Reversal by Metabolic Regulators of CO_2-Induced Inhibition of Mammalian Spermatozoa.* (24877)

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The causes of quiescence of mammalian spermatozoa in the epididymis and of their acquisition of active motility upon ejaculation have long been sought by physiologists. Lardy and his co-workers(5) found a metabolic regulator in epididymal spermatozoa of the bull, which diffused from the cells to stimulate them to markedly greater respiration. Bishop and Salisbury(2) after studying aerobic metabolic aspects of the "dilution effect" with ejaculated bull spermatozoa, had postulated a metabolic inhibitor in semen "associated with spermatozoa." They based their speculation on the fact that dilution of a standard number of cells with either seminal plasma or with 0.9% NaCl resulted in markedly increased rate of oxygen consumption/cell. At first inspection these observations, one dealing with a stimulator, the other with inhibitor, seem contradictory and no attempt has been made to reconcile them. However, recent studies indicate these observations to be part of the physiological change associated with maturation and production of fully active spermatozoa. Earlier identified as elemental sulfur by yeast-test then employed, the regulator contained that element(5) but its effects on respiration were duplicable only by cysteine, homocysteine, glutathione and inorganic sulfide and sulfite, the sulfite isolated as one of the important reducing substances in semen by Larson and Salisbury(6). Roedel in Germany verified the observations of the Wisconsin group and showed that epididymal spermatozoa contained a cysteine desulfhydrase which released sulfide from cysteine(8). The sulfide was effective in producing the same stimulation to respiration by epididymal spermatozoa as reported for diffusible substance containing sulfur. Sodium and chloride ions tend to stimulate metabolic activity of spermatozoa(2,9). K-ion at levels present in the epididymis inhibits respiration(4) and anaerobic glycolysis by ejaculated bull spermatozoa(12). Calcium ion interacts to counter the potassium effect(4). The p CO_2 above about 2% of atmosphere independently inhibits glycolysis(12), and at 100% CO_2 glycolysis is less than 10% of that occurring under N_2(11). Inhibition is reversible by replacement of CO_2 by air or by N_2(11). Evidence exists that epididymal sperm or ejaculated cells in a highly concentrated condition are low in chloride and high in CO_2(3,7) and on dilution a chloride shift occurs allowing CO_2 to be released from cells and chloride to enter them(7). Recent work reported here shows that sulfide or sulfite by-product of cysteine desulfhydrase activity by spermatozoa is fully capable of reversing inhibition of anaerobic glycolysis by CO_2. These suggest that diffusible substance in epididymal spermatozoa observed by Lardy *et al.*(5) and others(8) was probably not only a sulfhydryl decomposition product but possibly, in addition, CO_2, and that sulfide and sulfite from sulfhydryl component are catalysts of, if not active participants in, the reaction releasing CO_2 from the cell.

Procedure. The technic used has been described(12). Briefly it consisted of measurement of CO_2 evolution by acid metabolites from bicarbonate, or of lactic acid output, by ejaculated spermatozoa suspended in their own seminal plasma, and in IVT_{Na} diluent at 37°C for 4 hours under 3 p CO_2, of 5 and 50% in N_2 and 100% CO_2. The data, each a mean of 3 replicates, are reported as equivalent μg of lactic acid produced by 10^8 cells/hour during 4-hour incubation at 37°C. In 5 experiments the μg acid equivalent was calculated from μl CO_2 evolved from bicarbonate after correction for CO_2 and acid retention (12). In Exp. No. 3 in which levels of 0, 30, 300, and 600 μg of sulfur were added/ml diluent as glutathione, actual lactic acid determinations were made before and after the 4-hour period by method of Barker and Summerson(1). Motility of cells was determined

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TABLE I. Effect on Anaerobic Glycolysis and Livability of Bovine Spermatozoa during 4 Hr at 37°C of Various Amounts of Cysteine, Glutathione, Sodium Sulfide and Sodium Sulfite.

		Cysteine Exp. 1				Glutathione Exp. 2				Glutathione Exp. 3			
	pCO_2	μg S/ml				μg S/ml				μg S/ml			
	(%)	0	30	60	90	0	30	60	90	0	30	300	600
Z_{LA}*	5	148	141	132	133	156	155	150	144	82	90	67	70
	50	38	36	29	43	11	14	14	78	22	23	24	26
	100	15	14	11	15	3	9	10	9	13	10	10	18
Final	5	52	50	55	58	67	70	67	70	38	58	60	65
mot., %	50	57	60	57	48	80	73	73	70	52	35	63	65
	100	47	57	52	57	73	70	60	70	58	55	58	57

