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EFFECT OF DRUG-RESISTANCE FACTOR R ON THE F PROPERTIES OF *ESCHERICHIA COLI*

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ABSTRACT

HIROTA, YUKINORI (Osaka University, Osaka, Japan), YUKINOBU NISHIMURA, FRITS ØRSKOV, AND IDA ØRSKOV. Effect of drug-resistance factor R on the F properties of *Escherichia coli*. J. Bacteriol. **87**:341-351. 1964.—Infection of *Escherichia coli* male cells (Hfr or F^+) with resistance factor R results in the co-ordinate inhibition of several distinct functions of F factor: mating capacity to transfer chromosome by conjugation, production of f^+ antigen, and formation of receptors for the male-specific bacteriophages, f1 and ribonucleic acid phage. The i^- mutant (R_{100-1}) of R factor, which was isolated from wild-type R factor (R_{100}), shows no inhibition of these F properties. Male R^+_{100-1} cells were autoagglutinable but the f^+ antigen was still present. When R-infected female cells had acquired the ability to form recombinants with an F^- strain, they also had become autoagglutinable. The question of the presence of f^+ antigen in these strains was not solved. The cause of the autoagglutinability is not known, but it is not the result of loss of O antigen (rough autoagglutinability). Sensitivity to a phage *tau*, which can form plaques on female cells only, is not affected by the presence or absence of R factor. No difference in the pattern of segregation of recombinants was observed between the cross of Hfr $R^- \times F^-$ and that of Hfr $R^+ \times F^-$. These results indicate that R factor controls a key mechanism in the synthesis of "F substances" formed on the cell surface by the F factor.

The sexuality of *Escherichia coli* K-12 is determined by the presence or absence of a fertility factor, F. Male bacteria (F^+) transfer their chromosome to the female (F^-) by a conjugation process (Lederberg, Cavalli, and Lederberg, 1952; Hayes, 1953). There are differences between the surfaces of male and female cells. Male strains produce the f^+ antigen (Ørskov and Ørskov, 1960), they have a specific substance called male substance which is possibly a polysaccharide (Sneath and Lederberg, 1961), and they also carry receptors for certain bacteriophages called f1 and f2 (Loeb, 1960; Loeb and Zinder, 1961). Furthermore, male cells are not lysed by a bacteriophage called *tau* which can form plaques on female bacteria (Hakura and Hirota, 1961).

It has been reported that the drug resistance factor R, which is known to be a contagious agent responsible for resistance to several antibiotics in Enterobacteriaceae (Ochiai et al., 1959; Akiba et al., 1960; Harada et al., 1960) also confers sex-compatibility to *E. coli* K-12 female strains (*R mating*) just as the F factor does (Sugino and Hirota, 1961).

However, if the analogous factors F and R are introduced together into a bacterial cell, they show several antagonistic activities. In male bacteria of *E. coli* K-12 (F^+, F'^+, or Hfr) which have acquired an R factor, the frequency of recombinations mediated by F (*F mating*) in a cross with female bacteria is reduced (Nakaya, Nakamura, and Murata, 1960; Yoshikawa and Akiba, 1961; Watanabe and Fukasawa, 1961), and adsorption and lysis by the phage f1 or f2 specific for male strains of *E. coli* K-12 are inhibited by the presence of R (Egawa and Hirota, 1962; Watanabe, Fukasawa, and Takano, 1962).

Mutants of the R factor which have lost the inhibitory effect on fertility were found recently (Egawa and Hirota, 1962).

The present report is concerned with the immunological and genetic analysis of the effect of R on the mating system of *E. coli* strains with O antigen 100 (Ørskov and Ørskov, 1960). This strain is preferable for use in analysis of the changes in surface antigens caused by the introduction of F or R factors. Diverse forms of R have been reported by many investigators. For designation of R factors, the terminology used by Sugino and Hirota (1962) and Egawa and Hirota (1962) was adopted in the present study. Immunological terminology, such as f^+ antigen and notation of genotypes of bacteria, are in accordance with the reports of Ørskov and Ørskov (1960, 1961), and

TABLE 1. *Bacterial strains used*

Strain	F state	R state[a]	Genotype	Serotype
K12				O⁻:K?:H48
JE 51	F^-	R^+_{100} (i^+; SM, CM, TC, SA)	Lac^-_{11D3}, Mal_5^-, S^r	
JE170	F^-	R^+_{100-1} (i^-, SM, CM, TC, SA)	Lac^-_{11D3}, Mal_5^-, S^r	
W4573	F^-	R^-	Lac^-_{85}, Gal_2^-, Ara_2^-, Xyl_2^-, Mal_1^-, Mtl^-, S^r	
W3132	F^-	R^-	M^-	
W1895	Hfr_1	R^-	M^-	
JE346	F^-	R^-	Lac^-_{85}, Gal_2^-, Ara_2^-, Xyl_2^-, Mal_1^-, Mtl^-, S^r, Try^-, Pur_1^-[b]	
H509a				O100:K(B):H2
D169	$F^+(f^+)^+$	R^-	His^-, $Isol^-$	
W3478	$F^-(f^+)^-$	R^-	His^-, $Isol^-$, Mal^-[c]	
W3468	$F^-(f^+)^-$	R^-	Try^-, Leu^-	
JG 18	$Hfr\left[\begin{matrix}\text{Try, Leu}\\ \text{Lac, Gal}\end{matrix}\right]$[d]	R^-	His^-, $Isol^-$	
JG 19	$Hfr\left[\begin{matrix}\text{Try, Leu}\\ \text{Xyl, Mal}\end{matrix}\right]$	R^-	His^-, $Isol^-$	

