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The uptake of [^{3}H]uridine in normal and filamentous forms of *Escherichia coli* infected with T-2 bacteriophage

HERSHEY[1] and VOLKIN AND ASTRACHAN[2] have shown that although net RNA synthesis almost comes to a complete halt during T-2 phage infection, a small amount of ^{32}P becomes incorporated into RNA nucleotides, indicating the occurrence of some RNA turnover. In the course of investigating sites of RNA synthesis in *E. coli* the existence of a concurrent uptake of uridine was established. Some features of this uptake will be described here.

We first considered the uptake of [^{3}H]uridine (obtained from New England Nuclear Corp.) by a culture of *E. coli* B in minimal A-1 medium[3], aerated at 37°, and infected with approximately 20 T-2 bacteriophages per cell. [^{3}H]uridine, at a concentration of 50 μC/ml (specific activity, 680 mC/mmole) was added to the culture 3 min after infection. The final cell concentration was $3.5 \cdot 10^7$ cells/ml. Colony counts following 10^4 dilution of the aliquots of this culture revealed that at this point less than 1 % of the cells were non-infected. Samples (5 μl in volume) from the culture were taken at regular intervals and placed on stainless-steel planchets in a 10 % aq. solution of formalin containing a high concentration of unlabeled uridine to reduce adsorption of label to the metal surface. The cells were fixed for 10 min, dried, fixed again for 10 min in Carnoy solution, transferred to distilled water through graded alcohols, and dried again. Counts were made in a windowless flow Geiger counter. A correction was made for background due to adsorption of label to the planchet and other causes.

Fig. 1 shows that the uridine content of the cells rises at a linear rate for about 10 min, then reaches a plateau for the next 20 min. The initial rate of uptake is much higher than that expected for the non-infected cells present at that time. It must be concluded therefore that this uptake takes place in the infected cells. It has been shown that in normal cells 90 to 95 % of the incorporated [^{3}H]uridine appears as RNA uridine and cytidine, the rest appearing as DNA deoxycytidine[4]. Treatment with RNAase of the phage-infected cells removed approx. 90 % of the label. It is, therefore, inferred that [^{3}H]uridine uptake is an indication of RNA synthesis or turnover. One difference should be noted between our results and those of VOLKIN AND ASTRACHAN[2]. They found that in minimal medium phosphorus turnover in RNA continues throughout the entire latent period, while in our experiment the

Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid.

[^{3}H]uridine uptake seems to stop after about 10 min (behavior corresponding to the turnover of phosphorus in peptone broth). Whether this reflects differences in experimental conditions or a basic difference between the uptakes of uridine and phosphorus has not been ascertained here. Experiments conducted on *E. coli* B in ANDERSON'S M-9 medium or in nutrient broth and on filamentous forms of *E. coli* B gave results similar to those expressed in Fig. 1, but with greatly varying initial rates of uptake and final amounts of incorporation.

The uptake of [^{3}H]uridine by T-2 bacteriophage-infected normal cells and filaments was also studied by radioautography. In one experiment *E. coli* B filaments (obtained by irradiation with a ultraviolet dose of 20 ergs/mm^2 at 265 mμ), averaging about 15 μ in length, and growing exponentially, were infected with approx. 50 T-2 bacteriophages per cell and, after 5 min, inoculated with 12 μC/ml [^{3}H]uridine. 6 min later the cells were placed on a prepared glass slide on a drop of 10 % formalin in water containing a high concentration of unlabeled uridine, fixed for 10 min, dried, placed in osmium tetroxide vapors for 5 min, washed 3 times in distilled water and dried. They were radioautographed, using the stripping-film technique[5], with Kodak Ltd. AR-10 emulsion. After suitable exposure, and development of the slides, they were examined in a phase-contrast microscope, the cell length measured by means of a micrometer disc, and the number of photographic grains associated with each cell counted in bright field. This number of grains was assumed to be proportional to the [^{3}H]uridine uptake of the cell[6]. Fig. 2 presents the results of this experiment. It can be seen that the uptake of uridine in T-2-infected filaments is directly proportional to length.

