Dependence of virus-induced membrane fusion on membrane potential revealed by these experiments confirms the writers' previous hypothesis [2] that electrical breakdown of the cell membrane plays an important role in the initiation of membrane fusion.

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TRANSPOSON CONTENT OF NONCONJUGATIVE PLASMIDS OF *Escherichia coli*

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Cells of strains of *Escherichia coli* isolated from different natural sources may contain both conjugative and nonconjugative plasmids, and often in the form of complexes consisting of more than one plasmid [2]. Meanwhile, if several different plasmids are present in the same bacterial cell, the question of their transposon content arises, i.e., whether all plasmids in the complex contain the same transposon.

To answer this question we made a search for nonconjugative plasmids in antibiotic-resistant natural strains of *E. coli* and then studied the transposon content of these plasmids, contained in complexes.

EXPERIMENTAL METHOD

Strains C-6, C-8, C-19, and C-25 of *E. coli*, isolated from different natural sources and possessing resistance to different antibiotics and to trimethoprim, and also standard strains *E. coli* AP115 Lac$^-$, resistant to nalidixic acid, and *E. coli* C600 Lac$^-$, resistant to streptomycin and rifampicin, were used. The ability of nonconjugative plasmids to be mobilized for transfer to conjugative plasmids was studied in "three-parent" crosses [1]. The search for transposons in the plasmids was undertaken by a method in which bacterial cells containing one of the nonconjugative plasmids were treated with sodium dodecylsulfate in a concentration of 5% for 18 h, after which they were seeded on media with the addition of antibiotics, and clones characterized by monoresistance were then selected. Cells of these clones were used as intermediate recipients in the "three-parent" crosses, in which the donors were cells containing conjugative plasmid pAP42 or pAP43, and the final recipients were *E. coli* C600 Rif^r Lac$^-$ cells. In similar experiments cells with nonconjugative plasmids were introduced into "three-parent" crosses without preliminary treatment of the cultures with sodium dodecylsulfate. The sensitivity of the bacteria to phage MS2 was determined by the agar layers method and treatment with ethidium bromide was carried out by the usual method [3]. DNA was isolated from plasmids pAP42 and pAP42-1 by the method in [10] with some modifications. Plasmid DNAs were restricted by endonucleases Eco RI and Hind III. The restriction fragments were frac-

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TABLE 1. Drug Resistance Determined by Nonconjugative Plasmids

Original strain	Resistance markers of original strain	Selective marker	Nonselective markers	Resistance markers of nonconjugative plasmids
C-6	Ap Sm Tp	Ap	Sm Tp	pMS6 (Ap Sm Tp)
		Sm	Ap Tp	
		Tp	Ap Sm	
C-8	Ap Sm Lm Tc Tp	Ap	Lm Sm Tc Tp	pMS8 (Ap Lm Sm Tc Tp)
		Lm	Ap Sm Tc Tp	
		Sm	Ap Lm Tc Tp	
		Tc	Ap Lm Sm Tp	
		Tp	Ap Lm Sm Tc	
C-19	Ap Lm Tp	Ap	Tp; Lm Tp	pMS19-1 (Ap Tp)
		Lm	Ap Tp	pMC19-2 (Ap Lm Tp)
		Tp	Ap; Lm Ap	
C-25	Ap Lm Sm Tc Tp	Ap	Lm Sm Tc Tp; Lm Sm Tc	pMS25-1 (Ap Lm Sm Tc Tp)
		Lm	Ap Sm Tc Tp; Ap Sm Tc	pMS25-2 (Sm Tp)
		Sm	Ap Lm Tc Tp; Ap Lm Tc; Tp	pMS25-3 (Ap Lm Sm Tc)
		Tc	Ap Lm Sm Tp; Ap Lm Sm	
		Tp	Ap Lm Sm Tc; Sm	

TABLE 2. Ability of Nonconjugative Plasmids to be Mobilized for Transfer by Conjugative Plasmids

