Lysis Inhibition in *Escherichia coli* Infected with Bacteriophage T4

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A technique of continuous filtration of T4-infected *Escherichia coli* has been devised to study the phenomenon of lysis inhibition. Studies using this technique revealed that the length of the lysis delay caused by superinfection can attain only certain discrete values, which for low average multiplicity of superinfection is thought to be a reflection of the actual number of superinfecting particles per cell. The time interval between primary and superinfection had little effect on the length of lysis delay. With increasing rate of superinfection, the length of lysis delay decreased. In superinfected cells, the concentration of endolysin exceeded the final concentration in nonsuperinfected cells. Superinfection of a lysing culture induced lysis inhibition immediately. Temperature-shift experiments, with cells primarily infected by a temperature-sensitive endolysin mutant, revealed that after the normal latent period superinfection was unable to induce lysis inhibition. Amber-restrictive cells, which were primarily infected by an endolysin negative amber mutant, released adenosine triphosphate (ATP) at the end of the normal latent period although lysis did not occur. Superinfection reduced the loss of ATP markedly. The hypothetical role of the cytoplasmic membrane in lysis inhibition is discussed.

Cultures of *Escherichia coli* infected by wild-type (r^+) T-even bacteriophage do not lyse for several hours if the infection is initiated and maintained at a cell concentration of about 4×10^7 cells/ml or higher. This phenomenon was termed "lysis inhibition" by Doermann (2), who demonstrated that secondary infection or superinfection by the phage present in the medium caused the inhibition. One-step growth experiments at cell concentrations lower than 10^6 cells/ml do not show long-term lysis inhibition, but lysis is delayed for about 10 min if the cells have been superinfected with multiplicities of superinfection ranging from 2 to 50 (at 37°C). The length of the delay of lysis was found by Doermann (2) to be constant and independent of the time of superinfection. Rutberg and Rutberg (7), on the contrary, observed an increase of the length of delay when they increased the interval between primary and superinfection.

Our experiments have been undertaken to clarify the discrepancy in the published data and to find new approaches to the phenomenon of lysis inhibition in an attempt to understand it.

MATERIALS AND METHODS

Bacterial and phage strains. *E. coli* S/6/5; T4D wild type (r^+), the late amber mutants am N54, am H11 (gene 31), and am 8-82 (endolysin negative),

and ts e 3-90 (temperature-sensitive endolysin) were kindly provided by R. S. Edgar.

Media. Hershey's enriched broth (HB) contained: 8 g of Nutrient Broth (Difco), 5 g of peptone (Difco), 6 g of NaCl, 1 g of glucose, and 1,000 ml of demineralized water. PAGFe contained: 18 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 3.0 g of KH_2PO_4 , 1.0 g of NH_4Cl , 5.0 g of glucose, 0.25 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0 mg of FeCl_3 , and 1,000 ml of demineralized water.

Phage assays. Phage samples were plated as described by Chase and Doermann (1). When large numbers of samples had to be plated, the amount of top agar was reduced to 0.7 ml and two samples were plated in one petri dish.

Standard procedure for experiments on lysis inhibition. Aerated overnight cultures of S/6/5 were diluted 1:100 into fresh medium, aerated for 70 min at 37°C, and then concentrated by centrifugation to 2×10^9 cells/ml.

Primary infection was performed at 2×10^9 cells/ml; 2 min later a 1:100 dilution followed. Superinfection was carried out at 2×10^7 cells/ml whenever possible in order to distribute the superinfection events evenly over the period during which superinfection was carried out. During 3 min of superinfection the infection rate decreased by about 30%, from 39% at the start of infection to 28% at the end. (Infection rate = phages adsorbed per min per phages adsorbed during the entire infection period.) In 3 min 28% of the input phage was adsorbed. Superinfection was stopped either by T4 antiserum or by further dilution.

The adsorption constant was determined from three independent experiments to be $k = 5.0 \times 10^{-9}$ ml/min. This value was used to compute the phage concentrations necessary to establish the desired actual multiplicities of infection (MOI) or superinfection (MOSI).

