UPTAKE OF TRITIATED THYMIDINE AND URIDINE BY *PSEUDOMONAS AERUGINOSA*-INFECTED HELA CELLS

by

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ABSTRACT

Radioautographic and liquid scintillation techniques using ^{3}H-uridine and ^{3}H-thymidine were performed on Pseudomonas aeruginosa-infected HeLa cells to study the uptake of these labeled nucleic acid precursors. Herpes simplex and poliovirus type 2, as well as Salmonella typhimurium, were used as controls in this study.

New evidence relating to the mechanism of plaque formation by P. aeruginosa in HeLa cell cultures is presented in this paper. Radioautographic studies showed an increased uptake of ^{3}H-uridine by HeLa cells around the peripheries of the plaques and in areas where bacteria were visible prior to the appearance of plaques. Liquid scintillation experiments confirm the increase in ^{3}H-uridine uptake by the infected HeLa cells. Conversely, there was no increase in the uptake of ^{3}H-thymidine by the P. aeruginosa-infected HeLa cells as shown by the radioautographic studies. The liquid scintillation experiments showed a large increase in the uptake of ^{3}H-thymidine by the infected cells, but this was shown experimentally to be uptake by the bacteria themselves.

INTRODUCTION

In 1965, Ludovici and Christian first reported the formation of virus-like plaques by bacteria in HeLa monolayer cultures (1). Initially viral contamination was suspected but on continued incubation the fluid became turbid indicating the presence of bacteria.

Further investigation into this phenomenon revealed that Pseudomonas aeruginosa was the organism responsible for the virus-like plaques in tissue culture (2). This bacterial contaminant was traced to a technician whose child had developed summer diarrhea. It was found that strains of Pseudomonas fluorescens and Alcaligenes fecalis as well as P. aeruginosa were able to produce virus-like plaques on a variety of established cell lines. Plaque production was absent when strains of Salmonella typhosa, Escherichia coli, and Staphylococcus aureus were tested.

This same study showed that newborn calf serum (NBCS) was required in the media for plaque formation and that low concentrations (1 to 2%) permitted earlier plaque formation than high concentrations (5 to 20%). There was a linear relationship between the number of bacteria added and the number of plaques produced. The authors presented preliminary evidence suggesting that these organisms multiply intracellularly.

Coleman, Janssen, and Ludovici (3) investigated toxins produced by P. aeruginosa which were cytopathogenic for HeLa monolayers. They found that two types of toxin were produced. One type of toxin, designated Y, was produced in the absence of NBCS but was neutralized by the addition of 10% serum. Another, designated Z, was produced in the presence of 10% NBCS. Since NBCS was required for plaque formation, toxin Z was implicated as a possible factor in the formation of virus-like plaques in HeLa monolayers.

The similarity of plaques caused by P. aeruginosa and those caused by viruses and the disadvantages of plaquing in a liquid system led Wexlar, Moore, and Ludovici (4) to a study of the agar overlay plaquing technique commonly used for virus titrations. It was found that plaques would appear if NBCS was present in both the adsorption fluid and the agar overlay medium. The absence of serum permitted colonial growth but no plaques. It was suggested that NBCS might play a dual role in plaque production, first stimulating bacterial phagocytosis by the HeLa cells and second acting as a nutrient factor required by the host cell for possible intracellular multiplication of the bacteria.

Viruses that form plaques are known to have an intracellular relationship with their host cells. The virus takes over the host cell's machinery and manufactures more virus particles by synthesizing the appropriate nucleic acid cores and protein coats. The search for

evidence to determine whether or not P. aeruginosa is actually phagocytized by the HeLa cells and multiplies from intracellular loci to produce plaques led to this study of the uptake of RNA and DNA precursors by infected cells.

The purpose of this research was to determine whether the uptake of tritiated uridine (^{3}H-uridine) or tritiated thymidine (^{3}H-thymidine), two specific labeled precursors for RNA and DNA respectively, by the HeLa cell would be affected under the influence of P. aeruginosa infection. Two methods were chosen to determine the uptake of the labeled precursors, so that a comparison might be made. The two methods were radioautography (using a liquid emulsion dipping technique) and liquid scintillation.

MATERIALS AND METHODS

Bacteria. The strain of P. aeruginosa used was isolated by Ludovici and Christian (1) and stored in the frozen state in liquid nitrogen at -196° C. It was maintained on nutrient agar slants throughout the course of these experiments. Prior to use, the organism was added to tubes containing 10 ml of 5% NBCS, Eagle's basal medium (EBM) with Earle's balanced salt solution (BSS) without antibiotics and incubated at 37° C for 6 to 8 hr. Duplicate plate counts using Difco plate count agar were made to determine the number of organisms per ml. The bacterial suspensions were held at 4° C to prevent further multiplication, until they were used within two days.

Viruses. The viruses used include an RNA virus, poliovirus type 2, Lansing strain, and a DNA virus, Herpes simplex, Mayo 1814. Virus stocks were made by growing the viruses on HeLa S_3 monolayers in 32 oz prescription bottles. After repeated freezing and thawing, the cell-virus suspension was uniformly dispersed and placed in ampules and subsequently frozen at -65° C. The virus titer of representative ampules was determined by the test tube dilution procedure and calculated by the method of Reed and Muench (5).

Cell culture. The propagation of stock HeLa S_3 was done in 6 oz prescription bottles using 10% NBCS, EBM with BSS supplemented

with 100 units of penicillin and 0.1 mg of streptomycin per ml. Twenty-four hr before preparing coverslip or test tube cultures, the medium was changed to 5% NBCS, EBM without antibiotics. A 0.25% trypsin solution was used to release the cells from the glass for subculture.

Coverslip cultures for the radioautography experiments were prepared by using a slight modification of the technique of Miller and Ludovici (6). The 11 X 22 mm, No. 1 coverslips were seeded with 1.2×10^5 cells per 0.4 ml of the medium previously described. The cell suspension was trapped between the coverslips and tubes. The tubes were allowed to stand horizontally in this manner at room temperature for 1 hr to allow the cells to settle and attach to the glass coverslips. The coverslips were then turned over and dropped to the lower surface of the horizontal tubes so that the cells could be covered with an additional 0.6 ml of medium. The tubes were incubated for 2 to 3 days at 37° C. Prior to their use in the radioautography experiments, the coverslips were examined microscopically for healthy monolayer sheets and the media was changed to 5% NBCS, EBM with BSS without antibiotics.

Tube monolayer cultures used in the liquid scintillation counter experiments were prepared in 16 X 125 mm disposable Pyrex culture tubes. The tubes were seeded with 1 ml of the same medium containing 10^5 cells. The tubes were incubated at 37° C for 2 to 3

days until a confluent monolayer developed, at which time the media was changed to 5% NBCS, EBM with BSS without antibiotics.

Isotopic compounds used. The tritiated uridine used was obtained as 5-^{3}H-uridine from New England Nuclear (specific activity, 26.6 c/mmole). A stock solution of 10 uc/mM was made using sterile double distilled water. For the radioautography experiments the stock ^{3}H-uridine was diluted to 0.1 uc/0.1 ml and 0.1 ml of this dilution was added to each tube to be labeled. In the liquid scintillation counter experiments, 0.1 ml (containing 1 uc) was added to each tube to be labeled.