[a] Symbols: i = a genetic trait of the R factor concerning the inhibition of *F mating*; + or − indicates the inhibition or noninhibition of *F mating*, respectively. SM, CM, TC, or SA indicate the resistance to streptomycin (20 μg/ml), chloramphenicol (25 μg/ml), tetracycline (25 μg/ml), or sulfanilamide (200 μ, g/ml), respectively.

[b] Pur_1^- means the requirement of adenine and thiamine.

[c] W3478 was originally Mal^+. The Mal^- mutant was accidentally subcultured from a stab culture.

[d] Markers in brackets denote the genes belonging to the high fertility region of the Hfr chromosome.

for notation of the other markers Lederberg's designations are used (Lederberg et al., 1951).

MATERIALS AND METHODS

Bacterial and phage strains. E. coli K-12 carrying R was employed as source of R factors. Use was made of R_{100} which inhibits *F mating*, and a mutant of R derived from R_{100}, R_{100-1}, which does not inhibit *F mating* (Egawa and Hirota, 1962).

Experiments were performed with derivatives of the test strain of *E. coli* O antigen 100:H509a. These are preferable for use in immunological studies, and are fertile in crosses either with each other or with K-12.

The strains used in this experiment are summarized in Table 1. Phage f1, originating from N. D. Zinder, The Rockefeller Institute, New York, N.Y., and ribonucleic acid (RNA) phage, MS2 (Davis et al., 1961; Strauss and Sinsheimer, 1963) from E. T. Bolton, Carnegie Institution of Washington, Washington, D.C., were used for testing sensitivity to bacteriophages specific for male bacteria. Both phage lysates were prepared by the agar-layer method (Adams, 1950) with the medium described by Loeb and Zinder (1961).

Media. Brain Heart Infusion (BHI) broth (Difco) or beef broth was used for the liquid media, BHI broth or ox heart infusion plus 1.6% agar was used for the plates. Scoring of drug-resistant colonies was carried out on Eosin Methylene Blue (EMB) sugar agar (Lederberg, 1950) or modified Drigalski plates plus bromothymol blue (BTB) as indicator (Kauffmann, 1954), with the addition of one of the drugs, such as 20 μg/ml of streptomycin (SM), 25 μg/ml of chloramphenicol (CM), 25 μg/ml of tetracycline (TC), or 200 μg/ml of sulfanilamide (SA).

Infection with R factor, R mating, F mating, preparation of antisera, antigen-antibody reactions, and absorption of sera. The procedures reported previously were employed in the present study (Sugino and Hirota, 1962; Kauffmann, 1954;

Ørskov and Ørskov, 1960). Additional information is given in footnotes to the tables.

RESULTS

Effect of R infection of H509a on F mating and sensitivity to the f1 and RNA phages. Five sublines of the strain H509a, i.e., D169F$^+$, two F$^-$ derivatives, W3478 and W3468, and two kinds of newly isolated Hfr strains (JG18 and JG19), were used as recipients of R_{100} and R_{100-1}. From each of these sublines infected with R factors, five to six clones were chosen, and their inhibition of *F mating* in F$^+$R$^+$ and HfrR$^+$, *R mating* in F$^-$R$^+$, and their sensitivity to phage f1 and to RNA phage were tested (Tables 2 and 3). The male strains (F$^+$ and Hfr) of H509a carrying R_{100}, a wild-type R factor inhibiting *F mating* in K-12 males, showed decreased frequency of *F mating* in the cross with F$^-$ strains as in K-12 (Nakaya, Nakamura, and Murata, 1960). The sensitivity to phage f1 and RNA phage was also lost after infection with R_{100}. This is in agreement with the findings of several investigators who used K-12 (Egawa and Hirota, 1962; Watanabe et al., 1962). When the males were infected with a noninhibiting mutant of R factor, R_{100-1}, there was no inhibition of maleness of the host, i.e., transfer of chromosome. Sensitivity to the specific bacteriophages was also present in contrast to R_{100}-infected cells (Egawa and Hirota, 1962).

