on the spermatozoa was eliminated by examining its effect on anaerobic break-down of fructose. Small tubes containing suspensions of washed spermatozoa (2 ml.), fructose (1 ml. $\equiv$ 1 mg.) and various concentrations of fluoroacetate (0.001–0.1 M) were sealed with liquid paraffin and incubated at 37° for 3 hr. In four replicate experiments the mean values of fructose broken down by 10⁸ spermatozoa in the presence of

10⁻³, 10⁻² and 10⁻¹ M-fluoroacetate were 140, 134 and 132 μ g. respectively, compared with 140 μ g. in the control. Fructose break-down was clearly little affected by fluoroacetate.

The endogenous respiration of washed ram spermatozoa was reduced to 20–25% by fluoroacetate (10⁻² M). Malonate (10⁻² M) usually reduced the oxygen uptake of ram spermatozoa in the presence of acetate to 70–80% of the control values, but the effect was not significant (at the 0.05% level) for three ejaculates (Table 4).

Production of fatty acids. Examination of the unchanged fatty acids remaining after incubation indicated that with bull, fowl and dog spermatozoa negligible amounts of fatty acids were produced. Ram spermatozoa gave rise to small amounts of acetate when incubated with fatty acids of chain length C₆–C₈ and to propionate from C₆ and C₇ acids. The composition of the residual acids

Table 1. Oxidation of [1-¹⁴C]labelled C₁–C₈ fatty acids by spermatozoa

Washed spermatozoa were incubated at 37° in air for 2–3 hr. The amounts of acids in the Warburg flasks were for C₁–C₄ acids, 54 μ moles, 100 μ mc; for C₅ acid, 54 μ moles, 190 μ mc, except in Expt. C with the ram, when 10.8 μ moles, 20 μ mc were used. The numbers of spermatozoa ($\times 10^8$)/flask were: ram, 8.1–10.2; bull, 12.2–15.7; dog, 1.4–1.5; fowl, 7.2–7.9. Values are the means of two (ram, dog and fowl) or three (bull) ejaculates and are expressed/10⁸ spermatozoa. The letters (A, B, C) given in the Table designate the particular experiments. The amount of acid oxidized has been calculated both from the oxygen uptake and ¹⁴CO₂ recovered.

Substrate	Ram										Bull			
	Oxygen uptake (μ l.)	Acid oxidized (μ moles)		Molecular percentage of residual acids								Oxygen uptake (μ l.)	Acid oxidized (μ mole)	
		$^{14}\text{CO}_2$	O_2	C_2	C_3	C_4	C_5	C_6	C_7	C_8	$^{14}\text{CO}_2$		O_2	
Control	10.2 (A)	—	—	—	—	—	—	—	—	—	—	—	—	
	14.3 (B)	—	—	—	—	—	—	—	—	—	7.4	—	—	
	9.8 (C)	—	—	—	—	—	—	—	—	—	—	—	—	
Formate	12.3 (A)	0.52	1.00	—	—	—	—	—	—	—	7.3	0.56	0.64	
Acetate	73.8† (B)	1.01	1.64	—	—	—	—	—	—	—	21.7†	0.39	0.47	
Propionate	18.3 (B)	0.22	0.23	—	—	—	—	—	—	—	10.0	0.20	0.13	
n-Butyrate	37.3† (B)	0.37	0.33	—	—	—	—	—	—	—	7.0	0.10	0.06	
n-Valerate	29.9† (B)	0.23	0.21	5	8	—	87	—	—	—	4.8	0.01	0.03	
n-Hexanoate	39.9† (B)	0.27	0.26	4	—	—	—	96	—	—	5.1	0.02	0.03	
n-Heptanoate	25.6† (B)	0.24	0.11	3	7	—	—	—	90	—	6.6	0.009	0.03	
n-Octanoate	5.6 (B)	—	—	—	—	—	—	—	—	—	—	—	—	
	27.3† (C)	0.06	0.18	2	—	—	—	—	—	98	6.9	0.003	0.03	

