An Erythrocytic Virus of the Brazilian Tree-frog, *Phrynohyas venulosa*

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Blood erythrocytes of Brazilian tree-frogs, Phrynohyas venulosa *were found to frequently contain single, small, densely staining inclusions. Electron microscopy showed these to be icosahedral viral particles which measured from 250-280 nm in diameter: they were devoid of an envelope, and thus differed from previously described viruses of frog erythrocytes. The infected erythrocytes lacked a crystalline body.*

Key words: viral particles - erythrocytes - *Phrynohyas venulosa* - tree-frogs - Brazil

Erythrocytic icosahedral viruses have been found in anurans from North America (Gruia-Gray et al. 1989), Africa (Alves de Matos & Paperna 1993) and neotropical America. In the latter geographic region they have been recorded in the frog *Leptodactylus ocellatus* from the State of Rio de Janeiro, Brazil (Sousa & Weigel 1976) and the toad *Bufo marinus* from Costa Rica (Speare et al. 1991). In this communication we report the finding of erythrocytic icosahedral virus in the tree-frog, *Phrynohyas venulosa* from the State of Pará, North Brazil.

MATERIALS AND METHODS

Blood was collected from a clipped toe, and thin films were air-dried, fixed in absolute methyl alcohol, and stained with Giemsa (30 drops of stain to 15.0 ml of distilled water buffered to pH 7.4) for 1 hr. Blood collected into a glass capillary tube was allowed to coagulate before extraction and fixation in 2.5% glutaraldehyde in 0.1m cacodylate buffer (pH 7.4) for 24 hr at 4°C. After post-fixation in 1.0% osmium tetroxide in the same buffer, and consecutive rinsing in the buffer and distilled water, the material was stained, en-bloc, in uranyl acetate. The clot was then rinsed in distilled water, dehydrated in graded ethanol, and embedded in Agar 812 medium (Agar Company, U.K.). Thin sections, cut on a LKBIII microtome with a diamond knife, were stained on grids with uranyl acetate and lead citrate and examined with a JEOL 100S transmission electron microscope.

RESULTS

Densely stained, purple-red inclusions were found in the erythrocytes of 7 out of 33 tree-frogs collected in Capanema, 50 km East of Belém, Pará, between October and December 1992. Among the positive frogs, three developed heavy parasitaemias.

The inclusions were usually single, and uniformly small (about 1.5-2.5 µm in diameter). The erythrocytic vacuoles associated with the inclusions were also small (Figs 1-3) and unlike the large ones described for erythrocytic viral infections in other anurans. Crytalline bodies were absent.

Electron-microscopic examination revealed virions 250-280 nm in diameter, of hexagonal outline and compatible with an icosahedral shape (Figs 4-7). The virion nucleoid was either uniformly dense (Fig. 7), or made up of concentric dense and lucent layers (Fig. 6). Some others possessed a dotted pattern (Fig. 8). The polygonal shell (capsid) was closely applied, but a number of empty or even peeled-off capsids were seen (Figs 5-7).

Virions occurred in an intranuclear inclusion (Fig. 5) or in the cytoplasm (Figs 4,6). One cytoplasmic inclusion assemblage contained empty and defective particles, and also one with a long extension derived from the capsid (Fig. 4). Virosomes without virus had an internal fibrilar area resembling accumulated DNA (Fig. 9). Membranous inclusions occurred in the cytoplasm of some cells, but the virion-associated envelopes demonstrated in other anuran erythrocytic viruses were lacking (Fig. 10).

