SOME BIOLOGICAL PROPERTIES AND MORPHOLOGICAL CHARACTERISTICS OF A TRANSPLANTABLE HAMSTER TUMOR INDUCED BY TYPE 3 BOVINE ADENOVIRUS

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From a primary hamster tumor induced by type 3 bovine adenovirus, tumors (to which the name BATH was given), transplantable in vivo, were obtained. These tumors possess high transplantability, giving 100% of successful tumor cells in hamsters of any age very quickly (after 5-6 days). The morphological characteristics of the original tumor (a malignant hemanigopericytoma) and of the tumors derived from it by passage are given. No transplantation antigens were found in the BATH tumors. The BATH cells went through more than 60 passages in hamsters and form a convenient model for the study of more aspects of virus carcinogenesis.

Type 3 adenovirus was isolated in 1965 from a healthy cow [3] and its oncogenicity to golden hamsters was demonstrated [2]. This was confirmed later by Gilden et al. [4] and by Strizhachenko et al. [1].

A study of the properties of tumors (BATH) induced by this virus and transplantable in vivo is described blow.

EXPERIMENTAL METHOD

Oncogenic strain WBR-1, generously provided by Darbyshire, was used. The virus was propagated and titrated in a primary calf kidney culture. The titer of the virus varied from 3.5 to 4.5 log TCD_{50}/ml.

Primary tumors were induced by injecting 0.2 ml of the virus subcutaneously into newborn hamsters into the dorsal and cervical region. For transplantation into the newborn and adult animals, 10^6 tumor cells were injected in a volume of 0.2 ml. The hamsters were immunized intramuscularly at the age of 2-3 months with 2 ml of a mixture of equal parts of virus with Freund's incomplete adjuvant (9 parts mineral oil, 1 part anhydrous lanoline). Three weeks later, 2 ml of the virus was injected intraperitoneally. One week after the end of immunization, the immunized and control animals received injections of serial tenfold dilutions of tumor cells. Pieces of the tumors were fixed in formalin and embedded in paraffin wax. Sections were stained with hematoxylin-eosin, picrofuchsin, Heidenhain's azan, for polysaccharides (the PAS reaction) with an amylase control, and impregnated by Gomori's method. Frozen sections were prepared from some tumors and stained for lipids with Sudan.

EXPERIMENTAL RESULTS

Transplantability of the Tumors. A primary tumor developed in the hamster 9 weeks after inoculation and it was removed for transplantation after 14 weeks. Passages were carried out through newborn and adult hamsters. In the first transplantation experiments tumors appeared after 2 weeks, while later the latent period was shortened to 5-6 days, and tumors developed in all the infected animals. At the 28th

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TABLE 1. Frequency and Time of Appearance of Transplantable Tumors Depending on Dose of Transplanted BATH Cells

Number of cells	Number of hamsters with tumors	Time of appearance of tumors (in days)
10^1	0	–
10^2	1	37
10^3	4	14
10^4	3	6
10^5	4	14
10^6	4	6

TABLE 2. Formation of Tumors in Hamsters Immunized with Virus Depending on Number of Transplanted BATH Cells

Number of cells	Immune hamsters		Control hamsters	
	frequency of discovery of tumors	latent period (in days)	frequency of discovery of tumors	latent period (in days)
10^1	–	–	0/4	–
10^2	2/5	13	1/4	37
10^3	4/5	6	3/4	14
10^4	5/5	13	3/4	14
10^5	5/5	6	4/4	14
10^6	5/5	6	4/4	6

passage the minimum number of tumor cells inducing a tumor was determined. Hamsters were injected with tenfold decreasing numbers of cells from 10^6 to 10^1. Four animals were infected with each dose of cells and kept under observation for 2 months. The results are given in Table 1.

As Table 1 shows, injection of 100 cells was sufficient to induce tumors in single animals. The latent period shortened as the number of transplanted cells was increased.

The high oncogenic activity of the BATH cells when inoculated into animals could be dependent on low activity of the transplantation antigen in the tumor cells. To test this hypothesis, a transplantation test was carried out. Its results are given in Table 2.

The results confirmed the hypothesis that transplantation antigen is either absent or extremely weak in the tumors studied.

Macroscopic Picture. Primary tumors were found at the site of injection of the virus. In the overwhelming majority of cases the tumor was of solid type, and rarely it was cystic. The commonest type of solid tumors were multiple large nodules, frequently in a conglomeration, adherent to the skin and invading the underlying tissues, soft or soft-elastic in consistency, pinkish-white on section, and with foci of necrosis and hemorrhages. This was the appearance of the tumor originally used for passage.

The tumors obtained by passage were indistinguishable from the original tumor but the foci of necrosis in them were larger, despite their lower age.