For continuous filtering the infected and superinfected cells were pipetted into a jacketed filter-holder containing a membrane filter type B-6 (Schleicher & Schuell Co., Keene, N.H.). Before use, the filters were washed repeatedly in saline and finally soaked in 30% bovine serum albumin solution. The filtrate was pumped by a peristaltic pump model E (The Zero-Max Co., Minneapolis, Minn.) into test tubes at a flow-rate of 0.5 ml per min. Samples were collected continuously for 0.5 to 2 min periods. Depending on the phage titer in individual samples, 0.05 or 0.1 ml was plated on S/6/5. During the experiment, the filter-holder was heated with water of 38.5 C, which kept the medium inside the filter-holder at 37 C. Pumped out medium was continuously replaced by prewarmed medium. The pump can accommodate four filter holders; thus four experiments could be run in parallel. About 1,000 cells were routinely placed onto each filter.

Endolysin assay. The method of Sekiguchi and Cohen (9) was employed. One unit of endolysin activity is defined by a decrease of 0.1 absorbance per min of the substrate in a Spectronic-20 colorimeter (Bausch and Lomb, Inc., Rochester, N.Y.).

Adenosine triphosphate (ATP) assay. The ATP concentration released from cells into the medium was measured by recording the light output of the firefly enzyme system (5) in a Packard scintillation counter, set on 54.5 gain, window 0.30 $-\infty$. This method was suggested by E. K. F. Bautz. Two-ml samples containing 2×10^8 cells were pipetted into 2 ml of glycine buffer (0.05 M, pH 7.4) with 0.02 M MgSO_4 , 0.02 M Na_2HAsO_4 (stock solution adjusted to pH 7.4), and 250 μg of firefly enzyme extract (Worthington Biochemical Corp., Freehold, N.J.), and were immediately placed into the counter. Each sample was counted at least eight times for 0.5 min. The counts were plotted on semilog paper against the number of countings. The intersection of the straight part of the curves and the abscissa was chosen as characteristic value of the curves. The ATP concentration per sample was obtained by comparing the characteristic values of samples and standards. The characteristic value of standards was obtained by adding known amounts of ATP (disodium salt; Sigma Chemical Co., St. Louis, Mo.) to the reaction mixture.

RESULTS

Effect of variation of time and multiplicity of superinfection on the delay of lysis in one-step growth experiments. The time of lysis of an infected culture can be described either by the time at which the rate of lysis (the first derivative of the one-step growth curve) passes through its maximum or by the interval from beginning to termination of lysis. Thus it was desirable to

obtain one-step growth curves which showed well-defined plateaus and inflection points. (One-step growth experiments actually measure release of phage. Since the rate of phage maturation to burst size is small compared to the average rate of lysis, the kinetics of released phage and of lysis must be very similar.)

It turned out that the shape of lysis curves is dependent on the temperature, the medium used, the state of the bacteria, the multiplicity of superinfection, and the time of superinfection. In PAGFe medium, for instance, the length of the delay of lysis increased with decreasing concentrations of supplementing Vitamin Free Casamino Acids, whereas the cessation of phage maturation was independent of the type of medium used. Optimal lysis curves, with termination of phage maturation and lysis coinciding, were obtained with *E. coli* S/6/5 in exponential growth phase using Hershey's enriched broth (HB) at 37 C.

The main effects of varying time and multiplicity of superinfection of lysis can be seen in Fig. 1; they are summarized in Table 1. The curves we obtained did not permit a reliable determination of the time at which the maximal rate of lysis occurred.

The results suggest that increasing the MOSI or the interval between primary and superinfection, shifts lysis to later times. Doermann (2) obtained the same effect with varying the MOSI, but he did not comment on it. Superinfection at two different times 10 min apart did

FIG. 1. Effect of varying time and multiplicity of superinfection on the delay of lysis: Δ , no superinfection; $\circ$, superinfection 5 min after primary infection, MOSI = 2; $\square$, superinfection at 5 min, MOSI = 20; $\bullet$, superinfection at 15 min, MOSI = 2; $\blacksquare$, superinfection at 15 min, MOSI = 20.

TABLE 1. *Effect of time and multiplicity of superinfection on the timing of lysis*

Time of superinfection (min after primary infection)	Beginning and end of lysis ^a	
	MOSI 2	MOSI 20
5	26 ^b to 35 ^c	30 ^b to 42 ^c
15	28 ^b to 40 ^c	30 ^b to 48 ^c

^a Multiplicity of primary infection = 10. Without superinfection lysis commenced at 20 min, stopped for the first time at 28 min, started again, and stopped definitely at 40 min.

