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A Possible Iridovirus in Erythrocytes of *Bufo marinus* in Costa Rica

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ABSTRACT: Icosahedral viral particles were found in the cytoplasm of erythrocytes and splenic reticular cells of a marine toad (*Bufo marinus*) collected from Costa Rica. Capsids had a maximum diameter of 312 nm and a spherical core with biphasic electron density. Viruses in erythrocytes were associated with cytoplasmic assembly areas and vacuoles in cytoplasm. Nuclei had finely granular material of decreased electron density located centrally, but contained no viral particles. A group of unenveloped viral particles was seen extracellularly in a splenic vessel. The virus was consistent with an iridovirus.

In a blood smear stained with Giemsa round basophilic bodies with average diameters of 1.70 μm and morphologically similar to *Pirhemocyton* sp. were seen in the cytoplasm of erythrocytes and occasionally in the cytoplasm of monocytes or extracellularly. Erythrocytes containing these bodies had vacuoles and irregular pale-staining areas in the cytoplasm and pale-staining areas in the nucleus. These changes corresponded to the viral particles, assembly areas, vacuoles and nuclear changes at the ultrastructural level.

Key words: *Bufo marinus*, iridovirus-like, marine toad, icosahedral viral particles, morphology.

Concern about the effect of the introduced toad *Bufo marinus* on the Australian fauna prompted a study into its diseases. As part of this study, 32 toads were collected in Costa Rica (11°18′N, 85°21′W), killed by pithing, the body cavity opened ventrally and the carcase immersed in 10% formalin. A single blood smear was made from each toad at the time of collection. The preserved carcases subsequently were examined in Australia for gross and microscopic pathology using standard techniques. Blood smears were stained using Giemsa. Using routine techniques, formalin-fixed spleen was examined by transmission electron miscroscopy. This communication reports the finding of a virus in one of these toads.

The toad was a mature female weighing 339 g with snout–urostyle length of 137 mm collected from Estacion Maritza, Gramacaste Province, Costa Rica. There were no gross pathological changes. Fat bodies and liver comprised about 1 and 3%, respectively, of the total body weight.

On histological examination of sections stained with haematoxylin and eosin, many erythrocytes were noted to have nuclear abnormalities consisting of a homogeneous, strongly eosinophilic centrally located region with margination of nuclear chromatin. These changes were particularly noticeable in erythrocytes in the spleen. The cytoplasm of erythrocytes also contained bodies, but these were not as obvious in histological sections as the nuclear changes. The cytoplasmic bodies stained positively with Fuelgen stain, but the intensity of staining was greater at the periphery and almost absent centrally in some bodies. There were no other microscopic pathologic changes. These abnormalities of erythrocytes were not seen in histological sections from the other 32 toads.

In the blood smear 21% of red blood cells (n = 526) contained small, roughly spherical, basophilic bodies in the cytoplasm (Fig. 1). Size was 0.81 to 3.24 μm (1.70 ± 0.39 μm; n = 50) and 96% of infected cells contained one body, while 4% contained two. Variations from this pattern were uncommon, but included poorly staining cytoplasmic masses with a clear center and an ill-defined periphery, and irregularly shaped basophilic masses of smaller size than the spherical bodies. Occasionally, basophilic bodies of the same appearance and dimensions as the cytoplasmic bodies were seen free of red cells. Pale oval, circular or irregularly shaped

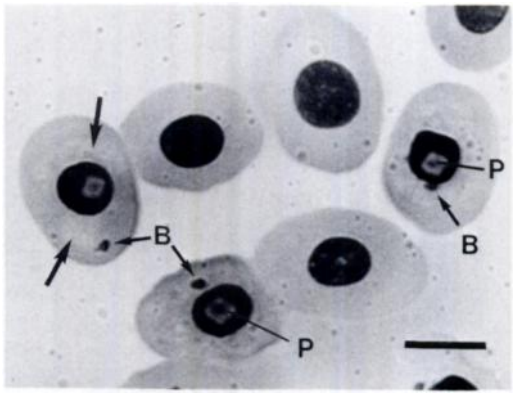

FIGURE 1. Basophilic bodies (B) in the cytoplasm of erythrocytes of a blood smear from *Bufo marinus*. Irregular pale areas are present in the cytoplasm of infected erythrocytes (arrows). Note the central nuclear palor (P). Giemsa. Scale line 10 μm.

