Metabolism of amphibian spermatozoa in relation to their motility[1]

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Summary. *Bufo arenarum* spermatozoa were able to sustain motility both under aerobic and anaerobic conditions. In aerobiosis, the oxygen consumption varies between 2.6 and 4.2 µl $O_2/10^8$ cells/h at 30 °C. The synthesis of lactic acid by anaerobic spermatozoa demonstrated the existence of an active glycolytic pathway.

Despite the fact that a great deal of work has been devoted to amphibian fertilization and development, little attention has been paid to sperm metabolism among these vertebrates. Besides the initial observations of Engelmann[2] about the influence of anaerobiosis on frog sperm, we have not found any other contributions but those reported by Bernstein[3] and Valdez de Moreno and Barbieri[4]. Bernstein - in a short paper - summarized the influence of some metabolic poisons as well as the motility of *Rana pipiens* and *R. clamitans* sperm. Valdez de Moreno and Barbieri have estimated the oxygen uptake of *Bufo arenarum* spermatozoa under several experimental conditions. Anyway these experiments were carried out with unwashed spermatozoa - i.e. in the presence of testicular fluid - thus a reservation seems pertinent[5].

Accordingly, the present paper intends to determine: a) the Z_{O_2}value (µl $O_2/10^8$ spermatozoa/h)[6]; b) glycolytic activity estimated as the rate of lactic acid synthesis under anaerobic conditions; and c) the influence of anoxia on sperm motility.

Material and methods. Cells were obtained by macerating adult *B. arenarum* testes in a glass homogenizer handled up and down (3 strokes) in the presence of Ringer solution, where they remained immotile. The suspensions were then filtered through glass wool in order to remove large cell debris and centrifuged at 250 × g for 5 min. The supernatant containing almost exclusively sperm cells, was shaken in a vortex-mix to disaggregate agglutinated spermatozoa and centrifugated at 1000 × g for 10 min more.

The supernatant obtained was discarded and the precipitate resuspended in 10% Ringer solution at pH 7.4 with 745 IU/ml streptomycin and 100 IU/penicillin added[7]. The solution containing the spermatozoa was once more shaken and filtered through glass wool to remove sperm aggregations and avoid errors in sperm count. Cells were counted by means of a hemocytometer. Final sperm suspensions, which were microscopically examined before and after each experiment, were found to contain 2-3% immature cells.

Motility was estimated visually, between slide and cover glass, under high microscopic magnification to distinguish between spermatozoa that were motile, motile in situ and immotile. Motility is always expressed by percentages. The observations were performed at 30-min intervals. Since spermatozoan motility is affected by temperature[8], all these experiments were carried out at 30 °C.

The anaerobic medium was obtained by gassing nitrogen into the sperm suspension. Nitrogen (99.9%) was further purified by bubbling in through an alkaline pyrogallol-sodium metavanadate-zinc amalgam mixture[9]. The purity of the gas was checked by chemical, biological and bacteriological methods. In this atmosphere ferrous salts did not oxidize to the ferric form; *B. arenarum* eggs failed to gastrulate as compared with control batches kept in open air; and *Clostridium perfingens,* an anaerobic bacterium, exhibited development.

In order to estimate sperm motility under anaerobic conditions, 2 procedures were used. On one hand, one drop of sperm suspension was placed between slide and cover glass, and the borders were sealed with liquid vaseline in order to avoid air contact. On the other, a modification of Sergin's technique for the estimation of spermatic reductasimetry was used[10]. Nitrogen was bubbled through a sperm suspension for 10 min and capillary tubes of uniform diameter were loaded with this suspension. The ends were sealed, first with molding clay, and then with paraffin wax, and the tubes were kept floating in a bath at 30 °C. Microscopic observations were performed at 30-min intervals. Sperm motility was determined and expressed in the same way as described for aerobic spermatozoa.

Oxygen uptake was estimated by means of a Gilson polarograph with Clark electrode; 1.6 ml of the sperm suspension was placed in the cell compartment and the rate of oxygen uptake was measured for 20 min at 30 °C.

Lactic acid content was determined according to the technique proposed by Loomis[11] and modified by Peterson and Freund for human spermatozoa[12].

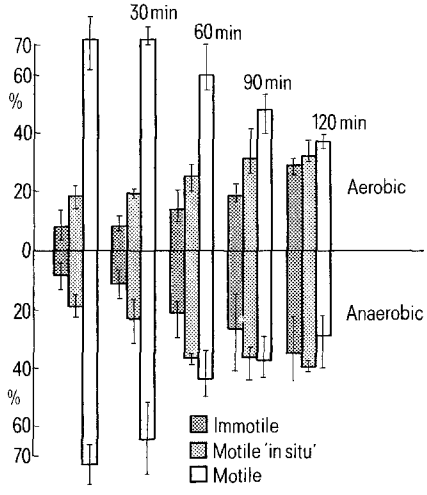

Table 1. Lactic acid production

Experiment	Cells/ml (× 10^8)	Time of anoxia (min)	Lactic acid
1	0.80	120	0.44
2	0.20	120	0.32
3	0.50	120	0.39

Lactic acid production was determined as described in the text, at 30 °C. The lactic acid formed is expressed in µmoles/10^8 sperm/h.

