INHIBITORY AND LETHAL EFFECTS OF DNA ON TRANSFORMABLE STREPTOCOCCI

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It is generally and implicitly assumed that the sole effect of transforming DNA on competent cell cultures is the transmission of new genetic traits. During attempts to increase the transformation frequency of group H Streptococci, it was observed that concentrations of DNA above saturation (5 μg/ml.) affected the growth characteristics of recipient strains (Piechowska, Żiżina and Shugar, 1966). Two facts prompted a more detailed study of this phenomenon: (a) a close examination of the literature showed that analogous effects have been noted by others (see below); and (b) the group H Streptococcal system has been developed to give transformation frequencies, in viable units, in excess of 50 to 60% (sum of 3 non-linked markers) (Piechowska and Shugar, 1966); the consequent established ability of a large proportion of the cell population to incorporate DNA made it feasible to examine the influence of the latter by comparison of the growth of a DNA-treated population relative to that of a non-treated control.

A lag in growth of transformants has been noted with the D. pneumoniae (Hotchkiss, 1957; Ephrussi-Taylor, 1959) and B. subtilis (Nester and Stocker, 1963) systems. Fig. 1 exhibits growth curves for a low-competent recipient culture of S. Challis, and for the corresponding dihydrostreptomycin-resistant transformants, both obtained by means of the overlay technique (Alexander and Leidy, 1953). Growth of the entire recipient culture began after a lag phase of 30 mins., with a generation time of about 35 mins. during the log phase; by contrast, the transformants showed no growth for a period corresponding to three generations of the entire recipient culture (isolation of DNA, preparation of competent cultures and transformation conditions are described in detail elsewhere (Piechowska and Shugar, 1966)). It is difficult to say whether this effect is due to transient inhibition of cell division of transformants by incorporated DNA for several reasons, such as the tendency of S. Challis cells to grow in clusters, the possibility of DNA integration in only one of the recipient strands, and the possible

appearance in the transformant progeny of untransformed cells (Stocker, 1963; Ephrussi-Taylor, 1966; Piechowska and Shugar, 1966).

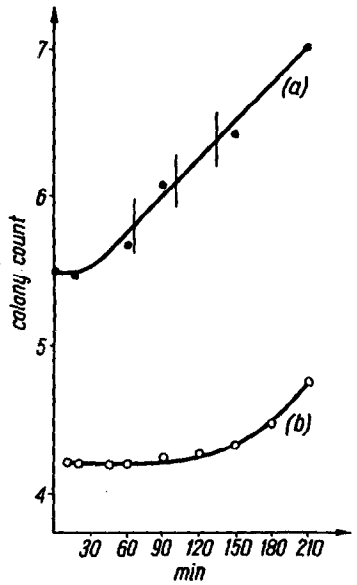

Fig. 1

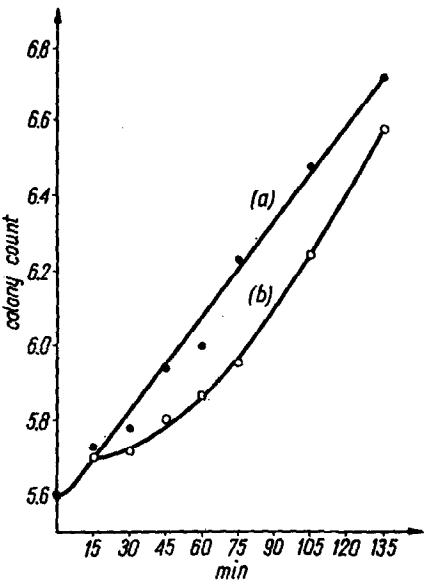

Fig. 2

Fig. 1: Growth curves for: (a) S. Challis group H low-competent recipient culture (transformation frequency for one marker, dihydrostreptomycin-resistance, 5%); and (b) dihydrostreptomycin-resistant transformants from the same population.

