Effect of Semliki Forest Virus on Rabbit Myxoma.* (19464)

DAVID R. GINDER AND WILLIAM F. FRIEDEWALD.

From the Department of Bacteriology and Immunology, Emory University School of Medicine, Atlanta, Georgia.

Although there is much evidence that indicates that rabbit fibroma virus is closely related to rabbit myxoma virus, the end results of infection caused by these viruses are diametrically opposite(1). Myxoma virus causes a disseminated disease that is usually 100% fatal, while fibroma virus causes localized, benign lesions that regress in 20 to 30 days(2). The destructive effect of Semliki Forest virus (SFV) on rabbit fibromas has recently been described(3). Virus-induced fibromas of domestic rabbits became necrotic and regressed rapidly after the intramuscular injection of SFV. Subsequently, the effect of SFV on rabbit myxoma was studied. This paper describes the results of these studies and the multiplication of SFV in tissue cultures of myxoma subcutaneous tissue.

Methods. SFV was prepared as 20% suspensions of infected mouse brain. The intracerebral LD_{50} titer in 15 g mice varied from $10^{-7.5}$ to $10^{-9.5}$. The myxoma virus, originally isolated by Dr. A. Moses, was obtained from Dr. M. H. D. Smith. In these experiments, this strain of myxoma virus killed all 200 control rabbits that were infected with 10 to 10000000 LD_{50} virus. Death usually occurred 10 to 12 days after infection. Myxoma infection was usually induced by injecting 0.1 ml of a $10^{-4.0}$ to $10^{-5.0}$ dilution of virus (containing 10 to 100 LD_{50}) intradermally on the abdomen of rabbits. New Zealand white rabbits were used for all experiments. The effect of SFV on myxoma was studied by injecting intramuscularly 1.25 ml of a 20% extract of mouse brain SFV or a 20% extract of myxoma-passed SFV (that also contained myxoma virus) 24 hours after infecting the rabbits with myxoma. Rabbits were housed in an air-conditioned room maintained at 20°C. The development of myxoma lesions was observed daily. Rabbits were discarded and considered recovered from myxoma infection when all the lesions had disappeared 21 to 55 days after the initiation of infection. Weighed portions of myxoma lesions were extracted, centrifuged and titrated intradermally on the abdomens of normal rabbits using 3 to 4 inoculations per dilution and a 0.1 ml inoculation. Weighed blood clot was titrated in a similar manner when quantitative estimations were made of myxoma viremia. SFV rabbit antiserum was added to extracts of myxoma tissues that contained SFV before myxoma titrations were performed. Myxoma virus dilution neutralization tests were performed by incubating equal amounts of serum and virus one hour at 24°C, and titrating intradermally on the abdomens of rabbits. The tissue culture technic has been previously described(3).

Results. Multiplication of SFV in tissue culture. The multiplication of SFV in minced myxoma subcutaneous tissue resembled that of SFV in fibroma tissue. After the inoculation of 10 to 400 LD_{50} SFV, maximum titers of $10^{-5.5}$ to $10^{-6.5}$ developed in 48 to 96 hours and persisted at least 8 days. However, little or no multiplication of SFV was demonstrated in 15% of the experiments. Detailed studies were undertaken to investigate these inconsistencies in the growth of SFV. SFV multiplied equally well in 0.2 and 0.4 g amounts of 4 to 9 day subcutaneous myxoma tissue. The yield of SFV was not affected by the passage of myxoma virus used to initiate the myxoma tissue. Myxoma-passed SFV did not multiply better than mouse brain SFV. Both the white, homogenous, upper part and the lower, gelatinous part of the myxoma growth supported equally well the multiplication of SFV. The actual cause for irregular SFV multiplication was never completey revealed. However, best growth was observed when the inoculum of SFV was of the order of 200-400 LD_{50} and when myxoma tissue was minced into 1 x 2

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ml rather than 0.05 to 1 ml pieces.

Effect of mouse brain SFV on course of myxoma. In preliminary experiments, SFV from infected mouse brain was injected intramuscularly into rabbits 1 to 3 days after the intradermal inoculation of 1000 to 100000 LD_{50} myxoma virus. In some of the SFV-injected rabbits, certain observations suggested that myxoma was modified by SFV even though all the rabbits died with signs of generalized myxoma infection. On occasion, death was prolonged 4 to 8 days beyond the controls' usual survival period. The initial lesions that developed at the site of myxoma virus inoculation became smaller and occasionally necrotic, and the ear lesions were sometimes circumscribed. When mouse brain SFV was injected intramuscularly one day after intracutaneous injection of 10 to 100 LD_{50} myxoma virus, 5 to 10% of the rabbits survived after undergoing a generalized myxoma infection. Delayed death, initial lesion involution, and circumscribed ear lesions were observed more commonly when 10 to 100 LD_{50} myxoma inocula were employed. The effect of SFV on myxoma was more clearly demonstrated in normal rabbits by a combined intramuscular injection of 1.25 ml of 20% mouse brain SFV and 20% myxoma (Table I). Eight of 21 injected rabbits recovered completely after developing generalized myxoma.

