Effect of Rifampin on the Development of Ribonucleic Acid Bacteriophage Q β

JADWIGA PASSENT¹ AND PAUL KAESBERG

Biophysics Laboratory and Biochemistry Department, University of Wisconsin, Madison, Wisconsin 53706

Received for publication 29 April 1971

The drug rifampin, when added at the time of infection, inhibits synthesis of the phage Q β . Both viral ribonucleic acids and viral proteins are made in nearly the same amount as in the absence of rifampin, but the rate of assembly into phage particles is low.

Rifampin (formerly known as rifampicin) is 3-(4-methyl-1-piperazinyl)imino methyl rifamycin SV (14). It is known to inhibit specifically the deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) synthesis in *Escherichia coli* (5) by forming a stable complex with RNA polymerase (19). Rifampin acts also as an antiviral agent. It inhibits vaccinia virus replication (6, 17) by preventing the formation of the core polypeptide from a larger precursor (10).

According to Fromageot and Zinder (2), rifampin at a concentration of 45 μ g/ml, when added at the time of infection, severely limits f2 phage growth in *E. coli* K225. However, when added 5 min after infection, growth proceeds in nearly normal fashion. Friesen (1) noted a similar inhibitory effect although he observed that addition of the antibiotic 15 min after infection caused a threefold reduction of f2 plaque-forming units (PFU). The extent and cause of inhibition resulting from rifampin addition at time of infection are not known. We have found that rifampin inhibits the synthesis of phage Q β to an even greater extent than it does f2. We have studied the nature of this early inhibition in some detail and conclude that early events, phage RNA and protein synthesis, proceed normally but that a late event, phage assembly, is markedly inhibited. Thus, both PFU and physical particles are made in only small amounts.

MATERIALS AND METHODS

Virus and bacteria. Phage Q β was originally obtained from C. Weissmann and phage R17 from A. Graham. *E. coli* Q13 strain, obtained from T. Sugiyama, was used throughout our work.

Growth and assay conditions. Cells were grown over-

night at 37 C in tryptone broth. Portions of such cultures were diluted into tryptone broth containing 1mM CaCl₂ to optical density at 590 nm (OD₅₉₀) between 0.05 and 0.07, and growth was allowed to continue. Cultures were infected with phage Q β at OD₅₉₀ = 0.25, except as noted, and at multiplicity of infection (MOI) = 5. Rifampin (Schwarz/Mann, Orangeburg, N.Y.) was used at a concentration of 100 μ g per ml of culture. (i) To measure the number of infectious phage particles, 0.1-ml samples were withdrawn from the cultures at appropriate times and combined with 10 μ liters of 0.1 M tris(hydroxymethyl)aminomethane (Tris) buffer, pH 8.0, containing 5 μ g of lysozyme and 0.005 M ethylenediaminetetraacetic acid (EDTA). After 15 min of shaking at 37 C, cell debris was removed by centrifugation and phage titer was determined by the standard agar overlay technique. (ii) To measure uracil incorporation, 1 μ Ci of ¹⁴C-labeled uracil (specific activity, 52 Ci/mole; Schwarz/Mann) was added per 1 ml of culture at the time of infection. Duplicate 50- μ liter samples were transferred at appropriate times to glass-fiber filters treated previously with 0.1 ml of 1% sodium dodecyl sulfate (SDS), and cold trichloroacetic acid-insoluble radioactivity was assayed. (iii) To measure lysine incorporation, 10 μ Ci of ³H-lysine (specific activity, 15 Ci/mmole; Schwarz/Mann) was added per 1 ml of culture at time of infection. Duplicate samples of 50 μ liters were handled as in (ii) and were subsequently assayed for hot trichloroacetic acid-insoluble radioactivity by the method of Mans and Novelli (15). (iv) To determine the proportion of uracil incorporated into viral and cellular RNA, 10-ml portions of *E. coli* Q13 were infected with and without addition of rifampin. A 20- μ Ci amount of ¹⁴C-uracil was added to each portion at time of infection. After 40 min, the cultures were cooled in a dry ice-ethanol bath, and cells were collected by centrifugation (5 min at 5,000 $\times$ g). To each sample, 0.2 ml of a bentonite suspension (8% bentonite, 0.001 M EDTA, pH 7.5) and 0.1 ml of 10% SDS were added. Nucleic acids were extracted by the phenol method and precipitated from the aqueous layer with 2.5 volumes of absolute

