During prolonged incubation of the cells (up to 12 h or more) a decrease in the sedimentation rate of DNA was observed after removal of the thiophosphamide. This may be evidence that mouse cells can repair DNA injuries induced by thiophosphamide. Restoration of the molecular mass of DNA took place more rapidly in cells of the CBA mice than in those of the 101/H mice. Maximal differences in the degree of repair of DNA injuries between these lines were found 12 h after removal of the thiophosphamide (Table 1, Figs. 1 and 2). At that time the molecular mass of DNA in the CBA cells was close to the control, whereas in the 101/H cells it was twice as high as the control. The molecular mass of DNA of fibroblasts of the CBA mice after 24 h had virtually reached the control value ($P > 0.05$), but in the 101/H mice the values of this parameter differed significantly in the experiment and control ($P < 0.01$). Repair of DNA cross-linkages in cells of 101/H mice thus takes place much less efficiently than in cells of CBA mice.

The data are evidence that DNA injuries induced by thiophosphamide appear and increase in the course of time. The sites of injury are evidently DNA cross-linkages between strands. Mouse cells also were found to be capable of repairing DNA injuries induced by thiophosphamide, and differences were found in the rate of repair of DNA cross-linkages between 101/H and CBA mice. These results, together with evidence of the relatively high sensitivity of mice of 101/H line to the mutagenic action of thiophosphamide, point to the presence of effective repair of thiophosphamide-induced DNA injuries in the cells of this line of mice.

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ABILITY OF CONJUGATIVE PLASMIDS OF SEROLOGICALLY TYPED *E. coli* TO INHIBIT GENETIC TRANSFER FUNCTIONS OF DEREPRESSED Flac AND pAP22-4 PLASMIDS

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In the classification of the various plasmids detectable in natural bacterial populations, determination of their ability to inhibit genetic transfer function (Tra function) of the F plasmid of *E. coli* K-12 is widely used [10, 11]. This property (the Fin^+ character) is probably controlled by different genetic systems of plasmids, which determine the synthesis of specific protein inhibitors, depressing the functions of transfer genes (tra genes) of the F plasmid [8].

To assess the functional properties of these systems, the inhibitory properties of a group of plasmids identified previously [1, 4-6] by the writer in cells of serologically typed strains of *E. coli* isolated from man and from livestock were investigated with respect to genetic transfer functions of the Flac plasmid (a substituted variant of the F plasmid), and also of the F-like plasmid pAP22-4, derepressed for these functions and discovered by the writer in cells of strain AP1 [2], and marked by incorporation of the transposon Tn1 in its structure [5].

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TABLE 1. Characteristics of Clone Strains of *E. coli* AP132 Containing the Test Conjugative Plasmids

Plasmid	Genetic markers (plasmid)	Sensitivity to phage MS2		PTRI MS2	Frequency of transmission of plasmid to cells of strain C600
		agar layers method	PTRT		
R1	Ap, Km, Lm, Sm, Su	—	+	$0{,}5\cdot10^{1}$—$2{,}5\cdot10^{1}$	$3{,}9\cdot10^{-4}$—$4{,}9\cdot10^{-4}$
pAP17-1	Hly	—	—	0,4—1,0	$1{,}3\cdot10^{-3}$—$4{,}6\cdot10^{-3}$
pAP20	Hly	—	+	1,0 10^{4}—$6{,}1\cdot10^{4}$	$1{,}0\cdot10^{-2}$—$2{,}5\cdot10^{-2}$
pAP22-1::Tn1	Ap	—	+	$0{,}4\cdot10^{5}$—$5{,}3\cdot10^{5}$	$0{,}2\cdot10^{-1}$—$6{,}1\cdot10^{-1}$
pAP22-4::Tn1	Ap	+	+	$0{,}5\cdot10^{8}$—$3{,}4\cdot10^{8}$	1,9—2,9
pAP27	Ap, Lm, Sm, Tc, Su	—	+	$0{,}6\cdot10^{6}$—$4{,}7\cdot10^{6}$	0,8—2,5
pAP38::Tn9	Lm	—	+	$0{,}2\cdot10^{6}$—$1{,}4\cdot10^{6}$	$1{,}3\cdot10^{-1}$—$3{,}6\cdot10^{-1}$
pAP41::Tn9	Lm	—	+	$0{,}6\cdot10^{5}$—$3{,}4\cdot10^{5}$	$1{,}2\cdot10^{-2}$—$1{,}4\cdot10^{-2}$

Legend. In each case no fewer than three clones of transconjugants obtained by transfer of the corresponding plasmid into AP132 cells were tested. Values of PTRI for cells of plasmid-free strain AP132 were 0.3-0.9. Positive or negative results of determination of sensitivity of the bacteria to phage are indicated by + or — signs respectively.

