ponents have been found in avilamycin, curamycin, and everninomycin, which confirms the close relationship between those 4 antibiotics[9].

Flambamycin exhibits a very low toxicity. The aqueous suspension is practically non-toxic orally in the mouse and its LD_{50} is 2,500 mg/kg by the s.c. route.

The bacteriostatic activity of flambamycin against some organisms is shown in Table II. The minimum inhibitory concentration (MIC) determinations were carried out by the dilution method in the appropriate medium for each organism and after incubation for 18 h at 37 °C. Flambamycin is mainly active in vitro against gram-positive or gram-negative cocci and some gram-positive bacilli. It is practically inactive against gram-negative bacilli, yeasts and filamentous fungi.

In vivo it retains its activity against the organisms already shown to be sensitive in vitro. It therefore has an excellent therapeutic activity in mice infected experimentally with staphylococcus, streptococcus and meningococcus. However, as shown in Table III, flambamycin is active only by the s.c. route, since it is not assimilated through the intestinal tract.

Given by the s.c. route, flambamycin is inactive against several animal parasitic infections: e.g. chickens infected with *Eimeria tenella* or *Plasmodium gallinaceum* and mice infected with *Plasmodium berghei*.

In conclusion, flambamycin, a new member of the heterosidic antibiotics derived from dichloroisoeverninic acid, exhibits in vitro excellent activity against gram-positive or gram-negative cocci. The same activity is found in vivo when the antibiotic is given parenterally.

Résumé. La flambamycine, nouvel antibiotique produit par *Streptomyces hygroscopicus* DS 23 230, est un hétéroside dont l'aglycone est l'acide dichloroisoéverninique, précédemment mis en évidence dans l'avilamycine, la curamycine et l'éverninomycine. Tant in vitro qu'in vivo elle inhibe fortement la croissance des cocci gram-positifs ou -négatifs, mais thérapeutiquement elle n'est utilisable que par la voie parentérale.

Table III. Curative doses of flambamycin in the mouse[a]

Infecting organism	CD_{50} (mg/kg/day)
Staphylococcus aureus (strain Smith)	13
Streptococcus pyogenes hemolyticus (strain Dig 7)	18
Neisseria meningitidis (strain IP 5 813)	2

[a] s.c. route for 2 days.

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In vitro and in vivo Inhibitory Action of 2-Amino-4,6-Dichloropyrimidine on Polio and Herpes Virus

2-Amino-4, 6-dichloropyrimidine (Py 11) prevents the growth of poliovirus in aminoacid-free medium, by impairing the assembly of virus RNA and proteins into complete particles[1]. Due to the antagonism exerted by glutamine and cysteine[2] Py 11 has been found to be scarcely active, in complete medium, on poliovirus and other unrelated, viral agents, which require complete medium for growth. Data herein referred to show that Py 11 is able to inhibit the growth of both polio and *Herpes simplex* virus in complete, glutamine and cysteine containing medium, provided that mercaptoethanolamine is also present in that medium. Evidence is also given that combined treatment with Py 11 and mercaptoethanolamine has a protective effect against herpes keratitis in rabbits.

Material and methods. Colchicine (Simes); β-mercaptoethanolamine HCl (Sigma); 2-amino-4, 6-dichloropyrimidine (Py 11, Istituto Chemioterapico Italiano); 5-fluoro-

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Table I. Potentiating effect of non-inhibitory doses of mercaptoethanolamine on the antiviral action of Py 11 in HEp 2 cell cultures

Culture medium	Drugs in the medium (μg/ml)		Virus yield at 24 h (input: 10 infectious units/cell)		
			Polio	Herpes	Vaccinia
AFE		—	2.6×10^7	—	—
AFE	Py 11	30	3×10^4	—	—
MEM		—	3.4×10^7	1.2×10^7	1.5×10^7
MEM	Py 11	90	7.8×10^6	9.5×10^6	8.6×10^6
MEM	Mercapt.	30	2.1×10^7	8.9×10^6	1.2×10^7
MEM	Mercapt.	15	1.6×10^7	1.3×10^7	2.1×10^7
MEM	Py 11	30			
	+ Mercapt.	15	6.6×10^4	1.1×10^5	6.2×10^6

2-deoxyuridine (FUdR, kindly provided by N.I.H. Bethesda).

