Spontaneous transformation in *Bacillus subtilis*

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1. INTRODUCTION

Extracellular DNA has been found in cultures of a variety of micro-organisms (Smithies & Gibbons, 1955; Catlin, 1956, 1960; Ottolenghi & Hotchkiss, 1960; Campbell, Evans, Perry & Niven, 1961; Takahashi, 1962; Demain, Burg & Hendlin, 1965). However, little is known about the biological significance of released DNA or of the mechanism by which it is released from the cells. In bacterial species amenable to genetic transformation by DNA molecules, such as *Neisseria meningitidis* (Catlin, 1960), *Diplococcus pneumoniae* (Ottolenghi & Hotchkiss, 1960) and *Bacillus subtillis* (Takahashi, 1962), it has been shown that extracellular DNA spontaneously released by untreated cultures is genetically active. In *N. meningitidis*, biologically active extracellular DNA accumulated in ageing cultures as a result of cell lysis (Catlin, 1960). A detailed study of the genetic activity and mode of release of extracellular DNA by cultures of *D. pneumoniae* revealed that highly active transforming DNA was found only in young logarithmic cultures (Ottolenghi & Hotchkiss, 1962). The present communication describes the biological efficiency of spontaneously released DNA, the pattern of DNA release during the growth cycle of *B. subtilis* and the possible physiological mechanisms involved.

2. METHODS

(i) *Bacterial strains*

The following derivatives of strain 168 of *Bacillus subtilis* were used in transformation experiments: $31(try_2^- his_5^-)$, $26(try_2^- his_8^-)$ (Ephrati-Elizur, Srinivasan & Zamenhof, 1961), SB 58$(try_2^- ade^-)$ (kindly supplied by Dr D. Green) 58S (an adenine-requiring smooth variant, isolated from strain SB 58 and transformed to tryptophan independence), and MT$(try_2^- met^-)$. The mutation to methionine requirement was induced by tri-ethylene melamine (Iyer & Szybalski, 1958). Strain AMT was obtained by transforming strain MT to adenine dependence, using strain SB 58 as the DNA donor. W 168 (SA), a prototrophic strain, resistant to 200 $\mu g/ml$ of sulphanilamide, served as a source of wild-type DNA carrying the gene determining sulphanilamide resistance.

(ii) *Transformation procedure*

Transformation with purified DNA and preparation of competent cultures was carried out according to Ephrati-Elizur (1965) or to Kelner (1964). Transformation experiments in a donor–recipient system with spontaneously released DNA were done with strain 58S as DNA donor. Strain 58S is poorly transformable when grown in transformation medium (0·001 %) and non-transformable when grown as DNA donor in Difco Penassay broth. Unless otherwise stated, about 10^6/ml spores of the donor strain were inoculated into Penassay broth (Difco) and the culture incubated overnight with aeration. The culture was diluted 1/20 in fresh Penassay broth and 0·1 ml (about 10^7 cells) mixed with 2 ml (about 2×10^8 cells) of a transformable competent culture of a differently marked auxotrophic mutant. The mixture was incubated with aeration at 37 °C for 60 min and the reactions ended by the addition of 20 μg DNase/ml. Transformants to amino acid independence were scored on the appropriately supplemented minimal agar plates (Spizizen, 1958). Double transformants were scored on minimal agar. Transformants to sulphanilamide resistance were scored on minimal agar plates supplemented with 200 μg sulphanilamide/ml and 20 μg/ml of both tryptophan and histidine. Wild-type DNA was prepared from strain W 168 according to Marmur (1961).

(iii) *Bacterial growth*

Bacterial growth was followed by optical measurements in a Klett-Summerson colorimeter with filter 42 or by viable counts on Blood Agar Base (Difco). Culture supernatant fluids (CSF) were obtained by centrifugation at 10 000 rev/min for 15 min or by filtration through membrane filters of 0·45 μ porosity (Millipore Filter Corporation).

(iv) *Proneness to lysis*

This was measured by a slight modification of the method of Mohan *et al.* (1965). A culture was washed and suspended in 0·05 M tris (hydroxymethyl) aminomethane (Tris) buffer, pH 7·5, at an optical density of about 100 Klett units (filter 42). The bacterial suspension was incubated at 37 °C with aeration, and lysis was determined by measuring the fall in optical density.

