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.

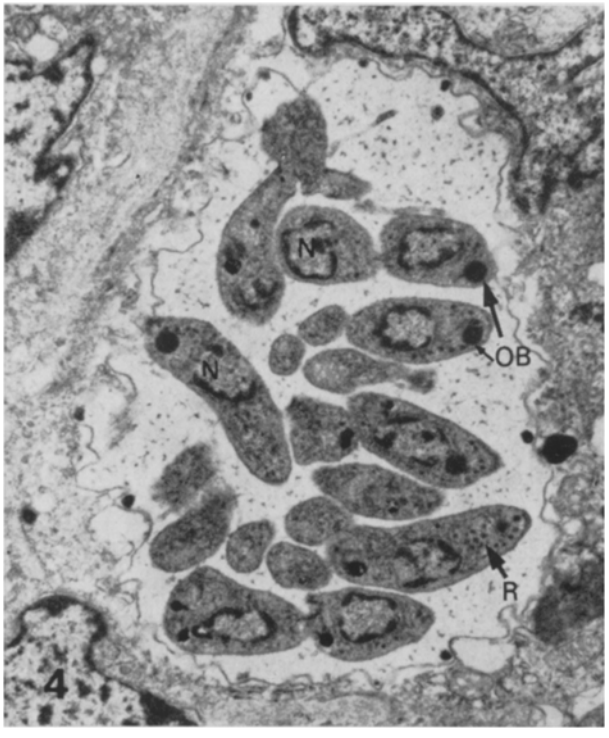


Fig. 4. Final phase of cycle with parasites (merozoites) in cell vacuole. N, nucleus; OB, oval body; R, rhoptry (Toxoneme). ×10.00.

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.

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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]

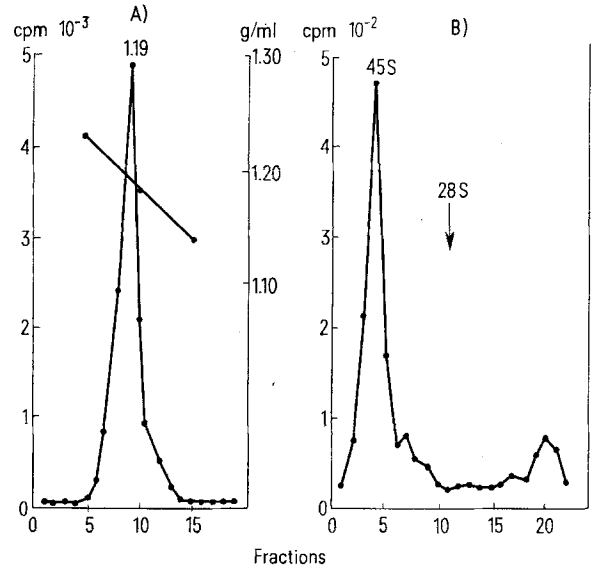


Fig. 1. A) Isopycnic banding of TBEV in a sucrose density gradient 20–60%. $^3$H uridine-labeled virus was layered on the gradient and centrifuged in a SW 27.1 rotor of a Spinco L3 centrifuge at 25000 rpm for 5 h. B) Sedimentation properties of RNA extracted from TBEV in a sucrose density gradient 5–20%. $^3$H uridine-labelled RNA was layered on the gradient and centrifuged in the same rotor at 20000 rpm for 18 h. The position of ribosomal 28 S marker RNA is shown by the arrow.

may also induce DNA synthesis using a foreign RNA as a template[7], and such DNA transcript may be incorporated into the cellular genome. Therefore it was worthwhile to study HEp2-Sof culture chronically infected with TBEV in search of DNA sequences complementary to the viral RNA.

The Sof strain of TBEV was propagated in a continuous tissue culture of pig kidney (SPEV) labelled with $^3$H-uridine (10 μCi/ml, specific activity 26 Ci/mmol), the culture medium was collected, the cell debris was removed by centrifugation at 15,000 *g* for 20 min, the virus was sedimented at 100,000 *g* for 3 h, resuspended in TNA buffer (*Tris* HCl pH 7.4 0.01 *M*, NaCl 0.1 *M*, EDTA 0.001 *M*) and purified by equilibrium centrifugation (25,000 rpm for 5 h in a SW 27.1 rotor of a Spinco L3


