Zusammenfassung. Elektronenmikroskopische Untersuchungen des Analorgans bei Drosophilalarven zeigten, dass die cuticulare Oberfläche dieses Organs durch Einstülpungen der Epicuticula vergrössert wird. Die an die Cuticula angrenzende Plasmamembran der Hypodermiszellen bildet zahlreiche Faltungen. Je nach der Salinität des Aussenmediums weist die Anzahl der Plasmamembranfaltungen und der im Cytoplasma lokalisierten Mitochondrien regulatorische Veränderungen auf.

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Accumulation of Ribonucleoprotein Structures in Chronically Virus-Infected Cells

A line of HEp2 cells chronically infected with tick-borne encephalitis (the strain Sofyin) has been obtained which was designated HEp2-Sof[1]. This culture is characterized by a retarded cell growth, the resistance to superinfection and the release into the medium of infectious material in a low concentration[2]. We describe in this paper virus-specific structures revealed in this system.

Experiments were conducted with the HEp2-Sof cell line, which within 13 years, has undergone about 400 passages. In 5 to 7 days after reimplantation into medium 199 with 10% bovine serum, a monolayer was formed, which was used for the study. Actonomycin D (5 μg/ml) was added for inhibition of the synthesis of cellular RNA: in the control non-infected HEp2 culture, the RNA synthesis was inhibited to 97–99%. Newly synthesized viral RNA in the HEp2-Sof cells was labelled by introduction of ^{3}H uridine (5 μCi/ml, specific activity 1.5 Ci/mmole), 2 h after the antibiotics had been added, for 5 to 6 h. At the end of this period the culture was rapidly chilled and washed with the cold Hanks' solution. The cells were collected mechanically into STE buffer (*Tris* HCl 0.01 *M* pH 7.4, NaCl 0.1 *M*, EDTA 0.001 *M*), washed in a centrifuge (1200 × *g*, 10 min), resuspended in RSB (*Tris* HCl 0.01 *M* pH 7.4, NaCl 0.01 *M*, $MgCl_2$ 0.0015 *M*), the cells were disrupted in a Dounce homogenizer, the nuclei were removed by centrifugation (1200 × *g*, 10 min) and cell homogenates thus obtained were studied.

Figure 1 presents sedimentograms of virus-specific structures revealed in sucrose density gradients. It is seen that these structures sediment with the fraction of mitochondria and membranes and are separated from them by the treatment of the cell homogenates with a non-ionic detergent (NP40), being non-sensitive to the EDTA treatment. Therefore these structures are true ribonucleoproteins, but not polyribosomes. Two types of ribonucleoproteins are revealed according to their sedimentation properties, having the sedimentation coefficients 180 S and 140 S. It is worthwhile to note that Sendai virus ribonucleoprotein, labelled with P^{32}, was used as a marker, whose sedimentation coefficient is known to be 210 S[3]. In the given conditions of centrifugation, the fraction of mitochondria and membranes occupies an equilibrium position that corresponds to fraction with the density 1.18 g/ml, i.e. 50% sucrose, while the structures with the density more than 1.24 g/ml (60% sucrose) sediment and do not occupy the equilibrium position.

To determine the buoyant density of ribonucleoprotein structures, both peaks with the sedimentation coefficients 180 and 140 S were collected, fixed in 8% formaldehyde

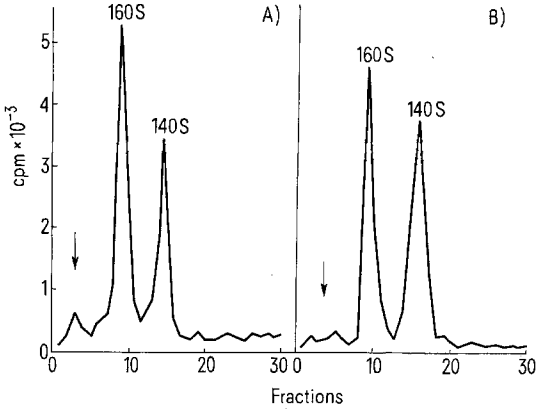

Fig. 1. Sedimentigrams of homogenates of HEp2-Sof cells after centrifugation in sucrose density gradients 15/60% in a rotor SW 27.1 of a Spinco L3 centrifuge at 22,500 rpm for 1 h 45 min. Conditions of the experiment are described in the text. A) non-treated cell homogenate; B) treated with 0.5% NP40 and 0.03 *M* EDTA. The position of the marker peak of P^{32} Sendai virus labelled ribonucleoprotein is shown by arrows.

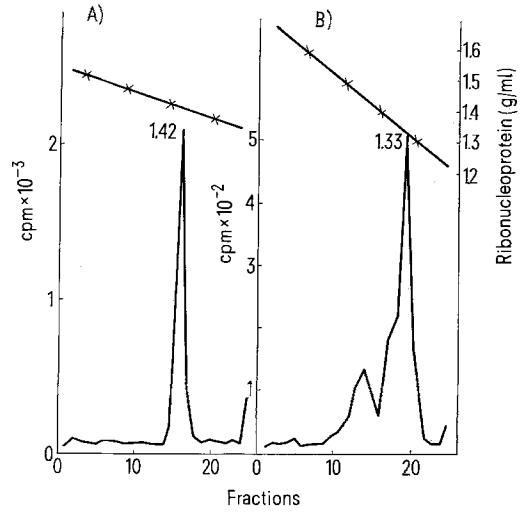

Fig. 2. Density distribution of ribonucleoproteins after equilibrium centrifugation in Caesium chloride density gradients in a SW50 rotor of a Spinco L3 centrifuge at 45,000 rpm for 3 h. A) sucrose gradient fractions that contain 180 S ribonucleoprotein; B) sucrose gradient fractions that contain 140 S ribonucleoprotein.

