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THE VIRAL NATURE OF *TODDIA* FRANÇA, 1912

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SYNOPSIS. *Toddia* França, 1912 under the light microscope occurs as inclusion corpuscles in the cytoplasm of erythrocytes of cold-blooded vertebrates sometimes accompanied by crystalloid bodies. Its position among the protozoans or the viruses has been discussed by some authors, but remained unclear. To elucidate this problem we studied *Toddia* from a Brazilian frog *(Leptodactylus ocellatus)* by electron microscopy. In the cytoplasm of the infected cells we found no protozoan, but rather virus-like particles often hexagonal in outline, averaging 195 nm excluding their two involving membranes, and presenting a central area of variable electron density. Particles at different stages of development were generally found around or on an area of lighter density than the cytoplasm, which resembled a virus synthesis site. At high magnification, the nuclear or cytoplasmic crystals allied to *Toddia* resembled the crystalline lattice of the inclusion bodies associated with the polyhedrosis viruses and poxviruses from insects, of the capsules of the granulosis viruses and of other protein crystals in ultrathin sections. Cytochemical tests in *Toddia* corpuscles displayed exclusively the presence of deoxyribonucleic acid. These findings indicate that *Toddia* is not a protozoan and demonstrate that it is in all probability a viral inclusion corpuscle. Taking into account the nucleic acid type found in its structure (DNA) and the hexagonal shape usually shown in ultrathin sections by its component particles, which have a cytoplasmic site of synthesis and assembly, we tentatively relate *Toddia* with the so-called "Icosahedral Cytoplasmic Deoxyriboviruses". We believe that the present paper gives the first report of virus-like particles in *L. ocellatus*.

Dutton et al. (1907) were the first to find an "unidentified parasite" occurring as red-staining round masses associated with crystalloid bodies in the erythrocytes of African frogs. França (1912) created the genus *Toddia* for it and called *Toddia bufonis* the similar parasite he found in the toad *Bufo regularis*. Subsequently, *Toddia* was found in red blood cells of other cold-blooded vertebrates, mainly amphibians (Mathis & Léger, 1911; Scorza & Boyer, 1956; Pereira et al., 1973) and reptiles (Marquardt & Yaeger, 1967; Pessôa, 1967; Arcay de Peraza et al., 1971; Sousa et al., 1973) and more rarely in fish (Arcay de Peraza & McLure, 1971). On account of its small size and delicate internal structure, till then exclusively studied by light microscopy, the problem of the true nature of *Toddia* remained controversial. It has been considered either a protozoan (Scorza & Boyer, 1956; Arcay de Peraza & McLure, 1971; Arcay de Peraza & Roca, 1971; Arcay de Peraza et al., 1971) or a probable virus (Marquardt & Yaeger, 1967; Pereira et al., 1973; Sousa et al., 1973); in addition, in classical treatises of Protozoology (Wenyon, 1926) and Zoology (Poisson, 1953) *Toddia* was reported together with *Cytamoeba*

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bacterifera, another intraerythrocytic corpuscle, among the parasites of doubtful nature. To clarify this problem we studied *Toddia* from a common Brazilian frog *(Leptodactylus ocellatus)* by electron microscopy, besides doing cytochemical tests for nucleic acids.

MATERIAL AND METHODS

May Grünwald-Giemsa stained blood smears of several specimens of *Leptodactylus ocellatus* from Rio de Janeiro State (Brazil) were examined under the light microscope. One frog heavily infected by *Toddia* was selected to study this parasite by electron microscopy. This frog was anesthetized with chloroform and ventrally incised; blood of its heart was collected with a Pasteur pipette and immediately fixed for 4 hrs in 2.5% (v/v) glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2; during the first 10 min of fixation the blood was centrifuged at low speed. The resulting pellet of cells was postfixed for 40 min in 0.5% (w/v) osmium acid also in 0.1 M cacodylate buffer, pH 7.2, rinsed in distilled water and placed in 1.0% (w/v) uranyl acetate for 12 hr. Then the pellet was dehydrated in increasing gradients of ethanol, passed three times in acetone and embedded in polylite T-208 resin (Weigl & Kisielius, 1972)*. Ultrathin sections were made with glass or diamond knives on a Sorvall Porter-Blum MT-2 microtome and collected on 300-mesh grids previously covered with a Formvar pellicle which was discarded if more resolution was desired. These sections were stained with lead (Reynolds, 1963) and examined with a Philips EM-300 microscope, 60 or 80 KV. All measurements were taken directly on the electronmicrographs.

Some blood smears from the selected frog were fixed in ethanol-ether (1:1) for 10 min and submitted to the Feulgen and methyl green-pyronin methods (De Tomasi, 1936; Lison, 1960). Some May Grünwald-Giemsa stained blood slides from this animal were used to observe the peculiarities of *Toddia* by light microscopy and to establish the correlation with the results from the electron microscopy. Measurements of *Toddia* were made with an Olympus micrometer eyepiece and photographs with an Orthomat automatic camera on an Orthoplan microscope, Leitz, adapted to xenon illumination.

RESULTS

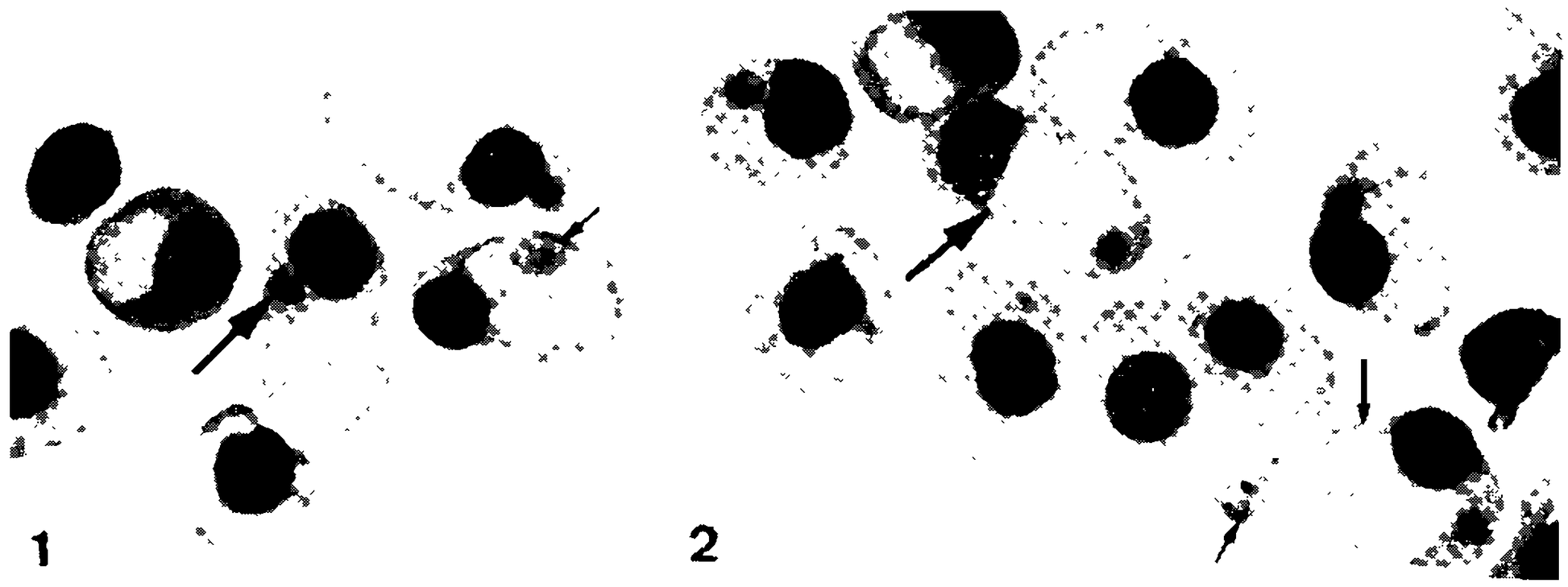
Light Microscopy

In blood films stained by May Grünwald-Giemsa, *Toddia* appeared basically as purple cytoplasmic inclusion corpuscles mainly in erythrocytes and more rarely in immature red blood cells (Figs. 5, 6). Its internal structure could be sometimes compact or diffuse (Figs. 1, 5) and more frequently heterogeneous displaying some compact granules (Figs. 1, 2, 9) and portions more condensed than others (Fig. 2). *Toddia* corpuscles were often nearly circular or oval in shape; they averaged 2.7 x 2.2 μm, ranging in length from 0.9 to 5.3 μm and in width from 0.8 to 3.5 μm.

The parasitized cells sometimes showed one or more crystalloid bodies either in their nucleus and/or cytoplasm (Figs. 1-9). The intranuclear crystals usually had a regular or irregular hexagonal outline (Figs. 7, 8, 9), whereas the intracytoplasmic ones were often roundish (Fig. 5) or ovoid (Figs. 2, 3) although at times could present some straight sides (Figs. 1, 4, 6). Such crystals displayed a homogeneous structure and stained bluish gray. The nuclear inclusion bodies averaged 7.1 x 5.6 μm, ranging in length from 4.2 to 11.7 μm and in width from 3.2 to 8.5 μm; the cytoplasmic ones measured on an average 9.8 x 8.1 μm and ranged in length from 4.8 to 12.3 μm and in width from 4.1 to 10.7 μm. There was no apparent connection between the crystalloid bodies and the *Toddia* corpuscles.

