sorption was distributed throughout the bowel in both groups, was quantitatively the greatest in the colon, and was increased in the deficient animals. The relative distribution of absorption did not appear to be significantly altered, indicating a generalized enhancement in the uptake of Mg from the alimentary tract in magnesium deficiency.

1. Chutkow, J. G., J. Lab. and Clin. Med., 1965, v65, 912.
2. ———, ibid., 1964, v63, 80.
3. Orange, M., Rhein, H. C., J. Biol. Chem., 1951, v189, 379.
4. Andreason, E., Scand. J. Clin. Lab. Invest., 1957, v9, 138.
5. Brownlee, K. A., Statistical Theory and Methodology in Science and Engineering, John Wiley & Sons, New York, 1960, p252.
6. Snedecor, G. W., Statistical Methods Applied to Experiments in Agriculture and Biology, Iowa State Coll. Press, Ames, Iowa, 1956.
7. Chutkow, J. G., J. Lab. and Clin. Med., 1964, v63, 71.
8. Aikawa, J. K., Proc. Soc. Exp. Biol. and Med., 1959, v100, 293.
9. Aikawa, J. K., Rhoades, E. L., Gordon, G. S., ibid., 1958, v98, 29.
10. Graham, L. A., Caesar, J. J., Burgen, A. S. V., Metabolism, 1960, v9, 646.
11. Terkildsen, T. C., Acta Pharmacol. et Toxicol., 1952, v8, 374.
12. Nicolaysen, R., Skandinav. Arch. Physiol., 1936, v73, 75.
13. Fawcett, D. W., Gens, J. P., J.A.M.A., 1943, v123, 1028.
14. Collins, E. N., Russell, P. W., Cleveland Clin. Quart., 1949, v16, 162.
15. Stevens, A. R., Wolff, H. G., Arch. Neurol. and Psychiat., 1950, v63, 749.
16. Hendrix, J. Z., Alcock, N. W., Archibald, R. M., Clin. Chem., 1963, v9, 734.
17. Smith, R. H., Nature, 1959, v184, 821.

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Production of Interferon in Embryonated Eggs and in Cell Cultures Infected with Simian Virus 5.* (31619)

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Simian virus 5 (SV5), a strain of parainfluenza 5, is a common myxovirus contaminant in uninoculated monkey kidney tissue cultures(1). Previous studies showed that SV5 was incapable of interfering with the multiplication of poliovirus in the monkey kidney cell system(2,3). In addition, Choppin found that SV5 infected monkey cell cultures did not interfere with the growth of several other viruses including Coxsackie, ECHO, vaccinia, and vesicular stomatitis virus(2).

Embryonated eggs have been commonly used for the propagation of most myxovirus types but the avian host system has not been generally applied to the cultivation of SV5. In our laboratory, attempts were made to adapt SV5 to growth in avian cells both in embryonated eggs and in tissue culture. After serial passages of SV5 in the avian host system it was noted that an inhibitor, subsequently identified as an interferon, was obtained. The results of these studies are reported here.

Materials and methods. Virus strains and infectivity assay. A newly isolated strain of SV5 was obtained from an uninoculated rhesus kidney tissue culture. Infectivity titrations were made by the hemadsorption technique, essentially as originally described by Shelokov *et al*(4).

Sindbis virus, strain AR339 M1318 Egg 2, was obtained through the courtesy of Dr. J. R. Henderson of Yale University School of Medicine. Infectivity of the Sindbis virus was determined by the plaque technique using chick fibroblast cultures in 3 oz prescription bottles as previously described(5).

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Tissue cultures. Primary chick embryo (CE) fibroblast cell culture and African Green (AG) or rhesus (Rh) monkey kidney cell cultures were prepared by standard methods. With the CE cells, complete monolayers were obtained 2-3 days after seeding, while the monkey cells were used 7-8 days after seeding.