This proportionality could be linked to either the number of bacteriophages

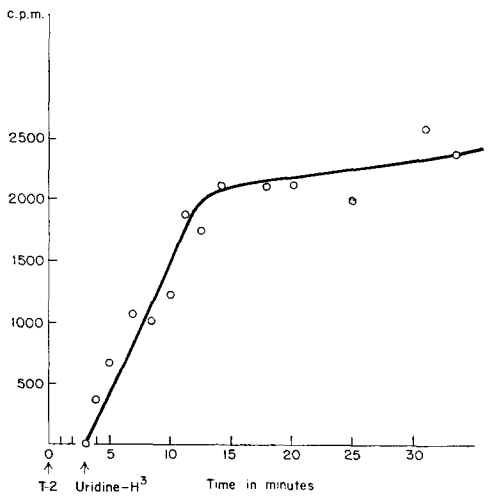

Fig. 1. Uptake of [^{3}H]uridine by *E. coli* B infected with T-2 bacteriophage. The [^{3}H]uridine was added 3 min after inoculation with 20 bacteriophages/cell.

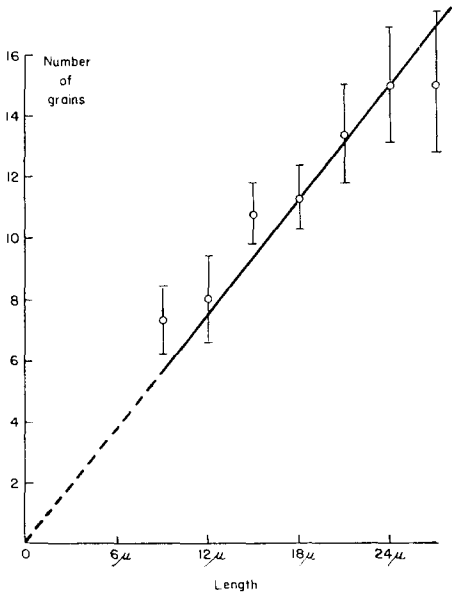

Fig. 2. Uptake of [^{3}H]uridine by filamentous forms of *E. coli* B infected with T-2 bacteriophage, *versus* cell length. Each point indicates the average number of radioautographic grain counts for cells of a given length class. A few non-infected cells present in the preparation were not included in calculating the averages.

