sugars(13). Yet, the marked increase in blood glucose of fasting (30 hrs) animals, significantly above the control changes (probably due to experimental stress) and maintained for a relatively long period of time, suggests that some mechanism for glucose release may be triggered by quinidine. Studies on the effect of quinidine on isolated perfused livers have been initiated.

The differences observed between the fasting and non-fasting animals tend to indicate an inhibitory effect of quinidine on potassium transport at the gut level. At present, this aspect needs further investigation.

The electrocardiographic evidence suggests the possibility that the action of quinidine upon the heart may be mediated via its effect on the K ion, since dogs rendered hypokalemic either by quinidine, vivodialysis or vomiting associated with intestinal obstruction(14) show a striking similarity in cardiac changes.

Summary. Evidence is presented to demonstrate that quinidine, when administered as described here, produces a significant and progressive hypopotassemia; with a non-significant variation in the arteriovenous potassium concentration, denoting that the drug does not alter the normal efflux or influx of the potassium ion at the somatic muscle cell level. The drug also effects a marked hyperglycemia and an apparent inhibition of K transport at the gut level. The electrocardiographic evidence suggests the possibility that the mechanism of action of quinidine may be directly related to the latter's effect on the potassium ion.

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Influence of 5-Fluorodeoxyuridine on Growth Characteristics of Rubella Virus.* (29595)

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The action of 5-Fluorodeoxyuridine (5-FUDR) on DNA synthesis by its inhibitory effect on thymidine synthetase has been the subject of many reports(1,2,3). Salzman (4) first demonstrated that 5-FUDR inhibits DNA, but not RNA viruses, and proposed that the use of 5-FUDR might provide a method for typing the nucleic acid moiety of

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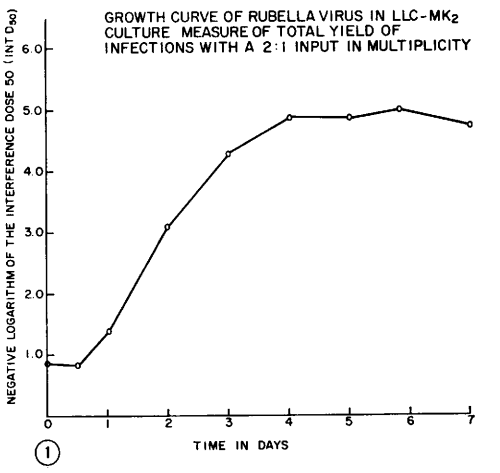
viruses. In the present paper, data will be presented to show that 5-FUDR does not alter the yield of infectious rubella virus in tissue culture. Hence, pending direct analysis of nucleic acids of purified preparations of rubella virus, it may be said that rubella virus appears to be of the ribonucleic acid (RNA) type.

Materials and methods. Virus. The WM strain of rubella virus isolated in this laboratory(5) was used throughout our studies.

Growth curve. The cells used were a continuous line of rhesus monkey kidney (LLC-MK_2) obtained from Dr. Robert Hull of Eli Lilly Research Laboratories(6). They were grown and maintained in medium 199 with 1% horse serum. To obtain monolayers, 3 ml of suspensions containing 75,000 cells/ml were placed in 2-ounce prescription bottles. After 5 days, growth media were discarded and $10^{4.5}$ 50% interfering dose (Int.D_{50}) of virus in 2 ml was added. The virus was allowed to adsorb for 5 hours at 36°C. The inoculum was removed and 3 cc of media were added for maintenance. At selected intervals up to the 6th day after infection, duplicate cultures were used for infectivity titration. To measure total infectivity, cultures were sonicated for 5 minutes, the cellular debris centrifuged and supernate was titrated for combined extra- and cell-associated virus. As indicated in the text, in certain experiments extracellular virus was measured in the supernate, while cell-associated virus was measured in sonicated cells resuspended to the original volume.