¹ Present address: Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland.

ethanol. After overnight storage at -20°C , RNA was collected by centrifugation and dissolved in 0.1 ml of 0.25 M Tris buffer, pH 8.0. Samples containing about 16,000 counts/min were run on polyacrylamide gels containing SDS by the method of Lane and Kaesberg (12). Gels were crushed with an automatic gel crusher (1 fraction = 1 mm of gel). After addition of 0.1 μg of ribonuclease, each fraction was incubated with shaking for 3 hr at room temperature. Scintillation fluid [ethoxyethanol-toluene = 1:1, containing 5 g of 1-2,5-diphenyloxazole (PPO) per liter and 0.1 g of 1,4-bis-2-4-methyl-5-phenyloxazoly]-benzene (Me_2POPOP) per liter] was added, and radioactivity was assayed. (v) To measure synthesis of phage proteins, rifampin was added to one culture at time of infection and to a second culture 30 min after infection. Reconstituted ^{14}C -algal protein hydrolysate (10 μCi) and ^{14}C -lysine (10 μCi , 231 Ci/mole, both from Schwarz/Mann) were added to each culture 40 min after infection. Fifteen minutes later, the cultures were cooled in a dry ice-ethanol bath and cells were harvested by centrifugation. Cells were solubilized as described by Jockusch et al. (8). Samples were electrophoresed on gels containing SDS, as described by Glowacki-Strauss and Kaesberg (3) and fractionated with an automatic gel crusher. Gel fractions were left overnight with 10 ml of scintillation fluid consisting of one part of Beckman BBS-3 solubilizer and 10 parts of toluene containing 5 g of PPO and 0.1 g of Me_2POPOP per liter and were assayed for radioactivity.

All radioactivity measurements above were made with a Packard Tri-Carb liquid scintillation counter at an efficiency of about 80% for solubilized ^{14}C -samples, about 60% for ^{14}C -samples on filters, and about 25% for ^3H -samples on filters.

The ratio of viable particles to physical particles in phage lysates was obtained as follows. One-liter portions of *E. coli* Q13 were grown and infected in the presence or absence of rifampin. After 40 min of infection, the cultures were cooled in a dry ice-ethanol bath and cells were collected by centrifugation for 10 min at $6,000 \times g$. They were suspended in 1.5 ml of 0.1 M Tris buffer (pH 8.0) containing 0.005 M EDTA and 300 μg of lysozyme per ml. The suspensions were frozen and thawed three times. One-tenth volume of 0.1 MgSO_4 and 10 μg of deoxyribonuclease per ml were added, and the suspension was incubated for 10 min at 37°C . It was then centrifuged for 30 min at $30,000 \times g$. The pellet was extracted with 0.5 ml of Tris-EDTA buffer containing lysozyme, and the extracting liquid was added to the supernatant fluid. Plaque-forming titer was determined. Antigen-antibody precipitation was performed with bovine anti-Q β serum. To a portion of supernatant fluid containing 3×10^{10} PFU (a quantity far below that required to saturate the antiserum) were added lysing medium to a volume of 350 μliters , 2 M NaCl to a final concentration of 0.15 M, and 0.2 ml of four-fold-diluted anti-Q β serum. The diluted antiserum had been preadsorbed previously with an *E. coli* Q13 cell extract. The reaction was allowed to proceed overnight at 4°C . Precipitates were collected by centrifugation for 10 min at $5,000 \times g$ and washed twice

with 0.15 M NaCl. Their protein content was assayed by the method of Lowry et al. (13).

Preparation of a rifampin-resistant mutant. *E. coli* Q13 cells were grown overnight in tryptone broth medium containing 100 μg of rifampin per ml. Resistant cells were repeatedly cultured in the same medium. They were then plated on agar in the presence of 100 μg of rifampin per ml. One clone, designated RQ13, was isolated.

