THE INFLUENCE OF THE FEMALE GENITAL TRACT ON THE METABOLISM OF RABBIT SPERMATOZOA

I. DIRECT EFFECT OF TUBAL AND UTERINE FLUIDS, BICARBONATE, AND OTHER FACTORS

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Summary

The metabolism of glucose and lactate by washed rabbit spermatozoa has been studied in the presence of tubal and uterine fluids, bicarbonate, and other factors.

Uterine fluid stimulates spermatozoal respiration (particularly in the presence of glucose) and aerobic glycolysis; it is also beneficial to the maintenance of motility. Oviduct fluid and blood serum similarly stimulate metabolism but α- and β-amylase have little effect. Uterine fluid contains 60 μg of glucose and 40 μg of lactic acid per millilitre. In the presence of a CO_2, absorbent (KOH) uterine fluid depresses the oxygen uptake of spermatozoa when lactate is substrate. This is due to a rise in pH resulting from loss of CO_2, and spermatozoal respiration in the presence of uterine fluid is high if CO_2 loss is prevented. A rise in pH does not occur when uterine fluid and glucose are incubated together because lactic acid accumulates.

Bicarbonate is present in uterine fluid at a concentration of 45 μmoles/ml and, when incubated with spermatozoa, stimulates respiration and glycolysis. The stimulation of oxygen uptake is greatest in the presence of added glucose or lactate. Bicarbonate has no effect on the maintenance of spermatozoal motility but lactate is particularly effective.

Acidification and dialysis removes the heat-stable stimulating factor from uterine fluid and suggests that it is bicarbonate.

I. INTRODUCTION

The uterine and oviduct mucosa possess secretory glands which constantly secrete fluid into the lumen of the reproductive tract. In rabbits it has long been known that these fluids will accumulate if the fallopian tube or uterus are ligated (Woskressensky 1891; Bond 1898) and in recent years, this technique has been repeatedly used to collect luminal fluids for study (Shih, Kennedy, and Huggins 1940; Vishwakarma 1962; Stevens, Hafs, and Hunter 1964; Stevens, Hafs, and Kirton 1964).

Since the luminal fluids of the female genital tract probably serve as a vehicle and nutrient medium for the gametes (see VanDemark 1958; Bishop 1961; Restall

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1967), it is possible they may influence the capacitation of spermatozoa and related physiological processes. At all events Hamner and Williams (1963) have reported that tubal fluid enhances the respiration of rabbit spermatozoa and have suggested that the stimulating factor may be bicarbonate (Hamner and Williams 1964).

Since no information appears to be available on the metabolism of rabbit spermatozoa in the presence of uterine fluid, the present study was initiated to investigate the effects of this fluid and of other factors, particularly bicarbonate, on the metabolism of glucose and lactate by the spermatozoa. It was hoped in this way to shed more light on the physiological significance of the environment in the female tract during sperm transport.

II. Materials and Methods

(*a*) *Genital Tract Fluids and Blood Serum*

For experiments in which oviduct and uterine fluids were compared, oestrous does were anaesthetized with Nembutal and the genital tract brought up through a mid-abdominal incision. Ligatures were placed just anterior to the cervix, at the uterotubal junction, and at the fimbriated end of the fallopian tubes, and fluids in the lumen of the uterus and oviducts were aspirated after 72 hr. In most experiments uterine secretion was the only genital tract fluid studied and was collected by ligating the uteri at the cervical end only and aspirating the fluid after accumulation for 1 or 2 weeks. Where possible, the fluids were used in manometric experiments within 30 min of collection. Samples not used on the same day of collection were stored at −15°C until the experiments could be performed. For experiment 7 uterine fluid was dialysed for 24 hr in Visking dialysis tubing against 100 vol. of 0·9% NaCl. The saline was then replaced by 100 vol. of fresh solution and dialysis continued for a further 24 hr. For this experiment, uterine fluid was also heated at 100°C for 10 min in a stoppered tube, or acidified by adding 0·05 ml of 2N HCl per millilitre of fluid, after which the pH was readjusted.

After closing the incision, rabbits were given 100,000 units of procaine penicillin G and 0·125 g of streptomycin sulphate intramuscularly.

