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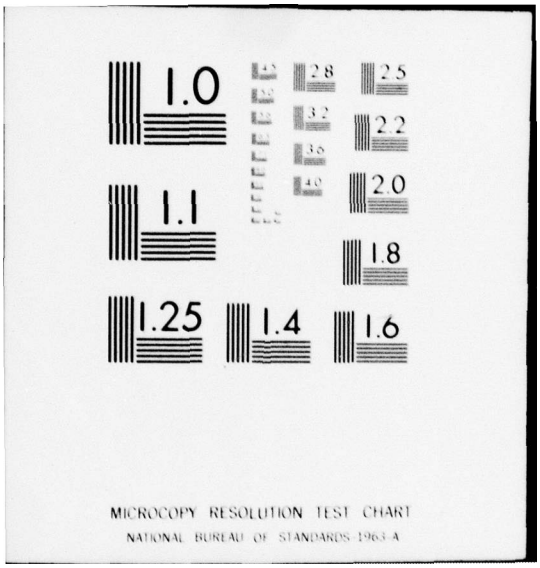
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Radiometric Methods for Rapid Diagnosis of Viral Infection

Final Report

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Three radiometric techniques were investigated for monitoring the effects of herpes simplex virus type I and II and cytomegalovirus on the metabolism of human embryonic lung fibroblast (WI-38) monolayers. The study was based on the hypothesis that (1) early metabolic effects of virus on the cell culture can be used as an indication for the presence of virus; (2) the specificity can be achieved by neutrolization of virus effects with specific antiserum; and (3) radiometric technique can be used to measure these metabolic effects of virus.

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Glucose oxidation by infected and uninfected cells was measured by the output of C-14 CO_2 using Bactec; DNA synthesis was monitored by H-3 thymidine incorporation measured by liquid scintillation counting or by I-125 iododeoxyuridine incorporation measured non-destructively by gamma scintillation counting. Radiometric results were compared to those obtained from visual examination for cytopathic effects in the same cell line. Herpes simplex virus type I inhibited glucose oxidation by WI-38 cells. Herpes simplex virus type I and II, and cytomegalovirus stimulated DNA synthesis by WI-38 cells. These metabolic effects were observed before any signs of cytopathic effects and could be neutrolized with antiserum. These radiometric techniques for detection of viral effect on cellular metabolism are simple, objective and quantitative.

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INTRODUCTION

Direct laboratory isolation of virus is important for the understanding of clinical and epidemiological characteristics of viral diseases (1,2). Although there are many techniques for the detection of viruses (1,3), clinical diagnosis of viral infection continues to be difficult and impractical. With the promise of antiviral agents for the treatment of viral infection (4), the importance of early diagnosis and identification of the virus becomes more apparent. Radioactive tracers are among the most sensitive substances detectable by modern technology. Recent effort has focused on the development of radioimmunoassays for the quantification of viral antigens and antibodies (5-7). Few studies have been done utilizing radioisotopes for the assay of biologically active viruses (8-10). Our studies are based on the hypothesis that 1) early metabolic effects of virus on the cell culture can be used as an indication for the presence of virus; 2) the specificity can be achieved by neutralization of viral effects with specific antiserum; and 3) radiometric technique can be used to measure these metabolic effects of virus. We have studied the detection of herpes simplex virus type 1 (HSV-1), type 2 (HSV-2) and cytomegalovirus using three different techniques (11-13). The results of these form the basis of this report.

MATERIALS AND METHODS

Cells. We planted WI-38 cells in the 20th to 24th passages (HEM Research, Inc., Rockville, Md.) at a concentration of 100,000 cells/ml in a total volume of 2 ml. The cells were grown 2-4 days as stationary

monolayers in Eagle's basal medium, consisting of Earle's base plus 10% fetal calf serum, 25 mM herpes buffer, and 100 units of potassium penicillin G, 100 µg of streptomycin, and 100 µg of kanamycin per milliliter. Cells were then changed to Minimal Essential Medium Eagle (Modified) with Earl's salts (Flow Lab.), 3% fetal calf serum and antibiotics ($MEM_{97}FC_3$), and were used the following day.

