NOTES

In Vitro Induction of a Herpes-Type Virus in "Summer-Phase" Lucké Tumor Explants

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A herpes-type virus was induced in explants of "summer-phase" Lucké tumor maintained on agar slants at 7.5 C for 13 to 14 weeks.

The cells of the Lucké tumor (a spontaneous renal adenocarcinoma of the leopard frog, *Rana pipiens*) exist in two phases (4, 5, 7). In the "winter-phase" tumor, whether from frogs captured upon emerging from natural hibernation or from frogs maintained for 3 to 4 months in the laboratory at or below 7.5 C, cells with enlarged nuclei, marginated chromatin, and Cowdry type A inclusions can be seen by light microscopy, and virus particles are visualized by electron microscopy. The tumor cells of the "summer-phase," whether from frogs collected in the field during the summer months or from frogs maintained in the laboratory at 22 to 26 C, lack inclusions and virus particles cannot be demonstrated (2, 6, 8).

Herpes-type virus has been identified in transplants of "summer-phase" tumors maintained for more than 2 months in the anterior eye-chambers of *R. pipiens* held at 7.5 C (4). Similar in vivo results have been obtained when "summer-phase" tumor transplants were maintained in the eye-chamber of *Bufo americanus* or *R. clamitans* held at 7.5 C, species in which the tumor does not occur naturally (3). These data have supported the hypothesis that the herpes-type viral genome exists in "summer-phase" tumors in a latent state (5) and that virus is induced when the tumor is maintained in vivo at reduced temperature for several weeks.

We now report the results of an in vitro experiment which employed "summer-phase" kidney tumors from four frogs maintained in the labora-

tory at 22 to 26 C. After a frog was killed by decapitation, its tumor was removed aseptically and placed in modified Leibovitz L-15 medium (1). A nodule from each tumor was further subdivided into small fragments (approximately 2 mm³). Single fragments were incubated on the surface of agar slants in screw cap tubes (15 by 150 mm; reference 9). The slants consisted of equal volumes of 2% special Noble agar and modified Leibovitz L-15 medium with an additional 1.5 ml of the latter placed at the base of each slant. For the first 24 hr, explants were maintained at 25 C; thereafter the incubation temperature was either 7.5, 11.5, or 15.5 C. The explants were fed twice weekly with the same medium. Serial sections of fragments from each tumor were examined by light microscopy, and approximately 200 cells from each tumor were examined by electron microscopy. The light microscopic findings were typical of those seen by previous workers in "summer-phase" Lucké tumors (2, 4, 5). No virus was detected by electron microscopy (Fig. 1) in any of the original nodules used for explantation.

Sixty-three explants have been studied by light microscopy. The 46 explants which were cultured at 15.5, 11.5, and for less than 12 weeks at 7.5 C appeared similar in morphology to the original tumor tissue. Mitotic figures were observed at each temperature of incubation. After 12 weeks at 7.5 C, in four of six explants, 3 to 5% of the epithelioid cells contained enlarged nuclei with marginated chromatin. After 14 weeks at 7.5 C, in four of six explants, 5 to 10% of the epithelioid cell nuclei were pyknotic, 3 to 5% of the nuclei were enlarged with marginated chromatin, and 1% had type A inclusions.

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FIG. 1. Electron micrograph from a "summer-phase" tumor which was subsequently used for explanation. Chromatin is evenly dispersed and herpes-type virus is absent. $\times 26,000$.

The results of electron microscopic detection of herpes-type virus are summarized in Table 1. Explants incubated for less than 12 weeks were found to be "virus-free." After 13 weeks in vitro at 7.5 C, in one of five explants, 1% of the observed cells were seen to contain virus. Among explants held 14 weeks at 7.5 C, six of eight were found to contain virus in from 1 to 5% of the cells examined. Empty or complete herpes-type

virus particles, or both, were present in the nuclei, and enveloped forms were present in the cytoplasm (Fig. 2).

In summary, in tumors from three of four frogs which were followed individually, in temporal sequence over the period of 1 year, virus particles were demonstrated after 14 weeks at 7.5 C. That the herpes-type viral genome may be present in

FIG. 2. Explant from a "summer-phase" Lucké tumor which has been cultured *in vitro* for 14 weeks at 7.5 C. Note empty capsids in the nucleus and cytoplasm (arrows). $\times 22,000$. Insert shows several enveloped, complete herpes-type virus particles which are enclosed within a membrane, in the cytoplasm of another cell from the same preparation. $\times 47,000$.

a latent form within some of the "summer-phase" Lucké tumor cells has been demonstrated by its induction *in vitro*; factors which are present only in the frog host are not required for the induction of virus. The mechanism whereby prolonged exposure to lowered temperature results in the change from "virus-free" to overtly

virus-containing state is still unclear; the effect of temperature on such factors as enzyme activity, metabolic rate, and nutritional state of the neoplastic cells has yet to be determined. However, virus induction in the *in vitro* system now provides a basis for further investigation of various aspects of this virus-tumor relationship.

TABLE 1. *Presence of herpes-type virus as detected by electron microscopic examination of Lucké tumor explants after in vitro culture at various times and temperatures*

Time in culture (weeks)	Temp of incubation		
	15.5 C	11.5 C	7.5 C
8	0/4 ^a	0/3	0/4
10	0/4	0/3	0/9
12	0/2	0/2	0/6
13	0/2	NS ^b	1/5
14	0/3	0/2	6/8 ^c

^a Number of explants in which herpes-type virus was detected over the number of explants observed.

^b No sample.

^c Explants from four frogs were examined.

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