Infectivity titrations. Cell sheets were formed in 5 days in tissue culture tubes seeded with 75,000 cells. Sets of 4 tubes were each inoculated with 1 ml of serial 10-fold dilutions of virus in media and fed every 3 days. At 11 days 100 $TCID_{50}$ of ECHO-11 virus was added in 1 ml. Readings were taken when the ECHO-11 CPE was complete in control tubes. Titers were recorded as the reciprocal of log dilution showing 50% interference.

Results. Growth characteristics of the virus. The growth curve of rubella virus was obtained using a 2:1 input multiplicity. The



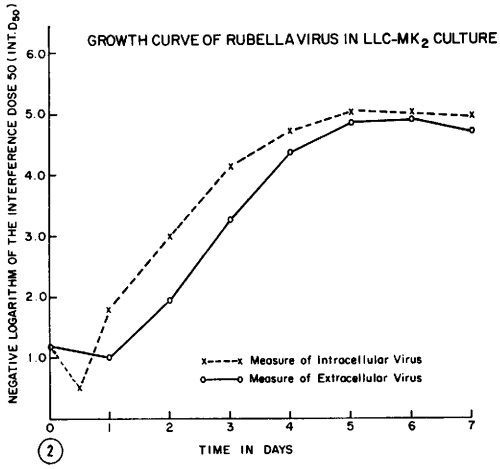


FIG. 1. Growth curve of rubella virus in LLC-MK_2 culture measure of total yield of infections with a 2:1 input in multiplicity.

FIG. 2. Growth curve of rubella virus in LLC-MK_2 culture.

results are presented in Fig. 1. There was little increase in titer in the first 24 hours after infection. The first marked increase in total virus occurred between 24 and 48 hours after infection; the increase continued for an additional 48 hours. By the fourth day the maximum infectious titer of rubella had been reached. The characteristics of rubella virus in both intracellular and extracellular phases are shown in Fig. 2. Intracellular virus increase is detectable as early as 24 hours. This finding accounts for the increase in total virus found at that same interval (Fig. 1). For the first 4 days, titers of intracellular virus clearly exceed those of extra-

TABLE I. Effect of 5-FUDR on Multiplication of LLC-MK_2 Cells.

Conc in molarity of 5-FUDR	Days of sampling 0	3	6	Yield at 6 days
None	2.5×10^5	5.0×10^5	8.0×10^5	3.3-fold increase
10^{-4}M	2.0×10^5	$.5 \times 10^5$	$.1 \times 10^5$	20 " decrease
10^{-5}M	1.8×10^5	1.5×10^5	$.5 \times 10^5$	3.6 " "
10^{-6}M	2.3×10^5	2.0×10^5	1.3×10^5	1.9 " "
10^{-7}M	1.8×10^5	2.0×10^5	1.7×10^5	No change

cellular virus after an eclipse phase. Extracellular virus increase was detected at 48 hours, and by the 5th day the levels found were almost equal to those measured in the cells. Veronelli(7), on using 100:1 input multiplicity, found that in the extra-cellular phase rubella virus was released at the 19th hour and by 40-58 hours maximum infectious yield of $10^{-6.5}$ Int.D_{50} was reached.

Because the difference in virus titer found in cells and supernates is small, and because the curves of intra and extracellular virus concentration are so nearly parallel, it seems probable that, like influenza virus in chick kidney tissue culture(8), rubella virus is promptly released after formation. The form of the growth curve indicates that for studies of inhibition of virus growth, the 3rd and the 6th day intervals were optimal.

Toxicity of 5-FUDR. Table I shows the effect of FUDR on the multiplication of LLC-MK_2 line using various concentrations of drug.

In a 6-day period, the control culture showed the usual increase in cell number. At 10^{-4}M there was a marked decrease in the number of cells even after 3 days of treatment. In addition there was extreme toxicity as judged by visible morphological alterations. At 10^{-5}M, which was the concentration used throughout the present experiments, there was a slight decrease in number of cells at the 3rd day; at the 6th day there was a 3.6-fold decrease and occasional cell rounding was seen throughout the sheet. Despite the slight toxicity of the drug, at the 6th day, no effect was seen on yield of virus. At 10^{-6}M cell decrease was less while at 10^{-7}M no numerical or visible alteration of cells occurred.