DNA-dependent RNA polymerase was assayed in crude extracts of *E. coli* Q13 and RQ13 as follows. Portions (50-ml) of RQ13 cells and Q13 cells were grown to $\text{OD}_{600} = 0.55$. Cells were collected and then disrupted by sonic oscillation in 0.01 M Tris buffer (pH 7.5) containing 0.01 M Mg^{2+} and 25% glycerol. The assay medium contained in 0.2 ml: uridine triphosphate, guanosine triphosphate, cytidine triphosphate, and adenosine triphosphate, 50 mmoles of each; phosphoenolpyruvic acid, 1 μmole ; pyruvate kinase, 2.5 μg ; Tris buffer (pH 8.0), 20 μmoles ; β -mercaptoethanol, 2.5 μmoles ; MgCl_2 , 2 μmoles ; ^3H -uridine triphosphate, 5 μCi (specific activity, 17.1 Ci/mole); and 20 μliters of the crude extract. Rifampin (1 μg) was added as indicated. Incubation was carried out for 10 min at 37°C and was stopped by addition of 3 ml of ice-cold 10% trichloroacetic acid. After 15 min, the cold precipitates were collected on glass-fiber filters and washed with $3 \times 5\text{-ml}$ portions of 10% trichloroacetic acid, absolute alcohol, and ether. The filters were dried, and radioactivity was assayed.

RESULTS

Preliminary experiments showed that for *E. coli* Q13 a somewhat higher rifampin concentration than used by Fromageot and Zinder (2) for *E. coli* K225 was required to inhibit cell growth. Therefore, in our experiments, we used 100 μg of rifampin per 1 ml of culture at cell concentrations of 2×10^8 to 3.5×10^8 cells/ml. Figure 1 shows that such rifampin concentration stopped *E. coli* Q13 cell growth within 5 min. Protein and RNA syntheses in uninfected cells were also rapidly suppressed.

Production of viable phage. Addition of rifampin at the time of Q β infection markedly affected development of progeny. Forty minutes after infection, the yield of Q β PFU grown in the presence of rifampin was 18% of that in the absence of rifampin. After that time phage yield with rifampin leveled off, whereas yield in its absence steadily increased. After 2 hr, the phage yield in the presence of rifampin was only 5% of the normal phage yield (Fig. 2).

Rifampin exerted a similar but quantitatively lesser effect on the multiplication of R17, a phage serologically related to f2. The yield of R17 PFU was about 20% of normal after 2 hr.

The absorbancy of cell cultures infected with Q β increased for about 50 min and then declined rapidly, signaling the onset of cell lysis (Fig. 1).

FIG. 1. Growth curves. (A) Infected cells, no rifampin (B) Infected cells, rifampin added 5 min after infection. (C) Infected cells, rifampin added at time of infection. (D) Infected cells, rifampin added 5 min before infection. (E) Uninfected cells. (F) Cells with rifampin added.

Lysis was complete within 120 min. Infected cells treated with rifampin showed no substantial increase of absorbancy, and there was only a small decline indicative of lysis. Nevertheless, in the absence of artificial lysis, 90% of the phage had been released into the medium within 120 min.

The yield of Q β PFU varied with the time of addition of rifampin. It approached normal yield when the drug was added 30 min after infection. Upon earlier addition, the yield varied approximately exponentially with the time of addition (Fig. 3). This would suggest that the "safe" time for addition of rifampin is substantially later for Q β infection than reported for f2. The inhibition of phage development by rifampin was irreversible. It may be seen (Fig. 4) that, when portions of cells infected in the presence of rifampin were transferred at various times to a medium without rifampin, essentially complete inhibition of phage yield was found if the removal occurred later than 20 min.

RNA synthesis. Despite the low yield of PFU upon early rifampin addition, there was substantial production of both RNA and viral proteins prior to the time of lysis and, as we will show below, the RNA and proteins were functional. Figure 5 shows kinetics of ^{14}C -uracil incorpora-

FIG. 2. Effect of rifampin (rifampicin) addition on, phage yield. Rifampin was added at the time of infection.

FIG. 3. Effect of time rifampin (rifampicin) addition on phage yield plotted as a per cent of PFU obtained in the absence of rifampin.

tion into RNA. It may be seen that incorporation is nil for uninfected cells treated with rifampin. Infected cells treated with rifampin 5 min after infection, at infection, and 5 min prior to infection synthesize RNA beginning about 10 min

FIG. 4. Effect of the time of rifampin (rifampicin) removal on phage yield. Rifampin was added at the time of infection. Portions were removed after 10 and 20 min; cells were collected and transferred to medium without rifampin. After 90 min of incubation, cells were lysed and phage titer was assayed. PFU in culture infected without rifampin was taken as 100%.

