The fact that PB loses its ability to induce microsomal enzymes on conjugation with albumin, but at the same time acquires ability to induce an immune response specific for PB, in the writers' view, reflects the ability of the two defensive systems of the body — hydroxylase and immune — to interact with foreign compounds of low and high molecular weight. The cytochrome P-450-hydroxylase system of the liver and other tissues is designed to protect the body against the action of hydrophobic low-molecular-weight compounds. The immune system is responsible for the protective effect against high-molecular-weight foreign substances.

LITERATURE CITED

1. A. I. Archakov, Microsomal Oxidation [in Russian], Moscow (1975), p. 245.
2. I. I. Karuzina and A. I. Archakov, in: Modern Methods in Biochemistry [in Russian], Moscow (1977), p. 49.
3. I. E. Kovalev, Khim.-Farmats. Zh., No. 12, 3 (1977).
4. I. E. Kovalev, É. F. Lavretskaya, S. E. Metkalova, et al., Khim.-Farmats. Zh., No. 6, 7 (1977).
5. I. E. Kovalev, O. Yu. Polevaya, N. P. Danilova, et al., Khim.-Farmats. Zh., No. 10, 7 (1976).
6. I. E. Kovalev, V. V. Shaidrov, O. Yu. Polevaya, et al., Farmakol. Toksikol., No. 3, 331 (1975).
7. S. Matsumara and T. Omura, J. Biochem. (Tokyo), 73, 407 (1973).
8. T. Omura and R. Sata, J. Biol. Chem., 239, 2370 (1964).
9. O. B. Zaalberg, Nature, 202, 1231 (1964).

ADSORPTION OF PHAGE λ DNA ON *Escherichia coli* CELLS TREATED WITH Ca^{++} IONS AND ON FROZEN AND THAWED BACTERIA

T. F. Moiseeva, S. Ya. Dityatkin, A. A. Kim, and B. N. Il'yashenko

UDC 576.858.9.098.396.332:576.851.48

Investigation of adsorption of biologically active tritiated phage λ DNA on *Escherichia coli* cells treated with Ca^{++} ions in the cold and on frozen-thawed bacteria revealed no correlation between the increase in adsorption and the efficiency of transfection. The level of adsorption of infectious DNA, for instance, was unchanged by freezing and thawing the *E. coli* cells, whereas after treatment with Ca^{++} ions in the cold it was increased tenfold; the level of transfection of phage λ DNA on both types of recipients was the same.

KEY WORDS: phage; adsorption; DNA; *Escherichia coli*.

Treatment of *Escherichia coli* cells with calcium cations in the cold, and also freezing and thawing the cells are known to form a state of competence in the recipient with respect to isolated phage, plasmid, and chromosomal DNA [1, 3-5]. However, the mechanism of induction of such competence is not yet clear. It has been suggested that as a result of the treatment mentioned above the permeability of the cell membranes is increased on account of structural changes arising therein. Such changes have been found by the fluorescent probe method in cells treated with Ca^{++} cations. Nevertheless it is not yet clear how these changes affect interaction between molecules of infectious DNA and recipient bacteria.

The object of this investigation was to study adsorption of isolated, biologically active phage λ DNA on *E. coli* cells treated with Ca^{++} cations in the cold and on frozen and thawed cells.

Laboratory of Molecular Microbiology, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 3, pp. 324-326, March, 1980. Original article submitted March 15, 1979.

TABLE 1. Adsorption of Phage λ DNA on *E. coli* HfrH Cells (in % of quantity added; M±m)

Treatment with DNase	Native bacteria	Bacteria + Ca^{++}	Frozen and thawed bacteria
Carried out	5,2±1,8	41,0±10,0	6,1±0,9
Not carried out	4,5±1,1	5,9±1,7	5,1±1,9

EXPERIMENTAL METHOD

DNA-^{3}H from bacteriophage λ C1857 was used for adsorption. Phage labeled by DNA-^{3}H was prepared by temperature-induction of a culture of *E. coli* CR-34 (C1857/B_1; obtained from Boldurh, USA), which is lysogenic for that phage, grown in synthetic medium with thymidine-^{3}H (5 μCi/ml) [7]. The DNA-^{3}H of phage λ was isolated by double phenolization followed by dialysis against 0.01 M tris-HCl buffer, pH 7.8. Strain *E. coli* HfrH (B_1, λ^-, S^s) was used as the recipient. The cells were treated with Ca^{++} ions by the method of Mandel and Higa [4], using 0.1 M CaCl solution. The bacteria and phage λ DNA were frozen concurrently in liquid nitrogen to −196°C according to a one-stage program, at a rate of freezing of 400°C/min. The samples were kept for 3 min at −196°C and then thawed at 42°C at a heating rate of 150-200°C/min. After adsorption for 10 min at 39°C the samples were centrifuged twice or treated with 20 μg/ml DNase (Worthington) for 10 min at 39°C to remove unadsorbed DNA. Aliquots of the residues and supernatant were taken for radiometry. Radioactivity of the samples was measured on a Beckman SL-250 (USA) liquid scintillation counter. Standard scintillation fluid (0.6% PPO and 0.01% POPOP in toluene) was used for counting radioactivity on the filters (pore size 0.45 μ).

