All of these results led to the conclusion that if modifications are induced by a guanidine-dependence to the viral capsid, these modifications do not noteably affect the density and isoelectric point of the virion[6].

Riassunto. La densità in gradiente di saccarosio ed il punto isoelettrico delle particelle virali di poliovirus guanidino-dipendenti non differiscono da quelle del virus guanidino-sensibile di origine.

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Thiopyrimidines: Specific Inhibitors of Poliovirus Induced Early Cell Damages

It has been observed that several thiopyrimidine derivatives inhibit specifically the in vitro development of polioviruses[1-4]. The researches reported here show that one of these compounds is also able to prevent the virus-induced blockade of cell protein synthesis.

Materials. Ethyl-2-methylthio-4-methyl-5-pyrimidine carboxylate (S-7) was kindly provided by Dr. Masuzo Kanamori of the Toyama Chemical Co., Tokyo. Guanidine HCl[5] (Eastman Kodak), D-penicillamine[6] (Dista) and α-hydroxybenzylbenzimidazole[7] (HBB, kindly given by Dr. Igor Tamm, Rockefeller University, New York) were also used. Experiments were carried out by employing human aneuploid HEp2 cells (American type culture collection, Rockville, USA), a strain of poliovirus 1 Brunenders and a S-7 resistant variant of the same strain, obtained through serial transplants in the presence of the thiopyrimidine, up to a maximum of 250 μg/ml.

Method. Cell cultures (10^6 cells/petri dish, 3 petri dishes/sample) were infected with 50 plaque forming units (PFU) of either viruses at +4 °C × 1 h. Cell monolayers were then washed three times with Hank's BSS (pH 7.2) and incubated at 37 °C in the same buffer containing 2 μg/ml of actinomycin D, in order to inhibit nuclear transcriptions. The inhibitors were added, soon after infection, at the maximum concentration which had been previously found to be inactive on the incorporation of RNA and protein precursors in uninfected cells. Infectious progeny was titrated as PFU, according to the Dulbecco and Vogt method[8]. Overall protein synthesis and virus RNA replication were established by measuring, respectively, the uptake under acidinsoluble form of H^3 leucine (Amersham, 15.2 Ci/m*M*, 0.1 μCi/ml, 1 h pulses) and of H^3 uridine (Amersham, 25 Ci/m*M*, 0.2 μCi/ml, from time 0).

Cytopathic effect (CPE) was evaluated by measuring the ability of cell to incorporate vital stains (neutral red 100 μg/ml of medium, 90 min pulses). Cells were then solubilized with sodium deoxycholate 1% in H_2O and neu-

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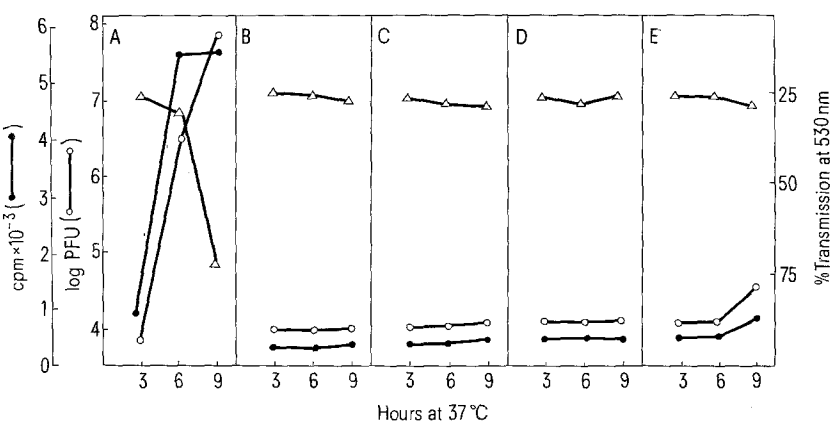

Fig. 1. Effect of several inhibitors on polivirus synthesis and CPE. HEp2 cell monolayers were infected with 50 PFU/cell and incubated at 37 °C in Hank's BSS supplemented with AMD 2 μg/ml (A) or in the same medium + guanidine HCl 300 μg/ml (B), + D-penicillamine 150 μg/ml (C), + HBB 150 μg/ml (D), + S-7 250 μg/ml (E). Infectious virus yield (○—○) was titrated as PFU produced, according to the Dulbecco and Vogt[8] method; viral RNA synthesis (●—●) was measured on the basis of H^3 uridine uptake (0.3 μCi/ml from time 0); CPE was evaluated on the basis of intracellular incorporation of neutral red, solubilized with DOC 1% and read at 530 nm (△—△).

