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Metabolism of Glycine by Bovine Spermatozoa

Early attempts to improve bovine semen diluents by the incorporation of glycine were unsuccessful (*1*), in contrast to the favorable response noted with sea urchin and fowl spermatozoa (*2*). Recently, however, glycine solutions have been used in combination with egg yolk or milk to increase the survival time of bovine spermatozoa at 5°C (*3, 4*).

Recent studies (*5*) in this laboratory with glycine-1,2-C^{14} have provided evidence that glycine is metabolized by freshly collected bovine spermatozoa. Previously, a metabolic role for glycine had received little consideration, since it apparently was not utilized (*2, 6*).

In a preliminary trial, 1.7 ml of whole semen containing 2×10^9 spermatozoa was incubated in a diluent of heated skimmilk and 0.5*M* aqueous glycine (1/1) (*4*) containing 4 μc of glycine-C^{14}. Respiratory CO_2, collected in alkali during 3 hours of incubation at 37°C, was converted to barium carbonate and yielded a total of 11,700 count/min.

Subsequent trials involved twice-washed spermatozoa suspended in Ringer-phosphate buffer containing 0.001*M* adenosine triphosphate. In experiment 1, 0.01*M* sodium formate was added as a metabolic trap (*7*); 0.01*M* glyoxylic acid was used similarly in experiment 2. Formate was recovered by steam distillation and was oxidized with mercuric oxide. Respiratory CO_2 and formate were radioassayed as solid barium carbonate. Glyoxylate was recovered as the 2,4-dinitrophenylhydrazone and was radioassayed as such. Results of these two experiments are presented in Table 1. Obviously, spermatozoa are capable of metabolizing glycine. The products recovered indicate that the pathways involved may be similar to those observed in other mammalian tissue (*7*).

Further evidence of the nature of glycine metabolism was sought by determining the glycine and glucose oxidation when one and when both substrates were available. Uniformly labeled glucose or glycine was employed in 0.01*M* concentration with 10^6 count/min, per flask. Osmotic pressure was within normal limits for these cells (*8*). Each flask contained 10^9 spermatozoa in a final volume of 2 ml. The mean results of three trials, presented in Table 2, indicate that each substrate is utilized in the presence of the other under the conditions employed. Recent work has shown that glycine at 0.01*M* concentration is detrimental to bovine spermatozoan survival (*4*) and therefore might be expected to reduce the production of CO_2 from glucose-C^{14}. However, 0.1*M* glycine has been shown to increase spermatozoan survival (*3, 4*).

The action of glycine in reducing CO_2 production from glucose-C^{14} without a reduction in total CO_2 production and the observation that glycine is metabolized lead to the postulation that glycine utilization may have a sparing effect on glycolysis in bovine spermatozoa. Obviously such an effect may not account for all of the benefit derived from glycine.

These studies are being extended to obtain additional information regarding the metabolism of glycine by bovine spermatozoa, particularly with regard to the nature of other possible intermediates and end-products.

Table 1. Formation of labeled products from C^{14}-labeled glycine by bovine spermatozoa. Yields expressed per 10^9 cells for 2 hours.

Expt.	Product			
	CO_2		Formate	Glyoxylate
	μ mole	(count /min)	(count /min)	(count /min)
No. 1 4 μcurie glycine + formate	13	4240	2300	
No. 2 2 μcurie glycine	28	2680		
2 μcurie glycine + glyoxylate	10	1700		1040

Table 2. Oxidation of glucose and glycine in suspensions of bovine spermatozoa in Ringer-phosphate buffer, *p*H 7.1. Results expressed per 10^9 cells for 2 hours.

Substrate	Respiratory CO_2	
	count/min	μmoles
Glycine-C^{14}	5295	32
Glycine-C^{14} + unlabeled glucose (0.01*M*)	4085	31
Glucose-C^{14}	7305	33
Glucose-C^{14} + unlabeled glycine (0.01*M*)	6935	30
Glucose-C^{14} + unlabeled glycine (0.1*M*)	6150	34

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Maternal and Sexual Behavior Induced by Intracranial Chemical Stimulation

A technique permitting chemical or electric stimulation, or both, of restricted brain areas in unanesthetized rats, and electroencephalographic (EEG) recording from these areas, has been developed and found to be of value (*1*).

Implants are prepared as follows. Two Tygon-insulated copper or silver wires (0.1 mm in diameter) are baked along the outside of No. 22 hypodermic tubing extending from 2 to 9 mm below the base of a plastic holder (*2*). The wires lead from contact points on the holder and terminate at opposite sides of the end of the shaft as a bipolar stimulating-and-recording electrode. The implant shaft is permanently inserted in the brain while the anesthetized rat is held in a stereotaxic instrument. Four holes in the base of the holder permit rigid attachment to the skull with jeweler's screws.

Two or more days later, rats are placed in 3- by 3- by 2.5-ft boxes for stimulation testing. A small clip connects the implant to light overhead leads from a 0- to 12-v, 60 cy/sec stimulator, or to an EEG machine. The clip also contains a