Substrate	Dog			Fowl		
	Oxygen uptake (μ l.)	Acid oxidized (μ moles)		Oxygen uptake (μ l.)	Acid oxidized (μ mole)	
		$^{14}\text{CO}_2$	O_2		$^{14}\text{CO}_2$	O_2
Control	—	—	—	6.8 (A)	—	—
	23.1	—	—	8.2 (B)	—	—
Formate	29.8	3.19	2.68	10.2* (A)	0.88	0.85
Acetate	59.5†	1.60	1.33	9.6* (A)	0.15	0.21
Propionate	54.8†	2.19	0.67	10.5* (A)	0.33	0.13
n-Butyrate	48.3†	1.80	0.44	10.3* (A)	0.18	0.09
n-Valerate	33.7*	0.62	0.23	9.4* (B)	0.05	0.06
n-Hexanoate	36.4*	0.48	0.21	12.5† (B)	0.07	0.07
n-Heptanoate	37.3†	0.31	0.18	12.7† (B)	0.03	0.06
n-Octanoate	42.9†	0.009	0.18	18.1† (B)	0.05	0.07

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

† Highly significantly different from the control, $P < 0.01$.

Table 2. *Oxygen uptake of spermatozoa metabolizing C₁-C₈ fatty acids*

For details of procedure see Table 1. All values are expressed as $\mu\text{L}/10^8$ spermatozoa and are the means of two (ram, dog and fowl) or three (bull) ejaculates.

Substrate	Ram				Bull			
	Min. ...	30	60	120	30	60	120	180
Control	Expt. A	3.6	5.7	10.2	3.9	5.4	6.5	7.4
	B	4.6	8.2	14.3	—	—	—	—
	C	3.0	5.6	9.8	—	—	—	—
Formate	A	3.4	7.0	12.3	3.5	5.2	6.8	7.3
Acetate	B	17.7	30.2	73.8	6.3	10.7	17.3	21.7
Propionate	B	5.8	10.7	18.3	5.6	7.4	9.5	10.0
n-Butyrate	B	12.7	21.7	37.3	2.8	4.8	6.3	7.0
n-Valerate	B	8.7	16.0	29.9	1.9	3.0	4.2	4.8
n-Hexanoate	B	11.0	20.5	39.9	1.6	2.8	4.2	5.1
n-Heptanoate	B	7.7	14.5	25.6	3.2	4.6	6.0	6.6
n-Octanoate	B	—	—	5.6	—	—	—	—
	C	6.7	12.7	27.3	2.0	3.4	5.5	6.9

Substrate	Dog				Fowl					
	Min. ...	30	60	120	180	Min. ...	30	60	120	180
Control	Expt. A	3.6	7.4	17.2	23.1	Expt. A	1.0	1.9	4.7	6.8
	B	—	—	—	—	B	1.6	2.8	5.7	8.2
Formate	A	7.8	11.8	23.5	29.8	A	1.9	3.7	7.3	10.2
Acetate	B	13.6	22.4	44.8	59.5	A	1.6	3.2	7.0	9.6
Propionate	B	19.5	27.3	44.6	54.8	A	1.4	3.6	7.5	10.5
n-Butyrate	B	10.4	16.5	34.5	48.3	A	0.8	2.9	6.8	10.3
n-Valerate	B	5.3	9.8	24.5	33.7	B	1.7	3.3	6.5	9.4
n-Hexanoate	B	5.7	11.2	26.3	36.4	B	2.0	3.6	8.3	12.5
n-Heptanoate	B	4.1	10.5	26.1	37.3	B	2.1	4.2	8.5	12.7
n-Octanoate	C	4.1	10.9	28.4	42.9	B	3.1	6.1	11.9	18.1

Table 3a. *Acetate and propionate oxidation by spermatozoa*

Spermatozoa were incubated at 37° in air for 3 hr. The amount of each acid in the Warburg flasks was 27 μ -moles (50 μmc). The numbers of spermatozoa ($\times 10^8$)/flask were 2.8–10.4 (ram) and 1.6–2.4 (dog). Values in the Table are the means of two ejaculates and are expressed/ 10^8 spermatozoa. The amount of acid oxidized has been calculated from the $^{14}\text{CO}_2$ recovered.

Substrate	Oxygen uptake (μL)		Acid oxidized (μmoles)	
	Ram	Dog	Ram	Dog
Nil	34.6	27.9	—	—
[1- ^{14}C]Acetate	129.6	55.9	2.01	0.95
[1- ^{14}C]Propionate	31.5	39.1	0.25	1.05
[1- ^{14}C]Acetate + propionate	103.3	73.7	1.76	0.99
[1- ^{14}C]Propionate + acetate	108.0	70.6	0.20	0.56

Table 3b. *Analysis of variance for oxygen-uptake data*

Degrees of freedom (D.F.) are reduced by one to compensate for inserting a missing value. The residual mean square is given in *italics* at the base of the variance-ratio column.