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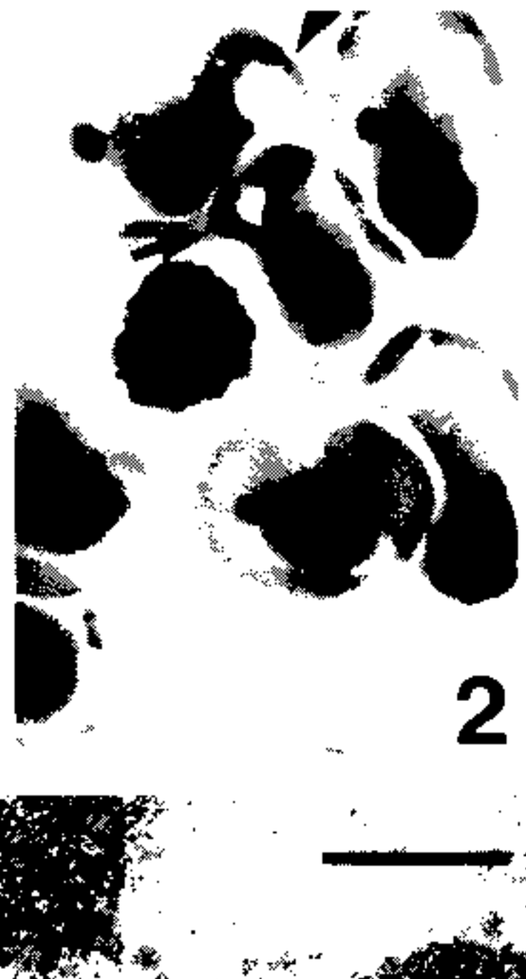