TABLE 2. Ability of Test Plasmids to Inhibit Conjugation Transfer of Standard (derepressed) Flac and pAP22-4::Tn1 Plasmids from Cells of Strain AP132, and also Synthesis of Specific Sex Fimbriae Controlled by Them

Plasmid introduced (tested)	Resident (standard) plasmid	Frequency of formation of double plasmid transconjugant in AP132 cells	Results of analysis of clones of plasmid transconjugants of AP132 cells			
			frequency of transfer of standard plasmid into cells of strain C600	PTRI MS2	index of reduction in transfer of standard plasmid	index of reduction in PTRI
—	Flac	—	0,9—2,2	$0{,}3\cdot10^{8}$—$2{,}5\cdot10^{8}$	—	—
R1	Flac	$1{,}2\cdot10^{-4}$	$5{,}9\cdot10^{-3}$—$9{,}0\cdot10^{-3}$	$6{,}7\cdot10^{4}$—$7{,}8\cdot10^{4}$	$2{,}2\cdot10^{2}$—$3{,}4\cdot10^{2}$	$5{,}5\cdot10^{2}$—$6{,}4\cdot10^{2}$
pAP17-1	Flac	$1{,}2\cdot10^{-2}$	$0{,}6\cdot10^{-5}$—$3{,}1\cdot10^{-5}$	$0{,}6\cdot10^{2}$—$1{,}1\cdot10^{2}$	$0{,}8\cdot10^{4}$—$3{,}7\cdot10^{4}$	$3{,}2\cdot10^{5}$—$5{,}5\cdot10^{5}$
pAP20	Flac	$1{,}7\cdot10^{-2}$	$1{,}6\cdot10^{-1}$—$2{,}1\cdot10^{-1}$	$0{,}6\cdot10^{6}$—$2{,}3\cdot10^{6}$	4,8—6,3	$1{,}6\cdot10^{1}$—$5{,}8\cdot10^{1}$
pAP22-1::Tn1	Flac	$1{,}5\cdot10^{-1}$	0,9—1,2	$1{,}4\cdot10^{7}$—$4{,}2\cdot10^{7}$	1,0—1,2	0,9—1,1
pAP22-4::Tn1	Flac	$4{,}3\cdot10^{-1}$	1,2—1,3	$1{,}0\cdot10^{7}$—$1{,}5\cdot10^{7}$	0,8—0,9	2,5—3,7
pAP27	Flac	$1{,}2\cdot10^{-1}$	0,7—1,2	$0{,}5\cdot10^{8}$—$0{,}7\cdot10^{8}$	1,0—1,7	0,5—1,1
pAP38::Tn9	Flac	$1{,}2\cdot10^{-2}$	$2{,}5\cdot10^{-3}$—$3{,}5\cdot10^{-3}$	$1{,}5\cdot10^{4}$—$3{,}4\cdot10^{4}$	$3{,}3\cdot10^{2}$—$5{,}2\cdot10^{2}$	$0{,}7\cdot10^{4}$—$1{,}7\cdot10^{4}$
pAP41::Tn9	Flac	$1{,}6\cdot10^{-2}$	$2{,}0\cdot10^{-2}$—$2{,}0\cdot10^{-3}$	$2{,}4\cdot10^{4}$—$2{,}8\cdot10^{4}$	$0{,}5\cdot10^{2}$—$5{,}0\cdot10^{2}$	$0{,}9\cdot10^{4}$—$1{,}0\cdot10^{4}$
—	pAP22-4::Tn1	—	1,9—2,9	$0{,}5\cdot10^{8}$—$3{,}4\cdot10^{8}$	—	—
R1	pAP22-4::Tn1	$1{,}3\cdot10^{-4}$	$1{,}8\cdot10^{-1}$—$3{,}9\cdot10^{-1}$	$2{,}0\cdot10^{6}$—$5{,}3\cdot10^{6}$	$0{,}7\cdot10^{1}$—$1{,}2\cdot10^{1}$	$0{,}9\cdot10^{1}$—$2{,}3\cdot10^{1}$
pAP17-1	pAP22-4::Tn1	$0{,}7\cdot10^{-2}$	$4{,}5\cdot10^{-4}$—$5{,}4\cdot10^{-4}$	$1{,}2\cdot10^{4}$—$1{,}4\cdot10^{4}$	$3{,}5\cdot10^{3}$—$4{,}2\cdot10^{3}$	$2{,}2\cdot10^{4}$—$2{,}4\cdot10^{4}$
pAP38::Tn9	pAP22-4::Tn1	$2{,}9\cdot10^{-1}$	$4{,}2\cdot10^{-2}$—$9{,}1\cdot10^{-2}$	$3{,}8\cdot10^{5}$—$6{,}1\cdot10^{5}$	$3{,}4\cdot10^{1}$—$7{,}4\cdot10^{1}$	$4{,}1\cdot10^{2}$—$6{,}6\cdot10^{2}$
pAP41:Tn9	pAP22-4::Tn1	1,8	$0{,}7\cdot10^{-1}$—$1{,}2\cdot10^{-1}$	$3{,}0\cdot10^{5}$—$3{,}7\cdot10^{5}$	$1{,}6\cdot10^{1}$—$2{,}6\cdot10^{1}$	$6{,}7\cdot10^{2}$—$8{,}3\cdot10^{2}$

Legend. In each case three clones of transconjugants containing two plasmids simultaneously — test and standard — were investigated.