The glucose oxidation method used sterile 10-ml serum vials with rubber liners (Johnston Laboratories, Cockeysville, Md.) and aluminum airtight seals (Wehaton Scientific, Millville, N.J.) for cell cultivation, the vials being incubated in a horizontal position at 37°C. The ^{3}H-thymidine incorporation technique utilized 1 dram culture vials (Wheaton Scientific, Millville, N.J.) containing 0.5 ml cell suspension. Culture vials routinely yielded 1-2 x 10^5 cells/monolayer as determined by direct counting in a hemacytometer. The ^{125}I-IdU incorporation technique used 16 x 125 mm glass culture tubes (Corning Glass Works, Corning, N.Y.) containing 1 ml cell suspension. Culture tubes yielded approximately 3.7 x 10^5 cells/monolayer.

Virus stock. A patient isolate of HSV-1 was obtained from the Johns Hopkins Hospital Virology Laboratory. Stock virus was prepared in WI-38 monolayers maintained on Eagle's minimal essential medium. Earle's base plus 3% fetal calf serum with buffer, and antibiotics as listed. The viral material was frozen and thawed twice and then stored at -70°C. The stock was sterility-tested and assayed for glucose concentration. The titer was determined by tube titration in WI-38 cells, and the 50% endpoint was calculated by the method of Reed and Muench (14), yielding 3,200,000 $TCID_{50}$ per 0.1 ml. Tube titration was confirmed by plaque

assay (11), which yielded 7 million plaque-forming units per 0.1 ml. HSV-2 stock (MS strain, ATCC 540) was prepared in a similar manner with a titer of 5.6×10^7 $TCID_{50}$/ml.

The AD-169 strain of human cytomegalovirus was obtained commercially at an infectivity titer of 630,000 $TCID_{50}$ per ml, and was maintained at -60°C until used.

Glucose oxidation system. Serum vials containing preconfluent monolayers with overlay medium removed were infected with 0.1 ml of virus stock, at a virus-to-cell ratio of approximately 10. Uninfected cells received 0.1 ml of an equalized glucose solution prepared in Earle's basal salts. Virus was exposed to the cells for 60 min at 37°C with mild agitation at 15-min intervals. Following the adsorption period, 1.9 ml of low-glucose maintenance medium (i.e., the maintenance medium without the usual 0.1% glucose) plus 4 µCi (60 mCi/mM) of ^{14}C-1-D-glucose (Amersham/Searle Corp., Arlington Heights, Ill.) was added per vial. All vials were prepared in quintuplet. Background controls consisted of an equal volume of ^{14}C-labeled medium without cells or virus.

Measurement of glucose oxidation. The $^{14}CO_2$ produced by cellular metabolism was measured with Bactec R-301 (Johnston Laboratories, Inc.). The $^{14}CO_2$ produced from ^{14}C substrate in the culture vial was flushed into the ionization chamber through two needles inserted in the septum and measured as ionic current. This measured radioactivity was expressed in index units (I.U.), where 100 I.U. ≃ 0.025 µCi. The atmosphere in the vial was replaced with 10% CO_2 culture gas. The $^{14}CO_2$ production from infected and uninfected cells was monitored at 2, 4, 6, 24, 48, and 72 hr after the ^{14}C-labeled medium was added.

Nucleic acid synthesis system. Stationary monolayers were infected as in the glucose oxidation system. One-tenth milliliter of phosphate-buffered saline (PBS) was added to the uninfected control cells. After the adsorption period, 1.9 ml of maintenance medium plus 1 μCi of ^{3}H-methylthymidine (52 Ci/mM) or ^{3}H-5,6-uridine (40 Ci/mM) (Amersham/Searle Corp.) was added to each infected and control vial. Background controls consisted of equal amounts of ^{3}H-labeled medium without cells or virus. All samples were prepared in duplicate and taken for measurement at 1, 2, 4, and 6 hr after infection.

Viral neutralization tests were performed as follows: herpes simplex human immune serum (Flow Laboratories, Rockville, Md.) was heat-inactivated at 56°C for 30 min, mixed with an equal amount of the virus stock, and incubated at 37°C for 60 min, and then 0.2 ml of the mixture was inoculated. Samples were assayed at 1, 2, 4, and 6 hr after infection.

Virus stock was diluted in maintenance medium to contain $\log_{10}$ quantities for dose-response determination. Then, 0.1 ml of the appropriate dilution was added per vial, and uninfected control cells received 0.1 ml of maintenance medium. In this group of experiments, the cells were cultured in one-dram vials (Wheaton Scientific), yielding 40,000 cells per vial as compared to ~ 400,000 cells per vial in previous experiments. Samples were prepared in triplicate and assayed 5 hr after infection.