The infected cells were usually enlarged and/or deformed (Figs. 1, 5, 6, 9). Their nucleus was often displaced showing an abnormally arranged chromatin, especially when

* Weigl, D. R. & Kisielius, J. J., A resina polylite na microscopia eletrônica. Presented at the "I Colóquio da Sociedade Brasileira de Microscopia Eletrônica" (1970), Ribeirão Preto, Brasil.



Figs. 1-9. Photomicrographs of *Toddia* in erythrocytes of *Leptodactylus ocellatus;* May Grünwald-Giemsa stain. Figs. 1-2, × 1,000; Figs. 3-9, × 940. Fig. 1. Note the heterogeneous (larger arrow) and diffuse (smaller arrow) structure of the *Toddia* corpuscles and the shape of the cytoplasmic crystalloid bodies in the infected cells. Fig. 2. Several aspects of the chromatic corpuscles; noteworthy
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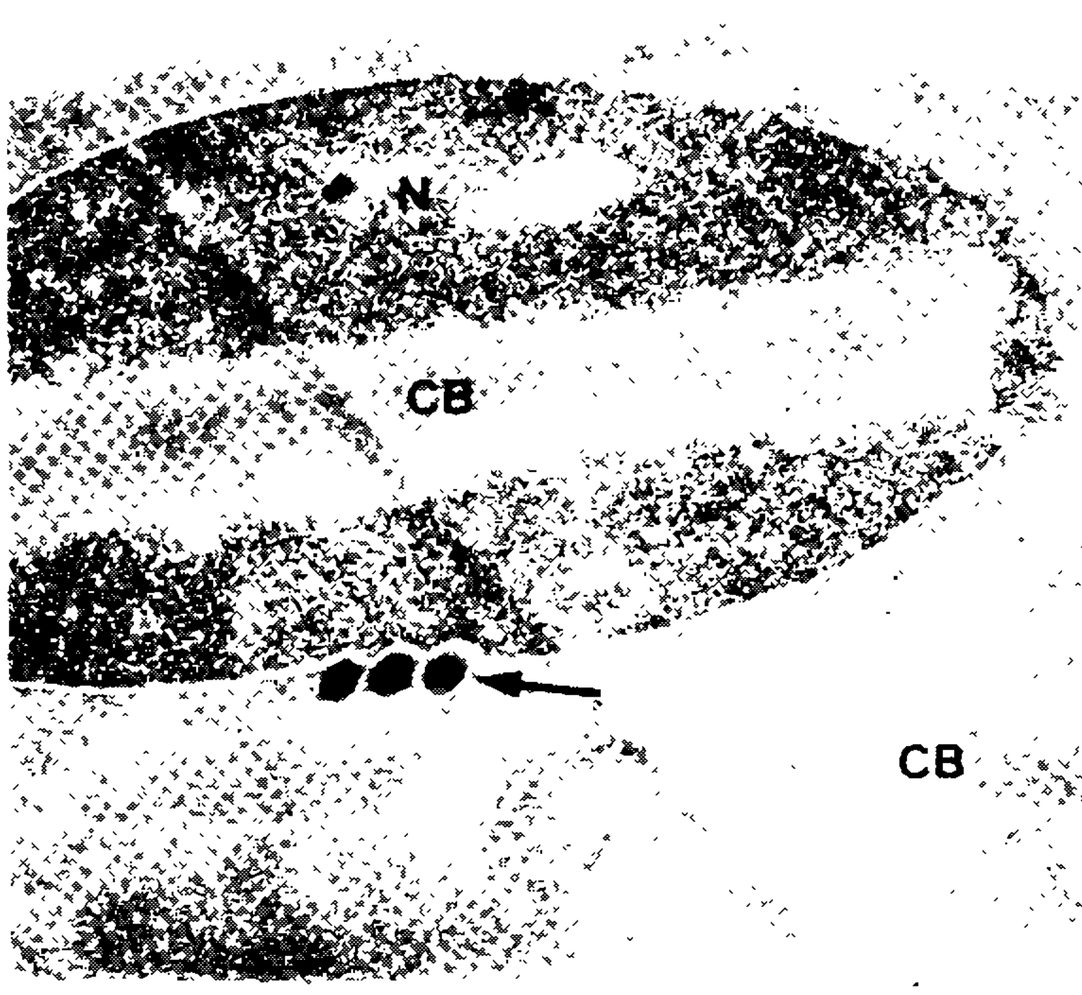
it contained one or more crystals (Figs. 2, 4, 5, 7, 8). Nonnucleated infected cells were sometimes found (Fig. 6). In some instances, the host cells seemed to have been disrupted by mechanical action of the crystals allied to *Toddia* (Fig. 9).

Electron Microscopy

Ultrathin sections of *Toddia* infected cells displayed no protozoan. Instead, numerous polygonal particles were found in their cytoplasm, sometimes accompanied by large bodies (CB) either in the nucleus or the cytoplasm, or in both (Fig. 10). These particles (Figs. 10-21, 24) were often hexagonal in profile, sometimes pentagonal (Figs. 12, 24) and more rarely circular; one (Figs. 12, 14, 19, 24) or two (Figs. 10, 11, 13, 14, 17, 18, 20, 21) membranes were usually surrounding them. The average diameter of the



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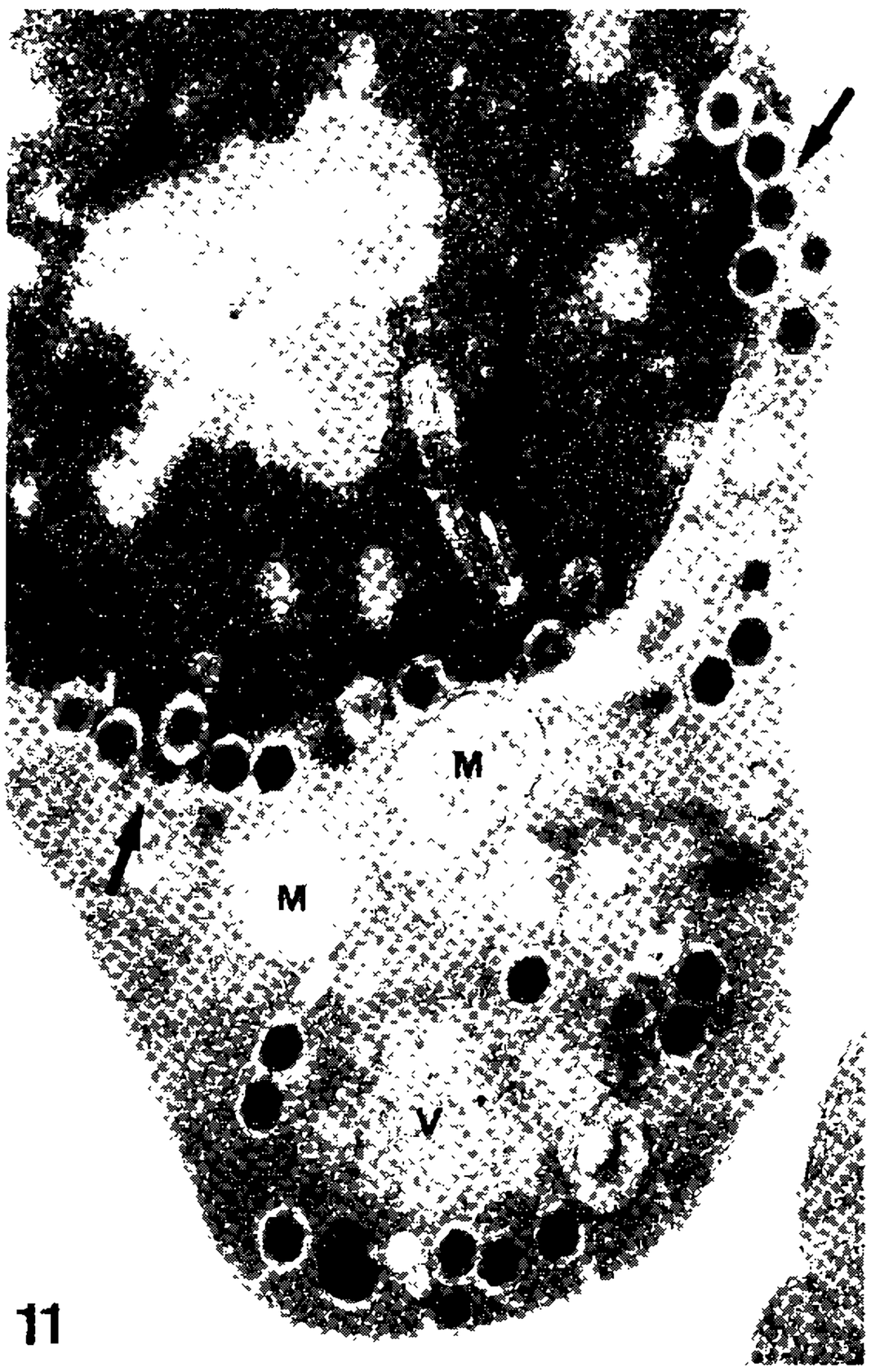