(v) *Protein and DNA determinations*

To 10 ml culture samples were added 2·5 ml of cold 50 % trichloracetic acid. The mixture was kept in the cold overnight and centrifuged. The pellets were suspended in 5 ml of 50 % TCA and heated at 100 °C for 30 min. After centrifugation, the supernatant fluid was assayed for DNA by the modified diphenylamine method (Burton, 1956). The pellet was dissolved in 1 N-NaOH at 90° for 30 min, and protein was determined by the method of Lowry, Rosbrough, Farr & Randall (1951).
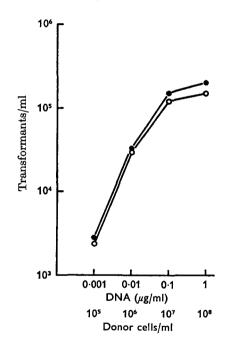
(vi) *Electron microscopy*

A drop of bacterial suspension was placed on a carbon-coated grid. The excess was blotted off by touching the edge with filter paper and the preparation was

briefly rinsed by floating on distilled water. The specimens were shadowed with platinum and examined in a Philips EM 200 electron microscope.

(vii) *Fluorescence microscopy*

The procedure was carried out according to Young & Smith (1963). A small volume of a suspension of *B. subtilis* was mixed with an equal volume of a fluorochrome euchrysine 3 R (1 : 2500 in buffered phosphate). Blue-violet light from a high-pressure mercury vapour lamp was used to activate fluorescence in the preparation.

(viii) *DNase treatment*

Unless otherwise stated samples were incubated at 37 °C for 10 min with 20 μg/ml of pancreatic DNase (Worthington).

Text-fig. 1. Transforming activity of cells of strain 58S and of purified DNA. A culture of strain 58S, grown overnight in Difco Penassay broth, was filtered, washed once and suspended in fresh broth. Viable counts were determined as described in Methods section. Transforming activity was assayed by incubating samples of 0·1 ml of different dilutions of the culture with 1 ml of a competent culture of strain 31 for 60 min at 37 °C. Transformants were scored for histidine independence. ○—○, Purified DNA; ●—●, donor cells.
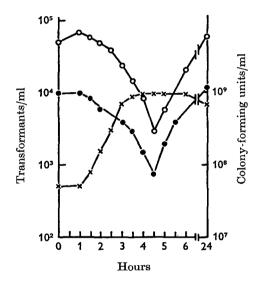
3. RESULTS

(i) *Donor cells and DNA response curve*

To determine the transforming activity associated with donor cells of *B. subtilis* as compared with that of purified DNA, competent recipient bacteria were exposed to increasing concentrations of cells and transforming DNA respectively. After

incubation, samples were plated and scored for transformation to histidine independence. Text-fig. 1 shows that the number of transformants rises steeply with the increasing number of donor cells. The curve reaches a plateau at about 10^7 cells/ml, i.e. at a transformant:donor ratio of about 1:50. When the same recipient culture was treated with purified DNA, a similar curve was obtained with a plateau at 0·1 μg/ml of DNA. It is of interest to note that the DNA content of donor cells under these conditions was approximately 0·1 μg per about 10^7 cells.

The addition of DNase (20 μg/ml) to the donor culture before mixing with the recipient culture inhibited the appearance of transformants, indicating that transformation was due to extracellular DNA.
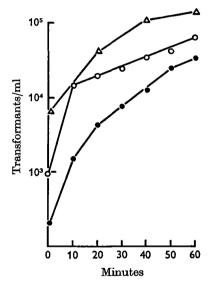

Text-fig. 2. Transforming activity of growing and stationary cultures of *Bacillus subtilis* 58 S. Strain 58 S grown overnight in Penassay broth was diluted into 10 ml fresh broth at an optical density of 50 Klett units and incubated with aeration at 37 °C. Culture aliquots were withdrawn at different stages of growth and about 10^7 cells and culture supernatant fluids were assayed for transforming activity (see Text-fig. 1). Transformants were scored for histidine independence. × — ×, Viable count; ○—○, transformants induced by 58 S culture; ●—●, transformants induced by 58 S supernatant fluids.

