

## NOTE ON THE PRESENCE OF VIRUS-LIKE AGGREGATES AND PARTICLES OF UNKNOWN ORIGIN IN THE TISSUES OF A COLUBRID SNAKE (*XENODON MERREMII*)

L. C. U. JUNQUEIRA (1)

### SUMMARY

Intracellular virus-like aggregates and extracellular particles of unknown origin were found during the electron microscopic study of tissues of the ophidian *Xenodon merremii*. In 11 snakes studied, 4 presented virus-like aggregates formed by crystal like oriented particles with an average diameter of 500 Å.

### INTRODUCTION

During the observation of snake tissues on the electron microscope a serie of organs were observed whose cells contained virus-like aggregates. As the frequency of these particles was relatively high and recently attention has been drawn to the fact that reptiles and amphibia can be virus reservoirs (for literature see BURTON et al.<sup>1</sup>) we thought it of interest to publish these results.

### MATERIAL AND METHODS

Eleven adults *Xenodon merremii* of both sexes collected at random in the State of São Paulo were used.

Small tissue fragments were fixed in MILLONIG's<sup>3</sup> osmium tetroxide phosphate buffer, dehydrated and embeded in Araldite according to LUFT<sup>2</sup>.

Thin sections were double stained in uranyl acetate and lead hydroxide.

Observation was performed in a Zeiss EM 9 electron microscope. Several sections of 2 to 4 fragments of each organ were observed.

### RESULTS AND DISCUSSION

The organs studied in the 11 snakes used were, the digestive glands and tract, adrenal gland, thyroid, hypophysis and lung. In these animals observed 4 presented aggregates with a virus-like morphology in different organs as shown in Table I.

TABLE I  
Occurrence of virus-like aggregates in the cells of some organs of *Xenodon merremii*

Snake number	Organ(s) with aggregate
3,459	Small intestine reabsorptive epithelium
3,555	Adrenal gland "cortical" cells and capillary endothelial cells
3,602	Adrenal gland "cortical" cells. Salivary gland epithelial cell. Lung endothelial cell. Large intestine epithelium.
3,624	Thyroid epithelial cell. Stomach capillary endothelium and superficial epithelium.

(1) Laboratório de Fisiologia Celular. Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brasil

