Inhibition of Poliovirus Growth by 2-Amino-4,6-Dichloropyrimidine

In the course of researches on the antiviral action of pyrimidine derivatives, one of these, 2-amino-4,6-dichloropyrimidine has been found particularly active on the growth of poliovirus 1.

Material. Actinomycin D (AMD) was provided by Merck; ^{3}H-thymidine (12.5 Ci/m*M*), ^{3}H-uridine (25 Ci/m*M*) and ^{3}H-leucine (14.5 Ci/m*M*) by Amersham. The pyrimidine derivative was synthesized by the Istituto Chemioterapico Italiano.

Methods. Experiments were carried out in HEp2 cell mono-layers (10^6 cells/small petri dish) which were infected with 30 plaque forming units (PFU) of poliovirus 1 Brunenders per cell, or mock infected (with Hank's BSS) and incubated in Hank's BSS (pH 7.3) at 37 °C. Cell damages were evidenced through the incorporation of both neutral red and labelled DNA, RNA and protein precursors. Virus progeny and infectious RNA (extracted according to the GIERER and SCHRAMM method[1]) were measured as PFU (DULBECCO and VOGT method[2]). ^{3}H-uridine and ^{3}H-leucine pulses were given to evaluate virus RNA protein synthesis, respectively, in cells whose nuclear transcriptions had been blocked by AMD, taking into account that most of the leucine uptake, after 3 h from cell infection with picornavirus, is due to virus proteins[3]. The assembly of virus particles and the participation in it of labelled RNA and proteins was established by spinning genetron purified culture pellets at 25,000 rpm (20 °C) in sucrose gradients (11 ml, 10–40%) in SW 40 Ti (Spinco). Details of technique have been reported previously[4–6].

Results. Data in Figure 1 show that the pyrimidine derivative completely prevents poliovirus growth at

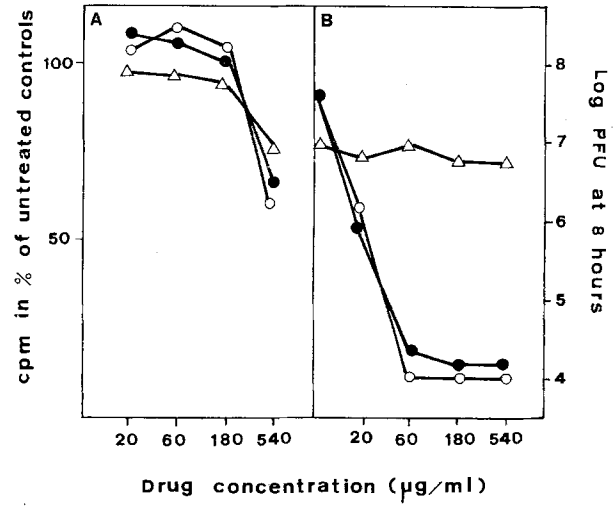

Fig. 1. Effect of 2-amino-4,6-dichloropyrimidine on the metabolism of uninfected cells (A), and on poliovirus growth (B). A) uninfected cell uptake, under acid-insoluble form, of ^{3}H-thymidine (o), ^{3}H-uridine (●), and ^{3}Hleucine (△), after 8 h of drug treatment (1 h pulses, 0.3 µCi/ml). B) infectious virus yield in cell monolayers drug-treated for 2 h, either before (○) or after (●) infection, and in cells infected with drug-pretreated virus suspensions; 2 h contacts at 37 °C; drug then removed by dialysis (△).

[1] A. GIERER and G. SCHRAMM, Z. Naturforsch. *11*b, 138 (1956).
[2] R. DULBECCO and M. VOGT, J. exp. Med. *99*, 167 (1954).
[3] S. PENMAN and D. SUMMERS, Virology *27*, 614 (1965).
[4] M. A. MARCIALIS, P. LA COLLA and B. LODDO, Experientia *28*, 1117 (1972).
[5] P. LA COLLA, M. A. MARCIALIS, G. P. MEREU and B. LODDO, J. gen. Virol. *17*, 13 (1972).
[6] A. M. CIOGLIA, C. DE GIULI, O. ZUFFARDI, F. PALMAS, M. A. MARCIALIS, P. LA COLLA, B. SCARPA and B. LODDO, Riv. Farmac. Terap. *11*, 61 (1971).