Table 2. Oxygen uptake in isolated amphibian spermatozoa

Experiment	Cells/ml (× 10^8)	Z_{O_2} (µl $O_2/10^8$ sperm/h) (average of duplicates)
1	0.64	4.2
2	1.20	2.6
3	1.20	3.0

Oxygen consumption of *Bufo arenarum* spermatozoa was followed in Gilson oxygraph as described in the text, at 30 °C.

Duplicate tubes contain 0.1 ml of sperm suspension were placed in boiling water for 1 min to stop their metabolism. 0.5 ml of buffer (glycylglycine-semicarbazide 0.1 M, pH 10) containing 0.05 mg NAD and 0.10 mg lactic dehydrogenase (from rabbit muscle) was added to the solution. Then it was incubated at 37 °C for 90 min. Finally, 4.4 ml of NaOH (with EDTA) was added. The fluorescence of the sample was read in an Aminco Bowman Spectrophotometer with excitatory light at 340 nm and set to pass emitted light at 460 nm. In all cases, parallel samples containing pure lactic acid were read.

Results and discussion. The histogram shows the motility of both control and anaerobic cells. Irrespective of the method employed, concordant results were obtained from a total of 13 experiments. The histogram shows the results of 8 experiments using capillary tube method. No differences between the 2 batches were observed when the data mentioned above were studied comparatively.

The production of small, but measurable amounts of lactic acid by anaerobic spermatozoa, demonstrated the existence of an active glycolytic pathway (table 1).

The anaerobic motility of washed spermatozoa and the presence of lactic acid in anoxia would demonstrate that the energy for motility in anaerobiosis, depends neither upon anaerobic oxidative processes nor upon the utilization of extracellular glycolyzable substrates, but probably upon the anaerobic utilization of endogenous substrates, such as glycogen. Anderson and Personne[13] located this polysaccharide in the residual cytoplasm of amphibian spermatozoa and, more recently, the presence of glycogen in the nucleus acrosome and middle piece of *R. clamitants* was cytochemically and morphologically demonstrated by Poirier[14].

In aerobiosis, the oxygen consumption varies between 2.6 and 4.2 µl $O_2/10^8$ cells/h at 30 °C (table 2). From results obtained in current experiments, it could be observed that the Z_{O_2} of *B. arenarum* spermatozoa are substantially changed when cells are incubated with substrates from Krebs' cycle such as succinate, or metabolites which alter oxygen consumption e.g. rotenone, dinitrophenol, oligomycin, antimycin, etc.[5]. These values were found to be below those reported for other species. Furthermore, a Z_{O_2} of 6.0 has been reported for *Echinus esculentus* at 15 °C, while for bull, cock, rabbit and ram Z_{O_2} values of 21, 7, 11 and 22, respectively, have been reported. Spermatozoa removed from *Loligo pealli* spermatophores exhibited a Z_{O_2} of 10 at 20–25 °C. The oxygen consumption of *B. arenarum* is higher than *Balanus balanus* spermatozoa, which was reported as only 0.18 µl $O_2/10^8$ cells/h at 10 °C[16].

According to the results of this first approach to the study of *B. arenarum* spermatozoa, it can be concluded that there are, in the cell, operative oxidative-glycolytic pathways which utilize endogenous substrate. Peterson and Freund[17] have demonstrated that the oxidative metabolism in human spermatozoa is less effective than glycolysis in maintaining ATP cellular levels or in supporting sperm motility. In the absence of glucose, ATP level declines concomitantly with a motility loss, while in the presence of glucose, ATP level remains constant for several hours and the decline in motility is low. These cellular levels of ATP did not maintain motility either when incubated with succinate, which is utilized at high rates, or with pyruvate, which is utilized at lower rates.

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Comparison of sterols from a phytophagous and predacious species of the family Coccinellidae

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Summary. The sterols of a phytophagous and a predacious species of the family Coccinellidae were found to be quite different from each other, indicating that adaptation to different diet regimens is reflected in the utilization and metabolism of dietary sterols.

The Mexican bean beetle, *Epilachna varivestis* Mulsant, which is one of a limited number of plant feeding insects in the otherwise predominantly predacious family Coccinellidae, was recently found to have unique pathways and final products of sterol utilization and metabolism[2,3]. This insect reduces most of its dietary C_{28}- and C_{29}-Δ^5-plant sterols to stanols, which are then dealkylated to the C_{27}-stanol cholestanol. Significant quantities of Δ^7-cholestenol (lathosterol) are then formed, and little, if any, of the plant sterol is metabolized to cholesterol. Saturated sterols (stanols) comprise 50–75% of the total sterols of this insect, but Δ^5-sterols, including cholesterol, are only minor sterol components in all stages of the Mexican bean beetle. To determine whether this unique pattern of sterol utilization and metabolism is characteristic of coccinellids in general or is peculiar to this particular phytophagous species, we compared the sterols of the Mexican bean beetle with those of a predacious coccinellid *Coccinella septempunctata* (L.), that feeds on phytophagous insects (aphids).

Mexican bean beetle adults were obtained from larvae reared as previously described[2] in 1-gal glass jars on bouquets of leaves of Clark variety soybean, *Glycine max*