Nonconjugative (mobilized) plasmid	Markers of nonconjugative plasmid	Conjugative (mobilizing) plasmid	Transconjugants (combinations of markers of nonconjugative plasmid)
pMS6	Ap Sm Tp	pAP42	I — Ap Sm
		pAP43	II — Sm Tp
			III — Ap Sm Tp
pMS8	Ap Lm Tp Sm Tc	pAP38::Tn9	I — Ap Lm Sm Tc Tp
		pAP42	II — Ap Lm Sm Tc
		pAP43	
pMS19-1	Ap Tp	pAP38:Tn9	I — Ap Tp
		pAP42	II — Ap
		pAP43	
pMS19-2	Ap Tp Lm	pAP42	I — Ap Lm Tp
		pAP43	II — Ap Lm
pMS25-1	Ap Lm Sm Tc Tp	pAP38::Tn1	I — Ap Tc Lm Sm Tp
			II — Ap Lm Sm Tc
pMS25-2	Sm Tp	pAP38::Tn1	I — Sm Tp
		pAP38::Tn9	II — Sm
		pAP42	
		pAP43	
pMS25-3	Ap Lm Sm Tc	pAP38::Tn1	I — Ap Tc Lm Sm
		pAP38::Tn9	
		pAP42	
		pAP43	

TABLE 3. Molecular Weights of Restriction Fragments of Plasmids pAP42 and pAP42-1

Plasmids	Enzymes	mol. wt. (megadaltons) of restriction fragments													Total
		f1	f2	f3	f4	f5	f6	f7	f8	f9	f10	f11	f12	f13	f14	
pAP42	EcoR I	15,7	6,7	6,0	5,1	5,0	4,4	3,8	3,3	2,5						52,5
pAP42-1	EcoR I	15,7	6,7	5,0	3,3	2,3	2,3									35,3
pAP42	Hind III	11,4	5,6	5,5	4,7	3,5	3,3	3,2	2,9	2,8	2,3	2,0	2,0	1,6	1,3	52,1
pAP42-1	Hind III	11,1	5,6	3,5	3,5	2,8	2,3	2,3	2,3	1,6	1,0					36,0

tionated in 0.65% agarose gel by horizontal slab electrophoresis. The dimensions of the restriction fragments of the plasmid DNase were determined by the use of restriction fragments of DNA from phage λ as molecular weight standards [12].

EXPERIMENTAL RESULTS

The reason why a search for conjugative plasmids was made in cells of *E. coli* C-6, C-8, C-19, and C-25 was that these cells exhibited antibiotic resistance, but they did not transfer this property during crosses to the recipient cells.

To discover whether cells of these strains contained nonconjugative plasmids, and if so, how many of them were present per cell, they were mobilized for transfer of the genetic transfer factor F'lac in "three-parent" crosses, in which the final recipients were C600 Rif^r cells. Transconjugants resistant to different antibiotics were obtained from all these crosses. After testing the transconjugants for the presence of nonselective markers it was found that cells of strains C-6 and C-8 each had one set of determinants of drug resistance mobilized by the trans-

fer factor, whereas cells of strains C-19 and C-25 had more than one set of mobilized resistance determinants (Table 1).

On the assumption that the identified genetic determinants are nonconjugative plasmids, they were designated by the symbols pMS6, pMS8, pMS19-1, pMS19-2, pMS25-1, pMS25-2 and pMS25-3. To confirm the data on the existence of several plasmids in one bacterial cell, in later experiments the F'lac plasmid was removed from the transconjugants with the aid of ethidium bromide (300 μg/ml) and the nonconjugative plasmids contained in them were mobilized in "three-parent" crosses for transfer by genetic transfer factors pAP38::Tn1, pAP38:: Tn9, pAP42, and pAP43 from cells of strain C600 Str^r into recipient cells *E. coli* AP115 Nal^r.

The results of the "three-parent" crosses showed (Table 2) that the nonconjugative plasmids studied could be mobilized for transfer by various transfer factors in low frequency (from $2 \cdot 10^{-6}$ to 10^{-5}). Since mobilization is known to be controlled [4-6] by the genes of the mobilized nonconjugative plasmids, but to depend also on the mobilizing plasmids [1], differences found in the ability of nonconjugative plasmids pMS19-1, pMS19-2, pMS25-1, pMS25-2, and pMS25-3 to be mobilized confirmed the simultaneous existence of two nonconjugative plasmids in the C-19 cells and three in C-25.

