phyllin, quercetin from quercetron, podophyllotoxinized resin and colchicine were determined in adult mice. In this species podophyllin and podophyllotoxin respectively are approximately 1/15 and 1/6 as toxic as colchicine. 2. A comparison of the median lethal doses of podophyllin and podophyllotoxin in young and adult rats demonstrated a greater degree of toxicity for young rats. 3. Cumulative toxic effects were produced in rats by administering podophyllin and podophyllotoxin multiple times a day in individual doses that were in the range of 1/6 and 1/5 of the LD_{50}. 4. Observations on the symptomatology and or pathologic alterations of rats poisoned with podophyllin, podophyllotoxin, podophyllic acid, picropodophyllin, quercetin and depodophyllotoxinized resin and colchicine are presented. 5. In animals that succumbed to subcutaneous injections of colchicine there was an accumulation of arrested mitoses in the skin (positive Dustin reaction); in animals killed by subcutaneous injections of podophyllin the Dustin reaction was negative as there were no epidermal changes.

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Effect of Semliki Forest Virus on Rabbit Fibroma.* (18747)

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Certain neurotropic viruses have been shown to parasitize transplantable mouse tumors and cause necrosis of the tumors(1-3). Similar antagonism of a transplantable lymphoid tumor of chickens by Russian spring-summer and other neutrotropic viruses has been reported(4). It has also been demonstrated that Virus III causes suppression or early regression of the rabbit fibroma(5). The inhibiting action was demonstrated only when Virus III was inoculated at the same site as the fibroma virus. The present paper describes the results of a study of Semliki Forest virus (SFV) in tissue cultures of normal and tumor cells and the destructive effect of this agent on virus-induced rabbit fibromas.

Methods. Viruses. SFV was prepared as 20% suspensions of infected mouse brain. The intracerebral LD_{50} titer in 10 g mice varied from $10^{-7.5}$ to $10^{-9.0}$. Fibroma virus was obtained from Dr. M. H. D. Smith. This strain of virus was isolated by Dr. R. E. Shope and is similar in its characteristics to the original OA strain. Fibromas were produced on the abdomens of groups of New Zealand white rabbits by injecting 0.5 cc of a 10% extract of testicular fibroma tissue intradermally into 6 to 8 sites. Simultaneously, or at various intervals thereafter, 1.25 ml of a 20% SFV extract was injected intramuscularly. The course of fibroma growth was observed daily or every second day and the diameter and height of each growth was measured and recorded. The volume of each growth was estimated in cubic centimeters (cm^3) by multiplying the height $\times \pi$ radius^2. Weighed portions of fibromas were extracted, centrifuged and titrated intradermally on the abdomens of normal domestic rabbits, using 3 to 4 inoculations for each dilution and a 0.10 cc inoculum. The titer of a given extract was recorded as the Infectious $Dose_{50}$ (ID_{50}) (6). When fibroma virus titrations were made with extracts of fibroma that contained

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1. Moore, A. E., *Cancer,* 1949, v2, 516.
2. Moore, A. E., *Cancer,* 1949, v2, 525.
3. Koprowski, H., and Norton, T. W., *Cancer,* 1950, v3, 874.
4. Sharpless, G. R., Davies, M. C., and Cox, H. R., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 270.
5. Andrewes, C. H., *J. Path. and Bact.,* 1940, v50, 227.
6. Reed, L. J., and Muench, H., *Am. J. Hyg.,* 1938, v27, 493.