(ii) *Release of transforming DNA during the growth cycle*

An experiment was carried out to examine whether the transforming activity of donor cells is associated with any particular stage of the growth cycle. Text-fig. 2 shows that high transforming activity was found mainly in lag, early-log. and stationary-phase cultures. Although the transforming activity per *c.* 10^7 cells examined is similar at time 0 (stationary cells) and at a 2 h growth, there had been a several-fold increase in viable count between 0 and 2 h so that the transforming activity must have increased proportionately. This means that during early growth DNA is released *de novo* and transforming activity is not due simply to DNA released by stationary cells. The fact that transforming DNA does not accumulate

in the supernatant fluids above 10–20 % of total indicates that the transforming DNA remains bound to the cell surface. A similar pattern of DNA release was obtained when the transformable strain MT was used as DNA donor.

To confirm that the transforming activity of cells is due to newly released DNA the following experiment was done. An early log. culture was divided into two, one part was incubated with DNase, washed and mixed with a recipient culture. The second part was mixed with a recipient without treatment. At the same time a similar recipient culture was treated with purified DNA. The frequency of transformation was determined at intervals in each mixture. Text-fig. 3 shows that the number of transformants induced by DNase-treated culture within 60 min of

Text-fig. 3. The effect of DNase and washing on the transforming activity of *Bacillus subtilis* 58S. An overnight culture in Penassay broth was diluted 1:25 into fresh broth and incubated for 90 min. Two 2 ml samples were withdrawn. To one sample DNase (10 μg/ml) was added and both samples were incubated for 10 min at 37 °C. The DNase-treated culture was filtered and washed 3 times in warm broth, and filter suspended in 2 ml fresh warm broth. Both culture samples were diluted 1:5 into a recipient culture and aliquots were removed at the indicated time and plated for transformants to histidine independence. Transformants induced by: ●—●, DNase-treated donor cells; ○—○, untreated donor cells; ▲—▲, purified DNA.

incubation is about 50 % of that induced by the untreated culture. This shows that a considerable amount of DNA is released by the cells during 60 min incubation. The reason for the reduced number of transformants in the treated culture may be the extensive washing that had to be carried out to remove the DNase. The fact that transformants induced by purified DNA appear at time 0 at a much higher rate than those induced by DNA released by either untreated or treated donor culture also indicates that *de novo* release of DNA occurs during incubation, since only a fraction of such DNA is available for transformation at time 0.

(iii) *Test for nuclease activity in culture supernatant fluid*

Extracellular nucleases were recently isolated from cultures of *B. subtilis* (Kerr, Pratt & Lehman, 1965; Birnboim, 1966) and might have been responsible for the decline in transforming activity observed during the late log. phase in Text-fig. 2. Samples were collected at hourly intervals ranging from lag to stationary phase and 0·1 ml of the supernatant fluid from each sample was incubated at 37 °C for 30 min with 0·1 ml (0·05 μg) DNA-SA (wild-type DNA carrying sulphanilamide-resistant marker). After incubation 0·8 ml of a competent culture of strain 31 was added and the mixture was incubated for another 60 min. Transformants to sulphanilamide resistance were scored. The results (not shown) revealed that incubation of DNA-SA with supernatant fluids from 58S cultures from various growth phases did not affect the transforming activity of the former, thus excluding the possibility that the appearance of nuclease activity at the late log. phase is the cause for the decline in transforming activity.