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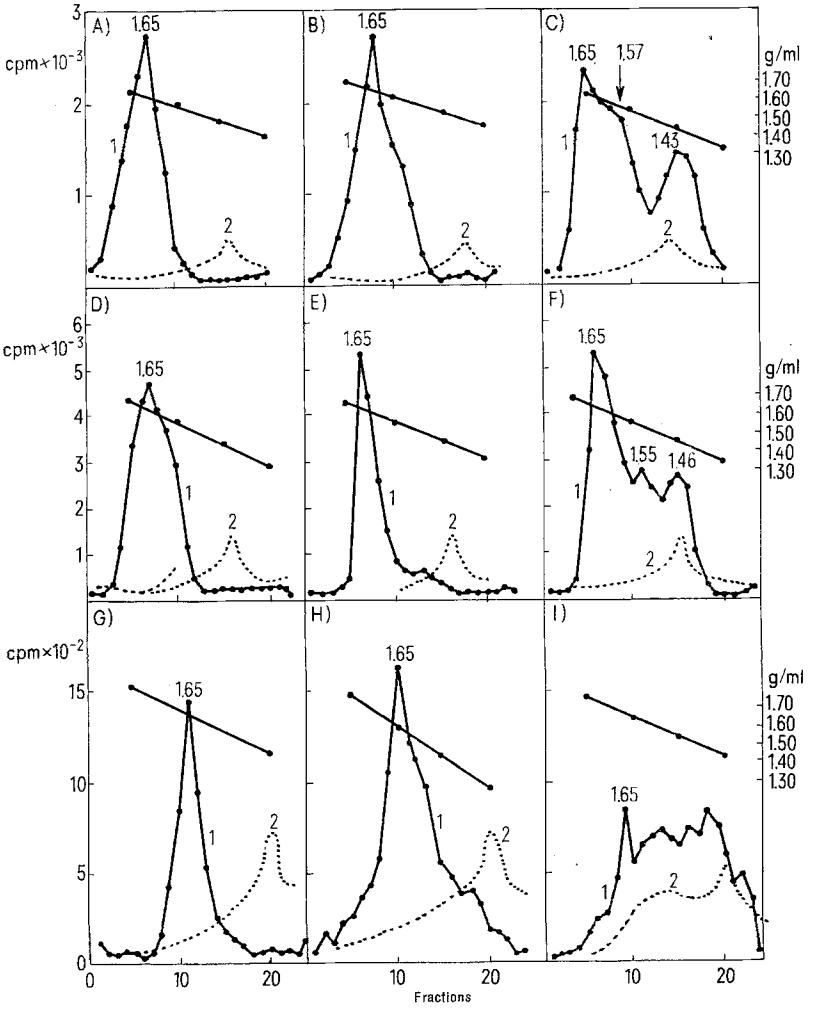


Fig. 2. Isopycnic banding of TBEV RNA hybridized with DNA from non-infected and chronically virus-infected HEp2 cells. $^3$H uridine labelled TBEV RNA (about 10000 cpm) was hybridized with 0.5 mg of DNA in 50% formamide with 0.4 *M* NaCl. The products of hybridization were centrifuged in caesium sulfate gradients in a Ti50 rotor of a Spinco L3 centrifuge at 35,000 rpm for 60 h. A), D), G)-RNA only; B), E), H)-RNA plus DNA from non-infected HEp2 cells; C), F), J)-RNA plus DNA from chronically virus-infected HEp2 cells.

centrifuge) in a linear sucrose density gradient 20–60% w/w prepared in TNE. The gradient was fractionated in a LKB Uvicord apparatus, aliquots of each fraction were taken and acid-insoluble radioactivity was measured in a Packard-Tricarb liquid scintillation counter. Figure 1A shows that the virus bands at the density of 1.19 g/ml.

The gradient fractions with the density of 1.18 to 1.20 g/ml were collected, diluted in TNE to the density of 1.10 g/ml, and the virus was sedimented at 150,000 *g* for 1 h RNA was extracted by phenol saturated by TNE, precipitated by ethanol with 20 μg/ml of carrier yeast RNA and stored at −20 °C. For characterization of the viral RNA, the latter was resuspended in TNE and centrifuged in a linear sucrose density gradient 5–20% prepared in TNE (20,000 rpm for 18 h in a SW 27.1 rotor of the same centrifuge). Figure 1B shows that RNA of TBEV sediments at 45 S.