Preparation of the interferon in embryonated eggs and in tissue culture. Embryonated eggs, 7-8 days old, were inoculated with 0.3 ml/egg of SV5, egg adapted strain, by the allantoic route. At various time intervals, the allantoic fluids from 3-5 eggs were pooled and were tested for virus infectivity, hemagglutinin titers, as well as interfering capacity. In order to produce interferon in tissue culture, SV5 stock virus was added to the freshly trypsinized cell suspension at a multiplicity of 10. Complete monolayers were obtained 2-3 days after seeding CE cells and 6-7 days for monkey cells. Supernatant fluids from these infected cultures were tested for their effectiveness as inhibitors.

Assay for interferon activity. Serial 2-fold dilutions of the SV5 infected egg fluid (EF) or tissue culture fluid were made in Hanks' balanced salt solution. CE bottle cultures, in duplicate or triplicate, were each inoculated with 0.5 ml of the serial dilutions of the SV5 infected fluids. After 3 hours adsorption at 37°C, 0.1 ml of Sindbis virus suspension containing 50-100 PFU was added. After an additional hour of incubation at 37°C, 10 ml of agar overlay medium was added to each inoculated CE bottle culture. All cultures were returned to the incubator after the agar was solidified. The numbers and size of the Sindbis virus plaques were recorded 2 days after inoculation. The highest dilution of the SV5 infected fluid which suppressed the growth of Sindbis virus plaques was considered to be the titer of the interferon tested.

Results. Growth of SV5 in avian cells. The growth of SV5 in embryonated eggs required adaptation. After 2-3 serial passages of growth in the amniotic cavity of 7-8-day-old embryos, SV5 could be propagated well in the allantoic cavity, reaching an infectivity titer of log 5.5 TCD_{50}/ml, 6-7 days after inoculation. The growth rate of SV5 in the embryonated eggs was slower as compared to

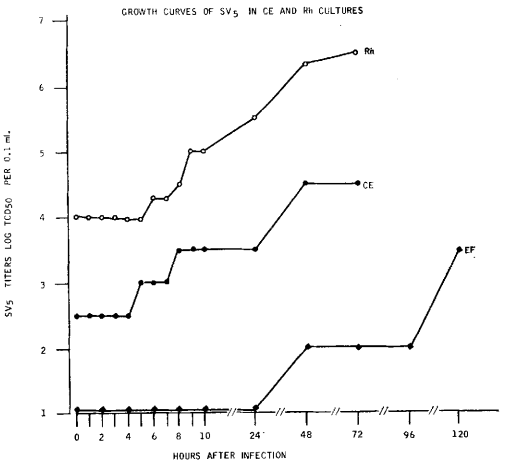


FIG. 1. Growth curves of SV5 in rhesus (Rh) monkey kidney cultures, chick embryo (CE) fibroblast culture, and embryonated eggs (EF).

that of the tissue culture systems (Fig. 1). In addition, 7-8-day-old embryos appeared to be more sensitive to SV5 infection than 10-12-day-old embryos, and were used throughout the study.

The growth of SV5 in CE tissue culture was easily obtained. Although the yield of virus in CE cells was lower than that in the Rh monkey kidney cells (Fig. 1), the rate and pattern of growth in both cell systems were similar. The yields of virus in the tissue culture systems were significantly higher than those in the embryonated eggs.

Demonstration of the presence of an interferon in allantoic fluids obtained from embryonated eggs infected with SV5. When the CE monolayer was first adsorbed with SV5 infected egg fluid and then superinfected with Sindbis virus, a marked reduction of Sindbis virus plaque was noted. An example is illustrated in Fig. 2. As the concentration of the SV5 egg fluid was decreased, an increase in number and size of Sindbis plaques was observed. The interfering capacity of the SV5 infected egg fluid was not affected by heating at 56°C for an hour, was non-dialyzable, and was resistant to acid treatment at pH 2.0. Specific SV5 antiserum showed no effect on the interfering property, while treatment with 0.25% trypsin abolished the interfering activity completely. All these properties indicated that an interferon was present in the allantoic fluid of embryonated eggs infected

