# Effect of Bicarbonate and Oviduct Fluid on Respiration of Spermatozoa. (32526)

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Bicarbonate and $CO_2$ have been reported to increase the inherently low respiration rate of spermatozoa of several species(1,2,3). Because of the complexities in measuring $O_2$ uptake in the presence of $CO_2$ in the Warburg apparatus, the necessity for rapid removal of sperm from seminal plasma and the relatively brief period of active respiration of sperm, the effect of bicarbonate or $CO_2$ on sperm respiration has been in dispute(4). The effect of bicarbonate may be of special significance since female tract secretions stimulate sperm respiration and are high in bicarbonate. The present investigation was undertaken to clarify the influence of bicarbonate on sperm respiration, using the Clark $O_2$ electrode and a fluid system eliminating the $CO_2$ atmosphere, and to determine if the metabolic stimulation of sperm by oviduct fluids is entirely due to bicarbonate or if additional stimulants are present.

*Materials and methods.* Throughout this paper we will refer to bicarbonate to represent $H_2CO_3$, $HCO_3^-$, $CO_3^=$ and $CO_2$, recognizing that one or more of these molecular species may be involved in the actual stimulation. Semen was obtained from rabbits by means of the artificial vagina(5), from rams by electroejaculation(6) and from boars by use of the gloved-hand technique(7). Semen samples from rabbits and rams were pooled in order to insure a sufficient number of washed spermatozoa. For repeat experiments semen from the same individual animals was used. Immediately after ejaculation the rabbit semen was added to 2 volumes of calcium-free Krebs-Ringer phosphate buffer and the spermatozoa washed free of seminal plasma as previously described(8). Following the removal of the gelatinous material from swine semen by straining through cheesecloth, the gel-free semen was centrifuged at 143 g for 5 minutes and the supernatant was removed by aspiration. The spermatozoa were resuspended in calcium-free Krebs-Ringer phosphate buffer. Centrifugation and aspiration were repeated twice. Ram spermatozoa were washed twice at 143 g for 5 minutes in the above buffer. The packed sperm cells from all 3 species were then resuspended at a ratio of 1 part spermatozoa to 5 parts buffer. The final mean concentration of sperm cells was 0.9, 0.74 and 0.24 $\times$ $10^8$/ml for the sheep, rabbit and pig respectively. One half ml of the sperm suspension was pipetted into vials with either 2.5 ml of warm KRP containing 3 mg glucose/ml and saturated with air (control) or 2.5 ml KRP-glucose plus bicarbonate at levels of 93, 185 or 283 $\mu$g/ml. Two minutes were allowed for equilibration. Sperm respiration was studied using the Yellow Springs biological monitor, model 53, and the Clark $O_2$ electrode. Change in % saturation of $O_2$ was recorded each minute over a 20 minute period and the $O_2$ consumed expressed as $\mu$l $O_2$ taken up/$10^8$ sperm/hour $=$ $ZO_2$. Initial $O_2$ concentrations were determined by the Alsterberg modification of the Winkler method(9). The pH in most vials was determined at the end of the experiment insuring that the medium had remained near pH 7.0. Initial and final motilities of sperm were checked.

Comparisons also were made between sperm samples incubated in the presence of 20.0 mM of succinate per 3 ml and in the presence of 283 $\mu$g bicarbonate per ml. The oviduct fluid from the rabbit was collected by a published method(10). The oviducts of estrous sows were ligated at both ends immediately prior to estrus, and 72 hours later the accumulated oviduct secretions aspirated from the distended oviducts. Oviduct fluids from the estrous ewe were collected as described by Perkins(11). The bicarbonate in the oviduct secretions was determined manometrically by releasing the $CO_2$ with acid(12). Semen samples used in the experiments concerning the metabolic stimulation of sperm by oviduct fluids were collected and treated as described

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above. Oviduct secretions were added to the vials at the expense of KRP, at levels of 0.5 and 0.2 ml per vial for swine studies and 0.5 ml per vial for sheep and rabbit. Control vials contained pure bicarbonate at levels equivalent to those contributed by oviduct secretions.

Statistical significance was determined by the method of Steel and Torrie(13) using the F test for means of paired observations. This eliminates the variance between pairs and measures the difference within pairs. This is justified in view of the recognized differences in physiological status of sperm from different collections(14,15).

