wiederholte Behandlung zustande kam. Bei einmaliger Behandlung war die Übertragbarkeit nur nach dem ersten Tag schwach herabgesetzt ($p < 0{,}005$), später entsprach sie der Kontrolle oder lag noch darüber.

Die Grössenverteilungen der Speicheldrüsenkerne vom A-Typ in Figur 2 sind bei der Kontrollgruppe (ausgezogene Linie) zweigipfelig, bei den behandelten Blattläusen dagegen stets eingipfelig. Die Hostacyclinbehandlungen haben demnach die Kernschwellungen und damit die Bildung des zweiten Teilkollektivs verhindert. Beide verwendeten Hostacyclinkonzentrationen zeigten darin kaum Unterschiede. Die Verteilungskurven der F-Zellkerne entsprachen denen des A-Typs. Es ist nach den bisherigen Ergebnissen auch anzunehmen, dass beide einem Zelltyp zuzuordnen sind (PONSEN[4]). In der Tabelle sind die errechneten Volumina aller Messungen vom A- und F-Typ gemittelt. Von beiden Teilkollektiven der Kontrolle ist nur das erste mit den Volumina der behandelten Blattläuse vergleichbar, das zweite, das etwa 50 % der Messungen enthält, beinhaltet Kerne mit etwa doppelten Volumina. Daneben wurden Zellkerne vom B- und C-Typ vermessen, die nach Aufenthalt der Blattläuse auf der Virusquelle keine Kernschwellungen zeigten. Ihre Volumina blieben auch durch die Hostacyclinbehandlung unbeeinflusst.

Insgesamt ergab sich, dass Schwellungen bestimmter Speicheldrüsenkerne, die durch Saugen der Blattläuse an PLRV-infizierten *P. floridana* hervorgerufen wurden, durch Tetracyclinbehandlungen unterdrückt wurden. Daneben wurde die Fähigkeit der Blattläuse, das PLRV zu übertragen, eingeschränkt. Es liegt deshalb nahe, zwischen beiden Ergebnissen einen Zusammenhang anzunehmen und Kernschwellungen der A/F-Zellen mit der Virusübertragung und damit möglicherweise mit der Virussynthese in Verbindung zu bringen. Allerdings wurde die Übertragungsfähigkeit der Blattläuse auch bei wiederholter Behandlung nicht völlig beeinträchtigt. Falls in den A/F-Zellen Virussynthese stattfindet, wird diese deshalb wohl nur in geringerem Masse zur PLRV-Produktion im Vektor beitragen, während andere Organe, die möglicherweise weniger von Tetracyclin beeinflusst werden, grössere Bedeutung haben. Nach neueren Ergebnissen (PONSEN[4]) kommen für die PLRV-Vermehrungen auch die Fettkörperzellen in Frage. Es ist deshalb zu vermuten, dass die Virusproduktion dieser oder anderer Zellen dazu beigetragen hat, die Übertragungsfähigkeit der behandelten Blattläuse teilweise zu erhalten.

Summary. Tetracycline hydrochloride treatment decreased the transmission rate of potato leafroll virus (PLRV) by *Myzus persicae*. It also prevented the PLRV-induced swellings of the A/F nuclei of the salivary gland. Apparently there is a connection between these two phenomena. The A/F cells may be a site of virus multiplication.

H. L. WEIDEMANN

Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für landwirtschaftliche Virusforschung, Messeweg 11/12, D-33 Braunschweig (Deutschland), 18. Juli 1973.

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On the Mechanism of the Inhibitory Action of 2-Amino-4,6-Dichloropyrimidine on Poliovirus Growth

We have reported previously that 2-amino-4,6-dichloropyrimidine (Py 11) prevents poliovirus 1 growth by impairing the ability of capsid precursors to organize virus particles[1]. The research here referred to has been carried out to investigate the irreversibility of this effect and, through the antagonism exerted on it by metabolic precursors, to shed some light on the mechanism of the antiviral action.

Materials and methods. Are mostly the same as previously described[1]. L- and D-aminoacids and metabolic precursors of nucleic acids were furnished by BDH and Merck.