after infection and continue at an increasing rate until the onset of lysis. Comparison of these kinetic curves with those of untreated, infected and uninfected (not shown) cells suggests that the synthesis of virus-induced RNA in rifampin-treated cells is comparable to that in untreated cells. To verify that it is, in fact, viral RNA that is synthesized, radioactive RNA was isolated 40 min after infection from cells treated with rifampin in the manner of Fig. 5 and was analyzed by polyacrylamide gel electrophoresis, (Fig. 6). The RNA from infected, untreated cells migrated as four well defined bands; the slowest moving corresponded to Q β RNA (sedimentation coefficient 30S) and the others corresponded to 23, 16, and 4 to 5S *E. coli* RNA. The infected, rifampin-treated cells produced predominantly viral-induced 30S RNA. The relative incorporation into the several bands is given in Table 1. Combining these data with those of Fig. 5 shows that in experiments corresponding to curves B, C, and D, 1.4, 1.7, and 1.8 times as much viral RNA was produced in rifampin-treated cells as in untreated cells. We may conclude that rifampin does not adversely affect the synthesis of Q β -induced RNA.

Protein synthesis. Apart from the evidence of the production of Q β RNA synthetase required to

FIG. 5. Kinetics of ^{14}C -uracil incorporation into infected and uninfected cells. (A) Infected cells, no rifampin. (B) Infected cells, rifampin added 5 min after infection. (C) Infected cells, rifampin added at time of infection. (D) Infected cells, rifampin added 5 min before infection. (E) Uninfected cells with rifampin. Cells were infected at $\text{OD}_{590} = 0.4$.

achieve the RNA synthesis (see above), it can be shown directly from kinetics of radioactive amino acid incorporation that there is substantial virus-induced protein synthesis in rifampin-treated cells. Figure 7 shows the time course of ^3H -lysine incorporation. It may be seen that in rifampin-treated cells incorporation is underway within 10 min after infection and continues throughout the entire period of virus production. Jockusch et al. (8) have shown that polypeptide I, the viral specific subunit of Q β synthetase, as well as maturation protein and coat protein are synthesized in infected cells treated with rifampin. We have measured the synthesis of these Q β -specific proteins in the presence and absence of rifampin under a variety of conditions, but quantitative comparisons are difficult to obtain because of the large contribution of cellular protein synthesis in the absence of the drug. Figure 8 shows gel patterns of ^{14}C -amino acid incorporation into proteins in the period 40 to 55 min after infection.

FIG. 6. Gel electrophoresis of RNA synthesized in infected cells. (A) No rifampin. (B) Rifampin added 5 min after infection. (C) Rifampin added at time of infection. (D) Rifampin added 5 min before infection.

TABLE 1. Distribution of radioactivity among the four RNA species^a

RNA	Rifampin addition			
	None	5 min after phage	With phage	5 min before phage
30S	13.5 ^b	47.6	56.3	59.1
23S	38.3	20.6	18.5	12.9
16S	25.1	13.4	8.9	9.6
4-5S	23.1	18.4	16.3	18.4

^a As calculated from Fig. 6.

^b Values expressed as percentages.

Without rifampin, only coat protein is clearly evident above the large background of cellular protein synthesis (Fig. 8). We calculate that about 26% of the total amino acid incorporation is into coat protein. With rifampin, the maturation protein band is evident as well and contains about 12% of the total gel radioactivity; the remaining 88% is in the coat protein band. From these data and a knowledge of the ratio of total ¹⁴C-amino acid incorporation corresponding to profiles A and B, we calculate that incorporation into coat protein in the presence of rifampin was 60% of that in its absence. From reasonable estimates of the amount of synthesis of cellular

FIG. 7. Kinetics of ³H-lysine incorporation into infected and uninfected cells. (A) Infected cells, no rifampin. (B) Infected cells, rifampin added at time of infection.

proteins, together with the data of Fig. 7, we can estimate the amount of coat protein synthesized during other time periods. With rifampin addition, we estimate a minimum of 50% of normal coat protein production during these times. Similarly we can compare the effect of rifampin addition at the time of infection with its addition 30 min after infection under which condition we obtain a nearly normal yield of PFU (Fig. 3). When the drug was added at infection, protein synthesis was approximately 60% of that when the drug was added 30 min after infection, and the ratio of maturation protein to coat protein was nearly identical in the two cases. From these data we conclude that coat protein and maturation protein are made in nearly normal amount in the presence of rifampin. No polypeptide I synthesis was detected during the 40- to 50-min incorporation period, presumably because of the repressive effect of coat protein. However, we know from gel patterns of proteins labeled at earlier times, as well as from the uracil-incorpora-

FIG. 8. Gel electrophoresis of proteins made upon infection. (A) No rifampin added. (B) Rifampin added at time of infection.

tion experiments, that polypeptide I had been synthesized.

