# Egg Cultivated Tumor Protects Embryo against Vaccinia Virus. (20455)

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It has been demonstrated that some viruses possess oncolytic activity against certain tumors(1-3). The basis for these viral antitumor effects is still unknown. In some instances, it appears to be a direct effect analogous to that which occurs in nontumor cells when a virus manifests its usual pathogenic effects. It is also possible that if the tumor process is the result of the reaction of a cell to a specific tumor agent, some of the effects observed may be due to the interference of one virus with the action of another.

The work done in the past on this problem has been concerned with how a particular virus affects a tumor. The present investigation considers the effect of a growing tumor on the toxicity of a virus to the host organism.

*Experimental.* Embryonated eggs bearing tumors implanted by the yolk sac method and viruses adapted to egg cultivation were used in these studies. Tests were made with a $C_3H$ mouse mammary adenocarcinoma, dba mouse mammary adenocarcinoma and a rat sarcoma. These tumors have been described elsewhere(4). Vaccinia was the principal virus utilized in the experiments, but tests were also made with Lee strain influenza. Seed viruses for the strains were obtained originally from Dr. McLean of Parke, Davis & Co. The eggs were implanted with tumor tissue on about the 4th day of incubation(5). The virus was introduced into the yolk sac on the 9th or 10th day, at which time the tumor tissue was well established but very small (0.02 g). At the conclusion of the tests the yolk sac tumors averaged 0.6 to 1.2 g. Vaccinia and influenza were used at concentrations which caused the death of the embryo in 4 and 3 days, respectively, in nontumor-bearing eggs. The tumor-bearing eggs were divided into 2 groups, one of which received injections of $H_2O$ into the yolk sac, while the other group was injected in the same way with the virus suspension. A third group, which consisted of nontumor-bearing eggs at the same stage of incubation, were injected with the virus suspension. The nontumor-bearing virus inoculated eggs served as the control for virus toxicity, and the $H_2O$ injected tumor-bearing eggs served as the control for embryo mortality due to tumor growth. In the work with vaccinia, 3 experiments involving 249 eggs were completed with the $C_3H$ mammary tumor, 2 experiments including 113 eggs with the rat sarcoma, and 4 experiments utilizing 253 eggs with the dba mammary tumor. The same tumors were used in experiments with influenza but since the results were negative, no further details are necessary.

*Results and discussion.* The results of the tests with vaccinia and the various tumors are contained in Table I and Fig. 1, 2, and 3. It will be noted that embryos supporting implants of the dba mammary tumors (Table I and Fig. 1) were protected against the usual toxic effect of the virus. Tumor growth was not appreciably affected.

Allantoic fluid of the embryos of the tumor-bearing eggs which survived vaccinia inoculation contained live virus. Extracts made from these eggs had the same virus concentration as those made up from the nontumor eggs which died in the usual 3-4 day period.

It will be observed in Table I and Fig. 2 that the $C_3H$ mammary tumor gave only a slight extension to the life of the embryo, while the rat sarcoma (Table I and Fig. 3) was ineffective. The $C_3H$ tumor grew more rapidly than the dba and for that reason was more toxic to the embryo. It may be that this accounts for the difference in the action of the 2 mammary tumors. The rat sarcoma, however, grew more slowly than either of the other two and was particularly nontoxic, so that many of the embryos of eggs inoculated with this tumor survived to hatching time.

The yolk sac implanted tumor is so situated that embryo and tumor grow together sharing a common blood stream but otherwise not in-

TABLE 1. Toxicity of Vaccinia to Embryos of Eggs Bearing dba Mouse Mammary Tumors, $C_3H$ Mouse Mammary Tumors, and Rat Sarcomas.

| | No. experiments | No. eggs | Mortality (days after virus inj.) 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | 7 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. eggs dead | % dead | No. eggs dead | % dead | No. eggs dead | % dead | No. eggs dead | % dead | No. eggs dead | % dead | No. eggs dead | % dead | No. eggs dead | % dead |
| dba tumor control | 4 | 85 | 20 | 24 | 23 | 27 | 29 | 34 | 37 | 44 | 44 | 52 | 55 | 65 | 73 | 86 |
| dba tumor + virus | 4 | 84 | 30 | 36 | 34 | 40 | 40 | 48 | 50 | 60 | 56 | 67 | 63 | 75 | 75 | 89 |
| Nontumor + virus | 4 | 84 | 14 | 17 | 16 | 19 | 33 | 39 | 79 | 94 | 84 | 100 | | | | |
| $C_3H$ tumor control | 3 | 95 | 18 | 19 | 30 | 32 | 36 | 38 | 45 | 47 | 51 | 54 | | | | |
| $C_3H$ tumor + virus | 3 | 95 | 12 | 13 | 18 | 19 | 48 | 51 | 84 | 88 | 95 | 100 | | | | |
| Nontumor + virus | 3 | 59 | 10 | 17 | 19 | 32 | 48 | 81 | 59 | 100 | | | | | | |
| Rat tumor control | 2 | 37 | 6 | 16 | 7 | 19 | 9 | 24 | 11 | 30 | 12 | 32 | | | | |
| Rat tumor + virus | 2 | 38 | 7 | 18 | 16 | 42 | 32 | 84 | 37 | 97 | 38 | 100 | | | | |
| Nontumor + virus | 2 | 38 | 6 | 16 | 9 | 24 | 34 | 90 | 38 | 100 | | | | | | |

