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Letter to the Editors

Synthesis of the Enzyme Galactozymase during the Transduction by Coliphage-λ (II)

In our previous paper we observed galactose metabolism in some strains of *Escherichia coli* K12. It is of interest to determine the conditions of galactozymase formation by populations of galactozymase negative (Gal$^-$) cells which are infected by the phageλ with the Gal$^+$- determinant controlling galactozymase synthesis.

This communication describes a preliminary experiment on the kinetics of the synthesis of galactozymase in such a system.

"Induced λ" had the character of high frequency transduction (HFT) was obtained from a heterogenotic strain of W 3094 of *E. coli* K12 by the methods of Morse *et al.* (1956).

The organism used was W 3094 (Gal$^-$, lysogenic for λ) which lacked inducible galactozymase (gal-1-P uridyl transferase). Cells grown in a penassay broth (Difco) with aeration at 37°C were harvested during the logarithmic-phase of growth, and resuspended in a similar fresh medium. The concentration of cell suspension was adjusted to ca 5.3×10^8 bacteria per ml and the infecting HFT-λ phage was concentrated to ca. 4.0×10^{10} particles per ml by ultracentrifugation at 25,000 r.p.m. for 30 minutes. Equal volumes (20 ml) of the bacterial culture and of the phage were mixed. After mixing with HFT-λ,the bacteria were centrifuged and resuspended in fresh penassay broth (40 ml) and regrown under the same conditions.

To maintain exponential growth, half of the culture was diluted 1 in 1 with the same medium every 30 minutes.

Each time the other half of the culture was removed, chilled to about 0°C, centrifuged in the cold and resuspended in cold phosphate buffer at an optical density of 0.55 (ca. 2.0×10^9 cells per ml). The enzyme activity was measured manometrically in the bacterial suspensions after adding galactose (10^{-2} M) and

Table 1

The appearance of galactozymase in Gal$^-$ cells during transduction by HFT-λ

Time after infection minutes		non-infected Gal$^-$ cells (control)	30	60	90	heterogenote[2] Gal$^+$ cells (control)
enzyme activity[1]	μl. O_2/10 min. per vessel	0.51	4.22	1.85	1.91	
	$\times10^{-8}$ μl. O_2/10 min. per transduced Gal$^+$ cell	—	2.09	1.51	1.52	1.92

1) The enzyme activity was the mean value per 10 min. 60–135 min. after the beginning of the assay.

2) This is a reference figure from an independent experiment.

yeast extract (0.01 per cent). The number of transduced Gal$^+$ cells was determined by plating on EMB-galactose agar.

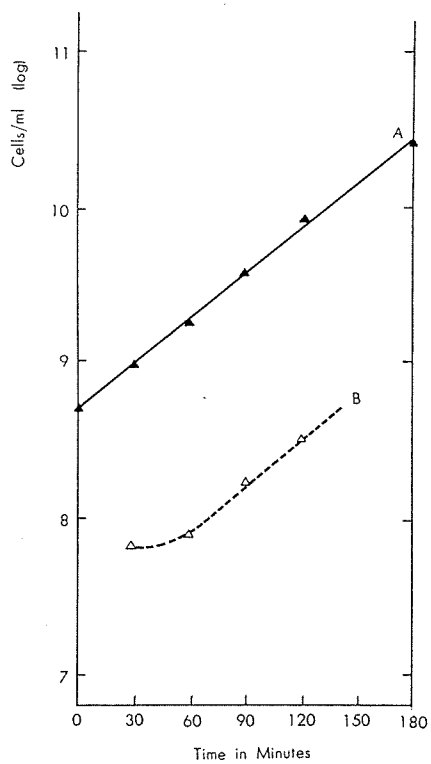
The results are shown in Fig. 1 and Table 1.

It is seen that the transduced Gal$^+$ cells do not increase during about the first 60 minutes (Fig. 1, curve B). In other words, there is a delay before the increase of transduced Gal$^+$ cells. Similar results were observed in the lysogenic conversion of *Salmonella anatum* 239 (Uetake *et al.* 1958) and the high frequency recombination (Hfr) of *E. coli* K 12 (Jacob, 1958). However this observation may also be explained by supposing that cells just introduced by Gal$^+$-determinant segregate the Gal$^-$ cells as seen in abortive transduction (Stocker *et al*, 1953).

Though the non-transduced Gal$^-$ cells cannot form galactozymase, the cells at 30 minutes after infection with HFT-λ can synthesize inducible galactozymase. Under our conditions, therefore, the enzyme-forming system must be established before the transduced Gal$^+$ cells begin to increase in number.

Furthermore, it is shown in Table 1 that the enzyme activity per population at 30 minutes is higher than that at subsequent times (60 and 90 min.). This is interpretable in the following way. Although the colonies counted as transduced Gal$^+$ cells had a Gal$^+$ sector originating from the center of the colony in this experiment and it was therefore difficult to determine exactly whether the Gal$^+$ colonies reflected immediately the number of cells with enzyme-forming ability, the transduced Gal$^+$ cells are not able to increase in number during about the first 60 minutes. They then begin to increase at the same rate as the non-transduced Gal$^-$ cells which can multiply exponentially from 0 time every 30 minutes (Fig. 1). Therefore it may appear as if there is a decrease of enzyme activity because of a decrease of Gal$^+$ cells per population.

Fig. 1. Number of transduced Gal$^+$ cells and total cells in bacterial culture infected by HFT-λ
Curve A.- Total numbr of cells.
Curve B.- Number of transduced Gal$^+$ cells.



However, after the enzyme-forming system had once been established, the enzyme activity per one Gal$^+$ cell was shown to be the same as that of a heteroge note of W 3094.

In conclusion, this experiment indicates that the introduction of genetic information by means of phage infection results in the formation of a particular

enzyme protein in the organism within the normal division time. Then, the enzyme activity per transduced Gal$^+$ cell is usually constant at various later times.

Addenda, in this experiment, the m.o.i. is ca 100. This seems to be exceedingly high, but it seemed from another experiment that enzyme synthesis is independent on the m.o.i.

Further research is now in progress. A later paper will show that the appearance of galactozymase begins about 5 minutes after infection of Gal$^-$ cells with λ-gal.

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REFERENCES

Jacob, F. (1958). Transfer and expression of genetic information in *Escherichia coli* K12. *Exptl. Cell Research Suppl.* **6**, 51–68.

Kurahashi, K. (1957). Enzyme formation in galactose-negative mutants of *Escherichia coli*. *Science* **125**, 114–116.

Matsushiro, A., Nagai K. and Mizobuchi, K. (1959). Synthesis of the enzyme galactozymase during the transduction by coliphage-λ (I). (1959). *Biken's J.* **2**(**3**), 227–228.

Morse, M. L., Lederberg, E. M. and Lederberg, J. (1956). Transductional heterogenotes *Escherichia coli*. Genetics **41**(**5**), 758–769.

Stocker, B. A. D., Zinder, N. D., and Lederberg, J., (1953). Transduction of flagellar characters in *Salmonella*. *J. Gen. Microbiol.* **9**(**3**), 410–433.

Uetake, H., Luria, S. E. and Burrous, J. W. (1958). Conversion of somatic antigens in *Salmonella* by phage infection leading to lysis or lysogeny. *Virology* **5**, 68–91.