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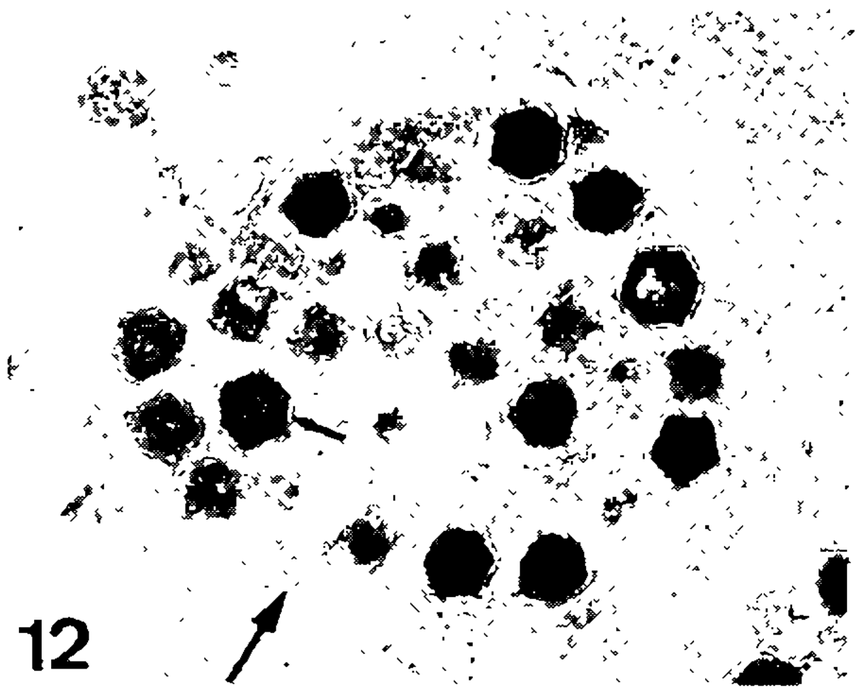

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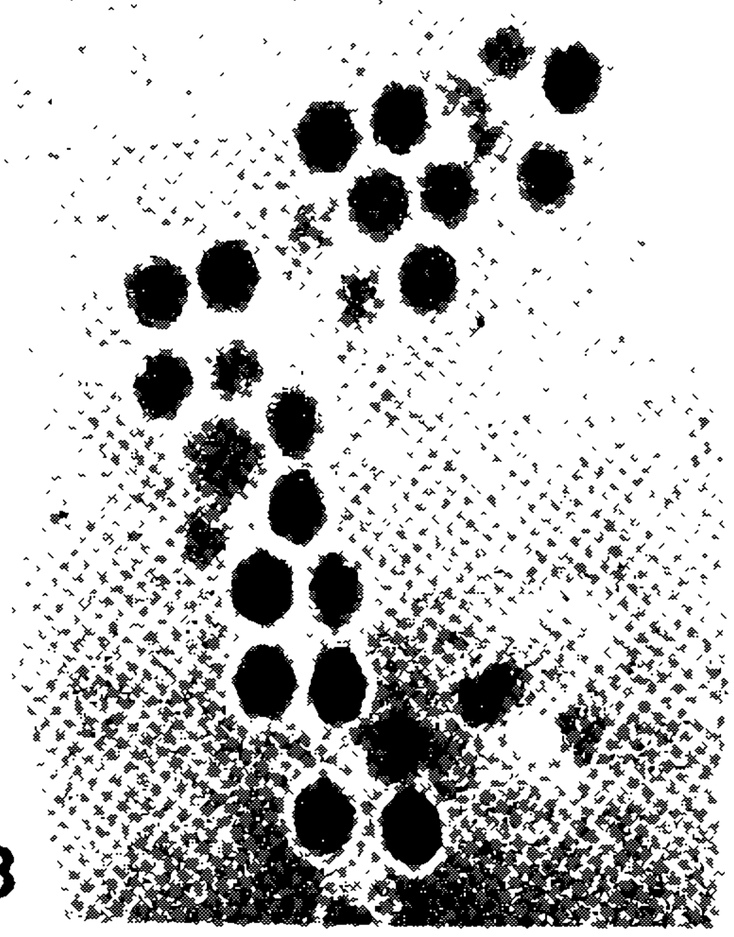

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Figs. 10-13. Electronmicrographs of *Toddia* infected cells. N, cell nucleus; M, mitochondria; CB, crystalloid body (also reported as inclusion body, crystalloid inclusion and crystal); V, viroplasm-

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regularly hexagonal particles was 195 nm, excluding any involving membranes, and 224 nm including the inner membrane.

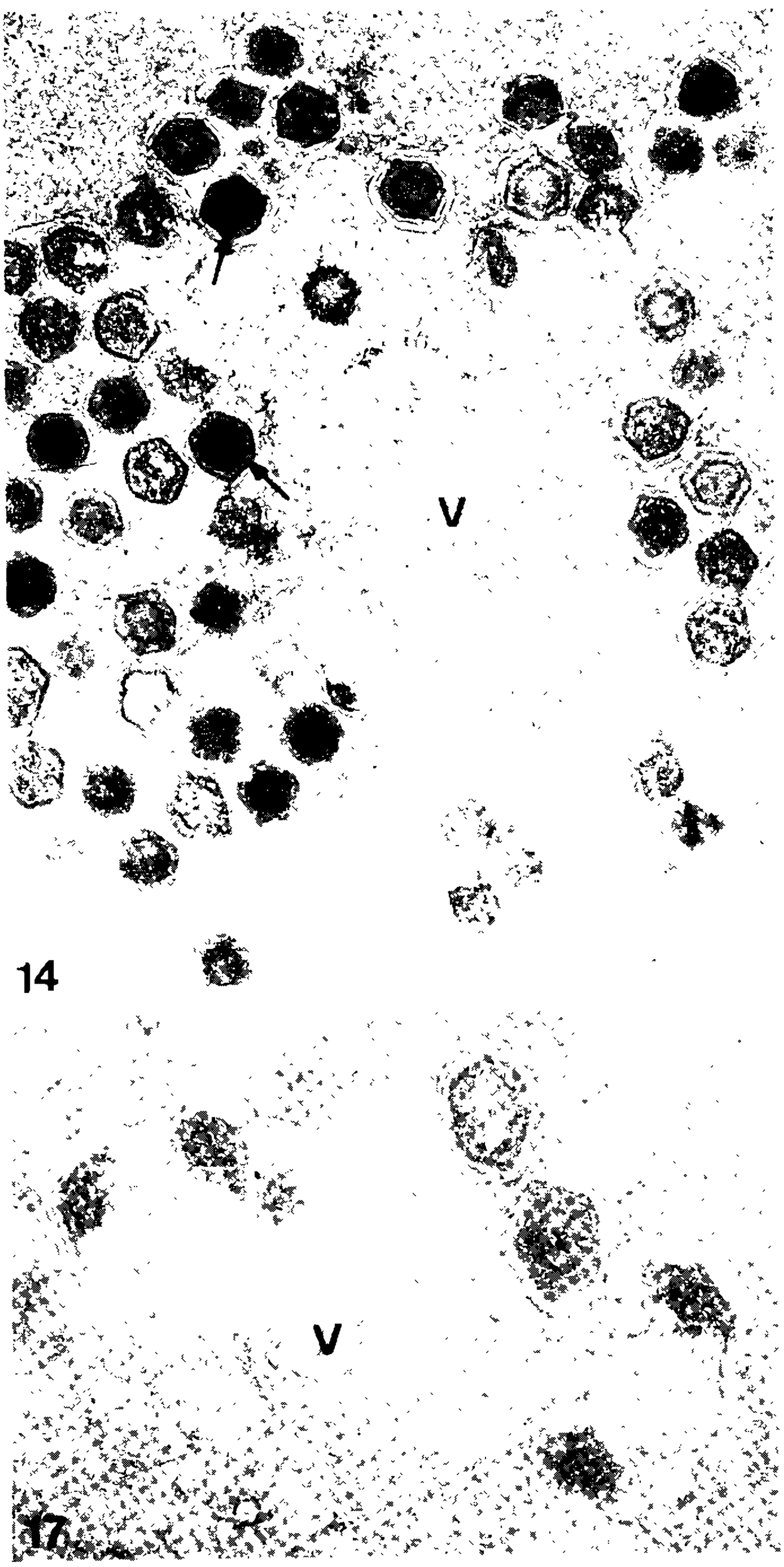
Within the host cell cytoplasm these particles sometimes appeared dispersed (Fig. 11) or grouped (Figs. 12, 13), but only rarely closely packed (Fig. 13). In some cases the particles were seen in the space between the two layers of the nuclear membrane (Figs. 10, 24), seeming to be budding to it, or simply close to the nucleus (Fig. 11). Particles apparently at different stages of development were generally found around or on an area (V) of lighter density than the cytoplasm (Figs. 10, 14-17) composed of granular and fibrillar material. Incomplete particles seemed to emerge from that area and to be simultaneously involved by membranes (Figs. 15-17). Such area averaged 2.1 x 1.5 μm; if the surrounding particles were also taken into account the average size was 3.0 x 2.3 μm. A noteworthy aspect is shown in Fig. 15, clearly suggesting that a particle at assembly stage is being involved by a double membrane, while material from the mentioned area (V) seems to be condensed and introduced into it.

