## The Mechanism of Genetic Expression of the New Marker in Transduction of *Salmonella typhimurium*

In studying the mechanism of transduction of galactose fermentation, it was found that the number of divisions of transduced cells is 2–4 less than that of divisions of nontransduced cells in the culture[1,2]. It was ascertained that during transformation of streptomycin-resistance[3], not only streptomycin-resistant, but also streptomycin-sensitive, cells may be selected after 1–2 divisions following incorporation of DNA. We therefore followed in further studies the type of division of potentially transduced cells during their first divisions in a cold-synchronized culture. Strains of *Salmonella typhimurium*, $LT_2$, $SW_{351}$[4] and $SW_{351}R$, the back mutant of the auxotrophic $SW_{351}$ strain were used. The multiplicity of the phage infection was about 1.

The original conception of the division of a potentially transduced cell during the first two generations was taken over from the work of EPHRUSSI-TAYLOR[5]. In her experiments, the number of transformed cells increases after the second division, and thereupon starts the regular reduplication and transmission of the marker. The reduplication of transforming DNA starts only after its integration into the genetic cell apparatus.

Our own results with the transduced cell do not correspond with this conception, irrespective of whether the difference between nontransduced and transduced cells was 2 or 4. In the first place, an analysis of the results of experiments where the difference of generations amounted to two, was carried out (Table I). Here it was found that the potentially transduced cell divides into two cells carrying the new marker ($gal^+$) already at the first division. The segregation of the cells of the original acceptor strain ($gal^-$) starts at the second division, and thus the potentially transduced cell acquires a delay of one generation. The second generation delay probably appears in the fourth generation. According to the original conception, the difference of generations should disappear after 1–2 divisions; the transduced cell should reduplicate the marker quite regularly, giving rise to $gal^+$ cells only. In our experiments, it was possible to observe after 2–3 divisions further ability of transduced cell to split off cells of the original acceptor ($gal^-$) strain.

Table I. The number of cell divisions of nontransduced and transduced cells in culture with the difference of generations 2

| Cultivation min | Number of cell divisions nontransduced $n_1$ | transduced $n_2$ | Difference in the number of generations $n_1 - n_2$ |
|---|---|---|---|
| 75 | 1.2 | 1.0 | 0.2 |
| 95 | 1.95 | 1.2 | 0.75 |
| 120 | 2.45 | 1.3 | 1.15 |
| 180 | 3.25 | 2.05 | 1.2 |

Table II. The number of cell divisions of nontransduced and transduced cells in culture with the difference of generations 4

| Cultivation min | Number of cell divisions nontransduced $n_1$ | transduced $n_2$ | Difference in the number of generations $n_1 - n_2$ |
|---|---|---|---|
| 60 | 0.3 | 0.15 | 0.15 |
| 70 | 0.6 | 0.2 | 0.4 |
| 80 | 1.5 | 0.45 | 1.05 |
| 100 | 1.8 | 0.4 | 1.4 |
| 120 | 2.4 | 0.35 | 2.05 |
| 150 | 3.0 | 1.0 | 2.0 |
| 180 | 3.6 | 0.9 | 2.7 |
| 220 | 4.2 | 1.15 | 3.05 |
| 260 | 4.85 | 1.5 | 3.35 |
| 300 | 5.2 | 1.9 | 3.3 |

At first sight, the original conception of the division of the potentially transduced cell, seems to agree with our own experiments where the difference of generations is four, and the transduced cell divides into two $gal^+$ cells only during the third division of the cells in the culture (Table II). It should be pointed out, however, that at this stage, the cell still has a delay of two generations, and that during further divisions, it splits off cells of the original acceptor ($gal^-$) strain in the same way as described above.

The mechanism of division of the potentially transduced cell, shows that in one cell suspension the transducing element apparently reduplicates at its first division, while in another at its third division, as an independent reduplicating unit without being attached to the genetic cell apparatus.

The existence of independently reduplicating particles in the transformation of cells by means of DNA is very improbable. The reduplication of transforming DNA depends on bacterial substances formed in the cell at a certain phase of cell division, and sets in only after the integration of DNA with the genetic cell apparatus[6]. The transformation with pure DNA differs, however, from virus infection, and especially from transduction with regard to the purity of the DNA which enters the cell. In infection with $T_2$ phage a non-DNA substance enters together with the DNA into the cell[7] and might thus initiate a heterocatalytic activity, or reduplication of the free DNA fragment in the cell. The transmission of a new character need not be regular during subsequent divisions.

In connection with the mechanism of division of the potentially transduced cell, it is of interest to note the experiments of STARLINGER[8], who studied the rate of synthesis of galactokinase after infection of *Escherichia coli* cells with HFT-lyzate of $\lambda$ dg phage. He found that the enzyme began to be formed 10 min after adsorption of the phage. During infection of $gal^+$ cells with normal nontransducing $\lambda$ phage, galactokinase began to be synthetized with a 14 min lag as compared with uninfected cells. As the actual infection with the phage causes a short lag in the metabolism of the cell, it may be assumed that the induced enzyme is formed immediately after the entrance of the transducing DNA into the cell. This fact can be well accounted for by the heterocatalytic activity of the entering DNA even before its reduplication and integration into the genetic apparatus of the cell.


