

Zusammenfassung. Es hat sich gezeigt, dass eine nahe Wechselbeziehung zwischen der Vermehrungsgeschwindigkeit der symbiotischen *Chloralla*-Bevölkerung im *Paramecium bursaria* und der Vermehrungsgeschwindigkeit des Wirts besteht, wenn man diese Kultur der Lichteinwirkung aussetzt. Die durchschnittliche (mittlere) An-

zahl von Algenzellen pro Protozoenzelle ist in Massenkulturen von Lichtstärke und Einwirkungszeit unabhängig.

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On the Presence of Multivesicular Bodies in *Leishmania donovani*

Single membrane limited cytoplasmic bodies containing small vesicles enclosed in a smooth membrane, called multivesicular bodies, are often found in the cytoplasm of many cellular types in metazoan animals (HRUBAN and RECHCIGL¹). The matrix of multivesicular bodies shows the activity of acid phosphatase (MOE et al.²). The role of these bodies in lytic processes has been demonstrated by BIBERFELD et al.³ and by SMITH and FARQUHAR⁴. It was suggested that multivesicular bodies arise by sequestration of the vesicles formed in the Golgi complex (BIBERFELD et al.³; ERICSSON and GLINSMANN⁵; SMITH and FARQUHAR⁴).

It is the purpose of this paper to report on the presence of multivesicular bodies in *Leishmania donovani*, a parasitic protozoan belonging to the family Trypanosomatidae. The relation of the multivesicular bodies of *Leishmania* to Golgi complex will also be discussed.

L. donovani was kept in vitro for several years in diphasic medium prepared according to TOBIE et al.⁶. For electron microscopy the cells were fixed for 5 h at 4°C in 3% glutaraldehyde buffered with *s*-collidine. The cells were postfixed in 4% unbuffered osmium tetroxide solution for additional 14 h at the same temperature. After dehydration in ethanol, the cells were embedded in Epon 812. The sections were stained with lead citrate.

All cells present in the culture medium were in the leptomonad form. For general ultrastructural data of these cells, see original papers by RUDZIŃSKA et al.⁷ and DJACZENKO et al.⁸. The Golgi complex of *Leishmania* was most frequently situated in a juxtannuclear position (Figure 1). It was composed of parallel arrays of flattened sacs and vacuoles. Some of them were slightly distended and contained accumulations of small smooth-walled vesicles with watery content. The amount of vesicles per single Golgi complex was variable. Multivesicular bodies of *L. donovani* (Figures 2 and 3) were limited by a single membrane 95 Å thick. The whole interior of the body was tightly packed with vesicles having similar

morphological properties as the vesicles seen in the Golgi complex. Some of the vesicles had the tendency to dissolve in the matrix of the body. In some instances the