Microscopic Picture. The original primary tumor consisted mainly of irregular or round, polymorphic cells (Fig. 1A). The cytoplasm of these cells was a narrow rim, poor in polysaccharides and almost free from lipids. The tumor cells showed a tendency toward angioplasia and they lay outside the argyrophilic membranes of the newly formed blood vessels. Most of these vessels were lined with endothelium. Some areas of the tumor consisted of spindle cells with acidophilic cytoplasm, and on staining with azocarmine they appeared orange-red in color, characteristic of muscle cell sarcoplasm. 'Cuffs" of delicate blue fibers could be seen around some of the cells, and they were also visible after impregnation with silver. Some cells contained specks or granules of glycogen. The cells showed a tendency to lie in bundles running in different directions. (Fig. 1B). These areas resembled a leiomyosarcoma. On the basis of all these features the tumor was described as an angiogenic sarcoma of the malignant hemagiopericytoma type. Its structure was particularly characteristic in tumors after the first passage in adult hamsters (Fig. 1C, D). During subsequent passage the size of the cells and the number of mitoses increased, the polymorphism became more marked, and the giant cells multiplied. The tendency for the tumor cells to form blood vessels persisted (Fig. 1E, F), although it diminished progressively. After the 50th passage this tendency could no longer be seen and the tumors were described as undifferentiated sarcomas. All the transplanted tumors grew by infiltration.

The BATH cells thus possessed high transplantability, as reflected by the rapid (5-6 days) and 100% successful transplantation of the tumor, possible on account of the very weak transplantation antigen. Comparison of the morphology of the original tumor and the tumors obtained after passage shows that despite the decrease in level of differentiation in the course of passage, the tumors retained the character of an angiogenic sarcoma for a long time. The BATH cells have gone through more than 60 passages in hamsters and they are a convenient model for the study of certain aspects of virus carcinogenesis.

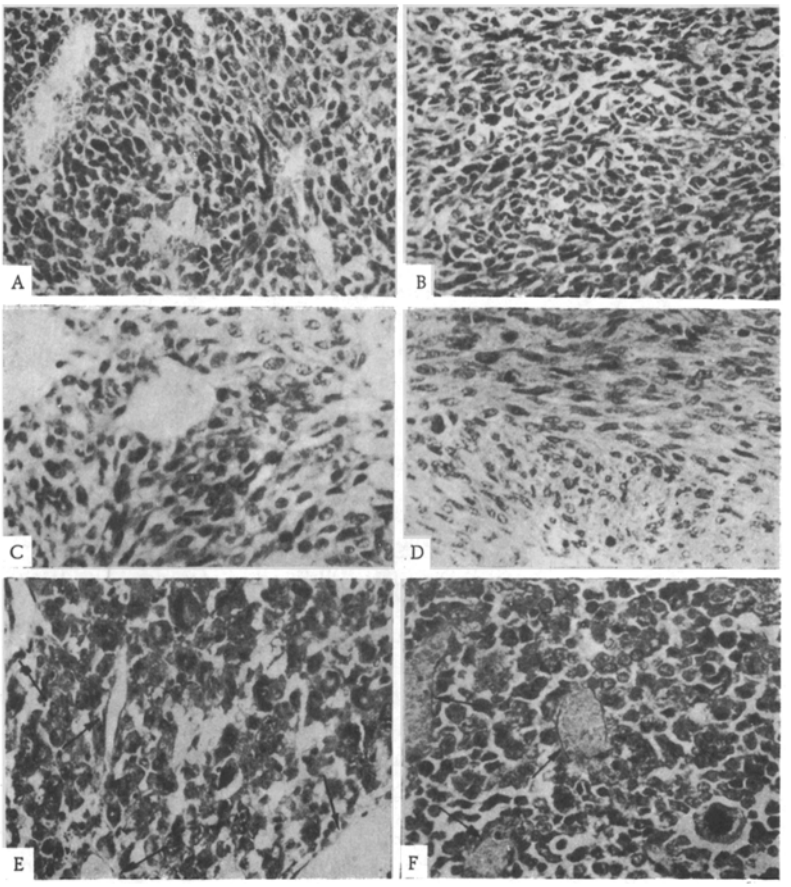

Fig. 1. Original primary tumor and subsequent passages. A) Original tumor, malignant hemangiopericytoma; B) an area of the same tumor with the structure of a leiomyosarcoma; C and D) tumor after first passage in adult hamsters, preserving structure of original tumor; E) tumor after 6th passage: tumor cells highly polymorphic but tendency toward angioplasia still present (blood vessels indicated by arrows); F) tumor after 23rd passage; despite marked anaplasia of the tumor cells, tendency toward formation of blood vessels still present (vessels indicated by arrows). Hematoxylin-eosin, 345 ×.

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