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and centriguged in Caesium chloride density gradients. In this case sucrose gradients were prepared not in STE buffer, but in a 0.1 *M* phosphate buffer, and instead of RSB a 0.1 *M* phosphate buffer with 0.0015 *M* $MgCl_2$ was used. This precaution was taken to prevent the formation of precipitates which occurs in *Tris* buffers.

Figure 2 presents density characteristics of both ribonucleoproteins. It is seen from the Figure that the 180 S ribonucleoprotein has buoyant density of 1.42 g/ml, while the 140 S ribonucleoprotein bands at 1.33 g/ml.

Gradient fractions that contain ribonucleoproteins with different sedimentation coefficients and buoyant densities were studied by the method of electrophoresio in poliacrylamide gels[4]. For this prupose chronically infected cultures were labelled with C14 aminoacid mixture (2 μCi/ml, specific activity 50 μCi/ml). One protein was detected in structures with the buoyant density of 1.42 g/ml that corresponded to nucleocapsid protein of the virus, while several proteins were isolated from the structures that banded at $\varrho = 1.33$ g/ml. (Figure 3). The latter may depend upon various amounts of protein molecules packed around the RNA thread. It should be noted that the parameters of the first ribonucleoprotein (180 S. 1.42 g/ml) resembles ribonucleoproteins that have been isolated from virions of arboviruses[4], while the other rather resembles hybrid particles formed of viral RNA and cellular ptoteins[5].

Thus, in HEp2 cells chronically infected with the tick-borne encephalitis virus, accumulation of viral ribonucleoproteins takes place in which viral RNA is associated with virus-specific and cellular proteins. Matured virions are probably formed in small amount and are therefore not revealed by the methods employed.

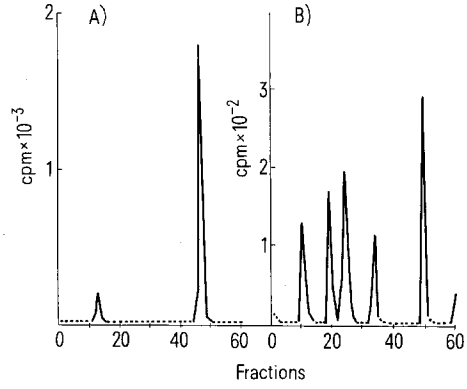

Fig. 3. Electrophoregrame of proteins from RNP structures that banded at 1.42 g/ml (A) and 1.33 g/ml (B).

ВЫВОДЫ. Проведено исследование гомогенатов клеток, хронически инфицированных вирусом клещевого энцефалита. В этих клетках обнаружены вирусные рибонуклеопротеиды, обладающие разными константами седиментации и плавучей плотностью (180S, 1.42 г/мл и 140S, 1.33 г/мл) отличающиеся между собой также по составу белков.

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Co-Existence of a Guanidine-Dependence and of a Thiopyrimidine Dependence in the Same Strain of Poliovirus

Polioviruses develop a dependence on certain specific inhibitors, such as guanidine and thiopyrimidines[1,2]. The purpose of the present research is to ascertain whether, in the absence of a cross dependence, a dependence on both inhibitors can coexist in the same strain.

Materials and methods. Guanidine (Eastman Kodak); ethyl-2-methylthio-4-methyl-5-pyrimidine carboxylate (S-7) kindly furnished by the Toyama Chemical Co., Tokyo; cells of the human aneuploid cell line HEp2 (American Type Culture Collection, Rockville USA); Brunenders Poliovirus 1 and its two variants, the guanidine-dependent variant (G 100) and the S-7 dependent variant (T 300) obtained through serial transplants in the presence of increasing concentrations of each of the inhibitors (up to a maximum concentration of 100 μg and 200 μg/ml of culture medium respectively) which had then been repeatedly cloned from single plaques according to the DULBECCO and VOGT method[3] in the presence of the above-mentioned drug concentrations.

Table I. Effect of guanidine and S-7 on the growth of sensitive and dependent poliovirus variants

Inhibitors in the medium (μg/ml)	PFU produced in 8 h at 37 °C		
	Sensitive strain[a]	Guanidine-dependent variant[b]	S-7 dependent variant[c]
—	2.6×10^{8}[d]	3×10^{4}	1.6×10^{5}
Guan HCl 33	4×10^{4}	2.1×10^{6}	3×10^{4}
Guan HCl 100	3×10^{4}	1.6×10^{8}[d]	$<10^{4}$
Guan HCl 300	4×10^{4}	8.5×10^{5}	2×10^{4}
S-7 100	2.5×10^{5}	3×10^{4}	5.8×10^{6}
S-7 200	6×10^{4}	3×10^{4}	7×10^{7}[d]
S-7 300	2×10^{4}	$<10^{4}$	3.9×10^{6}
Guan HCl 33 + S-7 100	$<10^{4}$	2×10^{4}	10^{4}
Guan HCl 100 + S-7 100	2×10^{4}	9×10^{4}	$<10^{4}$
Guan HCl 300 + S-7 100	$<10^{4}$	5×10^{4}	2×10^{4}
S-7 100 + Guan HCl 33	10^{4}	3×10^{4}	$<10^{4}$
S-7 200 + Guan HCl 33	2×10^{4}	$<10^{4}$	$<10^{4}$
S-7 300 + Guan HCl 33	2×10^{4}	2×10^{4}	3×10^{4}

[a] PFU titrated in drug free medium. [b] PFU titrated in the presence of guanidine HCl 100 μg/ml. [c] PFU titrated in the presence of S-7 200 μg/ml. [d] Maximum virus yelds.

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