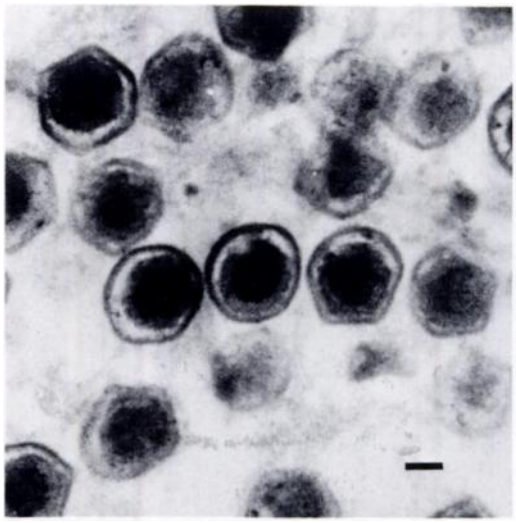

FIGURE 2. Icosahedral viral particles in the cytoplasm of a splenic reticular cell of *Bufo marinus*. TEM. Scale line 100 nm.

areas with abrupt transition from the normal cytoplasmic basophilia were present in the cytoplasm of many red cells (Fig. 1). A very narrow basophilic peripheral zone surrounded some of these paler-staining areas. Ninety four percent of cells containing distinct cytoplasmic bodies (n = 200) had these cytoplasmic changes compared with 2% of cells without cytoplasmic bodies (n = 200). This difference was highly statistically significant ($P < 0.0001$; χ^2) and indicated an association between basophilic bodies and focal cytoplasmic pallor. In blood smears from other toads, cytoplasmic pallor was not seen and the cytoplasm of erythrocytes either stained homogeneously or had diffuse less intensely stained areas with poorly defined margins in the outer two-thirds of the cytoplasm. The nuclei of 20% of red blood cells from the infected toad had circular, paler-staining areas (Fig. 1), measuring 1.62 to 4.86 μm (2.83 ± 0.60 μm; n = 50). These were single in 98% and double in 2% (n = 526). In 99% of instances the nuclear changes were associated with cytoplasmic bodies. Similar nuclear changes were not seen in the erythrocytes of other toads. The dimensions of red cells from the infected toad were 16.19 to 24.28 μm (19.51 ± 1.74 μm; n = 50) by 8.09 to 16.19 μm (13.57 ± 1.48 μm) for erythrocytes with cytoplasmic bodies and 16.19 to 24.28 μm (19.90 ± 1.65 μm; n = 50) by 9.71 to 17.00 μm (13.97 ± 1.31 μm) for erythrocytes without cytoplasmic bodies. There was no significant difference in red cell dimensions ($P > 0.1$; t-test) indicating that the virus was not associated with changes in red cell size.

Viral particles were seen in loose aggregations in the cytoplasm of erythrocytes and reticular cells in the spleen. The capsids of most were hexagonal in cross-section, with occasional pentagonal and circular outlines, indicating an icosahedral symmetry (Fig. 2). The core was spherical with a central spherical region of high electron density surrounded by a thinner outer layer of decreased electron density and was separated from the outer shell by an electron lucent region. The maximum diameters from vertex to vertex of the top 10% of 54 viral particles measured were 293 to 312 nm. Viruses in erythrocytes were associated with changes in the normal homogeneously electron-dense cytoplasm. Incomplete viral particles in various stages of assembly were found on the periphery of viroplasm, roughly circular or oval areas with electron-density less than the normal cytoplasm (Fig. 3). Particles were usually aligned with completed sides of the capsid

