Some Properties of Crystalline Inclusion Bodies in Oocytes of *Rana temporaria* and *Rana esculenta*

Ranidae are the only amphibia known to possess crystalline intramitochondrial inclusion bodies. By most authors these inclusion bodies are referred to as 'intramitochondrial yolk'[1,2]. Since intramitochondrial inclusion bodies have so far not been investigated biochemically, practically nothing is known about their chemical composition. Their crystalline lattice seems to be the only basis for comparison with the common amphibian yolk-platelet (vesicular yolk VY), which is composed of lipovitellin and phosvitin[3].

As is reported in a separate paper[4], both *Rana esculenta* and *Rana temporaria* mitochondria were found to possess two types of crystalline inclusions, one being located in the mitochondrial matrix (mitochondrial matrix yolk MMY) (Figure 1), the other in the cristae (intracristal yolk ICY) (Figure 2). As would have been expected, their crystalline lattice does not show an identical center to center spacing of the crystal-cylinders. MMY has a spacing of 165 Å as compared to 85 Å of ICY. In these two species (*Rana esculenta* and *Rana temporaria*) the common yolk-platelet (VY) has a spacing of 95 Å (Figure 3).

Since some authors[5,6] report an identical crystalline lattice for the vesicular yolk and the intramitochondrial yolk in *Rana pipiens*, differences as found in *Rana esculenta* and *Rana temporaria* are of great interest, because they indicate differences in composition as well. Microanalysis as carried out with the AEI Emma-4 on the 3 inclusion bodies revealed in fact a different composition.

Of all the elements expected to be present in the inclusion bodies, phosphorus was the easiest to detect with microanalysis. The figures in Table I show that the phosphorus measurements obtained from the 3 crystals differ considerably.

The peak background ratio of almost one in MMY is not due to a total absence of phosphorus but rather to the limited sensitivity of the Emma-4. For these measurements glutaraldehyde fixed, 2000 Å thick unstained sections were used. The spot size of the probe was approximately 1000 Å.

Table I. Counts per minute for phosphorus in the 3 different crystalline inclusion bodies

	Peak (cpm)	Background (cpm)	Peak/background (ratio)
VY	300	31	10
ICY	140	20	7
MMY	18	15	1,2

VY, vesicular yolk, ICY, intracristal yolk, MMY, mitochondrial matrix yolk.

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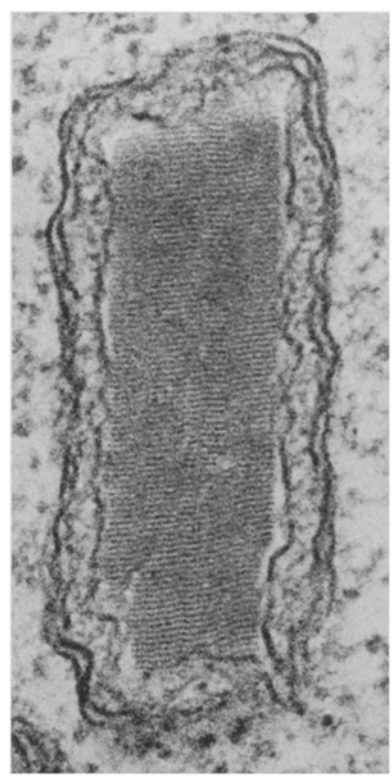

Fig. 1. Mitochondrial matrix yolk crystal (MMY). Center to center spacing of the crystalline lattice approx. 165 Å. ×75,000.
Fig. 2. Intracristal yolk crystal (ICY). Center to center spacing of the crystalline lattice approx. 85 Å. ×75,000.
Fig. 3. Vesicular yolk crystal (VY). Center to center spacing of the crystalline lattice approx. 95 Å. ×75,000.

Enzymatic digestion on ultrathin sections[7] further corroborated the results obtained through microanalysis.

After oxidation on periodic acid or H_2O_2 the sections were digested either on 0,5% pronase in distilled water which had been adjusted with NaOH to pH 7.4 or on 0.5% pepsin in 0, 1 *N* HCl. (Table II). When ultrathin sections were floated for 2 h on xylene practically all lipid was extracted, but the 3 crystalline inclusion bodies were left totally unaffected.

Table II. Digestion of the 3 crystalline bodies on pronase and on pepsin.

Time (h)	Pronase MMY	ICY	VY
1.5	0	0	++
3.0	0	0	+++
4.0	+	+	−−−
6.0	++	++	−−−
Time (h)	**Pepsin MMY**	**ICY**	**VY**
1.5	0	0	+
3.0	0	0	++
4.5	0	0	+++
6.0	+	+	−−−

0, no effect; +, slight effect; ++, clearly visible effect; +++, most material digested; −−−, all material digested, leaving holes in sections.