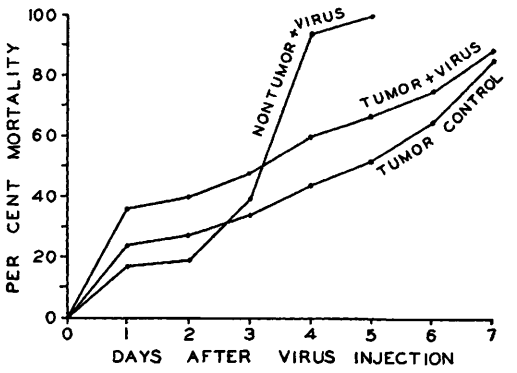


FIG. 1. Toxicity of vaccinia to embryos of eggs bearing dba mouse mammary tumors.

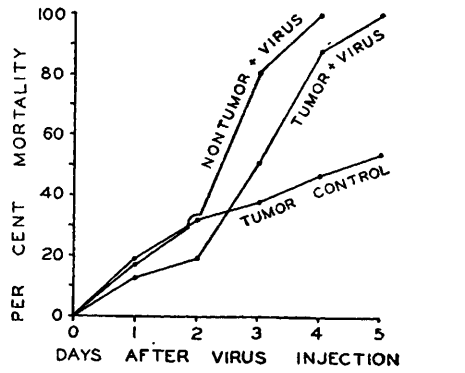


FIG. 2. Toxicity of vaccinia to embryos of eggs bearing $C_3H$ mouse mammary tumors.

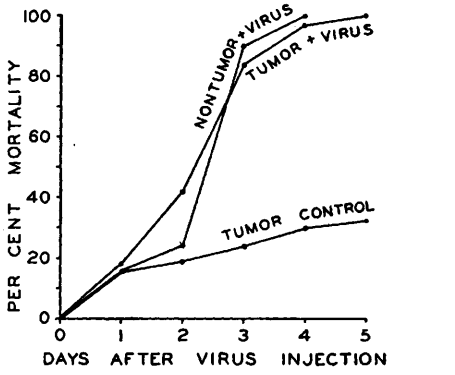


FIG. 3. Toxicity of vaccinia to embryos of eggs bearing rat sarcomas.

terfering with each other. The protection afforded the embryo by the dba tumor must have been due to something passed into the blood from the tumor tissue. Since the healthy, vigorous embryos continued to contain live virus in their fluids, it would appear that there was a constant neutralization of the virus toxicity.

Previous research with the dba mammary tumor used in this study has provided both direct and indirect evidence for the tumor agent or virus concept of tumor etiology(6-9). It is suggested that the present results contribute to the same hypothesis. The protection afforded the embryo against vaccinia by the implanted tumor could have been due to the well-known blocking effect which one virus may have on another.

*Summary.* 1. Yolk sac implants of a dba mouse mammary tumor protected the host embryos against vaccinia virus. A dosage of the virus which killed embryos of nontumor-bearing eggs in 3-4 days gave no evidence of toxicity in the embryos bearing the dba tumor 7 days after inoculation. Tests demonstrated that the virus was present in the fluids of these embryos. 2. Comparable tests with eggs bearing a $C_3H$ mammary tumor indicated the embryos were only slightly protected against the virus, and yolk sac implants of a rat sarcoma gave negative results in this respect. 3. None of the 3 tumors affected the toxicity of a strain of influenza. 4. It is suggested that the dba tumor's protective effect on the embryos was due to the neutralization of the vaccinia virus by the tumor agent.

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## Effect of Fatigue on Susceptibility of Mice to Poliomyelitis. (20456)

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It is often observed that the paralysis of poliomyelitis is preceded by a period of exhausting physical exercise and the belief is prevalent that fatigue increases susceptibility to paralysis in persons already infected. Statistical studies(1-6) tend to confirm these clinical impressions but can be accepted only with reservations. For example, the use of non-paralytic cases for control(4) is subject to considerable error because of the difficulty in making an accurate clinical diagnosis in the absence of paralysis(7). Variables inherent in estimation of the degree of paralysis make judgment difficult on this basis alone(1-3). In one series, fatigue was associated with an increased incidence of paralysis in adults, but no significant difference was found in children(5). The association of increased paralysis with the distance of transportation of patients(6) may be due to other factors than fatigue.

To our knowledge, there has been only one previous report concerning the susceptibility of fatigued animals to experimental poliomyelitis(8). In these experiments, fatigue was induced in monkeys by forcing the animals to swim until exhausted. However, under these conditions it was difficult to distinguish between the effects of fatigue and chilling and use of monkeys limited the numbers of animals in each experiment. In our experiments, fatigue was induced by a technic that avoided immersion in water and the availability of mice permitted the use of relatively larger numbers of animals.

*Materials and methods.* In the beginning,

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