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Immunofluorescent Studies of Virus-Induced Rabbit Papilloma (Shope) *in vitro*.* (32112)

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Virus-cell relationship in Shope virus-induced papillomas of rabbit skin has attracted the particular interest of investigators in the tumor virus field in respect to the "masking" of this virus in papillomas of domestic rabbits (1).

Recently Noyes and Mellors(2) have carried out immunofluorescent studies on the tissue sections of the neoplasia and have shown that the viral antigen could be clearly demonstrated in papillomas of cottontail rabbits while it was detectable but less conspicuous in the sections of domestic rabbit papillomas. On the other hand, it has been reported that papillomas of both cottontail and domestic rabbits can yield tumorigenic nucleic acid by phenolic extraction although the level of activity of the extracts from the latter tissue is much lower than that from the cottontails (3,4). These findings, however, were all obtained at the tissue level and little study has been done on the system at the cellular level.

The present article deals with immunofluorescent studies of cells derived from Shope virus-induced papillomas cultured *in vitro*, especially from a point of view of possible persistence of the virus-related antigenic substance(s) in these cells.

Materials and methods. Virus. Glycerinated papillomatous tissue of Kansas cottontail rabbits was employed as the source of Shope papilloma virus. A 10% aqueous extract was prepared and used as virus preparation. The virus was inoculated on to the skin of domestic rabbits by scarification method(4). Papillomatous growth appeared at the site of inoculation after 2 to 3 weeks.

Cell culture and media. A small piece of papillomatous tissue was removed from the skin of the animal and finely minced with

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surgical scissors. The minced tissue was washed twice with phosphate buffered saline and put on coverslips. After the tissue fragments stuck firmly to the coverslips, they were placed in Leighton tubes with culture medium, YLE (Earle's balanced salt solution containing 0.1% yeast extract and 0.5% lactalbumin hydrolysate) plus 20% calf serum and 10% tryptose phosphate broth, and incubated at 37°C in a stationary position.

Immunofluorescent staining procedure. The direct staining method was used for the immunofluorescent investigations. Coverslips with grown cells were dried and fixed in carbon tetrachloride for 30 minutes at room temperature. Fluorescein isothiocyanate (FITC)-conjugated serum (anti-Shope papilloma virus antiserum) globulin from a regressor cottontail rabbit was placed on the coverslips with fixed cells and allowed to stand for 50 minutes at 37°C. After washing thoroughly with phosphate buffered saline, the coverslips were mounted in 20% buffered glycerin. They were examined with a Nikon fluorescence microscope with a Corning No. 5840 or a Schott BG 12 exciter filter. The light source was an Osram HBO 200 lamp.

Results. Growth characteristics of papilloma cells in vitro. Two different types of cells grew out of papilloma explants approximately after 3 weeks of incubation. One was round or polygonal and the nuclei were usually large in size (Fig. 1). These cells showed a tendency to overgrow slowly, forming multilayered areas. Another type of cells was triangular or columnar. Nucleus was round and medium in size (Fig. 2). They showed a tendency to grow rapidly in criss-cross fashion. These two types of cells often showed a random growth pattern forming a mixed population of the cells (Fig. 3 and 4). Both types of cells occasionally exhibited considerable variation in size (Fig. 1 and 4).

Immunofluorescence of papilloma cells in vitro. More than 90% of round or polygonal cells strongly fluoresced in nucleus (Fig. 5). Specific nuclear fluorescence was also observed in some of triangular or columnar cells, but the intensity of the reaction was much weaker than the round-type cells. In the present study, anti-Shope papilloma antisera from 2 regressor cottontail rabbits were used. Both sera gave essentially the same results.

As controls, normal rabbit embryo kidney and skin cultures were treated with the FITC-conjugated sera. Cells from papilloma cultures were also treated with normal rabbit serum followed by treatment with FITC-conjugated sheep anti-rabbit globulin. All of the control sets gave completely negative results.