Blood serum was obtained by bleeding does from the marginal ear vein and allowing the blood to clot under paraffin. After centrifuging, the serum was drawn off and heated at 56°C for 10 min to destroy agglutinating and spermicidal factors (Chang 1947).

(*b*) *Semen*

Rabbit semen was collected with an artificial vagina using the apparatus of White (1955). Motility was scored by the system of Emmens (1947) and only samples of good initial motility were used. Spermatozoal counts were made in duplicate with a haemocytometer.

Ejaculates were pooled and the spermatozoa washed by diluting the semen with three volumes of calcium-free Krebs–Ringer phosphate buffer of pH 7·4 (Umbreit, Burris, and Stauffer 1959) and centrifuging for 10 min at 400 *g*. After aspirating the supernatant, the spermatozoa were redispersed in a convenient volume of the washing diluent for use in manometric experiments. The diluent contained streptomycin and penicillin (0·5 mg/ml of each).

(*c*) *Incubation of Spermatozoa*

Washed spermatozoal suspensions ($2 \cdot 0$–$4 \cdot 2 \times 10^8$ cells per flask) were incubated in Warburg flasks at 37°C and a shaking rate of 114 strokes/min. Saline (0·9%, w/v) was used to replace corresponding amounts of uterine or oviduct fluid in control flasks and for the dilution of the fluids. Oxygen uptake was measured in the presence of 20% (w/v) KOH in the centre well. For experi-

ments requiring anaerobic conditions, single-side-arm Warburg flasks (7 ml capacity) containing 20% (w/v) KOH in the centre well and yellow phosphorus in the side-arm were gassed for 10 min with nitrogen. Spermatozoal respiration in the presence of CO_2 was studied in double-side-arm Warburg flasks (about 18 ml capacity) by using the method of Pardee (1949) as modified by Krebs (1951). Appropriate amounts of Krebs' "CO_2 buffer" (4M diethanolamine with 0·1% thiourea) were added to one side-arm of each flask to give a 2% CO_2 atmosphere above the fluid after equilibration. The flasks were then gassed for 2–3 min with a mixture containing 5% CO_2 and 95% O_2 and allowed to equilibrate for 10 min with stopcocks open. The stopcocks were then closed and a further 15 min were allowed for the atmosphere above the fluid in the flask to reach the proper CO_2 concentration before the substrate was tipped in from the other side-arm. Control flasks, identical with the test flasks in every way except that they contained no spermatozoa, were also incubated to correct for any uptake of oxygen by the CO_2 buffer. Flasks having no CO_2 in the atmosphere had KOH in one side-arm and were closed for the entire 25 min period so that all CO_2 would be removed. At 37°C, the concentration of bicarbonate required to maintain the pH of the incubation medium at 7·4 under a 2% CO_2 atmosphere is 6·00 mM (see Umbreit, Burris, and Stauffer 1959). Preliminary tests with genital tract fluids alone indicated that their oxygen uptake was negligible when used in small quantities, and it was unnecessary to make any correction for their oxygen consumption in subsequent experiments. Further experimental details are given in Section III.

(*d*) *Analytical Methods*

Protein-free extracts of uterine fluid, of the spermatozoal suspension prior to incubation, and of the flask contents after incubation were prepared by precipitation with 0·3N $Ba(OH)_2$ and 5% (w/v) $ZnSO_4.7H_2O$, and glucose and lactate were estimated in the neutral filtrates by enzymic methods (Barker and Britton 1957; Huggett and Nixon 1957). The bicarbonate content of uterine fluid was determined manometrically (Van Slyke and Neill 1925).

(*e*) *Statistical Analyses*

Experiments were submitted to standard analysis of variance. All main effects and their first-order interactions were isolated and tested for significance. The residual mean squares are given in italics at the base of the variance ratio columns (Tables 2, 3, and 5).

In experiments where a number of independent treatments were compared with controls, the standard error of the difference between the means of each treatment and the control group has been calculated from the interaction mean square of the analysis of variance. The significance of the difference between the means has then been assessed by a *t*-test using the degrees of freedom associated with the interaction mean square.