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*Zusammenfassung.* Die Anzahl der Teilungsprozesse transduzierter Zellen im Vergleich zu nichttransduzierten Zellen nimmt während der ersten fünf bis sechs Zellteilungen in der Kultur um zwei bis vier Teilungen ab. Dieser Unterschied wird offensichtlich durch das regelmässige Abstossen von Zellen vom gleichen Typ wie beim Akzeptorstamm von potentiell transduzierten Zellen bewirkt. Die grundlegenden Mechanismen des Transduktionsprozesses werden diskutiert.

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## On the Fate of Rabbit Antibody/Antigen Complexes in the Presence of Normal Leucocytes *in vitro*[1]

Several investigators have shown by experiments *in vivo* that antigen is eliminated more rapidly in immune than in normal animals (for references see[2]), a fact presumably dependent upon the formation of variously composed antibody/antigen complexes. It has also been shown that passively transferred antibody is degraded faster when the injection of antibody is preceeded by an injection of antigen[3]. Both HUMPHREY[4] and WEIGLE[5] have also recently reported that when both components of antibody/antigen complexes are injected intravenously to normal rabbits, they are rapidly eliminated.

It has been demonstrated in earlier communications that rabbit antibody/antigen complexes are taken up and broken down by normal guinea pig leucocytes *in vitro*[2,6] and that spleen cells from immune animals adsorb considerably more antigen than cells from normal animals[7]. This latter fact is presumably mainly due to antibody fixed on cell surfaces.

The present note reports on the *in vitro* fate of trace-labelled rabbit antibody/antigen complexes in the presence of leucocytes from peritoneal exudates of normal guinea pigs and rabbits. The purpose of the investigation was to establish (1) whether both antigen and antibody are taken up and broken down, and (2) whether the fate of antibody in homologous cells was different to the fate in heterologous cells. Human serum albumin (HSA) was used as antigen. Two types of insoluble complexes were prepared: (a) A complex comprising both carrier and trace-labelled $I^{131}$-HSA and unlabelled rabbit antibody. (b) A complex comprising unlabelled HSA and internally labelled rabbit $C^{14}$-antibody. The $C^{14}$-antibody was obtained by incubating spleen fragments from hyperimmune rabbits (boosted 4 days previously with the antigen, HSA) with $C^{14}$-amino acids in Gey's balanced salt solution *in vitro*[7,8]. Rabbit $C^{14}$-antibody was isolated by precipitation with unlabelled HSA in the presence of carrier antibody. Both types of insoluble complexes contained similar amounts of antibody and antigen in the region of slight antigen excess.

Exudate cells were taken 3 days after intraperitoneal injection of glycogen in saline. For the study of uptake and breakdown identical amounts of the labelled antibody/antigen complexes (100 μg protein) were incubated with 0.5 ml of a 10% suspension of either guinea pig or rabbit cells in Gey's solution containing 10% homologous serum (Medium) for 2 h at 37°C. Controls contained Medium without cells. To exclude the possibility that the degradation of the complexes occurred extracellularly, controls were set up with supernatants from cell suspensions preincubated for 2 h at 37°C. The cells were centrifuged down and the supernatants incubated with the antibody/antigen complexes.

To terminate the experiments, the mixtures were centrifuged and 0.2 ml of a 25% trichloracetic acid solution was added to the clear supernatants. After 15 min the mixtures were centrifuged and the radioactivity of the supernatants determined. The results of a typical experiment are shown in the Table.

The controls show that incubation of cells for 2 h did not release sufficient proteolytic enzyme to degrade the antibody/antigen complexes extracellularly. The breakdown which occurred in the presence of the cells is therefore assumed to take place intracellularly following phagocytosis of the complex.

Catabolism of antibody/antigen complexes by leucocytes *in vitro*

| Type of cells | Type of antigen/antibody complex | Breakdown of antigen or of antibody(%)[a] |
|---|---|---|
| Rabbit leucocytes | Rabbit antibody/$I^{131}$-HSA | 13 |
| | Rabbit $C^{14}$-antibody/HSA | 9 |
| Supernatant control after cell removal | Rabbit antibody/$I^{131}$-HSA | 0 |
| | Rabbit $C^{14}$-antibody/HSA | 0 |
| Guinea pig leucocytes | Rabbit antibody/$I^{131}$-HSA | 6 |
| | Rabbit $C^{14}$-antibody/HSA | 5 |
| Supernatant control after cell removal | Rabbit antibody/$I^{131}$-HSA | 0 |
| | Rabbit $C^{14}$-antibody/HSA | 0 |
| Gey's 10% serum control | | 0 |

[a] These values were obtained by calculation from the radioactivity of the supernatants after precipitation by TCA.

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