^{3}H-thymidine incorporation technique. Measurement of ^{3}H-thymidine incorporation was performed as follows: confluent monolayers with overlay medium aspirated were infected with 0.1 ml of virus stock or suspected virus infected material. Uninfected control cells received 0.1 ml of

$MEM_{97}FC_3$. Inoculated cells were incubated at 37°C for 60 min, followed by addition of 0.5 ml/vial of $MEM_{97}FC_3$ containing 1 μCi of ^{3}H-methyl-thymidine (45Ci/mM, Amersham/Searle Corp., Arlington Heights, Ill). The amount of ^{3}H-thymidine incorporated by the cells was measured by liquid scintillation counting. At the designated time, ^{3}H-labeled medium was aspirated and the remaining cell monolayer was washed twice with 2 ml $MEM_{97}FC_3$. This procedure removed all the extracellular radioactivity and did not disrupt the monolayer. Caps of the washed vials were discarded and vials placed in 20-ml glass liquid scintillation vials (Wheaton Scientific) followed by the addition of 15 ml of Bray's solution. Samples were counted with a Tri-Carb scintillation spectrometer Model 3003 (Packard Instrument Co., Downers Grove, Ill).

^{125}I-IdU incorporation technique. Confluent monolayers with overlay medium decanted were infected with 0.1 ml of virus stock at a virus-to-cell ratio of approximately 15. One-tenth milliliter of $MEM_{97}FC_3$ was added to uninfected control cells. After the 60 min adsorption period, 1 ml of $MEM_{97}FC_3$/vial was added, followed by 1 uCi of 5-^{125}I-iododeoxy-uridine (2000Ci/mM, New England Nuclear, Boston, Ma) in 0.1 ml per tube. Cells were incubated at 37°C. Preliminary experiments indicated lower specific activity material did not give suitable sensitivity levels.

The amount of ^{125}I-IdU incorporated by the cells was measured by gamma scintillation counting. At the designated time, ^{125}I-labeled medium was decanted, the remaining monolayer washed twice with 4 ml of $MEM_{97}FC_3$, 1 ml unlabeled medium added, and tubes counted with a Auto-gamma scintillation spectrometer Model 5986 (Packard Instrument Co.). Tubes were relabeled by addition of 1 uCi/0.1 ml ^{125}I-IdU, and reincubated until time for subsequent measurements. Samples were prepared in quintuplet and radioactive measurements obtained at 6, 24, 48, and 72 hr after infection.

Dose response determination was performed as previously stated, except samples were prepared in quintuplet. Viral neutralization tests were performed as previously described above using herpes simplex human immune serum (Flow Lab.).

Examination for cytopathic effects. Radiometric techniques for viral detection were compared with visual detection of histological evidence of cell damage known as cytopathic effects (CPE) in corresponding test-tube monolayers maintained in triplicate.

Statistics. The calculation of statistical significance was based on pair differences (15).

RESULTS

Effect of HSV-1 on glucose oxidation. The effect of HSV-1 on ^{14}C-1-glucose oxidation by WI-38 cells is shown in Table 1. As early as 2 hours after infection there was a significant depression of glucose oxidation by cells infected with HSV-1. This effect was observed 16 hours before visual signs of CPE. The degree of the inhibition of glucose oxidation by HSV-1 infection continued to increase up to 72 hours (17% at 2 hours and 43% at 72 hours).

Effect of HSV-1 on nucleic acid synthesis. Table 2 shows the effect of HSV-1 on DNA synthesis by WI-38 cells. In virus-infected cells there was a marked stimulation of DNA synthesis: at 4 hours after infection, there was a fourfold stimulation. This was at least 14 hours before any signs of CPE were visible. In contrast, HSV-1 had no effect on RNA synthesis by WI-38 cells (Table 3). Thus, the tremendous stimulation of DNA synthesis by this DNA virus was not accompanied by a comparable stimulation in RNA synthesis.

Adding 10,000 bacteria of three different bacterial species (Staphylococcus epidermidis, Pseudomonas aeruginosa, and acinetobacter calcoaceticus var. anitratus) to each sample had no effect on the DNA synthesis of infected or control cells.

Effect of viral neutralization with specific antiserum on DNA synthesis. Neutralization of HSV-1 with specific antiserum resulted in complete elimination of the stimulation of DNA synthesis observed when HSV-1 is present alone (Fig. 1). Antiserum alone had no effect on DNA synthesis by WI-38 cells. Neutralization of virus with antiserum was confirmed by the absence of CPE over a 3-day period.