III. Experimental Procedure and Results

(*a*) *Experiment 1: Effect of Oviduct Fluid, Uterine Fluid, and Amylase on the Aerobic Metabolism of Glucose*

Washed spermatozoal suspensions (0·5 ml) were incubated in Warburg flasks (6 ml) with glucose (14 μmoles) and increasing amounts of oviduct and uterine fluids to give final dilutions of 1 : 3, 1 : 6, and 1 : 9. Since recent evidence suggests that amylase may be involved in the capacitation process (Kirton and Hafs 1965; Dukelow, Chernoff, and Williams 1966), the effects of α- and β-amylase (1·0 mg/100 ml) on the metabolism of rabbit spermatozoa were also investigated. The final volume in the main compartment of each flask was 1 ml.

Table 1 shows that spermatozoa incubated in the presence of oviduct or uterine fluids had a greater oxygen uptake and greater rate of glycolysis than the controls, the maximum response being obtained with fluids diluted least (i.e. 1 : 3). Uterine fluid appeared slightly more effective than oviduct fluid in stimulating the metabolism of rabbit spermatozoa. Amylase produced a small increase in the accumulation of lactate.

(*b*) *Experiment 2: Aerobic and Anaerobic Metabolism of Glucose in the Presence and Absence of Uterine Fluid*

This experiment was designed to investigate the effect of uterine fluid on anaerobic glycolysis. Warburg flasks contained 0·5 ml of washed spermatozoal suspension, 0·1 ml of glucose (14 μmoles) in diluent, and 0·4 ml of uterine fluid. The results and summary of the analysis of variance are given in Table 2. Uterine fluid markedly stimulated oxygen uptake and aerobic glycolysis but its stimulatory effect under anaerobic conditions was only slight. Glucose utilization and lactate production was greater in the presence of oxygen even in the control samples.

(*c*) *Experiment 3: Effect of Blood Serum on the Metabolism of Glucose*

In view of the response obtained with uterine and oviduct fluids, it was of interest to examine the effects of blood serum on the aerobic metabolism of glucose by spermatozoa.

Washed spermatozoal suspensions (0·5 ml) were incubated with 0·1 ml of glucose (14 μmoles) in diluent and with or without 0·4 ml of blood serum in 6-ml Warburg flasks. The results, given in the following tabulation, show that blood serum significantly stimulated oxygen uptake and the amount of glucose utilized and lactate produced. Values were calculated per 10^8 spermatozoa per 3 hr, and represent the means of four ejaculates. Standard errors of the differences between the means are given in parenthesis:

	Control	Blood Serum Added	
Oxygen uptake (μl)	16·8	29·3**	(2·25)
Glucose utilized (μmoles)	1·58	2·27*	(0·17)
Lactate produced (μmoles)	2·17	3·59*	(0·40)

* Significantly different from control, $P < 0·05$.

** Significantly different from control, $P < 0·01$.

(*d*) *Experiment 4: Effect of Uterine Fluid on the Metabolism of Glucose and Lactate*

The concentration of glucose and lactate in the uterine fluid from six oestrous does was found to be $60 \pm 20·1$ μg/ml and $40 \pm 12·2$ μg/ml respectively. Rabbit spermatozoa readily oxidize lactate (Murdoch and White 1966) and this may be a source of energy for their survival in the female reproductive tract. The effects of

TABLE 1

EFFECT OF OVIDUCT FLUID, UTERINE FLUID, AND AMYLASE ON THE METABOLISM OF GLUCOSE BY RABBIT SPERMATOZOA

Values are calculated per 10^8 spermatozoa per 3 hr and represent the means of three ejaculates

Material Added	Dilution	Oxygen Uptake (μl)	Glucose Utilized (μmoles)	Lactate Produced (μmoles)
Control		18·6	2·20	3·33
Oviduct fluid	1 : 3	25·1**	3·63**	5·49**
	1 : 6	21·2*	3·38**	4·76**
	1 : 9	21·4*	3·16**	4·52**
Uterine fluid	1 : 3	27·3**	3·84**	5·93**
	1 : 6	24·4**	3·65**	5·56**
	1 : 9	22·2**	3·39**	4·89**
α-Amylase	1 mg/100 ml	18·1	2·21	4·11*
β-Amylase	1 mg/100 ml	18·7	2·24	4·02*
Mean standard errors		1·04	0·31	0·31

* Significantly different from the control, $P < 0{\cdot}05$.