The R factors, both R_{100} and R_{100-1}, conferred the capacity of *R mating* on H590a F$^-$ derivatives, but with different frequencies. Similar results were obtained by Sugino and Hirota (1962) with K-12. Two clones from each F$^+$R$^+$ and F$^-$R$^+$ subline, JG6 $\sim$ JG17, were chosen for additional mating experiments and for slide agglutination tests with f^+ sera (Table 3). In Table 4, slide agglutination tests are compared with spot cross testing of R-infected strains from another series of experiments. Tables 3 and 4 show that all the F$^+$ cultures infected with R_{100}, which inhibits the fertility of its host bacterium, had lost their agglutinability in the f^+ test serum. All the F$^+$ clones infected with R_{100-1} were autoagglutinable; i.e., they agglutinated both in the anti-f^+ sera and in saline. This agglutination phenomenon could also be observed in broth culture after incubation at 37 C. To summarize, inhibition of *F mating*, inhibition of adsorption of phage f1 and RNA phage, and inhibition of production of f^+ antigen were observed when male bacteria were infected with R_{100}, whereas no inhibition was found when they were infected with R_{100-1}.

Effect of partial and full elimination of R factor from R$^+$ bacteria on F properties. To determine what influence a loss of R factor would have on the mating state and the serological properties, R$^-$ colonies were isolated by means of a penicillin screening method. This experiment comprised D169F$^+$R$^+_{100}$, D169F$^+$R$^+_{100-1}$, and W3478F$^-$R$^+_{100-1}$ (Table 5). All the three strains examined showed a restoration of the original serological state. The f^+ antigen manifested itself again in the D169 strains, and the spontaneous agglutinability disappeared from W3478. In other words, they behaved as they did before the R factors were introduced.

In cases where resistance to TC was retained while the strains had lost their resistance to SM and CM, the strains behaved as if they were still resistant to all drugs when tested with the slide agglutination test or crosses with F$^-$. It may be considered that the determinant is located on the R factor, close to the TC locus.

Serological properties of bacteria carrying R_{100-1}. Because of the autoagglutinability, the possibility that the R_{100-1}-infected strains had acquired a new surface antigen could not be tested by slide agglutination. These strains did not only agglutinate in 0.9% saline but also in distilled water and in saline of lower NaCl content; therefore, they agglutinated in homologous serum. What could be examined was the possibility that the f^+ antigen in D169F$^+$R$^+_{100-1}$ could be demonstrated by its ability to elicit the formation of f^+ antibodies in rabbits. In this case, the D169F$^+$-$(f^+)^+$ not infected with R would react in the F$^+$R$^+_{100-1}$ serum absorbed in the usual way.

Sera were prepared against the R-infected strains D169 and W3478: D338(F$^+$R$^+_{100-1}$), D339(F$^+$R$^+_{100}$), D340(F$^-$R$^+_{100}$), and D341-(F$^-$R$^+_{100-1}$). All four sera were absorbed with a boiled culture of the corresponding F$^-$R$^-$ strain, W3478, to remove O and the special kind of K antibody, B, present in the O100 antisera produced with living cultures. The absorbed sera were tested with F$^+$R$^-$, F$^-$R$^-$, F$^+$R$^+_{100}$, and F$^-$R$^+_{100}$ strains: the R_{100-1} infected strains could not be used because of their autoagglutinability. Furthermore, the sera were tested against an F$^+$R$^-$ and F$^-$R$^-$ strain of yet another O group, O38. This F$^+$ strain had been converted to the

F^+ state through contact with a K-12 strain and had thus acquired the f^+ antigen.

The results of the tube agglutination tests (Table 6) show that the $F^+R^+_{100-1}$ serum contains f^+ antibodies, since the f^+ antigen testers D169 and D179 agglutinate to a high titer in this absorbed serum.

Further absorption of this serum by living cultures of different O100 strains showed that the f^+ agglutinins could be removed by F^+R^- and $F^+R^+_{100-1}$ strains (D169 and D338), while this was not the case with $F^+R^+_{100}$, $F^-R^+_{100}$, $F^-R^+_{100-1}$, and F^-R^- (D339, D340, D341, and W3478).

A serum produced with the F^+R^- strain D169 was absorbed in the same way and tested with the same cultures mentioned above. The result was identical with that of serum D338.

TABLE 2. *Relationship of inhibition of F mating, sensitivity to phages, and R mating in H509a R^+ strains*[a]

