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Lung Tumors in Hamsters Produced by Polyoma Virus. (25785)

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Ease of transmission of polyoma infection in mouse colonies suggests that virus is spread by way of the respiratory tract(1). Rowe *et al.* reported that mice and hamsters can be infected with polyoma virus administered intranasally, although they did not describe the tumors(2). Eddy and associates described development of fibrosarcomas and angiomatous lesions in hamsters following subcutaneous injection of polyoma virus(3). Only a few animals in their study had lung neoplasms. The present report is part of study on pathogenesis of polyoma infection in the hamster and compares virus administration routes on development of lung tumors.

Materials and methods. Tissue Cultures. Embryos were removed from pregnant mice of ICR strain on 14th or 15th day of gestation. Trypsinized cultures of embryonic cells were prepared and cultivated as described by Stewart *et al.*(4). Cells were grown in 4 oz. prescription bottles and in roller tubes. Cultures were grown in 2% calf serum in medium 199 containing penicillin and streptomycin and maintained in 1% calf serum in medium 199 by weekly changes of fluids. The strain of virus in these studies was received from Dr. Bernice Eddy. Virus was carried through 7 passages in cultures of mouse embryo cells. Harvests for passage were usually collected 14 days after inoculation. Sixth and seventh passage viruses were used. For certain tests, virus was passed through Selas .03 filters. Syrian hamsters were obtained from Lakeview Hamster Colony, Lakeview, N. J. Pregnant mothers were received 3 or 4 days before parturition. Litters of hamsters less than 24 hours old were pooled, randomized and redistributed to dams. Virus or control fluids were instilled intranasally (IN) by placing a drop over noses and forcing inhalation by closing the mouth. For subcutaneous (SC) and intraperitoneal (IP) injections, each animal received 0.1 ml of undiluted culture fluids. For intracerebral (IC) 0.02 ml was injected. Preliminary tests showed that uninfected mouse cell suspensions were not tumorigenic by inoculation routes employed.

Results. Virus injected subcutaneously resulted in gross tumors in subcutis, liver, kidney, heart and, in one instance only, in lungs. Subcutaneous tumors often occurred at multiple sites. On occasion they reached massive proportions accounting for 25% of body weight. Growth of these tumors varied and was often rapid. In one animal a tumor weighing 21 g at necropsy was palpated a week earlier as 2-3 mm nodule. The neoplasms resembled sarcomas described by Eddy *et al.*(3). Those seen in other tissues except the liver were similar in appearance. Histologically the growths had the structure of fibrosarcoma. Microscopic tumors were found in certain tissues, including brain, not detected by gross examination. Liver involvement was angiomatous with lesions occasion-

TABLE I. Production of Lung Tumors as Influenced by Route of Infection.

Virus admin.	No. of hamsters with Gross tumors	Visceral tumors only	Lung & visceral tumors	Lung tumors only
Intranasally	38	9	14	15
All other routes	161	160	1	0

ally becoming quite extensive. Hemorrhages were frequently found in the peritoneal cavity.

Intracerebral route. Hamsters injected IC exhibited the same spectrum of visceral tumors as animals injected SC. In a few hamsters sarcomatous growths were observed at site of inoculation attached to periosteum on inner surface of cranium. Two animals had small angiomas apparently involving meninges covering cerebral hemisphere at inoculation site. These tumors were not studied histologically. No lung neoplasms were observed.

Intraperitoneal route. Animals injected IP developed only visceral tumors including angiomas of liver and sarcomata of heart and kidney. One hamster had tumorous masses on the omentum with attachment to wall of small intestine. Another had an extensive neoplasm of the ovary.

Intranasal route. Virus instilled IN induced tumor formation in tissues in the thoracic cavity. The relatively high percentage of animals developing tumors in lungs and surrounding tissues indicates that predilection was a function of method of inoculation (Table I). About one-half of animals developing lung tumors gave no evidence of other gross lesions. In the remainder there was visceral involvement in addition to pulmonary growths.

Unfiltered virus was employed for most IN studies; such preparations induced neoplasms in about 35 days. One experiment was conducted to determine if filtered fluids given IN would be tumorigenic. Two out of 19 hamsters developed lung tumors in 85 and 92 days respectively after receiving filtered virus.