Fig. 2: Influence of short treatment with transforming DNA on growth of S. Challis recipient culture with high competence (50% transformation frequency, in viable units, for three non-linked markers): (a) Control population; (b) Culture exposed to 10 μg/ml. DNA for 15 mins., DNase added to 2 μg/ml., and incubation continued.

In a highly competent culture, on the other hand, it might be anticipated that an increase in generation time of the transformants would be reflected in the growth curve of the entire population, of which the transformants form an important fraction. A thawed culture of recipient cells was diluted with fresh medium to a density of 8×10^5 cells/ml. (0.5 ml. samples used for plating) and DNA added to a concentration of 10 μg/ml. After 18 mins. at 37°, crystalline DNase was added to a concentration of 2 μg/ml. (only DNase was added to the control), and incubation continued. The resulting growth curve, from samples plated on agar, shows (Fig. 2) a net decline in the number of viable units in the DNA-treated culture, clearly visible after 30 mins., to one-half that of the control after 75 mins. The course of the curve suggests a transient inhibition of cell division in part of the population, followed by its subsequent participation in the normal cycle of cell division.

An increase in DNA concentration to 50 µg/ml., and prolongation of time of contact with recipient cells (by omission of DNase), appreciably accentuated the above effect, to the point where the colony count frequently decreased below the initial, as shown in Fig. 3 (curve b), in which the count dropped to 35% of initial in 60 mins. At 90 mins. the count began to increase, parallel to the control and, after 240 minutes, was 7% that of the control. Fig. 3 (curve a) shows that, during the initial 60 mins. incubation, the count of the control increased 2-fold. It follows that, during this period, not 65%, but at least 65%, of the viable units in the DNA-treated culture failed to survive, since what is observed is probably the difference between cell multiplication and cell death. The fraction of cells killed must be greater than 65% since only one viable cell per cluster will produce a colony. With the high transformation frequency of this system, transformants should also be affected; this is shown to be so by curve c of Fig. 3.

The foregoing indicates that the effect of exogenous DNA on the bacterial cells is bactericidal. Occasionally, usually when DNA was added during the log growth phase, high concentrations did not reduce the number of viable units below the initial value, but provoked a marked deviation of the growth curve from that of the control. In such instances the growth curves do not allow us to infer whether the effect of DNA is to increase the generation time of all the cells, or to act lethally on a certain proportion in successive generations.

In the experiment described in Fig. 3, the colony count in the DNA-treated culture after 240 mins. incubation was one-fourteenth that of the control. But the difference in optical densities of the two cultures was only 3-fold. This difference must be attributed to the presence in the DNA-treated culture of cells which have lost their viability, since a microscopic count at 240 mins. showed no difference in number distribution of cells per cluster between controls and DNA-treated populations.

That the foregoing effects are to be ascribed to DNA was shown by the fact that prior DNase treatment of DNA completely abolished its action on recipient cells. Possible union of two cells by single DNA molecules was eliminated by DNase addition after 60 mins. contact of the cells with DNA, with no alteration of the effect of the latter.

It follows that the lethal effect of DNA is related to its uptake by competent cells. This was confirmed with the help of S. Wicky, a non-transformable strain, which can take up DNA and undergo transformation in very high yield with S. Challis DNA (Piechowska and Shugar, 1966) only after addition to the culture of a "competence factor" isolated from an S. Challis