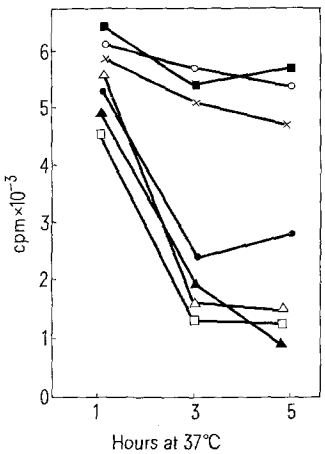

Fig. 2. Effect of poliovirus inhibitors on the leucine uptake by infected cells. HEp2 monolayers were infected with 50 PFU/cell and incubated at 37 °C. Inhibitors were added at time 0. Incorporation of H^3 leucine (0.1 μCi/ml, 1 h pulses) in uninfected cells (○—○) or infected cells (●—●), maintained in Hank's BSS supplemented with AMD 2 μg/ml and in infected cells maintained in the same medium containing guanidine HCl 300 μg/ml (△—△), D-penicillamine 150 μg/ml (□—□); HBB 150 μg/ml (△—△), S-7 250 μg/ml (■—■), and guanidine HCl 300 μg/ml + S-7 250 μg/ml (×—×).

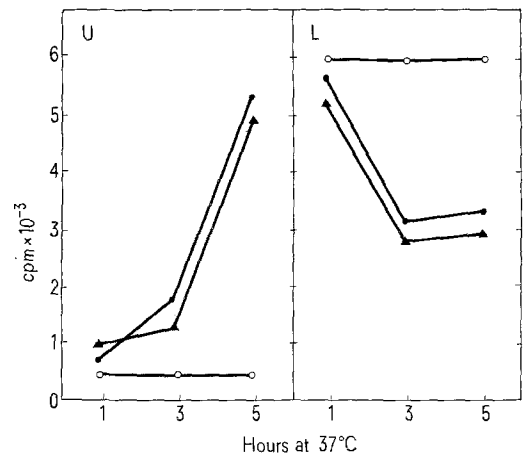

Fig. 3. Effect of S-7 on the blockade of cell protein synthesis induced by a S-7 resistant strain of poliovirus 1. HEp2 cell monolayers were infected with 50 PFU/cell and incubated a 37 °C in Hank's BSS containing AMD (2 μg/ml), with or without S-7 (250 μg/ml). Incorporation of H^3 Uridine (0.3 μCi/ml from time 0) (U) and of H^3 Leucine (0.1 μCi/ml, 1 h pulses) (L) in uninfected cells (○—○), in infected cells (●—●) and in infected cells maintained in S-7 250 μg/ml (▲—▲).

tral red was measured at 530 nm. For more details see previous papers[9].

Results. The data in Figure 1 show that, like guanidine, D-penicillamine and HBB, also S-7 prevents virus RNA replication, infectious virus production and CPE. However (Figure 2), only the thiopyrimidine is able to prevent the sudden decrease in leucine uptake which occurs within 3 h after infection and is ascribed to the sprogramming activity of virus proteins on cell polyribosomes[10]. Since the thiopyrimidine exerts this action also in cells in which virus proteins are reduced by guanidine, it can be assumed that S-7 prevents a virus-induced blockade of cell protein synthesis. The thiopyrimidine does not seem to exert this effect through a direct protection of cell polyribosomes, as other authors have observed for chloroquine in Newcastle Disease virus infected cells[11], but rather by acting on virus cycle. In fact no such effect can be seen in cells infected with the S-7 resistant variant of poliovirus (Figure 3).

The above results show that, contrary to what has been observed up to now for guanidine[12], D-penicillamine[12], and HBB[13], specific inhibitors of virus synthesis can also prevent the virus-induced damage in cell metabolism which causes cell death even when virus replication is inhibited[14].

Riassunto. Contrariamente ad altri inibitori specifici dei poliovirus che prevengono RNA-sintesi virale ed effetto citopatologico, ma non hanno effetto sui danni precoci che l'infezione induce nel metabolismo cellulare, un derivato tiopirimidinico è anche capace di impedire il blocco precoce, virus indotto, delle proteino-sintesi cellulari.

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Versuche zur Wirkungssteigerung der Kombination Sulfamethoxazol/Trimethoprim durch EDTA und Levallorphan an *Escherichia coli*

In letzter Zeit wurden verschiedentlich Versuche unternommen, die Wirkung von Antibiotika durch EDTA (Aethylendiamintetraessigsäure) zu steigern. Ein solcher Effekt, bestehend in einer verstärkten Wirkstoffaufnahme in die Bakterienzelle, ist durchaus zu erwarten, da durch die Bindung zweiwertiger Metallionen tiefgreifende Veränderungen in der Zellwand auftreten[1]. Eine synergistische Wirkung von EDTA mit verschiedenen Antibiotika konnte allerdings nicht generell festgestellt werden[2,3]. Die Ergebnisse variieren von Stamm zu Stamm und auch mit dem jeweils geprüften Antibiotikum.

RAWAL und OWEN[4] fanden, dass EDTA die Wirkung der Kombination Sulfamethoxazol/Trimethoprim (SMZ/TM) bei *Pseudomonas* verstärkt, bzw. dass erst durch diese Kombination primär resistente Keime in vitro ansprechen. Da andererseits EDTA auch bei *E. coli* zu einer Wir-