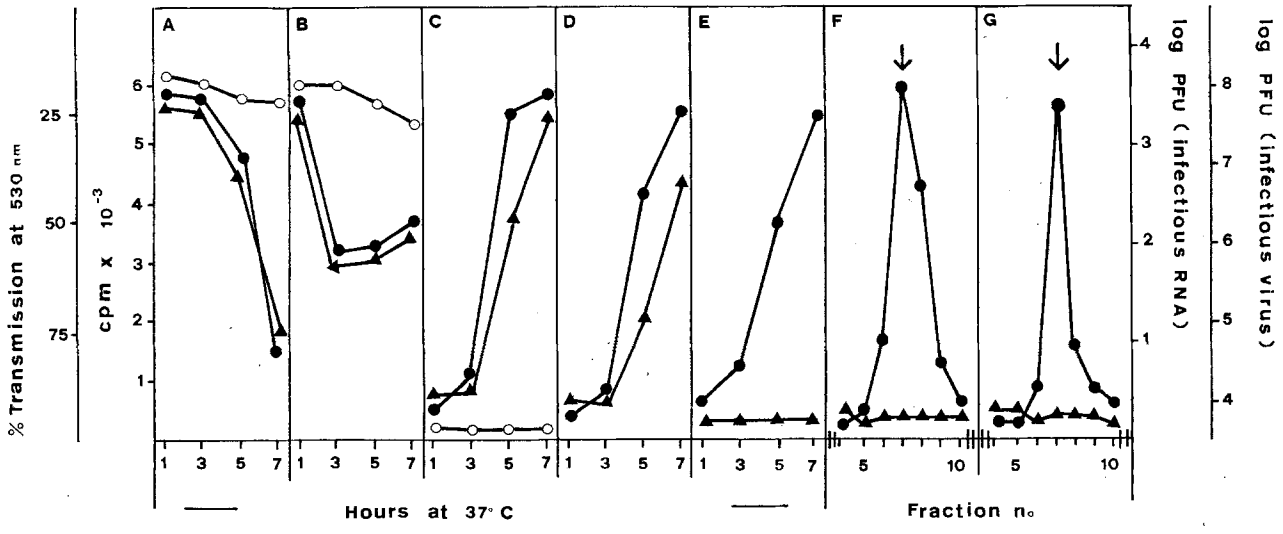

Fig. 2. Effect of 2-amino-4,6-dichloropyrimidine on poliovirus synthesis and poliovirus-induced cell damages. Infected (●) or mock infected (○) cells were incubated at 37 °C in Hank's BSS containing 2 µg/ml of AMD; 100 µg/ml of the pyrimidine derivative was added to half of the infected cultures (▲). A) Intracellular incorporation of neutral red (1%, 1 h pulses), liberated by 1% sodium deoxycholate and measured at 530 nm. B) ^{3}H-leucine uptake (0.2 µCi/ml, 1 h pulses) under acid-insoluble form. C) ^{3}H-uridine uptake (0.2 µCi/ml from time 0) under acid-insoluble form. D) Infectious RNA (as PFU). E) Infectious virus (as PFU). F) and G) Incorporation into virus particles of ^{3}H-leucine and ^{3}H-uridine respectively (2 µCi/ml, added at time 0 after infection). After 8 h, cell cultures were frozen and thawed (−70°: +20 °C) twice, treated with genetron, deprived of cell debris at 3,000 rpm 5 min, and pelletted at 40,000 rpm 2 h. Pellets were dissolved in 0.5 ml of Hank's BSS, layered on 10 ml of sucrose gradients (10–40%) and spinned at 25,000 rpm for 3 h at 20 °C. 5 ml fractions, obtained by puncturing the bottom of the tubes, were examined for radioactivity. Arrows indicate maximum infectivity.

concentrations which do not markedly affect the vitality of uninfected cells. The inhibition of virus growth is irreversible and takes place even when the drug treatment of cell cultures is restricted to 2 h, either before or after the infection period. No inhibition can be seen when the drug is allowed to act directly on virus particles before cell infection (Figure 1). Target of the pyrimidine action seems to be the assembly of virus particles, which is completely prevented, while the replication of infectious virus RNA and the net synthesis of virus proteins are scarcely affected as well the early virus-induced blockade of cell protein synthesis and the cytopathic effect (Figure 2). The pyrimidine analogue does not act on virus assembly directly, but rather by impairing the RNA coating ability of virus proteins made in its presence: when a 3-h period in drug-free medium is allowed to elapse between virus infection and drug treatment, proteins synthesized in that period assemble into complete, infectious virus, the RNA synthesized later in the presence of the drug (Figure 2). Researches are in progress to better define the intimate mechanism of action of 2-amino-4, 6-dichloropyrimidine, as well as the specificity of the antiviral effect[7].