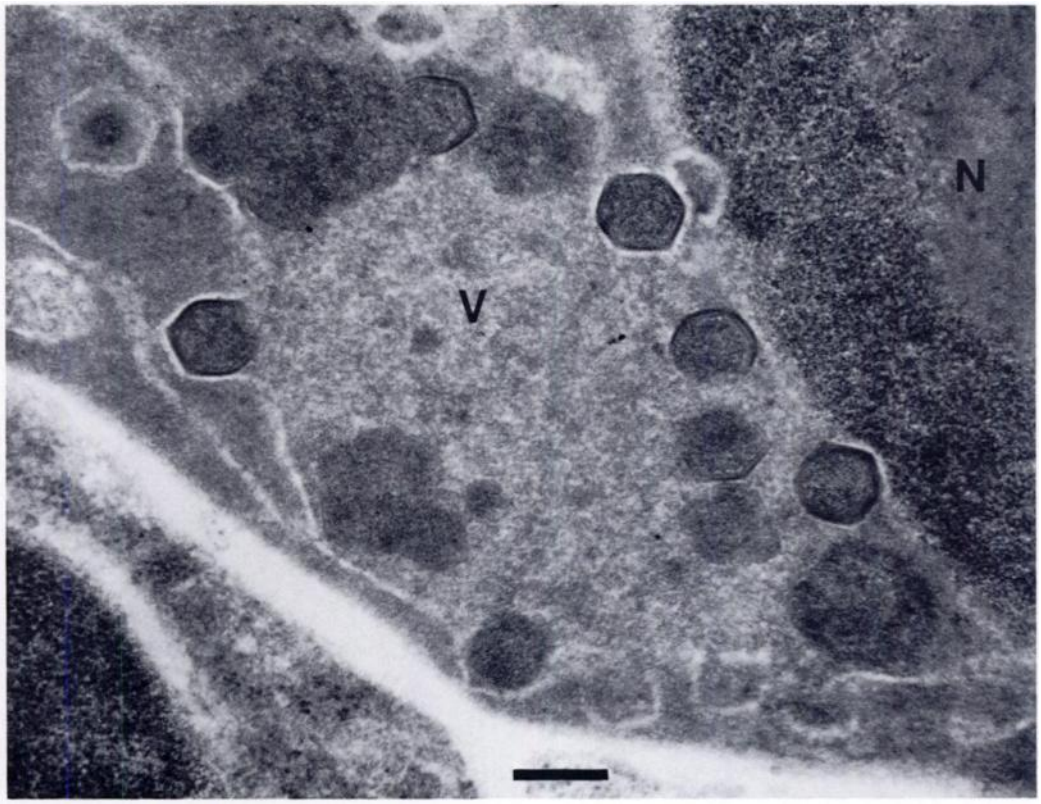

FIGURE 3. Assembly site for viral particles in the cytoplasm of erythrocyte of *Bufo marinus*. Note the incompletely formed capsids surrounding a finely granular viroplasm (V) adjacent to the nucleus (N). TEM. Scale line 500 nm.

away from the area. Completely assembled particles were usually surrounded by a narrow zone of electron-dense material enclosed within a vacuole (Fig. 4). Often these vacuoles were themselves contained within larger vacuoles to form irregular masses of cytoplasmic material. Occasionally particles were aligned longitudinally and enclosed in a cylindrical mass which often appeared to terminate just below the red cell membrane. Membranes surrounding the particles could not be discerned, but cell membranes were also indistinct, possibly as a result of the initial formalin fixation. Viruses in splenic reticular cells were grouped in the cytoplasm and were not associated with any cellular modification.

The nuclei of affected erthrocytes had chromatin of normal electron density peripherally, but with a finer granularity and decreased electron-density centrally (Fig. 4). Usually this transition was quite distinct. Occasionally irregularly shaped vacuoles containing electron dense material were seen in this centrally modified region of the red cell nucleus. No viral particles were observed in the nuclei of erythrocytes or reticular cells. Reticular cells containing viruses had normal nuclei.

A group of viral particles was found extracellularly in a splenic vessel (Fig. 5). The viruses were surrounded by short fibres consistent with fibrin and were not enveloped.

This is the first report of a virus from *B. marinus* (Speare, 1990). The icosahedral symmetry, the spherical core with biphasic electron density, and the dimensions of the particles from *B. marinus* are consistent with the nucleocapsids of Iridoviridae (Gruia-Gray et al., 1989b). Al-