Strain	Response to phage[b]		No. of recombinants in crosses with	
	f1	MS2	H509a F^{-c}	W4573
D169 F^+R^-	s	s	155	272
D169 $F^+R^+_{100}$				
#1 (JG6)	r	r	0	2
#2 (JG7)	r	r	0	4
#3[d]	s	s	100	188
#4	r	r	0	1
#5	r	r	0	0
D169 $F^+R^+_{100-1}$				
#1	s	s	61	161
#2 (JG8)	s	s	284	395
#3	s	s	230	517
#4 (JG9)	s	s	322	231
#5	s	s	238	352
#6	s	s	63	171
W3478 F^-R^-	r	r	0	0
W3478 $F^-R^+_{100}$				
#1 (JG10)	r	r	0	21
#2	r	r	32	202
#3	r	r	30	247
#4	r	r	4	112
#5	r	r	59	376
#6 (JG11)	r	r	30	306
W3478 $F^-R^+_{100-1}$				
#1	r	r	9	141
#2 (JG12)	r	r	7	733
#3	r	r	7	111
#4 (JG13)	r	r	22	100
#5	r	r	14	563
#6	r	r	29	192
W3468 F^-R^-	r	r	0	0
W3468 $F^-R^+_{100}$				
#1 (JG14)[e]	s	r	13	13
#2 (JG15)	r	r	0	0
#3	r	r	—	0
#4	r	r	3	6
#5	r	r	7	0
W3468 $F^-R^+_{100-1}$				
#1 (JG16)	r	r	5	11
#2 (JG17)	r	r	3	6
#3	r	r	5	13
#4	r	r	5	9
#5	r	r	7	10
JG18HfrR$^-$	s	s	702	445
JG18HfrR$^+_{100}$				
#1	r	r	3	0
#3	r	r	0	1
#4	r	r	0	1
#5	r	r	—	10
#6	r	r	2	0
JG18HfrR$^+_{100-1}$				
#1	s	s	460	214
#3	s	s	335	375
#4	s	s	610	—
#5	s	s	373	41
#6	s	s	446	80
JG19HfrR$^-$	s	s	273	55
JG19HfrR$^+_{100}$				
#2	r	r	0	1
#3	r	r	0	2
#4	r	r	0	2
#5	r	r	1	1
#6	r	r	0	0
JG19HfrR$^+_{100-1}$				
#3	s	s	150	38
#5	s	s	41	92
#7	s	s	203	94
#9	s	s	39	7
#12	s	s	—	—
W4573 F^-R^-	—	—	—	0

TABLE 2—*Continued*

[a] The procedure for infection with R factors was as follows. One loopful of each of the overnight cultures in BHI broth of donor and recipient bacteria was mixed in 1 ml of broth and incubated overnight at 37 C. The mixed culture was then streaked on to EMB-lactose agar containing 25 μg/ml of CM. A few Lac-positive and CM-resistant colonies were isolated from this plate and purified on BHI Agar. Each of the purified colonies was inoculated into 1 ml of broth and stored at 5 C as a new R^+ clone after retesting the drug resistance and the other genetic markers. When an Hfr mutant was used as recipient of the R factor, the mixture of donor and recipient was incubated for 2 hr, and thereafter several $HfrR^+$ clones were obtained in the same way. Mating experiments were performed as follows. Quantities of 1 ml of overnight cultures of donor and recipient were mixed. After 2 hr of incubation at 37 C, 0.1 ml of the mixed culture was plated onto either Davis-minimal agar in the cross with $H509aF^-$ or EM-lactose agar in the cross with W4573. The plate was then incubated for 2 days at 37 C after which the number of recombinant colonies was counted. As a control test, the parent strains were always seeded separately on to the same medium. In the case of the cross $HfrR^+ \times F^-R^-$, quantities of 0.05 ml of overnight cultures in nutrient broth of the parents were mixed and incubated for 2 hr at 37 C, followed by plating of 0.1 ml of the mixed culture, diluted 1:100, onto an appropriate selective medium, e.g., either Davis-minimal agar or EM-lactose (-xylose) agar for $JG18R^+$ ($JG19R^+$) with amino acids or sugar marker, respectively, as the selective markers. After 2 days of incubation at 37 C, the number of recombinants was counted.

[b] Symbols: s or r indicates the sensitivity or resistance to phages f1 or MS2.

[c] The $H509aF^-$ strain used as recipient in crosses with $D169F^+R^+$, $W3478F^-R^+$, and $HfrR^+$ is W3468, and that used in crosses with $W3468F^-R^+$ is W3478.

[d] The R factor in this clone seems to be an i^- mutant because the host of the R factor showed no inhibition of *F mating* and was sensitive to the test phages. Moreover, no reduction of frequency of *F mating* was observed in the cross of W1895Hfr tester infected with this R factor with $W4573F^-$ recipient.

[e] A f1 sensitive mutant; see Table 8.

In $F^+R^+_{100}$ (D339) serum absorbed by boiled F^-R^- culture, none of the f^+ testers agglutinated, indicating that the introduction of R_{100} factor was followed by disappearance of the f^+ antigen.

Similarly, the $F^-R^+_{100}$ strain did not cause production of f^+ agglutinins, while the problem as to whether $F^-R^+_{100-1}$ (D341) can induce formation of such antibodies is not yet solved. The f^+ antigen testers agglutinated to some extent in the serum produced with that strain, even after absorption by the boiled F^-R^- culture. Additional absorption by live cultures did not remove these agglutinins except when the F^+R^-, $F^+R^+_{100-1}$, or $F^-R^+_{100-1}$ cultures were used. (The fact that the latter strain, D341, could remove the antibodies in this case but not in the other case, shown in Table 6, may be due to quantitative differences.)

As regards the question of a special R factor antigen, no results were obtained which denoted the presence of such. The R_{100-1}-infected strains were spontaneously agglutinable and, therefore, could not be used in agglutination experiments. The existence of a special antigen cannot, therefore, be excluded. The R_{100}-infected strains did not agglutinate in their homologous sera absorbed by boiled F^-R^- culture, thus indicating that an antigen characteristic for this kind of strain was not present.

