of guinea pigs, rabbits, rats and mice prior to the immune response may differ greatly from rates of elimination of homologous serum proteins. While elimination of homologous proteins, particularly albumins, appears to be related primarily to the metabolic rate of the host, elimination of heterologous proteins apparently reflects, in addition, the physiological and metabolic suitability of the protein to the particular host. Rabbit gamma globulin is unique in that it persists in foreign hosts as long or longer than do the homologous proteins. The half-lives of serum proteins in guinea pig are independent of the methods used for their separation (alcohol and ammonium sulfate fractionation).

1. Armstrong, S. H., Bronsky, D., and Hershman, J., *J. Lab. Clin. Med.*, 1955, v46, 857.

2. McFarlane, A. S., *Biochem. J.*, 1956, v62, 135.

3. Cohen, S., Halloway, R. C., Matthews, C., and McFarlane, A. S., *ibid.*, 1956, v62, 143.

4. Knox, W. C., and Endicott, F. C., *J. Immunol.*, 1950, v65, 523.

5. Melcher, L. R., and Masouredis, S. P., *ibid.*, 1951, v67, 393.

6. Dixon, F. J., Talmage, D. W., Maurer, P. H., and Deichmiller, M. P., *J. Exp. Med.*, 1952, v96, 313.

7. Dixon, F. J., Maurer, P. H., and Deichmiller, M. P., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 287.

8. Dixon, F. J., *J. Allergy*, 1953, v24, 547.

9. Talmage, D. W., Dixon, F. J., Bukantz, S. C., and Dammin, G. J., *J. Immunol.*, 1951, v67, 243.

10. Masouredis, S. P., Melcher, L. R., and Shimkin, M. B., *ibid.*, 1953, v71, 268.

11. Dixon, F. J., Maurer, P. H., and Weigle, W. O., *ibid.*, 1955, v74, 188.

12. Melcher, L. R., Masouredis, S. P., and Shimkin, M. B., *ibid.*, 1953, v71, 275.

13. Weigle, W. O., Dixon, F. J., to be published in *J. Immunol.*

14. McFadden, M. L., and Smith, E. L., *J. Am. Chem. Soc.*, 1953, v75, 2784.

15. Putnam, F. W., *ibid.*, 1953, v75, 2785.

16. McFadden, M. L., and Smith, E. L., *J. Biol. Chem.*, 1955, v214, 185.

Received October 29, 1956. P.S.E.B.M., 1957, v94.

Viral Agents Oncolytic for Human Tumors in Heterologous Host. Oncolytic Effect of Coxsackie B Viruses.* (22931)

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Ability of many viral agents of low pathogenicity for man to proliferate in and destroy neoplastic human cells in tissue culture, has intensified the search for agents that produce such an effect *in vivo*(1-5). Several viruses produced oncolytic effects in animal tumors (6,7) and particular attention has been given to their effects on tumors of ascites and lymphoma types(8,9). The possibility of adapting a virus to multiply and later to become oncolytic in a tumor system which it initially failed to infect, has also been demonstrated (10,11). However, search for agents which are oncolytic for cancer in man thus far has made only very limited progress(12-14), possibly for want of adequate method of screening and assaying virus effects directly on human cancer cells *in vivo*. The high degree of cell and species specificity of most mammalian viruses makes it, *a priori*, unlikely that an animal tumor system will provide more than a model of specific virus-tumor relationships. Likewise, it is unwarranted to assume that behavior of a virus in tissue culture is necessarily a guide to its behavior on similar cells *in vivo*(8,15). However, a combination of the two approaches, transplantation of tissue culture grown human tumor cells into animals, might come closer to providing a practical method for *in vivo* study

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of viruses in human cancer tissue. Such a technic, of transplanting human carcinomata into irradiated and/or cortisone treated animals, has been developed by Toolan(16-17) and shown applicable for this purpose (18,19). Unfortunately, use of operative human tumor specimens injected subcutaneously in rats and into cheek pouches of hamsters, has not led, so far, to regular supply of tumor-bearing animals suitable for carrying out controlled experiments in adequate numbers. In the present study, a variation of this technic has been employed to establish on a large scale, relatively uniform transplants of human carcinomata from stable, tissue culture cell lines (HeLa and KB). The purpose of this report is to show (a) the application of this method to selection and adaptation of viral agents oncolytic for solid tumors of human cell origin, and, (b) to report in some detail the behavior of one of these agents.