Results. Data in Table I show that as little as 1 h incubation of HEp2 cell cultures under a thin layer of drug containing Hank's BSS medium is enough to impair irreversibly the ability of cells to produce infectious virus and, on the other hand, to deprive the medium of

[1] A. M. MARCIALIS, M. L. SCHIVO, P. UCCHEDDU, A. GARZIA and B. LODDO, Experientia, submitted for publication.

Table I. Intracellular incorporation of antiviral amounts of Py 11

A Py 11 (μg/ml)	B PFU produced by cells incubated in Py 11 media × 1 h and then in a drug-free medium for 11 h	C PFU produced by cells incubated × 12 h in the Py 11 media already used to incubate cells B	D PFU produced by cells incubated × 12 h in extracts obtained from drug-treated cells B
60	10^4	8.2×10^6	6.1×10^6
30	8×10^6	2.1×10^7	9.5×10^6
15	1.5×10^7	1.6×10^7	2.1×10^7
—	1.2×10^7	1.8×10^7	1.4×10^7

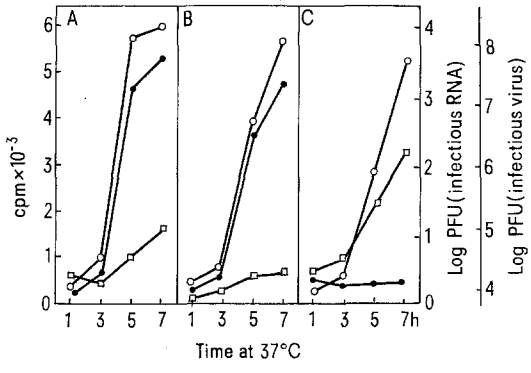
5×10^7 HEp 2 cell monolayers were infected × 1 h at 4 °C with 50 PFU/cell of poliovirus 1 Brunenders, washed 3 times in Hank's BBS and incubated at 37 °C × 1 h under a thin layer (1 mm deep) of the Py 11 media listed in A. The cultures were treated as follows: 10^6 cell samples were washed in Hank's BSS and reincubated at 37 °C in a drug-free medium (B); the drug-medium was recovered and used again for incubating new infected cells (10^6 cells/sample) (C); the treated cells were frozen and thawed 3 times. The extracts thus obtained were used as maintenance media for incubating new infected 10^6 cells/sample (D).

Table II. Antagonism of metabolic precursors of proteins and RNA on the antiviral action of Py11

Metabolites added to the cell cultures soon after infection, together with Py11 (Py11:100 μg/ml; each metabolite: 50 μg/ml; inoculum: 50 PFU/cell)	PFU produced after 12 h at 37 °C
Drug-free medium	3.5×10^7
Py11 only	1.2×10^4
Each one of the proteinogenous aminoacids	$2.2–4.6 \times 10^4$
All the proteinogenous aminoacids	1.2×10^7
All the proteinogenous aminoacids minus cysteine (and cystine)	4.5×10^4
All the proteinogenous aminoacids minus glutamine	3.2×10^4
All the proteinogenous aminoacids minus glutamic acid	1.8×10^7
L-cysteine + L-glutamic acid	4.1×10^4
L-cystine + L-glutamic acid	3.8×10^4
L-cysteine + L-glutamine	1.2×10^7
L-cystine + L-glutamine	3.1×10^7
D-cysteine + D-glutamine	1.6×10^4
D-cystine + D-glutamine	3.1×10^4
Adenine + guanine + cytosine + uracil	3.8×10^4
Adenosine + guanosine + cytidine + uridine	4.1×10^4

almost all of its antiviral activity. These data strongly support the idea that the substituted pyrimidine is avidly incorporated and firmly retained by cells, thus accounting for both the irreversibility of the antiviral action and the effectiveness of drug treatments limited to the pre-infection period[1]. The fact that extracts from drug-treated cells lack any inhibitory effect when added to infected cell cultures might depend either on bindings of the drug to cell structures and macromolecules or, more probably, on a metabolic transformation of the substituted pyrimidine into compound(s) unable to pass through the cell membranes.

