reliability of this medium for the growth of the trypanosomes, 4 different individuals, including the author, maintained subcultures of the trypanosomes in this medium.

Results and discussion. The autoclaved dialysate medium for *T. cruzi* supports growth and subcultivation of the organisms. After 10-14 days of incubation the cultures yielded a 10- to 20-fold increase in population. Microscopic examination revealed counts of the order of 2.0 to 5.0 $\times$ 10^6 trypanosomes per ml. *T. cruzi* and *T. ranarum* have been maintained in this medium by subcultivation every 14 days for over 8 months. Tobie and Rees(2) grew *T. cruzi* in a dialysate medium but other species of trypanosomes could not be carried beyond a few transfers. Zeledon(7) was unable to grow *T. rangeli* in dialysate medium although *T. cruzi* grew well. This is suggestive of divergent nutritional requirements among the mammalian trypanosomes.

Clearly, these investigations indicate that *T. cruzi* may be grown without proteins or other non-dialyzable substances. Furthermore, it is apparent that *T. cruzi* does not require heat-labile substances for growth.

The medium described here has many advantages. It is simple to prepare and may be sterilized in its entirety by autoclaving procedures. The medium is stable and may be stored for many months without a loss of growth-supporting properties. The medium is a protein-free dialysate which promotes excellent growth of the trypanosomes over long periods. Large quantities of the parasite may be harvested with ease.

This medium has definite advantges compared to other media alreary described particularly in the production and testing of the antigens of *T. cruzi.* Currently I am using this medium to determine the trypanocidal activity of several chemical agents. It is hoped that more accurate trypanocidal assays will be possible using this medium.

Summary. A protein-free dialysate medium that can be sterilized by autoclaving procedures is described for the growth and subcultivation of *T. cruzi.*

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Inhibition of the Replication of a Bacterial DNA Virus by Chloroquine and Other 4-Aminoquinoline Drugs. (32204)

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Chloroquine [7-chloro-4(4-diethylamino-1-methylbutylamino)-quinoline] has found extensive use as an antimalarial and on a smaller scale in a variety of other clinical conditions. Although many studies have been published on *in vitro* effects of chloroquine, none has gained wide acceptance as a satisfactory explanation for the action of the drug. Some years ago Irvin and Parker demonstrated that this quinoline antimalarial could interact with nucleic acids, and suggested that this might represent a possible mechanism of drug action(1,2,3). Recent studies on the interaction between chloroquine and DNA indicate that the drug binds more strongly to the purine moieties in DNA, its binding is antagonized by divalent cations, and it binds more strongly to the double helical form of DNA(4). As a consequence of such binding

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TABLE I. Effect of Chloroquine on Replication of ΦX-174. Experiment performed as described in text.

Chloroquine	Increase in titer at 30 min
0	40×
3×10^{-4} M	34×
5×10^{-4} M	20×
1×10^{-3} M	19×
3×10^{-3} M	10×

TABLE II. Test for Reversibility of Chloroquine Effect. Experimental details are described in text.

Chloroquine		Plaque count	
Before dilution	After dilution	0 time	1 hr
0	0	130	2,840
3×10^{-3} M	3×10^{-3} M	50	90
3×10^{-3} M	3×10^{-5} M	80	70

chloroquine stabilizes the double helical form of DNA(4,5) and inhibits the DNA primed synthesis of DNA catalyzed by "DNA polymerase" prepared from *E. coli*(6). In contrast to actinomycin D, chloroquine is only a weak inhibitor of the DNA primed synthesis of RNA(6). It was therefore proposed that this ability to interfere with the DNA directed synthesis of DNA could represent the biological mode of action for the drug(6). In exploring the host parasite relationship it is pertinent to inquire whether DNA dependent functions in a parasite could be more susceptible to the action of such a drug than that of the host. It appeared that replication of a DNA bacteriophage would represent an interesting system in which to explore this question. Such an experiment also suggests an approach to the problem of virus chemotherapy, that is, by seeking a drug that could interact with viral nucleic acid and interfere with virus directed synthesis of nucleic acid. The present communication describes an inhibitory effect of chloroquine on the replication of a DNA bacteriophage of *E. coli* c.