The tritiated thymidine was obtained as 5-methyl-^{3}H-thymidine from the same source (specific activity, 16.1 c/mmole). A stock solution was made in the same manner and was diluted to 0.1 uc/0.1 ml for the radioautography experiments and 2 uc/0.1 ml for the liquid scintillation counter experiments.

Radioautographic technique. The method used was basically that found in the Handbook of Cell and Organ Culture (7). The media was removed from all coverslip tubes and the cultures were infected with 0.3 ml of bacteria or virus suspension. During the adsorption period of 1 hr at 37° C, the 0.3 ml of infecting suspension was trapped between the monolayer of cells on the coverslip and the glass surface of the tubes. After this adsorption period, 0.7 ml of medium was added to each tube and the coverslips were dropped to the lower surface of the horizontal tubes as described previously.

Isotopes were added to cultures 20 min prior to fixing the coverslips. This gave a 20 min pulse-label. The cells were incubated at 37° C during the pulse-labeling period, after which the coverslips were removed from the tubes with forceps and placed in Columbia staining jars to be washed in four changes of warm, sterile BSS. Non-labeled control coverslips were washed separately. All coverslips were fixed for ten min in absolute methanol. After air drying they were fixed to the end of 1 X 3 inch slides with Permount (cell monolayer up) and allowed to dry at room temperature for several days.

Kodak NTB3 Liquid Nuclear Track Emulsion was used. The slides were coated with emulsion, air dried, placed in tape-sealed, light tight slide boxes and stored at 4° C for the proper exposure time. Exposure times were four days with ^{3}H-thymidine and three days with ^{3}H-uridine.

Exposed slides were developed for five min in Kodak D-19 developer, placed in SB-5a stop bath for 15 to 20 sec, and fixed for five min in Kodak fixer. All solutions were kept at 21° C. The development procedure was carried out in a darkroom equipped with a Wratten No. 2 safelight, at a minimum distance of four feet.

The developed slides were washed for 20 min in tap water, rinsed in distilled water and immediately stained in 0.25% toluidine blue for three min. They then were rinsed two to three sec in 70% ethanol

and allowed to air dry. A second coverslip was mounted over the original coverslip to protect the emulsion.

Isotope labeling counts. Two methods of determining the uptake of radioactive isotopes were utilized. The first was actual grain counts to determine the average number of grains per cell. This technique was used for cells labeled with ^{3}H-uridine when labeling was light. A micrometer grid was used in the eyepiece of the microscope to assist in these counts. The total number of grains and cells within the grid area was determined for four randomly selected high power (X450) fields per coverslip. From these values the average number of grains per cell for each coverslip was computed.

A different method was used for cells labeled with ^{3}H-thymidine because some cells were too heavily labeled to get an accurate grain count. The procedure used counted all cells having a grain count above background (five to seven grains per cell). The percentage of labeled cells was computed from the number of labeled cells divided by the total number of cells in five randomly selected high power (X450) fields per coverslip.

Liquid scintillation technique. Monolayer cultures of HeLa cells were prepared as previously described. After the monolayers were washed with 5% NBCS, EBM with BSS without antibiotics, they were infected with 0.5 ml of the bacterial suspension and incubated for

one hr at 37° C to permit adsorption of the bacteria. After this period, 0.5 ml of medium was added and the tubes reincubated.

At periodic intervals the tubes were pulse labeled for 20 min with the appropriate isotope. The medium was then removed and the cell monolayers washed three times with warm BSS. Each monolayer was treated with 0.5 ml of 0.25% trypsin for 5 to 10 min at 37° C to release the cells from the glass. The trypsinized cells were centrifuged for seven min at 1200 RPM in an International Model V Type 2 centrifuge, supernatent fluid discarded and the cells resuspended in 2 ml of 5% trichloracetic acid. The tubes were centrifuged and washed again with trichloracetic acid in the same fashion. The cells were digested by the addition of 0.5 ml of Hyamine (Packard Instrument Company, Inc., Downers Grove, Illinois) and incubated at room temperature in the dark for 24 hr. The contents of each tube were transferred to a scintillation vial using two washes of absolute ethanol (0.6 ml total). The vials were incubated for 1 hr at 70 to 75° C and cooled to room temperature before the addition of 15 ml of the scintillation solution (containing 400 g naphthalene, 1.2 g 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]- benzene (dimethyl POPOP), 28 g 2,5-diphenyloxazole (PPO), made up to 3.8 liters with dioxane). The vials were analyzed in a Model 314EX Packard Tri-Carb liquid scintillation spectrometer and the results expressed as counts per 10 min. The above procedure was similar to the one used by Singhal and Richter (8).

RESULTS

Radioautographic studies.

Effect of P. aeruginosa on the uptake of ^{3}H-uridine. These experiments were designed to determine the uptake of ^{3}H-uridine by HeLa cells infected with two different dilutions of P. aeruginosa. The two bacterial suspensions contained approximately 197 or 113,000 organisms per 0.3 ml. This gave a multiplicity of infection (MOI) of 0.0015 for the high and 0.87 for the low dilution. A low inoculum of organisms produced distinct plaques; while a high inoculum caused a more generalized cytopathogenic effect (CPE).

Table 1 lists the general results of these experiments. Plaques began to appear at about 13 hr after infection with the low bacterial concentration, while there was a 50% CPE of the HeLa monolayer at 12 hr with the higher concentration. Between plaques, areas with heavier labeling were noted, which upon closer inspection revealed the presence of bacteria. The cells around the periphery of the plaques contained much more label than cells farther away from the plaque. Fig. 1a shows an uninfected HeLa monolayer and Fig. 1b-d show plaque development in an infected HeLa monolayer.

Table 1. Time of appearance of P. aeruginosa plaques and characteristics of the uridine labeling.

P. aeruginosa	HOURS			
	8	12	13	15
MOI 0.0015	Plaques absent. 4 to 6 areas with heavier labeling where the monolayer is denser.	Plaques absent. Some heavy labeling in dense spots.	Very few plaques. Bacteria noted in some areas with heavier labeling. Heavier labeling around plaque periphery.	8 to 10 plaques. Many dense areas with heavier labeling. Also find heavy label around plaque periphery.
MOI 0.87	Plaques TNTC.* Heavier labeling in denser cells around plaque periphery.	Plaques TNTC. 50 to 75% CPE. Heavy satellite plaquing. Heavy labeling around plaque periphery.	Complete CPE.	Complete CPE.

*TNTC--too numerous to count.

Control uninfected cultures developed no plaques. There were some dense cell areas with slightly more uridine labeling. However, there were no bacteria present in these areas.