		Sulfide Exp. 4				Sulfide Exp. 5				Sulfite Exp. 6			
	pCO_2	μg S/ml				μg S/ml				μg S/ml			
	(%)	0	5	20	80	0	160	320	480	0	30	60	90
Z_{LA}*	5	168	158	155	161	142	116	174	173	130	171	160	153
	50	28	23	18	25	18	37	110	121	17	89	78	77
	100	10	12	14	18	3	45	105	129	6	94	89	85
Final	5	53	57	53	50	63	47	40	40	67	65	63	58
mot., %	50	53	57	63	63	60	53	20	30	63	53	52	60
	100	50	53	50	60	67	47	24	47	65	60	58	53

* Z_{LA} = μg lactic acid produced by 10^8 cells/hr, directly determined in Exp. 3, and calculated from CO_2 evolution from bicarbonate in the 5 other experiments.

at end of 4-hour incubation by direct microscopic examination after aeration of cells following their removal from Warburg flasks. Dilution rate was one part of semen to 4 parts of IVT_{Na} diluent. The buffer of this diluent was composed of sodium citrate and sodium bicarbonate (0.025M). Various quantities of cysteine and glutathione, as representative of sulfhydryl components in the epididymis, and of sodium sulfide, sulfite and sulfate, as representative of their breakdown products after desulfhydrase action(8), were added to the IVT semen diluent.

Results. The results of 6 experiments (Table I) clearly show the previously reported statistically and biologically highly significant inhibition of glycolysis by increasing p CO_2 in the gas phase surrounding the spermatozoa. Neither cysteine nor glutathione at levels employed, which were higher in the case of glutathione than the total non-carbohydrate reducing substances found in semen(10), had any measurable influence on glycolysis of ejaculated spermatozoa. There are, of course, variations in mean glycolysis values but, except for the effect of CO_2, none of the treatments approached statistical significance. In Exp. 3, however, ability of cells to withstand incubation as indicated by final motilities was improved by the glutathione.

Both sulfide and sulfite, on the other hand, each exerted a marked influence on inhibition of glycolysis by CO_2. The effect was to release inhibition by CO_2. In 100% CO_2, for example, without added sulfide, glycolysis was less than 3% of that occurring under 5% CO_2. However, in the presence of 480 μg S/ml as sulfide, glycolysis was approximately 75% as great as with only 5% CO_2. This level of sulfide was several times higher in sulfur equivalent than the levels reported in semen. However, sulfite at physiological levels(6) stimulated anaerobic glycolysis in 5% CO_2 and partially, though not completely, reversed inhibition by 50 and 100% CO_2. Additional sulfite over minimum level of an equivalent of 30 μg S/ml did not increase the response. All levels of sulfide in Exp. 5 depressed final motility.

A final experiment was replicated 3 times with 0, 30, 60 and 90 μg of S/ml as sodium sulfate added to diluent. It resulted in no measurable effect of the sulfate ion on motility nor on glycolysis, measured as acid equivalents of CO_2 evolved from bicarbonate, as hexose uptake, or as lactic acid produced.

These results and earlier observations lead to the speculation that phenomena of this sort are involved in initiation of motility of spermatozoa at ejaculation. Spermatozoa are inhibited by high p CO_2 and high K concentration in the epididymis. The enzyme or enzymes of spermatozoa responsible for cleavage of sulfide from the substrate, probably also from the cells, are probably activated by the citrate added to cells on admixture of accessory gland fluids at ejaculation. After activation, the sulfide diffuses from the cells to be converted to sulfite. The CO_2-release stimulated by these compounds, along with dilution of K by sodium chloride and uptake of hexose, result in reversal of the metabolic inhibition found in the epididymis.

Summary. Neither cysteine, glutathione nor sulfate exhibit a measurable effect on anaerobic glycolysis nor on its inhibition by high p CO_2. Sulfide, at higher than physiological levels, and sulfite, in amounts found in semen, stimulate anaerobic glycolysis and reverse inhibition of glycolysis by CO_2.

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Effect of Plasma from Wounded Donors on Nitrogen and Sulfur Excretion.* (24878)

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Several laboratories have presented data which seem to indicate that injured cells produce or cause production of a factor or factors having ability to alter rate of healing of wounds. These data have been obtained by observing the effects of extracts of crushed tissues when applied directly to healing wounds or when administered parenterally to wounded animals(1-3). Sandblom(4) and Auerbach(5) reported that the presence of injured tissue in the body causes production of factors appearing in the circulation, which affect rate of healing. They also observed that, when 2 wounds are consecutively inflicted in the same animal, rate of healing of the second wound appears affected by presence of the previously inflicted wound. Metabolism of nitrogen and sulfur have been shown to be markedly altered during healing of experimental wounds. As a result of wounding, the amount of urinary nitrogen increases sharply, while at the same time there is a relative retention of sulfur compounds by the wounded animal(6-9). At this time also, proteins rich in cystine and methionine are synthesized in regenerating wound tissue; these newly synthesized proteins must be considered to be distinct from collagen, since this latter protein contains no cystine and very little, if any, methionine(14). In this study, the effects of factor(s) released into blood by the stimulus of wounding, as they affect me-

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