shapes of the mitochondria are oval or illipsoid in contour. Some are irregular forms with focal expansion and constrictions. Elongated forms sometimes display a terminal illipsoid (Figure 1 and 3). Branching mitochondria are also seen (Figure 2). Occasionally, a portion of the mitochondria forms rings. Giant mitochondria are sometimes increased to a few hundred times in volume compared with the normal mitochondria (Figure 6).

The most striking feature of the mitochondria from tumor cells is the alteration of cristae. Some of the cristae are in parallel fashion (Figure 4) while others are in concentrically paired membranes (Figure 5). A few even show a mixed irregular bizzare arrangement (Figures 6 and 7). Dense bodies are occasionally observed within the mitochondria (Figure 8).

Mitochondria, as the 'power house of the cell', are actively involved in cellular oxidation processes. The transport of molecules across the membranes of mitochondria and their localization within particular organelles are a complicated process requiring specificity of an exceedingly high order. Because mitochondria are extremely sensitive indicators of the state of health of cells, morphological modifications may be as results of pathological conditions. At the present time, we still do not understand the mechanism of mitochondrial alteration in the tumor cells; however, stress of any kind may be expected to produce notable structural changes[8]. Pleormorphism is also a feature of this organelle even within the same cell.

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Anaerobic Fertilization of Amphibian (*Bufo arenarum*) Eggs

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Summary. Mature *Bufo arenarum* eggs, as well as body cavity oocytes of the same species, have been found to be fertilizable under anaerobic conditions. Anaerobic fertilization was obtained in a medium in which oxygen was replaced by purified nitrogen and in the presence of antimycin in concentrations assuring the complete blockage of respiration.

It has been repeatedly pointed out that, under anaerobic conditions, amphibian eggs can develop and show an intense glycolytic activity[2–7]. On the other hand, recent work has shown that oocytes of the amphibian *Bufo arenarum* show intense glycolytic activity during development to mature eggs (LEGNAME, personal communication). Similar observations have been made by FITCH and MERRICH[8] working with *Rana pipiens* eggs. As regards spermatozoa, ENGELMAN[9] reported as early as 1868 that amphibian spermatozoa are motile in the absence of oxygen. Our laboratory has been interested in the relation of metabolism to motility in spermatozoa from the species *Bufo arenarum*. These cells remain viable for considerable periods in the absence of air (DEL RÍO, unpublished). Since the viability of gametes was maintained under anaerobic conditions, it was of further interest to determine whether fertilization could also occur under these conditions.

Material and methods. *Bufo arenarum* oocytes were obtained from females stimulated to ovulate by injection of a suspension of homologous hypophysis and maintained in 10% Ringer solution at pH 7.4 and room temperature. Body cavity eggs were obtained in the same manner, except that the animal was sacrificed 8 h after the injection. In this case, the eggs remained in the body cavity and did not enter the oviducts. These eggs, which do not contain the gelatinous coats ordinarily present on the eggs that have passed into the oviduct, were kept in 5 m*M* Ringer *Tris* buffer at pH 7.4 until the expulsion of the first polar body was observed. The eggs were incubated for 30 min in an oviduct extract (pars recta) according to the method of RAISMAN (personal communication).

Spermatozoa were obtained by macerating adult *Bufo arenarum* testes and were suspended in 2–3 ml of 10% Ringer solution at pH 7.4. Anaerobiosis was achieved by a flow of nitrogen purified by passage through pyrogallol, vanadous sulfate-amalgamated zinc[10] or by incubating

Table I. Effect of anaerobiosis on fertilization of mature eggs

	Fertilization (%)	
	Anaerobic	Control
1	82 (400)	94 (360)
2	100 (400)	100 (400)
3	47 (400)	100 (400)
4	78 (300)	92 (300)
5	68 (230)	100 (230)
6	100 (270)	100 (270)
Mean ± SE	79 ± 8.4	97 ± 1.5

The number in parentheses indicate the number of eggs used in each experiment. The sperm concentration was 10^8 sperm/ml. Each experiment was performed on eggs and sperm obtained from different animals.

