Riassunto. Le piastrine di 3 diversi ceppi di ratti, contrariamente alle piastrine umane e di cavia, non sono aggregate (sia in vitro che in vivo) nè dalla ristocetina nè dal fattore von Willebrand bovino, porcino o umano, quest'ultimo reso aggregante previo trattamento enzimatico. Tale insensibilità, di cui si è potuta escludere l'origine plasmatica, potrebbe derivare dall'assenza, sulle piastrine di ratto, di un recettore per il fattore von Willebrand (acido sialico?); potrebbe essere così spiegata anche la mancata risposta delle piastrine alla ristocetina. Questi risultati suggeriscono che le piastrine di ratto possono essere un utile modello sperimentale per lo studio della sindrome di Bernard-Soulier nell'uomo.

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Low Virulence and Immunogenicity in Mice and in Rabbits of Variants of *Herpes simplex* Virus Resistant to 5-Iodo-2-Deoxyuridine

Previous research from this and other laboratories[1-3] demonstrated that the resistance to the inhibitory action of 5-iodo-2-deoxyuridine (IUdR) may be accompanied, in *Pox* viruses, by a marked decrease of virulence for receptive animals. Data here referred to show that this also holds for *Herpes simplex* viruses, and suggest a possible use of IUdR-resistant variants of *Herpes* virus in prophylaxis.

Material and methods. Human aneuploid HEp 2 cells (American type culture collection) and primary cultures of mouse embryo cells, which were grown in Eagle's MEM containing 7% calf serum, were used for in vitro experiments. Virus strains were *Herpes simplex* 1 (NIH) and one of its IUdR-resistant variants, obtained by serial passages in HEp 2 cells in the presence of increasing concentrations of IUdR.

In vitro experiments were carried out in cell monolayers (10^6 cells per sample) which were infected at 20 °C for 1 h with 5 infectious units (IU) per cell of either virus strain, washed 3 times in Eagle's MEM supplemented with 1% calf serum and incubated in the same medium at 37 °C. IUdR was added at time 0 after infection. 24 h later, cell cultures were frozen and thawed (−70 °C and +20 °C) 3 times and deprived of cell debris at 3,000 rpm for 5 min at 4 °C. The IU present in the supernatant liquid were titrated by the end point method (6 stationary tubes of HEp 2 cells per decimal dilution).

Part of the in vivo tests were carried out in young Swiss male mice, weighing 10 to 12 g, which were infected either i.p. (0.5 ml) or intracerebrally (0.02 ml). The onset of paralysis and lethality were registered.

Albino male rabbits weighing 2 to 2.5 kg were used in keratitis experiments. Eyes were anesthetized with 0.5% proparacaine, uniformly scratched 3 times with a hypodermic needle and infected with 2 drops of virus suspension or mock infected with Eagle's MEM. In rabbits infected twice, 2 or 30 days apart, the above technical procedure was repeated. The onset of conjunctivitis, keratitis, encephalitis and the lethality were registered. More details are given in the Tables.

Results. Data in Table I show that 10 serial passages in the presence of increasing concentrations of IUdR (from 0.1 up to 25 μg/ml) are sufficient to obtain from the original strain a variant which fully develops in the presence of IUdR concentrations 20 times higher than

Table II. Pathogenicity and immunogenicity of the IUdR-resistant variant of *Herpes simplex* virus in mice

1st injection (IU i.p.)[a]		2nd injection (IU i.c.)[b] (45 days apart)		Total No. mice paralyzed and dead / No. mice injected, at different times after the 2nd injection	
				10	30
—		HS[c]	10^3	18/18	18/18
—		HS	10^2	13/18	16/18
—		HS	10	3/18	4/18
—		HR[c]	10^3	6/18	8/18
—		HR	10^2	0/18	0/18
—		HR	10	0/18	0/18
HR[c]	10^6	HS[c]	10^3	0/18	0/18
	10^5	HS	10^3	0/18	0/18
	10^4	HS	10^3	0/18	0/18
	10^3	HS	10^3	3/18	3/18

[a] Tissue culture infectious units given i.p. [b] Tissue culture infectious units given intracerebrally. [c] See table 1.

Table I. Effect of IUdR on the growth of the IUdR-sensitive strain of *Herpes simplex* virus and one of its IUdR-resistant variants

Virus strain (5 IU per cell)[a]	Host cells	IU[a] produced in 24 h in the presence of IUdR (μg/ml)		
		0	2	20
Herpes simplex (HS)	HEp 2	3.3×10^7	$< 10^4$	$< 10^4$
IUdR-resistant HS variant (HR)[b]	HEp 2	1.6×10^7	5×10^7	3.3×10^7
Herpes simplex (HS)	Mouse embryo	1.6×10^6	$< 10^4$	$< 10^4$
IUdR-resistant HS variant (HR)[b]	Mouse embryo	8.3×10^5	1.6×10^6	3.3×10^6

[a] Infectious units, titrated in HEp 2 cells [b] Obtained by serially transplanting HS in HEp 2 cells, in the presence of IUdR (μg/ml): 0.1 – 0.2 – 0.4 – 0.8 – 1.6 – 3.2 – 6.4 – 12.8 – 25.6. The passages in the presence of 25.6 μg/ml were repeated 5 times and virus pool was then prepared by seeding the variant thus obtained in a IUdR – free Eagle's MEM.