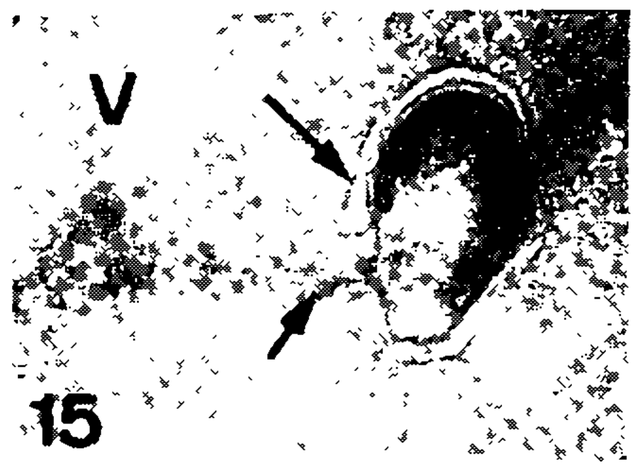
The apparently mature particles displayed an electron dense central area (Figs. 14, 19, 20) averaging 117 nm in diameter and similar in shape to the particle itself. This area was surrounded by another of lighter density delimited by a triple layered structure similar to a unit membrane (Fig. 19, shorter arrow), which also could be seen even in immature particles (Fig. 19, medium arrow) or in the partially empty ones (Fig. 21, longer arrow). The portion circumscribed by such membrane in the immature forms revealed the following aspects: it could be total or partially filled by fibrillar or granular or fibrillogranular material (Figs. 14, 17, 19), and in some cases it showed the central area irregularly condensed (Fig. 20) or limited by a dense ring (Figs. 14, 20). On the surface of the above-mentioned unit membrane several small and little differentiated elements (Fig. 21, smaller arrows) seemed to form a layer, around which one (Fig. 19, longer arrow) or two (Fig. 20, shorter arrows; Fig. 21, thicker arrows) other membranes were often seen. The inner of these membranes could be rippled (Figs. 17, 18, 20) and remained closely contiguous to the mentioned layer (Figs. 20, 21), thus seeming to limit a whole particle. There was an electron lucent space between the inner and the outer involving membranes (Figs. 14, 20, 21) and the outer one looked rather to delimit a vacuole where a particle remained included (Figs. 14, 20, 21); in some cases it circumscribed a larger vacuole containing two (Fig. 18) or three particles. In addition, when a group of particles had a single surrounding membrane there was always another membrane near them (Figs. 14, 22), sometimes enclosing all the group (Fig. 12). As shown in Fig. 22, the triple layered structure of these membranes was analogous to the others of the host cell.

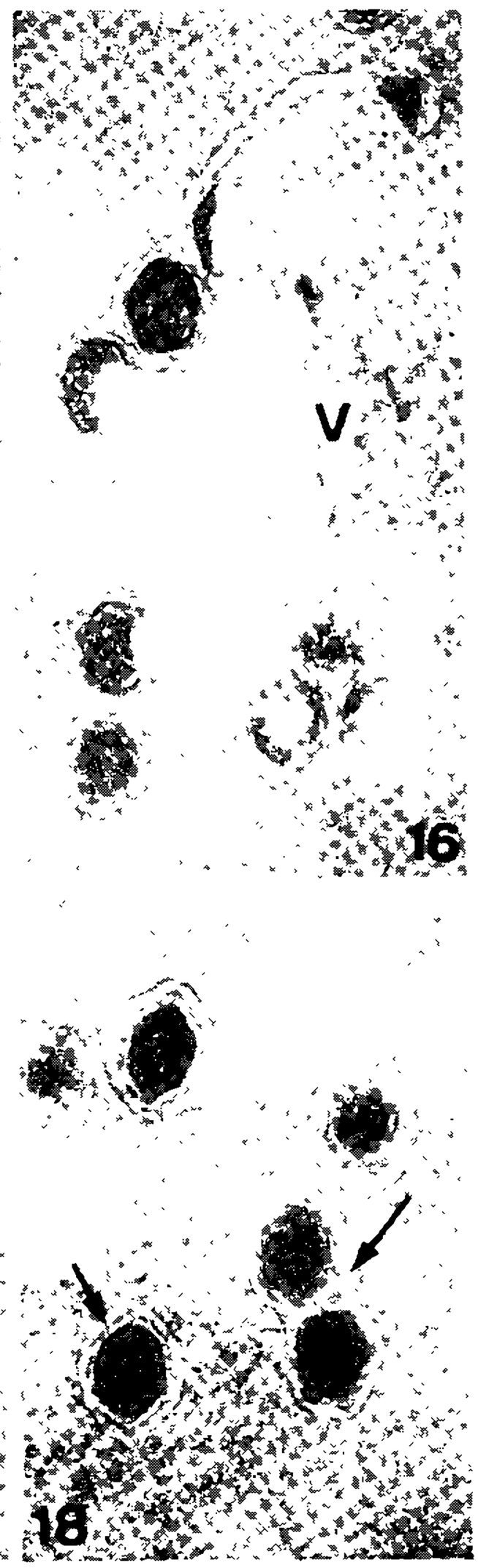
The large nuclear or cytoplasmic inclusion bodies (Figs. 10, 23-25; CB) found exclusively in infected cells displayed in ultrathin sections some variation in shape and electron density. They often appeared as elongated bars (Figs. 10, 24) with an outline usually almost parallelogrammic or trapezoidal and more rarely rectangular. Noteworthy is the nuclear inclusion (CB) shown in Fig. 23, since it presents a shape rarely achieved in ultrathin sections resembling the so-called crystalloid bodies seen under the light microscope (Fig. 7, arrow). The majority of these large inclusion bodies was almost as electron dense as the host cell cytoplasm, the orderliness of their internal structure being the chief differential feature. Internally, they usually displayed at higher magnification (Figs. 23 and 25) a linear periodic arrangement of very small units, resembling a crystalline lattice.

Cytochemical Tests

Toddia corpuscles gave weakly positive or negative Feulgen reaction and stained faint green with methyl green-pyronin, whereas the crystalloid inclusions remained unstained by the two methods.







Figs. 14-18. Electronmicrographs of *Toddia* infected cells. V, viroplasm-like area. Fig. 14. Particles at different developmental stages around the viroplasm-like area; some of them have a more electron dense central area (nucleoid) (arrows) × 48,970. Fig. 15. A particle at assembly stage is being involved by a double membrane (longer arrow), while material (shorter arrow) from the viroplasm-

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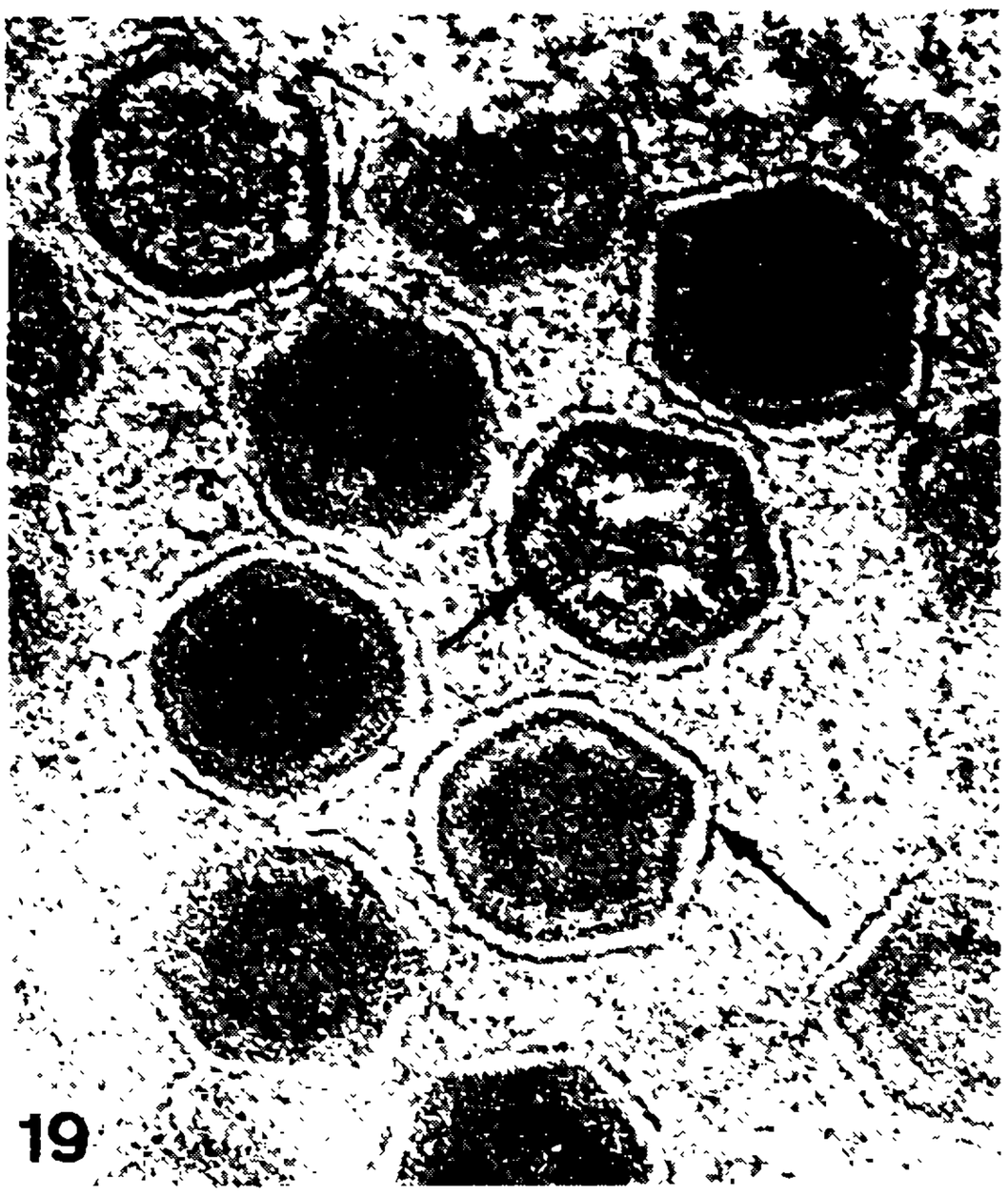
DISCUSSION