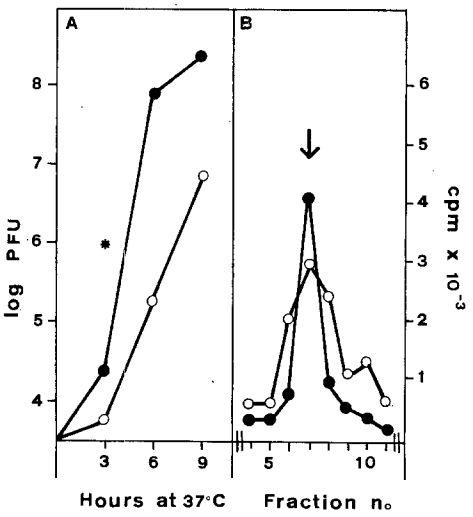

Fig. 3. Ability of virus proteins, synthesized before drug-treatment, to assemble into complete, infectious virus particles, RNA synthesized later in the presence of the drug. Infected cells were incubated for 3 h at 37 °C in Hank's BSS containing AMD 2 μg/ml, and then treated with the pyrimidine derivative. A) Production of infectious virus, titrated as PFU (○) and incorporation of ^{3}H-uridine, 0.3 μCi/ml from time 0 (●). B) Incorporation into virus particles of ^{3}H-leucine (5 μCi/ml) added to the medium from time 0 up to 3 h post-infection and of ^{3}H-uridine (3 μCi/ml) added to the medium 3 h after infection. Cells were harvested at the 8th h and treated as indicated in Figure 2. The asterisk in A) indicated the moment of drug addition to the culture. The arrow in B) indicates maximum infectivity in the sucrose gradient.

Riassunto. La 2-amino-4, 6-dicloropirimidina impedisce la formazione di proteine capsidiche capaci di organizzare con lo RNA virale particelle di poliovirus complete ed infettanti.

M. A. Marcialis, M. L. Schivo, P. Uccheddu, A. Garzia and B. Loddo

Istituto di Microbiologia II dell'Università, Via G. T. Porcell 12, Cagliari (Italia), and Istituto Chemioterapico Italiano, Lodi (Italia), 7 May 1973.

[7] This work has been supported by a grant of the Consiglio Nazionale delle Ricerche.

STUDIORUM PROGRESSUS

Kinetics and Na-Dependence of Riboflavin Absorption by Intestine in vivo

Introduction. Intestinal absorption of riboflavin has been studied both in vivo and in vivtro. In vivo absorption was related to urinary excretion of the vitamin which had previously been administered to the digestive tract. Obviously this approach to the problem is insufficient, since there are too many, barriers interposed in between and thus one cannot really draw conclusions on the mechanism of absorption of the vitamin across the intestinal barrier. However Levy and Jusko[1] were the first to affirm, using this experimental model, that the absorption was saturable in fasting subjects. Using analogous techniques, Stripp[2] had already come to the same conclusions. Since then, both Mayersohn et al.[3] and Christensen[4] have confirmed these results in both man and rat.

The results of these studies in vivo are in contradiction with the earlier work of Spencer and Zamchek[5], of Spencer and Bow[6] and of Turner and Hughes[7] who measured the absorption of riboflavin, using the technique of the everted sac. These authors concluded that there was a passive diffusion of the vitamin across the intestinal wall; they also showed that the rate of diffusion of the vitamin was the same in either the mucosal-serosal or the serosal-mucosal direction. These results are surprising and are possibly influenced by the external conditions used in vitro.

The aim of this work is to study the intestinal absorption of riboflavin in vivo, while adopting an approach to the problem which is different to previous authors. The technique used was the perfusion of a fixed segment of intestine in vivo. Using this method the kinetics of absorption of the vitamin were studied as well as the role played by sodium during absorption by the intestinal tract.

Material and methods. 6-to-10-week-old wistar rats were used in all experiments. They were fasted 24 h before the experiment and Nembutal was used as a narcotic. After abdominal incision, a segment (6–8cm) of

[1] G. Levy and W. J. Jusko, J. Pharm. Sci. *55*, 285 (1966).
[2] B. Stripp, Acta pharmac. tox. *22*, 353 (1965).
[3] M. Mayersohn, S. Feldman and M. Gibaldi, J. Nutr. *98*, 288 (1969)
[4] S. Christensen, Acta pharmac. tox. *27*, 27 (1969).
[5] R. P. Spencer and N. Zamcheck, Gastroenterology *40*, 794 (1961).
[6] R. P. Spencer and T. M. Bow, J. nucl. Med. *5*, 251 (1964).
[7] J. B. Turner and D. B. Hughes, Q. Jl. exp. Physiol. *47*, 107 (1962).