acid equivalents as determined by the orcinol reaction) and has an absorbance ratio of 1.40 at 2800 to 2600 Å (measured at a concentration of 0.5 mg/ml in 0.15*M* saline at *p*H 3.7).

Thus, the cationic protein fraction of rabbit PMN granules originally described by Zeya and Spitznagel (*6*) is capable, by itself, of producing an inflammatory reaction in rat and rabbit mesentery. This component of the granules is likely to be responsible for the adhesion and emigration of leukocytes produced by the complete granule lysate.

Besides sticking and emigration of leukocytes, another frequent event in the delayed-phase reaction to tissue insult (infection and thermal injury) is the participation of capillary vessels (in addition to venules) in the affected area. In view of the fact that this capillary phase of the reaction is not often observed after the introduction of the standard mediators of inflammation into tissues (*11*), the production of a capillary-phase reaction by cationic proteins extracted from leukocyte granules is of special significance. The presence of these substances in lysosomes (at least of leukocytes) provides further evidence of an important role of these cytoplasmic particles in tissue-injury reactions. The production, by rabbit PMN lysosomes, of leukocyte sticking and emigration in autologous tissue (donor mesentery) also supports the view that these particles take part in the pathophysiology of inflammation. Whether the sticking reaction produced by cationic protein involves an alteration of surface charges or a more complex mechanism is unknown.

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Phenotypic Repair of RNA-Bacteriophage Mutants by Streptomycin

Abstract. *A class of host-dependent mutants of RNA-phage f2 are dependent on streptomycin for growth in certain bacterial hosts. Maximum response to streptomycin occurred late in the growth cycle of phage, indicating phenotypic "repair" of a late phage function.*

Gorini and Kataja (*1*) have recently described a class of mutants of *Escherichia coli* which show a conditional dependence for streptomycin. One of the mutants, an arginine auxotroph, which possessed a defective structural gene for the enzyme ornithine transcarbamylase, synthesized functional enzyme when streptomycin (SM) was added to the growth medium; that is, the arginine auxotroph grew in a medium without arginine provided streptomycin was present. Because other amino-acid auxotrophs showed a similar response to streptomycin (*1*), Gorini and Kataja postulated a general streptomycin-activated suppression mechanism which might function to repair (correct) phenotypically mutations in a wide variety of cistrons.

Suppression of many different mutants by particular bacterial suppressor genes has been described by Benzer and Champe (*2*) for bacteriophage T4 rII mutants, by Garen and Siddiqi (*3*) for alkaline phosphatase mutants, by Campbell (*4*) for the class of bacteriophage lambda hd mutants, and by Epstein, *et al.*, (*5*) for the class of bacteriophage T4 amber mutants. Some of these suppressor-sensitive mutants have been shown to be phenotypically reverted by 5′-fluorouracil (*6*).

Using the Garen and Campbell strains, Zinder and Cooper (*7*) isolated a set of host-dependent mutants of the RNA-containing bacteriophage, f2. We are now reporting that one of the classes of these mutants is partially restored by streptomycin, and that the time during the phage's latent period in which the reagent was most effective can be determined. This serves to indicate the time when the gene represented by the mutation plays its most critical role.

Bacteriophage mutants of the class su-3 (*7*) are host-dependent mutants which produce plaques on strain K37 of *E. coli* (permissive for phosphatase mutants) but do not produce plaques on strain K19, (a derivative of C600, permissive for hd mutants of lambda bacteriophage) or on K38 (nonpermissive parent of K37). Our strain K19 was resistant to high concentrations of streptomycin. The genetic lesion in su-3 has not yet been completely defined; it is, however, a single-step mutation. Details of the characterization of su-3 are presented elsewhere. Infection of strain K38 by su-3 elicits no detectible response, implying an early block in virus synthesis. Infection of K19 with su-3 (multiple infection) results in a several-fold increment in the viral-induced RNA polymerase without significant production of phage particles, the lack implying a block in a late function. These two aspects of the su-3 mutation are not yet reconcilable. In-

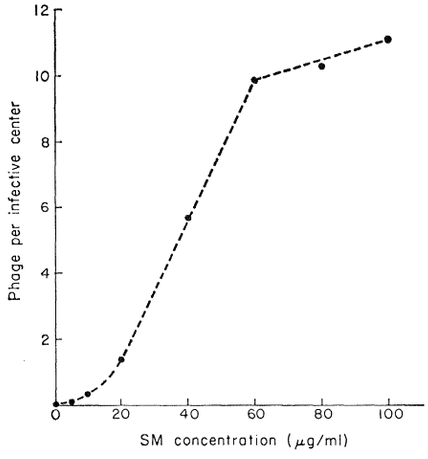

Fig. 1. Effect of streptomycin concentration on the growth of su-3 on K19. To K19 growing exponentially in tryptone broth (1.7×10^8 cells per milliliter) was added su-3 at 0.5 particles per bacterium. After 10 minutes of adsorption at 36°C, the cells were centrifuged and antiserum to bacteriophage f-2 was added to eliminate unadsorbed phage. The infected cells were diluted with tryptone broth (10^4 cells per milliliter) with different amounts of streptomycin (0 to 200 μg/ml) and incubated at 36°C for 50 minutes to allow development of phage. Chloroform and lysozyme were added to terminate growth, and the solution containing phage was plated on permissive and nonpermissive hosts.

Table 1. Single-cell analysis of bacteriophage su-3 on strain K19 of *E. coli* with and without the addition of streptomycin (SM). The figures are numbers of phages. The number of tubes was 31; the average number of bursts per tube was 0.7; and the number of doubles was 4 to 5.

