4. Hartley, Frederick, Jevons, F. R., *Proc. Biochem. Soc.* 1962, v82, 1.
5. Pigman, Ward, to be published.
6. Winzler, R. J., Devon, A. W., Mehl, J. W., Smyth, I. M., *J. Clin. Invest.*, 1948, v27, 609.
7. Markham, R. L., *Nature*, 1956, v177, 125.
8. Hcwes, E. L., Armitage, C. M., Mandl, I., *Surgical Forum*, 1956, v6, 54.

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Cancer Induction in Hamsters by Human Type 12 Adenovirus. Effect of Route of Injection.* (28324)

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We have recently reported induction of a high incidence of undifferentiated sarcomas, in short latent period, at the site of intrapulmonary injection of human adenovirus, type 12, into newborn hamsters(1,2). In our original systematic testing of the human adenoviruses for oncogenic effect in hamsters, the intrapulmonary (transthoracic) route of injection was selected as probably the most suitable for this group of predominantly respiratory viruses. Having obtained the initial positive results by this route of injection of adenovirus, type 12, the following comparison was then made of several routes of injection of this virus.

Methods. Human adenovirus, type 12, prototype strain Huie, was obtained originally from the American Type Culture Collection and propagated in our laboratory in HeLa cells as previously described(1). The virus was injected into newborn hamsters within 24 hours of birth by the intrapulmonary (through the chest wall), intrapleural, intraperitoneal, intracranial, intravenous, or subcutaneous routes, or was instilled intranasally. For intrapulmonary injection, the needle was inserted through the chest wall into the peripheral part of the lung. In most cases a small amount of the inoculum leaked from the nose or mouth, via the bronchi and trachea. For intrapleural injection the needle was inserted into the pleural cavity only, and not into the lung. Subcutaneous injection was made on the dorsum. Intravenous injection was by way of facial veins. All injections were made with a 30 gauge needle. All other materials, methods and precautions were as previously described(1).

Results and Discussion. Those hamsters injected with 50 times the minimum 100% tissue culture infectious dose ($MTCID_{100}$) of virus by the intrapulmonary, intrapleural, or intraperitoneal route, developed a high incidence of tumors at the site of injection in from 33 to 77 days (Table I). Three hamsters injected subcutaneously with the same virus fluid have not developed tumors in from 508 to 636 days to date. However, at the 500 $MTCID_{100}$ dose of virus, the subcutaneous route appeared as effective as any route tested. Of 17 hamsters injected with 500 $MTCID_{100}$ of virus by either the intrapulmonary, intrapleural, intravenous or subcutaneous route, all but 2 developed tumors within 98 days. By the intrapulmonary route, as little as 5 $MTCID_{100}$ produced tumors in 5 of 14 hamsters by 98 days. Of 25 hamsters injected intracranially with doses of from 2 to 200 $MTCID_{100}$ of virus, tumors arose in only one animal, of the 7 at the highest dose. This animal had no tumor at the site of intracranial injection, but had multiple abdominal tumor masses leading to death by 93 days. Hydrocephalus developed in 3 of the intracranially injected hamsters only, in from 36 to 58 days. Huebner *et al.*(3) have observed

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TABLE I. Tumor Induction in Hamsters Injected by Various Routes at Birth with Human Type 12 Adenovirus.

Adenovirus, Type 12										
Titer ($MTCID_{100}$/0.1 ml)	Dose ml	Dose $MTCID_{100}$	Inj route	Tumorous hamsters/ Inj hamsters*	Site of tumors and No. of hamsters		Age at death with tumor (days)	Death without tumor No.	Death without tumor Age (days)	Still alive without external evidence of tumor No.	Still alive without external evidence of tumor Age (days)
10	.02	2	Intracranial	0/17				2†	49, 58	8	406–427
								7	49–436		
"	.05	5	Intrapulmonary	5/14	Thorax	4	38–98	1	72	5	428–429
					" and liver	1		3	317–414		
100	"	50	"	2/2	Thorax	1	47				
					" and liver	1	44				
"	"	"	Intrapleural	3/4	Thorax	3	48–69	1	214		
"	"	"	Intraperitoneal	5/6	Abdomen	5	33–77			1	635
"	"	"	Subcutaneous	0/3				2	508, 632	1	636
1000	.005	"	Intranasal	0/7				1	233	6	246–287
100	.1	100	Intravenous	3/9	Inj site only	2	47, 85			6	105–107
					Liver only	1	38				
1000	.01	100	Intracranial	0/1				1	50		
"	.02	200	"	1/7	Abdomen‡	1	93	1†	36	4	372
								1	49		
"	.05	500	Intrapulmonary	4/5	Thorax and liver	4	42–45	1	210§		
"	"	"	Intrapleural	6/6	Thorax	5	29–45				
					" and liver	1					
"	"	"	Subcutaneous	3/3	Subcutaneous	2	86, 98				
					" & liver	1	37				
"	"	"	Intravenous	2/3	Subcut at inj site	1	70	1	387		
					Liver only	1	50				
"	.1	1000	Intravenous	7/11	Inj site only	1	99			4	107
					Inj site and liver	2	53, 90				
					" " & I.L.N.‖	1	44				
					Liver only	2	36, 103				
					Intraabdominal,¶ (rt. lower quad.)	1	93				

