muscle function in this disease. SZENTIVANYI[15] has thus suggested that in bronchial asthma there is a reduction of the sensitivity of β-adrenoceptors. It has also been demonstrated that leukocytes from patients with bronchial asthma have a decreased cyclic AMP response to β-adrenoceptor stimulators[16]. It is evident that most of the therapeutic agents used in bronchial asthma act via the cyclic AMP system. This applies to catecholamines[6], ACTH[17], as well as hydrocortisone which increase the cyclic AMP level in bronchial smooth muscle and decrease broncho-constriction.

Effect of hydrocortisone (6×10^{-5} *M*) on cyclic AMP content in bovine tracheal smooth muscle

Addition		Cyclic AMP (pmol/mg)
None (control)		0.85 ± 0.07
Hydrocortisone	1 min	1.02 ± 0.11 [a]
	5 min	0.97 ± 0.10 [a]
	10 min	1.01 ± 0.11 [a]

Each value represents the mean ± SEM of assays on 6 tissue samples.
[a] Differed from the control ($p < 0.05$).

Zusammenfassung. Untersuchung des Wirkungsmechanismus von Hydrocortison auf die Relaxation der Trachealmuskulatur von Kuh und Meerschweinchen, wobei stets eine Zunahme des zyklischen AMP-Gehalts gefunden wurde. Es wird angenommen, dass sich dadurch die intrazelluläre Sequestrierung von Calcium erhöht und der Hydrocortisoneffekt zum Teil von einer β-Rezeptorstimulation abhängig ist.

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Paracrystalline Inclusions in Mitochondria of Frog Oocytes

The main feature of amphibian oogenesis is certainly the formation of yolk platelets. In various amphibians, e.g. *Triturus viridescens, Rana temporaria, Rana esculenta* and *Triturus vulgaris*, the yolk platelet precursors are represented by multivesicular bodies which are as a rule formed through coalescense of pinocytotic vesicles[1–3]. Although it could be clearly demonstrated that the majority of vesicles incorporated into the yolk precursors is generally derived through pinocytotic activities, for most cases a participation of intraoocytic membrane systems in the formation of yolk could not be ruled out conclusively. Most ultrastructural investigations on amphibian oogenesis have been able clearly to demonstrate two or more different ways for the formation of yolk. In the case of *Xenopus laevis*, for instance, we too were able to demonstrate a dual mode of yolk formation[4]. One mode is represented by the transformation of mitochondria which comprises the disintegration of intramitochondrial membranes, the uptake of a large number of vesicles from various sources, and the formation of a paracrystalline lattice, resulting in yolk platelets whose mitochondrial origin is no longer depictable. In contrast to the formation of a paracrystalline lattice subsequent to the transformation of mitochondria, it is the direct intramitochondrial formation of paracrystalline lattices which is the subject of our present study carried out on a total of 10 different anuran species. (*Rana erythrea, R. graeca, R. adspersa, R. cyanophlictis, R. esculenta, R. temporaria, Rhacophorus maculatus, Hyla arborea, Bufo bufo, Bombina bombina*).

Intramitochondrial paracrystalline inclusion bodies (ICIB) were first described in 1958 by LANZAVECCHIA and LE COULTRE[5] in a study of embryogenesis in *Rana esculenta*. During the course of our investigation we were able to detect ICIB in 6 of the 10 species used. With respect to both the intramitochondrial localization and the centre to centre distance of the crystalline lattice, we were able to distinguish 2 different types of ICIBs. The main type of ICIB, occurring in all 6 species, is either formed in the intracristal or intermembrane space and has a centre to centre distance of the crystalline lattice which varies between 85 and 100 Å. In 5 of the 6 species mentioned, we were only able to observe the formation of ICIB's in the intracristal space. In *Rana erythrea* oocytes, the formation of ICIBs in the intermembrane space could, however, frequently be detected. On the basis of their morphology, that is the centre to centre spacing of the crystalline lattice and their size and shape, we were not able to detect any differences between the ICIBs that were formed in the intracristal space and those that were formed in the intermembrane space. Great differences were, however, found with respect to the largest crystals present in the different species. The size ranged between 0.2 μm × 0.5 μm in *Rana adspersa* and 1.5 μm × 5.0 μm in *Rana erythrea*. Although a large variety of different shapes of the ICIBs could be found even within the same oocyte, we were able to observe, in each of the 6 crystal-forming species, a crystal shape which appeared to be typical for the individual species, as can be seen in the Figure. Thus we found in *Rana adspersa* a large number of round to oval inclusions, in *Rana graeca* rectangular, in *Rana esculenta* and *temporaria* hexagonal, and in *Rana erythrea* most of the crystals were polymorphous. Quite outstanding were the crystals found in the intracristal space of *Rhacophorus maculatus*, and sometimes in *Rana esculenta* and *temporaria*, because of their rod-like shape. In *Rana temporaria* and *Rana esculenta*, as well as in *Rhacophorus maculatus*,