Effect of R infection on the pattern of segregation in F mating recombinations. An Hfr mutant JG18 [derived from $D169F^+$ (H509a), which transfers Try, Leu, Lac, Gal markers with high frequency] and $JE346F^-$ (K-12) were used. In crosses of $HfrR^-$ (JG18) or $HfrR^+$ ($JG18R^+_{100}$ and $JG18R^+_{100-1}$) with F^-R^- (JG346), His^+ $Isol^+$ Lac^+ recombinants were selected, and several unselected markers of the recombinants were tested by the replica-plating method (Lederberg and Lederberg, 1952). The results in Table 7 show that there is close similarity in the pattern of segregation of donor markers, irrespective of the presence or absence of R factor in the Hfr cells. This result might suggest that the inhibition of fertility by the R factor is due to some change in the cell surface and not to either disturbances in the order of chromosome transfer or to interference in the process of integration. In the cross of $JG18R^+_{100}$ and JE346, about 10% of the recombinants acquired R factors. These R factors were transferred to W1895, Standard Hfr Cavalli, and then the sensitivity to phage f1 and inhibition of fertility of W1895 R^+ were tested. Of 20 clones tested, 17 were sensitive to phage f1 and also

TABLE 3. *Slide agglutination tests compared with the yield of recombinants in crosses*[a]

Strain	Genotype	No. of recombinants in cross with			Slide agglutination		
		Expt 1		Expt 2	Sera		Saline
		W3132F⁻	H509aF⁻	H509aF⁻	$(f^+)^-$	$(f^+)^+$	
JG 6	$D169F^+R^+_{100}$ #1	24	6	3	−	−	−
7	#2	70	2	3	−	−	−
8	$D169F^+R^+_{100-1}$ #2	ca. 500	250	214	+	+	+
9	#4	ca. 500	ca. 300	ca. 1000	+	+	+
10[b]	$W3478F^-R^+_{100}$ #1	ca. 300	60	40	−	−	−
11[c]	#6	ca. 500	ca. 400	234	+	+	+
12	$W3478F^-R^+_{100-1}$ #2	ca. 500	150	100	+	+	+
13	#4	ca. 400	60	141	+	+	+
14[d]	$W3468F^-R^+_{100}$ #1	ca. 400	ca. 250	199	+	+	+
15	#2	1	1	4	−	−	−
16	$W3468F^-R^+_{100-1}$ #1	ca. 400	ca. 500	301	+	+	+
17	#2	100	50	500	+	+	+
D 169	F^+R^-	—	—	170	−	+	−
W3478	F^-R^-	0	0	0	−	−	−
W3468		1	—	2	−	−	−

[a] Procedure for mating in experiment 1: 20-hr broth cultures at 37 C were centrifuged, the supernatant was withdrawn, and the pellet was resuspended in 0.5 ml of distilled water; quantities of 0.05 ml of each partner were then mixed directly onto a plate and incubated for 48 hr. Procedure for mating in experiment 2: a mixture of one loopful of overnight cultures of donor and recipient was inoculated into 1 ml of BHI broth, and 0.2 ml of the mixed cultures was plated onto EM-lactose agar after overnight incubation; the number of recombinant colonies formed on the selective medium was counted after 2 days of incubation at 37 C. As recipient from the H509aF⁻ subline, W3468 was used for JG6-JG13 and W3478 for JG14-JG17.

[b] JG10 gave high fertility in *R mating* but no reaction in the f^+ test sera or saline. At the present stage of this investigation, it is impossible to say anything about this special phenomenon.

[c] JG11 showed exceptionally high frequency of *R mating* and at the same time became autoagglutinable. The R factor was transferred to the tester strain for *i*, W1895Hfr_1, and examined by the cross-brush method, both as regards the inhibition of *F mating* in the cross of Hfr_1R^+ with W4573F⁻ recipient and the sensitivity to f1 and MS2. The R factor was found to be i^-, since there was no inhibition of *F mating* and sensitivity to phages.

[d] JG14 carried a mutated R factor which conferred on the host cell susceptibility to phage f1 (see Table 8).

highly fertile when Lac^+ was used as the selected marker; i.e., 85% of the R factors isolated from those recombinants were i^- mutants. Similar results have been observed with K-12 (Egawa, *unpublished data*). This might indicate that the residual fertility of $JG18R^+_{100}$, an Hfr with an inhibiting R factor, may be interpreted by spontaneous mutation from i^+ to i^- of the R factor in the Hfr cells.

