Propagation of Theiler's Virus in Murine Ascitic Tumors.* (22540)

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Theiler's GD VII strain of mouse encephalomyelitis virus can be propagated in certain murine nervous tissue tumors but not in others(1). Sanders(2) has reported growth of this virus in ascitic forms of Bashford and Krebs carcinoma and in the S37 sarcoma. The present report gives the results of attempts to grow the virus in the latter tumors, the Ehrlich carcinoma, and in the ascitic form of two CNS murine tumors. Preliminary data were given elsewhere(3).

Methods. Virus passed more than 50 times in tissue cultures of newborn mouse brain was used. A mouse passage strain kindly sent by Dr. F. Rasmussen, U.C.L.A., also was used in some experiments. The Bashford 63 carcinoma and the Krebs 2 carcinoma passed in albino mice were kindly furnished by Dr. K. Sugiura, Sloan-Kettering Institute for Cancer Research. The sarcoma S37 was sent by Dr. J. L. Hartwell, National Cancer Institute, and was maintained in C57 mice. The ependymoma passed in albino A mice and the astrocytoma of C3H mice were those used previously(1). The Ehrlich carcinoma passed in albino mice was obtained from Dr. J. B. Field, of the Univ. of Southern Calif.

Virus was assayed by hemagglutination using serial 2-fold dilutions of virus with 0.5% human RBC. The endpoint was read from the sediment. Virus was also detected by intracerebral injection in mice with 0.03 ml of serial 10-fold dilutions of material tested. Ascitic forms of all tumors used were obtained by a method similar to that described by Klein and Klein(4). Intraperitoneal injections were made with tumor tissue broken into small fragments by passage through a perforated metal disc (1 mm holes) and a stainless steel screen (80 mesh). The tissue obtained was further broken up by suspension in Simms' solution with vigorous pipetting. After the larger clumps of cells were allowed to settle, the supernatant fluid contained small clumps of cells and single cells. The Krebs tumor was used only in solid form.

Solid forms of the tumors developed subcutaneously to approximately the size of 1 cm were inoculated, *in vivo,* with 0.01 to 0.03 ml of virus (100 intracerebral MLD_{50}); one week later the supernatant fluid from the centrifuged ground suspension of tumor was tested for viral content by intracerebral injection of mice. Minced tumor tissue mixed with virus was implanted subcutaneously in mice. The tumors developed after 7-14 days were likewise tested for virus. Minced tumor tissue mixed with virus was implanted to the chorio-allantoic membranes of 10-12-day chick embryos. After incubation of the eggs at 35°C for 8-10 days the amount of virus in the tumor tissue was determined by injection of mice.

Tissue cultures of tumor and virus were made in 50 ml stoppered Erlenmeyer flasks containing 3 ml of various media. After incubation for 2-3 days at 35°C the contents of 3 flasks were pooled, the tissue was sedimented and the supernatant fluid was tested for virus content. Cultures were also incubated for 7 days and tested for virus.

Mice with developed ascitic tumors were injected intraperitoneally with virus (100 MLD_{50}); 4 to 8 days later the ascitic cells were separated from the ascitic fluid, ground in a mortar with alundum and saline. After sedimentation of the debris, the saline supernate was tested for virus.

Results. No virus multiplication was found with Ehrlich, Krebs, Bashford or S37 tumors grown in mice and inoculated with virus or in tumor tissue inoculated with virus and grown in mice or in eggs. No virus proliferation was detected in tissue cultures of the above tumors in Simms' solution, lactal-