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these filamentous structures run parallel to the long axis of the mitochondria contrasting the cristae of mitochondria not participating in the formation of filamentous inclusions which are generally transversally orientated. Principally the cristae in mitochondria of *Rana graeca* oocytes do not exhibit a preferential orientation. Cristae of mitochondria participating in the formation of ICIBs, however, are always transversally orientated. Another typical feature of the crystal-forming mitochondria of *Rana graeca* appears to be the fact that crystalline bodies can sometimes be found in up to 12 cristae running parallel to each other. Dissolving of the cristal membranes and subsequent growth of a single crystal, as we were able to observe in *Rana erythrea*, or as is the case in *Rana pipiens* as reported by MASSOVER[6], could never be detected. The second type of formation of intramitochondrial crystalline bodies (ICIB) differs from the first one described in as much as it takes place neither in the intracristal nor in the intermembrane space but in the mitochondrial matrix. As reported already in a previous paper, this is the case for *Rana esculenta* and *Rana temporaria*[3]. In the course of the present study we were, however, able to detect that, in early stages of oocyte development, similar crystalline structures are formed in the mitochondrial matrix of *Rhacophorus maculatus* oocytes also. With a centre to centre distance of the crystalline lattice of 160 Å, the matrix crystals differ considerably from the lattice of the intracristal crystals, as well as from the lattice of the yolk platelets. Of great interest appears to be the fact, however, that in *Rhacophorus* matrix crystals can only be found in younger previtellogenic oocytes in which no yolk precursors were detectable. By the time the first multivesicular yolk precursors appeared, the matrix crystals had appearently been dissolved again. The rather temporary nature of the matrix crystals is corroborated by our observations made at the onset of embryogenesis in *Rana temporaria*. In this species the matrix crystals, which prior to ovulation could be found in all oocytes and mature eggs, had disappeared totally in newly cleaved two-cell stages. Another important feature of matrix crystals is the fact that frequently the cristal-membrane could be observed to be incorporated into the crystal lattice. This immediately raises questions as to the nature and composition of the crystalline bodies. As reported

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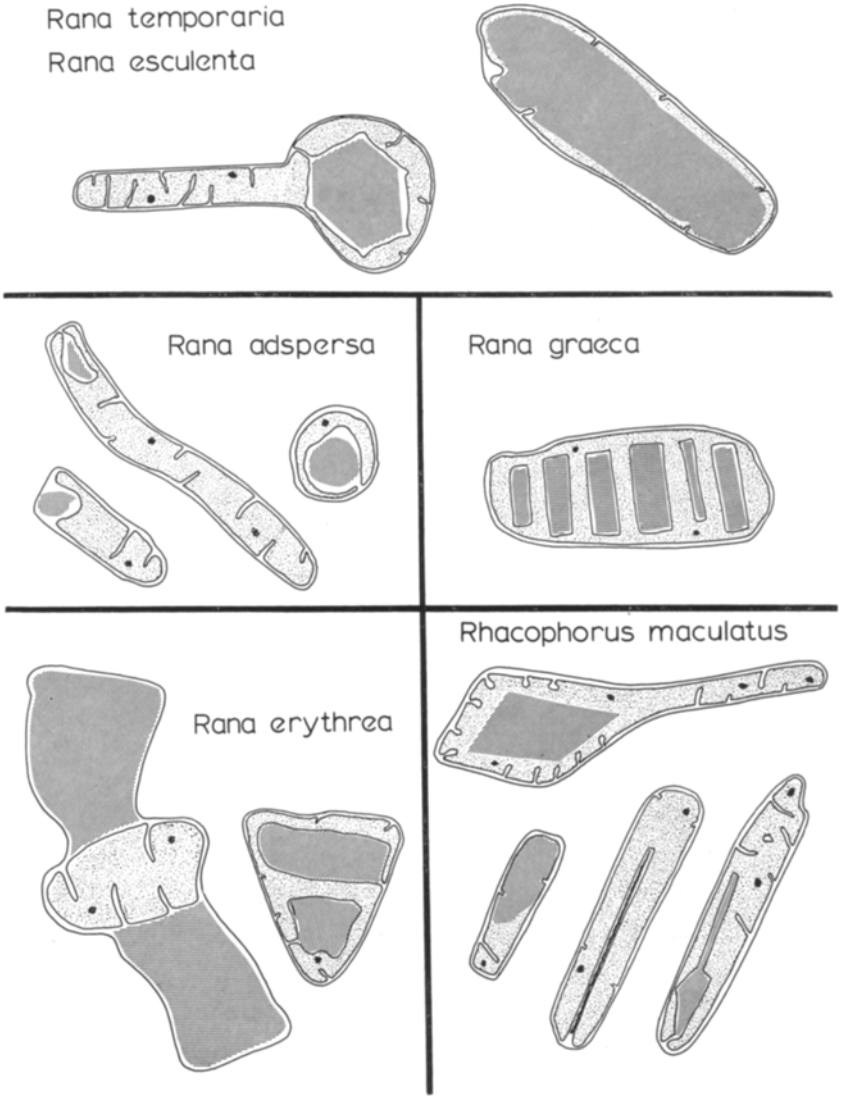