Materials and methods. Nutrient broth (Difco) was the standard culture media. Egg white lysozyme was purchased from Sigma and prepared fresh daily as a 4 mg/ml solution in 0.25 M tris buffer pH 7.5. Chloroquine phosphate was obtained from Sterling-Winthrop, Inc., and sterilized by autoclaving as a 0.1 M solution in 0.9% NaCl.

Stock solutions of ϕX-174 bacteriophage were grown on *E. coli* c in nutrient broth culture containing 10^{-3} M $CaCl_2$. After lysis these cultures were shaken with chloroform and stored at 5°. Bacteriophage titers were determined by plating with *E. coli* c in 0.7% nutrient agar according to the method described by Adams(7). For following phage replication, fresh overnight cultures of *E. coli* c were diluted 1:5 into fresh media containing 10^{-3} M $CaCl_2$ to give cell counts of about 10^8/ml, infected with 300-500 phage particles/ml, and aliquots taken for plaque counts at 0 time and at intervals as indicated.

Results. Table I illustrates a series of experiments showing that chloroquine at concentrations as low as 5×10^{-4} M interfered with ϕX-174 replication over time periods ranging from 30 minutes to 3 hours.

The experiments shown in Table II suggest that the drug is taken up by the bacterial cells in the inhibition process since preliminary exposure of the *E. coli* followed by 100-fold dilution into the infecting medium resulted in the same nature of inhibition. Similar preliminary exposure of the bacteriophage followed by dilution into the culture of *E. coli* c did not result in inhibition of phage replication.

Experiments were then directed toward determining the stage at which virus production was inhibited. Since chloroquine is positively charged within the pH range of these experiments, the possibility was considered that the drug could compete for Ca^{++} which is necessary for absorption of ϕX-174 to *E. coli*(8). Inhibition of phage replication was essentially constant whether Ca^{++} was limiting or in large excess, indicating that chloroquine was not simply displacing Ca^{++} and thus preventing absorption.

To inquire further whether the adsorption phase was involved, bacteriophage and *E. coli* were mixed and the drug added at various intervals thereafter. Table III shows that addition of chloroquine as long as 4 minutes after infection did not result in any decrease in inhibition. It has been reported that adsorption of the phage is essentially complete after 4 minutes(9).

TABLE III. Effect of Time of Addition of Chloroquine on ΦX-174 Growth. Experiment performed as described in text.

	Increase in phage titer at 30 min
Control	40×
Chloroquine 3×10^{-3} M	
Added at time of infection	10×
Added 4 min after infection	11×
Added 7 min after infection	24×

To check for accumulation of intracellular phage in the chloroquine inhibited cultures, bacterial cells were treated for 10 minutes in the broth culture with egg white lysozyme at an enzyme concentration of 0.8 mg/ml, frozen and thawed 4 times, and the resulting lysates plated for plaque counts. As shown in Table IV such lysates showed only a 4-fold increase in infective units over the unlysed cells, indicating that phage had not accumulated in the host cells.

Previous studies had shown that chloroquine can inhibit DPNH-cyto-chrome c reductase from animal tissues(10). Experiments were done, therefore, to determine whether the drug interfered with respiration of the *E. coli*. As shown in Table V, inhibition of oxygen uptake was observed at drug concentrations above 10^{-3} M. However, when the effect of the drug was examined on the growth curve of *E. coli* (Table VI) there was considerably less interference with bacterial growth. It was also found that

TABLE IV. Intracellular ΦX-174 in Chloroquine Inhibited Cultures. Experiment performed as described in text.

	Plaque count
0 time	250
1 hr infection with ΦX-174	300
1 hr infection, cells lysed	1290

TABLE V. Effect of Chloroquine on O_2 Uptake of *E. coli* c.

Chloroquine	O_2 uptake
0	32
5×10^{-4} M	26
1×10^{-3} M	20
1.5×10^{-3} M	15

Rate of oxygen uptake was measured using a Clarke O_2 electrode on a culture of *E. coli* at a cell density of about 10^8/ml in nutrient broth before and after addition of chloroquine as shown. Uptake is expressed in arbitrary graphic units.

phage inhibition was essentially the same whether the culture was grown under conditions of limited oxygen or with full aeration. Similarly, supplementation of the growth medium with up to 10% glucose did not decrease the level of drug inhibition.

