Table II. Effects of adenine on the inhibition of the growth of *E. coli* strain K12 caused by azaserine and DGE

	Azaserine concentration (μg/ml)					
Adenine	0	0.0001	0.001	0.01	0.1	1
–	14.6	8.9	28.1	82.3	100	–
+	0	0	6.2	14.6	91.5	100

	DGE concentration (μg/ml)				
Adenine	0	1	10	100	1000
–	14.6	8.9	14.6	35.4	91.5
+	0	3.1	14.6	49.6	91.5

The values are the % inhibition of the growth of bacteria in MM, supplemented when indicated with 0.38 m*M* adenine, in the presence of the drugs, in respect to the controls. Each value is the mean of 3 determinations.

Interactions of this type have been reported to occur when purified calf thymus DNA is incubated in vitro with DGA[11]. The lack of a differential sensitivity between W3110 and pol A⁻ strains suggested that these drugs did not react extensively with the bacterial DNA.

An interesting comparison can be made between the effects of these drugs on bacterial and mammalian cells. DGA and DGI, which do not affect the growth of *E. coli*, are very active immunodepressant and antitumour agents[2,6]. In contrast, DGE, which in this group of substances exhibits the greatest capacity of inhibiting bacterial growth, has no effect on tumour or immunocompetent cells. At first sight it might be suggested that the selective activity of these diazoacetyl-derivatives is related to their different liposolubility, and accordingly, to the diversity of surface structures of bacterial and mammalian cells. However, the analysis of the chemical structures and biological activities for some alkylating agents did not show any strict linear correlation between the partition coefficients and the antitumour properties for the drugs considered[12]. Further, the screening of various sulphonamide derivatives has shown that no relationship exists between partition coefficient and minimal concentration required for inhibition of bacterial growth in vitro[13].

In any case, the preliminary analysis of the properties of the drugs discussed in this paper seems to encourage the synthesis and the evaluation of the biological properties of new molecules characterized by the presence of the α-diazocarbonyl moiety. In this context, particularly attractive seem to be derivatives of metabolites involved in biosynthetic pathways peculiar to bacteria, since possibly drugs having the antibiotic effectiveness of azaserine, with a much lesser toxicity to the host, might result[14].

Riassunto. Gli effetti di una serie di N-diazoacetil derivati della glicina, alcuni dei quali possiedono notevole attività immunosoppressiva ed antineoplastica, sono stati studiati sulla crescita di *E. coli*. La diazoacetilglicina etilestere, praticamente priva di effetti farmacologici, si è dimostrata la più efficace nell'inibire la crescita batterica, con una DI_{50} di 50 μg/ml.

T. Giraldi, C. Monti Bragadin and R. della Loggia

Istituto di Farmacologia dell'Università, Via A. Valerio 32, I–34100 Trieste (Italy); and Istituto di Microbiologia dell'Università, Trieste (Italy), 23 November 1973.

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Plasmodium juxtanucleare: An Electron Microscopic Study of the Exoerythrocytic Stages[1,2]

Plasmodium juxtanucleare, a malarial parasite of the domestic fowl, was first described in Brazil by Versiani and Gomes[3]. Since the first studies it has been observed that this parasite provokes only a slight infection in the blood of chicks, which causes a weak immunity and very seldom gives exoery throcytic infection[4].

The exoerythrocytic stages, as an intermediate between the sporozoites from the mosquito and the erythrocytic phase, constitute an essential link in the cyclical development of a malarial parasite. Few ultrastructural studies have been done on these stages[5–9].

In the present paper preliminary results will be given of a study of the ultrastructure of *Plasmodium juxtanucleare* as it appeared in the spleen of a chick with exoerythrocytic infection.