Materials and methods. Irradiation: Weanling albino rats, Osborne-Mendel or Sprague-Dawley strains, weighing 40-60 g, were irradiated as follows: A single dose of 350 r was administered from two 200 KV tubes, one above and one below, at a distance of 54 cm, at 125 r/min, with 15 ma and 150 ohms, using .25 copper and .55 aluminum filters. An alternative procedure, using high voltage irradiation, was used in a few experiments where 425 r were given from a 2.5 million KV tube at 155 r/min, with a target distance of 150 cm. Cortisone acetate (Merck) 3 mg was injected subcutaneously on 2nd and again on 4th day after irradiation. Antibiotics were given prophylactically to all rats to minimize irradiation mortality. Penicillin 12,500 units, and streptomycin, 12.5 mg, were inoculated subcutaneously periodically, especially during 1st and 2nd week after irradiation. Chloramphenicol 0.6 mg/ml was added to drinking water during many experiments. Inoculum: HeLa cells† were grown in 32 oz. Blake bottles in medium consisting of 10% human serum in Eagle's basal medium(20). The cells were scraped from the flasks, pooled, centrifuged at 300 rpm for 10 minutes, and made into suspensions in Eagle's basal medium. This suspension was slowly agitated with magnetic stirrer, and inoculated intraperitoneally into rats on 4th or 5th day after irradiation. Each rat received 1 ml of cell suspension containing approximately 3-5 million cells, about ⅔ of cellular contents of 1 Blake bottle. Virus: The 4520 strain of Coxsackie B3, obtained from a case of pleurodynia in 1951(21), was studied intensively. The initial inoculum consisted of 10% suckling mouse carcass suspension of 8th suckling mouse passage. Titrations were performed in tissue culture tubes of monkey kidney cells‡ using 2 tubes per 10-fold dilution. Titration end point was considered the highest dilution which produced cytopathogenic effects or from which virus was recovered on passage. Neutralization procedures were carried out as described for the adenovirus group (2nd procedure) against 100 tissue culture doses(22). Virus inoculation was performed when growth of tumor was confirmed by abdominal palpation, about 9th day after implantation. Inocula were either virus-containing tumor suspensions, or HeLa cell or monkey kidney tissue culture passages thereof. These were injected routinely by intraperitoneal route, using 0.5-1 ml; intravenous injection through tail vein was also employed. Tumor suspensions were prepared as follows. Animals were sacrificed with chloroform, the tumors weighed and made into 10% or 20% suspensions in medium 199 (24) by mincing 3 minutes in chilled Waring blendor. All suspensions were frozen and thawed at least once, and clarified by centrifugation at 3000 rpm for 25 minutes. Tumor suspensions were inoculated as 10-fold ultracentrifugal concentrates in several instances. At autopsy, representative portions of tumor were removed for histological examination, fixed overnight in 10% formol-sublimate, and stained with hematoxylin-eosin.§ Growth of tumor was followed by frequent abdominal palpation, and finally by grading the tumor

† Seed cultures were obtained from Microbiological Associates, Inc., Bethesda, Md.

‡ Obtained from Microbiological Associates, Inc., Bethesda, Md.

§ In some cases, tissues were stained with Van Gieson; resorcin-fuchsin, and Barrett stain(23).

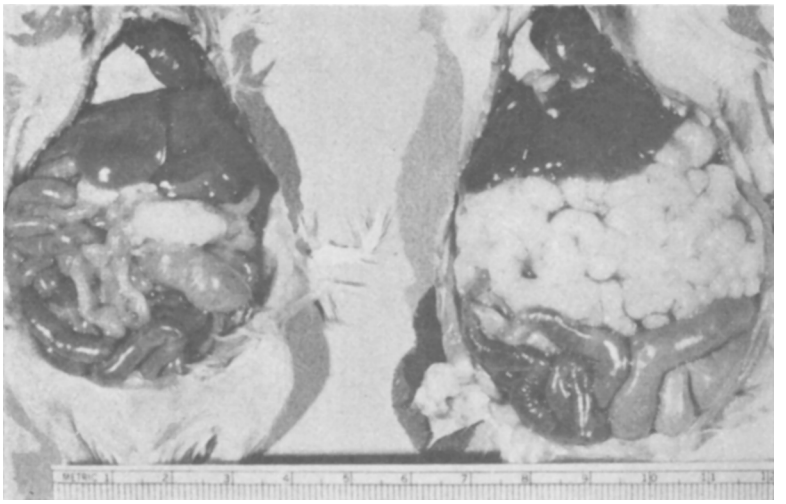


FIG. 1. Gross appearance of HeLa-tumor 6 days after intraperitoneal inoculation of 6 tissue culture log doses of Coxsackie B_3 passed through 6 previous rat-HeLa tumors plus 3 additional passages in monkey kidney tissue culture; comparison with an uninoculated control tumor; HeLa cells were implanted 15 days prior to autopsy.

at autopsy as follows: 1. abundant tumor: numerous pearly-grey, grape-like nodules scattered throughout omentum and mesentery; histologically, many islands of tumor cells with variable but usually inconspicuous evidence of necrosis (Fig. 1, 2). 2. no recognizable tumor: an irregularly shaped mass with areas of opaque white necrosis or fibrosis, in which no intact tumor cells could be detected microscopically (Fig. 1,3). 3. extensive necrosis and/or fibrosis: intermediate classification between 1 and 2, in which degeneration was obvious on gross and histological observation, but some intact tumor could still be demonstrated.