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the gametes in the presence of antimycin, a potent inhibitor of mitochondrial electron transport and respiration. The effect of these treatments on gamete respiration was determined using Warburg manometry to estimate oxygen uptake by eggs[11] and a Teflon covered Clark electrode (Yellow Spring Instrument Co) to estimate sperm respiration[12]. Fertilization was allowed to take place in a Warburg flask at 25 °C. Eggs were placed in the main compartment in 0.5–1.0 ml of 10% Ringer solution at pH 7.4. The side arm contained 0.7 ml of the sperm suspension.

As anaerobic experiments, the flasks were flushed with nitrogen for 10 min. The contents of the flasks were maintained under nitrogen for an additional 10 min before the spermatozoa in the side arm were tipped into the main compartment containing the eggs. Control experiments carried out under aerobic conditions were made simultaneously. At the appearance of the first embryo cleavage stage in the control flask, the contents of all flasks were removed and the number of fertilized ova counted.

Table II. Anaerobic fertilization of amphibian body-cavity eggs

	Fertilization (%)	
	Anaerobic	Control
1	56 (134)	48 (100)
2	100 (21)	100 (24)
3	75 (25)	98 (54)
4	68 (30)	89 (25)
5	63 (28)	91 (28)
Mean ± SE	72 ± 7.6	85 ± 9.6

Conditions were same to those indicated in Table I.

Table III. Effect of antimycin on the fertilization of mature eggs

	Fertilization (%)	
	Antimycin	Control
1	34 (32)	85 (59)
2	29 (68)	97 (64)
3	31 (54)	98 (60)
4	33 (62)	100 (70)
5	30 (54)	92 (68)
Mean ± SE	31 ± 0.9	94 ± 2.7

Concentration of spermatozoa was 10^8 sperm/ml. Concentration of antimycin was 1.0 µg/ml. The number in parentheses indicate the number of eggs used in each experiment. Each experiment was performed on eggs and sperm obtained from different animals.

Results and discussion. As is clear from the data in Table I, oocytes from the species *Bufo arenarum* are fertilized under anaerobic conditions. The fertilization of eggs taken from the body cavity (Table II) eliminates the possibility that the gelatinous coat found on eggs taken from the ovisac prevent anoxia when a nitrogen purge alone is used. Fertilization of eggs in the presence of antimycin in concentration that completely stops respirations (Table III), eliminates the possibility that technical errors were introduced in experiments using nitrogen only.

This is the first vertebrate species in which fertilization under anaerobic conditions has been observed. However, fertilization under these conditions has been observed in invertebrates and bacteria. GUZMAN BARRON[13] reported that the germinal cells of *Nereis* were unaffected by the lack of oxygen and were able to carry out fertilization 5 h after exposure to an oxygen-free environment; and more recently, STALLIUS and CURTIS[14] showed that chromosome transfer in *E. coli* occurs at high frequency under anaerobic conditions.

The lower rate of fertilization of eggs in the presence of antimycin, compared to the experiments in which nitrogen was used, suggested that the antibiotic may have other effect on fertilization not related to an effect on mitochondrial electron transport and respiration.

PETERSON and FREUND[15] have shown that high levels of ATP are maintained in human spermatozoa even in the absence of air. A similar situation appears to exist in amphibian gametes which generate sufficient energy anaerobically not only to maintain viability, but also to carry out fertilization.

Our studies showed no difference in the cleavage rate or any other anatomical character, which would indicate morphologic abnormality when fertilization in this species is carried out anaerobically.

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Specialized Membrane Junctions in the Avian Cerebellum[1]

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Summary. Pentalaminar specialized membrane junctions – tight junctions – are described in the granular layer of the pigeon cerebellum. The presence of these axo-dendritique and dendrosomatic contacts suggest the existence of electrotonic coupling in the pigeon cerebellum.

Specialized junctional zones between nerve cell processes, where membranes are in close apposition, have been described in the nervous system of many animal species[3]. BENNETT, PAPPAS et al.[4] demonstrated that these low resistant junctions are the morphological counterpart of electrotonic coupling, a phenomenon which now appears to be quite frequent in invertebrates as well as vertebrates. These intercellular contacts were

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