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Received September 3, 1963. P.S.E.B.M., 1964, v115.

Inhibition of Varicella Virus by 5-Iodo-2′-deoxyuridine. (28848)

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The demonstration that 5-iodo-2′-deoxyuridine (5-IDU) is a potent inhibitor *in vitro* of herpes simplex and vaccinia viruses(1) subsequently led to its successful use *in vivo* for treatment of keratitis of herpes simplex and vaccinia(2,3). Numerous publications have established that 5-IDU and certain related compounds inhibit only DNA-containing viruses. It has not been firmly established that varicella virus contains DNA exclusively; however, it does appear to share a number of characteristics with herpes viruses and usually is considered to be a member of this DNA-virus family(4).

The following studies were undertaken to determine if 5-IDU is a potent inhibitor of varicella virus, which would be indirect evidence that the virus contains DNA. Further, such a finding might suggest a potential use for this compound in selected individuals with chickenpox or herpes zoster.

Materials and methods. Tissue culture. Two strains of human diploid fibroblasts were used interchangeably, the one WI-38(5) was provided by Dr. Leonard Hayflick of Wistar Institute, and the other was derived from the chorionic villi of human placenta(6). Both strains were handled in the same manner(5), and no detectable differences between them were observed. Suspensions of trypsinized cells in Eagle's basal medium(7) with 10% fetal bovine serum and antibiotics (100 units of penicillin and 100 μg streptomycin per ml) were diluted to contain 1 to 2 $\times$ 10^5 cells/ml and 0.5 ml was planted in each culture tube. Tubes were incubated at 36°C in stationary racks slanted 5° until monolayers resulted (4 to 7 days). Then Eagle's medium was renewed but in this case it contained 0.01 M tris-buffer (pH 7.6).

Varicella virus. These studies involved a strain of varicella virus (Dr. E. Rosanoff, Wyeth Laboratories) originally isolated from a case of chickenpox and passed 24 times in human embryo kidney culture. It was maintained in this laboratory by continuous passage in human fibroblast cultures. Two additional strains of this virus, isolated from cases of chickenpox and herpes zoster, were also briefly studied. All virus strains were

passed as infected cells, that is, once cytopathic effects were observed, the entire cell sheet was removed from the glass with 0.2 ml of a mixture of 0.1% trypsin and 0.05% versene solution. The infected cell suspension was then mixed with 1 ml of medium to neutralize the trypsin and versene, and this suspension was used as the inoculum for further cultures(8).

5-Iodo-2'-deoxyuridine. 5-IDU (General Biochemicals) was dissolved in Eagle's medium (1 mg/ml) and stored at −15°C as a stock solution from which all test solutions were made fresh daily. Some recrystallization occurred on thawing the solution, but resolubilization was achieved at 56°C in a few minutes. Some consideration should be given, however, to the report that mishandling of this compound in solution might result in release of 5-iodouracil, very small amounts of which reverse 5-IDU activity(9). The antiviral activity of the preparation used in these studies would suggest that, if this compound was present at all, it was negligible in amount.

Detection of cytopathic effects. In human fibroblast cultures, varicella virus first produces a focal group of rounded cells which then slowly becomes a plaque-like area(10). These plaques can best be observed and counted, with the aid of a hand lens, if the cells are stained by addition of 0.1 ml of 0.3% 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride (Calbiochem) to each culture tube. Living cells usually produced a brilliant red-purple color in 12 to 24 hours (at 36°C); dead cells remained colorless. Estimates of the number of plaques in stained preparations agreed closely with estimates made during microscopic examination of unstained cultures.