Results. Production of tumor: Palpable

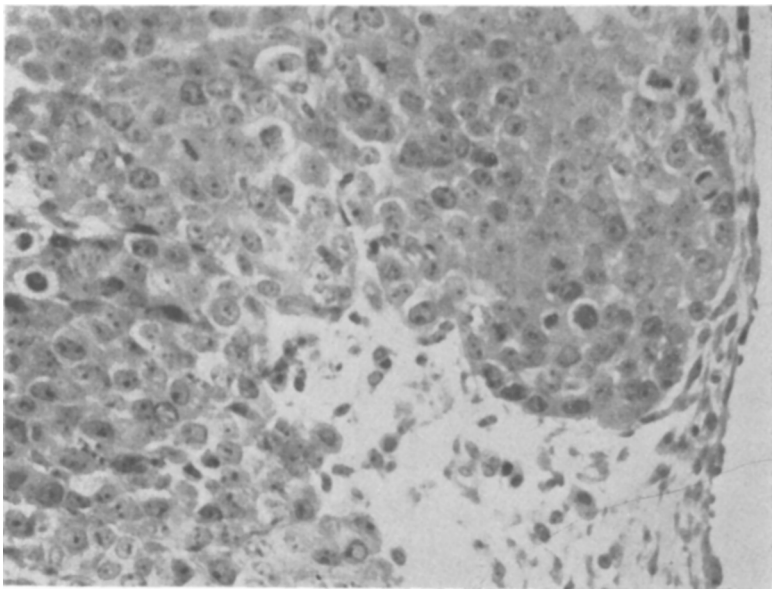


FIG. 2. Histological appearance of rat-HeLa tumor 12 days after implantation. Control tumor for Fig. 4, Barrett stain. 290 X.

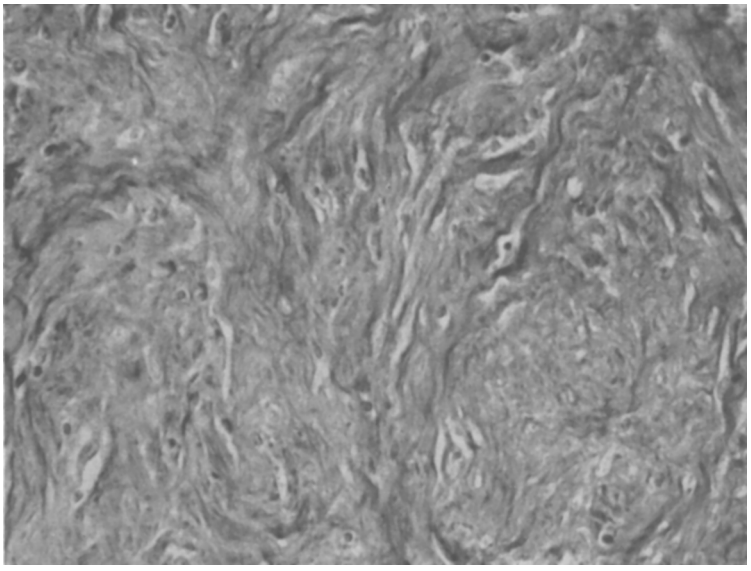


FIG. 3. End stage of tumor regression following intraperitoneal inoculation of Coxsackie B_3 passed through 6 rat-HeLa tumors. Van Gieson; resorcin-fuchsin. 290X.

tumor nodules 0.25 to 1 cm diameter were found in 93% (506/534) of rats surviving effects of whole body irradiation, at 7 to 9 days after cell inoculation. The presence of tumor was verified at autopsy in a total of 602/644 (93.6%) of rats inoculated. The tumors grew rapidly between the 7th and 18th days after implantation and their size could be readily estimated by palpation. The median weight of 65 tumors at 14 and 15 days was 13 g; at 17 and 18 days, the median weight of 41 tumors was 17.8 g. They appeared as pearly-grey, glistening solitary or conglomerate botryoid masses in omentum, mesentery, pancreas, and occasionally spreading over peritoneal surface of diaphragm, in-

