Table II. Percentage composition of PC and PE fatty acids of the embryonic chick lung

Age in days of incubation	⩽ 14	16:0	17:0	18:0	18:1	18:2	20:4	Total saturated	Total insaturated
Phosphatidylcholine (PC)									
14 – ♀	—	49.6	—	15.8	21.1	4.8	—	65.4	25.9
♂	—	39.5	—	19.6	30.2	4.1	—	59.1	34.3
16 – ♀	—	56.4	—	19.3	17.0	2.4	—	75.7	19.4
♂	—	42.9	—	28.7	20.8	4.6	—	71.6	25.4
18 – ♀	—	50.6	—	23.1	20.9	0.6	—	73.7	21.5
♂	—	51.0	—	25.3	17.9	2.3	—	76.3	20.2
20 – ♀	—	62.5	—	17.8	15.9	1.04	—	80.3	16.9
♂	—	54.7	—	21.5	19.5	2.0	—	76.2	21.5
Phosphatidylethanolamine (PE)									
14 – ♀	4.9	25.4	2.0	33.0	19.9	1.1	13.3	65.3	34.3
♂	7.6	23.3	1.4	30.1	17.4	5.1	10.6	62.4	33.1
16 – ♀	1.8	19.1	4.6	29.7	20.5	5.0	17.7	54.2	43.2
♂	3.3	16.5	4.1	31.4	23.8	6.7	6.8	55.3	37.3
18 – ♀	3.4	12.7	5.0	30.6	19.7	6.8	17.6	51.7	41.1
♂	2.0	11.9	5.8	27.2	18.6	8.4	18.7	46.9	45.7
20 – ♀	3.8	20.2	6.1	33.2	21.0	3.6	8.9	63.3	33.5
♂	4.2	13.3	7.6	32.5	21.8	6.1	12.7	57.6	40.6

Each value is a mean of results obtained on 3 separate extracts.

in males and females, and does not change in the course of development.

In conclusion, these results allow us to extend to the chick some of the data concerning mammalian foetal lung; first, as in mammals, the total phospholipid level, and, more precisely the PC and PE levels, rise significantly in the course of development[21]; then, the rapid increase in the total and individual phospholipids amounts observed between days 15 and 16, coincides with the time when granular pneumocytes start to differentiate. Finally, as has been described for rabbit and monkey[22, 23], the percentage of saturated fatty acids of lecithin increases during maturation.

Our results show also, and this for the first time, a distinct sexual dimorphism in the evolution of the lecithin level. This dimorphism, reflected in the evolution of the phospholipid content, is no longer perceptible in the level of total lipids, where it is probably masked by the presence of cholesterol, triglycerides, free fatty acids, etc, which we did not determine. At the present time, one can only speculate about the factors which cause this earlier differentiation of lungs in females, already observed in man by NAYE et al.[16] on a morphological basis. Since it is widely accepted that pulmonary maturation is influenced by corticosteroids[5–9], it would be of great interest to check whether this phenomenon can be related to a sex-linked difference in the onset of adrenocortical activity.

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Abnormal Mitochondria in Retinoblastoma