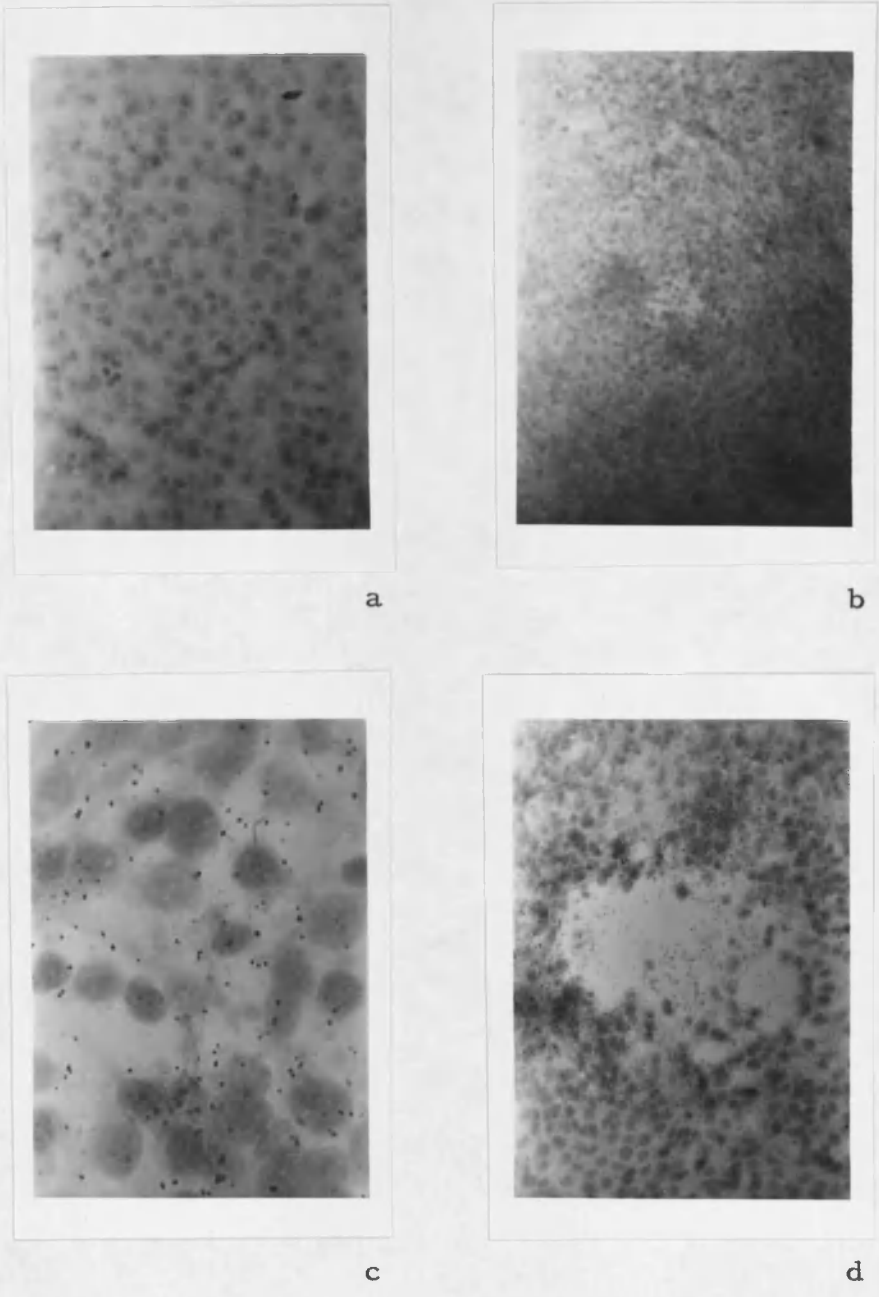


Figure 1. Plaque development in P. aeruginosa-infected HeLa cell monolayers labeled with ^{3}H-uridine.

(a) An uninfected HeLa cell monolayer. (b, c) Small plaque at 13 hr. (d) A mature 15 hr plaque with heavy labeling around the periphery. Note the bacteria in c and d. (a, d) X250; (b) X100; (c) X900.

The uninfected monolayers remained relatively stable in uptake of uridine, particularly after the first 2 hr. Grain counts on infected cultures were very erratic, probably due to the fact that there were some densely packed cell areas with slightly heavier labeling. These were found to some extent on uninfected cultures as well. Such areas would tend to shift or bias the counts since they were not present in all of the randomly picked fields. Examples of these types of labeling for both the infected and uninfected cultures appear in Fig. 2. However, note in the case of the infected monolayer, that there are bacteria present and that the labeling is much denser than in the uninfected monolayer. Apparently, such heavier labeled areas of infected cultures eventually give rise to plaques since denser labeling was also noted around the periphery of plaques as demonstrated in Fig. 3.

Effect of P. aeruginosa on the uptake of ^{3}H-thymidine. Two dilutions of bacteria were used to give a MOI of 0.0012 or 0.88. Plaque formation was noted at 12 hr with the lower dilution of bacteria followed by CPE at 16 hr while the higher dilution produced only a few plaques at 19 hr. Examples of an uninfected and infected monolayer as well as a 19 hr plaque are shown in Fig. 4. There was no difference in the percent of cells labeled (30 to 35%) for uninfected and infected cultures. Furthermore, as shown in Fig. 4c, the degenerated cells around the plaque periphery did not take up label.

