Influence of Hemagglutinating Viruses on Tumor Cell Suspensions: II. Newcastle Disease Virus and Ehrlich Carcinoma.* (19921)

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In a previous paper the inhibitory effect of Newcastle Disease virus (NDV) on the growth of cell suspensions of sarcoma 180 was reported(1). When equal parts of virus and cell suspension were mixed at 4° and room temperature (25°) immediate inoculation of the mixtures failed to result in tumor growth. When the same mixtures were kept at 4° and then transferred to room temperature, or if they were kept continuously at room temperature for one to 3 hours, their inoculation resulted in tumor growth.

It has been noted that NDV also has the power of acting on the cells of the Ehrlich carcinoma(1). By using this tumor, which produces ascites in mice, it was possible to compare the *in vivo* and *in vitro* action of NDV on the tumor cell. These studies are now presented. Since this work was completed Kausche, Landschutz and Sauthoff(2) have reported the effect of PR8 strain of influenza virus on the Ehrlich carcinoma. Our results with NDV have been similar to theirs and will be discussed later.

Materials and methods. Virus. The Massachusetts strain of NDV† was prepared by inoculation of 0.2 cc of a 10^{-3} dilution into 11-day fertile eggs and the clear allantoic fluid harvested 48 hours later. Pools were kept frozen until used. Normal allantoic fluid (NAF) pools were made in the same manner from uninoculated eggs. *Titrations.* Hemagglutination (HA) titrations were done by diluting the material to be tested in saline to make dilutions of 1-10, 1-100 and doubling dilutions thereafter. Equal quantities (0.5 cc) of ¼% washed adult chicken red blood cells were added and the tests were read after one hour at room temperature. The last tube showing complete hemagglutination was taken as the HA titer. Titrations in eggs were done by inoculating 0.2 cc of virus dilutions into the allantoic sac in groups of 3 to 5, 10-11-day-old fertile eggs. Embryo death was taken as the end point and the titer calculated according to the method of Reed and Muench(3). *Tumor suspensions.* Mice bearing Ehrlich carcinoma which had been implanted subcutaneously 10 to 14 days previously were killed and their tumors removed aseptically. The tissue was pushed through a 40-mesh monel metal screen with a pestle and made to 10% suspension in buffered Locke-Ringer (pH 7.2) to which 0.6% glucose had been added. After settling, a fairly uniform single cell suspension was obtained. For long experiments 100 units penicilllin and 2.5 mg streptomycin per cc were added.

Results. In vitro experiments. The general

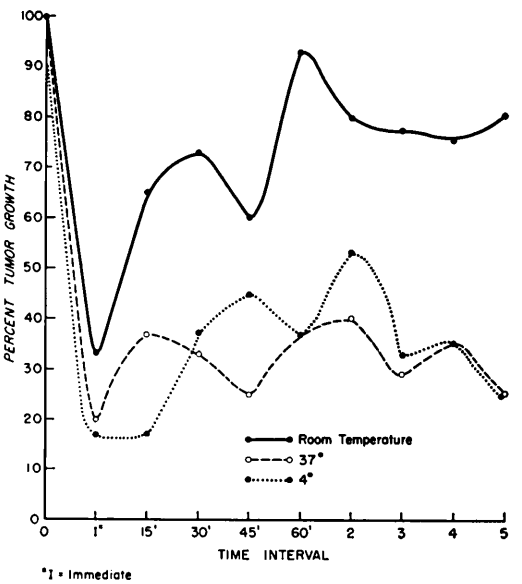

FIG. 1. Percentage growth of NDV and Ehrlich carcinoma cell suspensions mixed at 4°, 25° and 37° and inoculated subcut. at different time intervals.

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plan of experiments was the same as has been reported previously(1). Equal amounts of tumor suspension (10%) were mixed with equal amounts of undiluted NDV allantoic fluid. At different time intervals and at different temperatures 0.2 cc of the mixtures were inoculated subcutaneously into each flank of groups of 5 white mice. At the same time 1.0 cc of the mixture was rapidly sedimented in an angle-head centrifuge and a HA titration done on the supernatant. The animals were inspected at weekly intervals for 5 or 6 weeks and tracings of their tumors made. The results were recorded as the percentage of tumor growth regardless of size of the tumor. In a few experiments the mixtures were inoculated intraperitoneally and the time of appearance of ascites and day of death from tumor noted. In all instances the same tumor mixtures with normal allantoic fluid instead of NDV allantoic fluid were used as controls. For convenience of expressing the results of the experiments, failure of the growth of the tumor cell virus mixtures will be considered as the "adsorption" phase of the reaction and their subsequent growth as the "elution" phase. It is realized that the absolute proof that this is the explanation of the phenomenon must await further work.

