TABLE I. Number of Plaques and Calculated Plaque Forming Units (PFU) per ml for 4 Samples of Poliomyelitis Virus Assayed on Monkey Kidney and Human Amnion Plates in Various Dilutions.

Virus samples	Dilution	Cell layer from Monkey kidney Plaques/plate	Monkey kidney PFU/ml	Human amnion Plaques/plate	Human amnion PFU/ml
Mahoney	$10^{-5.5}$	19, 20, 21, 19	2.1×10^{7}		
	10^{-6}	9, 7, 5, 5	2.2×10^{7}	25, 24	8.2×10^{7}
	10^{-7}			0, 6	1.0×10^{8}
"	10^{-6}	38, 41, 43	1.4×10^{8}		
	$10^{-6.5}$	24, 17, 15	2.0×10^{8}		
	10^{-7}	2, 5, 4	1.2×10^{8}	26, 26	8.7×10^{8}
	$10^{-7.5}$			8, 11, 11	1.1×10^{9}
MEF-1	10^{-6}	25, 25, 34	8.9×10^{7}	113, 115	3.8×10^{8}
	$10^{-6.5}$	10, 10, 7	9.6×10^{7}		
	10^{-7}	2, 4, 3	1.0×10^{8}	10, 4	2.3×10^{8}
Saukett	10^{-4}			217, 189	6.8×10^{6}
	10^{-5}	88, 107, 90	3.2×10^{7}	85, 68	2.6×10^{7}
	$10^{-5.5}$	31, 32, 31	3.3×10^{7}		
	10^{-6}	3, 11	2.3×10^{7}	3, 5	1.3×10^{7}

Summary. Plaque formation of all 3 types of poliomyelitis virus has been obtained in monolayer cultures of human amnion cells. The plaque titer obtained on amnion was 3-6 times higher than on monkey kidney cell cultures for strains Mahoney and MEF-1.

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Propagation of Newcastle Disease Virus in Ehrlich Ascites Cells *In vitro* and *In vivo*.* (21945)

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(Introduced by Herald R. Cox.)

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Mengo encephalitis virus has been cultivated in the Ehrlich ascites carcinoma cell *in vitro*(1). While no cytopathogenic effect of Mengo virus—in spite of its multiplication—was visible in the test tube, its oncolytic property was readily demonstrated in the ascites cells *in vivo*. Some workers have reported the inhibition of Ehrlich ascites tumor growth when the inoculum was first mixed with Newcastle disease virus (NDV) either just prior to or at the time of inoculation(2). However, sustained multiplication of the NDV in the Ehrlich cells could not be demonstrated(3).

It seemed of interest to ascertain whether this virus could be adapted to grow in the Ehrlich ascites tumor and to determine whether viral multiplication would result in oncolysis *in vivo*.

Material and methods. Virus. The Newcastle disease virus was originally isolated in Massachusetts in 1945 by Dr. Van Roekel, and is designated MD20Z. It was received by Dr. Floyd Markham from the United States Bureau of Animal Industry as the fifth chick embryo passage of the original strain.