* No. of hamsters injected or instilled within 24 hr of birth and surviving more than 3 wk.
† Hydrocephalus.
‡ Died with tumors attaching to liver and diaphragm, but no intracranial tumor.
§ Abdominal abscess.
‖ I.L.N. = inguinal lymph node.
¶ Differs in histology from other tumors. Resembles lymphosarcoma.

intracranial tumors in 2 hamsters and hydrocephalus in several hamsters following intracranial injection of type 12 adenovirus. They have also obtained tumors by the subcutaneous and intraperitoneal routes. Although the hydrocephalus may arise on a traumatic or other such non-specific basis, a possible role of adenoviruses in hydrocephalus should be considered in view of the recovery of other types of adenoviruses from the cerebrospinal fluid of children(4).

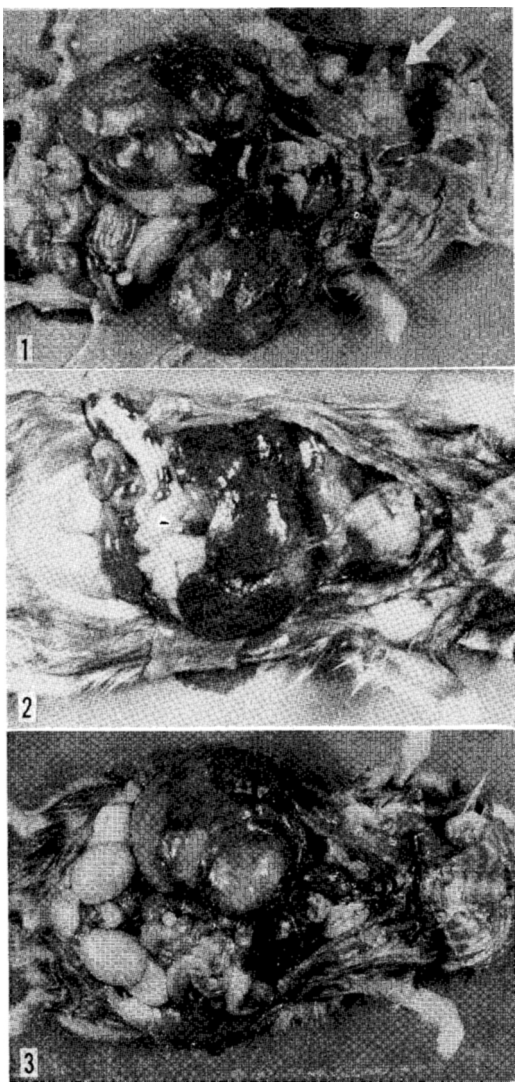

FIG. 1. Subcutaneous tumor (arrow) at site of injection, and remote tumors in liver of a hamster injected subcutaneously at birth with .05 ml of type 12 adenovirus (1,000 $MTCID_{100}$/0.1 ml).

FIG. 2. Local tumor filling thorax, and remote tumor diffusely disseminated in liver of hamster injected intrapulmonarily at birth with .05 ml of type 12 adenovirus (100 $MTCID_{100}$/0.1 ml).

FIG. 3. Intrahepatic tumor in a 50-day-old hamster injected intravenously by way of a facial vein at birth with type 12 adenovirus. No other tumor masses were found.

