RESISTANCE TRANSFER AGENTS IN SHIGELLA

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Approximately ten per cent of the Shigella strains isolated in Japan since 1958 have been recognized to have a high degree of resistance to chloramphenicol (CM or C), the tetracyclines (TC or T) and/or streptomycin (SM or S) at the time of their original isolation from human fecal specimens. The resistance patterns are exclusively /CTS, /CS, /TS, /T and /S, where /CTS, /CS and /TS denote the cross resistance to CM, TC and SM, to CM and SM, and to TC and SM, respectively (Nakaya, 1960). It was found by Ochiai et al. (1959) and Akiba et al. (1960) that the resistance can be transferred from the resistant strain to the sensitive by mixing the broth culture of the resistant with that of the sensitive, and it takes place not only within Shigella, but also between Shigella and E. coli. They reported that this phenomenon could be demonstrated only in the case when the living cells of a sensitive recipient were mixed with those of a resistant donor, while not when mixed with heat-killed culture, sterile culture filtrate and disrupted cells by freezing and thawing of the resistant.

The present communication deals with the nature of this resistance transfer phenomenon. From the results it is evidenced that for this phenomenon the resistance transfer

agents (Rta) are responsible.

The time course of resistance transfer was studied by the crossing experiment between Shigella/CTS and E. coli K-12. As illustrated in Fig. 1, it is clearly demonstrated that resistance transfer takes place very rapidly and in high frequency, suggesting it would occur by cell to cell contact. No decrease in the number of the resistant donor was observed.

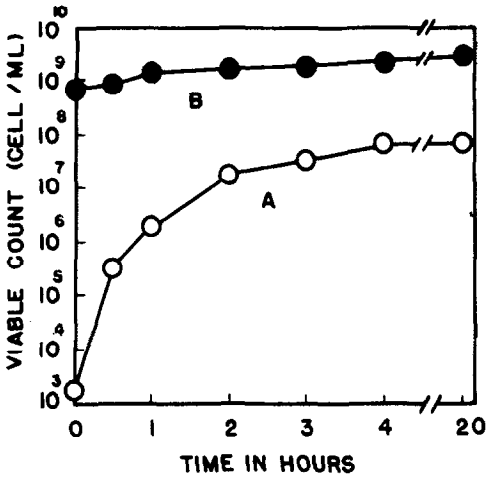

Fig. 1. The time course of resistance transfer. Overnight Penassay broth cultures of S. flexneri 2b, #222/CTS and E. coli K-12, CSH-2 were mixed at time 0 (9×10^8 #222/CTS cells/ml and 5×10^8 CSH-2 cells/ml) each in amounts of 5 ml and rocked in a L-shaped culture tube at 37° C for 4 hours in a water bath, followed by placing in a 37° C incubator without rocking. The differential characters of the two strains are; #222/CTS = CM-, TC-, SM-resistant, lactose$^-$, niacin$^-$, tryptophan$^-$, methionine$^-$ and CSH-2 = CM-, TC-, SM-sensitive, lactose$^+$, methionine$^-$. At different intervals of time samples were immediately plated, for the resistance transferred cells on lactose methionine minimal medium containing 50 µg CM/ml (/CTS transferred cells, curve A) and for CSH-2 cells on the similar medium without CM (total CSH-2 cells, curve B).

The resistance transfer was found to occur by contact from Shigella/CTS to many other sensitive strains of Enterobacteriaceae, including Salmonella, Arizona, E. coli, Citrobacter, Klebsiella, Enterobacter, Hafnia and Proteus. Most of them acquired simultaneously CM-, TC- and SM-resistance as well as the ability of its transfer to the other sensitives. This indicates a strikingly wide range of recipient bacterial genera, as in the transmission of the colicinogenic factors (Fredericq, 1958).

The resistance to all the antibiotics used could be

transferred not only to the sensitive strains, but also replaced the sensitivity of such a recipient Shigella strain as possessing the resistance pattern /CS, /TS, /T, or /S, which resulted in /CTS, /TS, or /CS after contact with an appropriate resistant donor. The result seems to suggest the presence of at least four independent Rtas, i.e., CS-Rta (CM- and SM-resistance transfer agent), TS-Rta, T-Rta, and S-Rta, and no interference between their transmissibility.

In order to compare Rta with the sex factor F of E. coli K-12, CTS-Rtas have been transferred from S. flexneri 2b, #222/CTS to the derivatives of E. coli K-12 by contact. The strains made /CTS were W-677 ($F^-T^-L^-$), CSH-2 (F^-M^-), 58-161 (F^+), W-2252 (Hfr_1), W-3780 (Hfr_2) and W-3753 (Hfr_4). In the course of isolation of these cultures, the growth of /CS and /T colonies were obtained, although infrequently. Thus the presence of two independent Rtas, i.e., CS-Rta and T-Rta in a /CTS strain is assumed. Furthermore, TS-Rta and S-Rta were recognized in a similar experiment, where Shigella strains possessing the resistance pattern /TS or /S were used as the donor. For the purpose to reveal the effect of Rta^+ status of F^- cells on its F^- status, a cross was performed between $F^-T^-L^-$ and F^-M^-/CTS strains along with the control cross of $F^-T^-L^-$ x F^-M^-. Since none of such characters as the synthesis of threonine (T) and leucine (L) or of carbohydrate utilization was jointly transferred with the resistance, it is conceivable that Rta^+ status cannot confer on F^- cells F^+ status. In crosses of Hfr_1 x $F^-T^-L^-$, where either one of them was made CTS-Rta^+ status, the Rtas were transferred from the Rta^+ strain to the Rta^- strain irrespective of the Hfr or the F^- status. Further, no close linkage of CTS-Rtas with ordinary bacterial characters was recognized. The results are presented

in Table I, from which it is obvious that Rta behaves as an independent agent from the sex factor F. In the crosses of Hfr/CTS x F^-, the frequency of transfer of the intrinsic chromosomal