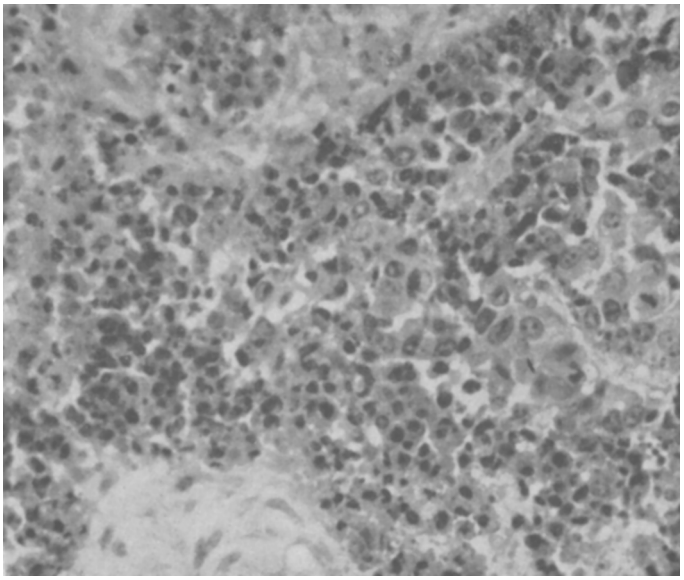
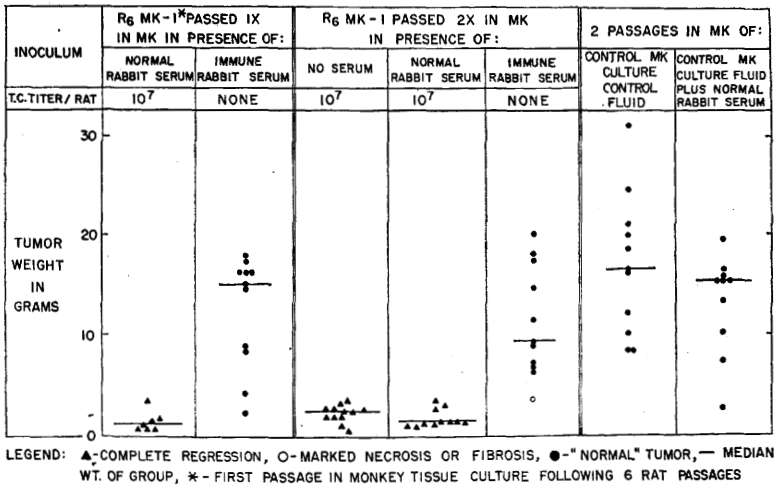


FIG. 4. Peripheral type necrosis seen 2 days following intraperitoneal inoculation of Coxsackie B_3 passed through 6 rat-HeLa tumor passages. Uninoculated control tumor in Fig. 2. 290X.

ONCOLYTIC EFFECT OF COXSACKIE B_3 VIRUS AFTER 6 RAT PASSAGES AND PASSAGE IN MONKEY KIDNEY TISSUE CULTURE. PREVENTION OF ONCOLYSIS BY IN VITRO NEUTRALIZATION



LEGEND: ▲-COMPLETE REGRESSION, O-MARKED NECROSIS OR FIBROSIS, ●-"NORMAL" TUMOR, — MEDIAN WT. OF GROUP, * - FIRST PASSAGE IN MONKEY TISSUE CULTURE FOLLOWING 6 RAT PASSAGES

FIG. 5. See text.

testine, liver, or stomach (Fig. 1). Discrete hemorrhages and opaque white and occasionally hemorrhagic areas of necrosis were seen in some tumor nodules.

Microscopically, in first few days following implantation, the tumors consisted of multiple nodules of cells separated and surrounded by delicate strands of areolar connective tissue (Fig. 2). Mitotic figures were abundant. Small areas of coagulation necrosis were present in center of some nodules and occasional isolated degenerated tumor cells were seen. Beginning about 2 weeks after implantation, necrosis of tumor cells became more widespread, and occasionally, minute areas of necrosis appeared at periphery of nodules. Necrotic changes were accompanied or followed by some inflammatory reaction, characterized by presence of macrophages, neutrophilic granulocytes and fibroblasts around and within the nodules. Many animals with massive tumors died around this time, often in a state of dyspnoea, and sometimes apparently from late effects of irradiation and secondary infection. Animals surviving beyond 3 weeks showed more widespread necrosis, phagocytosis of tumor cells, and proliferation of fibrous tissue. Five weeks after implantation, no recognizable tumor was found in 24 survivors out of original group of 40 rats. The overall mortality during the first three weeks after tumor implantation was 25% (216/814).‖

Screening for virus growth: Attempts have been made to carry a number of viruses in serial passage through tumor bearing rats. The following viruses could not be recovered after 1 or 2 passages: adenoviruses (APC) types 6 through 12, and Bertha; Coxsackie A9, and ECHO type 8. Adenovirus types 2, 3, and 4 were carried through 3, 5, and 2 serial passages respectively; no oncolytic effects were observed. Vaccinia, ECHO type 7, and vesicular stomatitis, types New Jersey and Indiana, were carried without difficulty through 4 to 6 serial passages, without production of oncolytic effects.