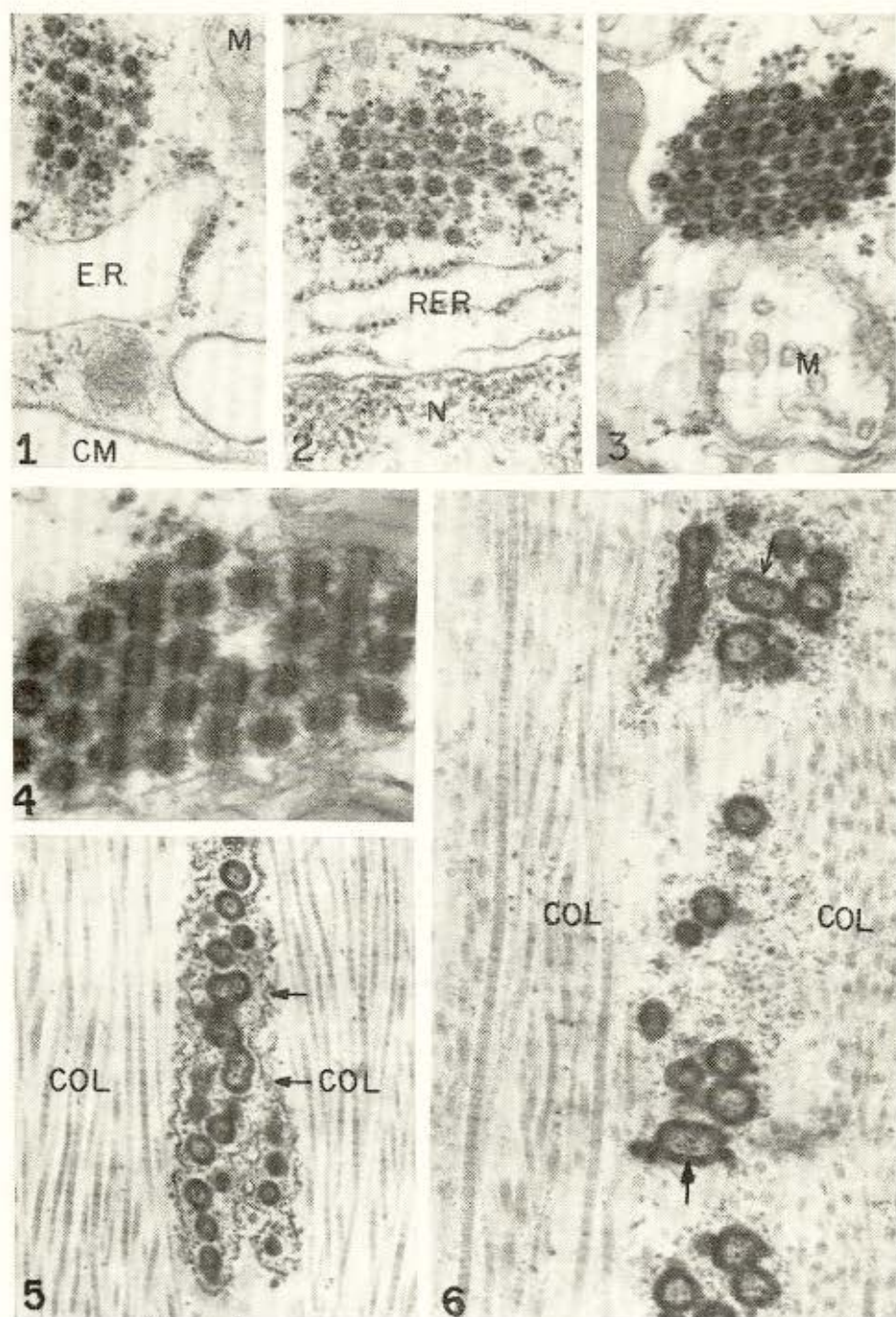


Fig. 1 — Small aggregate found in the endothelial cell of the stomach. 60,000 X. In M a mitochondrion, CM is cell membrane and ER endoplasmic reticulum. Fig. 2 — Aggregate in a thyroid epithelial cell. Observe in this fig. and the next the presence of an electron dense outer shell in the particles. Orderly arrangement of the particles with a crystalline like structure. The aggregate is in the outside of the abundant rough endoplasmic reticulum. 60,000 X. Fig. 3 — Aggregate found in a adrenal "cortex" cell. In M a mitochondrion and at the left of the inclusion a lipid droplet. 60,000 X. Fig. 4 — Another aggregate in an adrenal "cortex" cell. 120,000 X. Fig. 5 — Extracellular aggregate particles of unknown origin in the connective tissue of the stomach. Observe figures that might be due to binary fission (arrows). In COL collagenic fibers, 25,000 X. Fig. 6 — Another aggregate of the same particles found in the same tissue. 40,000 X.

As to the morphology of the aggregates, they always were formed by spherical particles measuring approximately 500 Å of diameter. The aggregates were of various sizes sometimes relatively small with 8 particles appearing in section but usually larger (Figs. 1-2-3 and 4). They were always intracellular and free in the cells ground cytoplasm, never surrounded by a membrane or in cellular organelles such as endoplasmic reticulum, nucleous, Golgi vacuoles, mitochondria, etc.

The spherical particles presented a distinct and relatively thick electron dense outer portion and usually were disposed in a orderly arrangement simulating a crystalline structure (Figs. 2-3 and 4).

Between the spherical particles, small round electron dense elements with morphology resembling ribosomes were constantly observed.

The highest incidence of aggregates was observed in capillary endothelial cells of the different organs studied.

The electron microscope is not well adapted to survey the presence of virus in animals collected in nature. Thus only some sections of 2 to 4 fragments of some but not all organs of the snakes we studied. We used therefore a poor sampling method. Despite this fact the incidence of the inclusions is relatively high and one is consequently led to suspect that it is probably much higher. It is interesting to correlate this observation with the data of BURTON et al.<sup>1</sup> who observed a high incidence (36.2%) of serum neutralizing antibodies to Western equine encephalitis in snakes of the genus *Thamnophis*. The Authors could also in some cases isolate the virus from the blood of these animals. They conclude that their data suggest a wide distribution of Western equine encephalitis in the snakes and frogs studied. These results agree with the observations that some cold blooded animals can be susceptible to encephalitis virus (THOMAS et al.<sup>4</sup> and VOROB' EVA & LEVKOVICH<sup>5</sup>).

In one of the snakes studied (n.º 3,624) extracellular particles were also observed in the connective tissue of the stomach's wall (Figs. 5 and 6). The particles here were of much larger size (diameter of approximately 2,000 Å) and were not aggregated in an orderly fashion. They presented a thick outer

portion and in some particles a dense granular content was visible. Some morphological aspects suggesting a binary fission were observable (arrows in Figs. 5 and 6). Their aspect and size suggest more the possibility of being a submicroscopic microorganism of non viral nature.

#### RESUMO

*Nota sobre a presença de agregados viróides e partículas de origem desconhecida nos tecidos de um ofídio (Xenodon merremii)*

O Autor descreve a presença de inclusões intracelulares com morfologia de vírus em tecidos do ofídio *Xenodon merremii*. As partículas se dispõem em arranjo cristalino e têm em média o diâmetro de 500 Å. Foram encontradas em 4 das 11 cobras estudadas ao microscópio eletrônico. Foram também descritas partículas extracelulares de origem desconhecida, com o diâmetro médio de 2.000 Å.

#### ACKNOWLEDGEMENT

The Author is grateful to the "Instituto Pinheiros" for donating the specimens used.

#### REFERENCES

1. BURTON, A. N.; Mc INTOCH, J. & REMPEL, J. G. — Western equine encephalitis Virus in Saskatchewan Garter Snakes and Leopard Frogs. *Science* 154:1029-1031, 1966.
2. LUFT, J. — Improvements in epoxy resin embedding methods. *J. Bioph. & Biochem. Cytol.* 9:409-414, 1961.
3. MILLONIG, G. — Advantages of a phosphate buffer for Os O<sub>4</sub> solutions in fixation. *J. Appl. Phys.* 32:1637, 1961.
4. THOMAS, L. A.; EKLUND, E. M. & RUSH, W. A. — Susceptibility of garter snakes (*Thamnophis*, sp) to WEE virus. *Proc. Soc. Exp. Biol. & Med.* 99:697-698, 1958.
5. VOROB'EVA, M. S. & LEVKOVICH, E. N. — Susceptibility of cold-blooded animals to thick-borne encephalitis virus. *In Fed. Proc. (Transl. Suppl.)* 24:936-938, 1965.

Recebido para publicação em 25/10/1967.