This figure illustrates the different shapes of typical inclusion bodies and their intramitochondrial localization, i.e. in the intracristal or intermembrane space and in the mitochondrial matrix.

in a previous paper, preliminary investigations on the composition of the ICIBs showed that not only to the matrix crystals and the intracristal crystals differ in their composition, but have apparently a composition different from that of the crystals of the yolk platelets[7]. For this reason we have hitherto avoided applying the term yolk to the intramitochondrial crystalline bodies. We soon hope to be able to elucidate their composition, since our present attempts to isolate the ICIBs are quite promising. Until it has been clearly established that their composition corresponds with the amphibian yolk characterized by WALLACE[8], which at the time being appears not very probable, we suggest the terms mitochondrial yolk and yolk inclusions be dropped. Because of the apparently limited coding capacity of the mitochondrial DNA, an intramitochondrial synthesis of ICIB proteins is not very likely, especially not if their molecular weight were of the same order as that of the yolk proteins lipovitellin and phosvitin. It therefore appears very probable that the ICIBs are composed of extramitochondrial material which has been incorporated into the mitochondria. In the light of this consideration, the fact that we were never able to observe any membrane discontinuities or invaginations of the mitochondrial membranes with subsequent uptake of the formed vesicles, seems to be of great interest, especially with respect to the transport activities of the mitochondrial membranes. Occasionally we were able to observe evaginations of the outer mitochondrial membranes which by some authors[9] are interpreted as contacts of the outer mitochondrial membrane with the endoplasmic reticulum. These evaginations were, however, seen so very rarely that it seems unlikely that they could account for the transport of material into crystal-forming mitochondria. With the single exception of *Rana graeca*, mitochondria containing crystalline inclusions are almost exclusively located in the peripheral region of the oocytes: in the same region which naturally has the highest concentration of extraoocytic material. Where there is a similar composition of the mitochondrial inclusions to that of the yolk proteins, their preferential localization in the cortex of the oocytes might be explained by the fact that yolk proteins in amphibians are mostly of an extraoocytic origin. The most important result of our investigation is, however, the fact that the 6 species which form intramitochondrial paracrystalline bodies all belong to the same suborder of Displasiocoela. In a number of other anuran or urodelan species that were used either in this or previous studies, and did not belong to the suborder of Displasiocoela, we were not able to detect any intramitochondrial paracrystalline bodies. For the time being we are not able to say whether or not intramitochondrial crystals are typical for the whole suborder of Displasiocoela, since our studies did not include species of the families Microhylidae or Phrynomeridae.

Despite these considerable morphological differences in the individual species, the formation of intramitochondrial crystals appears to be a specific trait of the family Ranidae. In addition to the 5 species of this family used in this study, there are to our knowledge at least 6 other species of the same family investigated by other authors which form intramitochondrial crystals too. There may be one exception in this respect, namely *Rana cyanophlictis*, because we have not been able so far to observe any ICIBs in oocytes of this species. Further studies appear, however, to be necessary since the only two animals available had strongly atretic ovaries which only contained either young previtellogenic oocytes or mature degenerated oocytes, both of which have never been shown so far to contain ICIBs in any of the other species of the family Ranidae.

The fact that oocytes of *Rhacophorus maculatus* contained intramitochondrial paracrystalline bodies corroborates the hypothesis that the family Rhacophoridae is descended from the family Ranidae[10]. Thus we hope to have demonstrated once more the importance of ultrastructural investigations for taxonomical studies.

Zusammenfassung. Intramitochondriale parakristalline Strukturen wurden in Froschoocyten bei 6 von 10 untersuchten Arten gefunden. Diese kristallinen Inklusionen lassen sich vom Ort der Entstehung innerhalb des Mitochondriums in zwei Arten unterteilen. Die eine Art entsteht im intracristalen oder intermembranalen Raum und hat in der Regel einen Gitterabstand von 85–100 Å. Die zweite Art entsteht in der mitochondrialen Matrix und hat einen Gitterabstand von ca. 160 Å. Die Tatsache, dass, bis auf eine Ausnahme, alle bekannten Arten mit intramitochondrialer Kristallbildung der gleichen Familie angehören, lässt den Schluss zu, dass es sich bei der Kristallbildung um ein spezifisches Merkmal der Familie Ranidae handelt. Die bei *Rhacophorus maculatus* gefundenen parakristallinen Kristalle stützen die Hypothese der Abstammung der Rhacophoriden von den Raniden.

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T-System and Couplings in Frog Myocardial Cells

It has been reported that there are some differences in ultrastructure in the cardiac muscle of cold-blooded animals (fishes, amphibians and reptiles) as compared with that of mammals[1–3].

The lack of transverse tubules (TT) and the absence of couplings in the cold-blooded animals may be related to a different mechanism of excitation-contraction coupling. However, the mechanism of activation of contractile elements is not yet clear in the cardiac muscle cell of cold-blooded species. For this reason we have chosen to report here some recent observations on ventricular cardiac muscle fine structure of Rana temporaria (RT).

Frog ventricle heart muscle was, in the first instance, fixed by perfusion with glutaraldehyde, postfixed with osmium tetroxide and embedded in araldite. In longitudi-

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