Effect of myxoma-passed SFV on course of myxoma. SFV was passed 45 times in myxomatous rabbits to determine whether adaptation of SFV to myxoma would enhance the effect of SFV on myxoma. 1.25 ml amounts of 20% SFV extract were injected intramuscularly into rabbits bearing 3- to 5-day-old abdominal growths. The abdominal myxoma lesions, harvested 2 days after the inoculation of SFV, were used as the source of the SFV for the next passage. Various passages were injected intramuscularly into normal rabbits and into rabbits infected 24 hours earlier with 10 to 100 LD_{50} myxoma virus. The results of the tests are depicted in Tables I and II. All tested extracts of myxoma-passed SFV caused a further significant reduction in the myxoma fatality rate when injected intramuscularly with myxoma into normal rabbits.

TABLE I. Result of Combined Intramuscular Injection of SFV and Myxoma Virus in Normal Rabbits.*

Inoculum	D/I†	Myxoma fatality rate, %
Control—Myxoma virus	15/15	100
Mouse brain SFV and myxoma	13/21	62
10th passage SFV in myxoma and myxoma	1/8	12
21st	4/18	22
31st	5/14	36
43rd	6/18	33
45th	1/10	10

* 1.25 ml of 20% SFV and 20% myxoma.
† Numerator=rabbits that died; denominator=rabbits inoculated.

TABLE II. Effect of SFV on Rabbits Infected with 10 to 100 LD_{50} Myxoma Virus.*

Inoculum	D/I†
Control—Myxoma virus	70/70
Myxoma passed SFV—3rd passage	2/4
10th	5/6
43rd	26/29
45th	18/35

* 1.25 ml of 20% SFV extracts were inj. intramusc. 24 hr after intracutaneous infection with myxoma virus. Extracts of myxoma passed SFV also contained myxoma virus.
† Numerator=rabbits that died; denominator=rabbits inoculated.

However, in rabbits previously infected with myxoma, only passage No. 45 substantially decreased the myxoma fatality rate. Subsequent tests confirmed this special effect of passage No. 45 SFV extract. No evidence was obtained that myxoma had become attenuated during the repeated passages of SFV. Injection of 0.1 ml of the 45th passage of SFV in myxoma, containing 10 LD_{50} myxoma virus and sufficient SFV antiserum to neutralize completely the SFV present, killed 19 of 19 rabbits in the usual 10- to 12-day period. During the passages in myxoma, the titer of SFV ranged from $10^{-7.5}$ to $10^{-9.5}$. SFV was separated satisfactorily from myxoma of passage No. 45 SFV-myxoma only by inoculating the virus mixture intraperitoneally in mice. When the brains were harvested, SFV was present in a titer of $10^{6.5}$ LD_{50}, but no myxoma virus was demonstrable. Intramuscular injection of this 20% SFV mouse brain extract did not prevent death in

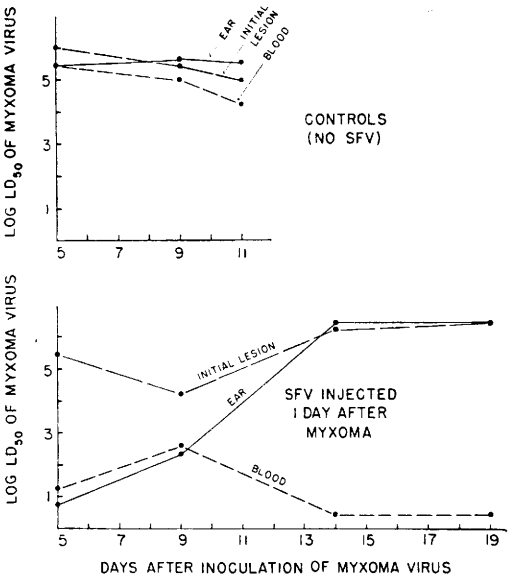

FIG. 1. Effect of SFV on recovery of myxoma virus. Each point represents average of myxoma virus concentrations in 3 or 4 rabbits. Determinations were terminated in control animals on the 11th day when the rabbits were dead or moribund.

17 of 17 domestic rabbits inoculated one day earlier with 50 LD_{50} myxoma virus.

Recovery of virus from myxomatous rabbits. Abdominal lesions, sections of ear, and blood were removed at intervals from a group of myxomatous rabbits inoculated with the 45th passage of SFV in myxoma and from a control group of myxomatous animals injected with 20% myxoma virus intramuscularly. Titrations were made for SFV and myxoma virus. SFV persisted 13 to 18 days in the abdominal lesions at titers of $10^{3.0}$ to $10^{4.5}$ LD_{50}, whereas no SFV was detected in blood or ear 4 days or later after the inoculation of SFV. Fig. 1 illustrates the findings with regard to myxoma virus.