Results. A brief examination of the effect of 5-IDU on the fibroblast host cell was undertaken and, even at a concentration of 1 mg/ml, no detectable cytotoxic effects were observed in 5 days. It was possible to show, however, that 5-IDU did influence cellular proliferation (Fig. 1). Concentrations of from 1.25 μg to 10 μg/ml all equally suppressed cellular multiplication but did not arrest it completely. At 0.62 μg/ml, 5-IDU produced

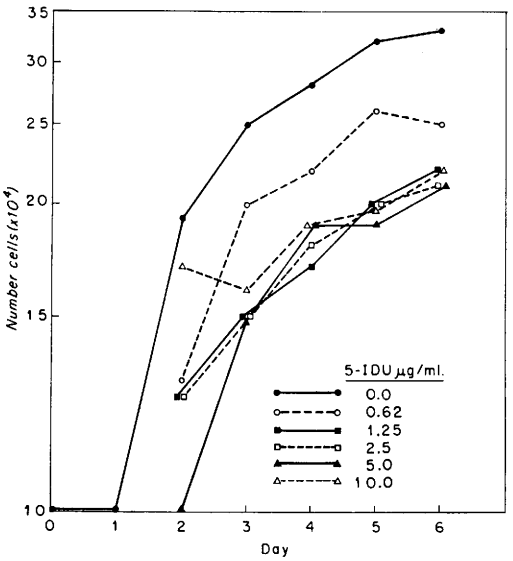

FIG. 1. Effect of 5-IDU on multiplication of cells of diploid human fibroblasts. Various concentrations of 5-IDU in Eagle's medium were added 24 hr after cells were planted. Each day cells from 4 tubes at each dose level were trypsinized and counted in hemocytometer. Each point represents average number of cells in 4 such cultures.

a somewhat less marked decrease in multiplication. These results are in agreement with other more sophisticated studies using human cell lines(11,12).

Fibroblast cultures were infected with 0.1 ml of an undiluted pool of varicella virus. Various concentrations of 5-IDU were added at various times before and after virus inoculation. Cultures were incubated at 36°C for 7 days in a slanted, stationary rack; they were stained with tetrazolium, and the plaques were counted. The data in Table I show that 5-IDU at 5 μg/ml completely suppressed plaque formation regardless of whether it was added 2 hours before or up to 24 hours after the virus. There is some indication that a concentration of 2.5 μg/ml also tended to decrease plaque formation. Fig. 2 presents the typical appearance of varicella virus-infected cultures with and without 5-IDU.

In an effort to determine if the cytopathic effect of varicella virus could be arrested once it had begun, as has been done with vaccinia virus(13), culture tubes were again infected with an undiluted pool of virus-

TABLE I. Effect of 5-IDU on Formation of Plaques by Varicella Virus.

Amt of 5-IDU in culture, μg/ml	Avg No. of plaques*—listed according to time (in hr) of 5-IDU administration relative to time of infection with virus							
	−2	0	+2	+4	+6	+8	+12	+24
0	36	39	36	38	34	37	35	38
.625	34	34	34	32	32†	34†	33‡	35
1.25	24	32	30‡	26	29	34‡	33	33
2.5	18	21	25	22	22	26	27	20
5.0	0	0	0	0	0	0	0	0

* Averages are based on 4 tubes except in those instances marked with a † where 2 tubes were used and in those marked with a ‡ where 3 tubes were used.

infected cells. After 3 days at 36°C cultures showed focal clusters of rounded cells and some small plaques. To 5 tubes, 10 μg of 5-IDU was added; 5 additional tubes served as controls. Cultures were incubated for 9 more days and then the cells were stained. As indicated by the example in Fig. 2, the extension of plaque formation was suppressed by addition of the 5-IDU.

Since the inhibition of plaque formation does not necessarily mean that virus production has been altered, another experiment was undertaken. An undiluted tissue-culture pool of varicella virus was inoculated into 30 tubes containing cultures of human fibroblasts. To 14 tubes, 5 μg of 5-IDU was added; another 14 tubes served as controls. The 2 remaining tubes were set aside, incubated for 7 days and then stained. An average of 16 plaques was observed indicating the original potency of the inoculum. Each day 2 tubes from each group were removed from the incubator, medium was aspirated, cell sheet washed once with fresh medium, fresh medium removed, and cells suspended in a solution of 0.1% trypsin and 0.05% versene at 36°C. Medium was added to neutralize trypsin and versene, and cells were pipetted vigorously. Contents of the 2 tubes from each group were combined and used as the inoculum for 4 tubes of fibroblasts. All 8 tubes from each day's determination were incubated 7 days, cell sheets were stained, and number of plaques was estimated. As seen in Table II, no plaque-forming particles could be detected in cultures previously treated with 5-IDU. In many cases with control cultures, rough counts of plaques had to be made, since many more plaques were produced than had been anticipated. Near the end of the experiment, much larger dilutions were used; unfortunately, these larger dilutions also were used with cultures containing 5-IDU. If a much smaller dilution