Table 1. *Co-transfer frequencies in transformation with extracted wild-type DNA and spontaneously released DNA (strain 58S)*

		Extracted DNA			Released DNA			
		Transformants/ml*		%	Donor	Transformants/ml*		%
Recipient strain	DNA (μg/ml)	met$^+$	met$^+$try$^+$	double†	cells/ml	met$^+$	met$^+$try^{+*}	double†
MT	0·1	$7{\cdot}5 \times 10^4$	$2{\cdot}5 \times 10^3$	3·2	10^7	$1{\cdot}7 \times 10^5$	$1{\cdot}0 \times 10^4$	5·9
	0·01	$1{\cdot}6 \times 10^4$	$7{\cdot}0 \times 10^1$	0·4	10^6	$5{\cdot}6 \times 10^4$	$2{\cdot}3 \times 10^3$	4·1
	0·001	$1{\cdot}2 \times 10^3$	0	0	10^5	$8{\cdot}7 \times 10^3$	$3{\cdot}3 \times 10^2$	3·8
		his$^+$	his$^+$try$^+$			his$^+$	his$^+$try$^+$	
26	0·1	$2{\cdot}0 \times 10^4$	$4{\cdot}0 \times 10^2$	2·0	10^7	$2{\cdot}8 \times 10^4$	$2{\cdot}2 \times 10^3$	8·0
	0·01	$3{\cdot}5 \times 10^3$	$2{\cdot}0 \times 10^1$	0·6	10^6	$4{\cdot}2 \times 10^3$	$1{\cdot}7 \times 10^2$	4·0
	0·001	$3{\cdot}3 \times 10^2$	0	0	10^5	$6{\cdot}2 \times 10^2$	$3{\cdot}5 \times 10^1$	5·7
31	0·1	$7{\cdot}5 \times 10^4$	$5{\cdot}1 \times 10^4$	68	10^7	$4{\cdot}6 \times 10^4$	$3{\cdot}3 \times 10^4$	72
	0·01	$1{\cdot}5 \times 10^4$	$1{\cdot}0 \times 10^4$	66	10^6	$5{\cdot}5 \times 10^3$	$3{\cdot}6 \times 10^3$	65
	0·001	$1{\cdot}3 \times 10^3$	$8{\cdot}0 \times 10^2$	61	10^5	$4{\cdot}7 \times 10^2$	$2{\cdot}8 \times 10^2$	60

* Transformation tubes were incubated without aeration.

† Frequencies of double transformants are given as percentage of met$^+$ in strain MT and his$^+$ in strains 26 and 31.

(iv) *Linkage studies with spontaneously released and purified DNA*

The molecular integrity of released DNA was examined in linkage studies by comparing frequencies of co-transfer by released and purified DNA, of several pairs of markers, one closely linked and two pairs of unlinked markers. The degree of co-transfer obtained for the closely linked $try_2^- his_5^-$ markers (strain 31) was similar when transferred by either released or purified DNA (Table 1). On the other hand, the apparent linkage between $try_2^- met^-$ (strain MT) or $try_2^- his_8^-$ (strain 26) which is known to be due to random double transformation disappears as expected with dilution of the DNA. In the spontaneous transformation there is
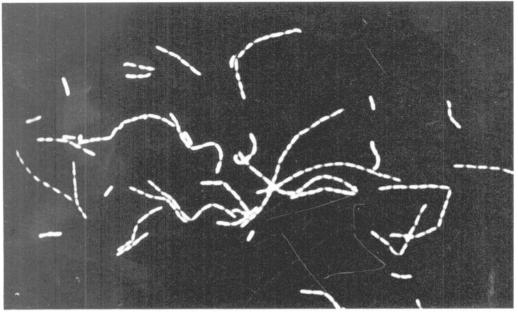
Fig. 1. Early log.-phase *Bacillus subtilis* 58S stained with euchrysine photographed on
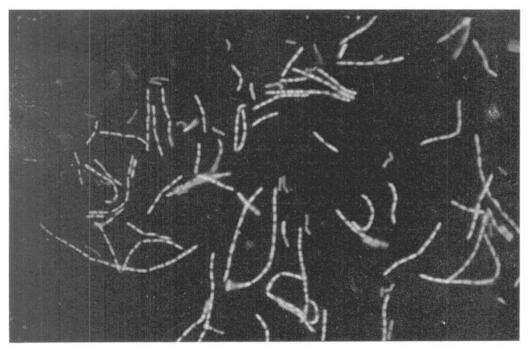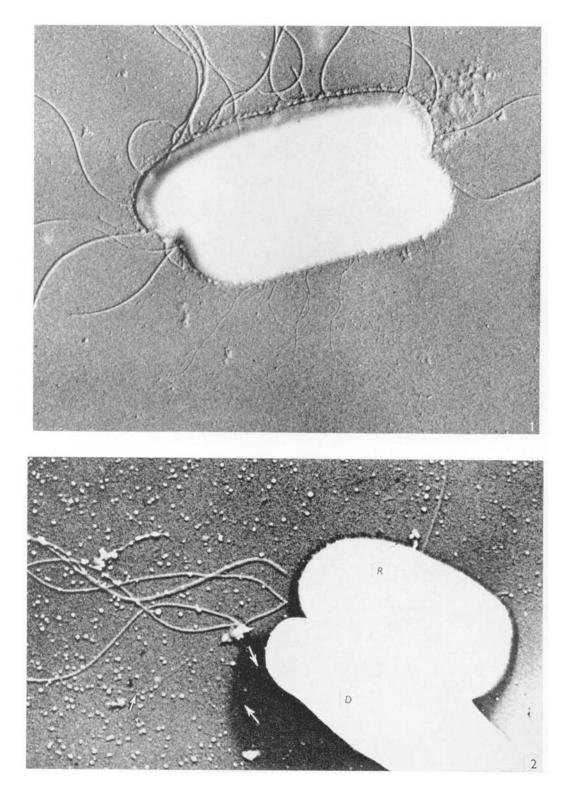3R Ektachrome high-speed daylight colour film. × 1200.