Yield of physical particles. The results of incorporation of RNA and protein precursors together with phage yield could suggest a normal yield of poorly viable particles. Thus we assayed the amount of Q β antigen in cell lysates after infection in the presence and absence of rifampin. Table 2 shows that the same quantity of viable particles resulted in formation of the same amount of antigen-antibody complex, regardless of the addition of rifampin and the consequent phage titer. We know from the work of Hung et al. (7) that unassembled components of Q β do not contribute significantly to the complex. It may be concluded then that decrease in the quantity of PFU upon addition of rifampin indicated a corresponding decrease in the number of physical phage particles.

Phage synthesis in a rifampin-resistant mutant. To exclude the possibility of a direct effect of rifampin on the assembly of Q β phage, we allowed phage to grow on a rifampin-resistant mutant, RQ13, derived from *E. coli* Q13. The resistance of RQ13 stems from the resistance of its RNA polymerase, as judged by in vitro assays (Table 3), rather than a permeability barrier to rifampin. After 2 hr of growth and overnight lysis, the phage were plated on both Q13 and RQ13. Table 4 shows that in the rifampin-resistant mutant Q β phage grew nearly as well in the presence of rifampin as in its absence.

Cell viability in the presence of rifampin. The

TABLE 2. Serological precipitation of Q β phage

Rifampin addition	Lysate (PFU/ml)	PFU used for reaction	Antigen-antibody precipitate (μ g of protein)
None.....	5.0×10^{12}	3×10^{10}	100
5 min before phage.....	8.6×10^{10}	3×10^{10}	109
With phage.....	7.1×10^{11}	3×10^{10}	99
5 min after phage.....	1.2×10^{12}	3×10^{10}	99

TABLE 3. DNA-dependent RNA polymerizing activity

Bacteria	Rifampin	Enzyme activity (counts/min)
Q13	—	33,100
	+	11,300
RQ13	—	35,000
	+	36,300

TABLE 4. Comparison of phage yield in rifampin-sensitive and rifampin-resistant strains of *Escherichia coli*

Phage grown on	Plating on Q13 (PFU/ml)	Plating on RQ13 (PFU/ml)
RQ13.....	3.6×10^{11}	3.4×10^{11}
RQ13 + rif....	2.7×10^{11}	2.3×10^{11}
Q13.....	3.8×10^{11}	3.6×10^{11}

low yield of PFU is not an obvious consequence of cell death induced by rifampin. To explain Fig. 3 and the incorporation data, there would have to be a lag period of about 30 min and then a rapid exponential cell demise after the addition of rifampin. We measured colony-forming ability of cultures grown in the presence and in the absence of rifampin. Figure 9 shows that cells in the presence of rifampin lose viability slowly and with no detectable lag period.

DISCUSSION

Rifampin, added at the time of infection, inhibits the production of Q β phage. Although the yield of PFU is only about 5% of normal, at least a normal quantity of phage RNA and a nearly normal quantity of phage proteins are made. The phage-induced RNA is indistinguishable from its rifampin-less counterpart. Almost all of it migrates in electrophoresis as a single band characteristic of undegraded RNA. Its increasing rate of synthesis indicates that it

FIG. 9. Colony-forming ability of *E. coli* Q13 cells in the presence and absence of rifampin (rifampicin).

serves as a template for viral RNA synthesis. The vigorous synthesis of phage proteins indicates that it functions as a messenger. The phage-induced proteins also show no aberrations. The induced RNA synthesis itself signifies the presence of Q β RNA synthetase, so we know that functional polypeptide I has been synthesized and that there exists an adequate pool of the cellular components of Q β RNA synthetase (9, 11). The small depression in yield of coat and maturation protein can be accounted for by the gradual loss of cells in the presence of rifampin (Fig. 9). From the cessation of polypeptide I synthesis late in infection, it is evident that coat protein plays its normal role of repressor (16). We have no assay for the biological activity of maturation protein, but by analogy with maturation protein amber mutants we know that even in the complete absence of maturation protein production of physical particles is not hindered.

Thus we judge that Q β phage precursors are made but they are not assembled into physical particles.