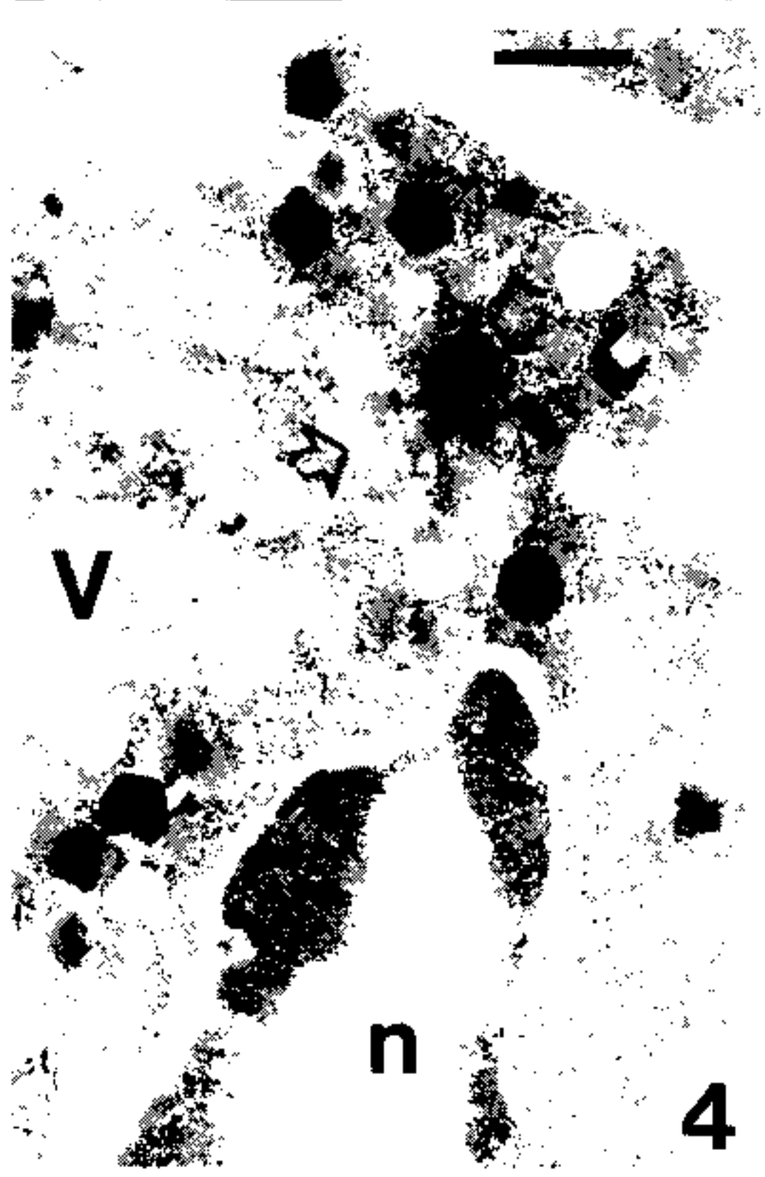
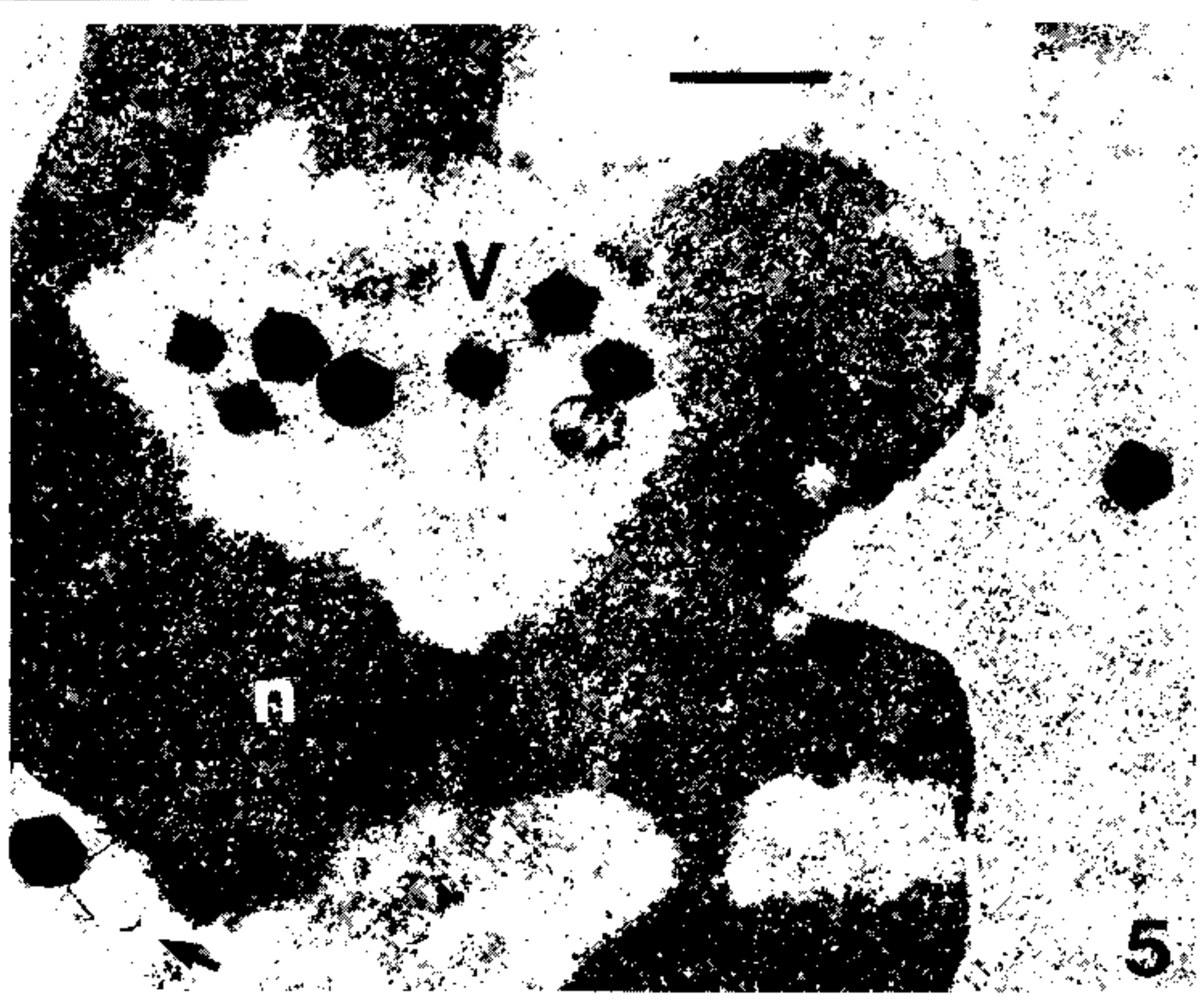
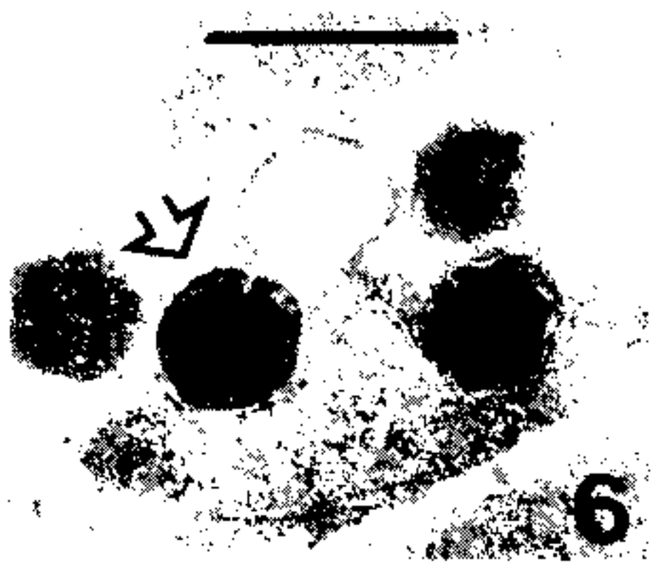