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In this laboratory it was passaged once in chick embryos, then 12 times in duck embryos, then twice in chick embryos. The present designation of MD20Z indicates the total passages of the virus to be 20. Undiluted allantoic fluids make up the virus pool. *Tumor.* The Ehrlich ascites tumor was received through the kindness of Dr. George Klein of the Karolinska Institute, Stockholm, Sweden. The tumor has been continuously passaged in this laboratory since 1950 in Swiss (or ICR) mice every 7 days. In order to test the effect of the virus preparations *in vivo,* female Swiss mice weighing 20-25 g were inoculated i.p. with 0.2 ml of pooled ascitic fluid containing 10-30 million tumor cells. *Virus-tumor cell suspension.* Ascites cells were collected 7-10 days after implantation, washed as previously described(1), resuspended in an arbitrary volume of Parker's synthetic medium 199(4) and the number of cells was counted in a standard bright-line Neubauer hemocytometer. The average of 4 counts was taken in order to determine the cell concentration. In all experiments the final concentration of Ehrlich ascites cells was adjusted with medium 199 to give 5 x 10^6 cells per ml. Penicillin and streptomycin were routinely added to the culture medium to give a final concentration of 100 units and 200 γ per ml respectively. Virus was added as noted in each experiment. Two ml aliquots of the cell-virus suspensions were transferred to 15 x 150 mm pyrex test tubes, which were closed with silicone stoppers, placed in a roller drum and incubated at 37°C for the desired time interval. After incubation the cultures were harvested, pooled and stored in rubber-stoppered vaccine bottles at –25°C until the virus was used in the next tissue culture passage or egg titration was performed. *Egg titrations.* Tenfold dilutions of tissue culture or ascites fluid were made in 2% N-Z† amine solution (Lederle) containing 2 mg of streptomycin per ml. This material was then injected in 0.2 ml amounts into the chorioallantoic cavities of 9-11-day-old embryonated chicken eggs. Eggs were incubated at 37°C, candled daily for seven days, and the number of dead embryos recorded. LD_{50} titers were calculated according to the method of Reed and Muench(5). *Virus identification.* Newcastle immune chicken serum of high titer, prepared in this laboratory, was diluted 1:2 with 2% N-Z amine solution containing 2 mg of streptomycin per ml; mixed with an equal volume of undiluted ascitic or tissue culture fluid and with 10-fold dilutions thereof; and incubated at room temperature for 60 minutes. Eggs were inoculated in groups of 5, incubated at 37°C, and candled as described above. Virus identification was confirmed when protection was afforded by the immune serum mixture in all the dilutions through 10° and no protection was afforded by normal serum in control egg inoculations performed simultaneously. *Mouse inoculation.* At frequent intervals aliquots of 0.5 ml of undiluted tissue culture passage material were inoculated i.p. into a number of Swiss mice which had received transplants of the Ehrlich ascites tumor 4 days earlier. A control group of tumor-bearing mice was given a similar inoculum of uninfected tissue culture material prepared in the same manner and at the same time as the infected cultures. *Analysis of oncolytic effect and viral growth.* Smears of the ascitic fluid from at least 2 experimental and control mice were prepared daily, stained by Leishman's method, and examined. Karyorrhexis of tumor cells or their disappearance from the fluid was regarded as cytological evidence of oncolysis(6). This assessment of oncolysis was confirmed by determination of the survival

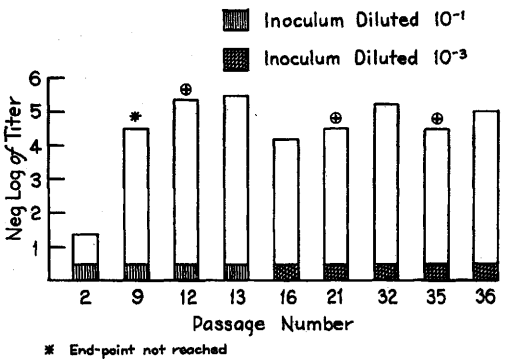

FIG. 1. Egg infectivity titer of NDV-Ehrlich cell tissue culture passages.

† Tryptic digest of casein (Sheffield Chemical Co., N. Y. City) 2% solution in distilled water.

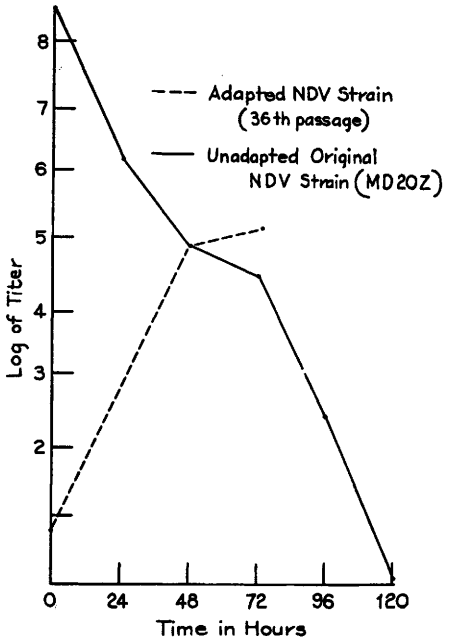

FIG. 2. Virus titer of NDV in Ehrlich cell cultures* after various intervals of incubation.

* Final dilution of MD20Z = 10^{-1} in 5 × 10^6 Ehrlich cells/ml.

ratio of experimental and control groups over a period of 30 days. In certain experiments 3 to 5 mice were killed, the ascitic fluid pooled, and egg infectivity titrations performed. In some instances neutralization tests were also done.

Results. Virus passage. The serial passage of NDV in the Ehrlich ascites cells *in vitro* was begun with a ratio of one egg LD_{50} of the MD20Z strain to 10,000 cells. After 96 hours incubation, the tube contents were harvested and pooled. This pool, in a final dilution of 10^{-1}, served as inoculum for the second passage, which was incubated as before. Starting with the third passage and continuing to the present (38th), incubation has been for 48 hours only. From the 13th passage on, each tissue culture inoculum was a 10^{-3} dilution of the preceding tissue culture passage.

