Journal of Bacteriology
Vol. 88, No. 6, p. 1573-1579 December, 1964
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RELATIONSHIP BETWEEN COLICINOGENIC FACTORS E_1 AND V AND AN F FACTOR IN *ESCHERICHIA COLI*

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Received for publication 18 July 1964

Abstract

Kahn, Phyllis L. (Princeton University, Princeton, N.J.), and Donald R. Helinski. Relationship between colicinogenic factors E_1 and V and an F factor in *Escherichia coli*. J. Bacteriol. 88:1573–1579. 1964.—Colicinogenic factors are the genetic determinants for colicin production in enteric bacteria. An examination of the relationship of two colicinogenic factors, *colE*$_1$ and *colV*, to the F factor of fertility in *Escherichia coli* revealed that the *colE*$_1$ factor does not have the properties of a fertility factor, whereas the *colV* factor appears to possess a genetic determinant of fertility (F_v). Cells containing *colE*$_1$ alone do not recombine with F^- strains, whereas $V^+F_v^+$ strains can recombine, but at a lower frequency for certain markers than cells containing the F factor from *E. coli*. Strains possessing the *colV* factor can transfer the V^+ and F_v^+ properties to an F^- recipient, whereas strains harboring *colE*$_1$ alone are not able to transfer this factor. When a $V^+F_v^+E_1^+$ strain is used as a donor, recipients show F_v^+ sexuality characteristics only if they also acquire the ability to produce colicin V. The *colV* factor is transferred at lower efficiency to F^+ than to F^- strains, whereas the transfer of *colE*$_1$ is equally efficient to both. In addition, the $V^+F_v^+F^+$ colonies isolated segregate $V^+F_v^+F^-$ and $V^-F_v^-F^+$ cells at high frequency, indicating an incompatibility between the *colV* and F factors. Finally, under the conditions employed, acridine orange does not remove the *colE*$_1$ factor, but will remove the *colV* factor from colicinogenic cells. In every case tested, the V^+ and F_v^+ characteristics are removed simultaneously by the acridine orange treatment.

Certain strains of enteric bacteria, designated colicinogenic or *col*$^+$ strains, are capable of producing substances, termed colicins, which are lethal to other bacterial strains. A large number of colicins have been distinguished on the basis of differences in their ability to kill a variety of sensitive strains. These colicins are genetically determined by factors which appear to be nonessential genetic elements acquired by cell contact and which are transmitted from cell to cell independently of the bacterial chromosome.

In *Escherichia coli* the genetic determinant of sexuality is an episomal element, the F factor. Cells possessing this factor, designated as F^+, are capable of acting as a genetic donor in crosses with strains not harboring the factor (F^- strains). Certain colicinogenic factors appear to be closely related to, or in some way associated with, an F factor of fertility, as judged by their ability to transmit themselves or chromosomal markers to F^- recipients (Smith and Stocker, 1962; Fredericq, 1963*a*). Specifically, a relationship between the colicinogenic factor determining colicin V and an F factor of fertility is suggested by the cotransfer of these properties to an F^- recipient (Fredericq, 1963*b*). This is not the case for the colicinogenic factor E_1, since cells possessing this factor are unable to transfer the ability to produce colicin E_1 or chromosomal markers unless the F factor of fertility is introduced into these cells (Fredericq, 1954; Clowes, 1963*a*). In this study, evidence is presented which demonstrates an association of a genetic determinant of fertility with the V colicinogenic factor and the lack of such an association in the case of the E_1 colicinogenic factor. In addition, some of the properties of these two types of colicinogenic factors are described.

Materials and Methods

Organisms. The bacterial strains used in this work are described in Table 1. Strains producing or resistant to a specific colicin were isolated, as described by Fredericq (1957). In general, K-30 was used as a donor for the E_1 colicinogenic factor and K-94 as a donor for V. Azide-resistant strains were selected by culturing on nutrient agar plates containing sodium azide at a concentration of 2×10^{-3} M.