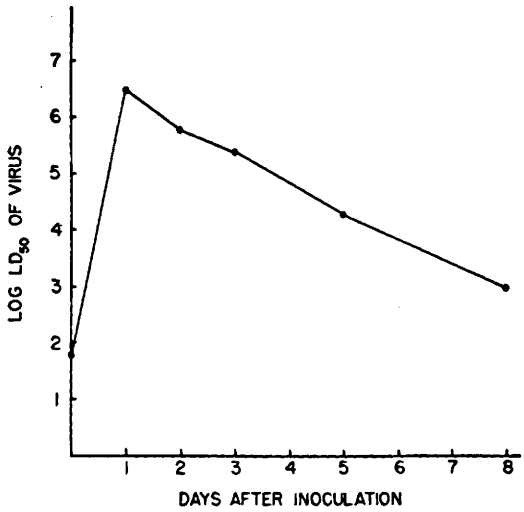

FIG. 1. Multiplication of SFV in tissue culture of chick embryo brain.

SFV as well as fibroma virus, SFV rabbit antiserum (that protected against 10,000 LD_{50} SFV virus) was added to the extracts. These mixtures were incubated 1 hour at room temperature before titration by the intracutaneous method in rabbits.

Tissue culture technic. Weighed portions (0.06 g or 0.2 g) of finely minced animal or human tissue were suspended in 6.0 ml of HS-X6 (10% horse serum in Simm's X6 solution) in a 50 ml Erlenmeyer flask fitted with a rubber stopper. Five hundred units of penicillin and 500 μg of streptomycin per ml were included in the medium of all cultures. SFV was added in 0.24 ml amounts of a $10^{-5.0}$ dilution of mouse brain extract. Cultures were incubated at 37°C for 2 days. The LD_{50} of SFV added to the cultures was determined at the time of each experiment by titrating the extract intracerebrally in mice. After incubation the tissue fragments were extracted in the liquid medium and titrated intracerebrally in mice.

Results. Multiplication of SFV in tissue cultures. SFV multiplied rapidly in tissue cultures containing minced brain or muscle from 8 to 16-day-old chick embryos. The results of a typical experiment employing 0.06 g of 9-day-old chick embryo brain are shown in Fig. 1. Maximum titers ($10^{-4.5}$ to $10^{-6.5}$) were attained within 24 to 48 hours after inoculation and the virus persisted for at least 8 days. The results of experiments designed to determine the capacity of SFV to multiply in tissue cultures of several animal tumors are summarized in Table I. Definite multiplication of virus in 48 hours occurred regularly in 6 to 9-day-old fibroma tissue when 0.2 g of tissue was employed. Growth of virus was not observed in 0.06 g of fibroma tissue. Systematic studies to determine the critical amount of tissue required were not performed. Further experiments revealed that the rate of multiplication of virus in the fibroma tissue was similar to that obtained with chick embryo brain shown in Fig. 1. No multiplication of SFV occurred in cultures of normal adult (6-month-old) domestic rabbit tissues, including brain, heart, kidney, testes, eye, voluntary muscle and subcutaneous tissue. In some instances, however, normal kidney, skin, testicle and subcutaneous tissue from young rabbits (16, 30 and 90 days old) supported the growth of SFV.

The ability of SFV to grow in tissue from several human tumors was also studied. As indicated in Table I, SFV multiplied only in a rhabdomyosarcoma. Of the nonmalignant human tumors studied, SFV grew in 1 of 2 fibromyomas of the uterus but in neither of 2 breast adenofibromas. In a survey of normal human tissue from adults 30 to 73 years old, SFV was also observed to multiply to LD_{50} titers of $10^{-3.0}$ to $10^{-5.3}$ in stomach muscle, hyperplastic thyroid, 1 of 3 endometrial tissues, and in 1 of 4 trials with voluntary muscle. Little or no growth was observed in uterine myometrium, stomach mu-

TABLE I. Multiplication of SFV in Tissue Culture of Certain Tumors.

No. of tests	Tumor	Yield of virus,* Log LD_{50}
13	Rabbit fibroma	4.2–6.6
7	" V_2 carcinoma	<1
16	Mouse sarcoma 180	<1–4.5
4	Hamster fibrosarcoma	<2
1	Human rhabdomyosarcoma	5.3
1	" fibromyosarcoma	<1
1	" lymphoma	<1
2	" breast adenocarcinoma	<1
1	" brain glioma	<2

* After 48 hr incubation at 37°C. Inoculum—log LD_{50} 1-2.6 of SFV.