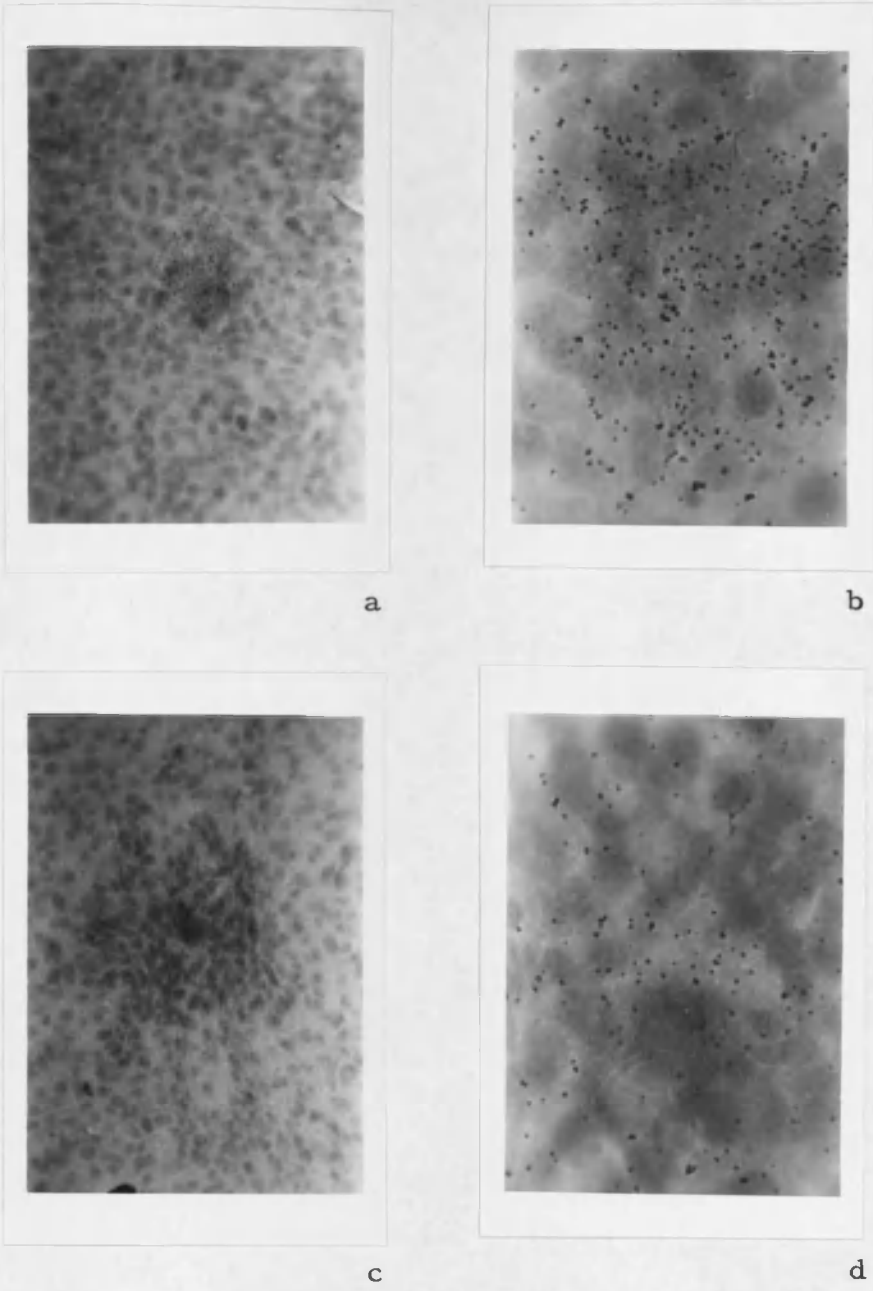
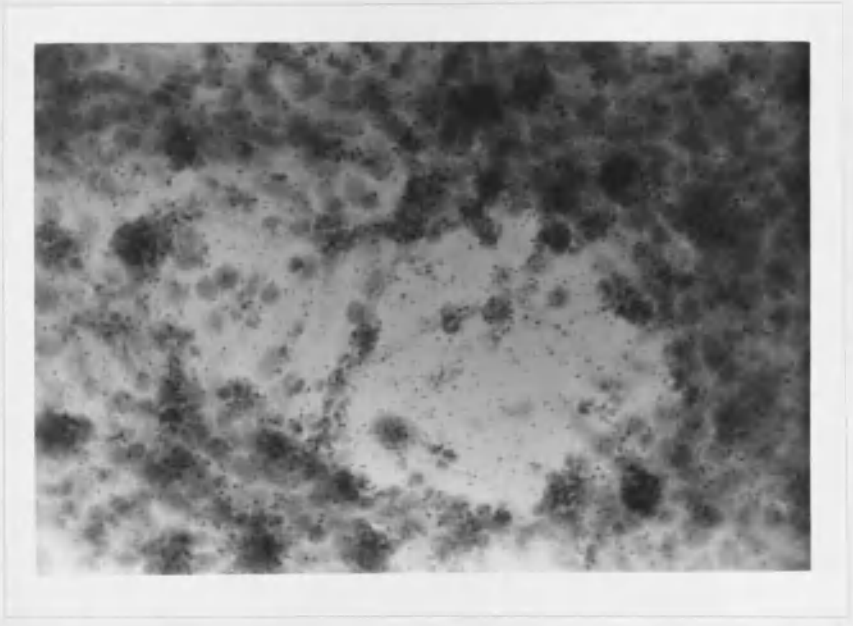
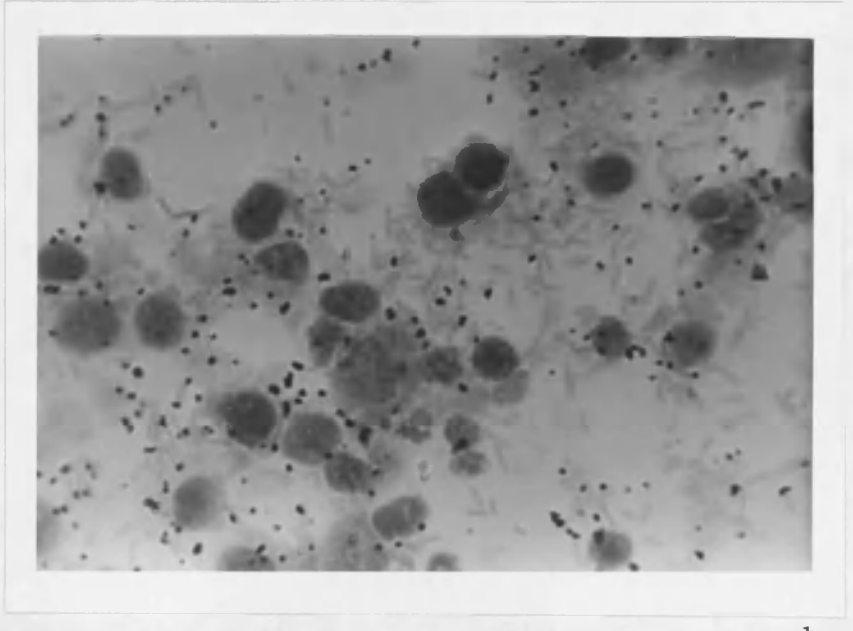


Figure 2. Comparison of heavy ^{3}H-uridine labeling in P. aeruginosa infected and uninfected HeLa cell monolayers at 12 hr.

(a, b) HeLa monolayers infected with MOI of 0.0015. (c, d) Uninfected HeLa cells. Note lightly stained bacteria in b. (a, c) X250; (b, d) X900.



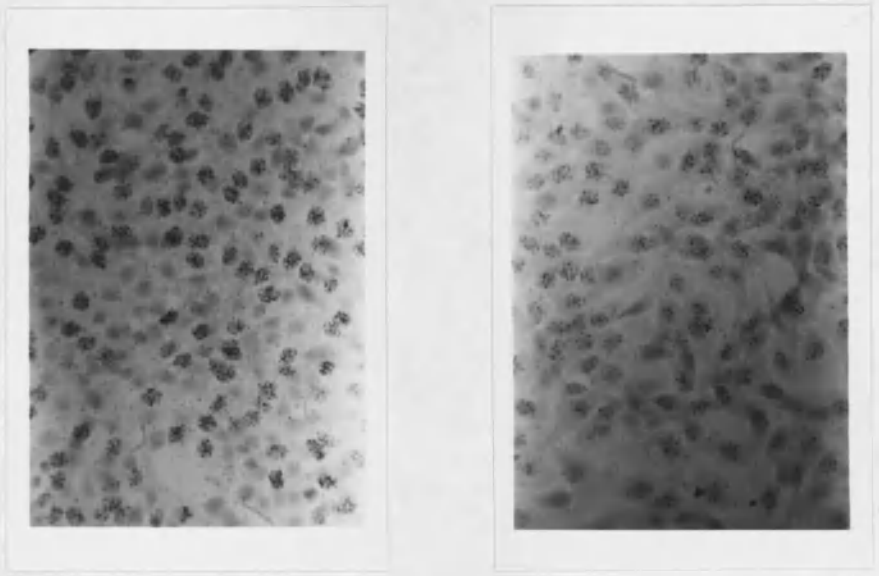
a



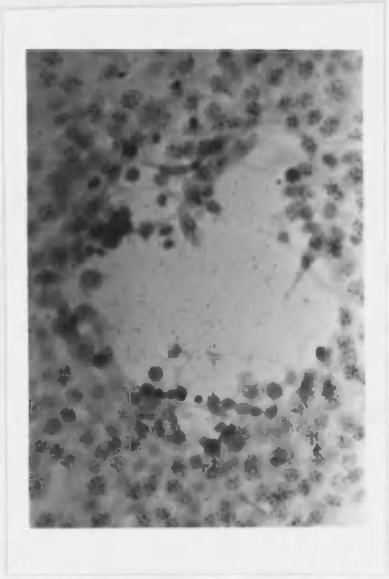
b

Figure 3. P. aeruginosa-infected HeLa cells at 12 hr demonstrating increased uridine labeling around plaque periphery.