Table III. Pathogenicity and immunogenicity of the IUdR-resistant variant of *Herpes simplex* virus in rabbits

1st infection (IU i.c.s.)[a]		2nd infection (IU i.c.s.)[a]		No. rabbits affected from Conjunctivitis	Keratitis	Encephalitis	No. rabbits dead within 15 days from the 2nd infection
—		HS[b]	10^5	12/12	12/12	12/12	12/12
—		HS	10^4	12/12	11/12	11/12	11/12
—		HR	5×10^6	4/12[c]	2/12[c]	0/12	0/12
—		HR	5×10^5	0/12	0/12	0/12	0/12
HR[b]	2×10^6	HS (2 days apart)	10^5	1/12	1/12	0/12	0/12
HR	2×10^6	HS (30 days apart)	10^5	0/12	0/12	0/12	0/12

[a] Tissue culture infectious units, injected in the conjunctival sac. [b] See Table I. [c] Lesions reversed within 6 days.

that inhibitory for the parental virus. It is noteworthy that this IUdR-resistance is evident both in HEp 2 and in primary mouse embryo cells normally endowed with thymidine kinase activity[4].

Data in Table II show that on the basis of the lethal effect produced by intracerebral injection in mice, the IUdR-resistant strain is about 200 times less virulent than the parental virus. In addition, when injected i.p., the IUdR-resistant variant protects mice from lethal inocula of the parental strain, injected intracerebrally 45 days apart.

Data obtained in rabbits are of more interest, in view of the analogies which exist between rabbit and human herpetic keratitis. As shown in Table III, eye infection with as little as 10^4 IU of the parental sensitive strain of *Herpes simplex* virus are sufficient to trigger a sequence of pathologic events (conjunctivitis, keratitis, encephalitis) which, almost in all cases, results in death. Eye treatments with 2×10^6 IU of the IUdR-resistant variant are, on the contrary, almost deprived of any pathological effect and, moreover, protect rabbits from highly active inocula (10^4 IU) of the parental sensitive strain, introduced by the same way either 3 or 30 days apart.

Research is in progress to establish whether and which relationship exists between IUdR-resistance and avirulence of the virus strain under study, and to ascertain what kind of mechanism, either interferonic or immune specific, or both, is at the basis of the protection this virus induces in animals[5].

Résumé. Par passage en série sur des cellules en culture et en présence de 5-iodo-2-desoxyuridine (IUdR) on a obtenu une souche de virus herpétique résistant à l'IUdR. Cette souche est très peu virulente chez la souris et le lapin, mais est capable de protéger ces animaux contre le virus pathogène d'origine.

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PRO EXPERIMENTIS

A Simple Fluorometric Assay for Ampicillin in Serum

A number of chemical methods for the determination of ampicillin are available. Some of them lack sensitivity (British Pharmacopoeia, 1963, addendum 1964, p. 2; Gravnetterova[1]; Bundgaard and Ilver[2]), or are not applicable to serum (Br. Pharmacopoeia; Smith et al.[3]; Bundgaard and Ilver[2]). Others are laborious because of an extraction step (Gravnetterova[1]; Jusko[4]) or titration procedures (Gravnetterova[1]). A fluorometric method (Jusko[4]) is sensitive and can be applied to serum but is not specific in that α-aminobenzylpenicilloic acid (ampicilloic acid) gives the same response as ampicillin itself.

A stable product with blue fluorescence is formed from ampicillin at pH 5.2 and 100 °C in the presence of low concentrations (< 1 m*M*) of uranyl acetate. The compound has an absorption maximum at 338 nm and an emission maximum at 415 nm at pH 5.2. Fluorescence intensity decreases below pH 3. Benzyl-penicillin does not give any fluorescence. An assay procedure for ampicillin in serum, based on these observations is proposed in the following. The method is simple because uranyl acetate achieves both deproteinization and colour development.

(a) *Direct method in serum* (in the absence of ampicilloic acid). In a polyethylene centrifuge tube, 9 parts of serum and 5 parts of a 3.2% (w/v) aqueous uranyl acetate (dihydrate) solution are mixed by vigorous shaking for 1 min. The precipitate is removed by centrifuging the tubes for 15 min at 4,500 rpm, the supernatant is filtered through paper (Schleicher and Schuell Nr. 589[3]) into glass test tubes and the clear and colourless filtrate is heated for 20 min to 100 °C, the tubes being covered with loosely fitting glass balls to minimize evaporation.

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