Discussion. It has been clearly shown in the present study that among two types of cells cultured from the papillomatous tissue of domestic rabbits *in vitro* a specific fluorescence is regularly demonstrable in the nucleus of round or polygonal cells. These observations differ somehow from the results obtained by previous workers(2). They could detect only occasional fluorescent staining of the cells of higher horny layer in the tissue sections of the domestic rabbit papillomas. In this connection, it is tempting to assume that the triangular or columnar cells with lower reactivity in fluorescence originated from the basal or para-basal cell layer of the skin and the round or polygonal cells came from the higher layers of more "differentiated" cells. We have recently established 2 cell strains from the papillomas of domestic rabbits, both of which were primarily composed of either triangular or columnar cells but later in aged cultures showed a tendency to grow in a mixed population of the two types of cells previously described(5).

Another alternative assumption is that due to some unknown factor the "de-repression" of functional expression of persisting viral genome, carrying the code for the synthesis of viral antigen, may have occurred when the cells were brought into *in vitro* conditions. Provided this is the case, the more frequent and intense immunofluorescent reactivity of the *in vitro* cultured papilloma cells than that demonstrable in the tissue sections of domestic rabbit papillomas(2) may be explained. However, data to substantiate these assumptions are not available.

Another point of importance is whether virus-specific tumor antigen or "neo-antigen" could be differentiated from viral antigen in this system by immunofluorescent and other techniques. This is now being studied.

Summary. Shope virus-induced papillomas of domestic rabbits were cultivated *in vitro* and were subjected to immunofluorescent investigation. Two morphologically different cells, round or polygonal cells and triangular or columnar cells, were observed to grow out