Materials and methods. The strain of *Plasmodium juxtanucleare* used in this work was isolated in our laboratory from the blood of a naturally infected fowl bought in the commercial market of Rio de Janeiro (Brazil). For about 5 years, the parasite has been maintained in chicks by successive blood passages. During this period, no exoerythrocytic infections were seen when

animals were tested. After this time, 1 chick was found with exoerythrocytic forms in the spleen. From this chick, the exoerythrocytic forms were then maintained by routine inoculations of infected spleen tissue into young chicks which died after about 2 weeks from exoerythrocytic infection.

Since the spleen in these chicks was always the organ which was most heavily infected, one of the inoculated chicks was sacrificed when it showed signs of infection 21 days after the inoculation. Small fragments of spleen were then removed and fixed in 2.5% glutaraldehyde buffered with phosphate 0.1 *M* pH 7.2 for 2 h. They were postfixed for 1 h in 1% Osmium tetroxide also buffered with phosphate. After the fixation they were washed in distilled water dehydrated in ethanol and embedded in Epon. Thin sections were obtained with a diamond knife

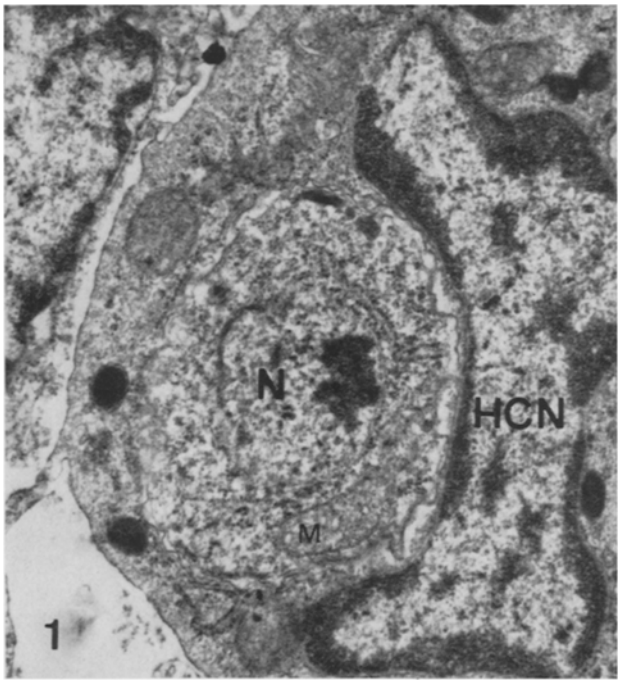

Fig. 1. Round-shaped parasite in endothelial cell showing nucleus (N), free ribosomes and mitochondria (M). HCN, host cell nucleus. ×20.000.

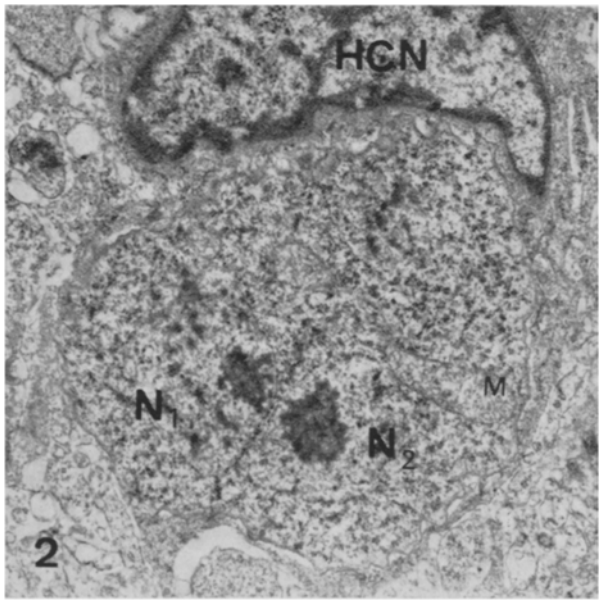

Fig. 2. Parasite after first nuclear division showing 2 nuclei (N_1, N_2) with nucleolus, free ribosomes and mitochondria (M) in the cytoplasm. HCN, host cell nucleus. ×9.700.