Effect of various numbers of HSV-1 on DNA synthesis. In order to determine the relative sensitivity of the nucleic acid synthesis system, various numbers of virions ranging from 10 to 3,200,000 $TCID_{50}$ units were tested. Since preliminary experiments indicated that an increase in the multiplicity of infection by a decrease in the number of Wi-38 cells increases the degree of stimulation of DNA synthesis, monolayers composed of only 40,000 WI-38 cells were used for this part of the study. As shown in Table 4, a significant stimulation of DNA synthesis by WI-38 cells could be detected with 10,000 virions at 5 hours after infection.

Effect of HSV-2 on ^{3}H-thymidine incorporation. In order to see whether HSV-2 also stimulates DNA synthesis, its effect on ^{3}H-thymidine incorporation by WI-38 cells was studied. As shown in table 5, HSV-2 (5.6×10^6 $_{TCID50}$) stimulated ^{3}H-thymidine incorporation by WI-38 cells. In virus-infected cells, there was a 2-fold increase in incorporation beginning 2 hours after infection, proceeding to 4-fold by 4 hours and 6-fold by 6 hours.

Effect of varying numbers of HSV-2 on ^{3}H-thymidine incorporation. Relative sensitivity was determined by addition of decreasing numbers of HSV-2 virions

ranging from $10^{6.8}$ to $10_{TDIC_{50}}$ doses. As shown in Fig. 2, the level of sensitivity is a function of time, and by 72 hours after infection even an initial inoculum of 10 virions is detectable. Attempts to distinguish between type 1 and type 2 have failed due to inability to obtain non-cross-reacting antisera.

Effect of IdU pretreatment on ^{3}H-thymidine incorporation. It has been reported that 5-iododeoxyuridine (IdU) potentiates the in vitro replication of several unrelated RNA and DNA viruses (16). Thus, the effect of pre-treatment with IdU on ^{3}H-thymidine incorporation by HSV-1 infected and un-infected Wi-38 cells was investigated to determine if detection time and/or sensitivity could be improved. WI-38 cells were pretreated with 0.5 ml of IdU (10 ug/ml) for 4 days. After removal of IdU, cells were infected with HSV-1 and ^{3}H-thymidine incorporation was determined as before. No significant effect was observed (data not shown).

HSV-1 infected mouse brain model. Experimentally induced HSV-1 encephalitis in weanling mice was employed to ascertain if the ^{3}H-thymidine incorporation technique has any possible efficacy in the detection of HSV in clinical speci-mens. Radiometric measurement of HSV-1 infected and uninfected mouse brains by ^{3}H-thymidine incorporation by WI-38 cells is shown in Table 6. Significant incorporation was observed with mice exhibiting minor to severe symptoms. Increased ^{3}H-thymidine incorporation was detected approximately 1 day before visual signs of CPE with material from mice with minor symptoms. Uninfected mouse brain homogenates had no effect on ^{3}H-thymidine incorporation.

Effect of varying numbers of HSV-1 on ^{125}I-IdU incorporation. The ^{3}H-thymidine incorporation method is a disruptive technique, since addition of scintillation fluid terminates the experiment. Thus, it requires multiple samples for measurement at different time intervals. The available quantity

of a clinical specimen is often insufficient to allow multiple samplings. Therefore, the incorporation of ^{125}I-IdU, a thymidine analogue, into infected and uninfected cells was investigated. ^{125}I is a gamma emitter. It can be counted non-destructively without addition of scintillation fluid, allowing repetative measurements on the same sample. Fig. 3 shows the dose response effect of HSV-1 on ^{125}I-IdU incorporation by WI-38 cells. Increased incorporation was observed 6 hours after infection with 10^5 -$10^{6.8}$ virions (p 0.005), 24 hours with 10^4 virions (p 0.005), 48 hours with 10^3 virions (p 0.001), and 72 hours with 10-10^2 virions (p 0.01, p 0.005). These radiometric measurements were not always more rapid than the appearance of early signs of characteristic CPE (Fig. 3).

Effect of viral neutralization with immune serum on ^{125}I-IdU incorporation. Neutralization of varying numbers of HSV-1 with human immune serum resulted in complete elimination of the increased ^{125}I-IdU incorporation observed when HSV-1 alone is present (Fig. 3). Immune serum alone did not cause any increase in incorporation. Viral neutralization was confirmed by absence of CPE over a 2 week period.

Since the availability of WI-38 cells is uncertain at present (17), another well characterized human embryonic lung fibroblast cell line, MRC-5, was evaluated. Similar results were obtained with HSV-1 infected MRC-5 cells.

