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.

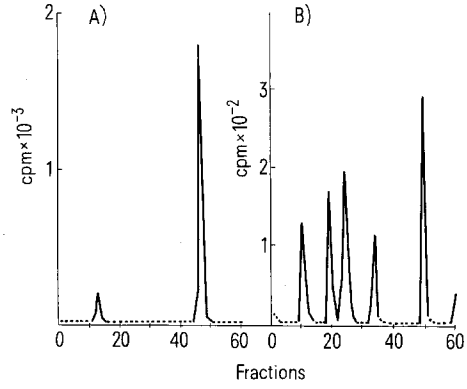

Fig. 3. Electrophoregrame of proteins from RNP structures that banded at 1.42 g/ml (A) and 1.33 g/ml (B).

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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Table II. Double drug-dependence induced to the poliovirus 1 Brunenders by serial passages in the presence of guanidine and S-7

Virus strain (25 PFU/cell)	Drugs in the growth medium (μg/ml)	Virus yield, titrated as PFU according to the DULBECCO and VOGT[3] technique, in the presence of (μg/ml)			
		—	Guanidine HCl 100	S7 200	Guanidine HCl 100 + S7 200
GT variant[a]	Guanidine HCl 100 + S7 200	10^4	$4{,}2 \times 10^5$	3×10^4	$1{,}8 \times 10^7$

[a] Obtained by transplanting the guanidine dependent strain (see text) in cell cultures containing both 100 μg/ml of guanidine HCl and increasing concentrations of S7 up to a maximum of 200 μg/ml.

Table III. Lack of genomatic recombination between a S-7 dependent variant and a guanidine dependent variant of poliovirus 1 Brunenders infecting the same cells

Virus strains (60 PFU/cell)	Drugs in the growth medium (μg/ml)	Virus yield, titrated as PFU according to the DULBECCO and VOGT[3] technique in the presence of (μg/ml)			
		—	Guanidine HCl 100	S7 200	Guanidine HCl 100 + S7 200
Guanidine dependent + S7 dependent	Guanidine HCl 100 + S7 200	1.5×10^3	10^4	10^3	$< 10^3$

Cell monolayers which had reached confluence after incubation for 16 h at 37 °C in Eagle's MEM (10^6 cells/sample) were deprived of the culture medium and infected for 1 h at +4 °C with 25 plaque-forming units (PFU) per cell. After removing the excess of inoculum by washings in Hank's BSS, the cells were incubated at 37 °C in the same buffer solution with or without the inhibitors. 8 h after infection the whole cultures were frozen and thawed (−70 °C: +20 °C) 3 times and cell debris was removed by centrifugation at 5,000 rpm for 5 min. The PFU produced were titrated according to the DULBECCO and VOGT method[3], adding the appropriate concentrations of the 2 inhibitors for the titration of the respective dependent variants.

Results. 25 μg/ml of guanidine or 50 μg/ml of S-7 are sufficient to inhibit the development of the original sensitive strain. The guanidine-dependent variant shows optimal development in the presence of 100 μg/ml of guanidine and fails to develop if guanidine is either lacking, substituted with S-7 or present together with S-7 in the culture medium. The S-7 dependent variant, on the other hand, requires 200 μg/ml. of the thiopyrimidine for optimal development and fails to develop if the thiopyrimidine is either lacking, substituted with guanidine or present together with guanidine in the culture medium (Table I).

Using these data, which indicate there is no cross dependence between the inhibitors, an attempt was made to induce a double dependence in the same virus strain by transplanting serially the guanidine-dependent strain in the presence of 100 μg/ml of guanidine and increasing concentrations of S-7 up to a maximum concentration of 200 μg/ml.

The strain obtained in this way (Table II) was dependent on both drugs in that it required guanidine and S-7 contemporaneously in the culture medium for optimal growth.

On the other hand, no evidence of double dependence was obtained by recombination, i.e. by infecting simultaneously the same cells with the guanidine dependent and the S-7 dependent variants (Table III).

Results and conclusions. Guanidine and S-7 markers occupy different positions in the virus genome. This excludes the possibility of a cross dependence and permits the coexistence of a double dependence in the same virus strain. The impossibility (or rather the difficulty) of obtaining such a double dependence through genomatic recombination tests seems to indicate that guanidine and S-7 markers are placed so close together in the virus genome that their separation during cross-over is unlikely[4,5].

Riassunto. Una doppia dipendenza nei confronti della guanidina e di una tiopirimidina (S-7) può essere indotta in uno stesso stipite di poliovirus 1.

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Use of Griseofulvin for the Isolation of Auxotrophic Mutants of *Rhodotorula* sp.

Several methods have been developed for isolating biochemical mutants of microorganisms by selective means which facilitate in an increase in the percentage of auxotrophic mutants among mixed surviving population. These include the use of penicillin[1–4], cycloserine[5], nystatin[6], tritium labelled thymidine[7], thymine or diaminopimelic acid[8,9], 2-deoxy glucose[10], 8-azaguanine[11], pentachlorophenol[12], 5-fluorouracil[13], tritium labelled