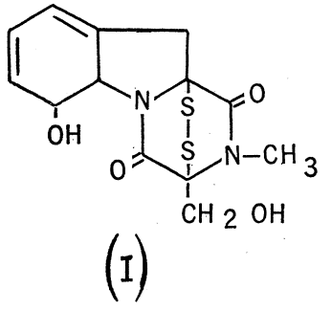
Specific Inhibition of Viral Ribonucleic Acid Replication by Gliotoxin

Abstract. *Gliotoxin inhibits intracellular replication of poliovirus in HeLa cells at a stage subsequent to adsorption and penetration of virus. The sensitive step is synthesis of viral RNA; synthesis of viral protein is unaffected except as a consequence of blockade of RNA synthesis. Concentrations of gliotoxin sufficient to block viral RNA synthesis completely do not affect cellular RNA synthesis.*

Gliotoxin is a common fungal metabolite (*1*) with the structure I (*2*). Although long known to possess diverse biological activities, such as inhibiting growth of bacteria, fungi, and transplantable neoplasms (*3*), it has only recently been recognized as an antiviral agent (*4*). Gliotoxin inhibits the process of intracellular virus synthesis at an early stage in the viral replication cycle (*4*). We now show that the process affected is that of viral RNA replication and that the inhibition is irreversible and specific. Concentrations of gliotoxin which inhibit viral replication are without effect on normal cell metabolism and, in particular, on RNA synthesis. Thus, the action of gliotoxin superficially resembles that of guanidine (*5*, *6*), except that gliotoxin inhibition is irreversible.



(I)

HeLa S_3 cells were maintained in monolayers and propagated in suspension culture by standard procedures. After infection at a multiplicity of 15 plaque-forming units per cell with poliovirus type 1 (Sabin strain), cells were suspended (2×10^5 cell/ml) in a modified Eagle's medium (Fig. 1, legend). A suspension of uninfected HeLa cells was similarly prepared. Protein and RNA syntheses were determined by measuring the incorporation of ^{14}C-labeled amino acids and ^{3}H-uridine, respectively, into material insoluble in trichloroacetic acid; this material was