Cytomegalovirus experiment: Figure 4 shows the effect of CMV on [^{3}H] thymidine incorporation in WI-38 cells when a viral inoculum of 63,000 $TCID_{50}$ was used. In virus-infected cells there was no significant effect at 24 hours after infection, but there was a marked stimulation of [^{3}H] thymidine uptake after 48 hours.

Table 7 summarizes the [^{3}H] thymidine incorporation by cells infected with various concentrations of CMV. The numbers represent percentage uptake in

infected cells compared with uninfected control cells (100%) at each viral concentration. Significant stimulation was noted with 1,000 $TCID_{50}$ 48 hours after infection. When CMV inocula of 100 $TCID_{50}$ or less were used, no significant stimulation was observed even after 72 hours.

Parallel experiments were performed to compare this radiometric method with microscopic observation of cytopathic effects. As shown in Table 8, at a high infectivity (63,000 $TCID_{50}$) CPE was noted as early as one day after infection, but significant [^{3}H] thymidine uptake was not observed until the second day. This contrasts with the results of a viral inoculum of 1,000 $TCID_{50}$, which showed significant increase in [^{3}H] thymidine incorporation 2 days after infection, whereas CPE was not observed until the fourth day. At viral concentrations below 1,000 $TCID_{50}$, no stimulation was seen up to 4 days, and no CPE was noted until 8 days after infection.

DISCUSSION

The data suggests that radiometric measurements of the effects of HSV on DNA synthesis by cultured cells can be used as a quantitative and objective assay for the active virus. Specificity can be afforded by neutralization of the virus with immune serum. Sensitivity is time-dependent with an initial inoculum of 10 virions being detectable by 72 hours after infection. These measurements are at least as fast, if not more rapid than the appearance of characteristic cytopathic effect. The ^{3}H-thymidine technique was shown to be efficacious for the assay of HSV-1 in infected mouse brains. Smith and Melnick (18) have reported that the concentration of HSV in vesicular fluid ranges from 3×10^9/ml to 7×10^{10}/ml. Thus, it is possible to detect HSV from vesicle fluid with our radiometric technique in a few hours.

The ^{125}I-IdU incorporation technique has distinct advantages over the ^{3}H-thymidine technique because it is non-destructive, thus allowing repeated measurements over extended periods and requiring a minimal quantity of samples. IdU is known to incorporate into DNA of mammalian cells (19) and into viruses (20). It has been shown to exhibit diverse effects from inhibition of cancer cells (21) and viruses (22) to enhancement of replication of unrelated viruses (16), conversion of non-permissive cells to a permissible state (23) and activation of RNA (24) and DNA (25) tumor viruses. The radionuclide ^{125}I-IdU has been employed in assays for tumor cells (26) and cell-mediated cytotoxicity (27), and may exhibit cell toxicity under certain conditions (28). In this study we showed that ^{125}I-IdU might also be employed as a simple, quantitative and objective assay of DNA virus such as herpes simplex.

The early inhibition of ^{14}C-1-glucose oxidation by HSV-I in WI-38 cells has not been previously demonstrated. Graves (15), utilizing a poliovirus-HeLa-cell system, was unable to detect any effect on glucose oxidation. Our method of measuring glucose oxidation by monitoring $^{14}CO_2$ release with an ionization chamber has the advantages of being non-destructive and of allowing repeated sampling from the same vial over extended periods. However, the presence of a relatively high concentration of glucose in biologic fluid, such as blood, poses a practical problem, since this nonlabeled glucose would compete with ^{14}C-glucose for oxidation.

Radiometric detection of HSV in cell culture as shown in this study depends on the metabolic effects of virus, although the metabolic effects of viruses on cell cultures are closely related to their cytopathic effect, a dissociation of these two effects may occur. The efficacy of these radiometric techniques in diagnostic virology and in monitoring noncytopathic

viruses and viruses which do not multiply in cell cultures, awaits further evaluation.

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TABLE 1. EFFECT OF HSV-1 ON ^{14}C-1-GLUCOSE OXIDATION BY WI-38 CELLS

	Time after infection (hr)					
	2	4	6	24	48	72
Control*	58 ± 8	226 ± 36	438 ± 74	1,222 ± 203	1,874 ± 253	2,310 ± 253
HSV-1*	44 ± 8	173 ± 33	326 ± 33	720 ± 125	1,078 ± 168	1,322 ± 187
% Control	83.4	76.6	74.4	59.0	57.5	57.2
p value	<0.01	<0.01	<0.005	<0.01	<0.005	<0.005

* The results are expressed as mean ± standard error (index units) of the cumulative $^{14}CO_2$ production. Each of four experiments was done in quintuplet and the results were averaged. The number of WI-38 cells used was 300,000–400,000, and the quantity of HSV-1 was 3,200,000 $TCID_{50}$ units.