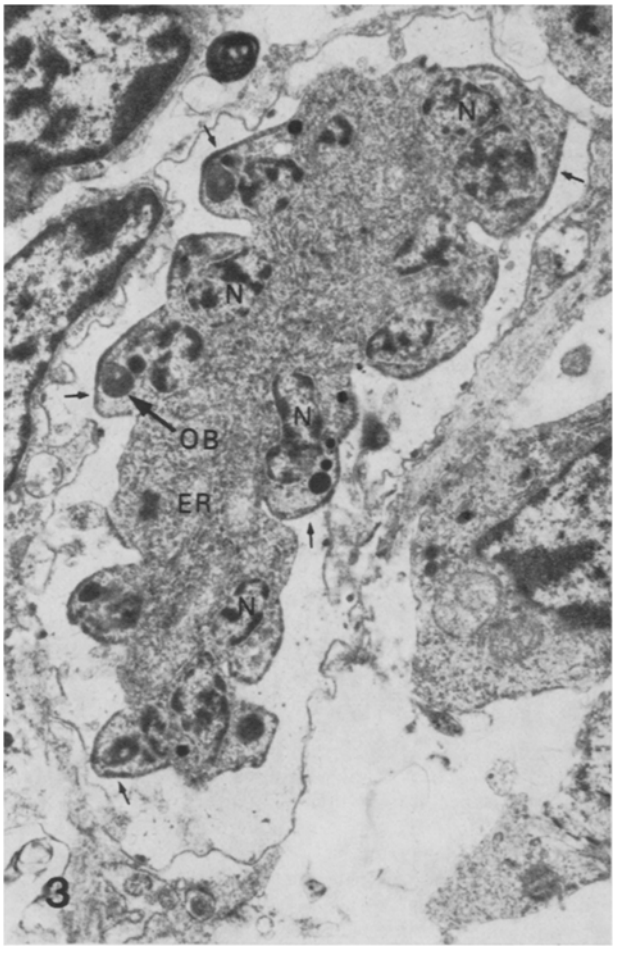

Fig. 3. Parasite in advanced stage of schizogony. Peripheral distribution of nuclei (N), appearance of thickened membrane (→) and oval bodies (OB); ribosomes and endoplasmic reticulum (ER) in central region. Parasite in vacuole limited by host cell membrane. ×11.200.

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on an ultramicrotome (Ultratome III, LKB), stained with uranyl acetate and lead citrate[10] and examined with an AEI EM6-B electron microscope.

Results and discussion. In the spleen tissue the most frequently infected cells seen were the endothelial cells. In the initial forms which have just penetrated such cells, the parasite shows an elongated shape with a nucleus in the center containing chromatin masses at its periphery. Mitochondria are seen and an endoplasmic reticulum; the cytoplasm is filled with ribosome particles. The parasite is covered with a double membrane in which sub-pellicular microtubules may be found occasionally. It is surrounded by a small vacuole but shows intimate contact with the cytoplasm of the host in many places.

The parasite soon loses its small elongated shape, becomes more rounded, and increases in size (Figure 1). The nucleus divides but during its division the nuclear membrane remains intact. Intranuclear microtubules may be observed occasionally. The division of the nucleus is not followed by division of the cytoplasm. In the cytoplasm the rough endoplasmic reticulum proliferates and many mitochondria are found (Figure 2). After repeated nuclear divisions and considerable growth of the cytoplasmic mass, the segmentation process, i.e., the formation of the new merozoites, begins.