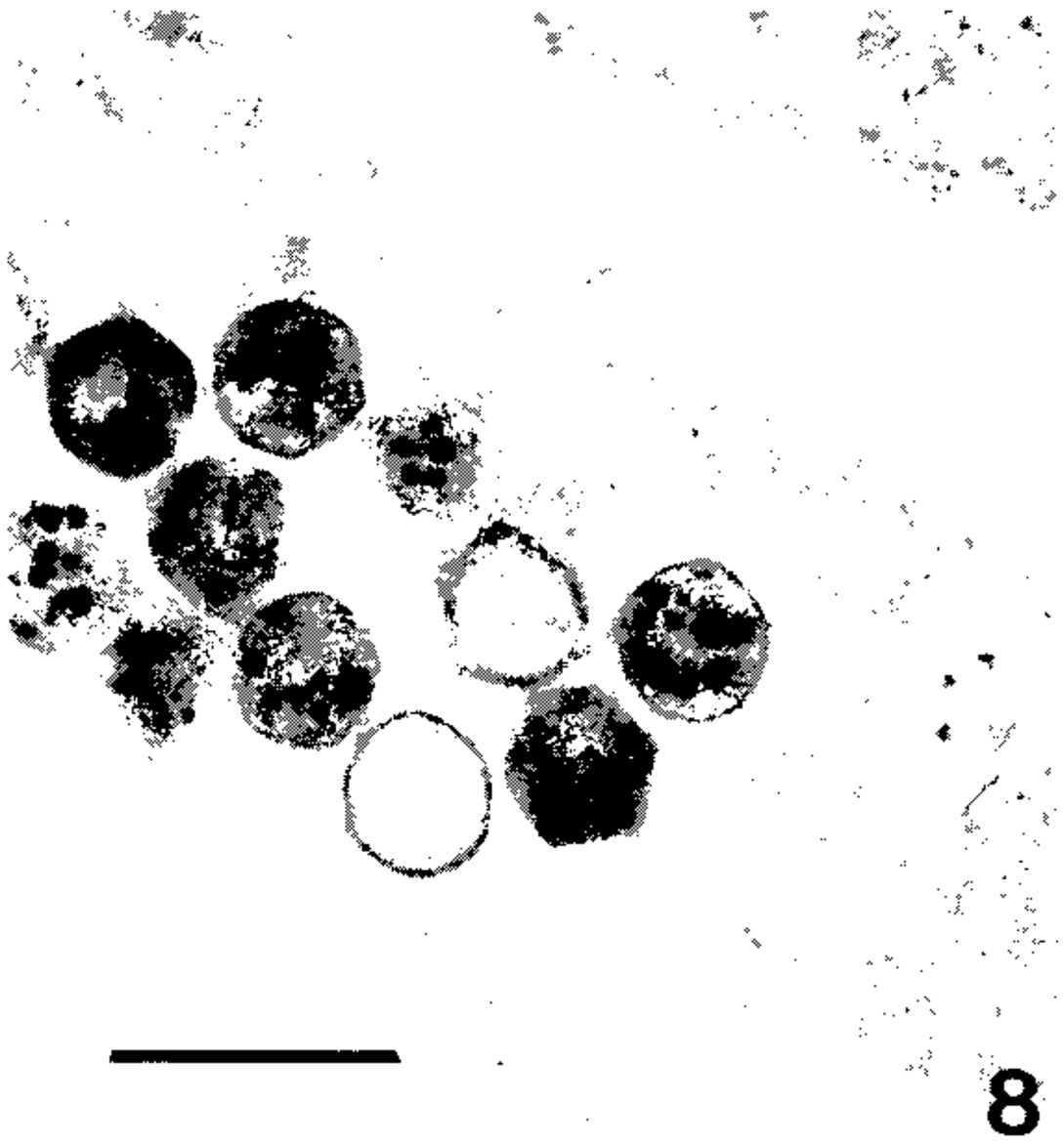




An erythrocytic virus of the Brazilian tree-frog, *Phrynohyas venulosa*. Figs 1-3: infected erythrocytes in a Giemsa-stained blood film: note the variable size of the vacuoles, some of which adjoin the viriosomes (arrows): scale bar = 10.0 µm. Figs 4-7: transmission electron-microscope view of infected erythrocytes (scale bar = 1.0 nm). Fig.4: with complete and empty virions and a capsid extension (arrowed): n = erythrocyte nucleus; V = virosome (inclusion). Fig.5: intranuclear viral inclusion, with a peeled-off virion shell (arrowed). Fig.6: virion with heterogenic nucleoid material (arrowed). Fig.7: enlarged view of virions in the intranuclear inclusion shown in Fig.5.