Mutation of R factor at a locus which directs receptor of a phage f1. A mutant of F^-R^+ bacteria which became sensitive to phage f1 was isolated during this experiment (Table 2). The mutated R factor was still infectious, and the introduction of this R factor into other F⁻ bacteria conferred sensitivity to phage f1 on the infected cells. The same results were observed when this R factor was transduced by phage Plkc. Removal of the R factor from the host bacterium after treatment with acridine orange resulted in resistance to the phage. In the acridine treatment experiment, a strain showing partial loss of the R factor was isolated: TC was retained, and SM, CM, and SA were lost. This strain was still sensitive to phage f1. The K-12 F⁻ bacteria which possessed the mutated R factor were resistant to RNA phage, and were sensitive to phage *tau* (Table 8). This R factor was transferred from F⁻ to F⁻ cells, and was not in contact with the F factor. These results suggest that the bacteria carrying the R

TABLE 4. *Slide agglutination test compared with spot cross test of R-infected strains**

Strain	No. of colonies tested	Spot cross with W3468	Slide agglutination in:		
			Sera†		Saline
			$(f^+)^-$	$(f^+)^+$	
$D169F^+R^+_{100}$	11 without selection	+	−	+	−
	43 selected from TC or SM or CM plate	−	−	−	−
$D169F^+R^+_{100-1}$	9 without selection	+	−	+	−
	51 selected from TC or SM or CM plate	+			+
$W3478F^-R^+_{100}$	12 without selection	−	−	−	−
	35 selected from TC or SM or CM plate	−	−	−	−
$W3478F^-R^+_{100-1}$	12 without selection	−	−	−	−
	48 selected from TC or SM or CM plate	+			+

* Infection with R factor: quantities of 0.1 ml of an overnight broth culture (37 C) of donor and recipient were mixed in 5 ml of broth and incubated at 37 C for 4 hr; the mixture was then plated onto three kinds of BTB-lactose agar containing either SM (10 μg/ml), CM (25 μg/ml), or TC (25 μg/ml). Lactose-positive and drug-resistant colonies were then purified. All the colonies isolated from the selective media were resistant to all the drugs examined. Lactose-positive colonies isolated on the same medium but without addition of the drug were used as a control. Procedure for mating: 20-hr cultures at 37 C were centrifuged, the supernatant withdrawn, and the pellet resuspended in 0.5 ml of distilled water. One loopful of the resuspended pellet of $W3468F^-$ culture was streaked across an EMS sugar agar plate. When the streak had dried, one loopful of the cultures to be examined for fertility was spotted on top of the streak. Slide agglutination tests in an $(f^+)^+$ and an $(f^+)^-$ serum were performed from a culture developed direct from the cultures used in the crosses.

† Sera: $(f^+)^-$ serum produced with strain $W1611F^-$ (O antigen 25); $(f^+)^+$ serum produced with strain W3703Hfr (O antigen 25).

TABLE 5. *Slide agglutination test compared with the yield of recombinants in crosses involving R-infected strains which have partly or totally lost the R factor**

Partly or totally sensitive mutants from	No. of colonies tested	Resistance to			No. of recombinants in crosses with W3468	Slide agglutination in		
		SM	CM	TC		Sera		Saline
						$(f^+)^-$	$(f^+)^+$	
$D169F^+R^+_{100}$	3	s	s	s	ca. 500	−	+	−
	13	s	s	r	1–11	−	−	−
$D169F^+R^+_{100-1}$	3	s	s	s	ca. 500	−	+	−
	3	s	s	r	ca. 500	+	+	+
$W3478F^-R^+_{100-1}$	12	s	s	s	0–5	−	−	−
	4	s	s	r	ca. 500	+	+	+

* Selection was made for colonies which had lost their resistance to CM. Procedure for mating: see Table 3, experiment 1; s or r indicates sensitivity or resistance to the drug.

factor have mutated in a region which directs synthesis of a substance working as receptor of phage f1, and that the mutant type of R factor may carry a locus or loci responsible for the production of receptor substance of phage f1.

DISCUSSION

The experimental results described above would indicate that infection of male cells with R factor causes the inhibition of the expression of several distinct properties of maleness endowed by F factor; i.e., (i) fertility, (ii) agglutinability in anti-f^+ sera, and (iii) sensitivity to phage f1 and RNA phage are simultaneously inhibited when F^+ or Hfr bacteria are infected with the R factor. The pleiotropic effect of inhibition is controlled by a locus *i* of the R factor (Egawa and Hirota, 1962). The position of *i* would probably be close

TABLE 6. *Results of tube agglutination tests performed with sera produced with R_{100-1}-infected cultures**

Strain (nonheated)	Sera							
	D 338($F^+R^+_{100-1}$)				D 341($F^-R^+_{100-1}$)			
	Unabsorbed	Absorbed by W 3478 (100 C)			Unabsorbed	Absorbed by W 3478 (100 C)		
		No further absorption	Further absorbed by live			No further absorption	Further absorbed by live	
			D 169 or D 338	D 339, 340 341 or W 3478			D 169, 338 or D 341	D 339, 340 or W 3478
O antigen 100:								
D169(F^+R^-)	++	++	0	++	+	+	0	+
D339($F^+R^+_{100}$)	+	0	0	0	+	0	0	0
W3478(F^-R^-)	+	0	0	0	+	0	0	0
D340($F^-R^+_{100}$)	+	0	0	0	+	0	0	0
O antigen 38:								
D179(F^+R^-)	++	++	0	++	+	+	0	+
D180(F^-R^-)	0	0	0	0	0	0	0	0

