215. On the r^+ Antigens of Bacteria, Having Drug Resistance Transfer Factor R

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Ochiai *et al.*¹⁾ and Akiba *et al.*^{2),3)} discovered independently that multiple drug resistance was transferred from a resistant strain to a sensitive strain in their mixed culture; and they confirmed that this did not take place from cell-free filtrates of the resistant strain. Later it was found by several investigators⁴⁾⁻⁷⁾ that the transfer of drug resistance was effected by the transfer of R factor,⁸⁾ the term proposed by one of the authors, Iseki. On the other hand, Ørskov and Ørskov⁹⁾ reported that f^+ antigen was present in F^+ and Hfr strains of *E. coli* group O 25. Furthermore, these investigators¹⁰⁾ clarified that productivity of K 88 antigen was transferred by K 88 transfer factor of episome from one strain to another of *E. coli*.

This paper deals with r^+ antigens found in R^+ strains of *E. coli* and *Salmonella*.

Materials and methods. 1. *Strains.* W3478 (His^- , Isol^- , Mal^-) and W3468 (Try^- , Leu^-) of *E. coli* H509a,⁹⁾ and JG 12 R_{100-1}^+ and JG 17 R_{100-1}^+ , which were produced from W3478 and W3468 by the transfer of the F factor (SM, TC, CM, SA), were supplied by Department of Genetics, School of Medicine, Osaka University. Besides these strains, the authors used, for immunization and absorption experiments, *S. paratyphi* B Sp-4 (Leu^-) and Sp-4 R_{100-1}^+ , the latter of which was produced from the former by transferring the R factor of *E. coli*.

2. *Transfer of the R factor.* Transfer of the R factor from the resistant to the sensitive strain was made by the method as reported by Harada *et al.*⁷⁾

3. *Antisera.* Anti- r^+ sera were prepared by immunizing rabbits with living cells of JG 12 R_{100-1}^+ of *E. coli* H509a and formalin-killed cells of *S. paratyphi* B Sp-4 R_{100-1}^+ , and then absorbing these antisera with R^- strains of these two, respectively. Anti-H (b:2) serum was prepared by the method as reported in the previous paper.¹¹⁾

4. *r^+ antigenic substance.* The methods of preparation of r^+ antigenic substance, its treatment with enzyme, and quantitative determination of its protein and sugar are same as reported in the

previous paper.¹¹⁾

Results. 1. When anti-*E. coli* JG 12 (R₁₀₀₋₁⁺) rabbit immune serum was absorbed with *E. coli* W3478 (R⁻), remained r⁺ antibody which agglutinated not only *E. coli* JG 12 (R⁺) and *E. coli* JG 17 (R⁺) but also *S. paratyphi* B Sp-4 (R⁺). After absorption of anti-*S. paratyphi* B Sp-4 (R₁₀₀₋₁⁺) rabbit immune serum with *S. paratyphi* B Sp-4 (R⁻), remained r⁺ antibody which agglutinated not only *S. paratyphi* B Sp-4 (R⁺) but also *E. coli* JG 12 (R⁺) and *E. coli* JG 17 (R⁺). It was consequently assumed that there must be common r⁺ antigen between R⁺ strains of *E. coli* and *Salmonella* (Table I).

Table I. Absorption of anti-*E. coli* (R₁₀₀₋₁⁺) and anti-*S. paratyphi* B (R₁₀₀₋₁⁺) rabbit immune sera with respective R⁻ strains (the figures denote agglutination titers)

Strains	Resistance character	Anti- <i>E. coli</i> (R ⁺) immune serum		Anti- <i>Salm.</i> (R ⁺) immune serum	
		Before absorption	After absorp. with W3478 (R ⁻)	Before absorption	After absorp. with Sp-4 (R ⁻)
<i>E. coli</i> H 509 a					
JG 12	R ⁺	2560	160	320	40
W3478	R ⁻	2560	—	320	—
JG 17	R ⁺	2560	160	320	40
W3468	R ⁻	1280	—	160	—
<i>S. paratyphi</i> B					
Sp-4	R ⁺	640	160	5120	320
Sp-4	R ⁻	320	—	2560	—

2. When anti-*E. coli* r⁺ serum, contained in anti-*E. coli* JG 12 (R₁₀₀₋₁⁺) rabbit immune serum, was absorbed with *S. paratyphi* B Sp-4 (R⁺), remained r⁺ antibody which agglutinated only *E. coli* JG 12 (R⁺) and *E. coli* JG 17 (R⁺). Furthermore, when anti-*S.* r⁺ serum in anti-*S. paratyphi* B Sp-4 (R₁₀₀₋₁⁺) rabbit immune serum was absorbed with *E. coli* JG 12 (R⁺), remained r⁺ antibody which agglutinated only *S. paratyphi* B Sp-4 (R⁺). It was consequently assumed that r⁺ antigens of *E. coli* and *Salmonella* possess their