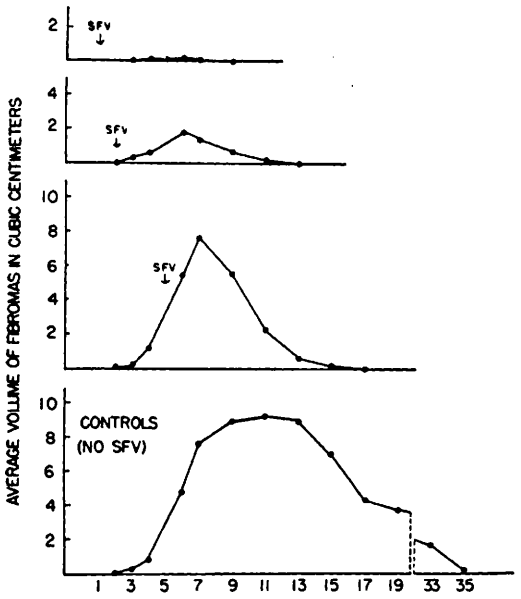

FIG. 2. Development and course of fibromas in control and SFV inoculated rabbits. Each point represents the avg volume of 12 to 16 fibroma growths.

cosa, skin, prostate, fat or tonsil.

Effect of SFV on course of fibromas. The effects of intramuscular injection of SFV on the development and course of rabbit fibromas were studied by inoculating groups of 3 to 4 rabbits with SFV at various intervals after intracutaneous injection of fibroma virus. The results of a comprehensive experiment are shown graphically in Fig. 2. When SFV was injected intramuscularly immediately after the intracutaneous inoculation of fibroma virus, no fibromas developed in about one-third of the inoculated areas. In the remaining areas small papules (2 to 3 mm in diameter) appeared which promptly regressed within 5 days. When SFV was given 24, 48, and 72 hours after fibroma virus inoculation progressively larger fibromas occurred and then disappeared 9, 11, and 13 days respectively after inoculation of fibroma virus. Injection of SFV 5 days after fibroma virus inoculation, when developing fibromas were present, generally caused a firm black scar to develop over the entire fibroma surface within 3 to 5 days. Furthermore, the tumors completely regressed within 13 to 17 days as compared to the average duration of 30 days (range 19-35 days) in the control rabbits. Microscopic examination of sections from the fibromas revealed areas of necrosis 2 days after inoculation of SFV. Within 4 to 7 days all but the deepest layers of fibroma tissue were destroyed. Later injection of SFV up to the 9th day caused similar necrosis of the fibroma surface and somewhat earlier tumor regression but the effect was difficult to demonstrate because of the natural regression of control fibromas. In other experiments it was found that as little as 1.0 cc of a $10^{-6.0}$ dilution of SFV injected 1 day after fibroma virus inoculation caused significant limitation of fibroma growth and regression within 14 days. Intramuscular injection of heat inactivated SFV and normal mouse brain extracts had no effect on the development and course of fibromas. The large amounts of SFV injected into the fibroma rabbits of these experiments was not observed to cause illness or death. The effect of SFV was also demonstrated in hanging drop preparations of fibroma tissue. Pieces of fibroma tissue about 1 mm in the greatest diameter were explanted in plasma hanging drop cultures. Each piece of tissue was embedded in a medium of equal parts of heparinized chicken plasma and 10% chick embryo extract. Six cultures were prepared in a Petri dish(7). Two days after inoculation of fibroma rabbits with SFV, 6 to 9-day fibroma tissue placed in hanging drop preparations showed no fibroblastic proliferations in contrast to moderate fibroblastic proliferation that were usually demonstrated with normal fibroma tissue of comparable age. Also, fibroma tissue fragments that had been incubated 30 to 60 minutes at room temperature in various dilutions of SFV ($10^{-1.0}$ to $10^{-9.0}$) failed to develop fibroblastic proliferation in the hanging drop preparations after exposure to concentrations of SFV from $10^{-1.0}$ to $10^{-5.0}$. This effect of SFV was specifically inhibited by SFV rabbit antiserum.