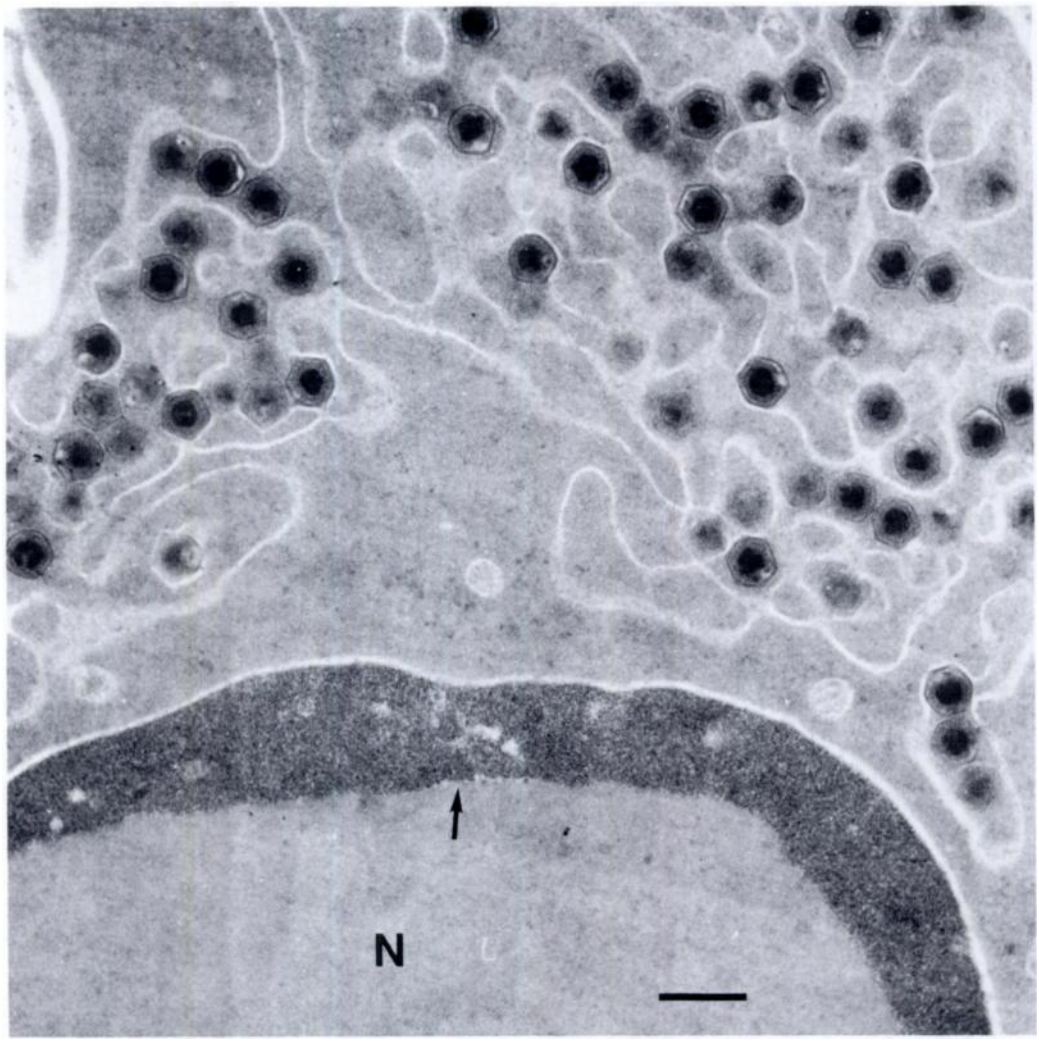

FIGURE 4. Viral particles in the cytoplasm of erythrocytes of *Bufo marinus* associated with extensive cytoplasmic vacuolation. A distinct demarcation (arrow) separates chromatin condensed under the nuclear membrane and less dense material centrally. N = nucleus. TEM. Scale line 500 nm.

though the positive Fuelgen reaction indicated that the virus contained DNA, accurate identification cannot be made without more convincing information on nucleic acid. A number of similar viruses have been reported from erythrocytes of amphibians (Bernard et al., 1969; Sousa and Weigl, 1975, 1976; Desser and Barta, 1984; Gruia-Gray et al., 1989a, b), but only frog erythrocytic virus (FEV) has been identified as an iridovirus from its biochemical properties (Gruia-Gray et al., 1989a, b). The virus from *B. marinus* is intermediate in size to the iridoviruses and other irido-like viruses from amphibians (Table 1). Unlike the virus reported here, these other erythrotropic viruses have not been seen in splenic reticular cells or free in the circulation. The virus from *B. marinus* is larger than the viscerotropic ranaviruses (Iridoviridae) with dimensions of 130–145 nm (Essani and Granoff, 1989). Identification of the virus reported here as an iridovirus is premature.