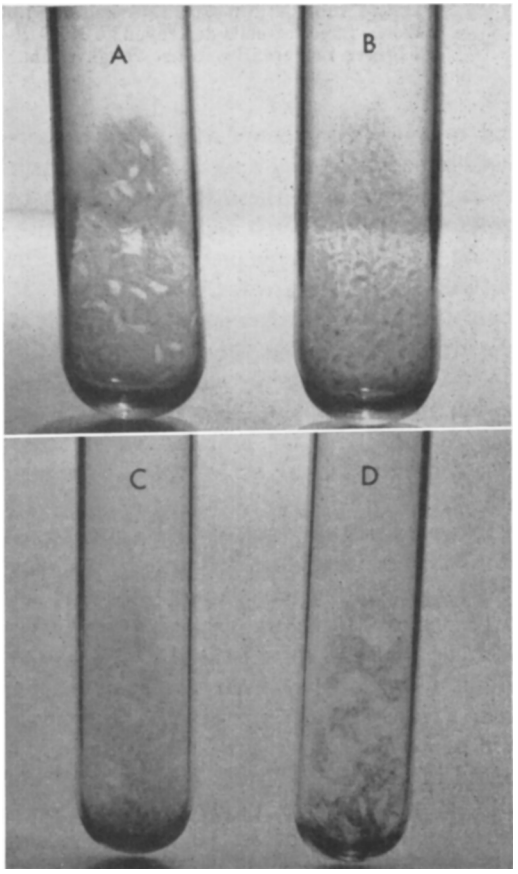

FIG. 2. Human fibroblast cultures infected with varicella virus and stained with tetrazolium 7 days after infection. Tube A contains infected control culture with no 5-IDU; tube B contains infected culture with 5 μg of 5-IDU; tube C contains infected culture to which 10 μg 5-IDU was added after development of small focal areas and plaques; tube D contains infected control culture with no 5-IDU.

TABLE II. Effect of 5-IDU on Production of Varicella-Virus Plaque-Forming Units (PFU).

Conc of 5-IDU, μg/ml	Day after infection	Trypsin-versene solution, ml/tube	Added medium, ml/tube	Plaque count according to size of inoculum .2 ml	Plaque count according to size of inoculum .1 ml	Total PFU, original tubes
0	1	.15	.5	5, 6	3, 5	40
5	1	.15	.5	0, 0	0, 0	0
0	2	1.5 *	.5	SD†	40,46	1720
5	2	1.5	.5	0, 0	0, 0	0
0	3	.15	.5	SD	SD,(100)‡	(1300)
5	3	.15	.5	0, 0	0, 0	0
0	4	.15	.5	TNTC†	(100),(100)	(1300)
5	4	.15	.5	0, 0	0, 0	0
0	5	1	3	98,101	57,66	4640
5	5	1	3	0, 0	0, 0	0
0	6	1	3	SD	(100),(100)	(8000)
5	6	1	3	1, 0	0, 0	1
0	7	1	5	85, 80	39,46	5040
5	7	1	5	0, 0	0, 0	0

* Only 1 tube used here; all other determinations involved 2 tubes.
† SD = Sheet of cells destroyed; TNTC = Too numerous to count.
‡ Figures in parentheses are rough estimates; plaque counts probably exceeded these numbers.

had been used on these latter cultures, perhaps some virus might have been detected. In any event, over the 7-day period, there appeared to have been a significant reduction in the synthesis of plaque-forming virus in cultures previously treated with 5-IDU. It could be postulated that some 5-IDU might be carried over within the cells used as the inoculum, but it certainly would be at a level so low that it would have little effect on plaque production; for, as indicated in Table I, a concentration of at least 5 μg/ml was required.