(a) Infected HeLa monolayer showing heavy labeling around periphery of plaque. (b) Similar plaque at higher magnification showing some labeled bacteria and cells. (a) X250; (b) X900.



a b



c

Figure 4. HeLa cell monolayers labeled with ^{3}H-thymidine.

(a) P. aeruginosa-infected HeLa cells at 8 hr. (b) Uninfected HeLa cells at 8 hr. (c) Mature 19 hr plaque demonstrating absence of label in degenerating cells. Note similarity in percent of cells labeled in infected and uninfected (a, b). (a, b, c) X250.

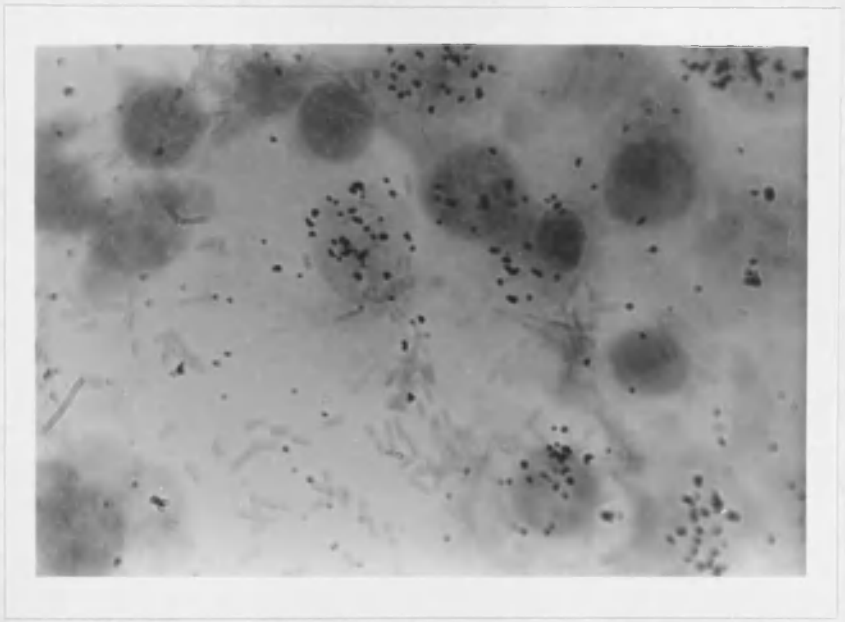
Bacteria took up some of the ^{3}H-thymidine. However, this was only a small percentage as illustrated by the photomicrographs in Fig. 5. Also Fig. 5 shows that the rounded, degenerating cells generally do not take up the label while the more normal appearing, elongated cells, do.

Effect of poliovirus on the uptake of ^{3}H-uridine. Poliovirus, an RNA virus, is known to cause an increase in RNA synthesis in infected HeLa cells and to produce plaques as well. For this reason, this virus was chosen as a positive control for the ^{3}H-uridine uptake studies to demonstrate the adequacy of the techniques used.

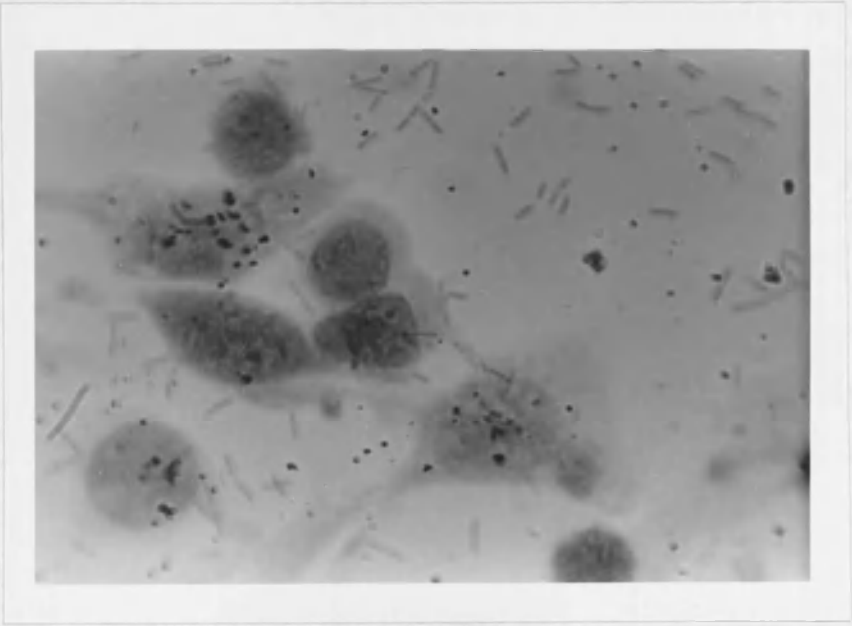
A dilution of poliovirus was prepared so that there were approximately 5,000 virus particles per coverslip, yielding a 0.038 MOI. This virus dilution produced plaques but was insufficient to permit a significant increase in the uptake of label by the poliovirus-infected cells (Fig. 6).

Effect of Herpes simplex on the uptake of ^{3}H-thymidine. This experiment was run as a positive control for the ^{3}H-thymidine studies. Herpesvirus, a DNA virus, produces plaques and an increase in DNA synthesis in infected HeLa cells.

Approximately 100,000 virus particles (MOI=0.77) were added to each coverslip and at designated intervals the coverslips were pulse-labeled with ^{3}H-thymidine. As is shown in Fig. 7, there was



a



b

Figure 5. Examples of P. aeruginosa-infected HeLa cells labeled with ^{3}H-thymidine.

(a, b) Periphery of 15 hr plaques showing numerous bacteria, some of which are labeled. Note how the rounded, degenerating cells do not take up the labeled thymidine. (a, b) X900.