** Significantly different from the control, $P < 0{\cdot}01$.

TABLE 2

EFFECT OF UTERINE FLUID ON THE AEROBIC AND ANAEROBIC METABOLISM OF GLUCOSE BY RABBIT SPERMATOZOA

Values are calculated per 10^8 spermatozoa per 2 hr and represent the means of three ejaculates

Gas Phase	Uterine Fluid	Oxygen Uptake (μl)	Glucose Utilized (μmoles)	Lactate Produced (μmoles)
Aerobic	−	12·7	1·22	1·70
	+	25·2	2·76	3·85
Anaerobic	−	—	0·77	1·25
	+	—	1·05	1·68

Summary of Analysis of Variance

Source of Variation	D.F.	Variance Ratios		
		Oxygen Uptake	Glucose Utilized	Lactate Produced
Gas phase (A)	1		25·7**	15·5**
Uterine fluid (B)	1	241·0**	18·1**	14·9**
Interaction $A \times B$	1		8·7*	6·7*
Ejaculates (C)	2	45·5*	5·2	7·9*
Residual	6	*1·00*†	*0·14*	*0·34*

* $P < 0{\cdot}05$. ** $P < 0{\cdot}01$. † 2 degrees of freedom.

uterine fluid on the metabolism and survival of rabbit spermatozoa when incubated for 6 hr in the presence and absence of glucose or lactate are shown in Table 3.

Spermatozoa were incubated in 15-ml Warburg flasks in 3 ml of diluent containing 0, 0·4, and 0·8 ml of uterine fluid with no substrate, glucose (27 μmoles), and lactate (18 μmoles).

TABLE 3

EFFECT OF UTERINE FLUID ON THE METABOLISM OF GLUCOSE AND LACTATE BY RABBIT SPERMATOZOA

Metabolic data are calculated per 10^8 spermatozoa per 6 hr and represent the means of four ejaculates

Uterine Fluid (ml)	Substrate	Oxygen Uptake (μl)	Lactate Utilized (μmoles)	Glucose Utilized (μmoles)	Lactate Produced (μmoles)	Motility	pH
0	Nil	7·8	—	—	—	0·4	7·4
	Glucose	35·6	—	3·37	4·92	0·8	6·9–7·1
	Lactate	74·6	0·90	—	—	2·1	7·4
0·4	Nil	11·8	—	—	—	1·1	8·3–8·5
	Glucose	49·2	—	5·55	7·15	2·5	6·9–7·1
	Lactate	48·2	0·56	—	—	2·9	8·3–8·5
0·8	Nil	15·8	—	—	—	0·6	8·5–8·8
	Glucose	61·0	—	6·22	7·89	2·6	6·9–7·1
	Lactate	38·5	0·36	—	—	1·1	8·5–8·8

Summary of Analysis of Variance

Source of Variation	D.F.	Variance Ratios				
		Oxygen Uptake	Lactate Utilized	Glucose Utilized	Lactate Produced	Motility
Uterine fluid (*A*)	(2)					
0 *v.* rest	1	1·6	55·7**	15·8**	27·7**	35·3**
0·4 *v.* 0·8	1	1·2	10·8*	0·8	1·7	19·1**
Substrate (*B*)	(2)					
Nil *v.* rest	1	648·4**				125·2**
Glucose *v.* lactate	1	7·8*				0·4
Ejaculates (*C*)	3	15·8**	18·7**	2·5	9·9**	8·7**
Interactions:						
$A \times B$	4	54·5**				22·8**
$A \times C$	6	1·6				0·7
$B \times C$	6	5·1**				1·1
Residual	12	*19·2*	*0·009*†	*1·07*†	*0·65*†	*0·09*

* $P < 0·05$. ** $P < 0·01$. † 6 degrees of freedom.

In the absence of uterine fluid, lactate was more effective than glucose in increasing oxygen uptake and in maintaining motility. In the presence of increasing amounts of uterine fluid, the oxygen uptake with lactate as substrate became progressively smaller, while the oxygen uptake and rate of glycolysis became progressively greater with glucose. Uterine fluid without added substrate was also effective in increasing the oxygen uptake of spermatozoa and was slightly beneficial in maintaining motility. In the presence of uterine fluid, glucose was as good or better than lactate in maintaining motility. At the end of the incubation period, the pH of the contents of all flasks containing uterine fluid but no added glucose was much higher than the others.