A variety of chloroquine analogs have been studied for their ability to bind to DNA (4), and to interfere with DNA primed synthesis of DNA(6). Several of these analogs were also examined for effects on φX-174 replication. It was found that compounds with 6,8-dichloro, 7 bromo, or 7 iodo in lieu of the chlorine at position 7, also inhibited phage replication. All these compounds were shown previously to inhibit the *in vitro* synthesis of DNA.

TABLE VI. Effect of Chloroquine on Growth of *E. coli* c.

Chloroquine M × 10^4	Increased turbidity 30 min	1 hr	1½ hr
0	10.0	21.1	42.1
.88	9.6	21.4	46.6
2.22	8.5	16.8	39.7
6.66	9.0	15.0	25.9
8.88	8.8	13.2	26.0
11.10	4.9	13.2	21.9

Exponentially growing culture diluted into fresh media at 37° and turbidity read immediately and at times indicated.

Discussion. The results of the present experiments clearly indicate that chloroquine is capable of interfering with the replication of φX-174 in cultures of *E. coli*. The fact that the drug appeared to work after preliminary adsorption of the virus to the bacteria would indicate that the drug affects some intracellular phase of the virus cycle. In view of the ability of the drug to inhibit DNA directed synthesis of DNA, this would seem a likely site of action for the drug in these studies; but the inhibition of O_2 uptake by the host cells serves as an alternate explanation.

These experiments demonstrate an example of selective inhibition of parasite replication under conditions where host growth continues. They further suggest the possibility of interfering selectively with virus replication by the use of agents which bind to nucleic acids and inhibit viral directed nucleic acid

synthesis. Although a drug does not exhibit a specificity *in vitro* for viral nucleic acid, the intracellular milieu of the host may protect its own DNA from binding while leaving the viral DNA exposed. For example, chloroquine is antagonized by the binding of cations to DNA(4), and the binding of actinomycin D was shown recently to be antagonized by nucleoprotein (11).

Summary. Chloroquine, a 4 aminoquinoline 'antimalarial' which binds to DNA, has been shown to inhibit replication of a DNA bacteriophage of *E. coli* under conditions where growth of the host continues. It is suggested that the ability of the drug to bind to DNA may account for this effect, but a more general metabolic effect cannot be ruled out.

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Protein Leakage from Mengovirus-Infected Cells.* (32205)

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Many viruses cause a rapid destruction of mammalian cells cultured *in vitro*. While the occurrence of cytopathic alterations affords a dramatic visualization of host cell-virus interaction the basic mechanism(s) responsible for these untoward effects is obscure. One of the manifestations of virus-induced cellular injury is the leakage of enzymes and proteins from cells(1,2). We have found the leakage of acid-insoluble proteins from mengovirus-infected cells to be a reliable and quantitative measure of cellular injury. The present investigation was undertaken to examine the kinetics of protein leakage from mengovirus-infected cells, and by the use of selective inhibitors of protein synthesis an attempt was made to delineate some of the factors which underlie the protein leakage phenomenon. The experiments presented here suggest that cellular protein leakage is conditioned by viral protein synthesis and not the suppression of host cell functions by the viral genome.

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Materials and methods. Cells and virus. An established line of guinea pig spleen cells (GPS) was cultivated in suspension in Eagle's medium (Grand Island Biological Co., MEM F-15) supplemented with 10% calf serum and 0.5% lactalbumin hydrolysate. Monolayers were prepared by plating out an appropriate cell suspension onto 6.0 cm plastic culture dishes (Falcon).

The mengovirus used in this study was obtained from E. Swim (Cleveland) and passaged several times on GPS cells.

Plaque titrations were carried out by plating 0.3 ml of an appropriate virus dilution onto GPS monolayers, and the subsequent addition of an agar overlay containing 200 μg/ml of protamine sulfate. After 48 hours 1:5000 neutral red, containing 0.01% acetic acid, was added to the dishes and the plaques were counted at 60 hours.