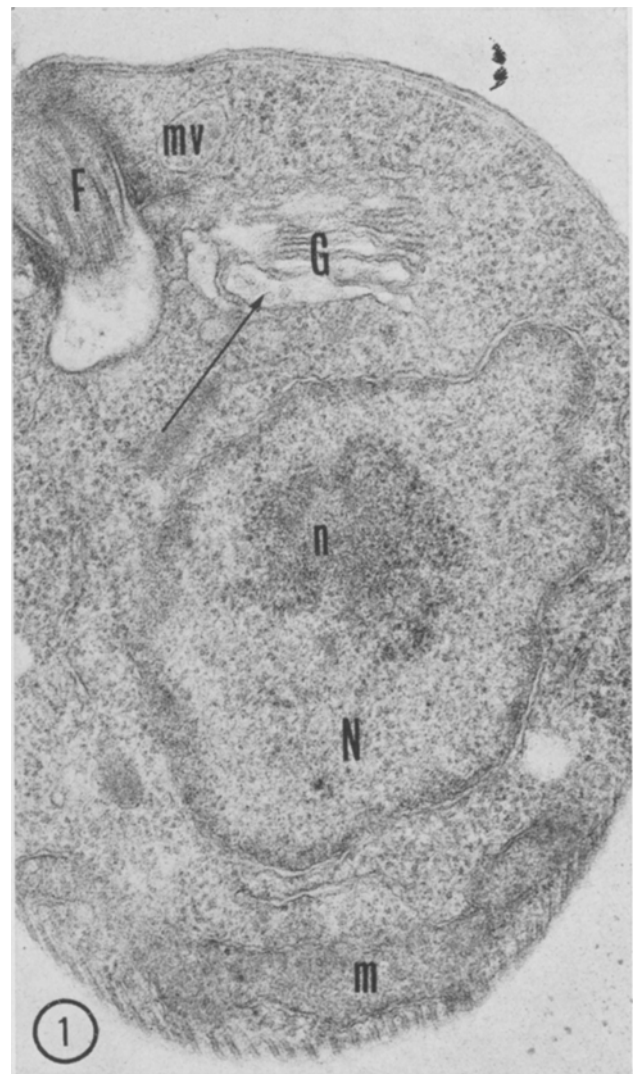


Fig. 1. Golgi complex (G) contains in one of its sacs small smooth vesicles (arrow). Multivesicular body (mv) is seen in the proximity of the Golgi complex. The nucleus (N) contains prominent nucleolus (n). At the cellular periphery is visible a mitochondrion (m). A part of the flagellum is marked (F). $\times 36,000$.

¹ Z. HRUBAN and M. RECHCIGL, Int. Rev. Cytol., in press (1969).

² H. MOE, J. ROSTGAARD and O. BEHNKE, J. Ultrastruct. Res. 12, 396 (1965).

³ P. BIBERFELD, J. L. E. ERICSSON, P. PERLMAN and M. RAFTELL, Z. Zellforsch. 71, 153 (1966).

⁴ R. E. SMITH and M. G. FARQUHAR, J. Cell Biol. 31, 319 (1966).

⁵ J. L. E. ERICSSON and W. H. GLINSMANN, Lab. Invest. 15, 750 (1966).

⁶ E. J. TOBIE, T. VON BRAND and B. MEHLMAN, J. Parasitol. 36, 48 (1950).

⁷ M. A. RUDZIŃSKA, P. A. D'ALESSANDRO and W. TRAGER, J. Protozool. 11, 160 (1964).

⁸ W. DJACZENKO, F. FILADORO and R. PEZZI, Riv. Biol., in press (1969).

multivesicular bodies contained membranous fragments (Figure 2). Whorls of membranes and lysosome-like particles were often seen in the proximity of the bodies (Figure 3).

There is no indication in the literature of the existence of multivesicular bodies in protozoa. Mitochondrial structures ordinarily tightly packed with vesicles and considered to be similar to multivesicular bodies of higher animals are the only reports on the subject (ANDERSON and BEAMS⁹; ANDERSON¹⁰; GAVIN et al.¹¹; WANKO et al.¹²). Detailed description of *L. donovani* by RUDZIŃSKA et al.⁷ does not contain any remark on the existence of multivesicular bodies in this species. This fact may be due to older preparative techniques used by these authors. These techniques probably were not suitable to preserve adequately the structures mentioned. Another explanation may be that RUDZIŃSKA et al.⁷ studying the transformation of *Leishmania* to leptomonad form of *L. donovani* limited their observations to the period of 21 h following the passage of cells from intracellular to extracellular biotopes. It is possible that during such a short period the multivesicular bodies had not yet appeared. Our observations show convincingly that vesicles of the multivesicular bodies have their origin in the Golgi complex. Mature multivesicular bodies contain also membranous fragments, which, according to BIBERFELD et al.³, can be considered as proof of their lytic activity. Close spatial relationship of multivesicular bodies to whorls of mem-

branes and primary lysosomes as seen in our material serves as an additional proof in favour of such an assumption.