No SM	SM
1	1
1	1
2	2
2	9
2	38
2	41
2	67
2	91
3	94
5	106
5	234
9	405
10	480
38	
53	

fection of K19 with su-3 under conditions of single infection results in the production of an infective center (yield of a few viable particles) from 10 to 20 percent of the infected bacteria. These particles are produced very late; for example, when the culture is incubated in broth for 60 minutes and artificially lysed, the particles are not yet present. Su-3, as the other single-mutant stocks of f2, contains about 10^{-3} revertants, and these each yield 250 to 500 wild-type progeny on K19. It is against this background that the effects to be described must be measured.

Addition of streptomycin (100 μg/ml) to agar-layer plates seeded with K19 did not enhance the production of visible plaques by su-3; instead, streptomycin tended to reduce the size of plaques produced by wild-type phage under these conditions and appeared to block phage penetration, in agreement with Brock (*8*). For this reason, all experiments were carried out in liquid culture (tryptone broth) with streptomycin (usually 100 μg/ml) being added only after a period sufficient for phage attachment. An actively growing culture was infected with su-3 (single infection) and then diluted into broth with or without the antibiotic. After incubation for 60 minutes, the cells were lysed with chloroform and lysozyme. The progeny phage was plated on permissive and nonpermissive hosts. Wild-type phage forms plaques on both indicators, and thus the yield of su-3 can be determined by the difference in the numbers. Figure 1 shows the yield of su-3 as a function of streptomycin concentration. A maximum response was observed with 100 μg/ml. Under these conditions, about 10 to 20 viable su-3 were produced per infective center. This represents at least a 20-fold increase over the control without SM, but is only a few percent of the yield on K37. Su-3 yields approximately 500 particles on K37. It is evident that streptomycin is not fully able to activate the system and mimic completely the permissive host.

The phage yield from individual bacteria growing in the presence of streptomycin (Table 1) showed a very wide variation with yields of from one to several hundred for different cells. As shown in Table 1, the percentage of cells that yield phage is equivalent to the number of potential infective centers produced by su-3 in the absence of streptomycin. This represents about 10 to 20 percent of the cells initially infected with phage. Streptomycin "rescue" apparently occurred only in the cells in which spontaneous "leakage" took place, that is, only "infective centers" responded to streptomycin. For this reason, all data have been normalized to the phage yielded per infective center rather than to the phage produced per infected cell.

This reversal of phenotype by streptomycin affords us the opportunity to determine the time during the phage's latent period when streptomycin is most effective. Two kinds of experiments were done. The antibiotic was added to cells at various times after infection and was allowed to remain to a fixed termination of about 60 minutes—the end of normal growth cycle. Infected cells were also diluted into or out of streptomycin, such that the latent period was divided into a series of 10 minute pulses with streptomycin. The results are presented in Table 2. Thus both experiments show that the streptomycin is most effective 30 to 40 minutes after infection. These results indicate that su-3 is blocked in its growth on K19 because of a mutation in a gene which functions most critically late in infection, and agree with the results previously obtained from analysis of enzyme production.

These findings focus attention on the role of streptomycin as an external suppressor substance. Superficially, the action of this antibiotic is similar to that produced by the pyrimidine analog, 5′-fluorouracil. Champe and Benzer (*6*) have shown that the maximum phenotypic response to 5-FU for the rescue of rII mutants occurred early during the period when synthesis of messenger RNA is assumed to take place. They conclude that 5-FU functions by incorporation into phage messenger RNA. Streptomycin obviously does not function in this way and probably as postulated by Gorini and Kataja acts at the time of messenger translation. Restoration of function by streptomycin should, therefore, be most effective at the period of greatest need for the protein.

Table 2. Effect of streptomycin (SM) on yield of su-3 infecting K19. Phage was added to bacteria in the log phase (2×10^8 cells/ml) at a multiplicity of 0.5. All of the cultures were lysed with chloroform and lysozyme 63 minutes after infection and plated for yield on K37 (permissive) and K38 (nonpermissive). Yield on K37 includes su-3 and revertants. The input phage contained 10^{-3} revertants. Ten percent of the infected bacteria gave an infective center, when plated on K37, 10 minutes after infection. Sufficient numbers of the plaques on K37 were tested for their mutant character in order to ensure the validity of the su-3 yield obtained by subtraction.

Time of SM pulse (min)	Phage yield		Yield su-3/infective center
	K38	K37	
	*Experiment A**		
13–63	2.9×10^7	4.8×10^8	23.7
23–63	2.5×10^7	4.4×10^8	22.1
33–63	3.4×10^7	4.6×10^8	22.4
43–63	3.1×10^7	1.7×10^8	7.3
53–63	3.1×10^7	8.0×10^7	2.6
	5.1×10^7	5.3×10^7	< 1
	Experiment B†		
13–23	5.2×10^7	4.7×10^7	0
23–33	4.0×10^7	7.7×10^7	1.9
33–43	4.0×10^7	1.3×10^8	4.7
43–53	3.6×10^7	1.0×10^8	3.3
53–63	4.1×10^7	5.2×10^7	0.5

* Experiment A: After adsorption of phage and treatment with antiphage serum, the bacteria were diluted 10^{-5}. Streptomycin was added to portions at the indicated times. † Experiment B: Bacteria were handled as in experiment A but diluted only 10^{-3} to medium without SM. Streptomycin was added at the indicated times and removed by a 1/100 dilution at the indicated times.

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