The male specific phage employed was phage μ (Dettori, Maccacaro, and Piccinin, 1961) and

TABLE 1. *List of strains**

Strain	Colicin produced	Auxotrophic characteristics	Resistance markers
C600	—	*thr⁻ leu⁻ thi⁻ lac⁻*	—
C600/ E_1VAz	—	*thr⁻ leu⁻ thi⁻ lac⁻*	Az, E_1, V
C600 $V^+F_v^+$	V	*thr⁻ leu⁻ thi⁻ lac⁻*	Az, E_1, V
C600 E_1^+	E_1	*thr⁻ leu⁻ thi⁻ lac⁻*	Az, E_1, V
C600 $E_1^+V^+$-F_v^+	E_1, V	*thr⁻ leu⁻ thi⁻ lac⁻*	Az, E_1, V
C600 S^R	—	*thr⁻ leu⁻ thi⁻ lac⁻*	S
C600 S^R/ E_1V	—	*thr⁻ leu⁻ thi⁻ lac⁻*	S, E_1, V
YS40	—	*his⁻ pro⁻*	S
YS40/V	—	*his⁻ pro⁻*	S, V
YS40/ E_1V	—	*his⁻ pro⁻*	S, E_1, V
K-94	V	—	—
K-30	E_1, V	—	—
HfrH	—	*thi⁻*	Az
HfrH/V	—	*thi⁻*	Az, V
HfrH/E_1	—	*thi⁻*	Az, E_1
HfrH/ E_1V	—	*thi⁻*	Az, E_1, V

* The following abbreviations are used: *his*, histidine; *pro*, proline; *thr*, threonine; *leu*, leucine; *thi*, thiamine; *lac*, lactose; S, streptomycin; Az, sodium azide; E_1, colicin E_1; V, colicin V; *colE*$_1$, colicinogenic factor determining E_1; *colV*, colicinogenic factor determining V; F_v^+, defined in text. C600, C600 S^R and HfrH were obtained from K. Fisher, YS40 from C. Yanofsky, and K-94 and K-30 from P. Fredericq. The parent strains were made colicinogenic or resistant to the indicated markers, as described in the text.

was obtained from K. Fisher. This phage absorbs to and lyses only *E. coli* male strains, harboring F either in the autonomous (F^+) or integrated (Hfr) state.

Media. Liquid cultures generally were prepared in 0.8% Difco nutrient broth, supplemented with 0.5% Difco peptone, 0.5% NaCl, 0.15% KH_2PO_4, 0.35% Na_2HPO_4, 0.1% glucose, and thiamine at a concentration of 5 μg/ml. Hard nutrient agar was prepared by solidification of the supplemented nutrient broth by the addition of 1.5% agar. Soft nutrient agar contained 0.65% agar. The minimal medium employed was that of Vogel and Bonner (*unpublished data*). Amino acid supplements were added at a final concentration of 30 μg/ml when necessary, streptomycin was added at a final concentration of 250 μg/ml, and sodium azide at a concentration of 2×10^{-3} M.

Transfer of colicinogenic factors. Exponential-growth cultures of donor and recipient strains were mixed at a 1:1 ratio and incubated at 37 C for the desired time. The cells were then diluted and the mating pairs were separated by 60-sec agitation with a Vortex Junior mixer. The diluted cells were then plated on nutrient agar containing either streptomycin or azide to counterselect the donors. The plates were covered with 8 ml of hard agar, and the colicinogenic colonies were detected by use of the double-layer technique of Fredericq (1957). When it was necessary to distinguish colonies producing both colicins E_1 and V from colonies producing one or the other, seeding with a double indicator was employed. The double indicator consisted of two strains, one sensitive to both colicins and one sensitive to E_1, but resistant to V. The ratio of colicin V-resistant indicator to doubly sensitive indicator used was 1:10. After incubating these plates at 37 C, a colony producing V alone was at the center of a large turbid zone, a colony producing E_1 alone was at the center of a small clear zone, and a colony producing both colicins was at the center of a small clear zone surrounded by a large turbid one. When it was desirable to test colonies for both colicinogeny and sexuality, the double-layer plates were incubated for 24 hr and the colonies were numbered, picked, and inoculated into 1 ml of nutrient broth and grown overnight for the sexuality test. The plates were then exposed to chloroform vapors, to kill cells brought to the top by this process, and seeded, to test for colicin production. The cultures of the few colonies that could not be scored unambiguously by this technique were stabbed on plates and incubated for 24 to 48 hr, and then the macrocolonies were exposed to chloroform vapors and seeded with a proper indicator strain (Fredericq, 1957). The cultures to be tested for susceptibility to phage μ were spread in small patches on a nutrient agar plate and a loopful of phage μ at a concentration of approximately 10^{12} phage per ml was added to the center of each patch. A positive test was indicated by a clear zone in the center of the spread culture after incubation at 37 C for several hours.