(*e*) *Experiment 5: Effect of 2% CO_2 Atmosphere on the Metabolism of Glucose and Lactate in the Presence of Uterine Fluid*

The pH of six samples of uterine fluid measured immediately on collection with a glass electrode was found to be $7 \cdot 7 \pm 0 \cdot 1$ and the bicarbonate content $44 \cdot 8 \pm 0 \cdot 9$ μmoles/ml, i.e. 1005 ± 22 μl CO_2/ml. In order to prevent loss of bicarbonate, spermatozoal suspensions in this experiment were incubated under a 2% CO_2 atmosphere.

Washed spermatozoal suspensions (0·5 ml) were incubated with 0·4 ml of uterine fluid and 0·6 ml of diluent containing glucose (18 μmoles) and lactate (14 μmoles) in double-side-arm Warburg flasks.

Table 4 shows that glycolysis and oxygen uptake with lactate as substrate were significantly enhanced by 2% CO_2. At the end of the experiment, the pH in flasks containing lactate but no CO_2 in the atmosphere was high.

TABLE 4

EFFECT OF A 2% CO_2 ATMOSPHERE ON THE METABOLISM OF GLUCOSE AND LACTATE BY RABBIT SPERMATOZOA IN THE PRESENCE OF UTERINE FLUID

Values are calculated per 10^8 spermatozoa per 3 hr and represent the means of three ejaculates

Atmosphere	Lactate as Substrate			Glucose as Substrate			
	Oxygen Uptake (μl)	Lactate Utilized (μmoles)	pH	Oxygen Uptake (μl)	Glucose Utilized (μmoles)	Lactate Produced (μmoles)	pH
$-CO_2$ (control)	23·8	0·79	8·3	28·1	3·07	4·72	7·1–7·2
$+CO_2$	38·6*	1·23	7·4	28·5	4·34*	6·32*	7·1–7·2
Mean standard errors	2·94	0·26		1·53	0·16	0·34	

* Significantly different from control, $P < 0 \cdot 05$.

(*f*) *Experiment 6: Metabolism of Glucose and Lactate in the Presence and Absence of Bicarbonate*

The results of the previous experiment suggested that bicarbonate might stimulate glycolysis and possibly also the oxidation of lactate. Spermatozoa were therefore incubated for 6 hr with and without sodium bicarbonate (6·00 mM), in the absence of substrate or in the presence of glucose (18 μmoles) or lactate (14 μmoles). Double-side-arm Warburg flasks containing 1·5 ml of diluent under a 2% CO_2 atmosphere were used. The results and the summary of the analysis of variance are presented in Table 5.

Lactate was again more effective than glucose in increasing the oxygen uptake of rabbit spermatozoa and bicarbonate stimulated oxygen uptake and glycolysis. The stimulation in oxygen utilization was greater with lactate than with glucose, which in turn was greater than when no substrate was added. Adding glucose and particularly lactate improved the motility of the spermatozoa. Bicarbonate had no significant effect on motility.

TABLE 5

EFFECT OF BICARBONATE (6·00 mM) ON THE METABOLISM OF GLUCOSE AND LACTATE BY RABBIT SPERMATOZOA

Metabolic data are calculated per 10^8 spermatozoa per 6 hr and represent the means of four ejaculates

Bicarbonate	Substrate	Oxygen Uptake (μl)	Lactate Utilized (μmoles)	Glucose Utilized (μmoles)	Lactate Produced (μmoles)	Motility
−	Nil	10·9	—	—	—	0·6
	Glucose	26·3	—	3·09	4·26	1·6
	Lactate	49·6	0·77	—	—	3·0
+	Nil	15·6	—	—	—	0·9
	Glucose	41·9	—	4·20	6·83	1·9
	Lactate	91·1	0·84	—	—	3·0

Summary of Analysis of Variance

Source of Variation	D.F.	Variance Ratios				
		Oxygen Uptake	Lactate Utilized	Glucose Utilized	Lactate Produced	Motility
Bicarbonate (*A*)	1	52·3**	0·06	145·8**	15·4*	1·4
Substrate (*B*)	(2)					
Nil *v.* rest	1	262·5**				126·8**
Glucose *v.* lactate	1	170·1**				56·3**
Interaction $A \times B$	2	23·5**				0·4
Ejaculates (*C*)	3	1·9	1·0	36·7**	1·3	0·2
Residual	15	*30·9*	*0·16*†	*0·017*†	*0·86*†	*0·111*

* $P < 0·05$. ** $P < 0·01$. † 3 degrees of freedom.