TABLE 2. EFFECT OF HSV-1 ON DNA SYNTHESIS BY WI-38 CELLS

	Time after infection (hr)			
	1	2	4	6
Control*	2,958 ± 678 (4)	3,700 ± 447 (4)	5,818 ± 685 (5)	7,245 ± 720 (3)
HSV-1*	2,789 ± 479 (4)	8,341 ± 1,930 (4)	26,482 ± 3,392 (5)	43,298 ± 7,882 (3)
% Control	94.3	225.4	455.2	597.6
p value	>0.5	>0.05	<0.005	<0.05

* The results are expressed as mean ± standard error (cpm). Each experiment was done in duplicate and the results averaged; the number in parenthesis indicates the number of experiments. The number of WI-38 cells used was 300,000–400,000, and the quantity of HSV-1 was 3,200,000 $TCID_{50}$ units.

TABLE 3. EFFECT OF HSV-1 ON RNA SYNTHESIS BY WI-38 CELLS

	Time after infection (hr)			
	1	2	4	6
Control*	5,242 ± 2,475	10,086 ± 4,324	17,007 ± 8,498	28,328 ± 11,607
HSV-1*	5,305 ± 2,503	10,158 ± 4,610	20,274 ± 9,392	30,351 ± 13,603
% Control	101.2	100.7	119.2	107.1
p value	>0.5	>0.5	>0.1	>0.4

* The results are expressed on the same basis as in Table 2. Three experiments were performed.

TABLE 4. EFFECT OF VARIOUS NUMBERS OF HSV-1 ON DNA SYNTHESIS BY WI-38 CELLS 5 HR AFTER INFECTION

	Infectivity ($TCID_{50}$/sample)*						
	3.2×10^6	10^6	10^5	10^4	10^3	10^2	10^1
% Control	844 ± 79 (3)	741 ± 12 (3)	437 ± 65 (4)	198 ± 14 (4)	117 ± 17 (4)	126 ± 7 (3)	125 ± 17 (4)
p value	<0.025	<0.001	<0.025	<0.01	>0.2	>0.2	>0.2

* The results are expressed as mean percent of control ± standard error (cpm). Each experiment was done in triplicate and the results were averaged; number in parenthesis indicates the number of experiments. The number of WI-38 cells used was 40,000.

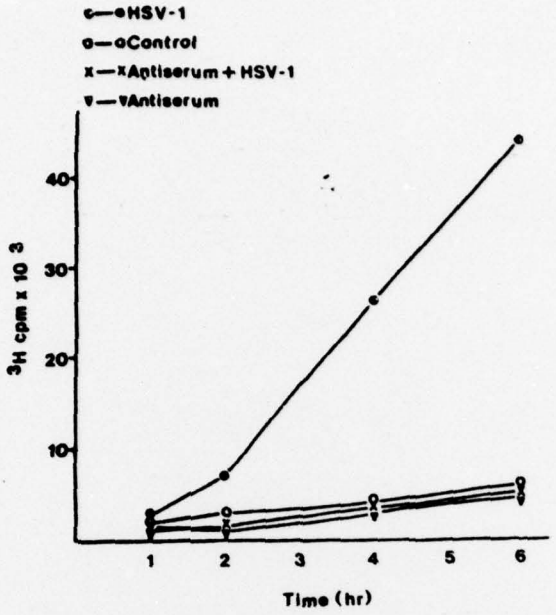

FIG. 1. Effect of HSV-1 neutralization by specific human immune serum on DNA synthesis by WI-38 cells. Each point represents mean of duplicate samples from typical experiment.