Fig. 2. As fig. 1. Culture treated with DNase before staining.

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also an apparent linkage between these pairs of markers, but this linkage is not affected by dilution of the donor culture.

(v) *The association of extracellular DNA with intact cells*

The following experiments were designed to show whether the DNA associated with the cellular fraction was released by intact cells. Euchrysine has been successfully used to distinguish between nuclei, which give green fluorescence, and other cell constituents which stain yellow or red (Young & Smith, 1963). This technique was used to detect extracellular DNA in a growing culture by direct observation of a large number of cells. Plate 1, figs. 1 and 2, show early log.-phase donor cells before and after treatment with DNase. It can be seen that cells from untreated cultures are bright, whereas, in preparations treated with DNase, the cells have lost their fluorescence and are dull in appearance. Diffuse fluorescence was not seen in cultures taken from late log. phase (not shown). These results indicate that at the early phase of growth, extracellular DNA is associated with the surface of the bacterial cell.

Donor cells were examined by electron microscopy. Plate 2, fig. 1, shows two donor cells with flagella and, in addition, fine strands which seem to be leaking out from one cell. Numerous such cells were observed. Similar strands were not seen in cell preparations treated with DNase, nor in cells taken from late log. phase cultures. Plate 2, fig. 2, shows a pair from a donor–recipient mixture, consisting of a donor and a recipient cell which can be distinguished by their shape and size (which differ because of differences in their ages and growth conditions). Fine strands are coming out of the donor cell. There is a no decisive evidence at present that these strands are indeed DNA; however, they are similar to DNA molecules seen in several other electron micrographs (Lang, Kleinschmidt & Zahn, 1964; MacHattie & Thomas, 1964).

(vi) *Absorption of DNA by donor cells*

The source of extracellular DNA shown to be associated with the surface of the donor cells (Plate 1) could either be the fluorescing cells themselves or lysed cells which released their DNA into the medium. To distinguish between these alternatives the ability of donor cultures to bind and act as donors of externally added DNA was determined. The donor–recepient system consisted of an *ade*⁻ mutant (strain 58S) as DNA donor and strain AMT carrying the same *ade*⁻ mutation as recipient. Early and late log. donor cultures were each mixed with 0·5 μg/ml wild-

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type DNA. After 30 min incubation at 37 °C the whole cultures, supernatant fluids and cellular fractions obtained after filtration and suspension to the original volume in Penassay broth were each diluted 1:10 into a recipient AMT culture and examined for transformation to adenine independence. Table 2 shows that the cellular fraction of the donor cultures transferred the adenine marker poorly, most of the transforming activity remaining in the supernant fluids. In addition, no difference was observed in the ability of early and late log. phase cultures to bind DNA. On the other hand, when the activity of spontaneously released DNA was determined using the same donor cultures without the addition of DNA, a different picture was obtained. In this case transformation to methionine independence was scored for using strain MT as recipient. Table 2 shows that most of the transformation

