Pidot et al. [11] found wide individual variations between IN titers in leukocyte cultures obtained from different individuals and stimulated by Newcastle disease virus. Considerable variations in IN titers also were observed in the same subjects during a long (18 months) period of observation, possibly in connection with the functional state of the lymphocytes producing IN. The role of the genotypic characteristics of the individual in the manifestation of this process [1] has been confirmed in genetic studies on inbred mice with transplantation of bone marrow cells [10].

Taking into account the data in the literature and the results of the present experiments, a final conclusion on the value of the interferon formation test as a means of estimating functional activity of human lymphocytes can be drawn when the results of investigation of the same subjects at different time intervals are available. Work aimed at obtaining such results is now in progress.

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AMOUNT OF EXOGENOUS LINEAR DNA TAKEN UP BY *Escherichia coli* CELLS TREATED WITH Ca^{++} CATIONS

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Treatment of *Escherichia coli* cells with Ca^{++} cations, first used by Mandel and Higa [3], enables exogenous DNA to be introduced into this naturally untransformable microorganism. However, the frequency of chromosomal transformation obtained by what is currently the most widely used technique is extremely low, at best 10^{-7}-10^{-8} [1]. Meanwhile the quantity of irreversibly adsorbed DNA (i.e., not removed by washing out and resistant to the action of deoxyribonuclease) on Ca^{++}-treated cells is quite considerable [4]. It can thus be tentatively suggested that only a small proportion of this DNA penetrates into the cytoplasm. It is also possible that penetration of DNA into the cytoplasm takes place effectively, but the DNA is then quickly broken down by the action of intracellular enzymes and loses its transforming activity. The possible influence of various postrecombination processes on the appearance of the transformants likewise cannot be ruled out.

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TABLE 1. Adsorption of DNA on Cells Treated with Ca^{++} and on Spheroplasts Obtained from These Cells in Saturating DNA Concentrations (13 μg/ml)

Test object	DNA	IC 7623 recBC		AB 1157	
		daltons per cell	%	daltons per cell	%
Cells	DS	$3,9 \cdot 10^7$	3,4	$4,5 \cdot 10^7$	3,2
	SS	$6,4 \cdot 10^7$	5,6	$6,6 \cdot 10^7$	5,8
Spheroplasts	DS	$2,4 \cdot 10^7$	2,1	$2,1 \cdot 10^7$	1,8
	SS	$3,7 \cdot 10^7$	3,2	$3,2 \cdot 10^7$	2,8

Legend. DS) Double-stranded, SS) single-stranded DNA; total exogenous DNA added to the sample (3.9 μg) taken as 100%; results given are means of five independent measurements.

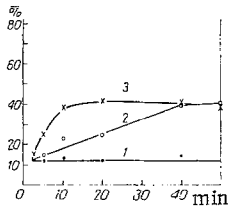

Fig. 1. Kinetics of accumulation of acid-soluble fraction of exogenous DNA in E. coli cells. Abscissa, time of incubation of cells with DNA at 37°C; ordinate, amount of acid-soluble DNA (in % of total DNA bound with spheroplasts, taken as 100%). 1) Control sample: 0.1 ml DNA incubated for 20 min at 0°C with 0.2 ml AB 1157 cells (not treated with Ca^{++}); 2) strain IC 7623; 3) strain AB 1157.

In this investigation an attempt was made to estimate the quantity of linear exogenous DNA entering E. coli cells treated with Ca^{++} cations by a direct method: by determining the degree of degradation of exogenous DNA taken up by the cells by intracellular nucleases.

EXPERIMENTAL METHOD

Strains K12IC 7623 (recB21, recC22, shcB15) and AB 1157 of E. coli were used as recipients. [^{14}C]DNA was isolated by using a thymine-dependent mutant of strain IC 7623 obtained with the aid of trimethoprim. DNA was isolated by the method of Cosloy and Oishi [1]. Some physicochemical parameters of this isolated DNA were: hyperchromic effect 21%, $OD_{260}/OD_{230} = 1.76$, specific activity 2×10^4 cpm/μg. Experiments to measure adsorption were carried out as described in [4, 5]. To obtain spheroplasts, twice-washed cells with DNA adsorbed on them were concentrated to 5×10^9 cells/ml and converted into spheroplasts by treatment with lysozyme-EDTA as described previously [4]. In some cases the spheroplasts were treated with deoxyribonuclease (20-40 μg/ml).

To investigate the kinetics of DNA degradation in E. coli cells treated with Ca^{++} cations, the mixture of cells and DNA was incubated for 20 min at 0°C and then at 37°C. At certain time intervals aliquots from the mixture were diluted with six volumes of cold L broth, the cells were sedimented by centrifugation in the cold, resuspended, and lysed with 0.1N NaOH, and after the addition of an equal volume of cold 12% TCA they were centrifuged. To determine acid-soluble radioactivity, aliquots (from 100 to 300 μl) of the supernatant were added to 4 ml of "minisolve" scintillator (from Koch-Light, England) and radioactivity was measured in a Mark III liquid scintillation counter.

EXPERIMENTAL RESULTS

For successful genetic transformation of E. coli strains defective for ATP-dependent exonuclease (recBC), in which no marked degradation of the entering DNA takes place, are used (for example, IC 7623). However, marked degradation of exogenous DNA can be a convenient indicator of the degree of penetration of DNA inside the recipient cells [2].

Data on absorption of DNA on cells and spheroplasts of two different strains recBC$^+$ (AB 1157) and recBC$^-$ (IC 7623) are given in Table 1. They show that both strains were similarly capable of adsorbing both double-stranded and single-stranded exogenous DNA. Denatured DNA, which has no transforming activity [1], was adsorbed on these strains just as well as native DNA. The different ability of these strains to undergo transformation was thus evidently entirely due to the different nuclease status of the recipients.

If penetration of DNA into the cytoplasm of the chosen strains differs just as little as adsorption, it must be expected that acid-soluble DNA would accumulate, as a result of the activity of intracellular nucleases, in strain AB 1157 (exoV$^+$) with an increase in the time of incubation of the cells with exogenous DNA at 37°C. It will be clear from Fig. 1 that this actually happened. The amount of acid-soluble products increased rapidly to reach a maximum after 5 min of incubation, when it was 40% of the total DNA absorbed on the spheroplasts. Accumulation of acid-soluble DNA also was observed in the case strain recBC$^-$ (IC 7623). In that case, however, accumulation of acid-soluble products took place more slowly. Whereas DNA degradation by intracellular nucleases began immediately after penetration, the process of DNA penetration ended very quickly – after 2-3 min. The amount of exogenous DNA which penetrated into the cell, as estimated by the method used, was evidently rather on the low side, for probably not all DNA entering the cell is hydrolyzed to acid-soluble fragments.

The results demonstrate that a large quantity of the DNA which remains bound to the spheroplasts penetrates into the cytoplasm. One of the main causes of the low transformability of E. coli is evidently the intensive degradation of exogenous DNA, even in recBC$^-$ strains.

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