Table I

Frequency of Rta transmission and recombination in crosses of Hfr x F^-

Selected marker	Cross (1) Hfr x F^-	(2) Hfr/CTS x F^-		(3) Hfr/CTS x F^-		(4) Hfr x F^-/CTS
/CM		$3/10^2$		$1/10^2$		$2/10^2$
/TC		$1/10^2$		$2/10^2$		$1/10^2$
/SM				$2/10^2$		
			Ratio*		Ratio*	
T^+L^+	$5/10^3$	$7/10^6$	1	$5/10^6$	1	
T^+L^+/CM		$2/10^6$	0.3	$0.2/10^6$	0.04	$9/10^3$
T^+L^+/TC		$1/10^6$	0.1	$0.5/10^6$	0.1	
Lac^+	$2/10^3$	$10/10^6$	1	$5/10^6$	1	
Lac^+/CM		$3/10^6$	0.3	$0.2/10^6$	0.04	$30/10^3$
Lac^+/TC		$3/10^6$	0.3	$0.5/10^6$	0.1	
Mal^+	$0.5/10^3$	$1/10^6$	1	$0.5/10^6$	1	
Mal^+/CM		$0.07/10^6$	0.07	$0.02/10^6$	0.04	$0.3/10^3$
Mal^+/TC		$0.03/10^6$	0.03	$0.02/10^6$	0.04	
Xyl^+	$0.5/10^3$	$3/10^6$	1			
Xyl^+/CM		$0.03/10^6$	0.01			$0.3/10^3$
Xyl^+/TC		$0.03/10^6$	0.01			

Hfr, strain W-2252 (Hfr_1 M^- Lac^+); F^-, strain W-677 (F^- T^- L^- B_1^- Lac^- Mal^- Gal_5^- Xyl^- Mtl^- Ara^-); /CM, CM-resistance; /TC, TC-resistance; /SM, SM-resistance; T^+L^+, synthesis of threonine and leucine; Lac^+, lactose utilization; Mal^+, maltose utilization; Xyl^+, xylose utilization.

Hfr cells were mixed with F^- cells in Penassay broth. The mixture was incubated for 60 minutes with aeration before selective plating. The mixtures contained 3×10^7 Hfr cells and 1.2×10^9 F^- cells/ml in the cross (1), 1×10^8 Hfr/CTS cells and 1.2×10^9 F^- cells/ml in the cross (2), 2.2×10^8 Hfr/CTS cells and 9×10^8 F^- cells/ml in the cross (3), and 2.7×10^7 Hfr cells and 7×10^7 F^-/CTS cells/ml in the cross (4). Frequencies are measured as the ratio of the number of the recombinant or resistance transferred cells to the number of the donor cells. In the crosses (1), (2) and (3) Hfr was the donor, while in the cross (4) Hfr was the acceptor of CM- and TC-resistance and the donor of the other characters.

* Ratio of the frequency of recombination accompanied with resistance transfer to the frequency of recombination of the given marker in total as presented by unity.

markers of Hfr decreased markedly, as if in a cross of $F^+ \times F^-$. This seems to suggest that the Rta in Hfr cells might interfere with the transfer of the intrinsic genomes of Hfr into F^- cells. The resistance transfer, however, took place readily in an equal frequency in any combination.

With bacteriophage P1kc (Lennox, 1955) grown on Shigella with the resistance pattern /CTS, /CS, /TS, or /S, the antibiotic-resistance was transduced into E. coli K-12 in a frequency of approximately 10^{-7} to 10^{-5} per phage particle adsorbed, that did not differ from the usual transduction frequency of ordinary characters. The transduction of CTS-Rtas was also successful among E. coli K-12 lines. The similar results were obtained as in the mixing experiments, confirming the following facts more evidently. No transductant resistant to CM alone was found, indicating the intimate linkage of CM-resistance to SM-resistance. In a transduction with phage grown on a certain /TS strain, it was also confirmed to exist TS-Rta which did not dissociate into T-Rta and S-Rta. The antibiotic-resistance and the ability of its transfer to the sensitives were found never to dissociate, indicating that the Rtas are an entity which possesses both properties.

From the results so far obtained it can be conceivable that the majority of the antibiotic-resistant Shigella strains isolated from human fecal specimens are infected with the resistance transfer agents. The evidences that characterize the agents as an entity which plays a main role in the resistance transfer phenomenon are as follows: one step mutation from sensitivity to a high level of resistance to CM or to TC that has never been observed in the selection of resistant mutants in vitro (Cavalli and Maccacaro, 1952); one step back mutation from resistance to sensitivity that could

be occasionally recognized in colony selection; the high frequency of the resistance transfer from a resistant strain to a sensitive as occurring by contact; the intimate linkage between CM- and SM-resistance or between TC- and SM-resistance that has never been observed in the mutants selected *in vitro*; the inseparable linkage between resistance and the ability of its transfer; a wide range of acceptor bacterial genera; transducibility by phage; no linkage to ordinary bacterial characters; no correlation to the sex factor F of *E. coli* K-12. The last two points indicate that Rta appears to differ from the episomes discussed by Jacob and Wollman (1958) because no evidence supporting its chromosomal cycle has been obtained yet, while in other respects one may be able to make the analogy to episomes by assuming a cytoplasmic status of Rta (Lederberg, 1952). Detailed data will be published elsewhere.

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