Table 2. *Test for transformation by adsorbed wild-type DNA and by spontaneously released DNA*

	Transformants/ml.			
	Adenine independence (purified wild-type DNA)		Methionine independence (spontaneously released DNA)	
	Early log. culture			
1. Culture	3.0×10^4	—	1.4×10^5	—
2. Supernatant	2.5×10^4	85 %	1.8×10^3	13 %
3. Cellular fraction	8.5×10^3	28 %	1.2×10^5	86 %
	Late log. culture			
1. Culture	2.8×10^4	—	2.2×10^4	—
2. Supernatant	2.5×10^4	90 %	6.0×10^3	27 %
3. Cellular fraction	6.0×10^3	24 %	1.9×10^4	86 %

induced by released DNA is associated with the cellular fraction and that early log culture is 7 times more active in transformation than late log. culture. These results do not support the notion that the transforming activity associated with the cellular fraction originated in adsorbed DNA, but rather suggest that the activity is due to DNA released by the fluorescing cells which remains bound to their surface.

(vii) *The effect of chloramphenicol on growth and on the release of transforming DNA*
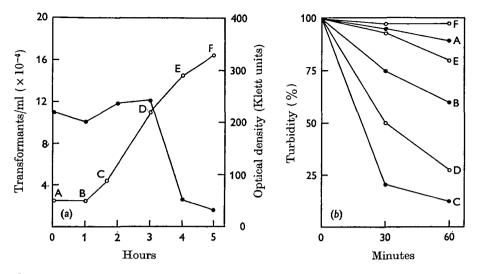
The next experiment was aimed at answering the question whether the release of DNA by young dividing bacteria was dependent on cell growth. Samples from an early log.-phase culture were treated with increasing amounts of chloramphenicol (CM) and the growth rate and transforming activity of untreated and CM-treated cultures recorded. Stationary donor cells were similarly treated. Text-fig. 4 shows that treatment of log. cells with CM (5 and 10 μg/ml) markedly lessened both their growth rate and transforming activity. On the other hand, CM had no depressing effect on the transforming activity of stationary cultures; in fact, their transforming activity was even enhanced. Thus, the release of transforming DNA by young cells seems to be correlated with growth activity and may differ in this respect from that by stationary cells.

Text-fig. 4. The effect of different concentrations of chloramphenicol on the transforming activity of logarithmic and stationary cultures of *Bacillus subtilis* 58S. Early logarithmic and stationary cultures (16 hr), were diluted into Penassay broth to an optical density of 50 Klett units. Culture samples were mixed with different concentrations of chloramphenicol, incubated for 60 min with aeration and their optical density recorded. About 10^7 cells were added to competent culture strain 31 and transforming activity assayed (Text-fig. 1). (Since CM had no effect on competent cells, CM-treated donor cells were not washed before mixing with the recipient culture). Transforming activity; O—O, log.-cells; ●—●, stationary-cells. Optical density: × --- ×, log.-cells; △ --- △, stationary-cells.

(viii) *A correlation between proneness to autolysis and the release of DNA by growing cells*

The active growth of several micro-organisms is associated with the activity of autolytic enzymes, which are thought to be involved in cell-wall growth. Proneness to lysis is high in the early log. phase and disappears towards the stationary phase of growth (Mohan *et al.* 1965; Mitchell & Moyle, 1957; Shockman, Kolb & Toennies, 1958; Shockman, 1965; Young, 1966). Experiments were carried out to test whether there is a correlation between the release of transforming DNA by strain 58S and its proneness to lysis. Samples of donor culture were collected at hourly intervals, starting with an overnight culture and going through the lag and log. phases of growth, and transforming activity and proneness to lysis determined. Text-fig. 5 shows that stationary-phase cells at the beginning of the lag phase (point A) released DNA but were resistant to lysis. Proneness to lysis was manifest at the end of the lag and is most pronounced at the early log. phase. The steep decline in transforming activity observed after 4 h of growth (point E) is paralleled by a considerable loss in proneness to lysis which disappears completely in the 5 h culture, when cells enter the stationary phase. These observations suggest that a correlation may exist between the release of DNA by young cells and their proneness to lysis. On the other hand, the release of DNA by stationary cells (point A) is not paralleled with a similar proneness to lysis.