TABLE 6

EFFECT OF WHOLE, HEATED, ACID-TREATED, AND DIALYSED UTERINE FLUID ON THE METABOLISM OF GLUCOSE BY RABBIT SPERMATOZOA

Values are calculated per 10^8 spermatozoa per 3 hr and represent the means of three ejaculates

Treatment	Oxygen Uptake (μl)	Glucose Utilized (μmoles)	Lactate Produced (μmoles)
Control	21·2	3·90	5·78
Whole fluid	33·4**	6·48**	8·76**
Heated fluid	30·9**	5·88**	8·38**
Acid-treated fluid	22·5	4·19	5·68
Dialysed fluid	21·1	4·31	6·07
Mean standard errors	2·20	0·47	0·49

** Significantly different from the control, $P < 0·01$.

(*g*) *Experiment 7: Metabolism of Glucose in the Presence of Whole, Heated, Acid-Treated, or Dialysed Uterine Fluid*

Aliquots of washed spermatozoal suspension (1 ml) were incubated in 15-ml Warburg flasks with 2·5 ml of glucose (18 μmoles) and whole, heated, acid-treated, or dialysed uterine fluid (0·5 ml). Oxygen uptake was measured with air as gas phase.

Table 6 shows that acidification and dialysis prevented the stimulating effect of uterine fluid on the metabolism of glucose by spermatozoa. Heating uterine fluid did not affect its activity.

IV. Discussion

There are relatively few references in the literature to the effects of the female genital tract secretions on spermatozoal metabolism. Olds and VanDemark (1957) reported that follicular and tubal fluid of the cow stimulated the respiration of bull spermatozoa, but uterine fluid was ineffective. In the absence of glucose, the genital fluids of the ewe also increase the oxygen uptake of ram spermatozoa (Restall 1964). By adding glucose to the incubation medium, however, this effect was abolished although the aerobic and anaerobic glycolysis of the spermatozoa was, in most cases, stimulated (Wales and Restall 1966). Hamner and Williams (1963), on the other hand, have reported that rabbit oviduct fluid alone does not stimulate the respiration of rabbit spermatozoa as much as when glucose is also present and suggest that the exogenous rather than the endogenous respiration of the spermatozoa is affected. The present investigation shows that rabbit uterine fluid also stimulates spermatozoal respiration, particularly in the presence of glucose, and has a profound effect on the rate of glycolysis, the stimulation being greater with greater concentration of fluid.

Oviduct and uterine fluids contain substantial amounts of bicarbonate (Shih, Kennedy, and Huggins 1940; Lutwak-Mann 1962; Vishwakarma 1962; Hamner and Williams 1964, 1965), and in the present study it was always found in uterine fluids in concentrations similar to those previously reported (Lutwak-Mann 1962). Bicarbonate appears to be actively secreted by the epithelial cells of the oviduct and uterus since its concentration in these fluids is about twice that of blood plasma (Vishwakarma 1962).

The results support the suggestion of Hamner and Williams (1964) that bicarbonate in the genital tract fluids of the doe is responsible for the stimulating effect on the respiration of rabbit spermatozoa in the presence of glucose. It may also account for the stimulating action of blood serum. Bicarbonate has been found to stimulate the spermatozoa of several other species, including epididymal bull spermatozoa (Henle and Zittle 1942) and ejaculated human, rooster, and bull spermatozoa (Hamner and Williams 1964). Bull spermatozoa in diluted semen have also been reported to respire at a more rapid rate in the presence of respired CO_2 than in its absence (Lodge and Salisbury 1963; Salisbury and Lodge 1963), but this observation could not be confirmed by Wales and O'Shea (1966). Although bicarbonate may, as Hamner and Williams (1964) suggest, stimulate respiration by increasing the intracellular

levels of tricarboxylic acid cycle intermediates, it seems probable that it may also have an effect on reactions of the Embden–Meyerhof pathway, since our results show that it greatly enhances the glycolytic activity of rabbit spermatozoa. The mechanism of this stimulation is, however, not clear and may be oxygen dependent since the effect of uterine fluid on glycolysis was greater under aerobic than under anaerobic conditions. Like human spermatozoa (MacLeod 1941; Murdoch and White 1968) the presence of oxygen does not appear to restrict the rate of glucose breakdown by rabbit spermatozoa, indicating the lack of a Pasteur effect.