Experiments on the possible antagonism exerted by metabolites on the antiviral action were carried out in cells maintained in Eagle MEM, lacking the amino acid supplement[2]. Drug and metabolites were added to the cell cultures soon after infection, simultaneously. Data in Table II show that the antiviral action of the substituted pyrimidine is strongly antagonized by the addition to the medium of an amino acid pool containing all the proteinogenous amino acids, while the addition of ribonucleic acid precursors is completely ineffective. Systematic researches have shown that no amino acid is active, if added alone to the cell cultures (data not reported in the Table), and that a marked antagonism is produced only by the combined addition of cysteine (or cystine) and glutamine (but not glutamic acid). All the other amino acid combinations, which do not include both cysteine (or cystine) and glutamine, have no effect. L-isomerism is essential. Finally, the antagonism is less marked if the active amino acids are added to the cell cultures some time after drug addition.



Inhibitory effects produced by a selective cysteine and glutamine starvation and by Py11 on the synthesis of poliovirus 1 Brunenders. HEP 2 cells (10^6 cells/sample) were infected at 4 °C with 50 plaque forming units (PFU) per cell of poliovirus 1 Brunenders. After 3 washings in Hank's BSS, the cells were incubated at 37 °C in Eagle MEM lacking all the amino acid supplement (○--○), in the same medium containing Py11 80 μg/ml (●--●), or in Eagle MEM lacking only cystine and glutamine (□--□). A) Virus RNA synthesis, evaluated by the intracellular incorporation, under acid insoluble form, of H^3-uridine (24 Ci/m*M*, 0,3 μCi/ml from time O) in the presence of actinomycin D (2 μg/ml). B) Synthesis of infectious RNA, measured as PFU by the method of DULBECCO and VOGT[3], in phenol extracts of infected cells, obtained by the technique of GIERER and SCHRAMM[4]. C) Production of infectious virus, measured as PFU, according to the method of DULBECCO and VOGT[3].

At this point, the question arises whether and how this antagonism is related to the antiviral action of the substituted pyrimidine. Cysteine and glutamine are essential amino acids in human cell cultures[2]. The possibility, remote indeed, that the drug inhibits virus growth by inducing an intracellular cysteine and glutamine starvation is to be ruled out, as expected, by the observation of the wide range effects which the omission of the above amino acids from the medium produces on virus synthesis in comparison with those, far more specific, produced by the drug (Figure). A more tenable interpretation of the antagonism exhibited by cysteine and glutamine is possible if the aspects of the antiviral action are taken into account. The substituted pyrimidine inhibits poliovirus growth by impairing the assembly of virus particles. For doing this the drug must be present in the cells from the beginning of virus protein synthesis. Later treatments do not prevent capsid proteins, synthesized previously in a drug free medium, from assembling virus RNA, produced in the presence of the drug, into infectious virus particles. On the other hand, the drug has no effect either on the net synthesis of cell and virus proteins or on certain virus protein related functions such as infectious RNA replication and cytopathic effects[1]. Finally the antiviral action of the substituted pyrimidine is antagonized by the combined addition to the cell cultures of both glutamine and cysteine, but not by either amino acid alone. Other amino acids have no effect, neither do nucleic acid precursors such as pyrimidine

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and purine bases and nucleosides. Considered together, these data led us to advance the working hypothesis that the substituted pyrimidine interacts with polipeptide chains soon after their synthesis in the polyribosomes, thus impairing the organization of these chains into normally active protein molecules. Glutamine and cysteine moieties, located in certain positions in the amino acid sequences of the same chain (or of different chains) would be the sites where this interaction occurs. The more frequent these amino acid sites are in the polypeptide chains (or the more favorable are their positions for interacting with the drug), the more intense will be the impairing effect of the drug on the chain structuring into active molecules. Viral capsids precursors would fulfill the above requirements, thereby representing a specific target for the drug action. Research is in progress to verify this hypothesis[5].