Early addition of rifampin is essential for inhibition of phage production, but the early events in phage protein and RNA synthesis, including the assembly of RNA synthetase from polypeptide I and cellular proteins, appear normal. It is a late function, phage assembly, that fails. This suggests that the effect of rifampin is indirect and probably is mediated through a cellular function. In cells carrying a rifampin-resistant *E. coli* RNA polymerase, Q β synthesis

proceeds normally in the presence of rifampin. Thus it is the inactivity of DNA-dependent RNA polymerase that results in the failure of phage assembly. We postulate that there must be a labile cellular site or factor essential for phage assembly, and in the presence of rifampin it is depleted.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grants AI-1466 and AI-21-942 from the National Institute of Allergy and Infectious Diseases and by grant AEC 1633 from the Division of Biology and Medicine, Atomic Energy Commission.

We are indebted to Andy Brugger for capable and conscientious technical assistance.

LITERATURE CITED

1. Friesen, J. D. 1969. Dependence of f2 bacteriophage RNA replication on amino acid. *J. Mol. Biol.* 46:349-353.
2. Fromageot, H. P. M., and N. D. Zinder. 1968. Growth of bacteriophage f2 in *E. coli* treated with rifampicin. *Proc. Nat. Acad. Sci. U.S.A.* 61:184-191.
3. Glowacki-Strauss, E., and P. Kaesberg. 1970. Acrylamide gel electrophoresis of bacteriophage Q β ; electrophoresis of the intact virions and of the viral proteins. *Virology* 42:437-452.
4. Godson, G. N., and R. L. Sinheimer. 1967. The replication of bacteriophage MS2. *J. Mol. Biol.* 23:495-521.
5. Hartmann, G., K. Hönikel, F. Knüsel, and J. Nüesch. 1967. The specific inhibition of the DNA-directed RNA synthesis by rifampicin. *Biochim. Biophys. Acta* 145:843-844.
6. Heller, E., M. Argaman, H. Levy, and N. Goldblum. 1969. Selective inhibition of vaccinia virus by the antibiotic rifampicin. *Nature (London)* 222:273-274.
7. Hung, P. P., E. M. Hale, and L. R. Overby. 1970. Comparative antigenicities of Q β phage, its structural components and reconstituted particles. *Virology* 38:703-706.
8. Jockusch, H., L. A. Ball, and P. Kaesberg. 1970. Synthesis of polypeptides directed by the RNA of phage Q β . *Virology* 42:401-414.
9. Kamen, R. 1970. Characterization of the subunits of Q β replicase. *Nature (London)* 228:527-533.
10. Katz, E., and B. Moss. 1970. Formation of vaccinia virus structural polypeptide from a higher molecular weight precursors: inhibition by rifampicin. *Proc. Nat. Acad. Sci. U.S.A.* 66:677-684.
11. Kondo, M. R., Gallerani, and C. Weissmann. 1970. Subunit structure of Q β replicase. *Nature (London)* 228:525-527.
12. Lane, L., and P. Kaesberg. 1971. Multiple genetic components in bromegrass mosaic virus. *Nature (London)* 232:40-43.
13. Lowry, O. H., N. J. Rosebrough, A. R. Farr, and R. J. Randall. 1951. Protein measurements with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.
14. Maggi, N., C. R. Pasqualucci, R. Ballotta, and P. Sensi. 1966. Rifampicin: a new orally active rifamycin. *Chemotherapy* 11:285-292.
15. Mans, R. J., and G. D. Novelli. 1961. Measurement of the incorporation of radioactive amino acids into protein by a filter-paper disc method. *Arch. Biochem.* 94:48-53.
16. Skogerson, L., D. Roufa, and P. Leder. 1971. Characterization of the initial peptide of Q β RNA polymerase and control of its synthesis. *Proc. Nat. Acad. Sci. U.S.A.* 68:276-279.
17. Subak-Sharpe, J. H., M. C. Timbury, and J. F. Williams. 1969. Rifampicin inhibits the growth of some mammalian viruses. *Nature (London)* 222:341-345.
18. Sugiyama, T. 1969. Translational control of MS2 RNA cistrons. *Cold Spring Harbor Symp. Quant. Biol.* 34:687-694.
19. Wehrli, W., R. Knüsel, K. Schmid, and M. Staehelin. 1968. Interaction of rifamycin with bacterial RNA polymerase. *Proc. Acad. Nat. Sci. U.S.A.* 61:668-673.