EXPERIMENTAL RESULTS

The experiments showed that freezing and thawing the bacteria, forming a state of competence in the recipient, did not affect absorption of infectious molecules of phage λ DNA, which amounted to about 4-5% of the quantity of phage DNA added, just as with the untreated bacteria (Table 1). Penetration of DNA into this type of recipient is evidently connected, not with an increase in absorption of infectious DNA, but with temporary reparable injuries to the *E. coli* membrane during freezing and thawing.

In the case of adsorption of phage λ DNA on bacteria treated with Ca^{++} cations in the cold, changes in adsorption at different stages of treatment were found to obey the following rule. If the *E. coli* cells were suspended in 0.1 M $CaCl_2$ solution at room temperature, absorption of phage λ DNA on them was increased three-four-fold compared with the control (up to 15-16% of the quantity added). However, such treatment was insufficient for transfection. Preincubation of the recipient, suspended in 0.1 M $CaCl_2$ solution, for 30 min in an ice bath increased the adsorption of DNA to 40% of the added amount. Under these circumstances transfection could take place. Subsequent incubation of the recipient with DNA at 0°C did not change the level of adsorption achieved, although it caused a tenfold increase in the efficiency of transfection. Treatment of the recipient after adsorption of DNA with a solution of DNase reduced the adsorption to the control level, evidence of the reversible character of binding of the DNA molecule with the recipient. The increase in adsorption of DNA following treatment of the recipient with Ca^{++} ions was evidently due to the cationic character of the treatment, by means of which the negative charge on the phospholipids of the outer membrane, an electrostatic obstacle to penetration of DNA molecules, could be neutralized. However, the role of calcium ions in the induction of competence during transfection is connected with their possible action on the structure of the membrane — condensation of phospholipids and a shift in the temperature of phase transitions in the membrane [6].

It can be concluded from all the available experimental data that there is no correlation between the efficiency of transfection of *E. coli* with DNA from bacteriophage λ and the degree of absorption of infectious DNA molecules of the recipient. Adsorption of DNA on frozen and thawed bacteria in fact did not exceed the level of control cells not subjected to transfection. In the case of treatment of the recipient with calcium cations in the cold, however, the level of adsorption was increased by an order of magnitude compared with the control, and subsequent treatment of the cells with DNase solution reduced the adsorption to

the control level. The efficiencies of transfection of both the above types of recipients under these circumstances were the same. Consequently, the increase in total absorption of infectious DNA molecules was evidently not the decisive factor in the formation of competence for these types of recipients and cannot in general be used as a prognostic criterion of the possibility of realization of the subsequent stages of transfection.

LITERATURE CITED

1. S. Ya. Dityatkin and B. N. Il'yashenko, Genetika, No. 10, 115 (1974).
2. A. G. Sabel'nikov, T. F. Moiseeva, A. V. Avdeeva, et al., Biofizika, No. 4, 640 (1977).
3. S. D. Cosloy and M. Oishi, Proc. Natl. Acad. Sci. USA, 70, 84 (1973).
4. M. Mandel and A. Higa, J. Molec. Biol., 23, 159 (1970).
5. A. I. Verbley, B. D. Knyff, P. H. J. Ververgaert, et al., Biochim. Biophys. Acta, 339, 432 (1974).
6. W. Wackernagel, Biochem. Biophys. Res. Commun., 51, 306 (1973).
7. V. Zgaga, M. Medic, E. Sabaj-Smic, et al., J. Mol. Biol., 79, 697 (1973).

CHANGES IN PROLIFERATIVE ACTIVITY OF HEMATOPOIETIC STEM CELLS AFTER ADRENALECTOMY

G. I. Bezin and O. O. Romashko

UDC 612.119-06:612.451

In the early stages after adrenalectomy a decrease in the total cell population and in the number of colony-forming units (CFU) in the bone marrow is found in (CBA×C57BL)F_1 mice and is accompanied by a significant increase in the number of proliferating stem cells in the bone marrow and spleen, determined by the "thymidine suicide" method. After normalization of the total number of CFU, followed by the total cell population of the bone marrow, the level of dividing stem cells returns to its initial value.

KEY WORDS: adrenalectomy; hematopoietic stem cells; proliferation; migration; bone marrow; spleen.

The level of endogenous glucocorticoids is an important component of the system controlling migration and recirculation of hematopoietic stem cells (colony-forming units, CFU_S) at the whole-body level [2]. Experiments on mice have shown a sharp decrease in the number of CFU_S in the bone marrow 24 h after adrenalectomy, with a return to their normal number by the end of the first week after the operation, whereas increased liberation of stem cells into the circulating blood and spleen was observed throughout this period [5]. Such rapid recovery of the numerical composition of CFU_S in the bone marrow could have been the result of stimulation of their proliferation [3]. The object of the present investigation was to test this hypothesis experimentally.

EXPERIMENTAL METHOD

At various times after bilateral adrenalectomy on (CBA×C57BL)F_1 mice the number of CFU_S in the bone marrow and spleen and their total cell population were determined by the method of exogenous colony formation. At the same time the proportion of proliferating CFU_S was determined as in [6]: Suspensions of hematopoietic cells were incubated at 37°C for 30 min and the specific activity of thymidine-^{3}H added was 200 μCi/ml. The results of the investigation were assessed by Student's t-test.

EXPERIMENTAL RESULTS

The number of CFU_S in the bone marrow 24 h after adrenalectomy was reduced almost by half

Institute of Biophysics, Ministry of Public Health of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 89, No. 3, pp. 326-327, March, 1980. Original article submitted March 6, 1979.

0007-4888/80/8903-0329$07.50