Coxsackie B viruses in rat-HeLa tumors: Coxsackie B_1 was passed without difficulty through 19 serial rat passages. Coxsackie B_3 was selected for more intensive study and has been carried through 22 consecutive rat passages. Up to 10^9 tissue culture doses of virus were recovered/g tumor, varying with time of harvest and state of tumor degeneration.

‖ Similar tumors were established on a large scale with equal success, using Eagle's KB cell(25) as inoculum (obtained from Microbiological Assoc., Bethesda, Md.)

TABLE I. Incidence of Tumor Degeneration in Infected and Uninfected Rat-HeLa Tumors as Seen in 11 Consecutive Experiments; Comparison of Gross and Histological Appearance. Inoculum consisted of Coxsackie B_3 virus passed through 4 to 8 rat-HeLa tumor passages; autopsies were performed 5 to 9 days after infection.

	Histological appearance											
	Infected tumors						Non-infected control tumors					
Gross appearance	Abundant tumor cells	Some tumor cells; severe necrosis and fibrosis	No tumor cells	Not examined	Total gross observ.		Abundant tumor cells	Some tumor cells; severe necrosis and fibrosis	No tumor cells	Not examined	Total gross observ.	
						%						%
Abundant tumor	2	1	0	0	3/162	1.9	55	0	0	38	93/106	87.7
Some tumor + severe degeneration	0	2	4	7	13/162	8.0	2	3	1	3	9/106	8.5
No tumor	0	8	80	58	146/162	90.1	0	2	2	0	4/106	3.8
Total histological observations	2/97 2.1%	11/97 11.3%	84/97 86.6%				57/65 87.8%	5/65 7.7%	3/65 4.6%			

TABLE II. Growth and Recovery of Coxsackie B_3 from Tumor-Bearing and Non-Tumor-Bearing Rats Inoculated Intraperitoneally with 7 Logs of Virus. (Titers are expressed in log doses/g omental mass, each figure representing 1 rat-HeLa tumor; figures with † and ‡ represent pools of tumors.)

		Time after inoculation											
Rats	Inoculum	30 min.	1 day	2 day	3 day	4 day	5 day	7 day	9 day	10 day	11 day	13 day	18 day	21 day
Tumor bearing	R_eMK_3*	10^4, 10^5	10^7, 10^8	10^8, 10^9	10^8, 10^8	10^5, 10^8	10^7, 10^8	10^4, 10^5		10^3, 10^3	0, 10^5	0, 10^7	0, 10^3	0
				——Period of tumor destruction——										
	Suckling mouse susp.		10^9		10^7†		10^9‡	10^5†	0†					
Non-tumor bearing	R_eMK_3						0, 0	0†						
	Suckling mouse susp.	10^5	10^3		0		0	0	0					

* Coxsackie B_3 (suckling mouse suspension) passed through 6 rat-HeLa tumors and 3 monkey kidney tissue culture passages.
† Pool of 2 rats. ‡ Pool of 3 rats.