Source of variation	Ram		Dog	
	D.F.	Variance ratio	D.F.	Variance ratio
Between ejaculates	1	33.5*	1	0.5
Effect of acetate	1	345.5†	1	178.4†
Effect of propionate	1	2.6	1	27.6*
Acetate-propionate interaction	1	15.3	1	0.1
Residual	2	4713	3	943

* $P < 0.05$.

† $P < 0.01$.

Table 4. *Effect of inhibitors on [1-¹⁴C]acetate oxidation by ram spermatozoa*

Washed spermatozoa were incubated at 37° for 2 hr. in air. The amount of acetate in the Warburg flasks was 54 μ moles (25 μ mc). The numbers of spermatozoa ($\times 10^6$)/flask were 4.3–7.7. Values are the means of three ejaculates and are expressed/10⁶ spermatozoa. The amount of acid oxidized has been calculated from the ¹⁴CO₂ recovered.

Inhibitor	Concn. (M)	Oxygen uptake (μ l.)	Acid oxidized (μ moles)
Fluoroacetate	0	80.2	1.66
	10 ⁻³	38.9	1.10
	10 ⁻²	8.4	0.25
	10 ⁻¹	4.4	0.06
Malonate	0	82.6	1.87
	10 ⁻³	68.4	1.52

obtained from the experiments are summarized in Table 1.

Production of ketone bodies. Ketone bodies were not produced during the incubation of butyrate and hexanoate with the spermatozoa of any of the four species examined.

Effects of carbon dioxide on the oxidation of propionate. Incubation of twice-washed ram spermatozoa, with propionate (8 μ moles/flask) in calcium-free Krebs–Ringer bicarbonate in an atmosphere of O₂ + CO₂ (95:5) for 3 hr. at 37°, failed to increase oxygen uptake, and no detectable lactate was produced.

DISCUSSION

The metabolism of short-chain fatty acids by isolated mammalian tissues has been extensively studied, and during the past decade a clear picture has emerged of the enzymic processes involved in their activation and subsequent oxidation (Lynen, 1955; Kennedy, 1957). Nevertheless, studies *in vitro* have revealed several striking differences in their metabolism by various tissues; for example, ketone bodies are produced from butyrate by sheep-rumen epithelium (Pennington, 1952), but not by sheep liver (Pritchard & Tove, 1960). In the present study major differences have been found in the pattern of oxidation of short-chain fatty acids by ram, bull, dog and fowl spermatozoa, as measured both by oxygen uptake and production of labelled carbon dioxide. Factors governing the rate of oxidation of an exogenous substrate by spermatozoa include the overall metabolic activity of the cell, the effective concentration of activating enzymes, possible loss of enzymes, coenzymes or cofactors during the preparation of spermatozoal suspensions, the permeability of the cell to the substrate, and competition between added and endogenous material.

The differences between the capacities of ram and bull spermatozoa to oxidize fatty acids were unexpected. Short-chain fatty acids play a dominant role in the metabolism of both ruminants, and no major difference in the fat or carbohydrate metabolism of these species has hitherto been reported. The limited capacity of bull spermatozoa to oxidize fatty acids may possibly be due to a greater susceptibility to the washing procedure used in the preparation of the cell suspensions. The absence of ketone bodies as products of butyrate metabolism indicates the effective activation and oxidation of this substrate, presumably by the tricarboxylic acid cycle in view of the susceptibility of respiratory activity to fluoroacetate.

The depression of oxygen uptake by high concentrations of octanoate could be due to a detergent-like action; ram spermatozoa are particularly susceptible to surface-active substances (Koefoed-Johnsen & Mann, 1954).

Dog and fowl spermatozoa, although of different overall metabolic activity, both showed a capacity to oxidize acetate, propionate and butyrate equally (Table 1). Acetate is normally present in both dog and fowl tissues, but the existence of body pools of propionate and butyrate in either species is unlikely. Lifson (1957) showed that propionate, like acetate (Lifson, Omachi & Cavert, 1951), was readily utilized by the isolated perfused dog gastrocnemius. Short-chain fatty acids (C₂–C₈) are also utilized by the isolated dog heart (Cavert & Johnson, 1956).