^b Beginning of lysis defined as the time when a burst size of 20 was reached.

^c End of lysis determined by the intersection of the extension of the slope and the upper plateau.

FIG. 2. Continuous filtering of infected cells, superinfected with varying multiplicities 15 min after primary infection. Cultures of infected (and superinfected) cells were pipetted onto membrane filters in jacketed filter holders (for details see Materials and Methods). The filtrate was continuously collected, and the sampling tubes were changed every 1 or 2 min. The figure shows the actual number of plaques scored per sample, to give an idea of the statistical reliability. (A) MOSI = 0.4; (B) MOSI = 1.6; (C) MOSI = 7.6; (D) no superinfection.

not shift the time of lysis for the same length, but only for 2 to 3 min.

Continuous filtering of infected cells. The resolution power of one-step growth experiments could be increased substantially by using a method which directly yields the number of released phage per unit of time. It consists mainly in continuous filtering of infected cells through a membrane filter, which is permeable to phage but not to cells, and sampling the filtrate at appropriate intervals.

Superinfection with varying multiplicity. In the following experiments, the multiplicity of primary infection was purposefully kept at only 0.5 in order to minimize the fraction of multiply infected cells. Without intentional superinfection, the release of phage started at about 22 to 23 min (Fig. 2); the rate of lysis reached a maximum at about 27 min. Superinfection at 15 min with varying multiplicities created a number of additional peaks. With increasing multiplicity of superinfection, the peak at 27 min became smaller, whereas, later peaks became larger. The first and second peak are about 5 min apart (27.5 min and 32.5 min); later peaks are spaced by 2 to 3 min. In Table 2 the areas under individual lysis maxima are compared with terms of the Poisson distribution, assuming that the first superinfection maximum (at 32.5 min) is created by lysis of cells which all have been superinfected by one phage, the second superinfection maximum by superinfection with two phage, and so forth. The comparison yielded good agreement except for MOSI = 0.4. It must be expected that at low MOSI the fraction of cells superinfected during primary infection can distort the data more than at higher MOSI.

TABLE 2. *Comparison of the relative size of the areas under lysis maxima in Fig. 2 with terms of the Poisson law*

MOSI and n^a	No. of lysis maximum and n^a				
	0	1	2	3	
0.4	0.67	0.27	0.05	0.007	A ^b
	0.4	0.5	0.1	0.03	B ^c
1.6	0.22	0.35	0.28	0.15	A ^b
	0.28	0.35	0.24	0.14	B ^c
7.6	—	0.07	0.26	0.67	A ^b
	—	0.065	0.43	0.49	B ^c

^a Variables of the Poisson law: $p_r = n^r e^{-n}/r!$

^b $p_r (r = 0, 1, 2, 3)/p_0 + p_1 + p_2 + p_3$.

^c Areas under lysis maxima 0, 1, 2, 3 in Fig. 2 (corrected for increase of the burst size with the time of lysis, rate of phage maturation = 14 phage/min was determined from Fig. 1) divided by the sum of these areas.

FIG. 3. Continuous filtering of infected cells, superinfected with varying multiplicities 5 min after primary infection. The cultures were pipetted onto filters, filtered, and sampled as described in the legend to Fig. 2. Symbols: $\circ$, MOSI = 0.3; $\square$, MOSI = 0.4; $\triangle$, MOSI = 1.6. The control without superinfection was left out for clarity of the graph; it is similar to (D) in Fig. 2.

Superinfection at 5 min after primary infection yielded curves with the same trend, namely, increasing multiplicity caused the peaks to appear later (Fig. 3). The time of appearance of corresponding peaks is different in the experiments of Fig. 2 and 3. After superinfection at 5 min, the first superinfection peak appeared at about 30 min; this is about 2.5 min earlier than with superinfection at 15 min. Thus, changing the time of superinfection did not shift the time of lysis by a comparable amount. We conclude that superinfected cells can lyse only at certain discrete times with constant intervals between. The MOSI per cell determines at which time an individual cell lyses.