1) *Effect of time and temperature on the growth of mixtures of NDV and Ehrlich carcinoma.* Fig. 1 represents the results of an average of 3 experiments. It can be seen that at all temperatures the inhibition of tumor growth took place immediately. The reaction was more complete and more sustained at both 4° and 37° than at 25°. There appeared to be some spontaneous elution at all temperatures but most marked at room temperature. The figures given for tumor growth at 5 hours may reflect a loss in viability of the tumor cells themselves as the normal allantoic fluid controls grew in only 90% of the instances at 25° and 37° in contrast to 100% for each of the other times and temperatures. The HA titers on the supernatant of the tumor cell virus mixtures which were done at the time of inoculation showed an initial drop immediately after mixing at all temperatures. This was followed by a gradual rise in titer so that after 3 to 5 hours the original titer was almost attained. As noted in the experiments with NDV and sarcoma 180 there was no absolute correlation between the rate of tumor growth and the HA titer. For example, although the HA titer for both mixtures at 4° and 37° had risen to almost the original value by 4 hours, tumor inhibition at these temperatures was still present.

TABLE I. Effect of Intraperitoneal Inoculation of Mixtures of NDV and 10% Ehrlich Carcinoma Held at 4° for 10 Min. 4 experiments.

NDV and Ehr.	NAF and Ehr.
3/10*	10/10
3/10	7/10
2/10	10/10
4/10	10/10

* Deaths from ascites over No. of mice inoc.

2) *Elution of NDV and Ehrlich tumor cell mixtures.* As mentioned above there was evidence of spontaneous elution (as measured by tumor growth), which was most prominent at 25°. When mixtures were kept at 4° for 10 minutes and then placed at 25°, elution also took place rapidly. In two different experiments no elution could be demonstrated when the mixtures were placed at 37° following adsorption at 4°.

3) *Effect of inoculating mixtures of tumor cells and NDV intraperitoneally.* When mixtures of equal parts of NDV and 10% Ehrlich carcinoma cells were held at 4° for 10 minutes and then inoculated intraperitoneally into mice, only a small per cent of those animals receiving the mixtures developed ascites, and it appeared long after the control animals had died. The results of these experiments are given in Table I.

In vivo experiments. It is apparent that inhibition of tumor growth took place when mixtures of Ehrlich carcinoma cells and NDV were inoculated after contact *in vitro*. We were therefore interested to see if any adverse effect could be demonstrated when the virus and tumor were inoculated separately or if the virus could be used as a therapeutic agent after ascites developed. Groups of 10 weighed mice were inoculated intraperitoneally with 0.2 cc of a 5% Ehrlich carcinoma suspension. They were then inoculated intraperitoneally with either NDV infected allantoic fluid or with normal allantoic fluid under the con-

TABLE II. Effect of Inoculating NDV Followed by Inoculation of Ehrlich Carcinoma Intraperitoneally, 4 Experiments.

Conditions of exp.	NDV	NAF	Avg day of death NDV	Avg day of death NAF
NDV				
1 cc & Ehr. immed.	6/10*	10/10	32.3	20.4
.2 cc " "	6/10	10/10	25.6	17
.2 cc " 10 min. later	8/10	10/10	33.4	15.5
.2 cc " 1 hr "	9/10	10/10	22.3	16

* Deaths from ascites over No. inoculated.

ditions specified in the experiments. Ten animals which had received virus only were included in each experiment. The mice were weighed individually at twice weekly intervals, to determine the time of development of the ascites. The day of death from ascites was also recorded. The results were judged on 1) the number of survivals 2) the average survival time and 3) the rate of development of the ascites.

Table II gives the results of the one category of experiments in which the virus in the form of infected allantoic fluid was given first. There were some survivors in all treated groups, the greatest number when virus and tumor cell suspension were inoculated almost simultaneously. The animals which received the virus developed ascites at a slower rate and survived longer than the controls. When the 0.2 cc of a 5% tumor cell suspension was given first and the same quantity of virus given immediately thereafter, there were no survivors although the NDV treated animals survived twice as long as the controls. When inoculation of virus was delayed for as much as 10 minutes after the tumor, no difference could be noted between virus and normal allantoic fluid treated animals. In other experiments daily inoculations of 0.2 cc of NDV failed to effect the development of ascites or failed to have an effect once the ascites had been allowed to develop.