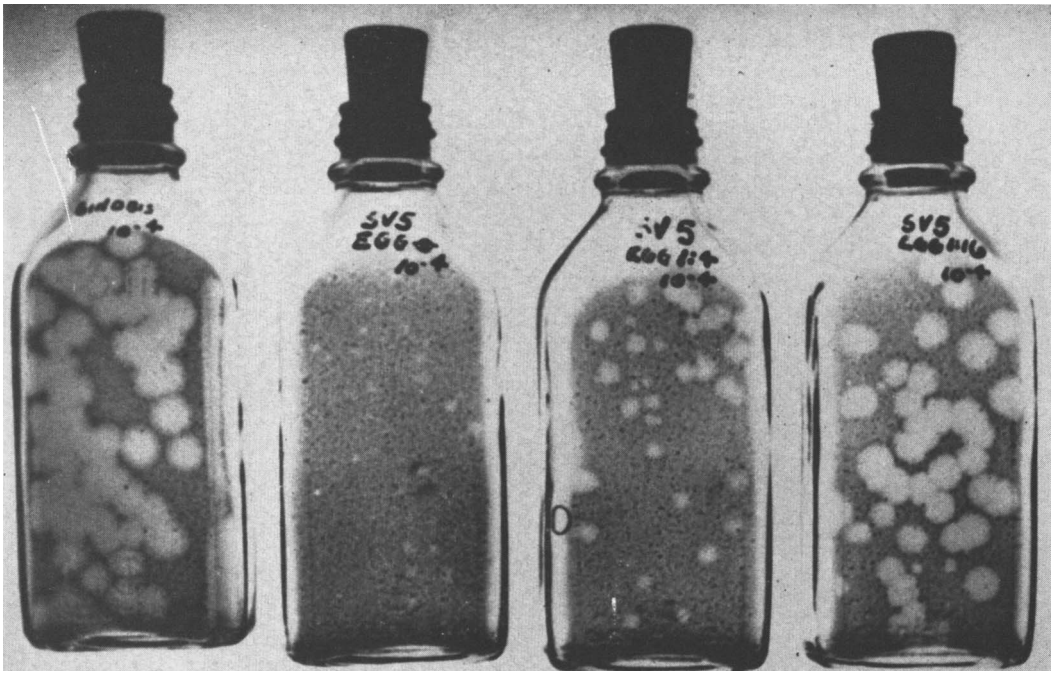


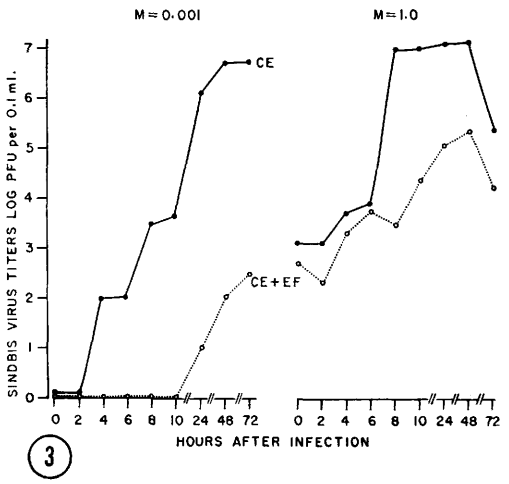
FIG. 2. Inhibition of Sindbis virus plaques by SV5 infected egg fluid. Bottle at left, Sindbis virus control. The 3 bottles at right were each inoculated with 0.5 ml of undiluted 1:4 or 1:16 dilution of SV5 infected egg fluid 3 hours prior to challenge with the same Sindbis virus suspension as shown in the bottle at left.

with SV5. Allantoic fluid obtained from non-infected eggs did not show an inhibitory effect.

Inhibition of the multiplication of Sindbis virus in CE fluid cultures previously treated with SV5 infected egg fluid was also demonstrated. The results of the two separate experiments are summarized in Fig. 3. During a single growth cycle of Sindbis in SV5 interferon treated cells, the multiplication of Sindbis virus was inhibited completely when the inoculum of the challenge virus was small, *i.e.*, at an input multiplicity of 0.001. However, Sindbis virus infectivity was detected in the interferon treated cultures 24-48 hours after challenge although the yield of the challenge virus in the treated cells was only 1/10,000 of that in the non-treated cultures. When the challenge virus dose was increased to a multiplicity of 1.0, the inhibition was never complete; although the yield of Sindbis virus was significantly lower in the treated cultures than the non-treated ones.