As evidenced by the results of this investigation, there is no reason to relate *Toddia* with the protozoans, as previously suggested by some workers (Scorza & Boyer, 1956; Arcay de Peraza & McLure, 1971; Arcay de Peraza & Roca, 1971; Arcay de Peraza et al., 1971). These authors were based on the supposition that *Toddia* corpuscles had nucleus and cytoplasm and underwent binary fission and schizogony; indeed, in a previous study of *Toddia* from Brazilian snakes (Sousa et al., 1973) we observed that some appearances of these corpuscles might erroneously give such impressions and then we discussed that they provided no conclusive evidence for a protozoan nature. The results of the present cytochemical tests confirm preceding observations (Scorza & Boyer, 1956; Pereira et al., 1973; Sousa et al., 1973) on the presence of DNA and the probable absence of RNA in *Toddia* corpuscles. On the other hand, the infectivity of *Toddia* (Pessôa, 1968-69; Pereira et al., 1973) having been proved, our observations on its fine structure confirms its suspected viral nature (Marquardt & Yaeger, 1967; Pereira et al., 1973; Sousa et al., 1973). The polygonal particles found in the cytoplasm of *Toddia* infected cells closely resemble the so-called "Icosahedral Cytoplasmic Deoxyriboviruses" (ICDV's) (Stoltz, 1971, 1973; Kelly & Robertson, 1973; McAuslan & Armentrout, 1974), also named "Iridoviruses" (Wildy, 1971; Andrews & Pereira, 1972; Fenner et al., 1974), and the results of the cytochemical tests support this assumption. In addition, some incomplete particles (Figs. 10, 14-17) were disposed around or on an area which, by its electron density and component material, resembles some virus synthesis sites of such viruses (Darlington et al., 1966; Granoff, 1969; Granoff et al. 1969; Zylber-Katz et al., 1974; Kelly, 1975), also called virogenic stroma, viroplasm, virus factory, etc. We consequently believe that *Toddia* corpuscles, previously reported as "chromatic" corpuscles (Sousa et al., 1973), as observed by light microscopy, are really composed of the viroplasm-like area and the particles around it (see the examples in Figs. 10 and 14, and some *Toddia* corpuscles in Figs. 1, 2 and 9). In fact, the mean sizes of both structures as ascertained by light and electron microscopy including the peripheral particles are practically the same. Moreover, it is probable that the variation in internal structure of *Toddia* corpuscles is a result not only of differences in degrees of grouping of the viral particles, but also of their arrangement relative to the viroplasm. It is also probable that at more advanced stages of the developmental cycle in the host cell, several integral particles randomly dispersed determine the diffuse appearance usually displayed by the largest "chromatic" corpuscles seen under the light microscope (Figs. 1, 5).

An attempt to explain some weakly positive or even negative results of the cytochemical tests for nucleic acids in *Toddia* from a toad (Pereira et al., 1973), which have also been observed in the present experiments, was advanced by Walker (personal communication), who suggested that such findings result from the dissimulation of the nucleic acid of the particles by the capsid "protection" or by "dilution" in the viral protein. We also believe that at advanced stages of the virus cycle in the host cell the increasing "consumption" of the viroplasm parallelly concurs to such results, since they are often found in the largest *Toddia* corpuscles commonly seen in severe infections.

According to Kelly & Robertson (1973), the qualifications required for a virus to be considered an ICDV are not stringent; the virus particles need to be icosahedral in shape, to replicate in the cytoplasm and to contain DNA. McAuslan & Armentrout (1974) mentioned that, among other characteristics, the virions also would need to have a minimum size of about 130 nm and a single structural unit membrane associated with the nucleoid. Although we did not prove that the particles found in the *Toddia* infected cells are icosahedrons, their three-dimensional form may be deduced from their profiles in ultrathin sections which are consistent with the icosahedral symmetry (Stoltz, 1971; Mattern et al., 1974). Moreover, viroplasm-like areas associated with particles apparently



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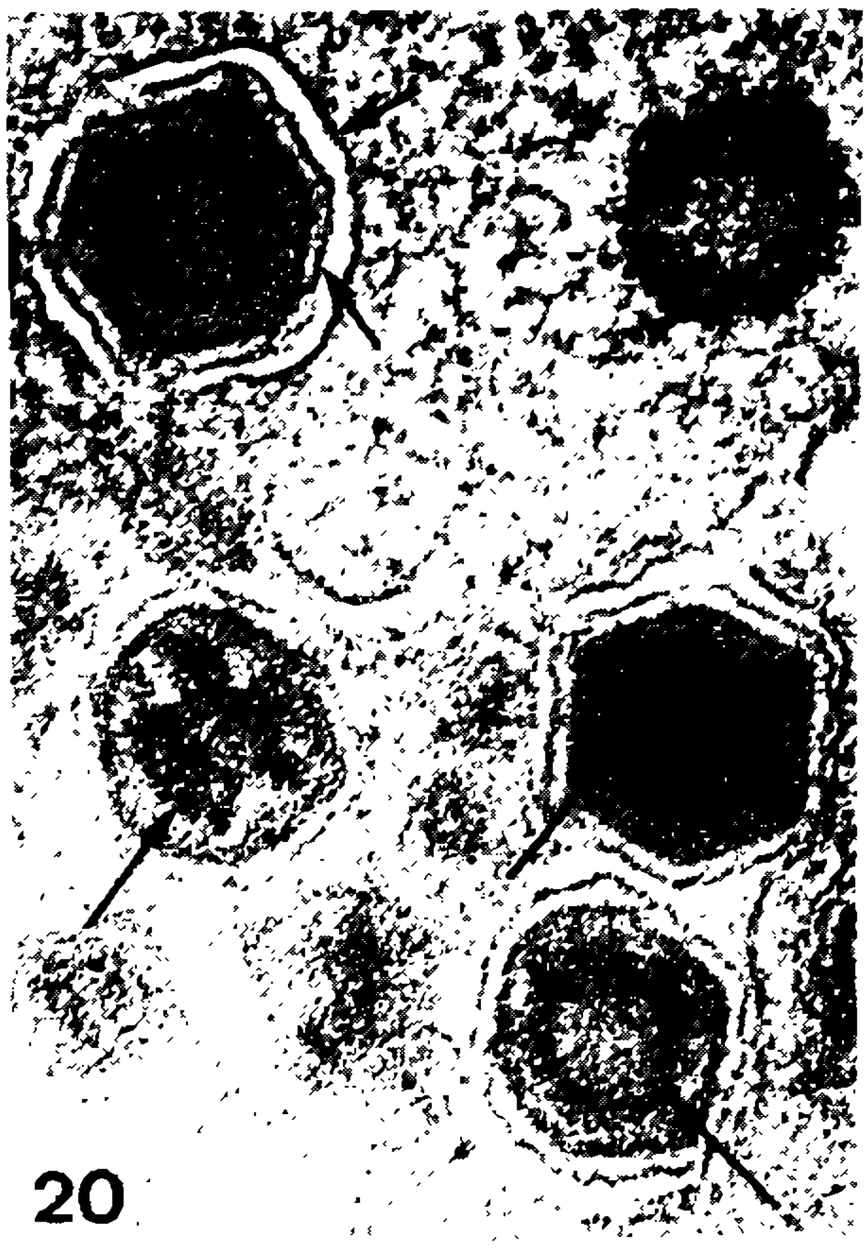

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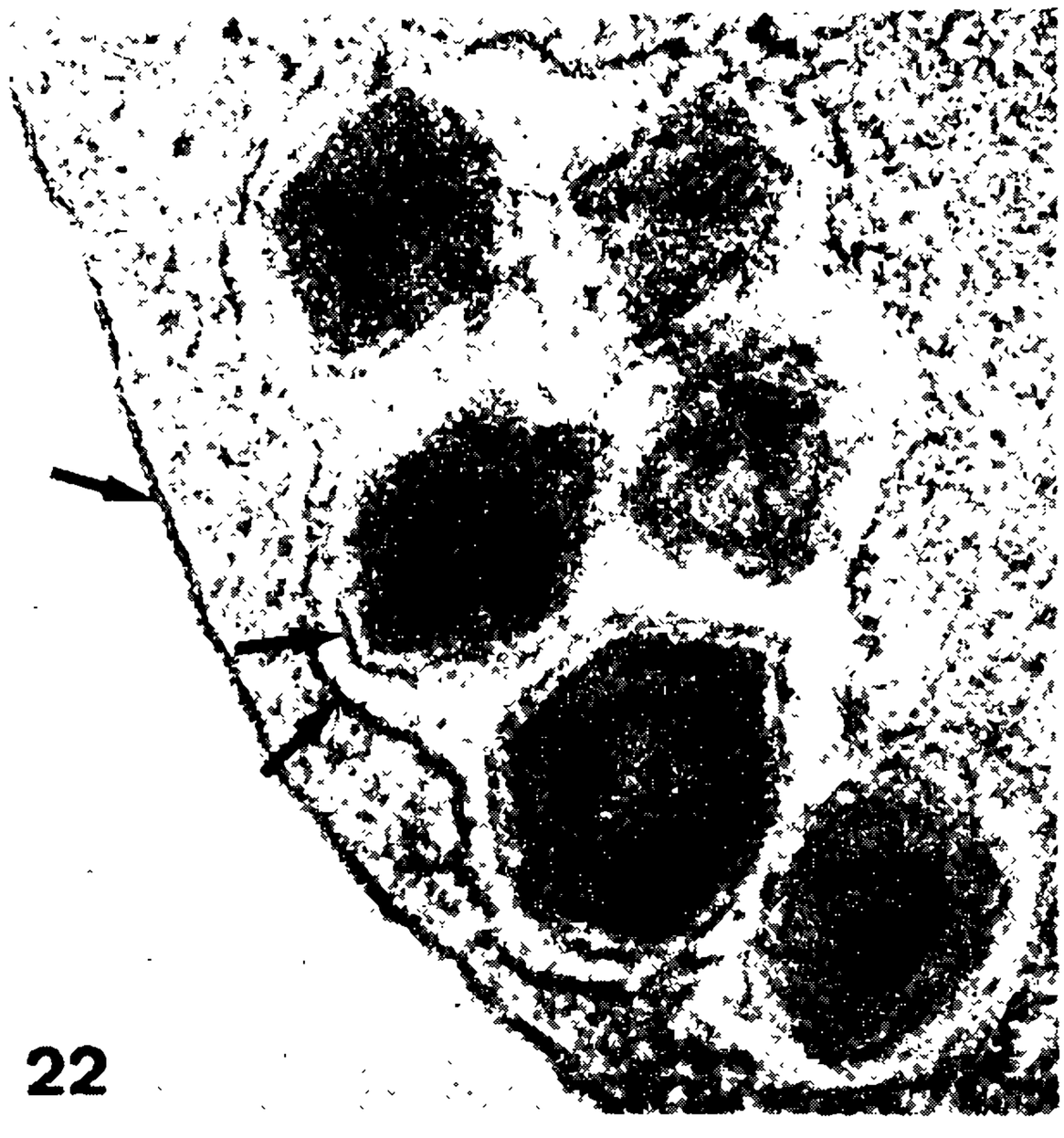