Text-fig. 5. Growth, transforming activity and proneness to lysis in *Bacillus subtilis*. An overnight culture on Blood Agar Base (Difco), was suspended in Penassay broth at an optical density of 50 Klett units. The culture was incubated at 35 °C with aeration. At hourly intervals, 10 ml samples A–F were withdrawn for the measurements of optical density, proneness to lysis and the assay of transforming activity (Text-fig. 1). (*a*) ●—●, Optical density; ○—○, transforming activity, (*b*) Autolysis of samples A–F in Text-fig. 5(*a*). The results are expressed as percentage of the turbidity of the samples at time 0.

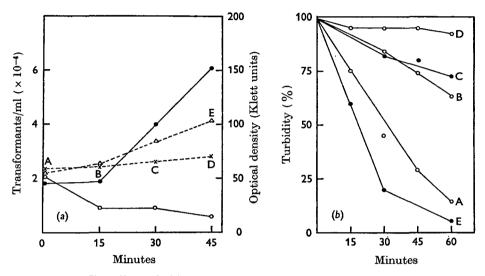### (ix) *The effect of chloramphenicol on proneness to lysis and release of DNA*

These observations suggested that CM inhibits DNA release in the early log. phase because it does not inhibit cell-wall synthesis (Mandelstam & Rogers, 1957). Unbalanced synthesis of cell wall results in thickened walls which are more resistant to wall lytic enzymes, as with *Streptococcus faecalis* (Shockman *et al.* 1958).

The following experiment was carried out to see whether inhibition of DNA release by CM is correlated with resistance to lysis. CM was added at a concentration of 10μg/ml to early log. phase donor cells 58S; samples removed at the indicated times were examined for proneness to lysis and transforming activity. Text-fig. 6 shows that within 15 min after adding CM, a marked decrease in transforming and lytic activities was observed. Within 45 min of incubation with CM, proneness to lysis was practically nil and transforming activity was about 10 % compared to the activity of a similar culture untreated with CM.

Treatment of cells with CM resulted in an immediate cessation of protein synthesis followed by a decline in transforming activity; DNA synthesis continued for about 30 min (Text-fig. 7). After removal of CM, there was an almost immediate resumption of protein synthesis and a 90 min lag before the resumption of DNA synthesis. The reappearance of transforming activity coincided with the resumption of protein synthesis.

4. DISCUSSION

The data show that high transforming activity is present in cultures of *Bacillus subtilis* at the beginning of exponential growth, declines towards the end of the log. phase and reappears at the stationary phase of the growth cycle. Cells collected at the proper stage serve as excellent donors of transforming DNA when mixed with a competent culture acting as DNA recipient. Genetic transformation
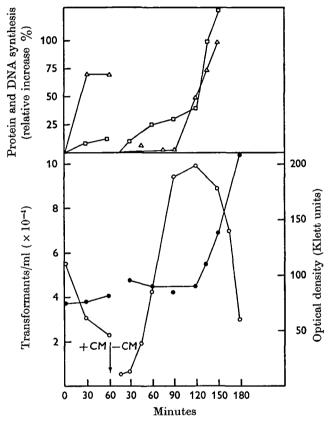

Text-fig. 6. The effect of chloramphenicol on growth, transforming activity and proneness to lysis of *Bacillus subtilis* 58S. A 3 h culture in Penassay broth was diluted into two flasks with fresh broth to optical density of 50 Klett units. To one flask 10 μg/ml chloramphenicol was added. The flasks were incubated with aeration. Samples A–E were drawn at the indicated time for measurements of optical density and proneness to lysis and for the assay of transforming activity. (*a*) ○—○, Transforming activity of CM-treated culture; ●—●, transforming activity of untreated culture; × - - - ×, optical density of treated culture; △ - - -△, optical density of untreated culture. (*b*) Autolysis. A–D, Treated culture; E, untreated culture (see Text-fig. 5).