Riassunto. La 2-amino-4, 6-dicloropirimidina inibisce lo sviluppo del Poliovirus rendendo i precursori capsidici incapaci di partecipare alla formazione di particelle infettanti. Questo effetto è irreversibile in quanto la sostanza è avidamente incorporata e ritenuta dalle cellule infette. L'antagonismo esercitato dall'azione combinata di glutamina e cisteina fa ritenere che la pirimidina sostituita svolga il suo effetto interreagendo con questi due aminoacidi dopo la loro incorporazione nei precursori capsidici.

M. A. Marcialis, M. L. Schivo, A. Atzeni, A. Garzia and B. Loddo

Istituto di Microbiologia II dell'Università Via G. T. Porcell 12, Cagliari (Italia), and Istituto Chemioterapico Italiano, Lodi (Italia),
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Irreversible Impairment Produced by Guanidine on the Functions of Poliovirus Proteins

It is still matter of controversy whether guanidine inhibits enterovirus growth by acting on RNA replication directly[1] or, rather, by impairing the functions of proteins[2]. To look into this question we have considered it of interest to study which effect guanidine has on the functions of capsid proteins of poliovirus. In detail, to establish whether capsid proteins synthesized in the presence of guanidine are able to assemble virus RNA, replicated later in a drug-free medium, into infectious virus.

In doing these experiments it was of crucial importance to rule out that any capsid proteins, synthesized after guanidine removal, could participate in virus assembly. To achieve this goal, use has been made of parafluorophenylalanine (FPA) and 2-amino-4, 6-dichloropyrimidine (Py11). Added in proper concentrations to cell cultures soon after infection, these substances prevent the assembly of poliovirus RNA into infections particles[3,4]. However, if drug treatment is delayed of some 2–3 h, capsid proteins made during that interval are able to assemble virus RNA into infectious particles[4]. As essential components for virus assembly, these capsid proteins can be used as 'targets' for evaluating the action of guanidine.

Material. Guanidine HCl was furnished by Eastman Kodak, parafluorophenylalanine (FPA) by Aldrich, actinomycin D (AMD) by Merck. 2-amino-4, 6-dichloropyrimidine (Py11) was synthesized by the Istituto

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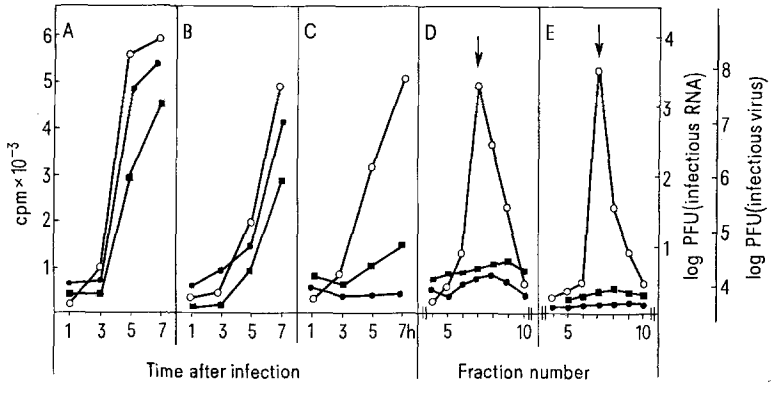

Fig. 1. Inhibition of poliovirus growth by early treatments with FPA or Py11 of infected cells. Infected cells were incubated at 37 °C in aminoacid-free Eagle MEM supplemented with AMD (2 μg/ml). FPA (25 μg/ml) or Py11 (100 μg/ml) were added to the cultures at time 0 after infection. A) Net synthesis of virus RNA, determined by the incorporation of [H³] uridine (0.2 μCi/ml from time 0); B) and C) synthesis of infectious virus RNA and production of infectious virus respectively, measured in PFU; D) and E) incorporation in virus particles of [H³] leucine and [H³] uridine, respectively (2 μCi/ml, cumulative pulses from time 0 up to 10 h after infection). ○— ○, untreaded cells; ■ — ■, FPA treated cells; ●— ●, Py11 treated cells. Arrows indicate maximum infectivity in the gradients.