Oncolytic effects of Coxsackie B_3. In the 1st 4 rat passages, there was inconclusive evidence that viral infection influenced growth of tumors; however, massive necrosis was produced in 98.1% of tumors inoculated with virus from 4th through 8th rat-HeLa tumor passage, resulting in the absence of tumor cells in 86.6% of those examined histologically (Table I). In uninoculated control tumors, grossly similar changes were seen in 12.3%. Comparison of virus-infected tumors with controls revealed a striking difference in amount and distribution of necrosis. Between 1st and 5th days after infection, there was conspicuous degeneration of tumor cells beginning around periphery of nodules and rapidly extending inwards (Fig. 4). During this period, only a very rare degenerated cell was observed around periphery of nodules in control tumors, and degeneration, when present, was almost entirely confined to center of lobules. Necrosis of tumor cells was followed by invasion by macrophages and neutrophilic granulocytes and fibroblastic proliferation. Occasional giant cells and scanty lymphocytes, plasma cells, mast cells, and eosinophils were present. Finally, tumor nodules were replaced by dense fibrous tissue indistinguishable from that observed in control tumors 4-5 weeks after implantation (Fig. 3). Thus, during first 5 days after inoculation, necrosis spread centripetally throughout the virus infected tumors, but was virtually absent in controls. During succeeding 10-12 days, almost all virus infected tumors were completely necrotic while intact tumor cells were abundant in control tumors; only small fringes of peripheral necrosis were seen in some uninfected tumors at this period. As shown in Table I, the state of regression, as evaluated on histologic grounds, correlated very well with the independent results of gross inspection. Oncolysis and marked reduction of tumor weights were observed following both intraperitoneal and intravenous inoculation. In a representative experiment rats were inoculated with 10^6 tissue culture dose of 6th rat-HeLa passed Coxsackie B_3 virus. Eight of 8 inoculated intraperitoneally, and 7 of 7 inoculated intravenously, showed only scar tissue with no evidence of tumor cells, whereas 8 of 9 uninfected control rats and 7 of 8 rats inoculated with ground, frozen and thawed suspension of healthy control tumor showed abundant tumor. Monkey kidney passage of the above virus inoculum also totally destroyed tumor in 8 of 8 rats. Frozen and thawed suspensions of regressed control tumors also produced no destructive effects in other experiments. Growth of virus and destruction of tumor has also been produced by feeding the virus inoculum.

Prevention of virus effects: Oncolytic effects were prevented by heating the virus to 56°C for 45 minutes and by neutralization with Coxsackie B_3 antiserum. *In vitro* neutralization was demonstrated by experiment shown in Fig. 5. The first monkey kidney tissue culture passage of virus passed through 6 rat-HeLa tumors was carried for 2 additional monkey kidney passages in 3 parallel series, one with no serum, one with a 1:20 dilution of normal rabbit serum, and one with a 1:20 dilution of Coxsackie B_3 antiserum. This serum was prepared by immunization of rabbits with mouse passage virus and had a neutralizing antibody titer of 1:80. Fluids were harvested and passage was made when virus control tubes showed complete cytopathogenic effects; no cytopathogenic changes were seen in cultures containing immune serum. Culture fluids containing virus without serum, and with normal rabbit serum, produced complete oncolysis, while fluids from passages with immune serum produced no effect. Also, fluid from serum control tubes (containing no virus) did not affect the tumor. Monkey kidney cell suspension inoculated as a further control in a subsequent experiment, failed to produce evident gross or microscopic changes. Inhibition of oncolysis was also demonstrated in a passive immunization experiment. Rats were inoculated subcutaneously, 2 hours before, and 2 days after virus challenge, with 1 ml of Coxsackie B_3 antiserum, produced in adult mice with virus grown in suckling mice. One ml of virus inoculum, consisting of 4 log doses of virus of 4th rat-HeLa tumor passage mixed with 0.2

TABLE III. Comparison of Oncolytic Properties of Coxsackie B_3 after Rat-HeLa Tumor Passage *In Vivo* as Compared with Passage in Tissue Culture. (Starting virus material was suckling mouse suspension, 8th passage.)

Exp. No.	Day of autopsy post inoc.	Passages of inoculum	T.C. dose/rat	Abundant tumor	Some tumor + severe degeneration	No tumor
32	6	HL_1	10^6	5	2	1
		R_6HL_1	10^6	0	0	8
		Uninoc.		8	1	0
43	6	MK_3	10^7	9	1	2
		R_6MK_3	10^7	0	1	10
		Uninoc.		7	1	1
47	7	HL_6	10^7	4	1	1
		R_6MK_3	10^7	0	1	9
		Uninoc.		1	2	0
63	7	MK_4	10^7	5	1	0
		R_6MK_3	10^8	0	0	6*
		HL_6	10^7	3	0	1
		R_6HL_6	10^7	0	2	4
		Uninoc.		4	0	0

R_6 = 6 rat-HeLa tumor passages. HL_6 = 6 HeLa-tissue culture passages. MK_3 = 3 monkey kidney tissue culture passages.

* Gross observation only.

ml of mouse immune serum, was inoculated intraperitoneally. A control group was inoculated in the same way with normal serum; all groups were sacrificed on the 6th day. Abundant tumor was found in 7 of 11 rats which were passively immunized, severe degeneration was seen in 2, and no tumor in one. In the virus control group without serum, of 13 rats, 11 showed no tumor, and 2, severe degeneration. The mouse immune serum, however, had a neutralizing titer of only 1:5. Virus was recovered from pooled tumors in each group, indicating that low-titered immune serum used produced incomplete *in vivo* neutralization.