DISCUSSION

Comparing the presently described material with that recorded from other anurans, the virus of *P. venulosa* approximates most closely to the size range (200-300 nm) of erythrocytic viruses from *Rana pipiens* (Bernard et al. 1968). These, and all other anuran erythrocytic viruses (with the exception of those from *B. marinus*, Speare et al. 1991), however, possess a virion-associated envelope, which is lacking in the organism from *P. venulosa*. Again, the infected erythrocytes harbouring the presently described virus have no crystalline body - commonly seen associated with



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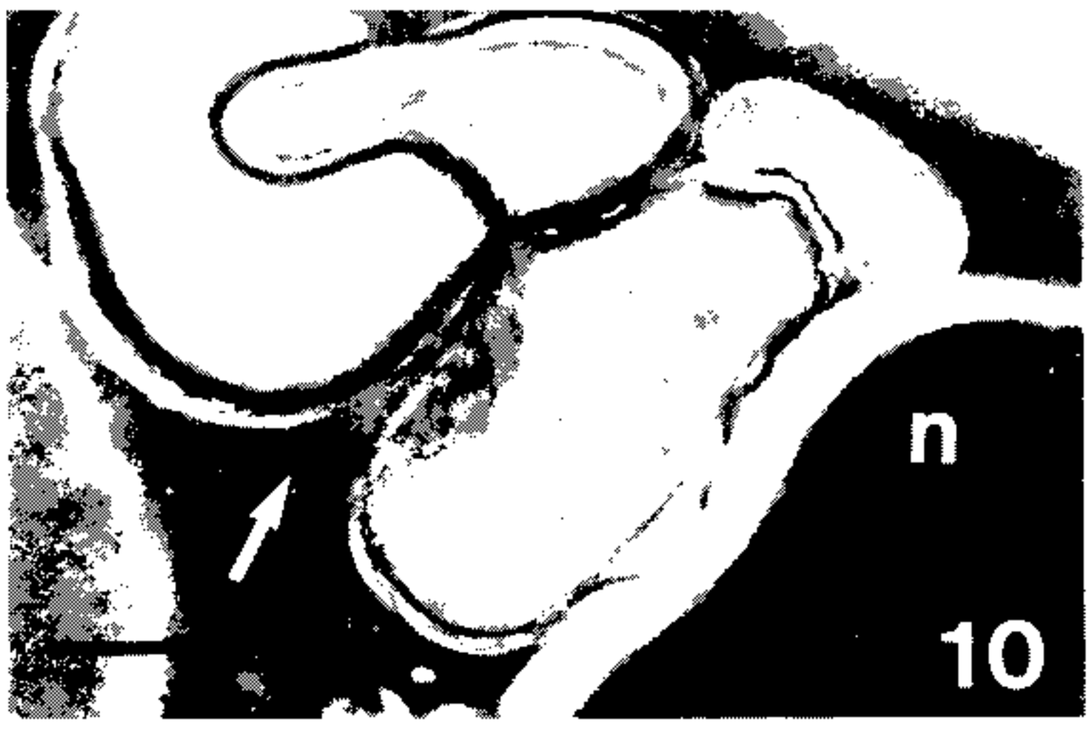

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An erythrocytic virus of *Phrynohyas venulosa* (scale bar = 1.0 nm). Fig. 8: virions with "dotted" nucleoids. Fig. 9: virosome with internal areas suggestive of DNA accumulation (arrowed). Fig.10: membrane-bound cytoplasmic inclusion (arrowed) in a virus-infected erythrocyte; n = erythrocyte nucleus.

the erythrocytic viruses of *L. ocellatus*, *Rana catesbeiana* and *Ptychadena anchietae* (Sousa & Weigel 1976, Gruia-Gray et al. 1989, Alves de Matos & Paperna 1993).

The absence of crystalline bodies, and the small size of the vacuoles in the infected erythrocytes, does not seem to reflect a particular stage of differentiation of the virus in *P. venulosa*, because these same characteristics were present in all the material collected from seven different, infected frogs, taken at various intervals during the months of October-December.

We conclude that frog erythrocytic viruses are probably host-specific, and that the crystalline body is not, as earlier implied (Alves de Matos & Paperna 1993), characteristic of all anuran infections, but is lacking in some.

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