Incubation of labelled acids with dog spermatozoa gave rise to considerably more labelled carbon dioxide than was expected from the oxygen uptake data (Table 1). This preferential liberation of carboxyl carbon implies incomplete oxidation of the carbon chain, but no evidence was obtained of ketone-body production or of break-down to lower homologues. Other possibilities are incorporation of carbon into spermatozoa, or failure to detect break-down products. Non-enzymic decarboxylation reactions of the type described by Geyer, Marshall, Ryan & Westhaver (1955) would seem to be excluded by the finding that production of labelled carbon dioxide is suppressed by fluoroacetate.

Howe & Flipse (1959) reported that fluoroacetate suppresses acetate oxidation by bull spermatozoa. A similar inhibition with ram spermatozoa was observed in the present study, suggesting that acetate is metabolized by the tricarboxylic acid cycle, but the demonstration of citrate accumulation would have provided more conclusive evidence. The suppression of endogenous respiration by fluoroacetate provides further evidence that the respiratory activity of

spermatozoa is based on the tricarboxylic acid cycle. The relative failure of malonate to inhibit tricarboxylic acid-cycle activity may be due to limited permeability of spermatozoa to dicarboxylic acids (Keofoed-Johnsen & Mann, 1954).

The spermatozoa of all four species showed some capacity to liberate carbon dioxide from formate. In most animal tissues formate is oxidized non-enzymically by a hydrogen peroxide-catalase complex (Nakada & Weinhouse, 1953; Oro & Rappoport, 1959), but the extremely low catalase content of mammalian semen suggests that this mechanism of formate oxidation is unlikely in spermatozoa. Spermatozoa may contain a formate dehydrogenase, but more detailed studies are required to confirm or exclude this possibility.

The recoveries of labelled carbon dioxide in the comparative study of acetate and propionate oxidation by dog and ram spermatozoa are of interest in relation to similar studies with other tissues. Dog spermatozoa metabolize propionate as rapidly as acetate, but, although propionate had no effect on acetate oxidation, propionate oxidation was reduced in the presence of acetate (Table 3). Similar results were obtained by Lifson (1957) with the isolated, perfused dog gastrocnemius. Ram spermatozoa showed only a low capacity to oxidize propionate relative to acetate, and there was little, if any, suppression of acetate oxidation by propionate (Table 3).

Since the endogenous metabolism of ram and dog spermatozoa is suppressed by exogenous substrate (Scott, White & Annisson, 1962), caution is needed in the interpretation of experiments where combinations of substrates are used. In the present study the yields of labelled carbon dioxide should be a more reliable criterion than oxygen uptake. The interrelation of acetate and propionate metabolism in ram spermatozoa was somewhat different from that observed by others in other sheep tissues. Production of labelled carbon dioxide from [$1^{14}C$]acetate by rumen epithelium was considerably reduced in the presence of propionate (Pennington, 1952), and similar results were obtained with sheep-liver slices (Pritchard & Tove, 1960). With the latter tissue, however, acetate markedly stimulated propionate oxidation (Pritchard & Tove, 1960). Propionate break-down in animal tissues, which may occur through β -hydroxypropionate (Kupiecki & Coon, 1957) or by carboxylation to form methylmalonyl-CoA (Flavin & Ochoa, 1957), is CoA-dependent. Acetate-propionate competition, first recognized by Grafflin & Green (1948), probably depends on competition for CoA, but in the absence of more knowledge on the pathway of propionate break-down by spermatozoa no conclusions can be drawn about the present observations.

SUMMARY

1. Ram, bull, dog and fowl spermatozoa metabolized [$1^{14}C$]fatty acids of chain length C_1 - C_8 as indicated by the production of labelled carbon dioxide. Acetate was most effective in stimulating respiration, except with fowl spermatozoa, where octanoate caused the greatest increase in oxygen uptake.

2. Ram spermatozoa oxidized acids with chains containing an even number of carbon atoms more readily than those containing an odd number; however, octanoate in high concentrations (18 μ -moles/ml.) suppressed oxygen uptake.