Superinfection for varying lengths of time. From unpublished experiments, we learned that raising the multiplicity of superinfection beyond 4 to 5 phage per cell was only partially effective in further delaying the time of lysis. This saturation of the system might be due to a limiting maximal rate of superinfection; i.e., beyond some number of superinfecting phage per unit time, additional phage cannot add to the length of the delay of lysis. To check this possibility, cells

FIG. 4. Continuous filtering of infected cells superinfected with constant multiplicity = 10 for different lengths of time. The cultures were pipetted onto filters, filtered, and sampled as described in the legend to Fig. 2. Superinfected from 15 to 16.5 min (1.5 min), $\circ$; superinfected from 14 to 17 min (3 min), $\square$; superinfected from 13 to 17.5 min (4.5 min), $\triangle$.

were superinfected around 15 min for varying lengths of time keeping the MOSI constant (Fig. 4). Inspection of Fig. 4 reveals that the shorter the period of superinfection the earlier

FIG. 5. Endolysin activity in once-infected and in superinfected cells. Cells were primarily infected with am N54 (MOSI = 10). Superinfection was stopped by adding T4 antiserum. Final cell concentration was 2×10^8 cells/ml, 35 C. Samples were filtered through membrane filters B-6 (Schleicher & Schuell). This filtrate is termed "primary filtrate." The loaded filters were then placed upside-down on the filter holder, and the cells on the filter were washed off with fresh medium of the same volume as the sample (5 ml); this suspension is termed "cells." Endolysin activity in "primary filtrate" and in "cells" was determined separately after chloroform had been added. Primary infection only: "cells," $\circ$; "primary filtrate," $\square$; sum of both, Δ . With superinfection: "cells," $\bullet$; "primary filtrate," $\blacksquare$; sum of both, $\blacktriangle$.

the lysis maxima appeared. In an attempt to quantitate the effect, we compared the number of phages released after a certain, rather arbitrarily chosen, time to the sum of all the phages released through the entire lysis period. Forty-two min was chosen as "line of demarcation" because at this time curve b (the experiment with intermediate MOSI) has dropped to about half of its maximum. The figures were: 0.42 (4.5 min adsorption), 0.17 (3 min adsorption), and 0.19 (1.5 min adsorption). The results of another experiment with the MOSI = 6, were: 0.34 (6 min adsorption), 0.25 (3 min adsorption), and 0.17 (1.5 min adsorption). These data suggest that the rate of superinfection can limit the efficiency of superinfection. More information has to be compiled before a quantitative evaluation can be made.

Superinfection at the end of the latent period. Endolysin appears about one-third of the way through the latent period and increases in concentration until the cells lyse (8). It is natural to inquire whether lysis inhibition might be due to

an effect of superinfection on endolysin synthesis. Several experiments were carried out to test this possibility. We found that superinfection slowed down the initial rate of endolysin synthesis (Fig. 5) as has been shown by Rutberg and Rutberg (7). Subsequently the rate of endolysin production increased and the endolysin concentration exceeded the concentration in nonsuperinfected cells. Since this experiment was performed at high cell concentration, superinfection resulted in a long period of lysis inhibition. This is also the case with cultures which had been primarily infected by a late amber mutant, as has been done in this experiment, and to which T4 antiserum had been added to inactivate the few progeny phage. The increase of endolysin activity in the primary filtrate of superinfected cells, and the constant activity after the end of the normal latent period in the cells which stayed on the filter, might be due to cell lysis as a result of stresses arising from the filtration procedure. Measuring the recovery of superinfected cells from filters directly supported this explanation (*unpublished data*). Since the concentration of endolysin in lysis-inhibited cells exceeds the final concentration in cells which are not superinfected, we conclude that the endolysin concentration does not determine the time of lysis.

We next did an experiment to determine whether there existed a point in time beyond which superinfection would be incapable of

FIG. 6. Superinfection of a lysing culture. The optical density of two cultures at 37 C primarily infected with am H11 (MOSI = 4) was recorded by measuring the absorbancy at 520 m μ . One culture ($\bullet$) was superinfected at the time indicated (SI) with am H11 (MOSI = 5). Dilution from superinfection caused the optical density to drop by 0.01 absorbance.