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Figs. 19-22. Electronmicrographs of *Toddia* infected cells. Fig. 19. The particles have only one surrounding membrane (longer arrow). The two shorter arrows indicate the trilaminar structure similar to the "core membrane". Note some aspects of the portion delimited by such membrane, especially the electron dense central area (nucleoid) of the particle near the upper right corner. x 118,770. Fig. 20. A complete particle with two membranes (shorter arrows) and a distinct electron lucent space between them; the inner membrane is closely contiguous to the capsid-like layer, while the outer one seems to delimit a vacuole where the particle included. The longer arrows show some

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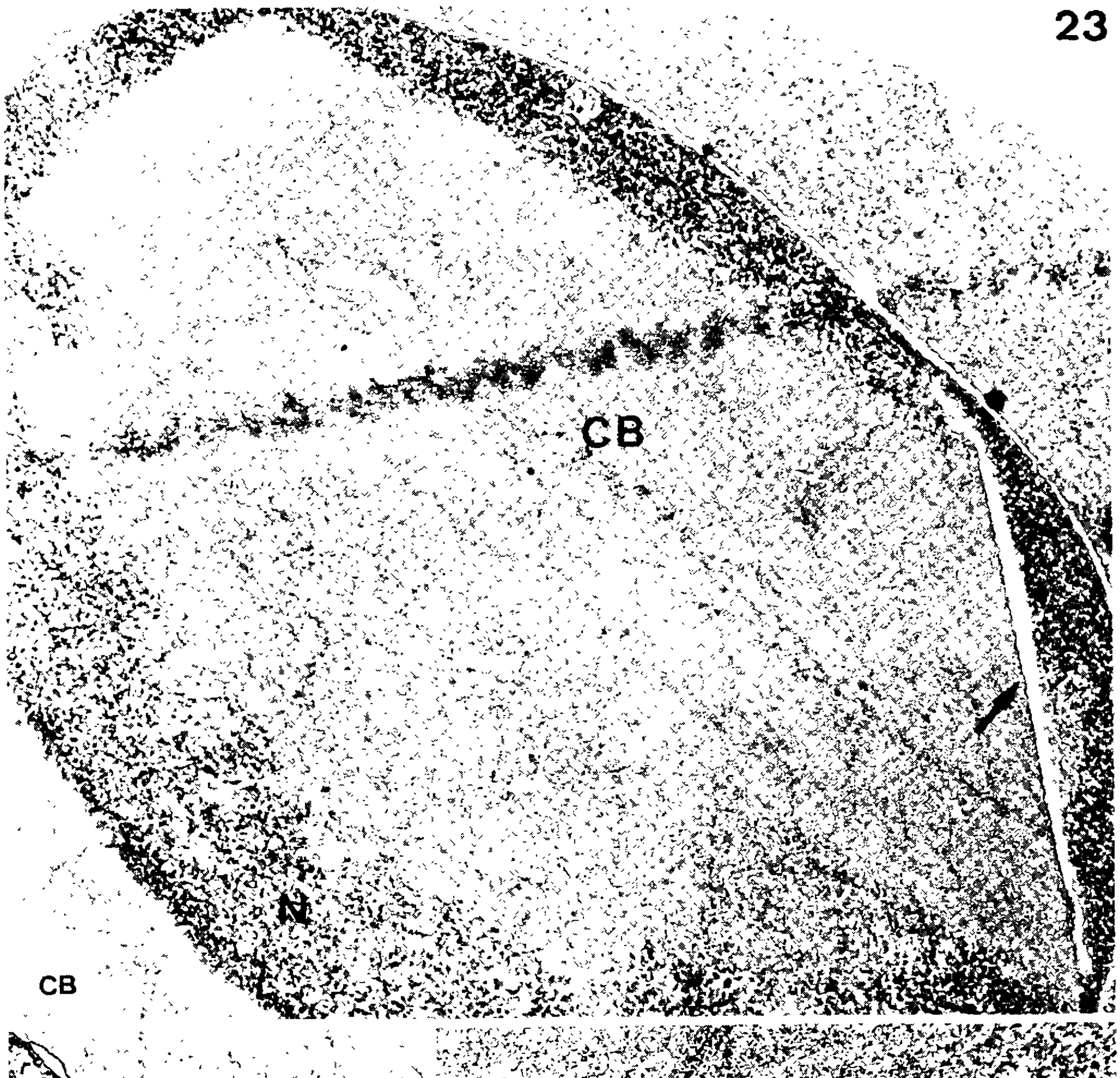
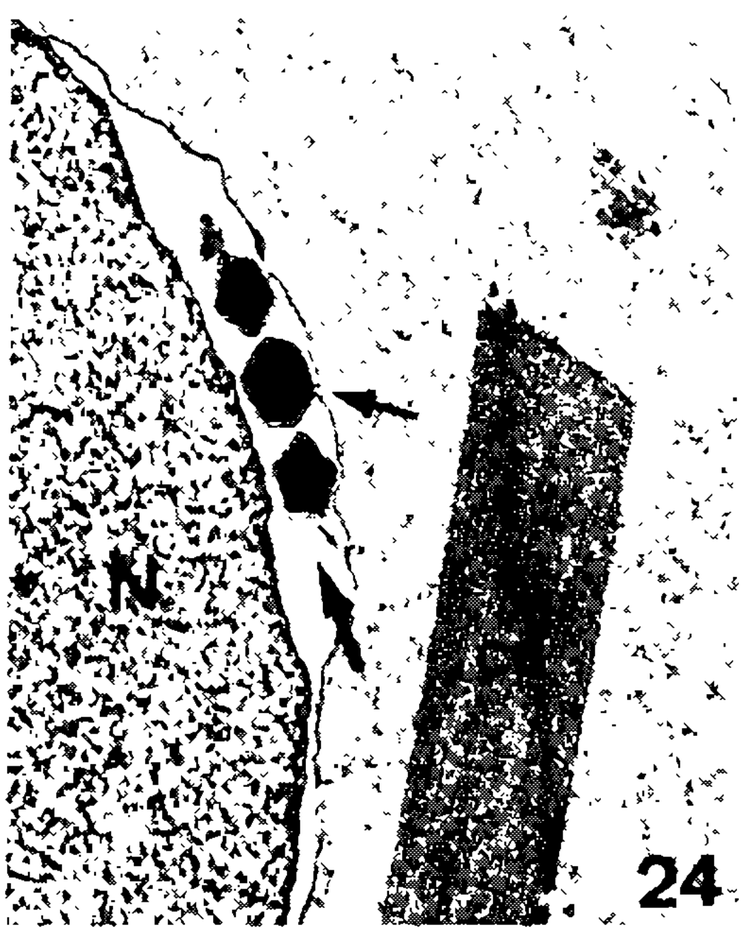
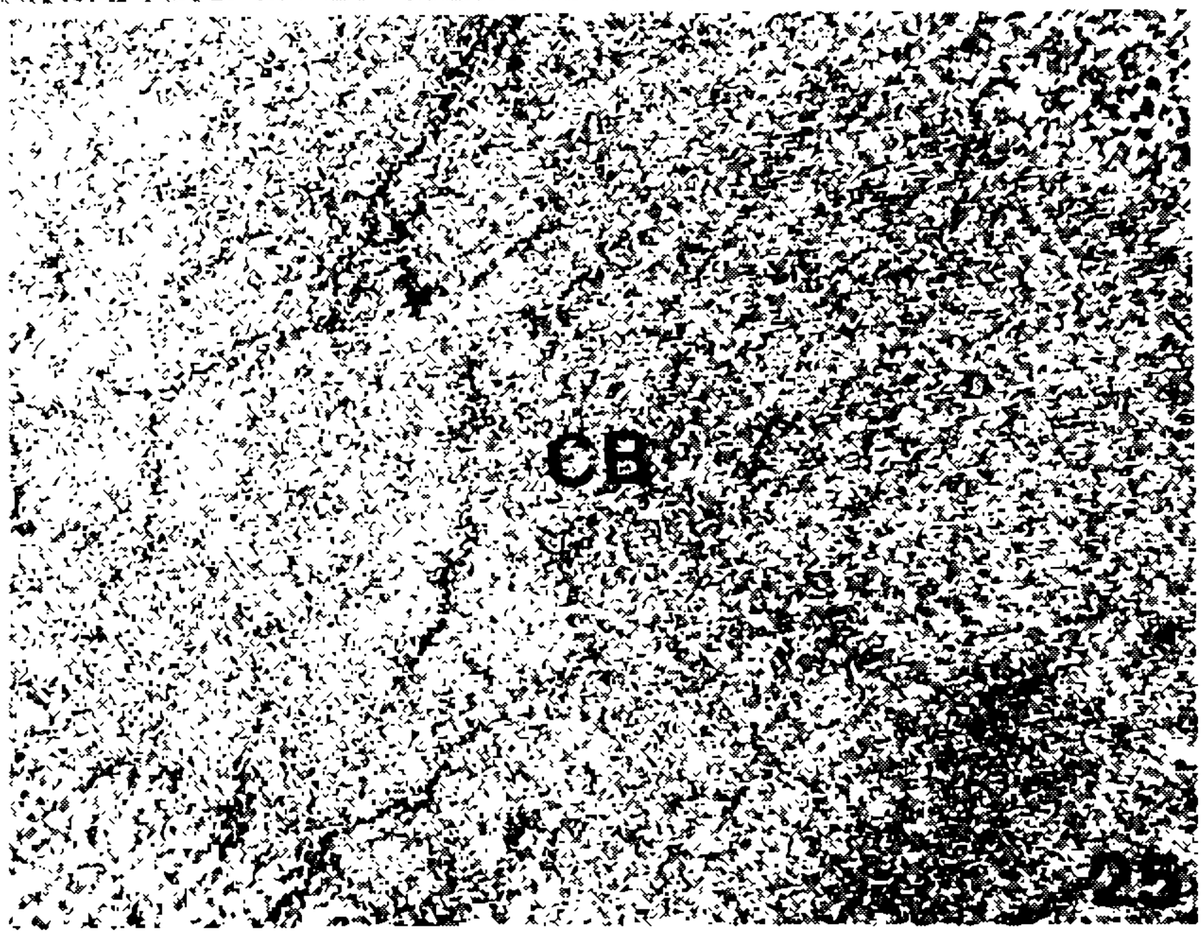
at various developmental stages were exclusively found in the cytoplasm of the infected cells and probably they are sites where virus replication occurs. Considering some positive cytochemical tests in *Toddia* corpuscles, it may be inferred that DNA occurs both in the central area (nucleoid) of the particles and in the viroplasm-like area. Since the mean diameter of these particles in ultrathin sections is 195 nm, also with respect to their dimensions they may be included among the large ICDV's, having a more approximate size to the African Swine Fever Virus (Breese & DeBoer, 1966), some Lymphocystis Disease Viruses (Walker, 1962; Walker & Wolf, 1962; Walker & Weissenberg, 1965; Midlige & Malsberger, 1968), the Regular Mosquito Iridescent Virus (Stoltz, 1971; Hall & Low, 1972), and the Aphelidium virus (Schnepf et al., 1970). Moreover, the internal structure of such particles is similar to that of the ICDV's, especially some of other frogs (Lunger & Came, 1966; Lunger, 1969; Stoltz, 1973; Kelly, 1975), some insects (Clark et al., 1965; Mercer & Day, 1965; Stoltz, 1973), a crustacean (Federici & Hazard, 1975) and a mollusk (Rungger et al., 1971), although they could differ from them with respect to size and some peculiarities. Finally, in these particles might be well identified a central portion usually electron dense similar to a nucleoid (Stoltz, 1973) (Figs. 14, 19, 20), which was surrounded by an area of lighter density delimited by a single trilaminar structure (Fig. 19, shorter arrows and 21, longer arrow) analogous to the "core membrane" defined by Stoltz (1973). Furthermore, on such membrane there was a layer of small and little differentiated elements (Figs. 19 and 21, smaller arrows) perhaps related to the capsid of other virions (Stoltz, 1973).