DNA from HEp2 cells non-infected and chronically infected with TBEV (Hep2-Sof system) was isolated as follows. The cells were suspended in TNE, disrupted in a Dounce homogenizer, the nuclei were sedimented at 1000 *g* for 5 min and thereafter washed twice by centrifugation (5000 *g*, 10 min) through 0.25 *M* sucrose prepared in TNE. DNA was extracted by phenol and 0.5% sodium dodecyl sulfate and precipitated by ethanol. Thereafter DNA was resuspended in TNE, passed several times through a tuberculin syringe and RNA admixture was hydrolyzed by 0.5 *M* NaOH at 37 °C for 16 h. After neutralization, DNA was precipitated by ethanol and stored at −20 °C.

RNA-DNA hybridization was performed in formamide with subsequent centrifugation of the products in caesium sulfate gradients[8]. DNA was taken in excess (0.3–1 mg) and was denaturated by heating at 90 °C for 2 min followed by cooling in ice-water. $^3$H labeled viral RNA was mixed with DNA in 50% formamide with 0.4 *M* NaCl in the volume of 0.2–0.4 ml, the mixture was incubated at 37 °C for 16 h and thereafter centrifuged in caesium sulfate density gradients (1.36–1.72 g/ml) in a Ti50 rotor of a Spinco 3 centrifuge at 35,000 rpm for 60 h .The gradients were fractionated and acid-insoluble radioactivity was counted as above.

Three repeated experiments gave similar results, one of which is shown in Figure 2. It is seen that TBEV RNA bands at the density of 1.65 g/ml in caesium sulfate gradients (Figure 2A, D G). Hybridization of the virus RNA with DNA from non-infected HEp2 cells does not change essentially the distribution of the radioactive label in the gradient (Figure 2B, E, H). Hybridization of the virus RNA with DNA from chronically virus-infected HEp2-Sof cells essentially changes distribution of the radioactive label: a part of it bands at the density of 1.45 g/ml that is characteristic for DNA, labelled (in our case) with fragments of RNA, and another part of the label occupies a zone with the densities of 1.60 to 1.52 g/ml that is characteristic for RNA: DNA hybrids (Figure 2C, F, J).

The data presented allow to conclude that the genome of HEp2-Sof cells chronically infected with TBEV contains DNA sequences homologous to the virus RNA, while such sequences are absent in the genome of non-infected HEp2 cells. Incorporation of DNA-transcripts of the virus RNA into the cellular genome may be an additional mechanism for the chronic persistence of the virus in the cells. In our case transcription of the virus RNA into double-stranded DNA might be due to the presence of a latent Oncornavirus in HEp2 cells, as has been already mentioned. Whether it is a peculiar phenomenon inherent in the system studied, or a more general mechanism of some chronic viral infectious, this is the subject of our further study.

ВЫВОДЫ. Исследована система клеток (HEp2-Соф), в которых более 13 лет поддерживается хроническая персистенция вируса клещевого энцефалита. В опытах молекулярной гибридизации РНК вируса клещевого энцефалита с ДНК из ядер клеток HEp2-Соф установлено наличие в геноме этих клеток последовательностей ДНК, гомологичных вирусной РНК. Эти последовательности отсутствуют в незараженных клетках HEp2.

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## The Velocity-Dependence of Myosin Cross-Bridge Movement and Tension Development in Oscillatory Contractions of Insect Fibrillar Muscle

Movement of myosin cross-bridges, pulling upon attachment to the actin the thin filaments towards the centre of the sarcomere, has been generally implicated in the mechanism of active tension generation[1–3]. Evidence for such an axial movement of the cross-bridges was derived from intensity changes in the 145 Å meriodonal reflection in activated vertebrate skeletal[4] and insect flight muscle[5,6]. When the 145 Å reflection was monitored during sinusoidal length changes at a power-producing frequency of 4 Hz it was found that the intensity varied inversely in phase with the active tension[6]. Thus, under the conditions investigated the extent of the axial movement of the cross-bridges was directly proportional to the amount of tension developed.

To study the kinetics of cross-bridge movement in relation to tension development insect fibrillar muscle has been allowed to perform sinusoidal extension-and-release cycles at different frequencies and amplitudes. By recording the intensity changes in the 145 Å reflection some information has been obtained as to the number of cross-bridges changing their angle with the myosin filament axis[4–6] at any particular moment in response to

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