* Symbols: 0 means negative in dilution 1:40; + means titers between 40 and 320; ++ means titers between 1,280 and 5,120. The titer is the reciprocal value of the highest serum dilution giving a reaction visible to the naked eye. After absorption with W3478 (100 C) no agglutinins for boiled cultures were left in any serum.

to the gene TC, which determines the resistance to TC, since both activities were lost at the same time (Table 5). The locus *i* can function in *trans* position and has nothing to do with the *status* of the F factor (cytoplasmic or chromosomal). The i^- mutant which has noninhibitory action on maleness mutates back to i^+ in a single step. The i^- state is recessive to i^+. These experimental results lead to the assumption that the locus *i* would be a kind of regulator gene which directs formation of hypothetical cytoplasmic repressor inhibiting the formation of F substances (Egawa and Hirota, 1962). Simultaneous inhibition of these functions, such as *F mating*, production of f^+ antigen, and formation of receptors of the male specific phages, would be caused by the prevention of synthesis of "F substance" (*see* Skaar, Richter, and Lederberg, 1957).

Several functions controlled by F factor can be separated by mutation. For example, some Hfr mutants and F^+ revertants from them have normal fertility but are not immune to phage *tau*. An F′ mutant which is resistant to the male-specific phages such as f1, Q, and RNA phage, in spite of having capacity of F duction and chromosome transfer, has been isolated and identified to be a mutant of F factor (Nishimura and Hirota, 1962). Moreover, mutants possessing a defective F factor have been found. F refractory mutants derived from an Hfr strain (Lederberg and Lederberg, 1956) were sterile but normal in response to phages, i.e., sensitive to phage f1 and immune to phage *tau*. Several sterile mutants possessing f^+ antigen (Ørskov and Ørskov, 1960) have also been isolated. A detailed analysis concerning these facts will be published separately.

These seemingly independent functions of the F factor are simultaneously inhibited by the R factor, and the fact that i^+ to i^- mutation removes this inhibition concurrently would suggest the existence of some mechanism of a pleiotropic inhibition. It might not be unreasonable to assume that these genes, corresponding to several processes of the mating mechanism, would constitute one operon (*see* Jacob and Monod, 1961).

It was pointed out previously that one explanation for the relationship between F factor and f^+ antigen may be that the transfer of F factor is followed by the uncovering of an already present f^+ antigen (Ørskov and Ørskov, 1960). The results presented here give some indication that this hypothesis is true, as an F^- strain infected with the noninhibiting R_{100-1} factor will elicit to some extent antibodies reacting with an f^+ antigen

TABLE 7. *Segregational pattern in the cross HfrR⁺ × F⁻**

Lac	His and Isol	Try	Pur	Gal	Mtl	Xyl	No. of recombinants in the cross		
							JG18R⁻ × JE346	JG18R$_{100}$⁺ × JE346	JG18R$_{100-1}$⁺ × JE346
1	0	1	1	1	1	1	1 (0.3%)	11 (3.9%) R⁺: 11 R⁻: 0	1 (0.33%) R⁺: 1 R⁻: 0
1	0	1	1	1	1	0	0	2 (0.7%) R⁺: 0 R⁻: 2	1 (0.33%) R⁺: 1 R⁻: 0
1	0	1	1	1	0	0	279 (93.0%)	264 (93.6%) R⁺: 22 R⁻: 242	283 (94.3%) R⁺: 261 R⁻: 22
1	0	1	1	0	0	0	0	1 (0.35%) R⁺: 0 R⁻: 1	0
1	0	1	1	1	0	1	0	3 (1.1%) R⁺: 0 R⁻: 3	1 (0.33%) R⁺: 0 R⁻: 1
1	0	0	1	1	0	0	12 (4.0%)	1 (0.35%) R⁺: 0 R⁻: 1	0
1	0	1	0	1	0	0	8 (2.7%)	0	14 (4.7%) R⁺: 12 R⁻: 2
R⁺								33 (11.7%)	275 (91.7%)
R⁻								249 (88.3%)	25 (8.3%)
Total							300	282	300
Recombinant frequency							ca. 0.2	ca. 0.0005	ca. 0.04

* JG18 Hfr Try⁺ Pur⁺ His⁻ Isol⁻ Lac⁺ Gal⁺ Mtl⁺ Xyl⁺: 1 1 1 1 1 1 1; JE346 F⁻ Try⁻ Pur$_1$⁻ His⁺ Isol⁺ Lac$_{85}^{-}$ Gal$^{-}_{2}$ Mtl⁻ Xyl$^{-}_{2}$: 0 0 0 0 0 0 0. Overnight cultures of donor and recipient were diluted with fresh broth 1:10 and shaken for 2 hr at 37 C. The shaken cultures were mixed and incubated for 2 hr at 37 C. After 2 hr, these mixtures were diluted appropriately and plated on to EM-lactose agar containing DL-tryptophan (40 μg/ml), adenine (20 μg/ml), and thiamine (5 μg/ml). In the case of the JG18R⁺ strain, the media for culture and for selection of recombinants were supplemented by 20 μg/ml of streptomycin to avoid spontaneous loss of R factor. After 2 days of incubation at 37 C, His⁺ Isol⁺ Lac⁺ recombinant colonies formed on the selective media were purified and transferred to a master plate and then replica-plated to check for unselected markers. Recombination frequency = number of recombinants per 100 donor cells.

tester (Table 6). These antibodies can be removed by the homologous strain, whereas this strain is incapable of removing the f^+ antibodies from an F⁺R⁻ or F⁺R$^{+}_{100-1}$ serum. However, extended absorptions were not carried out, and thus it is not known whether the differences between the f^+ antigen of the different strains mentioned are of a qualitative or quantitative nature.

