A New Host-Virus System.* (19897)

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The inability to manipulate animal cells constitutes one of the serious limitations in the investigation of the mechanism of viral propagation. Animal tissues which are susceptible to viral infection exist as organized aggregates of several types of cells. The production of a simultaneous uniform infection, the determination of cellular viability, or even the estimation of the number of cells involved most often is impossible. Ascitic tumor cells of a homogeneous type can be obtained, and when properly cultured in the mouse peritoneal cavity, they will grow as discrete cells which can be counted in a hemocytometer (1,2). Unlike most adult animal cells, their viability can be tested by implantation to a susceptible host. Because of these desirable properties, ascitic tumor cells were considered as a possible component of an experimental host-virus system.

In this preliminary report the adaptation of an influenza virus to culture in an ascitic form of a mouse tumor is described.

Materials and methods. Tissue. The Ehrlich mouse carcinoma used in these experiments was obtained in ascitic form from Dr. G. A. LePage. In this laboratory it has undergone 45 passages in Swiss white mice (Webster strain). For each passage ascitic fluid was removed from the peritoneal cavity of mice which had been inoculated with tumor cells 7 days previously. After counting the cells in a hemocytometer, appropriate dilutions were prepared in saline and mice were inoculated intraperitoneally with approximately 20 million cells. *Virus.* The WS strain of Type A influenza virus was chosen for these studies. This virus was maintained for several years by continuous passage in tissue culture and since has undergone 102 passages in mouse brain(3). It will multiply both in the brain and lungs of mice. *Virus titer.* The amount of virus was estimated by determining the infectious titer for eggs. Tenfold serial dilutions of the virus were prepared in broth and 4 eggs were inoculated with 0.1 ml of each dilution. After 3 days of incubation at 37°C, samples of allantoic fluid were removed from each egg. These fluids were tested for virus by the addition of chicken red blood cells. The 50% infectious titer was calculated using the method of Reed and Muench(4).

Results. Adaptation of influenza virus to the Ehrlich ascites tumor. Five mice, bearing 5-day-old ascitic tumors, were inoculated intraperitoneally with 0.2 ml of a 10% suspension of infected mouse brain which had an infectious viral titer for eggs of $10^{-5.0}$. Three days later ascitic fluid and cells were removed from these mice. One portion of the fluid was titered in eggs for virus content, and a second portion was used to infect a new group of mice which bore ascitic tumors. At 3-day intervals the process of serial passage was repeated. After each passage, the egg infectious titer of the ascitic fluid was found to be lower than the previous one until by the third passage no infectivity for eggs was demonstrable (Fig. 1). This material then was used to inoculate mice intracerebrally. When the brains from these mice were harvested, they showed an infectivity titer for eggs of $10^{-5.5}$. A 10% suspension of the brain was prepared and used to initiate a new series of serial passages in the ascitic tumors. By the third passage of this latter series, the infectivity titer had again decreased to $10^{-1.5}$. Thereafter, the titer of each successive passage increased until titers as high as $10^{-5.0}$ to $10^{-6.0}$ were steadily attained (Fig. 1). The virus has now undergone 38 serial passages in ascitic tumors.

Growth characteristics of influenza virus in ascitic culture. Mice bearing ascitic tumors were inoculated with 0.2 ml of a suspension

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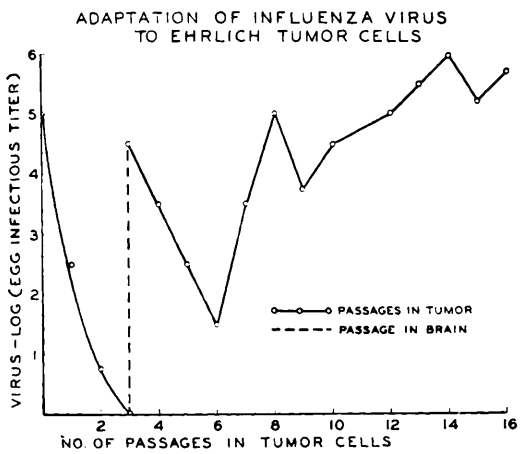

FIG. 1. Duration of each passage was 3 or 4 days. The virus was passed undiluted during the first 7 passages and thereafter at a dilution of 10^{-1}. All estimations of the amount of virus are based on infectious titers for eggs.

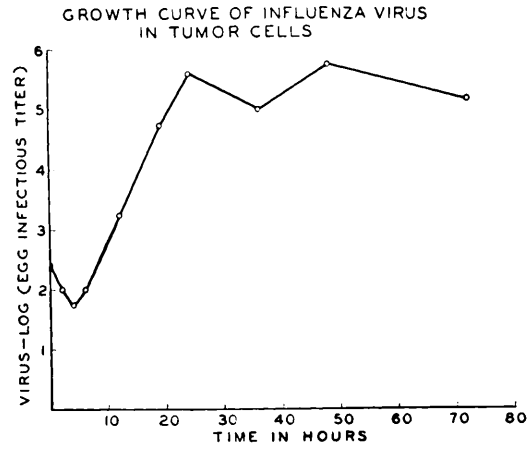

FIG. 2. Each mouse received .2 ml of an inoculum which had a titer of $10^{-5.4}$. This virus had undergone 33 previous passages in ascitic cells. The virus titers recorded are infectious titers for eggs.