Recombination. Mating to test for recombina-

tion between strains was done by mixing at a 1:1 ratio cells of the donor and recipient strains individually grown in nutrient broth to a concentration of about 2×10^8 cells per ml. The mating mixtures were incubated with shaking at 37 C for the designated time and then diluted and plated on a selective medium. When it was necessary to test a very large number of colonies, a modification of this method was used as a preliminary test of the recombining ability of a colony. In this case, overnight cultures of the colony to be tested and a suitable recipient were obtained, diluted 1:20 with nutrient broth, mixed at a ratio of 1:1, and incubated at 37 C for 4 hr with shaking. A sample from each tube was then removed with a wire loop delivering a volume of 10 μliters, added to a drop of saline solution on the selective plate, and spread. The plates were then incubated at 37 C for 2 days.

Exposure to acridine orange. To test the effect of acridine orange on the colicinogeny or sexuality of the strains, a variation on the method of Hirota (1960) was used. The strains to be tested were grown to stationary phase and inoculated into nutrient broth adjusted to pH 7.6 which contained acridine orange at a final concentration ranging from 25 to 40 μg/ml. The initial concentration of cells in the tubes containing acridine orange ranged from 10^2 to 10^4 per ml. These cultures were incubated at 37 C for 1 to 2 days, diluted, and then plated, to test the survivors for removal of the F and colicinogenic factors. When 2-day growth of the cultures was required, they were rediluted to keep them out of stationary phase.

RESULTS

Association of the V^+ and F_v^+ properties during transfer. The degree of association between colicinogenic factors E_1 and V and a genetic determinant of fertility, which we have designated F_v, was tested by transferring these factors between several different donor and recipient strains. Regardless of the combination of donor and recipient strains employed, the V^+ and F_v^+ characters were always transferred together to the recipient cell. In the case of the ability to produce colicin E_1, however, concomitant transfer with the F_v^+ characteristics was observed only in a fraction of the cases. This relationship is shown in Table 2, which summarizes the results of the transfer of these properties from C600 $E_1^+V^+F_v^+$ to YS40/E_1V. Of the 738 recipients tested, none exhibited either the F_v^+ or the V^+ functions alone, whereas approximately 25% of the recipients which received the E_1 factor did not receive the F_v^+ character.

TABLE 2. *Transfer of V^+, F_v^+, and E_1^+ characteristics**

Expt	No. of recipient cells acquiring characters indicated				
	$F_v^-V^-E_1^-$	$F_v^+V^+E_1^+$	$F_v^+V^+E_1^-$	$F_v^-V^-E_1^+$	$F_v^+V^-$ or $F_v^-V^+$
1	89	27	12	16	0
2	108	45	26	19	0
3	111	54	25	8	0
4	129	45	13	11	0
Totals	437	171	76	54	0
Per cent	59	23	10	8	0

* C600$E_1^+V^+F_v^+$ was the donor strain and YS40/E_1V the recipient strain. The strains were crossed for 20 min. The F_v^+ character of the recipients was determined by testing with phage μ.

Transfer of the V^+ and E_1^+ properties to F^+ and F^- recipients. An association between the V^+ and F_v^+ determined functions was further indicated by the difference in transfer of the *colV* factor to F^- and F^+ recipients. As shown in Table 3, the V colicinogenic factor with its associated F_v^+ character is transferred more efficiently to F^- than to F^+ recipients. Although consistently observed, the absolute magnitude of this difference varied, depending upon the particular donor and recipient strains employed. In contrast, *colE*$_1$ was transferred to approximately the same extent to either an F^+ or an F^- recipient. The efficient transfer of the *colE*$_1$ factor to either type suggests that the significantly lower *colV* transfer to an F^+ recipient is not due to lower efficiency in the formation of mating pairs, as compared with an F^- recipient.