Recovery of virus from fibroma tissue. Fibromas were removed at intervals after the intramuscular injection of SFV and titrated

7. Enders, J. F., and Florman, A. L., PROC. SOC. EXP. BIOL. AND MED., 1942, v49, 153.

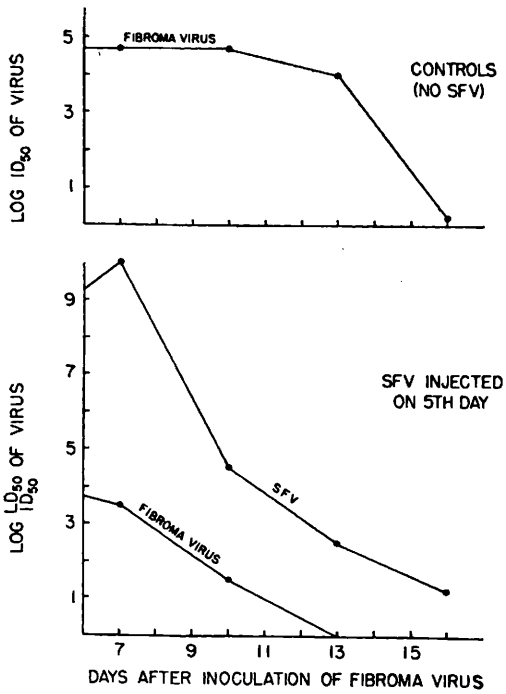

FIG. 3. Recovery of virus from fibromas.

for SFV and fibroma virus. Fig. 3 illustrates the findings in a typical experiment. In this and 2 other experiments SFV persisted until the fibromas completely regressed. The concentration of fibroma virus recovered seemed to parallel the rate of fibroma cell destruction. When necrosis of the fibroma tissue was delayed in the SFV inoculated rabbits, fibroma virus persisted as long as in control animals. This indicated that SFV multiplication *per se* was not associated with a decrease in fibroma virus.

In vitro it was also demonstrated that multiplication and survival of SFV for 8 days in cultures of fibroma tissue did not interfere with the recovery of fibroma virus in concentrations comparable to those obtained from control flasks containing only fibroma tissue. Furthermore, when SFV and fibroma virus were simultaneously added to cultures of voluntary muscle from normal rabbits multiplication of SFV occurred and fibroma virus could be recovered over a 3-day period. This finding is of interest since little or no multiplication of SFV was demonstrated in cultures containing only rabbit voluntary muscle. The results of this experiment suggest that fibroma virus formed fibroblasts or in some way modified muscle cells so that multiplication of SFV was supported.

In titrations for fibroma virus from fibroma tissue that also contained SFV, it was necessary to incubate the dilutions with SFV rabbit antiserum before titration. If this step was omitted SFV, by preventing fibroma growth, masked the presence of fibroma virus.

Discussion. The results of the present experiments demonstrate that Semliki Forest virus multiplies in the rabbit fibroma and destroys the tumor, although no multiplication could be demonstrated in normal subcutaneous tissue. It seems apparent that fibroma virus induces cellular changes in the subcutaneous tissue that are necessary for the growth of Semliki Forest virus. No evidence was obtained in these experiments that interference between the two viruses occurred. Fibroma virus could be obtained from the tumors despite the growth of SFV therein. Microscopic examination of the fibromas and studies of hanging drop preparations revealed that SFV probably exerted its effect by causing necrosis of the parasitized fibroma cells.