Removal of the heat-stable respiration-stimulating factor from uterine fluid by dialysis and acidification confirms the observations of Hamner and Williams (1964) on tubal fluid, and is further evidence that bicarbonate is involved. Yates and Olds (1959), on the other hand, have suggested that substances of high molecular weight may play some part in the stimulation of respiration by bovine follicular fluid. Although, in the present study, the metabolism of spermatozoa was stimulated by fluids incubated in flasks with KOH in the centre well, a greater stimulation of glycolysis occurred in an atmosphere containing CO_2 with diethanolamine in the side-arm. Shelby and Foley (1966) have also reported that the oxygen uptake and motility of boar spermatozoa incubated in the presence of diethanolamine is superior to that of spermatozoa incubated in the presence of KOH. By absorbing CO_2, KOH would tend to decrease the bicarbonate content of genital tract fluids and increase the pH. With glucose as substrate, the production of lactic acid by spermatozoa counteracts the rise in pH, but with sodium lactate or no substrate the pH is high (about 8·5). The results in Table 4 suggest that the upward drift in pH may explain the apparent depressing effect of uterine fluid on the metabolism of lactate by spermatozoa in Table 3. Even in a CO_2-free atmosphere sufficient bicarbonate apparently persists in uterine fluid to cause a maximal stimulation of glucose oxidation. Providing the bicarbonate concentration approximates to 6 mM, the presence of diethanolamine and a 2% CO_2 atmosphere was found to be effective in preventing loss of bicarbonate and maintaining the pH of the medium near 7·4. The pH of rabbit uterine fluid found immediately on collection (7·7) is in agreement with the observations of Vishwakarma (1962) and Stevens, Hafs, and Kirton (1964).

The stimulating effect of bicarbonate on respiration was even greater when lactate was used as substrate instead of glucose. Uterine and oviduct fluids contain variable amounts of lactate (Bishop 1957; Mastroianni and Wallach 1961; Hamner and Williams 1965; present study) and Lutwak-Mann (1962) has reported that its concentration in the uterine fluid of rabbits increases following mating. This may be of some importance to spermatozoa in the female genital tract since the oxygen tension in the uterus (Campbell 1932) and oviduct (Bishop 1957; Mastroianni and Jones 1965) is sufficient to support respiration. Glucose is also found in the genital tract fluids of the doe (Bishop 1956; Lutwak-Mann 1962; Hamner and Williams 1965; present study), while fructose (Mann 1964) and occasionally some glucose (Mann and Parsons 1950) occur in rabbit semen. The stimulating action of bicarbonate on the breakdown of these hexoses may also be of some physiological importance for spermatozoa in the female genital tract.

Emmens (1947) has shown that rabbit spermatozoa are adversely affected at pH values below 5·8. Vishwakarma (1962) has suggested that bicarbonate may prevent spermatozoa from deteriorating in the female tract by buffering the lactic acid produced by glycolysis. In the present study, lactate was found to be beneficial to the maintenance of sperm motility *in vitro* at pH 7·4, and may be a factor contributing to the survival of rabbit spermatozoa in the female tract. In the absence of exogenous substrate oxygen uptake and motility were much reduced.

Spermatozoa that have spent 6 hr in the female tract respire at a greater rate than the freshly ejaculated cells (Hamner and Williams 1963; Mounib and Chang 1964; Murdoch and White 1967) and it is tempting to suggest that stimulation of metabolism by the lumen fluids, and bicarbonate in particular, may in some way be associated with capacitation. Kirton and Hafs (1965), however, claim that capacitation can be achieved by incubating rabbit spermatozoa with β-amylase. If this is true, profound alterations in metabolic activity are not likely to be essential to the process of sperm capacitation since amylase, in the present investigation, did not stimulate the respiration of spermatozoa and only slightly altered the production of lactic acid.

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