EXPERIMENTAL METHOD

To determine plasmid genetic markers of resistance to ampicillin (Ap), kanamycin (Km), levomycetin (Lm), streptomycin (Sm), and tetracycline (Tc) cells of strains of *E. coli* K-12 containing plasmids were seeded on dishes with nutrient agar containing the appropriate antibiotic in a concentration of 25 μg/ml medium. Markers of resistance to sulfonamides (Su) were detected on agar with sulfathiazole (1 mg/ml). Hemolytic activity of the bacteria (Hly markers) were detected on agar containing 3% washed human erythrocytes.

Conjugation transmission of the plasmids was carried out in standard 2-h crosses [3], using strains of *E. coli* K-12 with chromosomal genes of resistance to streptomycin (AP106, C600) or to nalidixic acid (AP132), as donors or recipients. The frequency of transmission of plasmid markers was determined as the number of corresponding transconjugants, counted per cell of the donor strain of the conjugation mixture.

The sensitivity of bacteria to the donor-specific F-group phase (MS2) was studied by the agar layers method [9], and also by tests to determine the rise in titers of this stage (PTRT) in accordance with the scheme evolved previously [7]. In each case of determination

of PTRT, the phage titer rise index (PTRI) also was calculated as the ratio between the mean number of phage lysis zones found in the experimental feedings (incubation of the phage-bacterial mixture for 18 h) and their number in the control (before the beginning of incubation of the mixture).

EXPERIMENTAL RESULTS

Seven identified conjugative plasmids listed in Table 1, and also the Fin^+ plasmid R1 (the original strain containing this plasmid was obtained from Dr. Datta, England), were investigated.

As Table 1 shows, all the above-mentioned plasmids (except pAP17-1) determine synthesis of specific sex fimbriae (F-fimbriae) of bacterial cells which are receptors for adsorption of donor-specific phage MS2 [10], as is shown by positive PTRT. Meanwhile individual plasmids were characterized by considerable differences in the values of PTRI and also in the frequencies of their conjugation transfer into cells of recipient strain C600. For example, unlike most plasmids, which are characterized by some degree of repression of functions of the tra genes, two plasmids (pAP22-4::Tn1 and pAP27) behaved as derepressed for transfer functions into C600 cells; the first plasmid, moreover, was also derepressed for synthesis of specific sex fimbriae (Table 1).

To continue the study of the ability of the test plasmids to inhibit the Tra function of standard derepressed Flac plasmid each of them was transferred (by conjugation of the corresponding bacteria) into cells of strain AP132, containing the Flac plasmid (Table 2). Clones of double plasmid transconjugants thereby obtained, i.e., cells containing the test and standard plasmids simultaneously, were then studied in order to determine the frequency of transfer of the Flac plasmid into C600 cells and the level of synthesis of F fimbriae controlled by it, as reflected in the value of PTRI. Strain AP132, containing only the Flac plasmid, was used in each experiment as the control. For quantitative evaluation of the results obtained in each case the index of decrease in transmission of the standard plasmid and the index of decrease in PTRI were determined: These indices are the quotients obtained by dividing the frequency of transfer of this plasmid or the value of PTRI (respectively) for cells of the control strain AP132, containing Flac plasmid only, by the corresponding values obtained by testing double plasmid transconjugants.

It can be concluded from analysis of the parameters given in Table 2 that the Hly plasmid pAP17-1 was left the strongest inhibitory activity with respect to functions of the tra gene of the Flac plasmid. Rather weaker activity was observed in the case of plasmids R1, pAP38::Tn9, and pAP41::Tn9, whereas plasmid pAP20 had minimal inhibitory action. So far as plasmids pAP22-1::Tn1, pAP22-4::Tn1, and pAP27 are concerned, they were unable to inhibit the Flac plasmid, i.e., they had the Fin^- phenotype.

The results of similar experiments, in which plasmid pAP22-4::Tn1 was used as the standard, showed that it can be inhibited by various Fin^+ plasmids repressing the Tra function of Flac plasmid (Table 2). Consequently, plasmid pAP22-4::Tn1 is controlled by mechanisms of the genetic regulatory system of the tra operon, designated as the fin OP system [8]. Meanwhile the lower level of inhibition of this plasmid (compared with Flac) is probably a reflection on the specific features of function of its regulator fin P gene or tra genes. Allowing for its specificity, plasmid pAP22-4::Tn1 can be used in the classification system of new plasmids identified in natural populations of bacteria.

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