FIG. 7. Temperature shift (40 C $\rightarrow$ 27.5 C) experiment with cells primarily infected by a temperature sensitive endolysin mutant (*ts e 3-90*). Optical density was measured at 520 m μ . All cultures were primarily infected at 40 C (MOI = 4). The cultures represented by filled symbols were superinfected (MOSI = 10) shortly before they were shifted to 27.5 C. The time of the temperature shift is indicated in the graphs (TS).

arresting the lytic process. Optical density measurements of cultures infected by a late T4 amber mutant (to prevent autosuperinfection) indicated that even after lysis in a culture had started, superinfection could still induce lysis inhibition (Fig. 6). The almost instant response to superinfection suggests that the fraction of cells which has passed the critical time is very small or that the period between the critical time and actual lysis is very short.

Independent of the mechanism of lysis, one might assume that there has to be a critical time at which the cells become sensitive to endolysin.

It might be possible to separate this critical moment from actual lysis by reducing the endolysin concentration in the cells. When a temperature-sensitive endolysin mutant was used for primary infection, incubation at 40 C suppressed the endolysin concentration below a measurable level, and cells did not lyse at the end of the latent period. (T4 antiserum was added to exclude autosuperinfection.) Shifting to 27.5 C (the temperature sensitive endolysin is stable at this temperature), even after the end of the normal latent period, would result in accumulation of some active endolysin if protein

FIG. 8. Leakage of adenosine triphosphate from infected and superinfected cells into the medium. Symbols, ○●, primarily infected with am 8-82 (endolysin negative), MOI = 5; □■, primarily infected with am H11, MOI = 5; ●■, superinfected with am H11 at MOSI = 5. Temperature, 33 °C; medium, PAGFe + 0.5% peptone.

synthesis is not shut off but continues as long as templates, precursors, and energy are available. Unpublished experiments showed that lysis can be induced by shifting to low temperature after the end of the normal latent period. When the infected cultures were shifted later, the rate of lysis decreased. Now we can ask whether superinfection after the end of the normal latent period can still induce lysis inhibition. Results of the experiment to answer this question are presented in Fig. 7. Superinfection at 15 and 20 min (before the end of the latent period) did induce lysis inhibition, whereas superinfection at and after the end of the latent period (25 and 30 min) could not cause lysis inhibition. We conclude that at the end of the latent period cells can be lysed by subnormal concentrations of endolysin, and that at the same time they lose the ability to respond to superinfection.

Leakage of ATP from infected cells. ATP is the main energy intermediate in *E. coli*, as it is in other organisms. It is reasonable to assume that availability of ATP is a prerequisite for infected cells to stay intact. One step in the process which finally leads to lysis might be the release of ATP from the cells into the medium. We were interested in studying the behavior of

ATP before the cells lyse, and therefore chose conditions which precluded cell lysis. To achieve this, primary infection was performed with an endolysin-negative amber mutant infecting *E. coli* S/6/5, a restrictive host. Upon primary infection, cells commence to discharge ATP into the medium (Fig. 8). Thereafter, throughout the latent period, the cells continuously leak ATP at a moderate rate; near the end of the latent period the rate changes rapidly to a much larger value. Superinfection reduces the rate of leaking in this final stage by a factor of 5. Cells infected by an endolysin producing late amber mutant yielded similar results.

DISCUSSION

The resolution power of one-step growth experiments has been substantially increased in the present work by using a technique which measures directly the rate of phage release. With this technique, it was possible to resolve the lysis curves of superinfected cultures into several discrete subclasses of lysis maxima (Fig. 2 to 4). At low multiplicity of superinfection, individual lysis maxima result from simultaneous lysis of cells which very probably have been superinfected by the same number of phage. It became clear that the length of lysis delay is determined primarily by the actual MOSI per cell, whereas the time of superinfection influences the lysis delay only to a small extent. Experiments with constant MOSI and varying duration of superinfection revealed that the faster subsequent superinfections follow each other the less effective they become (Fig. 4). This result could be interpreted in at least two ways: (i) the number of superinfections per cell, or (ii) the amount of DNA or some other material of the superinfecting phage determines the length of lysis delay. The fraction of ^{32}P from superinfecting DNA which leaks into the medium is surprisingly constant (3). Applying the first hypothesis, the explanation of the rate effect would be as follows. The system which receives superinfections as lysis inhibition-inducing stimuli cannot recognize two subsequent superinfections (of one cell) as discrete stimuli if they follow each other too closely. According to the second hypothesis one must assume that, with increasing rate of superinfection, the maximal length of lysis delay which can be displayed by cells shortens, or that more superinfecting material gets lost. The second assumptions seem to us less plausible than the earlier one. The small effect of varying the time of superinfection on the length of lysis delay (Fig. 2 and 3) suggests a model in which the process of lysis delay starts at a constant time (reference point).