Transposons are often responsible for resistance of bacterial cells to the action of trimethoprim [7, 9, 11]. Since all nonconjugative plasmids except plasmid pMS25-3 contain determinants of resistance to trimethoprim, this suggested that a "trimethoprim" transposon is present in the genomes of these plasmids. To test this hypothesis, experiments were carried out to identify transposons in nonconjugative plasmids, for which purpose they were transferred into *E. coli* AP115 Nal^r cells, then treated with sodium dodecylsulfate. As a result of these experiments separate clones were obtained of strains AP115 (pMS-6) and AP115 (pMS19-1) of *E. coli*, resistant to the action of trimethoprim alone. This indicated that plasmids pMS6 and pMS19-1 contain transposons determining resistance to trimethoprim, and transferred after treatment with the eliminating agent into the chromosome of the host cell. These transposons were designated Yn6-1 and Tn19-1. In "three-parent" crosses they were transferred into the genomes of plasmids pAP42 and pAP43. As regards plasmids pMS19-2, pMS25-1, and pMS25-2, no clones of cells resistant to trimethoprim alone could be found after their treatment with the eliminating factor. However, this does not mean that they do not contain "trimethoprim" transposons in their genomes. On the contrary, the fact that they also contain transposons is confirmed by data on the structure of transconjugants isolated as a result of nonconjugative plasmids by different transfer factors (see Table 2). These results are thus evidence that nonconjugative plasmids found in C-19 cells resistant to trimethoprim are carriers of a "trimethoprim" transposon. An "ampicillin" transposon Tn6-2, contained in plasmid pMS6, was identified in other experiments.

Since introduction of plasmids containing transposons into cells may be followed by translocation of the latter into the chromosomes [8], we crossed C600 Rif^r pAP42::Tn19-1, C600 Rif^r pAP42::Tn6-1 and C600 Rif^r pAP42::Tn6-2 cells with AP115 recipient cells. The isolated transconjugants inherited only sensitivity to phage MS2, but not resistance to ampicillin or trimethoprim. This suggested that the transconjugants contain plasmids without transposons.

The results of comparative Eco RI- and Hind III-restriction analysis of DNA of plasmid pAP42 and plasmid pAP42-1, which contained and subsequently lost transposon Tn6-2, are illustrated in Table 3. As Table 3 shows, inclusion of transposon Tn6-2 and its exclusion from the genome of plasmid pAP42 are accompanied by multiple deletions of fragments. As regards the other nonconjugative plasmids, resistance to ampicillin determined by them is not transposon-, but plasmid-induced.

The general conclusions can be drawn from these observations that plasmids in a complex can all contain the same transposon (determining identical resistance).

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PROTEIN FERTILITY FACTOR AND CONGENITAL DEVELOPMENTAL DEFECTS

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No general agreement yet exists on a name for protein fertility factor [7]. An organ-specific placental α_2-globulin was first identified in 1976 in extracts of human placenta [1]. Later this globulin was discovered in organs of the reproductive system of both women and men [3, 5, 6] and its biosynthesis was shown to depend on hormonal contraceptive preparations [4]. On comparative immunodiffusion analysis the placental α_2-globulin was found to be similar to PP-14 [9], α-uterine protein [12], and progestin-endometrial protein [8]. The molecular weight of all these proteins lies within the range 25-50 kD, since the protein can exist in two forms or can form complexes with other serum or placental proteins [2, 5-8, 10, 12]. Since this α_2-globulin is synthesized not only by decidual cells and cells of the uterine mucosa in the secretory phase of the menstrual cycle, but also in the seminal vesicles of men [5, 11], we decided to propose a new name for this protein: "protein fertility factor" (PFF).

This paper gives the results of a semiquantitative analysis of PFF in human seminal fluid in order to reveal the connection between the PFF level in the sperm and birth of children with congenital developmental defects.

EXPERIMENTAL METHOD

Antiserum against PFF was obtained by immunization of rabbits with semipurified preparations of PFF isolated from extracts of abortion material or from amniotic fluid [1, 6]. Amniotic fluid, diluted to obtain the clearest precipitation line, was used as the standard antigen for immunodiffusion analysis. The antisera were additionally exhausted with dry plasma and with extracts of normal human organs. The sensitivity of the test system was about 2 mg/liter. The results of assay of PFF in the groups compared were subjected to statistical analysis by Student's test. Clinical observations were made on couples attending the Out-Patient Department in Ivanovo for genetic counseling. The men collected the sperm after coitus interruptus or by masturbation and sent it to the laboratory in glass bottles. The bottles were kept in the freezing compartment of domestic refrigerators and kept there until required for analysis. In all cases the karyotype of the husband and wife was determined, and no abnormality was found. Altogether 35 married couples with viable offspring (without any gross morphological developmental defects) and 18 couples who had given birth to children with various severe developmental defects, incompatible with life, were investigated.

EXPERIMENTAL RESULTS

The content of PFF in sperm obtained from men with normal children varied within quite wide limits — from 16 to 256 mg/liter (mean 45.9 mg/liter). Normally the PFF concentration in the sperm varied as a rule from 16 to 64 mg/liter. However, in 12 of the 18 men whose

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