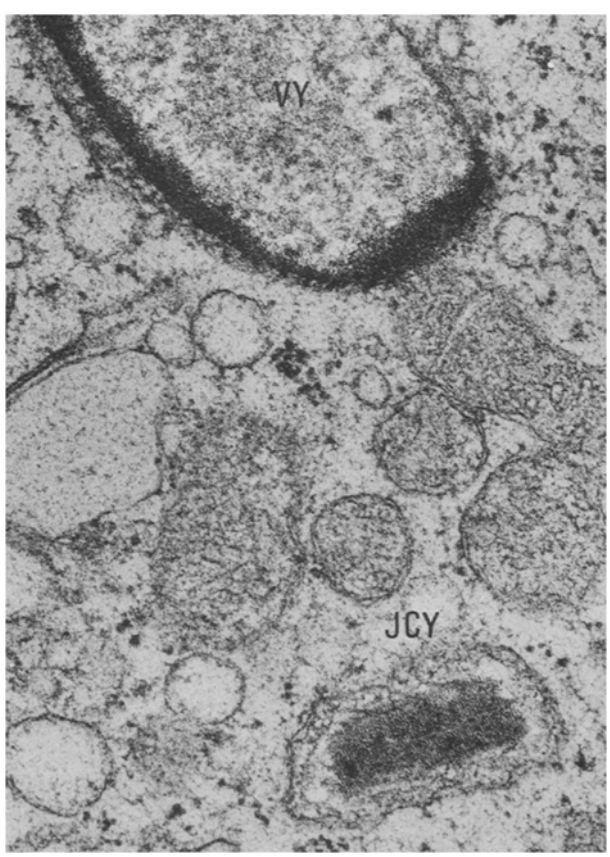

Fig. 4. After 3 h digestion on pronase the lattice of the vesicular yolk platelet (VY) is digested, while the lattice of the intracristal yolk (ICY) appears to be unaffected. ×44,000.

On the basis of the above findings it appears very probable that the intramitochondrial crystalline inclusion bodies (ICIB) are of a proteinaceous nature: They are not extracted through xylene-treatment, pepsin and pronase digestion attacks them to a certain extent.

Most authors, dealing with ICIB, take it as a fact that they have exactly the same composition as the vesicular yolk-platelets. This may perhaps be the case for some species. For *Rana temporaria* and *Rana esculenta*, however, this does not hold true. A number of facts speak against an identical composition. 1. Their crystalline lattice, even under the same fixing conditions and in the same oocyte is not identical with the lattice of the vesicular yolk. 2. Their phosphorus content is much lower. 3. Although MMY and ICY are attacked to a certain extent by enzymatic digestion, digestion takes place at a much slower rate than is the case in vesicular yolk, which is especially surprising in the case of pronase digestion (Figure 4), since pronase is one of the most unspecific proteolytic enzymes[8]. 4. MMY and ICY are much less electron dense than VY and can hardly be detected on unstained sections, MMY showing even less contrast than ICY.

Taking these four points into consideration, a different composition of the intramitochondrial inclusion bodies appears to be very likely. This would be of great interest particularly in view of a possible intraoocytic mechanism for the synthesis of ICIBs.

Although a means of transport for macromolecules across the mitochondrial membranes has not been reported so far, an intramitochondrial synthesis of the inclusion bodies was not believed to be very probable. The coding capacity of the mitochondrial DNA was considered to be too small to account for the synthesis of proteins with a relative high molecular weight, like lipovitellin (420,000) and phosvitin (40,000).

Phosvitin, containing about 8,4% phosphorus, represents most of the phosphorus of vesicular yolk-platelets. The high contrast of vesicular yolk in unstained sections is due to the high natural density of phosvitin. If the intramitochondrial yolk is at all composed of phosvitin and lipovitellin, than a varied composition with less phosvitin will have to be expected, especially since MMY and ICY are less electron dense and have a lower phosphorus content. Biochemical investigations on the nature of ICIB will be awaited with interest.

Zusammenfassung. Die drei in Oocyten von *Rana temporaria* und *Rana esculenta* vorkommenden, kristallinen Einschlusskörper wurden mit Elektronenstrahl-Röntgen-Mikroanalyse und enzymatischer Verdauung am Dünnschnitt untersucht. Die hierbei erhaltenen Ergebnisse lassen auf eine unterschiedliche chemische Zusammensetzung der bis jetzt als Dotterplättchen bezeichneten Strukturen schliessen, was im Gegensatz zu bisherigen Annahmen steht.

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