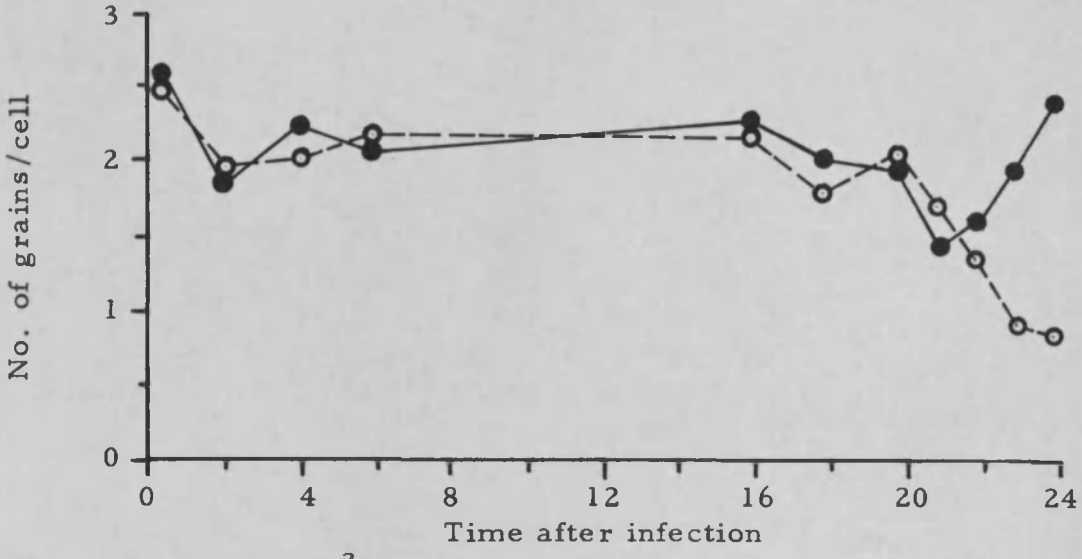
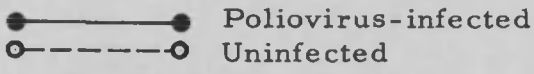


Figure 6. Uptake of ^{3}H-uridine by poliovirus-infected HeLa cells.



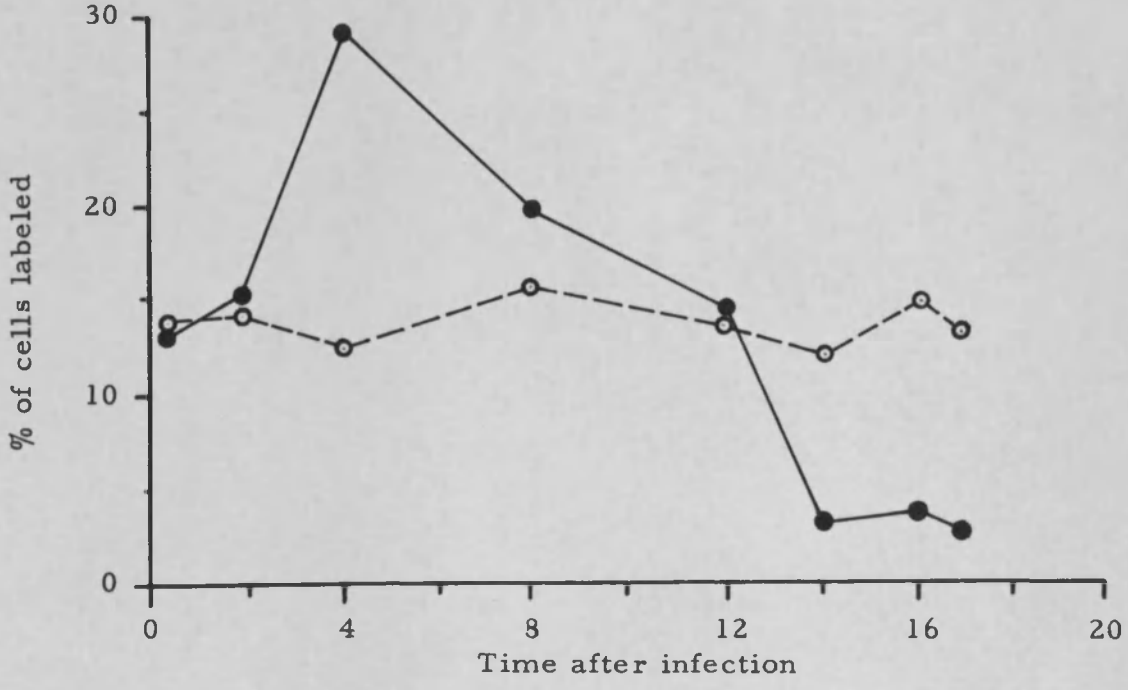
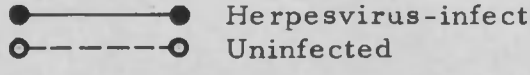


Figure 7. Uptake of ^{3}H-thymidine by herpesvirus-infected HeLa cells.



a significant increase in the uptake of ^{3}H-thymidine by the Herpes-infected cells with a peak at four hr post-infection, at which time the uptake slowly decreased. After 12 hr, when plaques first appeared, there was a marked decrease in the amount of label present. The percentage of label in the uninfected cultures remained between 13 and 19% throughout the course of the experiment.

Liquid scintillation studies.

Effect of P. aeruginosa and S. typhimurium infection on the uptake of labeled precursors. This experiment was designed to determine the uptake of ^{3}H-thymidine and ^{3}H-uridine by HeLa cells infected with P. aeruginosa or S. typhimurium. The liquid scintillation studies were performed to correlate and confirm the validity and significance of the results from the radioautographic work.

S. typhimurium was used because it is known to be phagocytized by and to multiply intracellularly in HeLa cells (9) without producing plaques, whereas definite proof for the phagocytosis and intracellular multiplication of P. aeruginosa by HeLa cells is lacking, yet it does produce plaques.

The MOI for this experiment was 9.6 for P. aeruginosa and 9.8 for S. typhimurium. A high MOI was used to insure that each cell had an opportunity to become infected. The data obtained from the scintillation counter was statistically analyzed by doing an analysis of

variance at the 95% confidence level. The confidence limits and means were computed and graphed. Fig. 8 and 9 show the results of this analysis.

The ^{3}H-uridine curves (Fig. 8) show an apparent increase in the uptake of the label in the P. aeruginosa-HeLa cell series after 2 hr, while the S. typhimurium-infected and uninfected cells remain steady. It appears from the graph that the bacteria are not taking up any of the labeled uridine since the three curves generally lie within the confidence limits imposed by the results.

The converse is true of the uptake curves for ^{3}H-thymidine (Fig. 9). It appears that there was definitely some uptake of the label by both bacteria because the infected cell uptake curves were so much different than the uninfected curve. The radioautographic study using ^{3}H-thymidine showed the uptake for the infected and uninfected cells to be the same. An explanation of this can probably be found in the high MOI used in the liquid scintillation work as opposed to the low MOI of the radioautographic studies.

Determination of the uptake of labeled precursors by the bacteria. Because the data of the preceding experiment indicates the possible uptake of labeled thymidine by the bacteria, an experiment was designed to determine how much of an uptake could be attributed to the organisms. The uptake of labeled precursors was measured both in the presence and absence of HeLa cells, using P. aeruginosa or S.