Of 35 hamsters with tumors at the site of injection, remote tumors in the liver were also found in 10 (Table I). They were of similar histology to the local tumors and usually occurred as nodular foci (Fig. 1), although one hamster had diffusely disseminated tumor in the liver (Fig. 2). While these tumors in the liver may represent metastatic foci from the primary at the site of injection the following facts suggest that at least some of them are, rather, distant primary tumors. (a) Most of them occurred in hamsters injected intrapulmonarily with larger doses of virus. (b) Similar liver tumors occurred in 4 intravenously injected hamsters without a tumor at the site of injection (Fig. 3). (c) Multiple abdominal tumors involving also the liver occurred in a hamster injected intracranially, without a tumor at the site of injection.

Histologically the tumors that arose at the various sites of injection were similar to the undifferentiated sarcomas reported earlier following intrapulmonary injection(1). The fact that tumors appear at many different sites of injection indicates that susceptible cells are widely disseminated throughout the body. If the remote tumors are indeed distant primaries, rather than metastases, their usual location in the liver would suggest the possible occurrence in the liver of a more susceptible cell type, or alternatively, localization or multiplication of the virus in the liver. The fact that the incidence of local tumors is directly dose dependent(2), and that the fewer remote tumors were usually seen following injection of larger doses of virus indicates that the local concentration of virus is an important factor in the malignant transformation that occurs.

All of the tumors reported in the present study became grossly apparent after a short latent period, resulting in death or sacrifice of the host because of tumor growth within 29 to 108 days. Trocar transplantation of such tumors to untreated hamsters leads to

death from tumor growth in from 13 to 139 days as previously reported(1). It is apparent that malignant transformation of cells at the site of injection occurs rapidly, probably within hours of the time of injection.

Of 7 hamsters administered 50 $MTCID_{100}$ of virus by intranasal instillation, none have died of tumors in approximately 9 months to date.

Summary. Tumors were induced at the site of injection of human type 12 adenovirus into newborn hamsters by each of the intrapulmonary, intrapleural, intraperitoneal, intravenous and subcutaneous routes, leading to death within 29 to 108 days. Whereas at higher doses of virus the subcutaneous route of injection was as effective as the other routes, at lower doses of virus it appeared less effective. For the intracranial route of injection, no local tumors were observed but one such hamster developed multiple abdominal tumors, and 3 developed hydrocephalus.

Of 35 hamsters with tumors at the site of injection by various routes, remote tumors in the liver also developed in 10. Similar liver tumors were also found in 4 intravenously injected hamsters without a tumor at the site of intravenous injection. Of 7 hamsters administered virus by intranasal instillation, none have died of tumors in approximately 9 months to date.

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1. Trentin, J. J., Yabe, Y., Taylor, G., *Science,* 1962, v137, 835.

2. Yabe, Y., Trentin, J. J., Taylor, G., PROC. SOC. EXP. BIOL. AND MED., 1962, v111, 343.

3. Huebner, R. J., Rowe, W. P., Lane, W. T., *Proc. Nat. Acad. Sci.,* 1962, v48, 2051.

4. Faulkner, R., Rooyen, C. E. van, *Canad. Med. Assn. J.,* 1962, v87, 1123.

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A Simplified Method for Virus-Tissue Culture Procedures in Microtitration Plates.* (28325)

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The method reported herein is a modification of the Takatsy(1) serological microtitration technique adapted to conventional tissue culture procedures, and recently described by Sever(2). Briefly, the titration plate (Fig. 1) simulates a series of micro tissue culture tubes in which cell monolayers are grown on the hemispherical bottom of the well compartment. Rapid serial dilution of virus or serum can be accurately accomplished by loop transfer.

Materials and methods. Microtitration apparatus was obtained from a commercial source† and consisted of Plexiglas plates moulded to contain 96 cups, each of which has a working capacity of approximately 0.2 ml; wire spiral loops with a calibrated transfer of 0.025 ml; and micropipets which deliver 0.025 ml/drop (Fig. 1).

Plates and micropipets were soaked in detergent and washed in tap water, followed by 3 rinses in triple distilled water. Virus-contaminated plasticware was soaked in a solution of 0.5% sodium hypochlorite and then washed as above. The utensils were then air-dried before further treatment.

Plates were irradiated with a 30W germici-

* The opinions and assertations contained herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

† Cooke Engineering Co., Alexandria, Va.