Table II. Absorption of r⁺ antibodies with R⁺ strains of *E. coli* and *Salmonella*

Antisera	Anti- <i>E. coli</i> r ⁺ serum			Anti- <i>S.</i> r ⁺ serum		
Strains	JG 12 (R ⁺)	JG 17 (R ⁺)	Sp-4 (R ⁺)	JG 12 (R ⁺)	JG 17 (R ⁺)	Sp-4 (R ⁺)
Before absorption	160	160	160	40	40	320
After absorption with						
JG 12 (R ⁺)	—	—	—	—	—	80
Sp-4 (R ⁺)	80	80	—	—	—	—

respective specific antigenicities besides common antigenicity (Table II).

These r^+ antibodies were not absorbed with F^+ strains of *E. coli* and *Salmonella* which have f^+ antigen. It was consequently considered that r^+ antigen is serologically different from f^+ antigen.

3. The bacterial cells after treatment in a blender or a sonicator were centrifuged, and the supernatant was recentrifuged twice, first at 10,000 r.p.m., and then at 38,000 r.p.m. The fraction which sedimented in the last centrifugation was found to have the strongest capacity to absorb r^+ antibody. This r^+ antigenic substance and the bacterial cells lost the capacity to absorb r^+ antibody when treated with proteinases such as trypsin, ficin, and bromelin.

Furthermore, when heated at 100°C for 30 minutes, the bacterial cells lost the absorbing capacity, as well as agglutinability. It was subsequently considered that the r^+ antigenic substance is probably a proteinic substance. However, about 2% sugar was demonstrated in the r^+ antigenic substance, which is considered to be principally glucose, and to be related with the somatic antigen.

4. Experiments were carried out for inhibitory action of the r^+ antibody in conjugational transfer of R factor from *S. paratyphi* B Sp-4 (R_{100-1}^+) to *E. coli* H509a W3468 (R^-).

It was found that nearly the same rate of inhibition was effected as by anti-H (b:2) antibody. The inhibition is considered to be attributed to agglutination.

Discussion. The r^+ antibody, contained in anti-*E. coli* JG 12 (R_{100-1}^+) rabbit immune serum, agglutinated not only *E. coli* JG 12 (R^+), used for the immunization, and *E. coli* JG 17 (R^+), but also *S. paratyphi* B Sp-4 (R^+), and the r^+ antibody, contained in anti-*S. paratyphi* B Sp-4 (R_{100-1}^+) rabbit immune serum, agglutinated not only Sp-4 (R^+), used for the immunization, but also *E. coli* JG 12 (R^+) and *E. coli* JG 17 (R^+). This indicates that as f^+ antigen⁹⁾ is produced in the strain, having fertility factor F , so r^+ antigen is produced in the strain, having drug resistance transfer factor R . Furthermore, *E. coli* (R^+) and *Salmonella* (R^+) into which R factor of *E. coli* had been transferred, possess their respective specific antigenicities besides common antigenicity to both of them. This seems to indicate that r^+ antigens, which are produced by the same R factor in both *E. coli* and *Salmonella*, may be serologically different from each other probably owing to difference in the processes of production.

The f^+ antigenic substance is destroyed either by heating⁹⁾ or by proteinase action.¹¹⁾ It is the same with r^+ antigenic substance. Therefore both are considered to have protein properties. Also K

88 antigen of *E. coli*, which is produced by transfer of K 88 transfer factor discovered by Ørskov and Ørskov,¹⁰⁾ is likewise a proteinic substance, destroyed by proteinases.¹²⁾

On the other hand, it is known that as F pili are found in the strains having F factor, so R pili are found in the strains having R factor.¹³⁾ Subsequently, r⁺ antigen is assumed to be present in R pili as f⁺ antigen is assumed by Brinton *et al.*¹⁴⁾ to be present in F pili.

Conclusions. The presence of specific antigen r⁺ was confirmed in *E. coli* and *Salmonella*, both of which possess drug resistance transfer factor R. The r⁺ antigens in *E. coli* and *Salmonella* possessed their respective specific antigenicities besides common antigenicity to both of them. The r⁺ antigenic substance is considered to be a proteinic substance.

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