Induction of Hepatomas in Hamsters by an Avian Adenovirus (CELO) (35591)

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Chicken-embryo-lethal-orphan (CELO) virus is the only known oncogenic avian adenovirus. Inoculation of newborn hamsters resulted in tumor formation, with a latent period of 3 to 6 months (1, 2). The tumors were diagnosed histologically as fibrosarcomas. Intracranial inoculation of CELO virus has been reported to induce ependymomas (3). This report describes the induction of hepatomas in hamsters with CELO virus. It is believed that this is the first report of viral hepatoma induction.

Methods and Results. Newborn of both random-bred hamsters and inbred LSH/LAK hamsters from an SPF colony (4) were inoculated by the subcutaneous route with the Phelps strain of CELO virus (5). Table I summarizes the results of these experiments. The random-bred animals appear to be slightly more susceptible to CELO virus oncogenesis than are the inbred strain. In each strain of hamsters, the latent period was the same, ranging from 58 to 252 days. Most of the tumors were found at the site of inoculation and were diagnosed histologically as fibrosarcomas. However, two inbred animals, after a latent period of 95 and 114 days were found to have abdominal tumors, with no neoplasm at the site of inoculation. In another experiment, after 56 days, a third inbred animal was found to have an abdominal tumor.

These tumors were confined to the liver and had extensive necrosis. In two animals, the spleens were greatly enlarged. Pieces of tissue were fixed in formalin and stained with hematoxylin and eosin.

Histologic examination revealed that the tumors were composed of small nodules of tumor cells separated by fine septa of connective tissue extending from the periphery of the tumor mass (Fig. 1). The surrounding hepatic tissue was compressed with no capsule separating it from the tumor. The peripheral areas of the tumor were infiltrated with lymphocytes and plasmacytes.

The cellular pattern was composed of large pleomorphic cells (Fig. 2). The nuclei were pleomorphic with marked chromatin clumping. Many cells had a partial or complete loss of the nuclear membrane. Multinucleated cells were common. The cells had abundant eosinophilic cytoplasm with varying degrees of vacuolation and granularity.

TABLE I. Tumor Incidence in Hamsters Inoculated with an Avian Adenovirus (CELO).

Hamster strain	Inoculum ($TCID_{50}$)	No. animals with tumor/no. animals weaned	Tumors (%)	Latent period (range; days)
Inbred LSH/LAK	$10^{7.9}$	8/15	53	58–241
	$10^{7.6}$	4/8[a]	50	
	$10^{6.9}$	3/9	33	
	$10^{6.6}$	9/11[a]	82	
Random-bred	$10^{7.9}$	19/21	91	60–252
	$10^{7.6}$	8/12	67	
	$10^{6.6}$	6/8	75	
Total		57/84	68	

[a] One animal bearing a hepatoma.

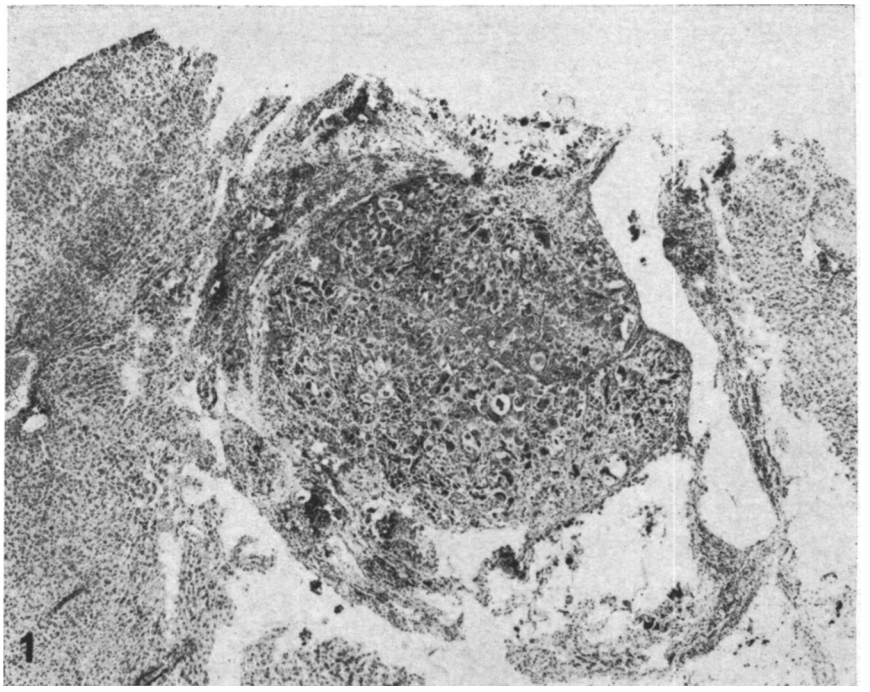

FIG. 1. A section through a tumor nodule diagnosed as a hepatocellular carcinoma or hepatoma: hematoxylin and eosin staining; ×ca. 41.

