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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nal sections (Figure 1a) of the fibres, invaginations of the sarcolemma at the Z-band level extend deeply into the cytoplasma. These invaginations were large (220–240 nm in diameter), though varying in size, and contain basement membrane material, while the wall shows micropinocytotic activity. Succeeding sections show clearly that the invaginations of the sarcolemma can be traced as tubules which run transversely into the interior of the fibre and demonstrate the continuity of the tubular lumen and the extra-cellular space. The shape in sections of the tubules depends on whether they have been cut straight across or down their length. The TT apertures at the level of the successive Z-bands are frequently visible in the same section, so that it is assumed that they are arranged in longitudinal rows. In transverse sections (Figure 1b), the TT were a prominent feature with walls composed of plasmalemma and basement membrane. They mostly lie in relation to the Z-line, starting as invaginations of the sarcolemma and extending deeply into the cytoplasma, often showing ramifications (Figure 1b). The continuity of the walls of these tubules with the sarcolemma could be clearly seen, so that we may assume that the lumen of the tubule, which is smaller at the sarcolemma and widening out inside the cell, communicates with the extracellular space.

Vesicle profiles at the rather extensive sarcoplasmic reticulum (SR) are situated either at some point in close apposition with the wall of the TT, or in close association with the sarcolemma showing the bridgelike structures joining the transverse tubular system (TTS) and SR membranes which are found in triads and dyads of mammalian cardiac muscle. Hence, both peripheral and interior couplings are observed in the ventricle myocardium of RT.

In order to refute any possibility of confusion as to whether or not a continuity is present between the space within the TT and the extracellular environment, some animals were injected i.v. previously to fixation with horseradish peroxidase (HRP) followed by visualization according to the method of GRAHAM and KARNOVSKY[4]. HRP could be seen adhering to the outside of the fibres and in T-tubules between the fibres (Figure 2a and b). Occasionally a small cluster of 2 or 3 tubules were sectioned closely together, possibly representing the branchings of a TT. Furthermore, in longitudinal sections, it can be clearly shown that longitudinal extensions of the T-System occurred between adjacent Z-bands. There is no penetration of HRP into the lumina of the SR.

Consequently, in the ventricular cardiac muscle of the frog, unlike the observations previously reported[1–3], sarcolemmal invaginations at the Z-band level resemble the TT in the mammalian cardiac muscle. Why other investigators were not able to demonstrate the existence of a TTS in the frog heart may be explained by the relative sparseness of these tubules. Moreover, peripheral as well as interior couplings are often present.

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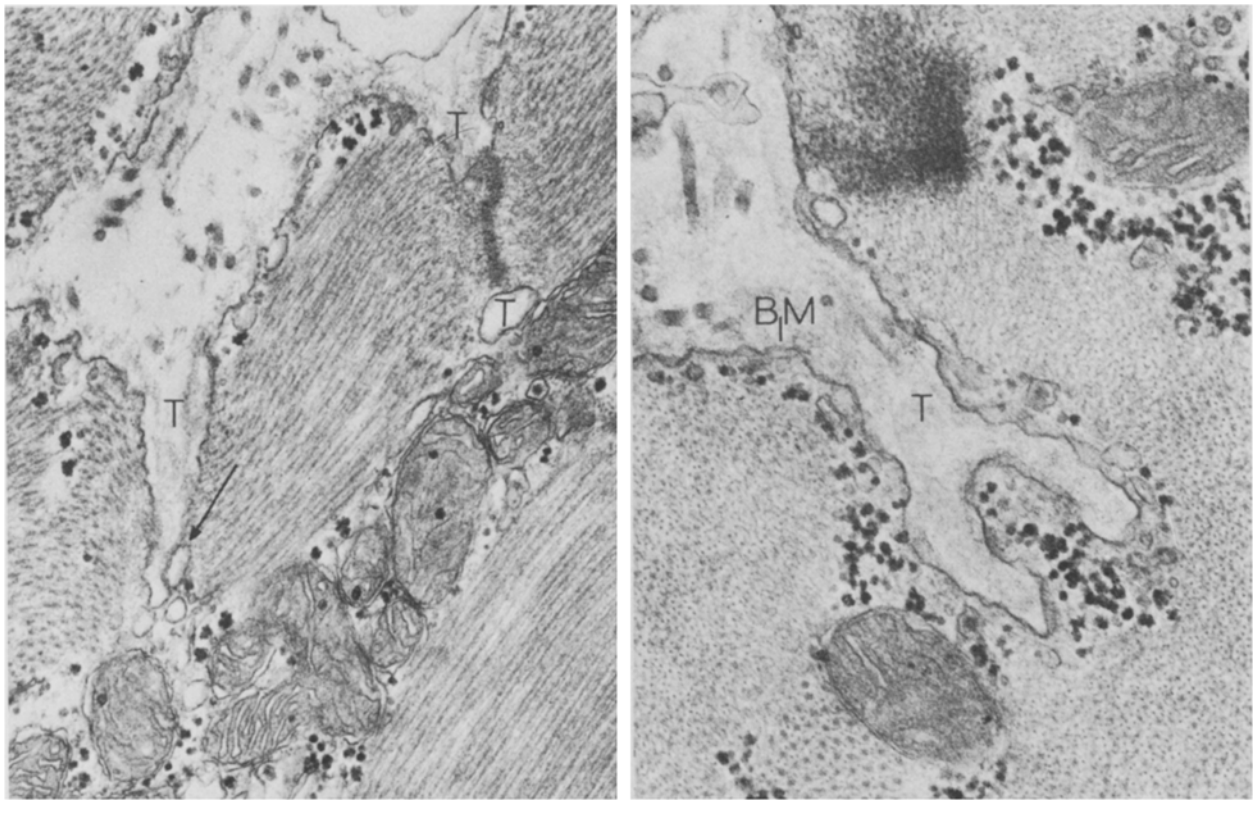

Fig. 1. a) A portion of a longitudinal section of the myocardium of *Rana temporaria*, showing the origin of 2 T-tubules (T) at the level of the Z-line. The left one is cut longitudinally very deeply into the myofibre, while the right one runs out of the plane of sectioning and then deeper into the myofibre where it is cut transversely at the Z-line level. Note the coupling (↓) with junctional processes extending between the opposed membranes of the tubular structures. ×36,000. b) Transverse section of the same muscle showing Z, I and A regions and a T-tubule, containing basement membrane material (BM), which deeply ramifies. ×44,000.