Animals with lung involvement generally were thinner than controls although this difference became apparent only when animals reached maturity. Preceding death, the only sign manifested by animals with lung tumors was polypnea. Coughing was not detected. Upon necropsy of dead or sacrificed hamsters, tumors in thoracic cavity varied in extent of involvement and in gross structure (Fig. 1, 2). Some growths almost completely filled the thoracic cavity to the exclusion of virtually all normal lung tissue. Others were comprised of 1 or more nodules occupying a portion of a lobe. In several animals neoplasms involved mediastinal tissues including the heart. Grossly pulmonary growths appeared similar to sarcomas and angiomas involving viscera of hamsters injected by other routes.

Histologically, lung tumors showed 2 types of changes. One was a fibrosarcoma with typical well-differentiated spindle-shaped fibroblasts arranged in whorls and palisade groupings. The pale staining nuclei were oval-

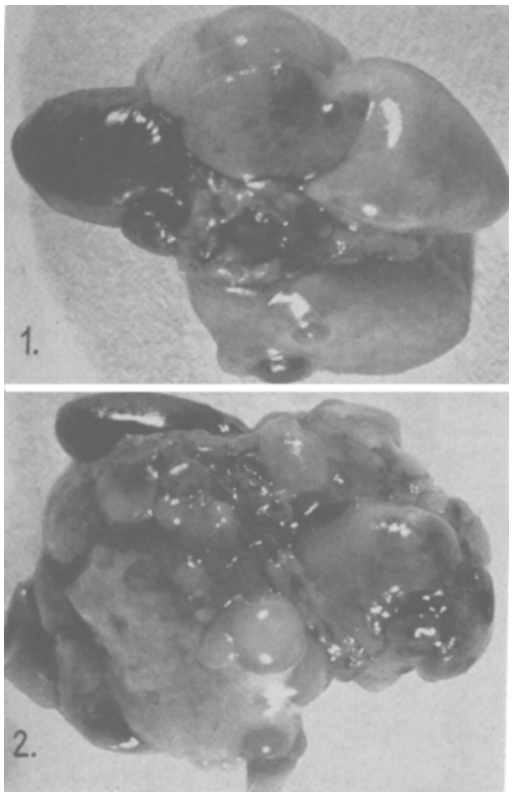

FIG. 1. Lungs from hamster sacrificed 33 days after IN infection with polyoma virus. Mediastinal and lung involvement. (Dark structure is heart.) ×15.

FIG. 2. Lungs from hamster dead 31 days after IN infection with polyoma virus. ×15.

shaped with pronounced nucleoli. Mitotic figures were rarely seen in these organized areas. Within the same tumorous nodule, focal areas of anaplasia were observed. Cells were polymorphous, exhibiting varying degrees of hyperchromatism. In anaplastic areas, a few more mitoses were seen. Lack of pattern and the wide intercellular spaces in anaplastic areas contrasted with the closely packed organized cells described above. The other main cellular change of frequent occurrence could not be identified and was presumably similar to changes described by Eddy *et al.*(3). These cells were ovoid or round with a very light staining nucleus and 1 or 2 prominent nucleoli. Cytoplasm was scanty. Cells were closely packed in rows or clusters with little stroma noted. Their identity as neoplastic cells must await further study.

An unusual amount of necrosis was seen in lung tumors. In some areas this was evidenced by extensive patches of homogenous eosinophilic matrix with accumulations of pyknotic and fragmented nuclei. In anaplastic areas, numerous necrotic cells were interspersed among actively growing tumor cells. Occasional foci or plaques of necrosis were seen in areas which otherwise had the usual neoplastic appearance. These foci were adjacent to several blood vessels, which suggests that the necrotic changes were not due to inadequate nutrition. Hemorrhage was another prominent feature of all tumors studied. Lung tissue adjacent to tumors was atelectatic. Infiltration of inflammatory cells was also noted. Occasional tongue-like processes from the tumors penetrated otherwise normal lung areas. In one area tumor cells were seen in a bronchiole.

Effects of cortisone. One experiment was conducted to test the influence of cortisone on development of tumors in polyoma-infected hamsters. Groups of randomized animals were inoculated with virus and control fluids by one of the following routes: SC, IP, IC and IN. Twenty-three days later one-half of the hamsters in each group was injected SC with 2 mg of cortisone. The experiment was terminated 15 days post-cortisone treatment, at which time surviving animals were sacrificed and examined for tumors. Observations recorded were the time that tumors were first noted, and their location. Histological studies were not made.