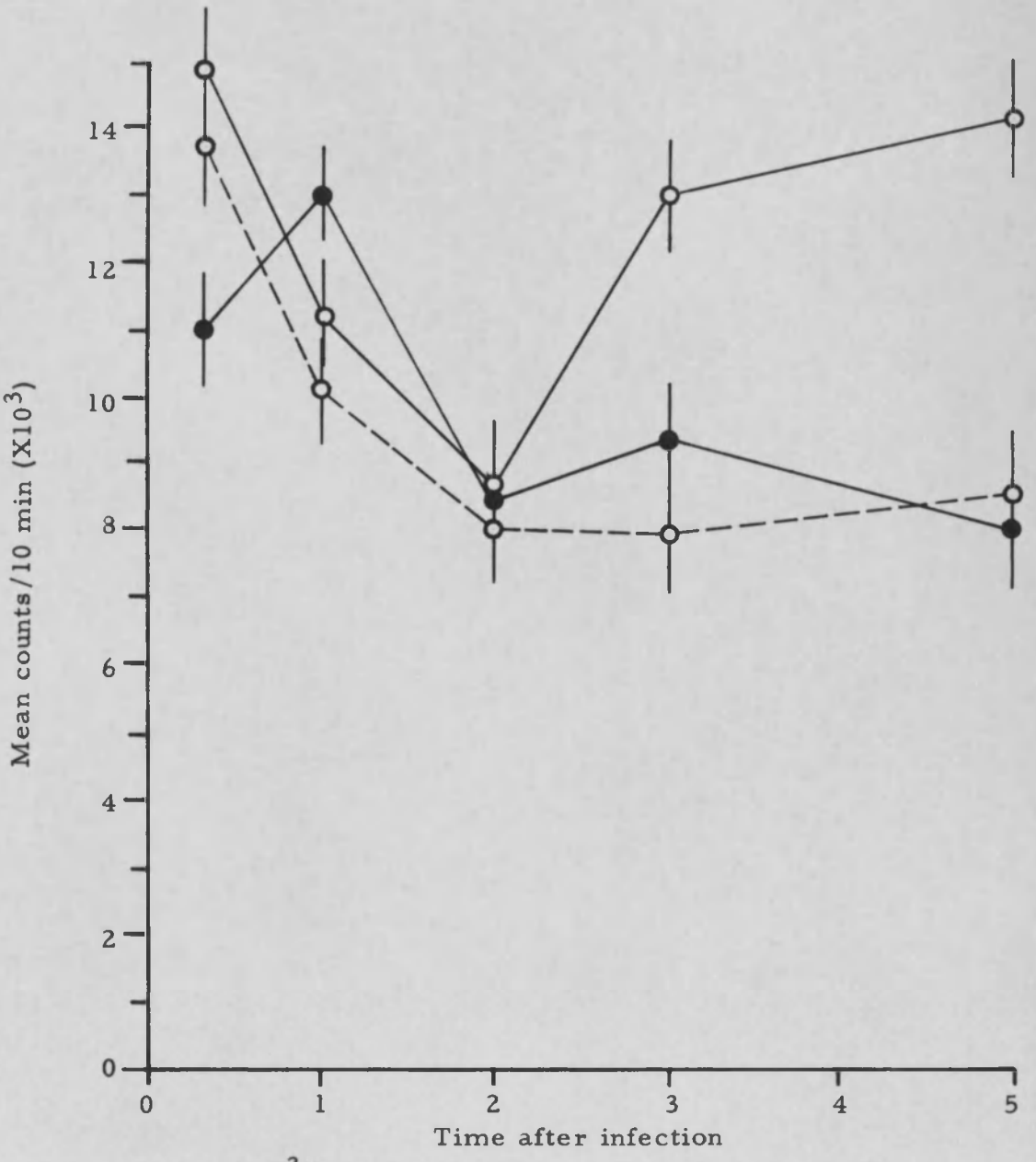


Figure 8. Uptake of ^{3}H-uridine by HeLa cells infected with P. aeruginosa or S. typhimurium.

●——● Uninfected HeLa monolayer labeled with ^{3}H-uridine.

○——○ P. aeruginosa-infected HeLa monolayer labeled with ^{3}H-uridine.

○- - - -○ S. typhimurium-infected HeLa monolayer labeled with ^{3}H-uridine.

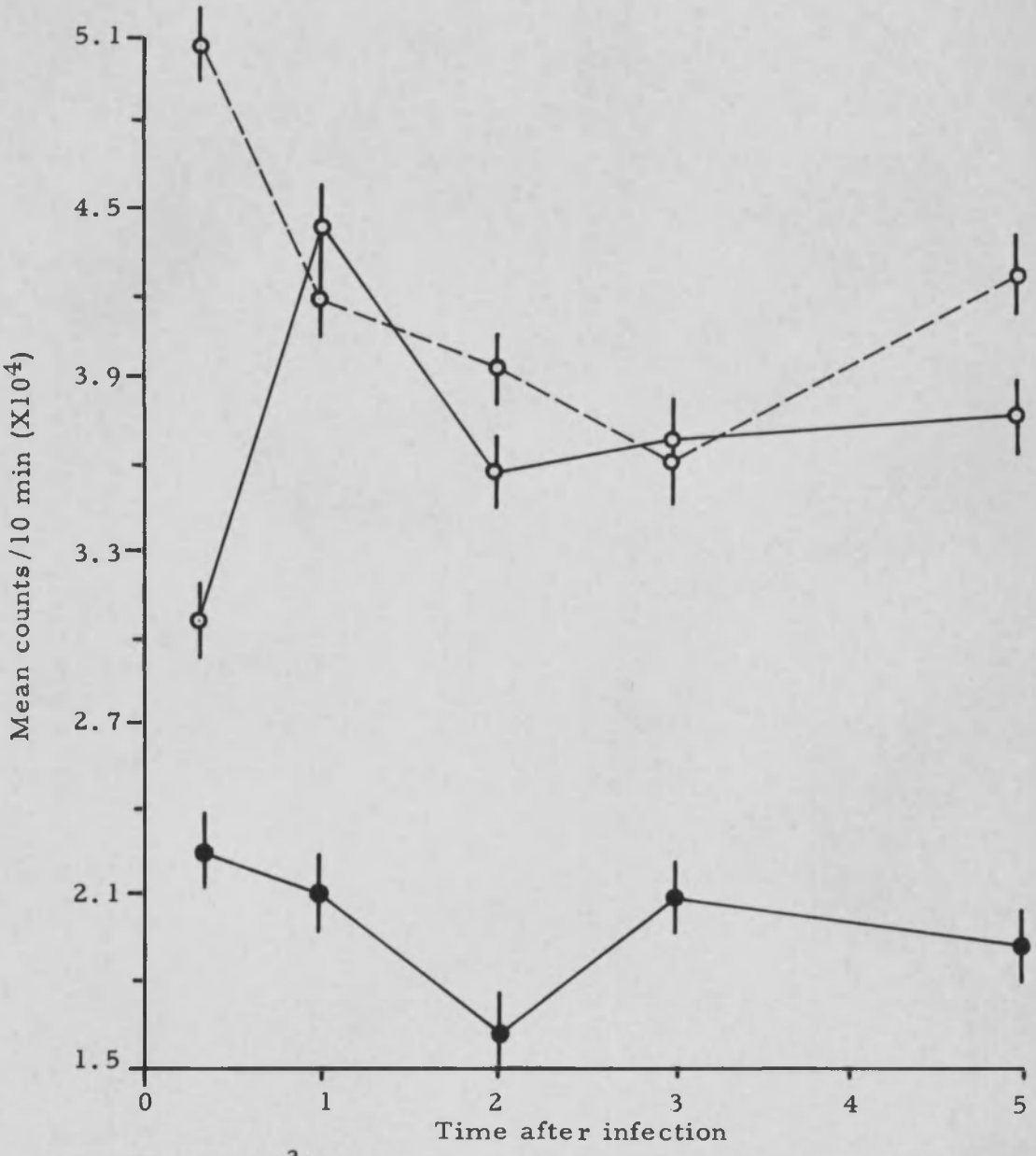


Figure 9. Uptake of ^{3}H-thymidine by HeLa cells infected with P. aeruginosa or S. typhimurium.