It would appear that 5-IDU suppresses formation of varicella virus and hence suppresses production of plaques. To aid in further efforts to determine if this were true of other varicella-virus isolates, 2 additional strains were obtained from vesicle fluid, one from a case of chickenpox and one from a case of herpes zoster. Each strain had been passed in fibroblast cultures only once. With both strains, plaque production was suppressed with 10 μg of 5-IDU added 2 hours prior to inoculation with the virus. Control tubes without the compound produced numerous well-formed plaques.

Discussion. Results presented here indicate that 5-IDU is a potent antiviral agent in tissue culture for varicella-zoster virus. Although the mode of action of 5-IDU is not known, it is thought to act by producing fraudulent DNA or by blocking thymidine kinase, thymidylic acid kinase or DNA polymerase(14), all of which are assumed to function in the synthesis of DNA viruses. Since no effective inhibition by this agent has ever been shown against any but DNA viruses, it is thought that these results provide strong, indirect evidence that the virus of chickenpox and of herpes zoster is in fact a DNA-containing virus.

Although the clinically successful use of 5-IDU has been confined to topical application in cases of infection with herpes simplex and vaccinia viruses, as indicated in numerous reports, Calabresi and co-workers (15) have shown it might also be useful when administered systemically. As first suggested by these authors, the systemic use of 5-IDU may be worthwhile in treatment of appropriate patients with varicella-zoster infections, for fatalities are not unknown. Patients who have serious illnesses due to vaccinia, smallpox, and herpes-B viruses may also benefit from treatment with 5-IDU.

Summary. Although 5-iodo-2′-deoxyuridine (5-IDU) has some effect on the multiplication of human fibroblasts in tissue culture, it also markedly prevents cytopathic effects of varicella-zoster virus even when added to the tissue culture in concentrations of 5 μg/ml

as long as 3 days after challenge with the virus. The compound will inhibit further plaque formation even after cytopathic effects are already evident. Production of plaque-forming virus particles is likewise suppressed by the compound. These findings provide indirect evidence that varicella-zoster virus is a DNA virus, and they suggest that perhaps this compound might find some use in cases of serious human disease produced by this virus.

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Received September 3, 1963. P.S.E.B.M., 1964, v115.

Insulin Action and Protein Synthesis in Diaphragm Muscle.* (28849)

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The recognition of the role of nucleic acids in regulation of protein synthesis has directed attention to the possibility that the diverse effects produced by a hormone in responsive cell types might all be the result of a single primary action of a hormone at the genetic locus. This view, which may be designated as the hormone-gene thesis, first advanced in the case of the sex hormones(1,2), has been suggested for other hormones as well, since in certain cases characteristic "target" tissue responses are abolished by actinomycin D, known to inhibit DNA dependent RNA synthesis(3,4) and/or puromycin which blocks protein synthesis(5,6) without inhibition of RNA synthesis(7). Based upon studies with these inhibitors, the hormones whose action at a cellular level appears to involve stimulation of protein synthesis, presumably by way of the synthesis of messenger RNA, include estradiol(7,8), corticoids(9,10,11) and thyroid hormones(12,13). The recent finding of Wool(14) that insulin increases the synthesis of RNA in muscle raises the possibility that the multiplicity of effects of this hormone upon cell metabolism and membrane transport(15) might all be secondary to an effect of insulin at the genetic locus which leads to increased protein synthesis. To test this view, studies were undertaken to determine whether puromycin inhibits characteristic effects of insulin in rat diaphragm muscle. We wish to report here that concentrations of puromycin which almost completely abolish peptide bond synthesis do not influence insulin action upon either D-xylose transfer, α-aminoisobutyrate (AIB) transport or glycogen synthesis in the isolated intact rat diaphragm preparation.

Experimental. Experiments to determine the minimal concentration of puromycin which could effectively block glycine-C^{14} incorporation into diaphragm protein revealed that concentration of 0.11 mM puromycin

* Aided in part by a grant from Nat. Science Foundation.