*Results.* The results of the present study are summarized in Fig. 1. Bicarbonate levels

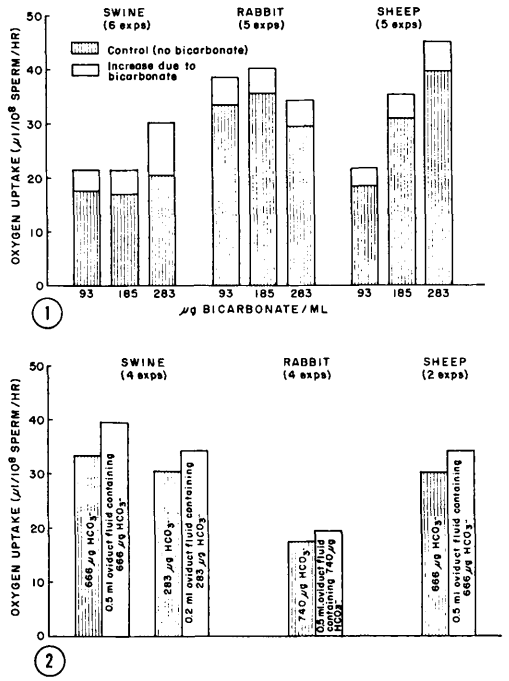


FIG. 1. Effect of different levels of bicarbonate on oxygen uptake of swine, rabbit and sheep spermatozoa.

FIG. 2. Comparison of stimulatory effect of pure bicarbonate and oviduct fluids on oxygen uptake of swine, rabbit and sheep spermatozoa.

of 93, 185 and 283 μg/ml stimulated $O_2$ uptake of swine sperm 17, 21 and 29% ($P<.01$) respectively over controls without bicarbonate for the 20 minute period studied to bicarbonate was approximately 14% (Fig. 1). With rabbit sperm the increase due ($P<.01$) for all levels. Bicarbonate increased ram spermatozoa respiration by 16, 13 and 13% ($P<.01$) respectively for the above mentioned levels.

One possible explanation for the increase with added bicarbonate is that added bicarbonate may increase the respiration by increasing the level of tricarboxylic acid cycle intermediates. When swine spermatozoa were incubated in the presence of succinate the spermatozoa consumed less oxygen than spermatozoa metabolizing in the presence of bicarbonate (22.8 μl/$10^8$ sperm/hr *vs* 25.8 μl). Rabbit sperm also utilized slightly less oxygen (25.7) in the presence of succinate than sperm incubated with $CO_2$ (28.3). Ram spermatozoa respired at a significantly more rapid rate in the presence of succinate (42.7) than bicarbonate (39.4).

The results for sperm incubated in the presence of oviduct secretions and compared to controls of equivalent amounts of bicarbonate are shown in Fig. 2. Boar sperm in the presence of 0.5 ml of oviduct secretions consumed 18% more oxygen than sperm incubated with pure bicarbonate ($P<.01$). Boar sperm incubated with 0.2 ml of secretions had an increased oxygen utilization of 11% ($P<.01$). Ram spermatozoa utilized 11% more oxygen in the presence of ewe tract secretions than the bicarbonate controls, and rabbit sperm consumed 12% ($P<.01$) more oxygen with rabbit oviduct secretions.

*Discussion.* Most investigations of aerobic metabolism of spermatozoa have involved manometric determination of the oxygen consumed by spermatozoa in seminal or various physiological fluids at 37°C in an enclosed system employing KOH to remove carbon dioxide. Potassium hydroxide irreversibly reacts with carbon dioxide producing an atmosphere that is free of carbon dioxide. Sperm of the human(13), bull(14,15), turkey(16) and swine(3) metabolizing in the presence of KOH have been reported to have lower rates of respiration and glycolysis than sperm in the presence of respired carbon dioxide. The stimulating effect of low concentrations of carbon dioxide on metabolism has been reported for human, chicken, rabbit and bull sperm(1), and for bull sperm(2). It has been reported(4) that incubation of ram sperma-