Growth of virus in tumor tissue: Table II illustrates growth of virus in tumor tissue in representative experiments. Maximum titers were obtained during the first 5 days in the experiment using 6th rat-HeLa tumor passed virus, which coincided with the period during which destruction of tumor occurred. Microscopic evidence of extensive necrosis was first seen on 2nd day after inoculation. The decline and gradual disappearance of virus could not be related to antibody production, for when sera of these rats were tested at a dilution of 1:5 for neutralizing antibody, only 1 rat serum, taken on seventh day, showed neutralization. It appears probable that destruction of tumor tissue was responsible for the decline in titer of virus. Growth of suckling mouse propagated virus in tumor bearing rats was also compared with its growth in irradiated, Cortisone-treated rats not inoculated with HeLa cells (Table II). Virus was recovered in high titer from the tumor tissue until the seventh day, but not after the first day, in omentum and pancreas of non-tumor bearing rats. Present evidence indicates that Coxsackie B_3 virus does not proliferate in tissue other than tumor in this system. When recovered from brain and muscle tissue in tumor bearing rats, it has been in lower concentrations than in tumor or serum.

Adaptation. Present evidence, gathered in experiments designed primarily for other purposes, suggests that repeated rat-HeLa tumor passage produced enhancement of oncolytic activity (Table III). Suckling mouse grown virus was used for initiating both rat-HeLa tumor and tissue culture passages. It is apparent that tumor destruction was more severe after *in vivo* passage in rat-HeLa tumor than after passage in tissue culture of either monkey kidney or HeLa cells. Experiments comparing virus passed through 13 rat-HeLa tissue culture passages have confirmed this difference between oncolytic properties of

virus passed *in vivo* and *in vitro*. The effect of repeated mouse passage has not been determined. Present evidence also indicates that rat-HeLa tumor passed Coxsackie B_3 virus also has an oncolytic effect on rat-KB tumors. Thus, Coxsackie B_3 virus, passed through 13 rat-HeLa passages (titer 10^{-6}), when injected into rats with KB tumor, was followed by disappearance of tumor in 10/11 rats in 6 days, 1/11 showing severe degeneration. Healthy tumor was seen in 9/10 uninoculated controls. Inoculations of the same quantity of virus which had been passed through 13 HeLa tissue culture passages was followed by severe degenerative changes in 2/8 KB tumors, but abundant tumor was seen in 6/8. Consistent destruction of HeLa cell tumors occurred after 5th rat-HeLa passage of Coxsackie B_1, after 4th passage of B_2, and 7th passage of Coxsackie B_5.

Discussion. The high degree of specificity for certain cell types shown by many viral agents, and the demonstrated possibility of altering such specificities by serial passage through different host systems, provides a theoretical background for supposing that some of these agents could be selected or adapted to produce oncolytic effects specifically in neoplastic cells(11,12). Our previous report (14) described the use of adenoviruses (APC) in experimental virotherapy of carcinoma of the cervix. In these studies, as well as those of Southam and Moore(12,13), the *in vitro* cytopathogenic effects of viral agents for cell types in tissue culture had little or no relationship to the specific cytologic effects, if any, produced in the same tissue *in vivo*. In furthering the search for agents oncolytic for human tumors, a practical laboratory method for comparing *in vivo* with *in vitro* viral effects seems most desirable. The previous work of others(16-19,26), especially that reported by Toolan and her associates, indicated that practical methods could be achieved for this purpose. The present investigations were, therefore, undertaken in an effort to develop an investigative tool which would be useful in selecting and adapting viral agents with oncolytic properties *in vivo*.

Our technic seems to serve this purpose by providing comparatively uniform, large carcinomata of human origin, capable of growing some prevalent viruses, namely, Coxsackie viruses, adenoviruses, ECHO viruses, and vaccinia virus. Tumor to tumor passage readily resulted in adapting at least 4 Coxsackie B viruses to produce profound oncolytic effects. A similar passage of one of these strains through the same line in tissue culture failed to produce such adaptation. This observation, as well as the fact that only some agents cytopathogenic in HeLa tissue culture grew in the transplant, and others grew without oncolytic activity, supports evidence available from studies in ascites tumors(8) that the oncolytic capacity of a virus *in vitro* does not necessarily correspond to its effect *in vivo*.

The mechanism of oncolysis in this system has not been determined; host factors may contribute to tumor destruction initiated by the virus(27), and further investigation of the mechanism of cell destruction is in progress. The application of viruses having oncolytic properties in animal-grown human cell tumors, to virotherapy of human cancer, depends, of course, on clinical trials and perhaps additional selection or adaptation by passages through the human host.