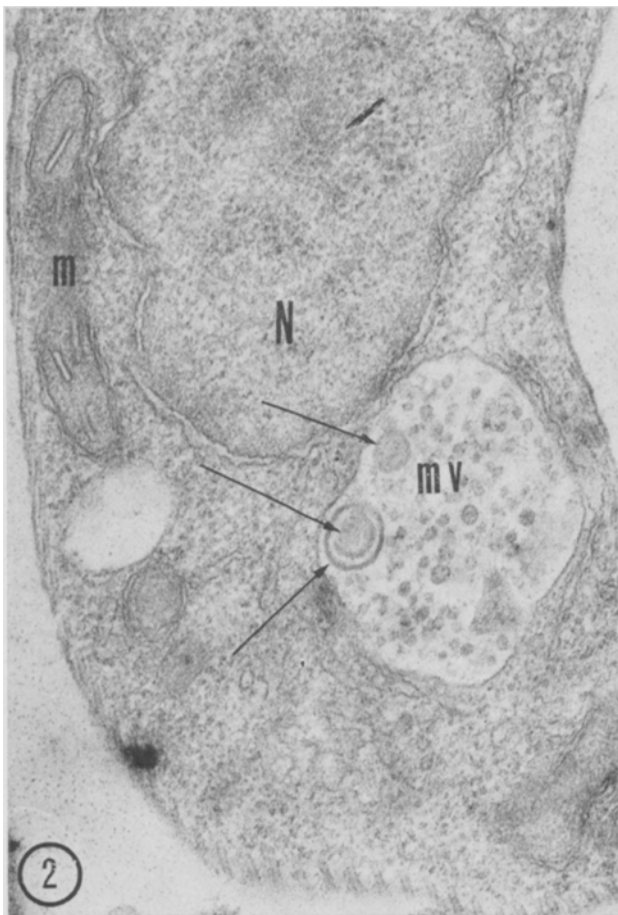


Fig. 2. The multivesicular body (mv) contains vesicles and fragments of cytoplasmic membranes (arrows). $\times 48,000$.

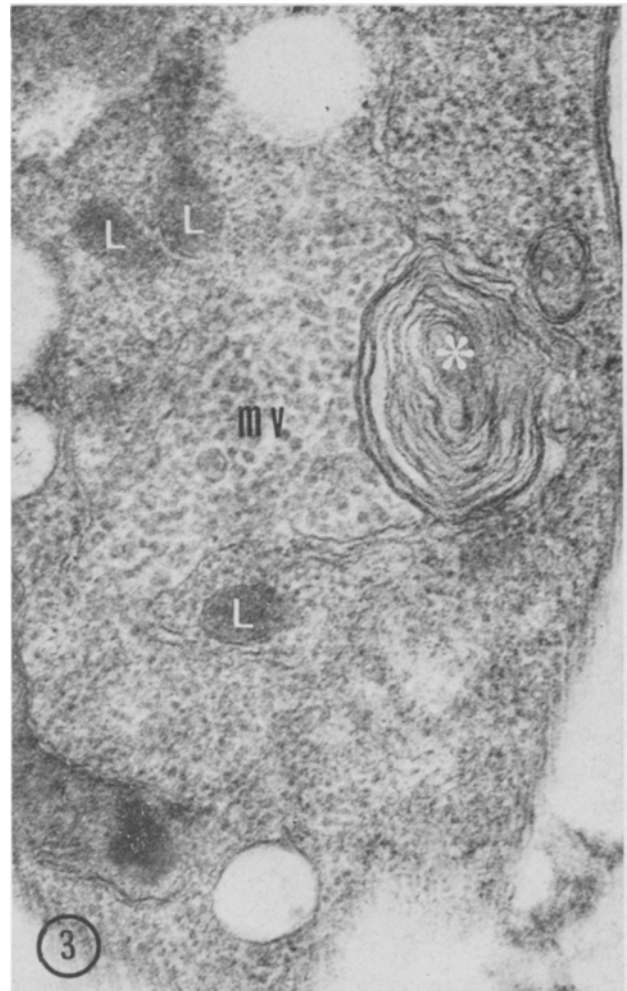


Fig. 3. The multivesicular body is located in the vicinity of the whorl of membranes (white asterisk). Many lysosome-like bodies (L) are easily discernible at the periphery of the multivesicular body. $\times 48,000$.

Riassunto. Nel presente lavoro gli autori hanno riferito la presenza di corpi multivescicolari in *Leishmania donovani*. Le vescicole di questi corpi si formano nei vacuoli dell'apparato di Golgi. Le immagini al microscopio elettronico mostrano chiaramente i segni dell'attività litica dei corpi multivescicolari.

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00100 Roma (Italy), 13 January 1969.*

⁹ E. ANDERSON and H. W. BEAMS, J. Protozool. 7, 190 (1960).

¹⁰ E. ANDERSON, in *Research in Protozoology* (Ed. TZE-TUAN CHEN; Pergamon Press, London 1967), p. 4.

¹¹ M. A. GAVIN, T. WANKO and L. JACOBS, J. Protozool. 9, 222 (1962).

¹² T. WANKO, L. JACOBS and M. A. GAVIN, J. Protozool. 9, 235 (1962).