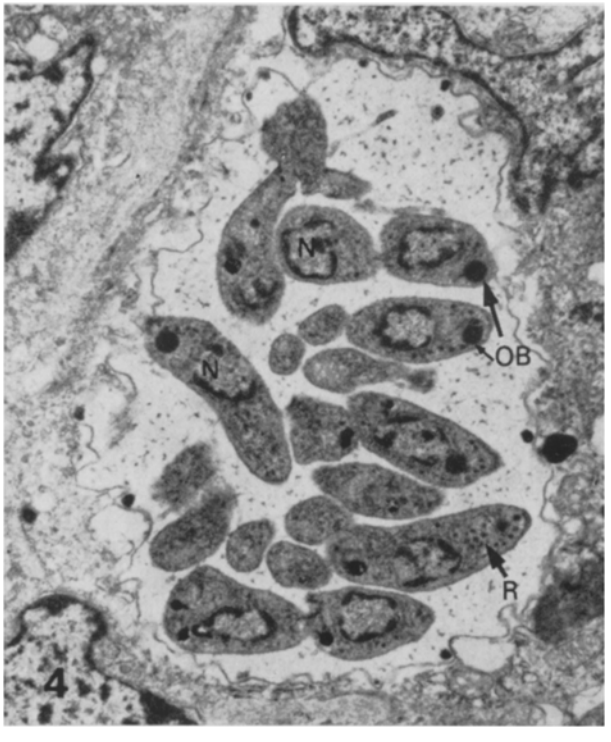

Fig. 4. Final phase of cycle with parasites (merozoites) in cell vacuole. N, nucleus; OB, oval body; R, rhoptry (Toxoneme). ×10.00.

The first signs of this process to be observed were a thickening of certain regions in the schizont membranes, nd icating the point which would be the anterior region in the future merozoite (Figure 3). The many nuclei are now situated at the periphery of the schizont, and in the center only a few mitochondria, endoplasmic reticulum and free ribosomes are left.

Below the thickened membrane one or more round or oval bodies are found similar to those described in *Plasmodium gallinaceum* and other malarial parasites and also structures which possibly represent the rhoptries (toxonemes) seen in other sporozoa[11,12].

In a more advanced stage of maturation, the thickened membrane invaginates profoundly, forming the future merozoites. In the final phase, when the formation is almost complete, the new merozoites are still attached to a small cytoplasmic piece of the schizont until they are eventually liberated.

During the whole process of schizogony, from the very beginning to the end, the parasite stays in a cell vacuole which is very evident in the later phase (Figures 3 and 4). It is limited by a membrane of host cell origin.

It has been seen that more than one parasite can infect the same cell, since cells with various schizonts can be found, each one developing in its individual vacuole. Multiple cell infection was previously observed in *Plasmodium fallax* infecting tissue culture cells. This fact has been considered by some authors as an anomaly due to the culturing of the parasites[5,13]. The results obtained here, however, show that in *Plasmodium juxtanucleare* multiple cell infection may occur in vivo, since cells are found with parasites in different phases of development, which indicates that a merozoite can infect an already parasitized cell.

Zusammenfassung. Die Feinstruktur von *Plasmodium juxtanucleare* wurde im Milzgewebe experimentell infizierter junger Hühnchen untersucht und die verschiedenen Veränderungen im Verlaufe des intrazellulären Entwicklungszyklus beschrieben.

M. DE OLIVEIRA MUSACCHIO and W. DE SOUZA

Instituto de Biofísica, Universidade Federal do Rio de Janeiro, Av. Pasteur 458, Rio de Janeiro GB ZC 82 (Brazil), 29 October 1973.

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Incorporation of Viral Genome in DNA of Chronically Infected Cells

In previous communications[1,2] we have shown that a line of HEp2 cells chronically infected with tick-borne encephalitis (TBEV) virus[3] does not produce mature virions, and that the persistance of the virus in the culture for 13 years is due to accumulation in the cells of viral ribonucleoprotein structures.

The study of HEp2 culture, which appeared to be virus-free and therefore is used for isolation and study of various viruses, has shown that at least some clones of this culture produce an Oncornavirus of type B[4,5]. It is well known that RNA-dependent DNA polymerase of Oncornaviruses usually associated with the virus RNA[6]