Summary. 1. The Toolan technic for growing cancer tissue in a heterologous host was applied to growth of tissue culture propagated HeLa and KB cells in the peritoneal cavity of rat. This method resulted in rapid development of large healthy explants of carcinoma on peritoneal surfaces, particularly of omentum. Ninety-three % of rats inoculated developed comparatively uniform tumor masses, thus allowing for adequately controlled studies of virus growth and oncolytic effects. 2. A preliminary survey of adenovirus, ECHO, and Coxsackie agents in rat-HeLa tumor system produced 3 types of reaction: 1. failure of some agents to grow. 2. growth of virus without oncolytic activity, and, 3. growth of virus with evidence of slight or incomplete oncolytic effects. Four Coxsackie agents, types B_1, B_2, B_3, and B_5, showed initial evidence of growth, and on serial passage through rat-HeLa tumors, in-

creased capacity for destroying solid carcinomata *in vivo*, producing characteristic histological changes. The oncolytic Coxsackie B_3 virus produced by rat-HeLa tumor passage *in vivo*, compared to the same strain passed in HeLa cells grown in tissue culture, revealed marked differences in oncolytic capacity. Use of this method illustrates the possibility of virus adaptation by tumor to tumor passage; that this principle has been successfully employed to destroy solid tumor transplants of human origin, suggests its possible application for future clinical studies.

1. Scherer, W. F., Syverton, J. T., and Gey, G., *J. Exp. Med.*, 1953, v97, 695.
2. Scherer, W. F., and Syverton, J. T., *Am. J. Path.*, 1954, v30, 1057.
3. Crowell, R. L., and Syverton, J. T., *J. Immunol.*, 1955, v74, 169.
4. Stulberg, C. S., Berman, L., and Ruddle, F. H., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 438.
5. Eagle, H., Habel, K., Rowe, W. P., and Huebner, R. J., *ibid.*, 1956, v91, 361.
6. Levaditi, C., and Nicolau, S., *C. R. Soc. Biol.*, 1922, v86, 928.
7. Moore, A. E., *Ann. Rev. Microbiol.*, 1954, v8, 393.
8. Koprowski, H., *Ann. N. Y. Acad. Sciences*, 1956, v63, 895.
9. Sharpless, G. R., Davies, M. C., and Cox, H. R., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 270.
10. Ackermann, W. W., and Kurtz, H., *ibid.*, 1952, v81, 421.
11. Flanagan, A. D., Love, R., and Tesar, W., *ibid.*, 1955, v90, 82.
12. Southam, C., and Moore, A. E., *Amer. J. Trop. Med. Hyg.*, 1954, v3, 19.
13. ———, *Cancer*, 1952, v5, 1025.
14. Smith, R. R., Huebner, R. J., Rowe, W. P., Schatten, W. E., and Thomas, L. R., *ibid.*, 1956, v9, 1211.
15. Kaplan, A. S., *Virology*, 1955, v1, 377.
16. Toolan, H. W., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 572.
17. ———, *Trans. N. Y. Acad. Sci.*, 1955, v17, 589.
18. Toolan, H. W., and Moore, A. E., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 697.
19. Moore, A. E., *Proc. Am. Assn. for Cancer Research*, 1956, v2, 135.
20. Eagle, H., *J. Exp. Med.*, 1955, v102, 37.
21. Huebner, R. J., Risser, J. A., Bell, J. A., Beeman, E. A., Beigelman, P. M., and Strong, J. C., *New England J. Med.*, 1953, v248, 267.
22. Rowe, W. P., Huebner, R. J., Hartley, J. W., Ward, T. G., and Parrott, R. H., *Am. J. Hyg.*, 1955, v61, 197.
23. Barrett, A. M., *J. Path. and Bact.*, 1944, v56, 133.
24. Morgan, J. R., Morton, H. J., and Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 1.
25. Eagle, H., *ibid.*, 1955, v89, 362.
26. Handler, A. H., and Foley, G. E., *ibid.*, 1956, v91, 237.
27. Love, R., and Sharpless, G. R., *Cancer Research*, 1954, v14, 640.

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Utilization of Vitamin A and Carotene by Selenium Poisoned Rats.* (22932)

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A previous study has shown that aqueous dispersions of carotene given intravenously to rats are readily converted to vit. A(1). In further experiments designed to obtain information about the site of conversion of carotene injected intravenously, attempts were made to block the reticuloendothelial system and also to damage the liver.

Methods. Weanling male rats of Holtzman strain were placed on synthetic, vit. A-free diet as follows: purified casein 18, salts 4(2), sucrose 74, cottonseed oil‡ 2, vit. mixture in sucrose 2(3). Half of the animals in each experiment received 12

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‡ Wesson oil.