Comparison between F- and colV-promoted recombination. Although the $V^+F_v^+$ strains are able to recombine with an F^- recipient, it was noted that the recombination rate was lower than that found in crosses promoted by the ordinary F of *E. coli*. This is particularly the case for the *his* marker which is donated at a frequency

TABLE 3. *Transfer of the V^+ and E_1^+ characteristics to F^+ and F^- recipients**

Expt	Donor	Recipient	Total†	Per cent V^+	Per cent E_1^+
1	K-94 (V^+)	C600 F^+/E_1VAz	1,292	1.8	—
		C600 F^-/E_1VAz	120	78.0	—
2	K-30 ($V^+E_1^+$)	C600 F^+S^r/E_1V	170	0.6	100
		C600 F^-S^r/E_1V	42	24.0	100
3	K-30 ($V^+E_1^+$)	YS40 F^+/E_1V	1,226	2.6	90
		YS40 F^-/E_1V	190	7.9	95

* The strains were crossed for 4 hr in experiments 1 and 2 and for 3 hr in experiment 3.

† This total refers to the number of recipient colonies tested for the production of colicins V and E_1.

TABLE 4. *Recombination of F^+ and col^+ strains**

Expt	Donor	*pro* recombinants		*his* recombinants	
		No.†	Per cent F^+	No.†	Per cent F^+
1	F^+	204	100	405	100
	$V^+F_v^+$	133	65	38	9
	$V^-F_v^-$	0	0	0	0
2	F^+	368	100	453	100
	$V^+F_v^+$	105	29	24	5
3	F^+	84	100	108	100
	$V^+F_v^+$	31	37	7	6
4	F^+	—	—	422	100
	$E_1^+F^+$	—	—	356	85
	$E_1^+F^-$	—	—	0	0
	$V^+F_v^+$	—	—	28	7

* Donor strain was C600/E_1VAz and recipient YS40/E_1V. The strains were crossed for 4 hr.

† A number of 100 would be equivalent to a recombination rate of approximately 10^{-5} recombinants per recipient.

of a factor of 10 or more lower than the F-promoted recombination of this marker, with YS40/E_1V as a recipient (Table 4). The absolute magnitude of this difference varied with the particular donor or recipient strains employed, and was independent of the time allowed for the mating, when tested over a 2-hr period. The lower recombination frequency with $V^+F_v^+$ strains does not appear to be caused by a lower ability of this strain to form a conjugating pair with an F^- strain, as *colV*-promoted transfer of the *colE*$_1$ factor is as efficient as the F-promoted transfer. *ColE*$_1$ alone was not able to promote recombination with an F^- strain of these and several other markers that were tested, nor did it appear to interfere with the F-promoted recombination.

Instability of $V^+F_v^+F^+$ strains. Upon transfer of *colV* to an F^+ recipient, a variable but marked incompatibility of the $V^+F_v^+$ and the F^+ characters in the recipient was observed. The extent and nature of this instability was examined by isolating and subculturing a number of the C600 F^+ recipients receiving *colV*. As shown in Fig. 1, subcultures of $V^+F_v^+F^+$ strains segregate $V^+F_v^+F^-$ and $V^-F_v^-F^+$ strains at a high frequency. The distinction between a $V^+F_v^+F^-$ strain and a $V^+F_v^+F^+$ strain is based on the difference in the number of *his*$^+$ recombinants in a cross, with YS40/E_1V as recipient. The number of *his*$^+$ recombinants with an F^+ donor is over ten times greater than with a $V^+F_v^+$ donor when this strain is used as recipient. Segregation of the $V^+F_v^+$ and F^+ characters continued even after three cycles of subculturing.

Removal of F and colicinogenic factors by acridine orange. Colicinogenic factors E_1 and V also showed a difference in susceptibility to removal by growth in the presence of acridine orange. Conditions that led to the curing of *colV* did not cure the *colE*$_1$ factor from colicinogenic strains (Table 5). The curing of the *colV* factor was not as efficient or reproducible as the curing of the F factor, and usually required 2 days of growth in the acridine orange medium with redilution to keep the culture out of stationary phase. From experiment 3, 20 $V^+E_1^+$ survivors were tested and proved to retain their F_v^+ character, as shown by susceptibility to phage μ and the ability to recombine at the F_v^+ level. In the same