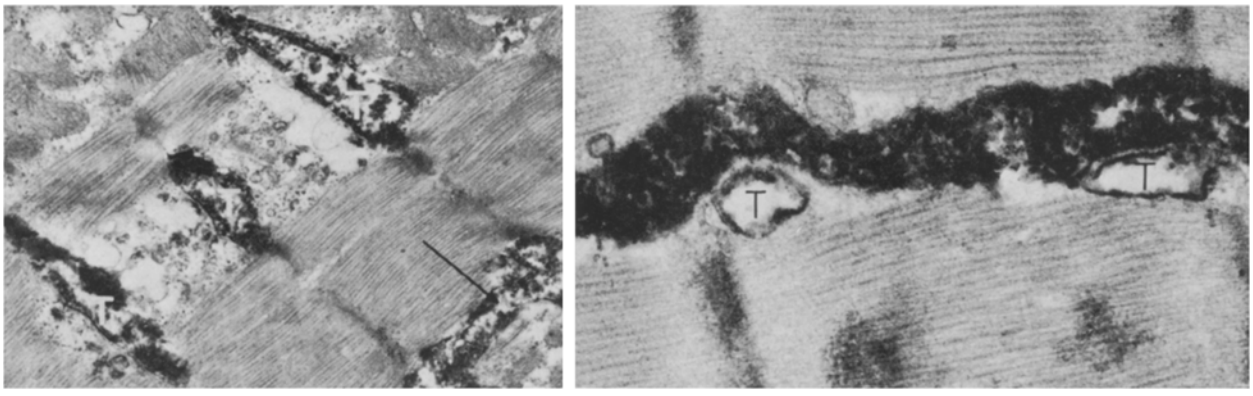

Fig. 2. a) and b) HRP-treated frog myofibres. Product of the enzymatic reaction is seen in the extracellular space (↑) and in the T-tubules (T), which run perpendicularly to the long axis of the myofibres at the level of successive Z-lines. In a) the T-tubules are cut longitudinally (×20,000); in b) the apertures of the TT (T) are cut transversely (×40,000).

In the mammalian cardiac muscle, couplings are regarded as a functional binding of the sarcolemma and the sarcoplasmic reticulum. Depolarization of the surface membrane, whether it is the wall of a transverse tubule or the peripheral envelope of the muscle fibre, leads to the release of calcium-ions stored in the SR. In the case of the ventricle of the frog, physiological experiments indicate the presence of an intra-cellular calcium store[5]. Hence, it would appear that, since the difficulty caused by the supposed absence of couplings has been eliminated, it is not necessary to consider a different mechanism of excitation-contraction coupling in the frog heart from that of the mammalian cardiac muscle.

Zusammenfassung. Auf Grund des grundsätzlich identischen Feinbaus (Vorhandensein von T-System und Koppelungen) des Myocards bei Frosch und Mammalia ist anzunehmen, dass in beiden Fällen auch die elektromechanischen Koppelungen identisch sind.

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Development of the Adrenergic Innervation in the Ureter and Vas Deferens in Rabbits

The peripheral sympathetic nervous system has been extensively studied with the fluorescence method of FALCK and HILLARP[1]. This highly specific histochemical approach has thrown new light on the morphological and functional aspects of the adrenergic transmitter. However, most investigations have been concerned with the completely developed adrenergic nervous system. At the present time only a few histochemical studies on the ontogenesis of the peripheral sympathetic nervous system have been reported[2-6]. In these investigations, various peripheral organs were studied but the development of the sympathetic innervation of the ureter was never considered.

We have investigated the development of the adrenergic innervation in the rabbit ureter and vas deferens, this latter organ being considered as a reference for fluorescence neurohistochemical studies. The appearance, distribution and subsequent development of catecholamine-containing nerves in these effector organs are reported.

Material and methods. 34 young male rabbits were studied at age 1, 2, 3, 4, 6, 8, 10, 14, 18, 21, 30 and 45 days. 12 embryos of the last week of gestation period were obtained by hysterotomy under nembutal anesthesia. Specimens consists of the distal parts of the ureter and vas deferens. All preparations were examined for the demonstration of fluorescent catecholamines according to the method of FALCK and HILLARP[1]. The sections were examined under a Zeiss fluorescent photomicroscope using a Philips high-pressure mercury lamp, a BG 12 excitation filter and a 470 u barrier filter.

All sections were studied at the same magnification and photomicrographs taken with the same exposure time. The number of fluorescent nerves were counted on these pictures.

Some tissues were incubated at 37 °C for 30 min in a modified Krebs-Ringer bicarbonate buffer containing norepinephrine in a concentration of 10^{-5} M[7]. The specificity of the fluorescence reaction was checked by omission of the paraformaldehyde reaction and supported by the fact that no fluorescence was observed after pretreatment of some animals with reserpine and α-methyl-metatyrosine[8].

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