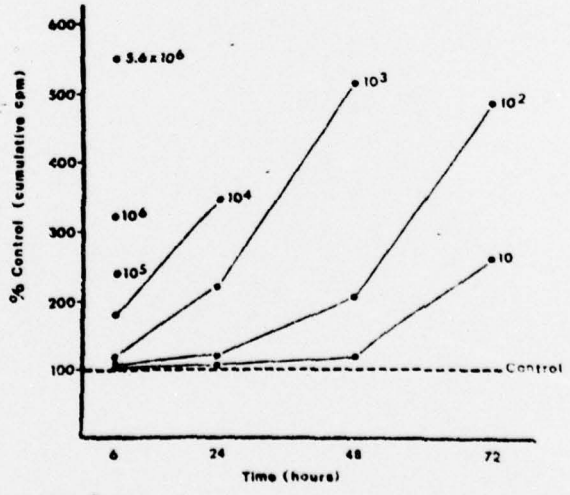

FIG. 2. Effect of varying numbers of HSV-2 virions on [^{3}H] thymidine incorporation by WI-38 cells. Results are expressed as percentage of control (control = 100%) for triplicate samples from a typical experiment. Number of WI-38 cells used was 100,000. Number of virions used ranged from 10 to 5.6 million $TCID_{50}$, as indicated.

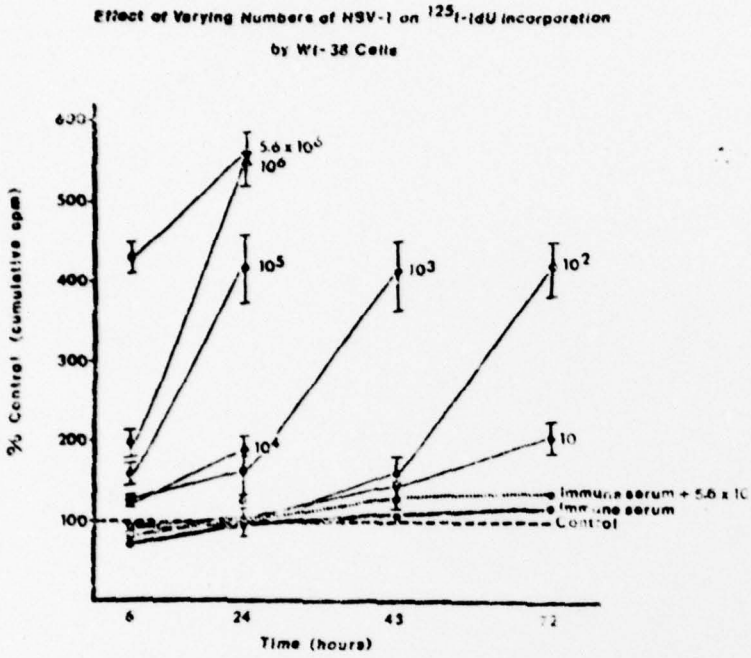

FIG. 3. Effect of varying numbers of HSV-1 virions, and neutralization by human immune serum, on [^{125}I] IdU incorporation by WI-38 cells. Results are expressed as percentage of control (mean and range). Each of three experiments was done in quintuplicate. The number of WI-38 cells used was approximately 3 × 10^5. Points designating viral neutralization are expressed as percentage of control for quintuplicate samples from a typical experiment.

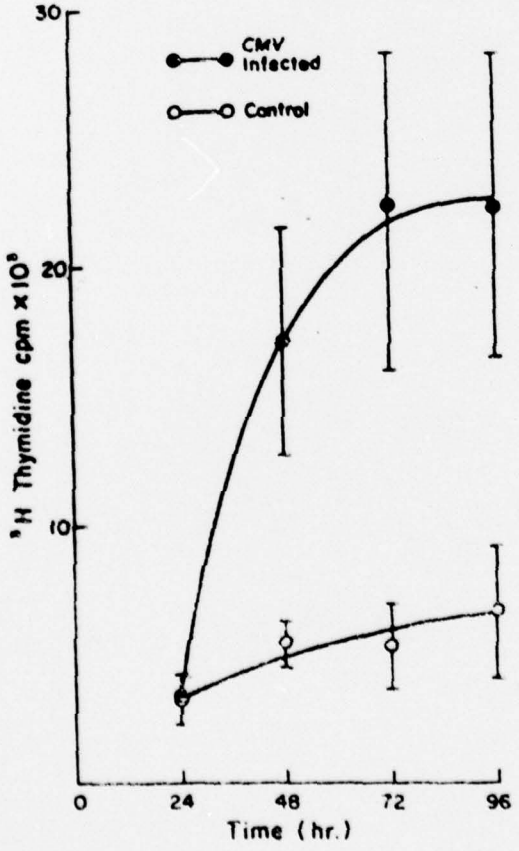

FIG. 4. Effect of CMV (AD 169) on [^{3}H] thymidine incorporation by WI 38 cells. Each point represents the mean of triplicate samples from three to six experiments using a viral inoculum of 63,000 $TCID_{50}$. Control cells were uninfected.