Some ICDV's, mainly from vertebrates and more rarely from invertebrates (Bellett & Mercer, 1964), acquire one envelope during the budding at the plasma membrane (Bellett & Mercer, 1964; Granoff et al., 1965, 1969; Breese & DeBoer, 1966; Darlington et al., 1966; Lunger & Came, 1966; Wolf et al., 1968; Kelly, 1975) or into cytoplasmic vacuoles (Granoff et al., 1965, 1969; Kelly, 1975). The particles found in *Toddia* corpuscles were also surrounded by membranes, but usually their envelopment was observed not during a budding process at the plasma membrane, but often during their assembly stage (Figs. 14-17, 22). Thus, one or generally two membranes (or even a double one, as suggested by Fig. 15) enclosed the particles; the inner one became in close association with the above-mentioned capsid-like layer and thus as an envelope apparently established the limit of a particle (Figs. 20, 21), while the outer one rather seemed to delimit a vacuole where one or more particles might be included (Figs. 18, 20, 21). On the other hand, some particles seemed to acquire their envelope by budding at the nuclear membrane and remained in the space between its two layers (Figs. 10, 24). We never observed particles leaving the host cell, but based on some observations under the light microscope (Fig. 9) we believe that this chiefly results from cellular lysis perhaps consequent to the increase of the crystalloid bodies in the infected cells.

The large nuclear or cytoplasmic bodies (Figs. 10, 24, 25; CB) found in infected cells rarely resembled in shape the so-called crystals seen under the light microscope. However, their large size, the similitude in outline of the nuclear inclusions shown in Figs. 23 and 7 (arrow), and the fact that no other structure within the infected cells might be related with the crystalloid bodies that typically accompany the *Toddia* corpuscles (Figs. 1-9), point to the sameness of both structures. From the observations of their profiles in stained smears and ultrathin sections it may be supposed that they are possibly tablet-like in shape. Thus, when sectioned parallel to their larger surfaces they would reveal the characteristic shapes observed under the light microscope and when sectioned perpendicularly to those same surfaces they would appear as rectangular bars whose width might well represent the thickness of the presumed tablets. We believe that the probabilities to get such section planes are few and for this reason certainly their resultant profiles would be hardly seen, as our results have confirmed. Then, we imagine that most shapes displayed by the crystalloid bodies result from oblique sections to their larger surfaces, whence the frequent appearance of elongated bars whose form was often







Figs. 23-25. Electronmicrographs of *Toddia* infected cells. N, cell nucleus; CG, crystalloid body. Fig. 23. Crystalloid body (intranuclear inclusion) whose shape is hardly got in ultrathin sections;
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nearly trapezoidal or parallelogrammic. The interesting outline of the nuclear inclusion shown in Fig. 23 seems to have resulted from an oblique section, but almost parallel to the larger sides of the inclusion.

At higher magnification the internal structure of such nuclear or cytoplasmic inclusion bodies (Figs. 23, 25; CB) resembled the crystalline lattice of the polyhedron associated with the polyhedrosis viruses from insects and crustaceans (Bergold, 1963 a, b; Arnott et al., 1968; Summers & Arnott, 1969; Longworth & Spilling, 1970; Couch, 1974; Federici & Hazard, 1975), the capsules of the granulosis viruses (Bergold, 1963 a; Arnott & Smith, 1968) and the inclusion bodies (spindles, spherules, etc.) allied to the poxviruses from insects (Vago et al., 1968, 1969; Hugher et al., 1970; Bergoin et al., 1971; Bird et al., 1971, Bird, 1974) whose protein nature has been often mentioned. However, unlike most inclusions associated with the above-mentioned viruses, those found in *Toddia* infected cells never occluded virus particles. The similarity between the internal arrangement of such bodies and other protein crystals in ultrathin sections (Gouranton, 1969; Hurez et al., 1972; Langer et al., 1975) is also noteworthy. Thus, regarding all these data it may be supposed that the crystals allied to the so-called *Toddia* are perhaps also proteinaceous bodies. Walker (personal communication) suggests that such crystals should be "composed of excess capsomere protein if there is disproportion in the synthetic rates for viral DNA and protein".

Possible affinities between *Toddia* and *Pirhemocyton* were sometimes mentioned in the literature, even without a knowledge of their true nature (Chatton & Blanc, 1916; Brumpt & Lavier, 1935; Pessôa & Campos, 1966; Marquardt & Yaeger, 1967; Arcay de Peraza et al., 1971; Arcay de Peraza & Roca, 1971; Pereira et al., 1973; Sousa et al., 1973). Separation of the two genera was only based on the shape of their associated inclusions, according to the original descriptions (Dutton et al., 1907; França, 1912; Chatton & Blanc, 1914, 1916): crystal-like in *Toddia* and globular, named "albuminoid inclusion" (Chatton & Blanc, 1916), in *Pirhemocyton*. Sousa et al. (1973) discussed the invalidity of such criterion since, like other authors (Pessôa & Campos, 1966; Marquardt & Yaeger, 1967; Pereira et al., 1973), they found the "chromatic" corpuscles associated with crystalloids, globules and intermediate forms. Confronting the fine structure of *Toddia* (present paper) and *Pirhemocyton* (Stehbens & Johnston, 1966) we now confirm their presumed affinity since both resemble the ICDV's; nevertheless, the virus-like particles of the gecko erythrocytes differ from those of *L. ocellatus* chiefly in size and some structural features. Stehbens & Johnston (1966) described the "albuminoid body" by electron microscopy as vacuoles of two types: one containing moderately electron dense material and the other almost empty; although the first type of vacuole was illustrated with an electronmicrograph of low magnification (X 6,000), which does not reveal details of its structure, we believe in its similarity to the crystalloid body of *Toddia* with respect to their chemical nature and internal arrangement.