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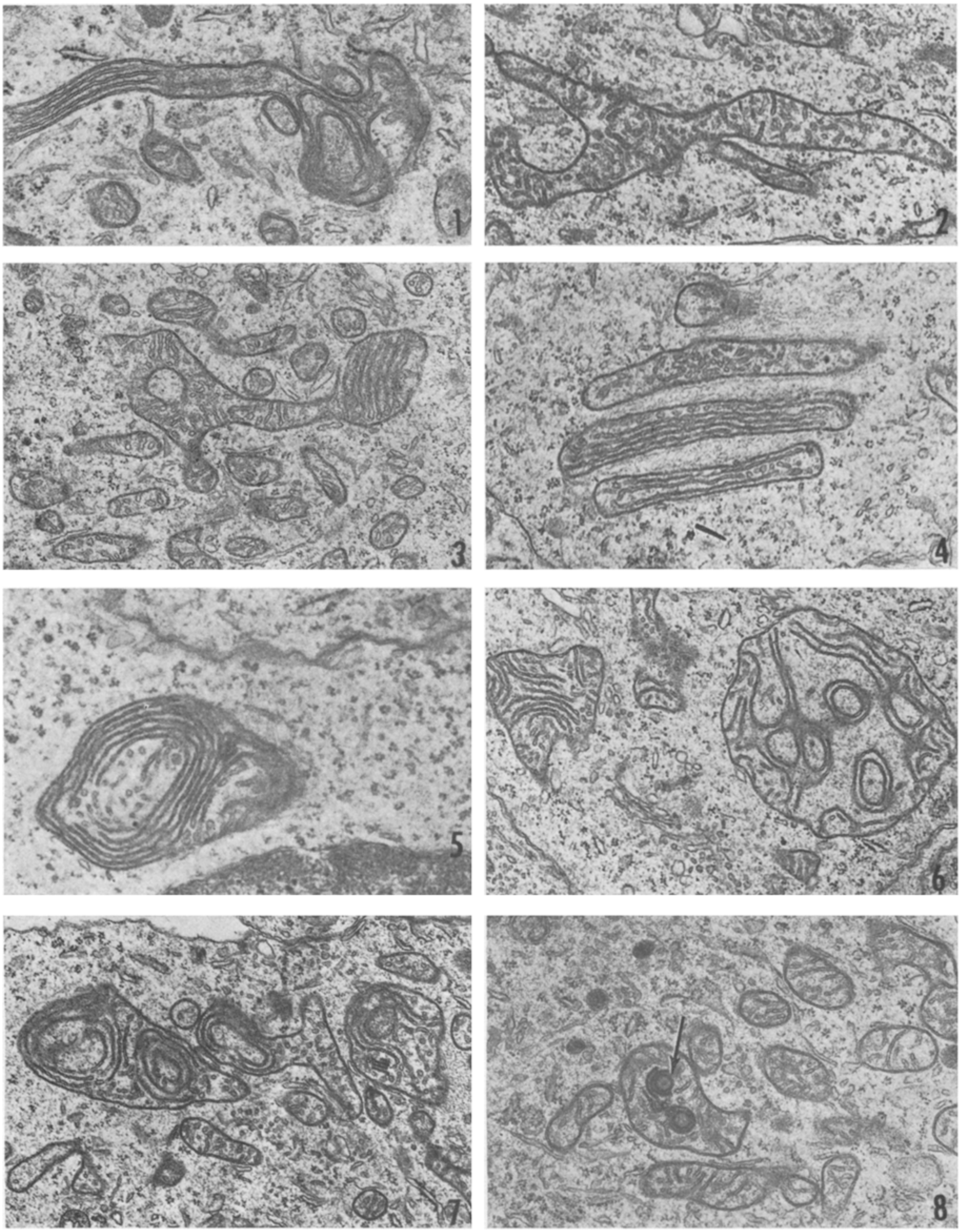
Summary. In examination of six retinoblastoma tumor specimens, bizzare mitochondria were often found. Some are irregular forms with focal expansion and constrictions. Occasionally, a portion of the mitochondria forms rings. Branching mitochondria are also seen. Other striking features of the mitochondria from tumor cells are the alteration of cristae. Dense bodies are also occasionally observed within the mitochondria. Morphological modifications of the mitochondria may be as results of pathological conditions of the tumor cells.

Although alterations of mitochondria have been observed in tumor cells such as: renal cell carcinoma[1, 2], oncocytoma[3, 4], and Warthin's tumor[5, 6], changes of mitochondria in the tumor cells of retinoblastoma have not previously been reported[7].

In examination of 6 retinoblastoma tumor specimens, bizzare mitochondria were often found. The tumor tissues were taken from a family of 3 children, 2 of which were fraternal twins. The ages of the children were from 12 to 21 months. Pieces of tumor tissue about 1 mm^3 were immediately placed in White's saline (pH 7) which contained 1% osmium tetroxide or Dalton's chrome-osmic fixative (pH 7.6) for 1 h. The sections were stained with uranyl acetate and lead citrate.

Mitochondria within the tumor cells varied considerably in size, ranging from 0.3 to 4 µm in diameter. The



Figures 1-8. Mitochondria from tumor cells of retinoblastoma are in different forms. Figs. 1, 2, 3, 5 and 8: ×20,000. Figs 4, 6 and 7: ×17,000.

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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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