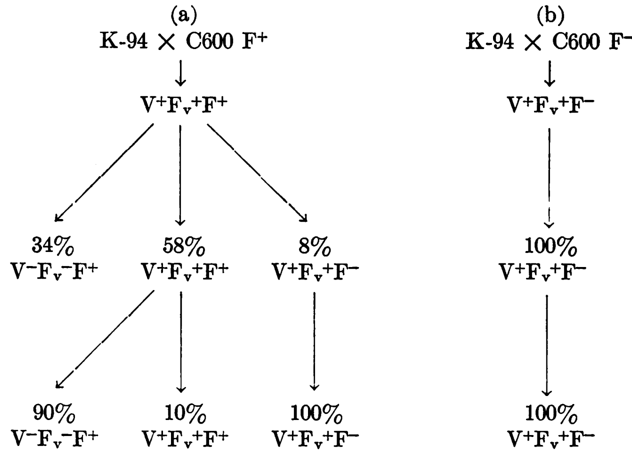

FIG. 1. *Instability of C600 $V^+F_v^+F^+$ strains. The $V^+F_v^+F^+$ recipients were isolated at low frequency from the original cross, whereas the $V^+F_v^+F^-$ recipients occurred at high frequency, as shown in Table 3.*

experiment, 20 $V^-E_1^+$ survivors were all resistant to phage μ and unable to recombine with an F^- recipient, indicating a loss of their F_v^+ characteristic. Retesting of $V^+F_v^+$ survivors after a 2-day treatment with acridine orange showed that these cells did not represent a special class of cells resistant to acridine orange treatment.

DISCUSSION

The experiments described above demonstrate an association between the genetic determinants of fertility and colicin V production and the lack of such an association in the case of the genetic determinant of colicin E_1 production.

Experiments on the transfer of the colicinogenic factors showed that $V^+F_v^+$ strains can transfer the *colV* factor and, if present in the same cell, *colE*$_1$ to an F^- recipient, whereas strains harboring the *colE*$_1$ factor alone were not able to effect its transfer. Furthermore, transfer of the ability to produce colicin V was always concomitant with the transfer of the F_v^+ characteristics, whereas transfer of the ability to produce colicin E_1 showed no such simultaneous transfer. The preponderance of the doubly colicinogenic state among the colicinogenic recipients does indicate, however, that the transfers of the two colicinogenic factors are not entirely independent events. A possible explanation for this is that, after formation of the mating pair, it is very likely that both colicinogenic factors are transferred. Smith, Ozeki, and Stocker (1963) have shown a similar result for the transfer of colicinogenic factors E_1, E_2, and I in *Salmonella typhimurium*. In this case, the fertility characteristics are associated with *colI* in that the two properties are inseparable through transfer to recipient strains.

TABLE 5. *Curing by acridine orange*

Strain	Acridine orange concn	Per cent removal of indicated factors		
		F	V	E_1
	µg/ml			
C600 $E_1^+F^+$	0	0 (10)*	—	0 (219)
	25	70 (20)	—	0 (213)
	30	93 (15)	—	0 (44)
	30	100 (20)	—	0 (356)
	35	—	—	0 (412)
	40	—	—	0 (69)
C600 $V^+F_v^+$	0	—	<1 (305)	—
	25	—	1 (77)	—
	30	—	7 (98)	—
	30	—	8 (117)	—
	35	—	85 (26)	—
	35	—	99 (168)	—
C600 $V^+F_v^+E_1^+$	0	—	0 (360)	0 (389)
	30	—	58 (202)	0 (241)
	30	—	58 (171)	0 (171)
	35	—	50 (86)	0 (86)

* Numbers in parentheses refer to actual number of clones examined.

An examination of the ability of the colicinogenic factors to promote genetic recombination revealed that, whereas E_1^+ strains are unable to

recombine with F^- strains, $V^+F_v^+$ strains are able, but at a lower frequency than that found for an F^+ strain. Smith and Stocker (1962) have similarly shown that a $colE_1$-containing *S. typhimurium* strain was unable to promote recombination with several recipients. Upon examining recombination with an $F^-his^-pro^-$ recipient (YS40/E_1V), the transfer of *pro* from a $V^+F_v^+$ strain varied from approximately 30 to 65% of an F^+ control, whereas the *his* transfer was approximately 5 to 10% of the F^+ control. In a similar investigation of recombination in *E. coli*, but mediated by colicinogenic factor I, Clowes (1961) found that, although the yield of prototrophic recombinants was about 100 times less than in an F-promoted cross, the relative frequency of the different recombinant classes was about the same. It should also be noted that the presence of the E_1 colicinogenic factor in cells does not make them susceptible to phage μ, a male specific ribonucleic acid phage (Dettori et al., 1961), whereas $V^+F_v^+$ cells show sensitivity to this phage. The fertility factor associated with I colicinogenicity does not elicit this sensitivity to μ (Clowes, 1963*b*).