TABLE II. Influence of Cortisone on Tumor Development.

	Route of admin.			
	SC	IP	IC	IN
Virus	7/7†(36)‡	6/6 (36)	6/7 (34)	2/11 (38)
Virus & cort.*	6/7 (33)	6/6 (33)	7/7 (33)	9/11 (36)

* Cortisone given subcut. 23 days after virus.
† Numerator = No. with tumors. Denominator = No. inoculated.
‡ Figures in parentheses represent No. of days before tumor was noted.

Results of this experiment (Table II) suggest that cortisone enhanced development of lung tumors in the infected IN group. Thus 9 of 11 hamsters receiving cortisone had extensive pulmonary neoplasms. In untreated infected IN group, only 2 had tumors and these were visceral angiomas. There was no significant effect of cortisone on groups infected by other routes except possibly for a slight acceleration of tumor development. The spectrum of tumors was similar to that described above. All controls were negative for tumors. There was no gross difference in appearance of tumors in treated and untreated groups.

Discussion. Although considerable work on production of lung tumors in mice by chemical carcinogens has been reported, few such studies have been made in the hamster. To our knowledge the only description of virus-induced lung tumors in hamsters has been that of Eddy *et al.*(3). Growths, primarily angiomatous, were observed in lungs of several animals inoculated subcutaneously. Lesions were apparently similar to those noted in our experiments. Virus-induced lung neoplasms have also been described by Kirschstein *et al.*(5) working with fibroma virus in rabbits and squirrels. The lesions recorded were epithelial and resembled those of adenomatosis as in man and sheep(6).

Lung lesions in our study were largely those of a fibro-sarcoma. However, all tumors had areas in which there were accumulations of cells of unidentifiable type. These did not have a true malignant appearance and likely

reflect the inherent nature of certain viruses to induce proliferative cellular response. Cellular necrosis observed in most of lung nodules is compatible with the fact that polyoma virus destroys cells grown in tissue culture.

Summary. Polyoma virus given intranasally produced primary lung tumors in hamsters. Some animals showed visceral involvement. Growths were largely fibrosarcomatous. Hamsters injected by other routes showed almost no lung neoplasia.

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Propagation of Trachoma Virus in Cultures of Human Tissues.* (25786)

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Although the etiological agent of trachoma was first described in 1907(1), technical limitations prevented a rapid evolution of the problem. T'ang *et al.*(2) reported on its physical characteristics and propagation in the yolk sac of chicken embryos. By omitting penicillin from the inoculum(2), virus isolation was confirmed by other workers(3-7). Subsequently, Gordon *et al.*(8) reported propagation of trachoma virus in tissue cultures of entodermal cells of chick embryos. This report describes adaptation and serial propagation of trachoma virus in tissue cultures of human origin.

Methods. The strain of trachoma virus (SA-2) was kindly provided by Dr. John C. Snyder, Harvard University(4). It had been passaged 7 times in yolk sacs of embryonated eggs. The virus was propagated by us in yolk sacs of embryonated eggs derived from a PPLO-free flock of chickens§ which were on an antibiotic-free diet. Infected yolk sacs were pooled, homogenized in a Ten Broeck grinder, and diluted 1:10 with PG medium (9). After centrifugation at 3000 rpm for 20 minutes, the aqueous supernatant, containing the virus, was stored in ampoules at dry ice temperature. Human amnion (FAM)(10) and human synovial (McCoy)(11 and personal communication) cell lines were propagated on cover slips in Leighton tubes. Tissue explants from 9-day-old chicken embryos were also used. Nutrient medium was made to volume with Mixture 199 and contained 5% heat-inactivated calf serum, 0.5% lactalbumin hydrolysate, 100 units/ml penicillin, and 0.1 mg/ml streptomycin. When confluent cell-sheets had formed and tissue explant growth was suitable, cultures were washed twice with Mixture 199 and 0.1 ml of the virus inoculum was added to each tube. Nutrient medium without antibiotics was then added to each tube (0.9 ml) and cultures were incubated at 37°C. Culture fluids were changed at 4-day intervals. Duplicate coverslip preparations were treated with Wright's plus Giemsa stain and with acridine orange dye. The latter preparations were examined by fluorescence microscopy(12). Other cultures were frozen and thawed twice, pooled, and inoculated (0.25 ml) into the yolk sac of

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