occurring in a donor recipient system proved to be at least as efficient as that induced by purified DNA (Text-fig. 1, Table 1). The results indicating co-transfer of distant markers by released DNA suggest that such DNA molecules are less fragmented than those obtained by conventional purification procedures (Berns & Thomas, 1965; Kelly & Pritchard, 1965). However, the finding that most of the transforming activity was associated with the cellular fraction raises an equally plausible possibility, that two DNA fragments attached to a single cell may bring about two independent transformation events, which are unaffected by dilution. The molecular integrity of released DNA seems to deserve further study, as the ease with which various donor–recipient systems can be constructed make it a useful tool for genetic and other studies.

The presence of extracellular DNA in young exponential cultures and the reproducible pattern of DNA release during the growth cycle raised the question

whether DNA is released by dead lysing cells or by living cells. The data obtained support the notion that most of the transforming activity found in exponential cultures can be attributed to DNA release by living cells. Treatment of such cultures with CM (Text-fig. 4), novobiocin or sodium azide (unpublished data), inhibited both the growth of the culture and its transforming activity. In addition, fluorescence microscopy showed that extracellular DNA was attached to the

Text-fig. 7. Effect of protein and DNA synthesis on transforming activity of *Bacillus subtilis* 58S. The culture was treated with chloramphenicol as in Text-fig. 6. After treatment the culture was filtered, washed and suspended in warm Penassay broth. Ten ml. samples withdrawn at the indicated times were examined for transforming activity, optical density, protein and DNA synthesis. ○—○, Transforming activity; ●—●, optical density; □—□, protein synthesis, relative increase; △—△, DNA synthesis, relative increase.

surface of a great part of the bacterial population. The data in Table 2 exclude the possibility that the DNA was absorbed by the cells, thus implying that DNA is released by what seems to be intact cells. An explanation for this phenomenon may perhaps be sought in the correlation observed between proneness to lysis of early log.-phase cultures and their transforming activity. It has been suggested that at this stage of growth weakness in cell wall structure may result from un-balanced synthesis of cytoplasmic and cell wall macromolecules (Shockman, 1965)

and the appearance of activity of lytic enzymes presumably associated with cell wall synthesis (Young, 1966). The decline in transforming activity observed during late exponential growth was shown not to be due to nuclease activity but coincided with the disappearance of the cell's proneness to lysis (see Text-fig. 5). The inhibition of both DNA release and proneness to lysis by CM is in accord with the finding that CM does not inhibit cell wall synthesis (Mandelstam & Rogers, 1958) and that treatment with CM results in cell wall thickening (Shockman, 1965). Thus, it seems reasonable to suggest that DNA release by young cells may be a result of a temporary unbalanced cytoplasmic growth. Whether the release at this stage of growth is a function of DNA synthesis is being at present investigated. The release of DNA during the stationary phase may also be a result of changes in cell-wall structure since lytic activity was observed during this phase of growth in another strain of *B. subtilis* (Nomura & Hosoda, 1956). However, these changes are probably of a different nature since they do not lead to proneness to lysis under the experimental conditions used here.

The high efficiency of transformation occurring in a donor–recipient system of *B. subtilis*, lends further support to the idea that genetic exchange by extracellular DNA may take place in nature (Hotchkiss, 1951).

SUMMARY

Cultures of *Bacillus subtilis* release transforming DNA during the early exponential and stationary phases of growth. The pattern of release of transforming DNA was followed by measuring transformation in a system consisting of a nontransformable DNA donor and a differently marked transformable recipient. Transformation in this system seems to be at least as efficient as that induced by purified DNA. Fluorescence microscopy revealed that released DNA remained bound extracellular to intact cells. The release of DNA during early exponential growth seemed to be correlated with the cells' proneness to lysis; both DNA release and cell lysis were inhibited by chloramphenicol. In stationary cells, the release of DNA was neither correlated with a similar proneness to lysis nor inhibited by chloramphenicol.

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