Our inability to obtain stable $V^+F_v^+F^+$ strains is analogous to the inability of Scaife and Gross (1962) to isolate Hfr or F^+ strains also harboring an autonomous *F-lac* factor and to the inability of de Haan and Stouthamer (1963) to obtain a bacterial strain carrying both *F-lac* and *F-gal*. This instability of $V^+F_v^+F^+$ strains could account for the significantly lower *colV* factor transfer to an F^+ recipient, as compared with an F^- recipient. The results of the experiments on $colE_1$ transfer, which suggest that mating pairs can form approximately as well between an F^+ or F^- recipient and a $V^+F_v^+$ strain, further support this hypothesis.

The demonstration that the *colV* factor can be cured by the acridine orange treatment, whereas the $colE_1$ factor cannot, is also consistent with a relationship between *colV* and F properties. These results do not agree with those of Furness and Rowley (1957), who reported a separation of fertility and colicinogenic properties upon treatment of fertile I colicinogenic strains with cobalt chloride. It is difficult to evaluate this result, however, since their conclusion is based on the detection of one colicinogenic and nonfertile colony out of a large number of colonies that were tested after treatment with cobalt chloride. As indicated in Table 5, it was generally found that curing of *colV* is more difficult and less reproducible than F-factor curing. Nevertheless, in every case tested, μ phage susceptibility and the ability to recombine (F_v^+ properties) were removed simultaneously with the ability of the cell to produce colicin V, again indicating a firm association between the genetic determinants for these properties. Although no elimination of the $colE_1$ factor was observed, even from cells cured of F or *colV*, it is, of course, possible that suitable conditions for $colE_1$ removal have not been discovered.

In a comprehensive review on the inheritance of drug resistance in bacteria, Watanabe (1963) described many properties of the R factor, an episome carrying the multiple drug-resistance markers, which are similar to those reported here for the V colicinogenic factor. Specifically, the R factor can promote genetic recombination, although at a lower rate than the F factor of *E. coli;* it can be eliminated at low frequency with acridine dyes; and it is transferred at lower frequency to F^+ than to F^- recipients. In addition, Kato and Hanaoka (1962) have shown that transfer of R factor to a doubly colicinogenic (K^+X^+) strain of *E. coli* induced loss of the X or K colicinogenic factors from this strain. This loss appeared to be a true loss rather than just a suppression, since elimination of the R factor by acridine orange treatment did not restore colicinogenicity.

Jacob, Brenner, and Cuzin (1963) advanced a "replicon" hypothesis to account for the accumulated data on F transfer and chromosome transfer. According to this hypothesis, the specific F antigen is localized at a limited number of sites on the bacterial cell wall. At these sites, the F factor is attached inside the cell membrane, whereas additional sites of attachment are postulated for other episomes. The incompatibility between related episomes is explained on the basis of this hypothesis as a competition for a limited number of specific attachment sites. Thus, according to this view, it is the competition between *colV* and F for the same attachment site that accounts for their incompatibility. On the other hand, $colE_1$ is compatible with F or *colV* because of its attachment to a different site.

While several differences between the F_v^+ and the F^+ properties have been indicated throughout this paper, it is not clear whether they represent

fundamentally different fertility determinants or just an alteration of the F factor due to its association with the genetic determinant for the production of colicin V. Another question is the nature of the association between the genetic determinants of fertility and colicin V production. The data presented in this report are not inconsistent with these two phenotypic properties being determined by genes on the same episomal factor. To resolve these questions, attempts are being made to dissociate the genetic determinants of fertility and colicin V production and to examine them individually.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grant AI-05210-01 from the National Institute of Allergy and Infectious Diseases. Phyllis L. Kahn held a Post-doctoral Research Fellowship from the National Institute of Allergy and Infectious Diseases.

We wish to thank Carol Segina for invaluable technical assistance.

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