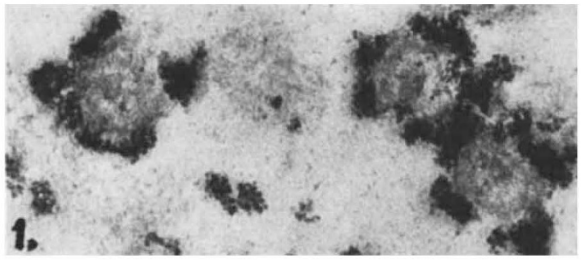

Fig. 1. Electron micrograph of a thin section cut through mature extracellular herpes virus particles which had been fixed with glutaraldehyde and stained for enzymes splitting adenosine triphosphate. An intensely opaque segmented band of enzyme reaction product (lead phosphate) lies close against the outer limiting membrane of each particle indicating the presence there of activity of adenosine triphosphatase-type. (× 90,000)

should be present in them outside the cell. This type of enzyme has never been reported in herpes virus and is in fact known only with the virus of fowl myeloblastosis[6,7].

To test for the presence of enzymes splitting adenosine triphosphate (ATP) in herpes virus, HeLa cultures were infected with heavy doses of the *HFEM* strain of this agent and were collected after 48 h when almost all the cells showed virus growth and maturation; the methods used have already been described[8]. The infected cultures were fixed by squirting from suspension into glutaraldehyde[9], were stained in suspension in slightly modified ATP-containing medium of Wachstein and Meisel[10,11], were post-osmicated, dehydrated, embedded in epoxy resin and sectioned for electron microscopy. The distribution of the opaque enzyme reaction product (lead phosphate) was investigated in this material and appropriate control preparations, with special reference to the mature extracellular virus particles.

On examination, such particles were present in profusion close to most of the infected cells. Where the cells showed surface activity and ATPase reaction product as a dense 30 mμ band of deposit at their plasma membranes, the extracellular virus associated with them was likewise similarly surrounded (Fig. 1). Virus particles lying beside cells without ATPase or in control preparations were always free of deposit. Using only ATP as substrate it is not possible exactly to define the nature of the enzymes involved[12,13], but the results demonstrate unequivocally that mature herpes virus carries activity of ATPase type when released through a cell membrane possessing this function. These findings and their significance will be reported and discussed in full elsewhere[14].

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Effect of 5-iodo-2′-deoxyuridine on Vaccinia Virus, *in vitro*

Several reports have appeared recently regarding the effect of various halogenated pyrimidines on herpes simplex virus[1–3]. 5-Iodo-2′-deoxyuridine (IUDR) and 5-fluoro-2′-deoxyuridine inhibit the herpes simplex virus growth, both *in vitro* and *in vivo*. These halogenated pyrimidines inhibit the biosynthesis of deoxyribonucleic acid by competing with thymidine in the synthesis of DNA-thymidine in mouse Ehrlich ascite cells.

This communication shows that IUDR inhibits vaccinia virus replication and is devoid of any inhibitory activity against polio 1 and coxsackie *B*3 viruses. Vaccinia virus is a DNA virus, like herpes simplex virus[3]; polio 1 and coxsackie *B*3 viruses are RNA viruses.

HeLa cells and culture media used have been described previously[4]. The viruses tested were: vaccinia virus (originally obtained from vaccinal lymph of Istituto Sieroterapico Milanese, Milano) sub-cultured 8 times in HeLa cells; polio 1 (Brunhenders) and coxsackie *B*3, sub-cultured 10 times in HeLa cells. 5-Iodo-2′-deoxyuridine was supplied by Farmila, Milano.

Table 1. *In vitro* Activity of IUDR against Viral Cytotoxic Effect

Concentration of IUDR in the medium (γ/ml.)	Virus	Cytopathic units inhibited	
10	Vaccinia	1,000	(2)*
3·3	Vaccinia	1,000	(2)
1	Vaccinia	< 10	(2)
333·3	Polio 1	< 10	(2)
333·3	Coxsackie *B*3	< 10	(2)

* In parentheses, the number of tests performed.

Table 2. *In vitro* Activity of IUDR against Virus Replication

Viral inoculum (CPU)		Concentration of IUDR in the medium (γ/ml.)	CPE and CPU produced in 5 days: CPE	CPU	No. of tests
Vaccinia	10,000	0	+ + +	10^6	2
Vaccinia	10,000	10	+	< 10	2
Vaccinia	10,000	3·3	+	10	2
Vaccinia	10,000	1	+ + +	10^6	2
Vaccinia	100	0	+ + +	10^5	2
Vaccinia	100	10	−	< 10	2
Vaccinia	100	3·3	−	< 10	2
Vaccinia	100	1	+ + +	10^5	2
Polio 1	100	0	+ + +	10^8	2
Polio 1	100	333·3	+ + +	10^8	2
Coxsackie *B*3	100	0	+ + +	10^7	1
Coxsackie *B*3	100	333·3	+ + +	10^7	1

CPE, Cytotoxic effect; CPU, cytopathic units.

Table 1 shows that IUDR in a concentration of 3·3 γ/ml. inhibits the cytotoxic effect of 1,000 cytopathic units of vaccinia virus. A concentration of IUDR 100 times higher does not inhibit the cytotoxic effects of 10 cytopathic units of polio 1 and coxsackie *B*3 viruses.

Table 2 shows that the cytotoxic inhibition is accompanied by an inhibition of complete cytopathic unit production. It appears that concentrations of IUDR ineffective in inhibiting the vaccinia virus cytotoxic effect inhibit, however, the production of a complete virus. The ineffectiveness of IUDR against polio 1 and coxsackie *B*3 cytotoxic effect is paralleled by a lack of inhibiting activity against complete virus production. In conclusion our results agree with previous reports[2,3] on the selective inhibitory activity of IUDR against DNA viruses. Moreover, they show that IUDR is able to inhibit vaccinia virus in concentrations probably obtainable *in vivo*.

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