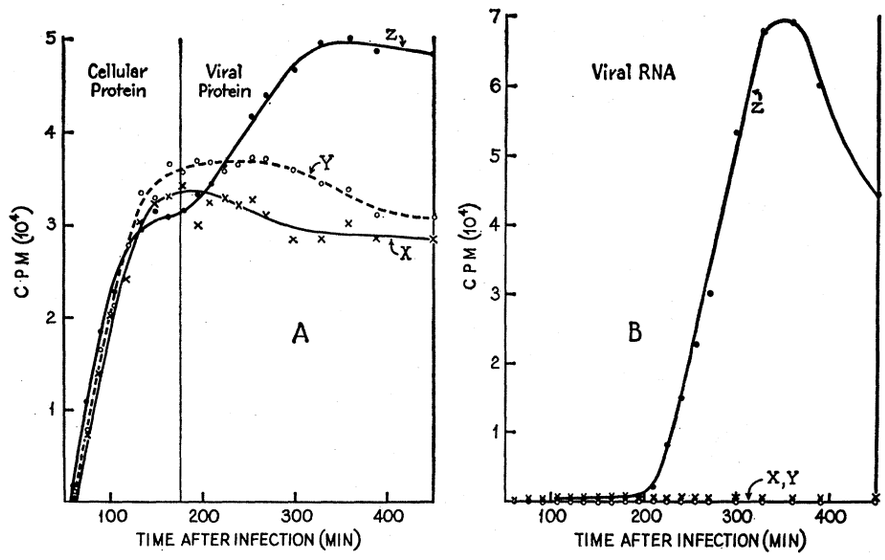


Fig. 1. Inhibition of poliovirus protein and RNA syntheses by gliotoxin added at the time of infection. Three samples of equal volume (*X*, *Y*, *Z*) of HeLa S_3 cells in suspension (4×10^6 cell/ml) were infected with type I poliovirus (15 plaque-forming units per cell) at 37°C in the presence (*X*, *Y*) and absence (*Z*, control) of gliotoxin (1.0 μg/ml). Thirty minutes after infection, samples *X* and *Z* were washed three times and then suspended (2×10^5 cell/ml) in Eagle's medium modified as follows: arginine, lysine, valine, and leucine were omitted; it was supplemented with bovine serum albumin (2 g per liter) and buffered with 25 m*M* *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (*9*) at *p*H 7.4. Sample *Y* was similarly washed and resuspended in the same medium containing 0.05 μg of gliotoxin per milliliter. The concentration of gliotoxin was kept constant according to the number of cells rather than the volume of cell suspension. Actinomycin D was present in all three cell suspensions at a final concentration of 2.5 μg/ml. Sixty minutes after infection, ^{3}H-uridine (21 c/mmole) and a mixture of ^{14}C-labeled arginine, lysine, valine, and leucine (average specific activity, 140 mc/mmole) were added to give final concentrations of 2.0 and 0.8 μc/ml, respectively. Samples (0.5 ml) of cell suspensions taken at timed intervals were transferred to Millipore filters, where the cells were washed immediately with two 5-ml portions of cold Hanks's balanced salt solution followed by four 5-ml portions of cold 5-percent trichloroacetic acid. The wet filters were transferred to scintillation vials and dissolved in 5 ml of scintillation solution [0.5 percent 2-(4'-*t*-butylphenyl)-5-(4"-biphenyl)-1,3,4-oxadiazole in methyl cellosolve : toluene, 1 : 1]. The undissolved trichloroacetic acid precipitate was then kept in suspension for counting by addition of 5 ml of a suspension (1 : 1 by volume) of Cab-O-Sil (colloidal silica) in the above scintillation solution.

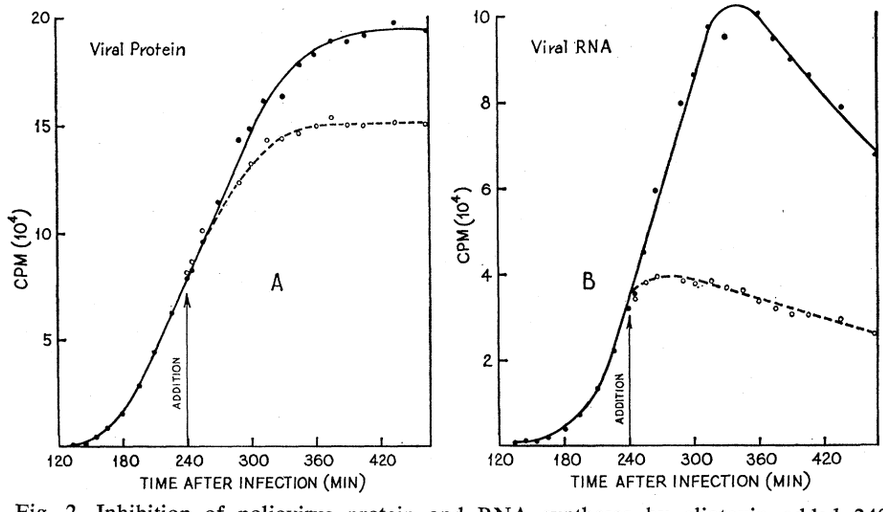


Fig. 2. Inhibition of poliovirus protein and RNA syntheses by gliotoxin added 240 minutes after infection. Infected cell suspensions containing radioactive precursors were prepared as described in the legend of Fig. 1, except that additions of radioactive compounds and gliotoxin were delayed until 135 and 240 minutes after infection, respectively. The final concentration of gliotoxin was 0.05 μg/ml. Sampling and preparation for radioassay were as described in the legend of Fig. 1. Closed circles, controls; open circles, gliotoxin added.

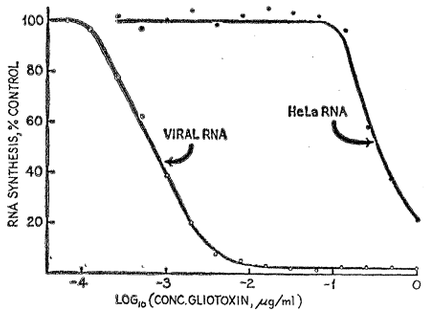


Fig. 3. Dose-response curves relating gliotoxin concentration with viral and cellular RNA synthesis. Poliovirus-infected HeLa cell suspensions were prepared as described for the control sample *Z* in the legend of Fig. 1, except that arginine, lysine, valine, and leucine were present in normal concentrations and no ^{14}C-labeled amino acids were added. The medium was also supplemented with calf serum (2.5 percent). Uninfected cell suspensions were prepared in this same medium, except that no actinomycin D was added. Infected cells were incubated with graded concentrations of gliotoxin for the period from 1 to 6 hours after infection. Uninfected cells were incubated with gliotoxin for 4 hours.

collected on Millipore filters. To measure viral RNA synthesis, cellular RNA synthesis was suppressed with actinomycin D (2.5 μg/ml). Viral protein synthesis is equated with the second phase of protein synthesis which begins approximately 3 hours after infection in poliovirus-infected cells (*6*) (Fig. 1A).