The reference point might be identical with the end of the normal latent period. Cells change drastically at this point even if lysis does not occur because of lack of endolysin. Oxygen uptake stops (6), ATP is released (Fig. 8), and the ability to respond to superinfection by lysis inhibition is lost (Fig. 7).

We favor a model in which the site of lysis inhibition is located in the cytoplasmic membrane, but at this stage of our knowledge of lysis inhibition other models cannot be excluded. In primarily infected cells, the membrane changes (conformational change) at the end of the latent period in such a way that ATP is released and respiratory enzymes become inactive. Because of lack of available energy, the membrane disintegrates and thus permits endolysin to attack the cell wall. A more direct possibility is that depletion of usable energy terminates repair of endolysin-induced cell-wall damage (in this case endolysin would have to penetrate the membrane before the end of the latent period).

If certain prerequisites are met, superinfection interferes with this chain of events. The prerequisites are primary infection by r^+ phage and protein synthesis after primary infection, i.e., expression of r^+ function (*unpublished data*). In successfully superinfected cells at the end of the normal latent period (the reference point), the very first changes of the membrane might also occur, but not the key one which is responsible for release of ATP (Fig. 8). At least two hypothetical mechanisms can be envisaged by which superinfection could change the behavior of the membrane after the end of the normal latent period: superinfection causes (i) a conformational change, or (ii) a change in the environment of the membrane (probably only in the interstitial space between membrane and wall). Both mechanisms must either slow down the normal lytic process, which starts with some minor initial change in the membrane and ends with lysis, or activate a different process which is typical for superinfected membranes. This process is affected in some way by the multiplicity of superinfection. The number of possible hypothetical explanations is still too large to be dis-

cussed here. An interesting possibility is suggested by Lehninger's studies (4) of swelling cycles of mitochondria in which the mitochondrial membrane and ATP play a central role.

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LITERATURE CITED

1. CHASE, M., AND A. H. DOERMANN. 1958. High negative interference over short segments of the genetic structure of bacteriophage T4. *Genetics* **43**:332-353.
2. DOERMANN, A. H. 1948. Lysis and lysis inhibition with *Escherichia coli* bacteriophage. *J. Bacteriol.* **55**:257-275.
3. FRENCH, R. C., S. M. LESLEY, A. F. GRAHAM, AND C. E. VAN ROOYAN. 1951. Studies on the relationship between virus and host cell. III. The breakdown of p^{32} labelled T2 r^+ bacteriophage adsorbed to *E. coli* previously infected by other coliphages of the T group. *Can. J. Med. Sci.* **29**:144-148.
4. LEHNINGER, A. L. 1965. The mitochondrion. W. A. Benjamin, Inc. New York.
5. McELROY, W. D. 1963. Crystalline firefly luciferase, p. 445-448. In S. P. Colowick and N. O. Kaplan [ed.], *Methods in enzymology*, vol. 6. Academic Press, Inc., New York.
6. MUKAI, F., G. STREISINGER, AND B. MILLER. 1963. Cited by S. P. Champe. *Ann. Rev. Microbiol.* **17**:87-114.
7. RUTBERG, B., AND L. RUTBERG. 1965. Role of superinfecting phage in lysis inhibition with phage T4 in *Escherichia coli*. *J. Bacteriol.* **90**: 891-894.
8. SECHAUD, J., AND G. STREISINGER. 1962. Cited by S. P. Champe. *Ann. Rev. Microbiol.* **17**:87-110.
9. SEKIGUCHI, M., AND S. S. COHEN. 1964. The synthesis of messenger RNA without protein synthesis. II. Synthesis of phage-induced RNA and sequential enzyme production. *J. Mol. Biol.* **8**:638-659.