Factors influencing the yield of SV5 interferon in embryonated eggs. It was noted that the titer of SV5 interferon varied greatly from experiment to experiment. These variables were found to be due to: 1) the amount of SV5 in the inoculum, *i.e.*, a minimum dosage for the initiation of interferon production was $10^{6.5}$ TCD_{50} per egg; 2) the age of the embryo inoculated, *i.e.*, 7-8-day-old embryos showed greatest susceptibility and gave maximum infectivity titers, as well as interferon titers; 3) number of days the embryos were infected. The latter experiments are summarized in Fig. 4. Each determination was based on a pool of 4-5 infected eggs. At an inoculum of 6.5 log TCD_{50}/egg, SV5 infectivity rose 2 days after infection. Interferon and hemagglutinin titers were both detectable on the fourth day, and reached maximum levels on the sixth day post-infection. Infectivity titers of SV5 were maintained at high levels in the infected eggs, while the titers of interferon decreased significantly 7-8 days post-inoculation.

Comparison of SV5 interferon derived from CE fibroblast cultures and from AG monkey kidney cultures. CE cultures treated with SV5 infected egg fluid or monolayer cultures



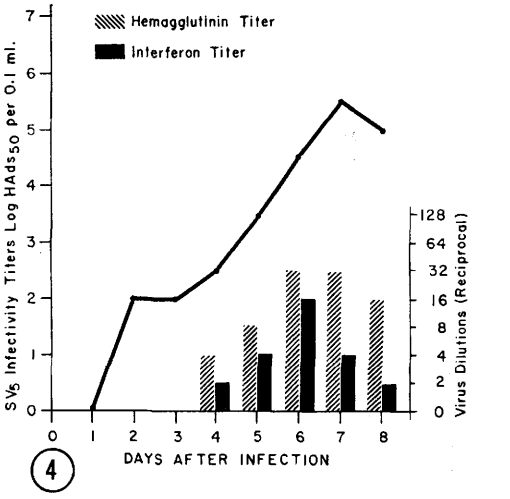


FIG. 3. Growth of Sindbis virus in fluid cultures of CE cells with or without treatment of SV5 infected egg fluid (EF), at input multicplicity of 0.001 (figures at left), and 1.0 (figures at right).

FIG. 4. Growth of SV5 in embryonated eggs, the yield of hemagglutinin and the production of interferon.

derived from SV5 infected CE cells, were completely resistant to a challenge dose of approximately 3000 PFU of Sindbis virus (Table I). Harvests from SV5 infected CE tissue culture fluid also gave protection to CE cultures superinfected with Sindbis virus. SV5 infected AGMK culture fluids containing 7.5 log TCD_{50}/ml of infectious virus did not, however, alter the susceptibility of CE monolayers to Sindbis virus infection, but did render AGMK monolayers resistant to superinfection by Sindbis virus. In a separate experiment, a 2 log reduction of Sindbis virus yield was noted in the fluid cultures of rhesus monkey cells infected with SV5. Such inhibition could not be demonstrated either in AG or Rh monkey kidney cells when the SV5 interferon produced in the avian cells was used, nor could the SV5 MK interferon protect monkey cultures from infection with poliovirus type 1.

Discussion. The experiments reported here demonstrated that harvests from embryonated eggs, CE cell cultures, AG or Rh monkey kidney cell cultures infected with SV5 induced an interferon which interfered with the multiplication of Sindbis virus both under agar overlay medium and in fluid cultures. Previous reports showed that in monkey kidney cell cultures, SV5 did not interfere with the multiplication of a variety of viruses including poliovirus, Coxsackie A9, ECHO 7, vaccinia, and vesicular stomatitis virus, but Sindbis virus had not been tested. The selective action of SV5 interferon upon Sindbis virus was worth noting but the mechanism was not clear. Apparently Sindbis virus was more sensitive to the interferon action than the enteroviruses tested. Thus minute amounts of interferon induced in monkey kidney cultures by SV5 were not sufficient to restrict the multiplication of enteroviruses or other viruses tested.