Failure of growth of NDV in Ehrlich tumor. When 0.1 cc of NDV was inoculated directly into Ehrlich tumor of 1.0 cm in diameter and mice killed at daily intervals, the virus titer showed a gradual decrease. Table III. The virus did not affect tumor growth. One attempt to propagate NDV in Maitland type tissue culture with the Ehrlich tumor as the tissue source gave no evidence of survival of virus after 3 passages although chick embryo control flasks showed good growth. In addition 7 mice with well developed ascites were inoculated with NDV (0.5 cc). Daily titrations of the ascitic fluid showed no increase in the amount of virus, Table III. Numerous attempts to keep the virus in passage at 3-day intervals in mice with ascitic fluid resulted in the loss of virus.

TABLE III.
Persistence of NDV in Ehrlich Carcinoma.

Days after inoc.	Solid tumor titer*	Ascitic fluid titer*
Orig.	9	9
Immed.	6.5	5.6
1	6	5
2	4	5
3	4	3
6	0	1.75
7	0	0

* Expressed as the reciprocal of the LD_{50}.

Discussion. The results of mixing NDV and Ehrlich carcinoma cell suspensions *in vitro* and *in vivo* have been reported. It has been seen that when the two are mixed in the test tube inhibition of tumor growth takes place immediately. Further inoculation of the mixtures at room temperature for one to 3 hours has resulted in growth of these mixtures. These results are entirely comparable to those obtained with the sarcoma 180 cell suspensions and NDV.

In contrast, when tumor suspension and virus were mixed in the animal in the same proportions employed in the *in vitro* experiments only a very slight inhibition of tumor growth could be demonstrated and then only when virus and tumor were inoculated simultaneously. Since it was shown that virus and tumor mixed *in vitro* at 37° show impaired growth on inoculation, we are forced to seek other explanations for its failure to occur in

the animal. It may be that the virus is so rapidly changed in the animal body that it has no chance to react with the tumor cell, or the known inhibitors present in all animal tissues may interfere. Perhaps much more virus is needed to produce the effect *in vivo* than *in vitro*.

The results reported are in complete agreement with those reported by Kausche, Landschutz and Sauthoff, who had observed the same phenomenon with the PR8 strain of influenza and Ehrlich tumor cell suspensions. Their explanation of the phenomenon, *i.e.* growth of the virus in the tumor cells, are at variance with ours since we have been unable to demonstrate any increase in viral titer in either the ascitic fluid or in the solid tumor following direct inoculations. It appears to us that the tumor inhibiting effect is more likely to be due to an adsorption of virus on the cell surface which for some unexplained reason is capable of retarding tumor growth when these mixtures are inoculated either intraperitoneally or subcutaneously into animals. The fact that these same mixtures on further incubation or on a change of temperature from 4° to 25° are capable of growing when inoculated, seems to indicate a surface phenomenon.

Summary. 1. When NDV and Ehrlich were mixed at 4°, 25° and 37° and inoculated immediately, a much smaller percentage of tumors grew than in the NAF control mixtures. When the same mixtures were held at 25° or transferred from 4° to 25° for one to 3 hours, inoculation resulted in tumor growth. 2. When virus and tumor were mixed *in vivo* no tumor inhibitory effect was noted unless the virus was given intraperitoneally first and the tumor suspensions by the same route immediately thereafter. The course of development of ascites in mice was not affected by daily inoculations of NDV. 3. No evidence could be found for multiplication of the virus in the tumor cells either in the animal or tissue culture.

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Dextran as a Source of Liver Glycogen and Blood Reducing Substance.* (19922)

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In earlier experiments in this laboratory it has been observed that dextran, fed to human subjects, is not recovered in the stools. Engstrom and Aberg(1) have suggested that some of the dextran administered intravenously might be excreted into the gastrointestinal tract where it could be destroyed by bacterial action. Our own observations on dextran incubated with stools suggested that dextran disappears only very slowly, but Hehre(2) has since shown that there are large numbers of anaerobic bacteria present in the intestinal flora, and that it is these, rather than the aerobes, that are capable of splitting dextran. Although ingested dextran may be in part degraded and consumed by the intestinal bacteria, it still seemed to us of interest to learn more about the disappearance of dextran fed by mouth. The following experiments show that dextran feeding leads to a sustained increase in blood sugar and a rise in liver glycogen in the fasted rat, and brings about an

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