When gliotoxin (0.05 μg/ml) was added at the time of infection (Fig. 1), it completely suppressed viral RNA and protein synthesis. The action of gliotoxin is irreversible in that repeated washing of the infected cells 30 minutes after infection did not relieve the inhibition (Fig. 1B). This inhibition is not caused by extracellular inactivation of virus, nor can it be attributed to interference with virus adsorption or penetration into the cell, because, in the presence of gliotoxin, shutoff of cellular protein synthesis coded for by the viral genome (*6*) still occurs (Fig. 1A). Rather, gliotoxin seems to block directly and specifically the processes by which viral protein and RNA are made within the cell. However, these processes are interdependent (*7*); thus, it is possible that only one of them is sensitive to gliotoxin, since specific inhibition of either one early in the infection would result in the suppression of both.

Therefore, in a second experiment, we added gliotoxin 240 minutes after infection, at a time when viral RNA and protein are both present in considerable amounts and are synthesized at a linear rate (Fig. 1). Labeled amino acids were not added until 135 minutes after infection, when all cellular protein synthesis had ceased (Fig. 1A) and viral protein synthesis was about to begin. Thus, the observed protein synthesis (Fig. 2A) is entirely viral. Gliotoxin had no effect on viral protein synthesis for at least 30 minutes after its addition. In contrast to this, it caused an immediate and striking inhibition of viral RNA synthesis (Fig. 2B). We conclude that viral RNA synthesis is the prime target of inhibition by gliotoxin and that the progressive inhibition of viral protein synthesis observed 30 to 120 minutes after addition occurs as a consequence of the primary action.

To show the specific inhibition of viral RNA synthesis of gliotoxin (Fig. 3), we incubated infected and uninfected cells with graded concentrations of gliotoxin. RNA was determined by an automated paper disk method (*8*). Approximately 1000 times more gliotoxin is required to achieve a 50-percent inhibition of HeLa RNA synthesis than to produce a similar inhibition of viral RNA synthesis.

Thus, gliotoxin appears to block RNA-dependent synthesis of RNA in poliovirus-infected cells and to be without effect on cellular, DNA-dependent synthesis of RNA. Whether this interesting specificity—the reverse of that shown by actinomycin D—is the result of the topographical separation of the two sites of RNA synthesis (*7*, *10*) or of specificity at the molecular level remains to be determined. Our recent experiment favor the latter alternative.

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Serial-Choice Reaction Time: Inadequacies of the Information Hypothesis

Abstract. *The results of an experiment on serial-choice reaction time, specifically designed as a critical test of the Information Hypothesis, lead to rejection of the hypothesis; information is found to be neither a necessary nor a sufficient condition to account for the data. Where previously information had been interpreted as a determinant of reaction time, it was usually confounded with the probability of nonrepetition of a signal. Thus, to the extent that this confounding is present in previous experiments, the inference attributing an increase in reaction time to an increase in information is logically invalid.*

Shortly after publication of Shannon's monograph (*1*), three reports (*2*) provided the basis for the Information Hypothesis in choice reaction time (RT). These studies provided compelling evidence that choice RT behaved as a linearly increasing function of transmitted information. During the following decade the Information Hypothesis served as the focal point for most studies of the problems of choice RT. As the results accumulated, it became increasingly evident that the scope of the Information Hypothesis was far more restricted than was originally thought; factors such as training, stimulus-response compatibility, coding, discriminability, and payoffs were all shown to be playing an important—often overriding—role in determining the characteristics of performance of RT tasks. Nevertheless a hard core of evidence remains, on the basis of which the Information Hypothesis is viable to this day. It is to that body of evidence and arguments that I address myself.

With rare exceptions it can be shown that, for a fixed number of signals, the