Species specificity of the antiviral action of interferon has been shown by previous investigators(6-9). The present study demonstrated that SV5 interferon induced either in avian cells or in primate cells was host specific. There was no evidence that heterologous activities could be exhibited even though the interferon samples used were crude preparations.

Embryonated eggs are the most susceptible host system for the cultivation of certain types of the myxovirus group but avian tissue has not been commonly used for the propagation of the parainfluenza viruses. Non-specific inhibitor for DA virus, a strain serologically identical to SV5, has been found in the normal egg fluid(10). Difficulties were encountered in the adaptation of DA growth in embryonated eggs when 10-day-old eggs were used. In the present study, 7-8-day-old eggs were

TABLE I. Species Specificity of SV5 Interferon Derived from Chick Embryo or African Green Monkey Cells.

Cell culture used for interferon assay	Treatment of test culture*	SV5 infectivity titers, log TCD_{50}/ml	Avg No. Sindbis plaque per bottle at dilutions: 10^{-5}	10^{-6}	10^{-7}	10^{-8}
Chick embryo fibroblast	None	0	Confl‡	300	36	4
	SV5-infected EF	3.5	0	0	0	0
	SV5-infected CE† monolayers	4.5	0	0	0	0
	SV5-infected CE tissue culture fluid	4.5	0	0	0	0
	SV5-infected AGMK tissue culture fluid	7.5	Confl	Confl	31	3
African green monkey kidney	None	0	100	29	ND	ND
	SV5-infected AGMK tissue culture fluid	7.5	0	0	0	ND
	SV5-infected EF	3.5	100	24	ND	ND

* Absorbed 3 hr with test fluid indicated.
† CE cells were infected at time of seeding.
‡ Confluent.
EF = egg fluid; CE = chick embryo; AGMK = African green monkey kidney; ND = not done.

found to be superior for the growth of SV5. Adaptation of DA virus as well as several other strains of SV5 were accomplished in the 7-8-day-old egg system (unpublished data). Apparently the age of the embryos used was an essential factor.

Summary. SV5, a strain of parainfluenza 5, was adapted to grow in 7-8-day-old embryonated eggs. SV5 infected egg fluid proved to be capable of inhibiting the growth of Sindbis virus in CE tissue culture both under agar overlay and in fluid medium. The inhibitor substance was found to be acid resistant, sensitive to trypsin digestion, but could not be neutralized by specific SV5 antiserum, and was characterized as an interferon. The yield of SV5 interferon in embryonated eggs was dependent on the dosage of inoculum, and the days of virus growth in the embryonated eggs. Chick embryo cell cultures infected with SV5 or CE fibroblast cultures adsorbed with SV5 supernatant fluid from infected CE cells were resistant to plaque formation by Sindbis virus. SV5 infected CE culture fluid did not protect AG monkey cultures from superinfection with Sindbis virus. AG monkey cells infected with SV5 were resistant to superinfection with Sindbis virus, but these SV5 infected monkey cells did not interfere with the multiplication of poliovirus type 1. SV5 produces interferon in both avian and primate cell systems but the presence of SV5 interferon requires a highly sensitive test system.

1. Hull, R. N., Minner, J. R., Smith, J. W., Am. J. Hyg., 1956, v63, 204.
2. Choppin, P. W., Virology, 1964, v23, 224.
3. Hsiung, G. D., Atoynatan, T., Gluck, L., Proc. Soc. Exp. Biol. and Med., 1966, v121, 562.
4. Shelokov, A., Vogel, J. E., Chi, L., ibid., 1958, v97, 802.
5. Hsiung, G. D., Diagnostic Virology, Yale Univ. Press, New Haven, 1964, 29.
6. Isaacs, A., Westwood, M. A., Lancet, 1959, v2, 324.
7. Wagner, R. R., Bact. Rev., 1960, v24, 151.
8. Baron, S., Buckler, C. E., Science, 1964, v145, 814.
9. Lampson, G. P., Tytell, A. A., Nemes, M. M., Hilleman, M. R., Proc. Soc. Exp. Biol. and Med., 1966, v121, 377.
10. David-West, T. S., J. Immunol., 1963, v91, 873.

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