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bumin hydrolysate-yeast extract medium with 10% horse serum(5) or with Simms' and ST199(6) in a 1:1 mixture. Virus grows readily in all these media containing minced newborn mouse brain. Mouse ascitic fluid successfully used in tissue culture by Sanders (2) could not be used, because as obtained from our stock mice it neutralized the virus. A mouse passage strain as well as a tissue culture passage strain of virus was used for the above experiments. It was reported previously(1) that Theiler's virus can be grown in solid tumors in mice as well as in tissue cultures of astrocytoma and ependymoma. In the present study ascitic forms of both tumors were developed. Both tumors produced a markedly bloody exudate and both had a tendency to form solid tumors on the peritoneum. Two to 3 weeks after they received an intraperitoneal implantation mice were found to have 3-7 ml of ascitic fluid which contained small and large clumps of cells in addition to approximately 5-14 million single cells per ml for ependymoma and 50-100 million for the astrocytoma. Virus grew readily to attain titers of 10^{-3}-10^{-4} in tissue cultures of such cells. Virus grew when injected intraperitoneally with ascitic astrocytoma cells and grew readily in the ascitic ependymoma if the latter tumor cells were first washed free of ascitic fluid and were left in contact with virus for 1½ hr in the refrigerator (4-6°C) before the cells were passed to the peritoneal cavity of a new mouse; virus could be recovered from the washed, ground tumor cells removed 2 weeks later from the mouse. The titer of virus obtained was 10^{-4} with ependymoma and 10^{-6} with astrocytoma, taking as 10° the dilution of virus in saline used to resuspend the ground cells in a volume equal to that of the removed ascitic fluid. No virus proliferation was found by this procedure with ascitic forms of Bashford or Sarcoma 37 tumors. Moreover, no tissue culture (mouse brain) virus was adsorbed from Simms' solution by washed cell suspensions of any of the 3 carcinomas or the sarcoma left in contact with virus for 1½ hr at 4-6° or at 37°C. Virus was adsorbed under these conditions by minced mouse brain and by ependymoma tissue but not by astrocytoma.

The ependymoma ordinarily grew readily in solid or in ascitic form in Albino A mice. It could not be grown in solid form for more than the initial passage in another inbred strain of albino mice [Albino 2 strain(1)] maintained in this laboratory. One of 15 attempts to pass the tumor in ascitic form in Albino 2 mice succeeded. This ascitic tumor has now been maintained for more than 30 passages. Starting with the 6th passage it changed in character in the following respects from the ascitic tumor maintained in Albino A mice: a. The cells never formed solid tumors on the peritoneal surface, neither did they grow as solid tumors when subcutaneously implanted in either strain of mice. b. There were about the same number of tumor cells found in the ascitic fluid that would be found in A mice but almost all the cells were single cells. c. When passed intraperitoneally in A mice the cells grew in the manner just described. d. There were usually few RBC in the exudate. e. The cells did not support growth of Theiler's virus when tested in tissue cultures. f. The cells appeared histologically identical with cells seen in the A mice when examined unstained or stained with H. E. or Giemsa stains. There was no marked difference in ploidy observed in the 2 cell lines although numerical distribution frequencies for the chromosomes were not determined. Evidently this strain of cells selected or produced as a mutant by passage in Albino 2 mice is a variant which differs biologically from the Albino A passage cells or whose presence was masked in the latter environment.

Discussion. The results obtained in this study suggest that Theiler's GD VII virus is not readily propagated in Bashford, Krebs, or Ehrlich carcinomas or in the S37 sarcoma. Why this should differ from the experience of Sanders(2) is not clear. The ascitic forms of astrocytoma and ependymoma which have been developed would be expected to support growth of virus since the parent tissue did so. Although tissue cultures of the solid form of astrocytoma did not yield as much virus as did newborn mouse brain(1), the ascitic cells

did. It has not been determined whether this is a result of the presence of a greater number of intact cells present in a given weight of ascitic cells than in a solid tumor minced or whether there has been a mutant line of cells developed during routine tumor passage which yields more virus. This is under study. The development of a variant strain of ependymoma cells by passage in a partially susceptible strain of mice is of interest in that it has provided a line of cells for further study which is insusceptible to virus and which lacks the capacity to invade host tissue in the sense that it does not establish itself on the peritoneum or in subcutaneous tissue. The capacity of washed ascitic cells to become infected with virus and to propagate it in a virus-inhibitory environment, the ascitic fluid, is of interest. It remains to be determined if the virus can persist in subsequent generations of the cells. This condition might be analogous to the lysogenic bacteria-bacteriophage relationship. Such a condition has been suggested as possible for psittacosis virus (7) and for APC viruses(8).

Summary. Ascitic forms of murine astrocytoma and ependymoma have been developed. These support growth of Theiler's GD VII virus, *in vitro,* and under suitable conditions, *in vivo.* A variant strain of ependymoma, not susceptible to virus was obtained by passage of tumor in a partially tumor-resistant strain of mice. Ehrlich, Bashford 63 and Krebs 2 carcinomas and S37 sarcoma were not susceptible to Theiler's GD VII virus, *in vitro,* or in mice.

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