HEp 2 cell monolayers (American type culture collection) (10^6 cells per sample) were infected at 4 °C for 1 h with 10 infectious units per cell of either poliovirus 1 (Brunenders) or vaccinia virus (I.S.M.) or *Herpes simplex* virus (N.I.H.). Cells were then washed 3 times in Hank's BSS and incubated at 37 °C either in complete Eagle's minimum essential medium (MEM), containing glutamine and cysteine, or in the same medium, deprived of the aminoacid supplemented (amino acid-free Eagle's: AFE), both containing 2% calf serum and brought up to pH 7.3 with $NaHCO_3$ and *Tris*. Poliovirus yield was titrated by the agar method[3], herpes and vaccinia virus yield was titrated by the end point method (6 stationary tubes per decimal dilution), starting, in both cases, from 24-h culture samples, which were frozen and thawed 3 times (−70 °C and +20 °C) and free of cell debris at 5,000 rpm for 5 min[4,5].

The effect of Py 11 and mercaptoethanolamine on the replication of uninfected cells was evaluated by the decrease in the number in mitotic figures accumulated in 30 h by colchicine (1 μg/ml in MEM supplemented with 5% calf serum). 5-fluoro-2-deoxyuridine was used as a reference inhibitor.

Albino male rabbits weighing 2 to 3 kg were used in keratitis experiments. Both eyes were anesthetized with 0.5% proparacaine. The corneal epithelium was then uniformly scratched 3 times with a needle and 2 drops of a suspension of *Herpes simplex* virus (approximately 1,000 tissue culture infectious doses – TCID) were added to each eye. Two drops of drugs in lanoline + vaseline oil (2 + 1) were administered to the right eye 3 times a day, at 8 h intervals, starting 4 h after infection. The left eye was similarly treated with drug free lanoline + vaseline oil, to serve as a control. Eyes were examined daily, and at 7th day the intensity of lesions was registered. Scores of — (no lesions) to +++ (maximal severity) were assigned.

Results. Data in Table I show that, at 30 μg/ml, Py 11 completely prevents poliovirus growth in aminoacid-free medium, while at 90 μg/ml has little or no effect on polio, herpes, and vaccinia viruses in complete, glutamine and cysteine containing medium. However, if the complete medium is supplemented with 15 μg/ml of mercaptoethanolamine (which is itself inactive to virus growth) 30 μg/ml of Py 11 is sufficient to inhibit, in that medium, the growth of both polio and herpes virus. Vaccinia virus is scarcely inhibited. The antiviral effect is not supported by apparent cell damages and can therefore be considered specific: as shown in Table II, combinations of Py 11 and mercaptoethanolamine which inhibit virus growth have no effect on the replication of uninfected cells. Data in Table III show, finally, that combined treatment with Py 11 and mercaptoethanolamine protects rabbits against herpes keratitis, while Py 11 alone has little or no effect.

Conclusions. Knowledge of the potentiating effect of mercaptoethanolamine resulted unsuspected from an investigation on a possible role of sulfhydril compounds on the antipolio action of Py 11. At present, it can only be hypothesized that mercaptoethanolamine interacts intracellularly with cysteine, thus depressing its antagonistic action on the antiviral effect of Py 11. It seems of interest that 2 unrelated viral agents, such as polio and herpes simplex virus, may be specifically inhibited by the same compound.

Table II. Lack of effect on uninfected cell replication of virus-active concentrations of Py 11 and mercaptoethanolamine

Drugs in the medium (μg/ml)	Mitoses accumulated in 30 h (%)
Colchicine 1	97
Py 11 30 + colchicine 1	98
Mercaptoethanolamine 15 + colchicine 1	95
Py 11 30 + mercaptoeth. 15 + colchicine 1	98
FUdR 5 + colchicine 1	3

Table III. Effect of topical treatments with Py 11 and mercaptoethanolamine on *Herpes simplex* keratitis in rabbits

Rabbit No.	Right eye treatments (% in lanoline + vaseline oil, 2 drops 3 times a day)	Herpetic lesions at the 7th day	
		left eye	right eye
1	Py 11 0.4	+++	+−−
2		+++	++−
3		+++	+−−
4		+++	++−
5		++−	+++
6		++−	+++
7	Mercaptoethanolamine 0.2	+++	+++
8		+++	+++
9		+++	+++
10		+++	+++
11		+++	+++
12		+++	+++
13	Py 11 0.2 + Mercaptoethanolamine 0.1	+++	+−−
14		+++	+−−
15		+++	−−−
16		+++	−−−
17		+++	−−−
18		++−	−−−

−−−, No lesions; +++, severe keratitis.

Résumé. La 2-amino-4, 6-dichloropyrimidine (Py 11) est capable d'inhiber la réplication du poliovirus et du virus *Herpes simplex* dans des milieux de culture complète, à condition que la mercaptoéthanolamine y soit aussi présente. Les combinaisons des deux composés sont actives, in vivo contre «l'herpes cornealis» du lapin.

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