Both F⁺ and F⁻ strains infected with this R$_{100-1}$ factor are autoagglutinable, i.e., agglutinable not only in all types of sera but also in saline and even in distilled water. Enteric bacteria devoid of their O antigen (the so-called rough strains) also show autoagglutinability in sera and saline but generally not in distilled water. Furthermore, ordinary rough strains keep their autoagglutinability after boiling, whereas these cultures lose the spontaneous agglutinability after heating to 100 C for 1 hr and are then agglutinated in their homologous O serum only. Another fact about this special characteristic is that the organisms lose their autoagglutinability after cultivation at 18 C. The agglutinability of the f^+ antigen and of many other K antigens of the L type is likewise not detectable after cultivation at 18 C (Ørskov et al., 1961). The question as to whether the autoagglutinability had something to do with the presence of the f^+ antigen or was an independent characteristic could not be solved by the technique used here.

The strains infected with the R$_{100}$ factor did not agglutinate in their homologous sera absorbed by boiled F⁻R⁻ culture, indicating the absence of an antigen characteristic for this kind of strain.

In crosses with Hfr donors, with and without R factor, the segregational patterns are the same. The inhibition of *F mating* by the R factor might not be due to any disturbance of the order of

TABLE 8. *Mutant of R which is sensitive to phage f1*

Host	R	Response to phage			Inhibition of *F-mating*	*R-mating*[a]	Agglutinability in		
		f1	MS2	*tau*			Sera		Saline
							$(f^+)^-$	$(f^+)^+$	
W3468F$^-$	$R^+_{f1}s$ (JG111)[b]	S	r	r	...	++	+	+	+
	R^-	r	r	r[c]	...	−	−	−	−
	R^+_{100} (JG15)	r	r	r	...	+	−	−	−
	R^+_{100-1} (JG16)	r	r	r	...	++	+	+	+
W1895Hfr$_1$ (infection)	$R^+_{f1}s$ (JE613)	s	s	imm.[d]	−	...	...	...	...
	R^-	s	s	imm.	...	...	...	...	...
	R^+_{100} (JE87)	r	r	imm.	+	...	...	...	...
	R^+_{100-1} (JE119)	s	s	imm.	−	...	...	...	...
W3876F$^-$ Lac$^-$ Mal$^-_5$ (infection)	$R^+_{f1}s$ (JE889)	S	r	s	...	+	...	...	...
	R^-	r	r	s	...	−	...	...	...
	R^+_{100} (JE51)	r	r	s	...	+	...	...	...
	R^+_{100-1} (JE170)	r	r	s	...	++	...	...	...
W3630F$^-$ Mal$^-_5$ (transduction)	$R^+_{f1}s$	S			...	+	...	...	...
	R^-	r			...	−	...	...	...
	R^+_{100} (JE111)				...	+	...	...	...
	R^+_{100-1} (JE177)				...	++	...	...	...
JG111 (AO treatment)	$R^-_{f1}s$	r			...				
	$R^+_{f1}s$ (TC)	s			...				

[a] *R mating* was checked for recombinant formation with W3637F$^-$M$^-$ as the tester. Selection was made for M$^+$Mal$^+$ recombinants.

[b] JG111 is a clone which was isolated from the culture of JG14.

[c] F$^-$ derivatives of H509a strains, such as W3468, were resistant to phage *tau*, which can lyse K-12 F$^-$ strains.

[d] imm. = immune to phage *tau*.

chromosome transfer, etc., but might be the effect of some change in the cell surface. In other words, the R factor would control a hypothetical switch to the mating process, which might correspond to a generator of the synthesis of surface substances. It would be interesting if this interpretation were comparable with the replicon hypothesis proposed by Jacob and Monod (1963) and Jacob et al. (*in press*), which states that the male substance on the cell surface takes part in replication and transfer of the host chromosome, and that the chromosome transfer necessitates replication of chromosome (Bouck and Adelberg, 1963; Jacob et al., *in press*). Should there be some mechanism which forms the trigger of chromosome duplication indispensable for chromosome transfer, then the R factor could be assumed to exert the action which restrains the trigger.

The isolation of a mutant of the R factor which [illegible] relationship may exist between these two factors. This mutant is still resistant to the RNA phage, thus suggesting that the receptors of phage f1 and RNA phage are separate structures.

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