Virus titers at various tissue culture passage levels. Egg infectivity titers were determined at various passage levels. Fig. 1 gives the results of representative titrations, and reveals the uniformity of infectious virus content at levels after the second passage. Thus the 13th and 16th passages contained approximately the same amounts of virus as subsequent passages, although the latter received smaller inocula. Fig. 2 shows the increase, during a period of 48 hours, in egg infectious virus in the 36th tissue culture passage. In contrast, a rapid drop in titer occurred when the unadapted parent NDV strain was inoculated into Ehrlich cell cultures. With the adapted strain there was an increase of approximately 4 logs in the period of 48 hours, whereas the drop in infectivity titer of the unadapted strain over the same period of incubation was 3.5 logs, and no virus was detectable after 120 hours.

Inoculation of ascites-bearing mice with

TABLE I. Summary of Experiments on Ascites-Bearing Mice Inoculated with Newcastle Disease Virus Tissue Culture.

Passage No.	Cytological evidence of oncolysis	Onset of karyorrhexis (days after inoculation of virus)	Survival ratio*	Virus titer after 96 hr
Parent strain	0	—		0
5	0	—	0/20	10^{-4}
10	+	7	0/20	$<10^{-4}$
12	0	—	0/20	$<10^{-4}$
18	+	7	2/20	N.D.
28	++	3	4/20	N.D.
29	+++	3	14/33	$10^{-6.0}$
37	+++	4	14/22	$10^{-7.5}$

* No. of mice surviving over No. of tumor-bearing mice.
N.D. = Not done.
All 150 control mice which did not receive virus inoculum died.
+ = Karyorrhexis, but tumor cells persisted until death. ++ = Complete disappearance of tumor cells in 50% of mice. +++ = Complete disappearance of tumor cells in all mice (see footnote ‡).