ICDV's or particles similar to them within erythrocytes of cold-blooded vertebrates have been reported sometimes in the literature (Stehbens & Johnston, 1966; Bernard et al, 1969; Walker, 1971; Johnston & Davies, 1973). Their occurrence in frog erythrocytes was observed only once by Bernard et al. (1969) in *Rana pipiens*. These authors made no mention to crystalloid or globular bodies in the infected cells; the virus-like particles, although usually hexagonal in profile, differ from those of *L. ocellatus* as to size, peculiarities of their internal structure and arrangement of the associated membranes. Since there are some *Toddia*-or *Pirhemocyton*-like corpuscles without associated crystalloid or globular bodies (Brumpt & Lavier, 1935; Bernard et al., 1969; Walker, 1971; Johnston & Davies, 1973), the possibility of such inclusion bodies being virus dependent or even host cell dependent deserves investigation.

According to these results it seems clear that *Toddia* is in all probability a viral inclusion corpuscle of the DNA type, such as *Pirhemocyton* (Stehbens & Johnston, 1966), and thus it should be named according to the viral nomenclature. On the other hand, it becomes confirmed that *Toddia* has no affinity with another intraerythrocytic corpuscle, *Cytamoeba bacterifera*, which often occurs in frogs including *L. ocellatus* and is an assemblage of bacterium-like organisms (Sousa & Weigl, 1975), although they had been mentioned together in classical treatises of Protozoology (Wenyon, 1926) and Zoology (Poisson, 1953). Further studies are necessary to characterize this unusual virus as well as to determine its true relationships with other members of the ICDV group (Kelly & Robertson, 1973; Stoltz, 1973; McAuslan & Armentrout, 1974). It is interesting to emphasize that so far no member of this group was found to be associated with crystalloid bodies.

RESUMO

A natureza virótica de Toddia França, 1912

Toddia França, 1912 pela microscopia ótica apresenta-se como corpúsculos de inclusão no citoplasma de hemácias de animais pecilotérmicos, às vezes acompanhados de corpos cristalóides. Sua relação com os protozoários ou os vírus tem sido discutida por alguns autores, mas não estava ainda esclarecida. Visando a elucidar este problema, fizemos a microscopia eletrônica de cortes ultrafinos do sangue de uma rã *(Leptodactylus ocellatus)* altamente infectada com *Toddia*. No citoplasma das células parasitadas não encontramos qualquer protozoário, mas partículas semelhantes a vírus, as quais eram geralmente hexagonais, mediam em média 195 nm de diâmetro não considerando suas duas membranas envolventes, e possuíam uma área central de densidade eletrônica variável. Partículas em diferentes estádios de desenvolvimento foram geralmente encontradas em volta ou sobre uma área menos densa que o citoplasma, a qual parecia um local para formação de vírus. Os cristais nucleares ou citoplasmáticos que acompanhavam os corpúsculos de *Toddia* pela microscopia eletrônica revelaram estrutura semelhante àquela observada nos corpos de inclusão associados com os vírus da poliedrose e poxvírus de insetos, nas cápsulas dos vírus da granulose e em outros cristais protéicos em cortes ultrafinos. Testes citoquímicos dos corpúsculos de *Toddia* demonstraram exclusivamente a presença de ácido desoxirribonucléico. Estas observações indicam que *Toddia* não é um protozoário e demonstram, com toda probabilidade, que ela é um corpúsculo de inclusão virótico. Levando em consideração o tipo de ácido nucléico encontrado em sua estrutura (ADN) e a forma hexagonal geralmente apresentada em cortes ultrafinos pelas suas partículas componentes, as quais, além disto, são formadas e reunidas no citoplasma, relacionamos *Toddia* com os chamados "Desoxirribovírus Icosaédricos Citoplasmáticos". Acreditamos que o presente trabalho dá a primeira referência a partículas semelhantes a vírus em *L. ocellatus*.

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Figs. 1-9. Photomicrographs of *Toddia* in erythrocytes of *Leptodactylus ocellatus;* May Grünwald-Giemsa stain. Figs. 1-2, x 1,000; Figs. 3-9, x 940. Fig. 1. Note the heterogeneous (larger arrow) and difuse (smaller arrow) structure of the *Toddia* corpuscles and the shape of the cytoplasmic crystalloid bodies in the infected cells. Fig. 2. Several aspects of the chromatic corpuscles; noteworthy is that indicated by the smaller arrow. The crystal indicated by the medium arrow is nearly oval, while that indicated by the larger one is possibly partially included in the cell nucleus and seems to determine the abnormal arrangement of its chromatin. Fig. 3. Two crystalloid bodies in the cytoplasm of the same cell (arrow); the larger is almost ovoid. Fig. 4. Two crystals appear to be at least partly in the nucleus (arrow). Fig. 5. An immature erythrocyte contains a large and diffuse chromatic corpuscle (medium arrow), while another cell (larger arrow) is intensely deformed on account of two inclusions, one placed in its nucleus and other in its cytoplasm. Two nearly round crystals are shown by smaller arrows. Fig. 6. The smaller arrows indicate infected erythrocytes without nucleus; the larger one points to an infected immature red cell. Fig. 7. Intranuclear crystalloid body (arrow) like that shown in Fig. 23. Fig. 8. An hexagonal and intranuclear crystal is forcing the nuclear membrane and determining the starry aspect in the chromatic material. Fig. 9. Soon after cellular lysis a diffuse *Toddia* corpuscle (smaller arrow), a karyolytic nucleus with a crystal (medium arrow) and other crystalloid inclusion (larger arrow) are near in the plasma. Note the heterogeneous structure of the chromatic corpuscle in the erythrocyte on the lower right corner.

Figs. 10-13. Electronmicrographs of *Toddia* infected cells. N, cell nucleus; M, mitochondria; CB, crystalloid body (also reported as inclusion body, crystalloid inclusion and crystal); V, viroplasm-like area. Fig. 10. General aspect of a *Toddia* infected cell. Note the viroplasm-like area with the surrounding particles; such ensemble certainly corresponds to most chromatic corpuscles seen under the light microscope; also observe the elongated shape usually shown by the nuclear and cytoplasmic crystalloid bodies in ultrathin sections. Noteworthy is the relationship of three particles (arrow) with the nuclear membrane, seeming to be acquiring their envelope during the budding to it. x 21,040. Fig. 11. Some particles are scattered in the cytoplasm while other are near the nucleus although their relationship with it is not clear. x 26,670. Fig. 12. A group of particles which have a single surrounding membrane (smaller arrow) is limited by a common membrane (larger arrow). x 32,600. Fig. 13. Observe the closely packed particles. x 32,600.

Figs. 14-18. Electronmicrographs of *Toddia* infected cells. V, viroplasm-like area. Fig. 14. Particles at different developmental stages around the viroplasm-like area; some of them have a more electron dense central area (nucleoid) (arrows) x 48,970. Fig. 15. A particle at assembly stage is being involved by a double membrane (longer arrow), while material (shorter arrow) from the viroplasm-like area seems to be condensed and introduced into it. x 53,350. Figs. 16 and 17. Particles seem to emerge from the viroplasm-like area and to be simultaneously involved by membranes. x 53,350 and x 65,210. respectively. Fig. 18. The shorter arrow points to the rippled envelope of a particle, the longer one to two particles enclosed in a common vacuole. x 53,350.

Figs. 19-22. Electronmicrographs of *Toddia* infected cells. Fig. 19. The particles have only one surrounding membrane (longer arrow). The two shorter arrows indicate the trilaminar structure similar to the "core membrane", Note some aspects of the portion delimited by such membrane, especially the electron dense central area (nucleoid) of the particle near the upper right corner. x 118,770. Fig. 20: A complete particle with two membranes (shorter arrows) and a distinct electron lucent space between them; the inner membrane is closely contignuous to the capsid-like layer, while the outer one seems to delimit a vacuole where the particle is included. The longer arrows show some condensation types of the central area (nucleoid) of a particle. x 118,770. Fig. 21. The smaller arrows point to the layer of small and little differentiated elements which suggest to be a capsid; the longer arrow shows the triple layered structure analogous to the "core membrane". The two membranes usually found around a particle are indicated by the thicker arrows. x 157,090. Fig. 22. Note the structural similarity between the plasma membrane and those that surround the particles (arrows). x 133,380.

Figs. 23-25. Electronmicrographs of *Toddia* infected cells. N, cell nucleus; CB, crystalloid body Fig. 23. Crystalloid body (intranuclear inclusion) whose shape is hardly got in ultrathin sections; it may be compared with that in Fig. 7 (arrow); one of its sides is limited by a membrane of nuclear origin. Note the structural orderliness of both intranuclear and cytoplasmic crystals. x 41,500. Fig. 24. Three particles are in the space between the two layers of the nuclear membrane (larger arrow); they seem to be acquiring an envelope by budding at the nuclear membrane (smaller arrow). Note the outline and electron density of the crystalloid body in this figure. x 32,600. Fig. 25. The internal periodicity of the crystal resembles a crystalline lattice. x 65,210.

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