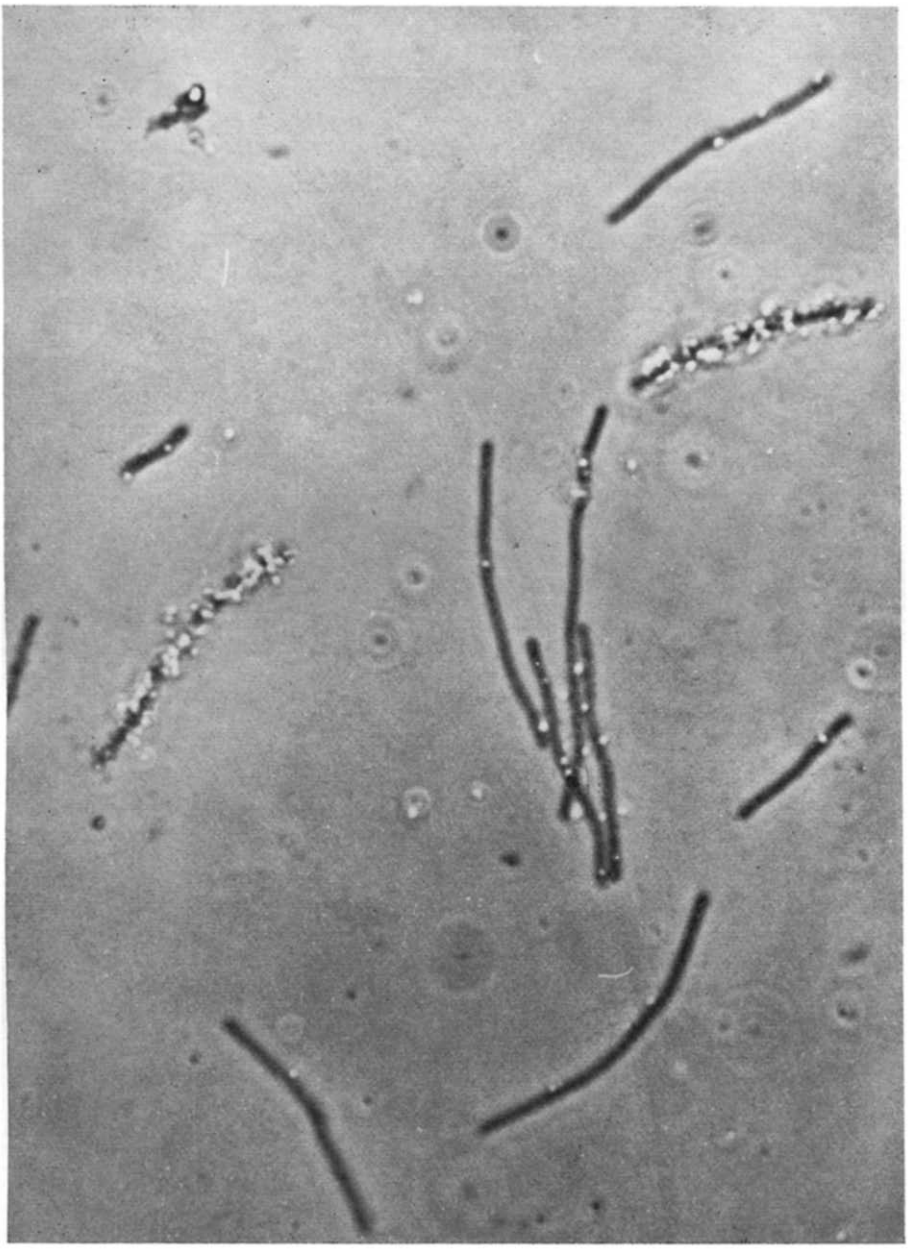

Fig. 3. Radioautograph of *E. coli* B filaments grown in the presence of [^{3}H]uridine following infection with one T-2 bacteriophage per cell. The photographic grains, seen slightly out of focus in phase contrast, appear as white spots. Two non-infected cells show a high uptake of [^{3}H]uridine. In the infected cells the grains appear in random places along the length of the filament.

infecting the cell or the general synthetic ability of the cell, both being proportional to length[3,4,7]. By considering cells infected with a low multiplicity of bacteriophages the first hypothesis can be eliminated. If filaments are infected with an average of one phage per cell, the rest of the procedure remaining the same, the average grain count per day per 6 μ of infected cell length is 0.41, compared to 0.39 grain/day/6 μ in the case of a multiplicity of 50 described above. Clearly the number of infective units does not determine the amount of uridine incorporated. Non-infected filaments labeled under similar conditions gave an average grain count of 6.8 grains/day/6 μ.

Some qualitative results can be obtained from the examination of such radioautographs (Fig. 3). In filaments infected with a low multiplicity of T-2 the percentage of non-infected cells (easily recognized by their heavy incorporation of [^{3}H]uridine) was, in one experiment, 24 %. Assuming a Poisson distribution of phage particles among cells it might be inferred that about 35 % of the cells were infected with only one phage. It was observed in this preparation that the reduction in uridine uptake related to phage infection was always extended to the entire length of the filament, even in very long cells (up to 30 μ). The reduced uptake in infected cells took place similarly, along the entire length of the cell. Thus, in regard to the two manifestations of phage infection studied here, decrease in uridine uptake and distribution of the small amount incorporated, even very long filaments behave as one single unit. This agrees with our findings on uridine uptake by non-infected filaments[4], and also with the results of DEERING[8], who reached a similar conclusion with regard to still another property of filaments (colony formation) from the results of radiation studies.

In conclusion, we found that T-2 phage-infected *E. coli* B and filamentous forms of this strain incorporate [^{3}H]uridine for a short period of time at a reduced rate and that the amount of label incorporated is proportional to the length of the cell but not to the multiplicity of infection. The reduction in uptake affects the entire length of the cell, even in long filaments, but similarly the uptake still present is not localized even when the cell is infected with one bacteriophage.

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