Before the mid 1970's the spherical bodies in the cytoplasm of erythrocytes of the

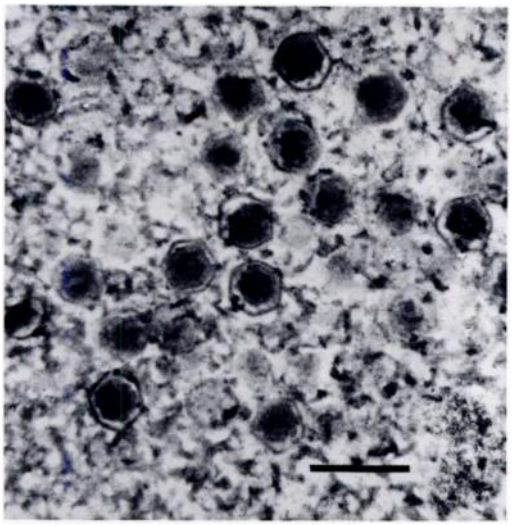

FIGURE 5. Group of viral particles free in the lumen of a splenic vessel of *Bufo marinus*. TEM. Scale line 500 nm.

toad would have been identified as paranuclear corpuscles and, owing to the presence of "globoids or albuminoid bodies" (the poorly staining regions in the cytoplasm), assigned to the protozoan genus *Pirhemocyton* (Marquardt and Yeager, 1967; Sousa et al., 1973). *Toddia*, another protozoan genus, has similar paranuclear corpuscles, but differs from the genus *Pirhemocyton* in that red cell cytoplasm and nucleus contains plate-like crystalline structures (Marquardt and Yeager, 1967; Sousa and Weigl, 1976). The distinction between *Pirhemocyton* and *Toddia* at the level of light microscopy was shown to be dubious (Marquardt and Yeager, 1967; Sousa et al., 1973) and all ultrastructural studies have shown members of these taxa to be viral (Stehbens and Johnston, 1966; Bernard et al., 1969; Johnston, 1975; Sousa and Weigl, 1976; Daly et al., 1980; Desser and Barta, 1984; Gruia-Gray et al., 1989a, b). FEV and the virus from *L. ocellatus* caused plate-like crystals in the cytoplasm of erythrocytes and were consistent with *Toddia* sp. (Sousa and Weigl, 1976; Gruia-Gray et al., 1989a, b). Like the virus from *B. marinus* the virus from *R. pipiens* lacked these structures and gave rise to changes consistent with *Pirhemocyton* (Bernard et al., 1969). There are insufficient data on whether differences in the red cell inclusions reflect taxonomically significant differences in the viruses. If they do, since a *Toddia* sp. was reported from *B. marinus* in Brazil (Pereira et al., 1973), *B. marinus* may possibly be host to two iridoviruses.

The toad infected with this virus had adequate fat stores, glycogen in hepatocytes and no obvious pathological changes. Infected red cells did not vary in size from those without cytoplasmic bodies. The vi-

TABLE 1. Iridoviruses and irido-like viruses of amphibians.

Virus	Diameter (nm)		Host	Site	Reference
	Core	Envelope			
Iridoviruses					
Ranaviruses	110–135	140–170	*R. catesbeiana* *R. pipiens* *T. viridescens*	vicerotropic	Granoff et al. (1965); Clark et al. (1969); Granhoff (1989)
FEV	300–370	360–450	*R. catesbeiana* *R. septentrionalis* *R. clamitans*	red cells	Gruia-Gray et al. (1989a, b)
Irido-like viruses					
—	140	200	*R. catesbeiana*	leucocytes	Briggs and Burton (1973)
Toddia sp.	195	224	*L. ocellatus*	red cells	Sousa and Wiegl (1976),
—	293–312	—	*B. marinus*	red cells RES, plasma	this paper
—	280–380	—	*R. pipiens*	red cells	Bernard et al. (1969)

— = unnamed or data not available.

rus did not appear to be pathogenic to this particular host. The ranaviruses are pathogenic and natural and experimental mortalities have been reported in tadpoles, juveniles and adults (Granoff, 1989). FEV causes a spherocytic anemia in *R. catesbeiana*, but does not appear to result in morbidity or mortality (Gruia-Gray et al., 1989a, b). The potential of the virus described here as a control agent for *B. marinus* is unknown and elucidation will require isolation of the virus and experimental testing.

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