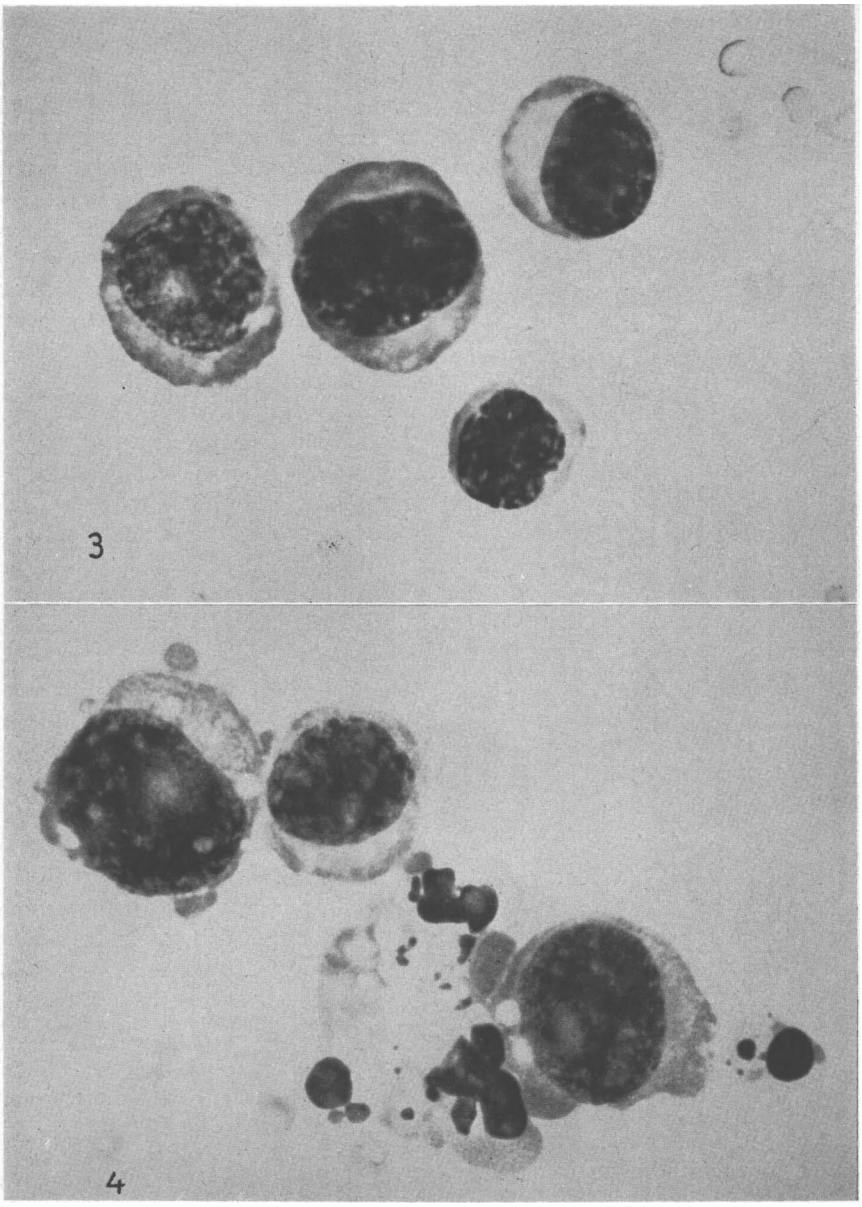

FIG. 3. Ehrlich ascites tumor cells 7 days after inoculation of uninfected tissue culture material. Leishman's stain × 1400.

FIG. 4. Karyorrhexis in 2 Ehrlich ascites tumor cells 7 days after inoculation of 10th passage of NDV in tissue culture. Leishman's stain × 1400.

NDV tissue culture. Virus from 7 different tissue culture passages was injected into mice bearing 4-day ascites tumors. Table I gives a summary of these experiments, which indicated that tissue culture passage of the Ehrlich-adapted strain of NDV enhanced its ability to produce oncolysis. Initially no oncolysis was evident, and it was not until the 10th passage that karyorrhexis was observed. This occurred 7 days after virus inoculation (Fig. 3 and 4). Inocula from later tissue culture passages produced more effective and more rapid oncolysis, and the survival ratios increased (Table I). Karyorrhexis was ob-

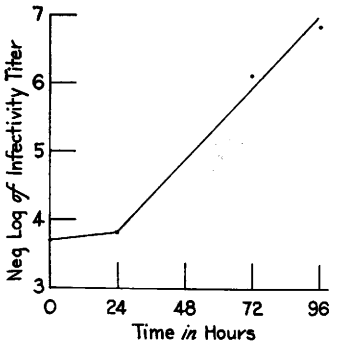

FIG. 5. Increase in egg infectivity titers of ascitic fluid of mice injected with adapted NDV.*

* 29th tissue culture passage.

served 4 days after injection of virus from the 37th tissue culture passage; no tumor cells were seen in the fluid withdrawn from any of the 4 mice on the 7th day after virus inoculation,‡ and the survival ratio was 14/22. No distinction was made between death from ascites and later death from solid tumors at the site of the original tumor injection. No virus could be detected in ascitic fluid obtained 96 hours after injection of the parent NDV strain; after 29 passages of the virus *in vitro* a comparable sample of ascitic fluid titrated to $10^{-6.8}$ in eggs. Fig. 5 shows a 3.5 log increase in egg-infectivity titer of the ascitic fluids obtained daily for 96 hours after injection of virus from the 29th tissue culture passage.

Virus identification. Undiluted tissue culture material from the 12th, 21st and 35th passages, as well as undiluted ascitic fluid obtained from mice 96 hours after injection with the 29th and 36th passages, was completely neutralized by NDV immune serum. Also chorioallantoic fluids withdrawn from embryonated chicken eggs injected with NDV-normal serum controls were spot tested, and gave positive hemagglutination which was specifically inhibited by high titer NDV immune serum.

Discussion. Inhibition of growth of the Ehrlich ascites tumor by the tissue culture adapted strain of NDV is different from the phenomenon described by Moore and Diamond(2). These workers regarded the effect of the virus on tumor cells as comparable to the hemagglutinating action of viruses on red cells(6). They did not observe any cytological evidence of oncolysis like that described above. The cytological changes which follow inoculation of the tissue culture adapted NDV are essentially the same as those which have been described during the destruction of Ehrlich tumor cells after infection with West Nile and Bunyamwera viruses(6,7). Since the latter are not hemagglutinating viruses, the cytolytic action of the adapted strain of NDV is probably of the same nature as that of other oncolytic viruses, and like them depends upon the multiplication of virus in the tumor. This was confirmed by the absence of cytological changes after inoculation of the original strain of NDV, when virus multiplication in the tumor could not be demonstrated; indeed, only the later passages of the adapted virus which multiplied to a high degree were capable of inducing consistent oncolysis.

Summary. The propagation of NDV in Ehrlich ascites tumor cells maintained *in vitro* is described. After prolonged serial passages *in vitro,* multiplication of the virus occurs in the tumor *in vivo,* and in the later passage levels is accompanied by cytological and biological evidence of oncolysis.

‡ Less than 1% of tumor cells was observed in one smear, but most of these showed advanced degenerative changes.

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