tozoa in an atmosphere containing 3.8% $CO_2$ had no effect on $O_2$ uptake and that the presence of respired $CO_2$ during incubation did not influence $O_2$ utilization. Our present results using a new system for oxygen measurement confirm previous findings(1,2,3), and show that bicarbonate does stimulate sperm respiration. The failure by others(4) to observe the stimulatory effect of bicarbonate may be caused by uptake of bicarbonate by the sperm during a longer period in semen before removal of seminal plasma. A special effort was made in our experiments to remove the seminal plasma which contains bicarbonate in all species as soon as possible after collection of the semen. Higher concentrations of sperm were used(4) sufficient to retain metabolic $CO_2$ and stimulate normal respiration(17,18). The percent increase for sperm respiration in this study due to bicarbonate was less than differences previously reported (1,3). This is not surprising since the earlier workers(1,3) incubated sperm cells over a 3 hour period, with the greatest difference between treatments occurring near the end of the study. The mean control $ZO_2$ values reported here of approximately 33, 19 and 30 respectively for rabbit, boar and ram spermatozoa are greater than the values usually reported in the literature with manometric techniques. $ZO_2$ values of 11 and 22 for rabbit and ram sperm have been reported(19). Hamner and Williams(1) found $ZO_2$ values of 4.2 for the rabbit. Mean $ZO_2$ values of 10, 7 and 7 have been reported for boar spermatozoa(3,20,21). A greater oxygen uptake with the oxygen electrode as compared with the manometric technique was also observed for uredospores of *Puccinia graminis* (22). Bush reported during early stages of germination a $QO_2$ of 33.6 when measured polarographically, but only 7.7 when measured manometrically(22). This suggests that oxygen uptake can be determined more rapidly and with greater sensitivity than with the Warburg method. The time between the collection of the semen and oxygen measurement was decreased by more than 30 minutes when the oxygen electrode was employed which allowed determination of the higher initial rates of respiration. Oxygen uptake was determined during the first 20 minutes as compared to periods of 1 to 3 hours with the Warburg apparatus.

Increased oxygen consumption by spermatozoa incubated in reproductive tract secretions has been reported for bull sperm (23), rabbit sperm(1) and rooster(24). Rabbit spermatozoa(25) were markedly stimulated by incubation for 6 hours in the uterus of an estrous rabbit or in oviduct fluid. Similar effects for boar sperm(26) incubated in the presence of sow reproductive tract secretions have been observed. It has been suggested that the sperm stimulation factor in rabbit oviduct secretions might be bicarbonate since bicarbonate seemed to duplicate the respiration stimulating effect of oviduct fluid(1). Our present study indicates that bicarbonate did account for a considerable portion of the increase in $O_2$ uptake but that additional stimulants may be present in female tract secretions. It would appear that this stimulation was due to something other than a carbohydrate substrate since adequate levels of glucose as substrate were present.

We have confirmed the observation(2) that succinate stimulates $O_2$ uptake to approximately the same degree as bicarbonate. Other tricarboxylic acid cycle intermediates were not tested because of the report that malate and oxalacetate are inactive(1) and because these and other intermediates do not enter the cell(14).

*Summary.* The stimulating effect of low levels of bicarbonate on the respiration rate of boar, ram and rabbit spermatozoa was studied using the oxygen electrode. Bicarbonate at levels of 93, 185 and 283 $\mu$g/ml was found to stimulate $O_2$ uptake of boar sperm 17, 21 and 29% ($P<.01$) respectively. With rabbit sperm the increase in $O_2$ uptake was approximately 14% ($P<.01$) for all levels. Corresponding values for ram spermatozoa were 16, 13 and 13% ($P<.01$). Without bicarbonate rabbit sperm respired at a rate of 33, boar sperm 19, and ram sperm 30 $\mu$l $O_2/10^8$ cells/hr. These values are higher than those found with manometric techniques. Succinate significantly stimulated ram sperm respiration above bicarbonate controls. Swine and rabbit spermatozoa utilized significantly more oxygen in the presence of bicarbonate than sperm incubated in the presence of succinate. Ad-

ditions of oviduct secretions from the sow, ewe and rabbit were found to increase $O_2$ uptake by 18, 11 and 12% respectively over the maximum rate obtained with bicarbonate suggesting the presence of additional stimulants in female tract secretions.

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## A Transient Fall in Pressure Precedes the Characteristic Intramammary Pressure Rise Following Mechanical Stimulation of the Mammary Gland.* (32527)

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The myoepithelium of mammary glands in goats(1), mice(2), rabbits(3,4) and rats(5) contracts when mechanical stimulation is applied to the skin overlying the mammary gland or directly to the exposed mammary tissue. This contraction causes a rise of intramammary pressure similar to that produced by oxytocin. It has been suggested that in some species stimulation of the myoepithelium by the kneading and butting activity of the suckling young may represent a subsidiary mechanism for milk ejection which reinforces the action of oxytocin(3,5). In the course of a study of the interaction of nervous, hormonal and mechanical influences on intramammary pressure, we noticed that mechanical stimulation of the rat mammary gland caused a transient fall of intramammary pres-

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