The rapid multiplication of SFV to high titer in cultures containing minced tissue provided a relatively simple method for studying the capacity of various tissues to support multiplication of the virus. The finding that SFV multiplied in fibroma tissue *in vitro* led to the present study demonstrating a destructive action of the virus on the tumor *in vivo*. Further studies are in progress to determine the effect of SFV on other tumors, particularly the rabbit myxoma which is so closely related to the rabbit fibroma.

Summary. Semliki Forest virus multiplied in tissue cultures of certain normal and tumor tissues with maximum titers of $10^{4.5}$ to $10^{6.5}$ LD_{50} within 24 to 48 hours. SFV multiplied regularly to high titer in cultures of minced fibroma. Following intramuscular injection of the virus into rabbits bearing virus-induced fibromas, SFV caused rapid necrosis of the tumors. The effect was most apparent when SFV was inoculated simultaneously or within 2 days of the inoculation of fibroma virus.

SFV did not multiply in cultures of normal adult rabbit tissue and caused no deaths or apparent illnesses in rabbits bearing fibromas.

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Factors Interfering with the Hemolytic Property of Mumps Virus.*† (18748)

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In earlier experiments(1), it was observed that amniotic fluids from embryonated eggs containing mumps virus exerted a more powerful and constant hemolytic activity than did virus-containing allantoic fluids from the same eggs(1-3). Allantoic fluids from uninfected eggs were shown to inhibit the hemolytic action of mumps virus and this inhibitory property was not destroyed by incubation with fresh mumps virus for 3 hours at 37°C(3). However, when infected allantoic fluids were dialyzed with phosphate-buffered saline in the cold, their hemolytic activity increased markedly to levels comparable to those obtained with amniotic fluids of similar virus content(3). Furthermore, in the course of earlier experiments(1), it had been observed that an isotonic sodium citrate buffer used as a diluent with infected allantoic fluids resulted in more active hemolysis than when an isotonic phosphate-buffered saline of similar pH was employed. These observations suggested that an inhibitory constituent for the mumps virus hemolysin was present in infected allantoic fluids and that this component was removed by dialysis or sodium citrate. Further studies on the properties and possible nature of this inhibitory factor are reported here.

Materials and methods. An egg-adapted strain of mumps virus was employed for the inoculation of 6- to 8-day-old embryonated eggs by the amniotic or allantoic routes(1). Following incubation at 35°C for 5 to 6 days, the extra-embryonic fluids were harvested separately, placed in glass ampoules, sealed and stored at —70°C. Where dialysis was used, these fluids were placed in cellophane tubing in a large volume of isotonic phosphate-buffered saline (pH 7.2) at 4°C for 24 hours. Hemagglutination and hemolysis tests with chicken erythrocytes were carried out as previously described(2).

Effect of dialysis and diluent. Dialysis of mumps virus-infected amniotic or allantoic fluids with phosphate-buffered saline at 4°C for 24 hours resulted in an increase in hemolytic activity, which was more marked with the allantoic fluids (Table I). Furthermore, the paradoxical increase in hemolysis observed in the first few serial dilutions of untreated allantoic fluids disappeared. An isotonic sodium citrate buffer used as diluent resulted in an increased hemolytic activity with undialyzed allantoic fluid, but had no effect on the dialyzed preparation (Table II). Neither of these procedures had any significant effect on the hemagglutinating activity of the virus.

Effect of an ion exchange resin. The effects observed following dialysis or use of the sodium citrate buffer suggested that some ionic constituent of the allantoic fluid might be re-

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1. Morgan, H. R., Enders, J. F., and Wagley, P. F., *J. Exp. Med.*, 1948, v88, 503.

2. Chu, L. W., and Morgan, H. R., *J. Exp. Med.*, 1950, v91, 393.

3. Chu, L. W., and Morgan, H. R., *J. Exp. Med.*, 1950, v91, 403.