Uninfected HeLa monolayer labeled with ^{3}H-thymidine.

P. aeruginosa-infected HeLa monolayer labeled with ^{3}H-thymidine.

S. typhimurium-infected HeLa monolayer labeled with ^{3}H-thymidine.

typhimurium. The label was added immediately upon the addition of the bacteria. In order to obtain a 20 min pulse-label, the HeLa cells were washed after 20 min. In the case where HeLa cells were absent, the tubes were placed in an ice-water bath after 20 minutes to arrest the uptake of label by the organism.

The results are shown in Table 2. The tubes without bacteria and cells acted as background control counts. Note that both bacteria showed a considerable uptake of ^{3}H-thymidine; between 25 and 30%. Only 9 to 12% uptake by the bacteria was noted in the case of ^{3}H-uridine.

Table 2. Determination of bacterial uptake of label.

	Uninfected		P. aeruginosa		S. typhimurium	
	^{3}H-Tdr	^{3}H-Urd	^{3}H-Tdr	^{3}H-Urd	^{3}H-Tdr	^{3}H-Urd
With HeLa Cells	22.5	12.0	30.5	13.5	43.5	13.0
Without HeLa Cells	0.9	0.75	9.0	1.7	17.5	1.2

*All numbers are times 10^{3} and are expressed as counts per 10 min.

DISCUSSION

The purpose of this investigation was to study the uptake of ^{3}H-uridine and ^{3}H-thymidine by P. aeruginosa-infected HeLa cells in an effort to gain new evidence on the mechanism of plaque formation by this bacterium. The evidence compiled by Ludovici and Christian (2) and Wexlar, Moore, and Ludovici (4) points towards phagocytosis and subsequent intracellular multiplication of the bacterium ultimately leading to cell death and plaque production. Obviously exotoxins may be involved in the plaque phenomenon. The role of toxins in plaque formation was studied by Coleman, Janssen, and Ludovici (3). They found two types of toxins, one of which (toxin Z) was implicated as a possible factor in the formation of plaques in HeLa monolayers. However, Coleman's (10) efforts to unequivocally prove (by the use of antiserum, complement, and bacteriophage) that the P. aeruginosa was being phagocytized met with mixed success. His attempts at inhibiting the bacteria with bacteriophage led to the discovery that the bacteriophage actually enhanced plaque formation by P. aeruginosa.

It appears from the results of my studies that P. aeruginosa in some manner affects the synthesis of RNA by HeLa cells. The radioautographic studies demonstrated an increased uptake of ^{3}H-uridine, an RNA precursor, by infected cells around the periphery of

plaques and by cells in areas where the number of visible bacteria suggest the development of a future plaque. (Fig. 2, 3 and Table 1). Supporting evidence for an increased uptake of ^{3}H-uridine by P. aeruginosa-infected HeLa cells is given by the liquid scintillation studies. They showed a statistically significant increase in the amount of uptake of label, when compared with both uninfected and S. typhimurium-infected cells.

The poliovirus-infected HeLa cells used as a positive control showed only a slight increase in ^{3}H-uridine uptake by the radioautographic technique. The reason for this finding was the low MOI of poliovirus used. This was necessary since a high MOI would cause generalized CPE without viral plaques in the liquid system used. From this standpoint the poliovirus cultures were not valid positive controls since increased uridine labeling was not demonstrable in cells surrounding the plaques. It was decided that S. typhimurium would be a more appropriate control in the liquid scintillation confirmation studies where a high MOI was necessary, since it is a bacterium which is known to be phagocytized by HeLa cells, yet does not produce plaques.

In retrospect, the increased uptake of ^{3}H-uridine by P. aeruginosa-infected HeLa cells would not necessarily imply that the bacterium is phagocytized by the HeLa cell and multiplies intracellularly, affecting the metabolism of the HeLa cell in such a manner that an increase in RNA synthesis occurs. It is also conceivable that the

organism might attach itself to or form a complex with the HeLa cell membrane from which, in some unknown manner, it might force the HeLa cell to increase its' synthesis of RNA. Further speculation could be made that, in either case, the RNA synthesized is messenger RNA made by the HeLa cells in response to the infection and for the purpose of producing substances required for the multiplication of P. aeruginosa. Such a confiscation of the HeLa cell metabolism might result, as it does with viruses, in degeneration of the cell accompanied by plaque formation in the monolayer.

The synthesis of DNA by the HeLa cells does not appear to be affected by the P. aeruginosa. Observations of the radioautographs of P. aeruginosa-infected HeLa cells indicate that there is no increase in the uptake of ^{3}H-thymidine by the HeLa cells. In the liquid scintillation studies with ^{3}H-thymidine, it was noted that both the P. aeruginosa and S. typhimurium-infected cultures had a much greater uptake than the uninfected cultures (Fig. 9). However, because of the high MOI used in the liquid scintillation experiments, it was conceivable that the bacteria might be taking up much of the labeled thymidine. Results of experiments (Table 2) to test this hypothesis indicated that there was considerable uptake of the label by both of these bacteria. Thus, it is clear that bacterial infection of HeLa cells had no effect on the DNA metabolism of the HeLa cells.

As in the case of the poliovirus-^{3}H-uridine studies, the herpesvirus was used basically as a check on the radioautographic technique for ^{3}H-thymidine labeling. The uptake of ^{3}H-thymidine in herpes-infected cultures increased as expected early in the infectious period and then decreased after the plaques were formed and cell degeneration occurred.

SUMMARY

Evidence relating to the plaque forming mechanism of P. aeruginosa in HeLa cell monolayers has been presented. Radioautographic and liquid scintillation studies have demonstrated an increase in the uptake of ^{3}H-uridine in P. aeruginosa-infected HeLa cells. The synthesis of DNA in HeLa cells was not affected by infection with P. aeruginosa. Both the radioautographic and liquid scintillation studies utilizing ^{3}H-thymidine, a labeled DNA precursor, support this conclusion. Further, it was found that when the MOI was high, the bacteria took up considerable ^{3}H-thymidine, but not ^{3}H-uridine.

It was suggested that this increased uptake of ^{3}H-uridine by infected cells does not imply that the bacterium is phagocytized and then multiplies intracellularly causing an affect on the metabolism of the HeLa cell, forcing it to increase synthesis of RNA. Conceivably the bacterium might initiate the increase in RNA synthesis in the HeLa cell from outside the cell, perhaps by attaching itself to the HeLa cell membrane.

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