Mitotic figures were frequent. No bile or hepatic ducts could be identified in any tumor. The tumors were diagnosed histologically as hepatic cell carcinomas or hepatomas. No spontaneous hepatomas have been observed in our hamster colony.

Using the indirect fluorescent antibody technique (FA), we found that sera from the hepatoma-bearing animals reacted with an intranuclear antigen in 3–5% of cells from hepatomas and in 5–10% of cells from fibrosarcomas when these tumors were grown for 3 passages *in vitro*. A similar antigen was detected by hepatoma sera (6, 7) in the early infection of chick kidney cells with CELO virus. The hepatoma sera did not react with the T antigens of SV40, human adenovirus types 7 and 12, polyoma or the group-specific antigen of the avian leukosis complex when tested by complement fixation (CF) or FA techniques. By CF, the hepatoma sera were negative for CELO virion antigen.

Electron microscopic examination of the hepatomas and cells grown in tissue culture has revealed the presence of hamster types A and C particles (Fig. 3) as first described by Stenback *et al.* (8, 9). Further studies are in progress to determine if an interaction between CELO virus and the virus particles is necessary for hepatoma induction in hamsters. These findings will be the subject of a future report. However, the serologic evidence indicates that the hepatomas were induced by CELO virus.

Summary. Hepatomas were induced in ap-

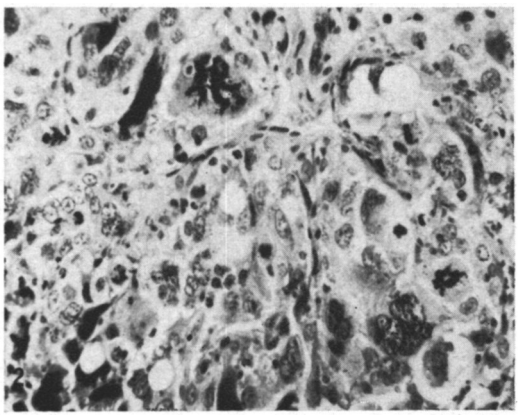

FIG. 2. Section of a hepatoma: note the chromatin clumping, multinucleated cells, and aberrant mitoses; hematoxylin and eosin staining; × ca. 324.

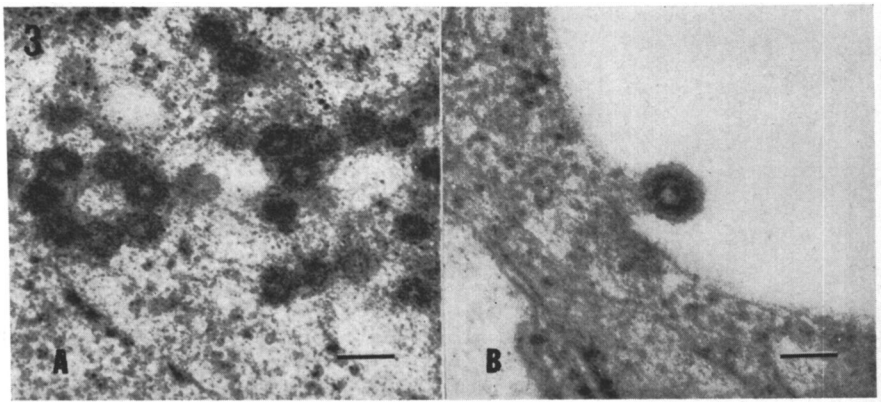

FIG. 3. Virus particles found both in hepatomas and in a cell line derived in vitro from one hepatoma: (A) cytoplasmic type A particles; (B) budding type C particle. Magnification bars represent 100 mμ.

proximately 4% of inbred LSH/LAK hamsters inoculated subcutaneously with CELO virus. The sera from these animals stained the CELO T antigen found in lytically infected chick kidney cells. The tumors contained hamster types A and C virus particles. The relationship between these indigenous hamster viruses and tumor induction by CELO virus is being investigated.

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