Mechanism of action of SFV on myxoma. Intramuscular injections of heat inactivated (65°C for one hour) SFV and normal mouse brain had no effect on the course of myxoma. Intramuscular injection of myxoma virus did not interfere with the development of generalized myxomatosis subsequent to the intracutaneous inoculation of myxoma. Because SFV did not significantly modify the initial body temperature response to myxoma infection, there is no evidence that elevated body temperature *per se* was responsible for the modification of myxoma(4). Earlier and more marked necrosis was seen in histologic sections of myxomatous lesions of SFV-myxoma inoculated rabbits than in tissues from control myxoma rabbits. Low titers of myxoma neutralizing antibody (neutralization of 1 to 2 log LD_{50} of myxoma virus) was detected 14 to 19 days after onset of infection.

Neurologic sequella of SFV injection. Thirty per cent of all rabbits injected intramuscularly with mouse brain or myxoma-passed SFV developed neurologic sequella, usually paralysis of one or more limbs. Ten per cent of all SFV-injected rabbits died from neurologic complications 5 to 6 days after injection of SFV. These deaths were easily distinguished from myxoma deaths and were excluded in determining myxoma fatality rates.

Discussion. The reported experiments demonstrate that intramuscular injection of SFV can reverse the usually 100% fatality rate of myxoma virus infections. Although this effect was demonstrated to some extent with SFV from infected mouse brain, it was best effected with myxoma-passed SFV. Efforts to study the mechanism of the superior myxoma destroying effect of myxoma-passed SFV were thwarted because SFV could not be separated from the myxoma without further modification of the SFV. SFV probably exerted its effect on myxoma infection by causing necrosis of myxoma tissue and, consequently, reducing myxoma viremia and metastatic myxoma lesions.

Summary. Semliki Forest virus multiplied in suspended cell cultures of myxoma tissue to titers of $10^{5.5}$ to $10^{6.5}$ LD_{50} in 48 to 96 hours. In contrast to the usual 100% mortality in myxoma infection, intramuscular inoculations of mouse brain SFV combined with myxoma reduced the myxoma fatality rate to 62%. Myxoma-passed SFV and myxoma injected together intramuscularly killed only 25% of the rabbits. In rabbits that had been infected intracutaneously with myxoma virus 24 hours earlier, intramuscular injection of the 45th passage of SFV in myxoma reduced the

myxoma fatality rate from 100% to 51%.

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Effect of Polyvinylpyrrolidone on Reticulo-Endothelial Storage. (19465)

KURT STERN.

From the Mount Sinai Medical Research Foundation, and the Department of Pathology, Chicago Medical School, Chicago, Ill.

Polyvinylpyrrolidone (PVP), a macromolecular substance first synthesized in Germany during World War II, has been used in Europe as a plasma substitute, reportedly with good results. Abstracts of the extensive European literature on PVP have been presented in a monograph(1) issued by the General Aniline and Film Corporation. Recently the possible use of PVP as "plasma expander" has been considered in this country. Hence biologic properties of PVP deserve continued investigation. Reports on storage of PVP in tissues are contradictory. Earlier negative findings were obtained on dogs and man (Korth and Heinlein(2)). On the other hand, Bargmann(3) observed storage in splenic macrophages of dogs and rats, and Barfuss and Eichler(4) noted transitory storage in rabbits, guinea pigs, and rats. Ammon and Müller(5) found evidence of PVP storage in rabbits, but not in infants treated with PVP, whereas Schoen(6) reported positive findings in such infants. Equivocal results were reported by Riedel and Zipf(7), and by Pellerat *et al.* (cit. 1, p. 50). Recently Nelson and Lusky(8) found in rabbits injected with PVP slight splenic enlargement, and presence of foam cells in spleen, lymph nodes, bone marrow, and other tissues. Bennhold, Schubert, and associates(9-12) reported an interesting property of PVP, namely its ability to promote renal excretion of dyes that are otherwise not excreted at all, or are excreted through the bile. Schubert(12) also found that PVP was capable of "washing out" dyes from tissues with subsequent renal excretion of the dye.

The present investigation is concerned with the effect of PVP on reticuloendothelial storage of dyes in mice. Since previous work (13-15) had shown that the extent of reticuloendothelial storage in mice may vary according to the strain, animals of several inbred strains were utilized for this study.

Materials and methods. Male and female mice, 3 to 6 months old, of strains C57 Black, dba, C3H, and CBA were used. The animals were free of tumors and any other obvious disease. They were kept on *ad libitum* diet of Rockland mouse pellets and water. The dyes used were 1) Erie Fast Rubin Conc. (EFR),* described in a previous communication(15), and 2) Chlorazol Black E (CBE). As a rule, 0.5 ml of 0.5% solutions of dye was injected subcutaneously 2 to 3 times weekly. This dosage was well tolerated by the animals, except in some instances where it was necessary to diminish the quantity of EFR. Controls and experimental animals received equal amounts of dye. PVP† was used as 3.5% solution in physiologic saline, of which 0.5 ml was injected daily subcutaneously, or intraperitoneally in some of the earlier experiments. The animals were weighed at least once weekly, and at the end of the experiments. They were sacrificed by decapitation, permit-

* This dye was obtained through cooperation of the National Aniline Division, Allied Chemical and Dye Corp.

† Mr. D. Witwer, General Aniline & Film Corp., furnished a generous supply of PVP. All experiments were carried out with a single lot (GA-PVP-118).