TABLE 5. EFFECT OF HSV-2 ON [^{3}H] THYMIDINE INCORPORATION BY WI-38 CELLS

	Time (hours after infection)					
	2		4		6	
	Mean	Range	Mean	Range	Mean	Range
Control*	16,652	11,549–20,500	25,617	13,552–41,556	43,765	23,985–52,553
HSV-2	37,192	26,891–61,067	112,834	94,773–175,356	177,841	122,891–227,667
% Control	218	178–307	432	225–699	565	436–763
p value		<0.05		<0.025		<0.01

* Results are expressed as mean and range (cpm). Each of four experiments was run in triplicate and the results averaged. The number of WI-38 cells used was 1×10^5, and the quantity of HSV-2 was 5.6×10^6 $TCID_{50}$.

TABLE 6. DETECTION OF HSV-1 IN INFECTED MOUSE BRAINS BY MEASUREMENT OF [^{3}H] THYMIDINE INCORPORATION INTO WI-38 CELLS

Clinical symptoms*	No. of mice	% control (20 hr after infection)†	First appearance of CPE (hr)	$TCID_{50}$/0.1 ml brain homogenate‡
Severe	5	606 (564–645)	20–36	>10^7
Minor	5	238 (212–260)	48–60	4.9×10^6 ($3.2–5.6 \times 10^6$)
None	4			
	(2)	124 (121–127)	72	5.6×10^5
	(2)	109 (103–112)	neg	neg

* Severe: tremors, contralateral hemiplegia; Minor: lethargic, ruffled fur.

† Results are mean cumulative cpm, expressed as percentage of control plus range. Quadruplicate samples were run, and the results averaged, for each infected mouse. Control values represent means of five uninfected mice. Each brain was run in quadruplicate.

‡ Results are expressed as mean and range of designated number of mice, each run in triplicate.

TABLE 7. EFFECT OF CMV CONCENTRATION ON [^{3}H] THYMIDINE INCORPORATION BY WI-38 CELLS

	Infectivity ($TCID_{50}$ per sample)*					
	63,000	10,000	1,000	100	10	1
48 hr after infection						
mean % of control	313 (6)	393 (5)	250 (3)	130 (3)	120 (3)	117 (2)
range (%)	188–390	240–627	233–291	113–162	85–161	99–134
72 hr after infection						
mean % of control	416 (3)	424 (5)	263 (4)	131 (3)	144 (3)	133 (2)
range (%)	310–477	242–666	194–350	96–170	113–178	114–151

* Each experiment was done in triplicate and results were averaged. Number in parentheses indicates number of experiments.

TABLE 8. COMPARISON OF [^{3}H] THYMIDINE INCORPORATION AND CPE IN CMV-INFECTED CELLS

Time after infection (days)	Infectivity ($TCID_{50}$ per sample)*					
	63,000	10,000	1,000	100	10	1
1	106	143	122	90	97	79
	(+)	(−)	(−)	(−)	(−)	(−)
2	313†	393†	250†	130	120	117
	(+)	(+)	(−)	(−)	(−)	(−)
3	416†	424†	263†	131	144	133
	(+)	(+)	(−)	(−)	(−)	(−)
4	331†	520†	220†	107	76	85
	(+)	(+)	(+)	(−)	(−)	(−)
8‡	(+)	(+)	(+)	(+)	(−)	(−)
10	(+)	(+)	(+)	(+)	(+)	(−)
15	(+)	(+)	(+)	(+)	(+)	(+)

* Numbers are mean percentage of control expressed on the same basis as in Table 1. (+) = positive CPE; (−) negative CPE. Significant mean percentages are indicated by †.

‡ Radiometric measurements were not made because cells detached from the vials.

Publications

1. D'Antonio N, Tsan MF, Charache P, et al: Simple radiometric techniques for rapid detection of herpes simplex virus type 1 in WI-38 cell culture. J Nucl Med 17:503-507, 1976.

2. D'Antonio NL, Tsan MF, Griffin DE, Charache PA and Wagner HN Jr: Radiometric detection of herpes simplex viruses. J Nucl Med 19:185-190, 1978.

3. Hurlburt EM, Ki PF, Wagner HN Jr: Effect of cytomegalovirus infection on metabolism of WI-38 cell cultures. J Nucl Med 19:191-194, 1978.

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