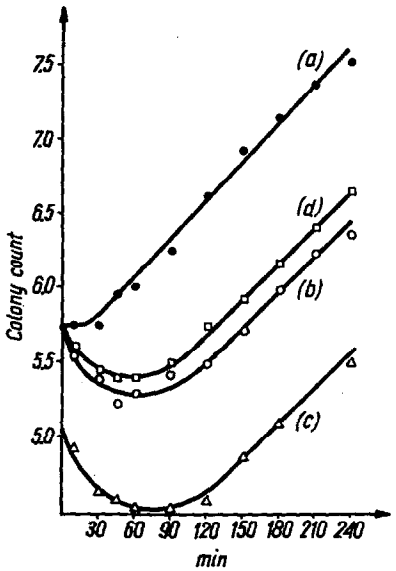

Fig. 3

Fig. 3: Effect of homologous and heterologous DNA (50 μg/ml.) on growth and viability of high competent culture of S. Challis, and of homologous DNA on dihydrostreptomycin-resistant transformants: (a) Control culture; (b) Culture to which S. Challis DNA added at time 0; (c) DNA-treated culture diluted with agar, plated, incubated for 3 hours, coated with a second layer of dihydrostreptomycin-agar, and incubated further; (d) As in (b), but with Challis DNA replaced by E. coli CR-34 DNA.

competent culture (Pakuła and Walczak, 1963; Osowiecki and Lancow, 1965; Dobrzański and Osowiecki, 1966). Under these conditions the growth of S. Wicky was affected by exogenous DNA in the same way as S. Challis; the same DNA was inert against a non-competent S. Wicky culture.

Reverting to Fig. 3, the number of cells in the control culture which lose their viability after 60 mins. contact with DNA is $\geq 65\%$ (curve b). The number of surviving transformants at this same time, for one marker, is 7% (curve c) of the number of viable units at zero time. With three non-linked markers this becomes 14% which, when corrected for the average number of cells per cluster, about 3, becomes 5% of the number of viable cells at zero time. Hence more than 70% of the cells must have taken up DNA.

The question then arose as to whether the lethal effect of exogenous DNA was linked to its transforming activity. Samples of DNA were therefore irradiated with increasing doses of UV to the point where the residual transforming activity was 10%, without impairment of ability to

undergo uptake by competent cells (Lerman and Tolmach, 1959; Notani and Goodgal, 1965). This treatment led to an enhancement of the adverse effect of DNA on cell viability of the order of 20-50%, which decreased, as expected, with higher irradiation doses.

Since the transforming activity of exogenous DNA did not appear to be involved, attention was next directed to tests with heterologous DNA, from E. coli CR-34T⁻, bacteriophage T2, and the poly-dAT fraction from Cancer magister DNA. All three of these, at concentrations of 50 μg/ml., affected the growth of S. Challis recipient cells to the same extent as S. Challis DNA (curve d, Fig. 3). E. coli DNA exhibited no visible influence on cell growth of E. coli B, in agreement with the known inability of the latter to take up DNA (Lerman and Tolmach, 1957). E. coli sRNA was inert in all the above systems.

The overall findings place in a new light the dependence of transformation frequency on DNA concentration and suggest that the maximum number of transformants may, in part, be the resultant of the transforming and lethal affects of DNA. With the S. Challis system, under appropriate conditions, we have in fact found that high saturation concentrations (50 - 80 μg/ml.) of DNA appreciably reduce the number of transformants below the initial plateau value (Piechowska and Shugar, 1966).

The influence of DNA on the growth and survival of competent bacteria is probably not limited to Streptococci, as testified to by studies on the growth of transformants of B. subtilis (Nester and Stocker, 1963; Kammen et al., 1966), of the entire population of S. aureus (Lambina, 1964) and the influence of 5-bromouracil-containing DNA on the growth of B. subtilis (Gimlin et al., 1966). The mechanism of this process remains to be clarified, but the fact that UV-inactivated and heterologous DNA samples exhibit the same effect implies that genetic recombination is not involved (Notani and Goodgal, 1965; Pene and Romig, 1964). Finally, this phenomenon may prove to be of wider significance in view of reports on the inhibitory effects of exogenous DNA on mammalian cells (Zahn and Tiesler, 1963; Glick and Goldberg, 1965; Halpern et al., 1966) and it is possible that it may prove useful in evaluating DNA uptake by mammalian cells.

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