3. Dog and fowl spermatozoa oxidized propionate as effectively as acetate, and the labelled carbon dioxide produced from C_3 - C_8 acids exceeded the theoretical amount of acid oxidized as calculated from the oxygen uptake for the two species. The recovery of labelled carbon dioxide suggests that acetate may suppress propionate oxidation by dog spermatozoa.

4. Formate caused a significant stimulation of oxygen uptake only with fowl spermatozoa.

5. Fluoroacetate inhibited acetate oxidation by ram spermatozoa, but had no effect on anaerobic break-down of fructose. The endogenous respiration of washed ram spermatozoa was greatly reduced by fluoroacetate ($10^{-2}M$). Malonate was a less effective inhibitor of acetate oxidation.

6. Examination of residual fatty acids by gas-liquid chromatography showed that only ram spermatozoa produced detectable amounts of acetate and propionate from the metabolism of C_4 - C_8 fatty acids.

7. Ketone bodies were not produced when butyrate and hexanoate were incubated with the spermatozoa of the species examined; carbon dioxide had no effect on propionate oxidation by twice-washed ram spermatozoa.

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REFERENCES

- Annisson, E. F. & White, R. R. (1961). *Biochem. J.* **80**, 162.
- Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.* **138**, 535.
- Cavert, H. M. & Johnson, J. A. (1956). *Amer. J. Physiol.* **184**, 582.
- Cochran, W. G. & Cox, C. M. (1950). *Experimental Design*. London: John Wiley and Sons.
- Flavin, M. & Ochoa, S. (1957). *J. biol. Chem.* **229**, 965.
- Flipse, R. J. (1960). *J. Dairy Sci.* **43**, 1.

- Geyer, R. P., Marshall, L., Ryan, M. T. & Westhaver, S. (1955). *Arch. Biochem. Biophys.* **56**, 549.
- Grafflin, A. L. & Green, D. E. (1948). *J. biol. Chem.* **176**, 95.
- Hartree, E. F. & Mann, T. (1959). *Biochem. J.* **71**, 423.
- Howe, G. R. & Flipse, R. J. (1959). *J. Dairy Sci.* **42**, 1325.
- Humphrey, G. F. & Mann, T. (1948). *Biochem. J.* **44**, 97.
- Kennedy, E. P. (1957). *Annu. Rev. Biochem.* **26**, 119.
- Koefoed-Johnsen, H. H. & Mann, T. (1954). *Biochem. J.* **57**, 406.
- Krebs, H. A. (1933). *Hoppe-Seyl. Z.* **217**, 191.
- Kupieccki, F. P. & Coon, M. J. (1957). *J. biol. Chem.* **229**, 743.
- Lardy, H. A. & Phillips, P. H. (1944). *Nature, Lond.*, **153**, 168.
- Lardy, H. A. & Phillips, P. H. (1945). *Arch. Biochem.* **6**, 53.
- Lifson, N. (1957). *Amer. J. Physiol.* **188**, 227.
- Lifson, N., Omachi, A. & Cavert, H. M. (1951). *Amer. J. Physiol.* **116**, 121.
- Lynen, F. (1955). *Annu. Rev. Biochem.* **24**, 653.
- Mann, T. (1954). *The Biochemistry of Semen*. London: Methuen and Co. Ltd.
- Nakada, H. I. & Weinhouse, S. (1953). *Arch. Biochem. Biophys.* **42**, 257.
- Oro, J. & Rappoport, D. A. (1959). *J. biol. Chem.* **234**, 1661.
- Pennington, R. J. (1952). *Biochem. J.* **51**, 251.
- Peters, R. A. (1952). *Proc. Soc. Roy. B.* **139**, 143.
- Pritchard, G. I. & Tove, S. B. (1960). *Biochim. biophys. Acta*, **41**, 130.
- Reid, R. L. (1960). *Analyst*, **85**, 265.
- Scott, T. W., White, I. G. & Annison, E. F. (1961). *Biochem. J.* **78**, 740.
- Scott, T. W., White, I. G. & Annison, E. F. (1962). *Biochem. J.* **83**, 398.
- Turner, C. (1960). *Amer. J. Physiol.* **198**, 48.
- Van Slyke, D. D. & Folch, J. (1940). *J. biol. Chem.* **136**, 509.
- Wales, R. G. & White, I. G. (1960). *Aust. J. biol. Sci.* **13**, 361.
- White, I. G. (1953). *J. exp. Biol.* **300**, 200.

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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.