of the adapted virus which had an infectivity titer for eggs of $10^{4.7}$. Five mice were sacrificed immediately, and the ascitic cells were found to have a titer of virus of $10^{-2.5}$. At appropriate intervals thereafter groups of mice containing 5 animals were sacrificed, and the ascitic cells removed. The titers of influenza virus of pooled samples of the tumor material were determined in eggs. By 4 hours after inoculation, the titer of the peritoneal fluid had decreased further to $10^{-1.7}$. An increase in virus titer was first detectable at 6 hours and by 12 hours was $10^{-3.5}$. The maximum titer ($10^{-5.5}$ to $10^{-6.0}$) was reached by 24 hours and remained relatively constant through 72 hours (Fig. 2).

Effect of influenza virus on Ehrlich tumor cells. Tumor cells were removed from the peritoneal cavity of mice which had been inoculated 2 days before with influenza virus. Examination under phase microscopy revealed no difference in gross appearance from the usual tumor cells. However, repeated attempts to initiate a new ascitic culture with these cells were without success. When an equal number of tumor cells uninfluenced by virus was employed, tumors were induced in all the animals. Thus, it appears that the infection of these cells is accompanied by a loss in viability of the host cell.

In view of this finding, it was of interest to determine the effect of the virus on the course of the development of an ascitic tumor culture which had been securely established prior to the introduction of the virus. One group of mice which had been implanted with tumor cells 5 days before was inoculated with 0.2 ml of a virus preparation having a titer of $10^{-4.5}$. When this group was compared with a control containing no virus, it was observed that in 2 days the amount of ascitic fluid was markedly reduced (controls containing 3 ml, while the virus treated contained 0.5 ml per mouse). Further, the ascitic cells in the infected animals had begun to aggregate and were found in the peritoneal cavity in large clumps. By the 7th day after the introduction of the virus, the control animals were greatly distended, while those with the virus appeared normal in size (Fig. 3). When the latter animals were sacrificed, none or only small aggregates of cells could be found in the peritoneal cavity. Thus, accompanying the inhibition of the multiplication of the tumor cells is an extensive regression of the tumor.

In several experiments a large number of mice died after the regression of the tumor presumably from the invasion of the virus. However, in other instances a considerable number of mice continued to survive until sacrificed 6 to 7 weeks later. A systematic examination of the variables which might produce these inconsistent results has not been completed. However, it will be noted that

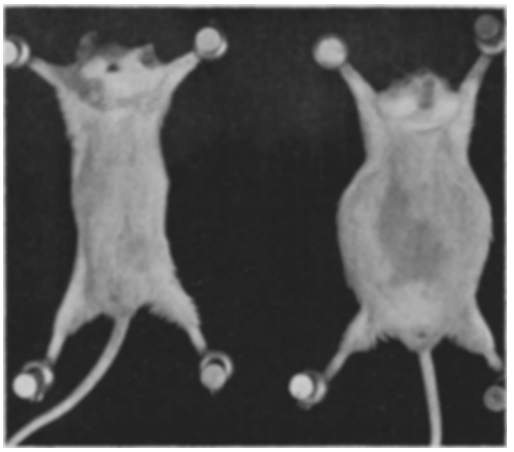

FIG. 3. Effect of virus on development of ascites tumor. Each mouse was inoculated with 20×10^6 Ehrlich tumor cells. Five days later the mouse on the left received intraper. .2 ml of a 10% suspension of virus which had undergone 15 passages in ascitic tumor cells. The photographs were taken 16 days after the viral inoculation.

the intraperitoneal inoculation of normal mice with this adapted strain of virus is never accompanied by fatalities.

Discussion. The multiplication of an adapted strain of influenza virus in an ascitic mouse tumor can be demonstrated by continuous serial passage or by following the growth curve during a single passage (Fig. 1 and 2). It appears that this viral multiplication in the tumor tissue is accompanied by a loss of viability of the host cells and a gross recession or destruction of the tumor itself (Fig. 3). As yet, experiments of long duration which would be necessary to evaluate thoroughly the use of this phenomenon in tumor therapy have not been completed.

This new experimental host-virus system should be of particular value in the study of the mechanism of viral infection, since it is possible to produce an infection simultaneously in a large number of cells of one type. Further, infected cells may be observed individually. While many new and interesting types of experiments can be performed with this system, accurate quantitative experimentation can best be done in tissue culture. Attempts to grow this virus in ascitic cells which are surviving in Warburg flasks are now in progress(5). How widely applicable this technic will be with regard to types of viruses and tumors also remains to be determined. However, the experiences of other workers with solid tumors may be indicative of the limitations to be encountered here(6,7).

Summary. A new host-virus system is described. The WS strain of Type A influenza virus was adapted to culture in the Ehrlich ascitic carcinoma of mice. This adapted strain of virus has been maintained through 38 serial passages in this tissue. The growth characteristics during a single passage have been described. Associated with the multiplication of this virus is a loss of viability of the host cell. The effect of this phenomenon on the course of development of an established ascitic tumor has been followed. The importance of this new host-virus system as a tool for the study of the mechanism of viral multiplication is discussed.

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