Effect of 5-FUDR on growth of rubella and control viruses. The yield of rubella virus in drug treated and control cultures is shown in Table II. Drug was added at zero time. It is quite evident that there is no delay or inhibition of growth of rubella virus at the 3rd and 6th day after infection. Similarly the growth of 2 known RNA viruses was not inhibited. In contrast, the growth of the DNA virus (vaccinia) was markedly impaired. 5-FUDR was not found to be directly harmful on rubella virus.

TABLE II. Inhibitory Effect of 10^{-5}M FUDR on Replication of Selected Viruses *in vitro*.

Virus		Day of sampling 0	3	6	Summary
Rubella	FUDR	1.0	2.7	4.5	No inhibition
	No FUDR	1.0	3.0	4.3	" "
Vaccinia	FUDR	1.5*	1.3	2.5	Inhibition
	No FUDR	1.3	4.5	6.0	No inhibition
ECHO-11	FUDR	1.3	6.5	6.7	No inhibition
	No FUDR	1.5	6.8	6.8	" "
Polio-1	FUDR	1.5	7.8	7.5	" "
	No FUDR	1.7	7.5	7.7	" "

* Expressed as negative log of infectious titer.

Pretreatment of cells with 5-FUDR. Table III illustrates the results obtained when cultures were pretreated with drug or drug plus thymidine for 12 hours prior to infection. At the 12th hour some of the cultures were infected with vaccinia virus and some with

TABLE III. Effect of Various Treatments on Yield of Vaccinia and Rubella Viruses.

0 hr	12th hr	Infectious yield of 6th day
Control	Vaccinia	6.3
5-FUDR	5-FUDR + vaccinia	2.3
5-FUDR + thymidine*	5-FUDR + thymidine + vaccinia	6.0
Control	Rubella	4.5
5-FUDR	5-FUDR + rubella	4.0

* Concentration 10^{-4}M.

rubella virus. Untreated control cultures were included in the experiment.

It is apparent that FUDR did inhibit vaccinia virus as expected, and that thymidine did reverse the inhibitory effect of the drug since by the 6th day the infectious yield was comparable to the untreated control. The yield of rubella was unaltered.

Since completion of this work, Cusumano (9) independently found that 5-IUDR (Iododeoxyuridine) failed to inhibit the yield of rubella virus in tissue culture. The evidence reported that 5-FUDR does not inhibit the growth of rubella virus indicates that rubella virus is an RNA virus. Purification of rubella virus for direct chemical analysis will be the proof of the type of nucleic acid of the virus.

Summary. Rubella virus grows to a maximum titer of approximately $10^{-5.0}$ Int.$D_{.50}$ in LLC-MK_2 cell cultures, with 2:1 input in multiplicity. There is always cell-associated virus which accounts for a small portion of the infectious yield. 5-FUDR did not affect in any way the yield of rubella virus as compared to the untreated control. Rubella virus therefore appears to belong to the RNA viruses.

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Effect of an Alpha-2 Globulin Fraction on Antibody Formation in Rabbits and Mice.* (29596)

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It has recently been reported by Mowbray that an alpha-2 globulin fraction obtained by DEAE cellulose chromatography called "fraction C" has the ability to prolong homograft rejection in rats(1) and to prevent antibody formation in rabbits(2). Treatment with fraction C was effective when started either before or with, but not after, injection of antigen. It was therefore suggested that fraction C may interfere with the induction phase of antibody production(2).

Fraction C contains a number of alpha-2 globulins and possesses RNAse as well as phosphatase activity(2,3). It could be extracted from the thymus, but not from other organs tested(3). Preparations obtained from both homologous and heterologous serum have been reported to be equally effective.

The experiments reported here were undertaken to study further the effect of bovine fraction C on antibody formation in rabbits and mice.

Materials and methods. Animals. Male

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