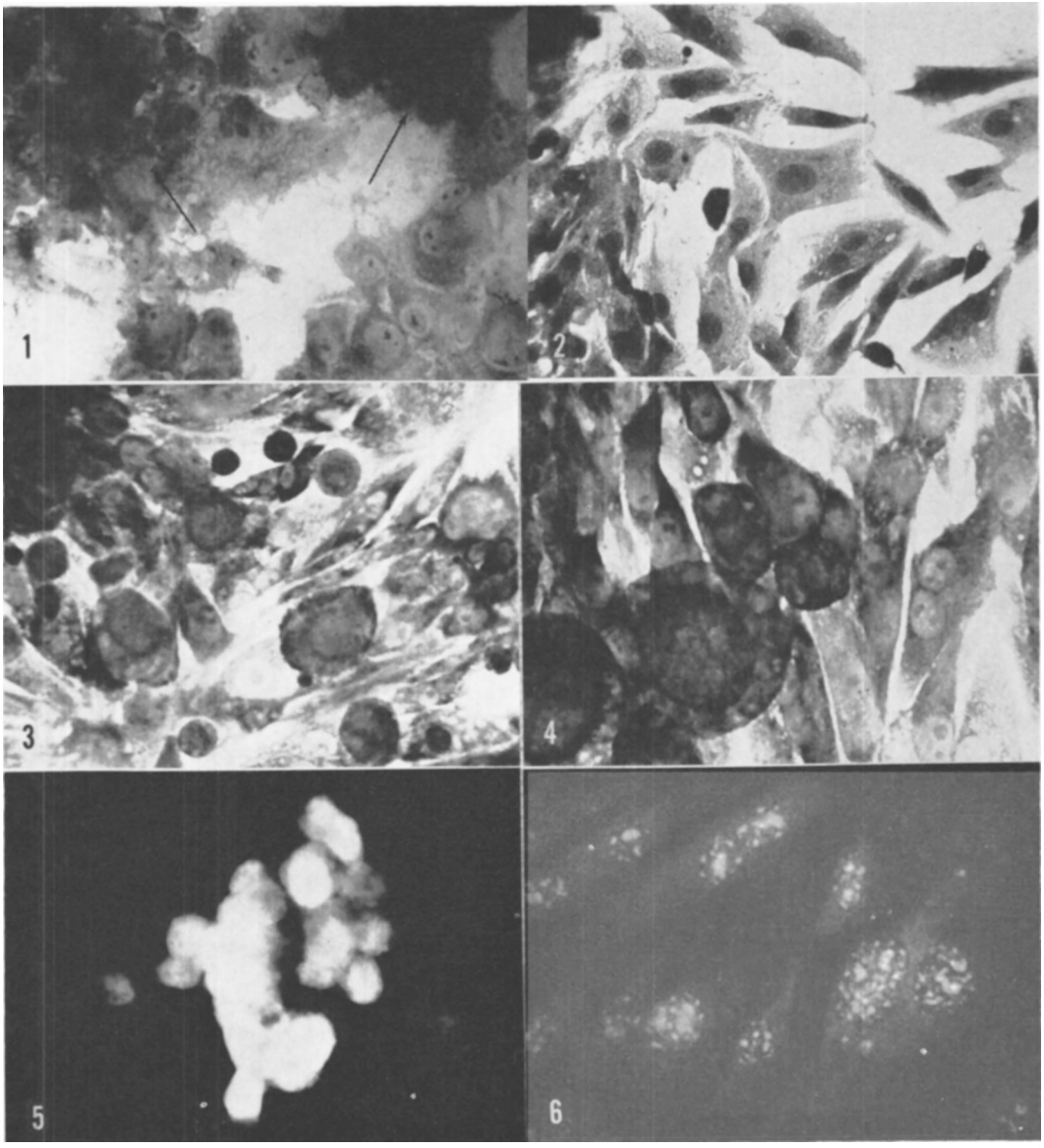

FIG. 1. Primary culture of Shope papilloma cells. A growth of the "polygonal cells" after 25 days in culture are shown. Arrows indicate multilayered areas as a result of piling-up of the cells. May-Giemsa stain. ×260.

FIG. 2. "Columnar cells" derived from the tissue explant of Shope papilloma growing for 25 days in culture. May-Giemsa stain. ×130.

FIG. 3. Mixed cell population of the two types of cells (25 days in culture). May-Giemsa stain. ×130.

FIG. 4. Higher magnification of the preparation shown in Fig. 3. May-Giemsa stain. ×260.

FIG. 5. Fluorescence photomicrograph of the "polygonal cells" (25 day culture). Note intense nuclear fluorescence. ×260.

FIG. 6. Fluorescence photomicrograph of "columnar cells" (25 day culture). Specific fluorescence in nuclei is shown. ×260.

of explanted papilloma tissues. Among them, the majority of round or polygonal cells exhibited intense fluorescence reaction in nucleus. A certain number of triangular or columnar cells also reacted with specific fluorescence in nucleus but the intensity of the staining was weaker and less conspicuous. The implication of these findings was discussed in connection with previous immunofluorescent studies on the tumorous tissues of the Shope system.

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Progesterone, 16α-Hydroxy-Progesterone, and Maintenance of Pregnancy In Mice.* (32113)

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The hormone 16α-hydroxy-progesterone has been demonstrated in extracts of human blood and corpora lutea(1), but little information on its endocrine activity seems to have been published. This study was undertaken to subject 16α-hydroxy-progesterone to the Hooker-Forbes bio-assay for progestin activity(2) and to investigate the effect on fetal survival of administration of pellets of this compound, alone or in combination with progesterone, in intact and ovariectomized mice.

Materials and methods. A sample of 16α-hydroxy-progesterone was assayed for progestin activity(2). Solutions for assay were injected into 40 uterine horns of ovariectomized adult CHI mice.

All other experiments were done with adult female Brown Belt mice. They were mated with males of this stock and were examined daily for vaginal plugs. The day on which a plug was found was recorded as day 1 of pregnancy. Mice found to be not pregnant, and mice that became ill, were excluded from the experiment.

Hormones were mixed by dissolving the appropriate amounts in ether, stirring, and recrystallizing. Cylindrical pellets of pure steroid(s) were prepared by a technique which subjected each pellet to the same amount of compression(3). The pellets, which weighed about 9 mg each, were implanted with a large hypodermic needle beneath the dorsal thoracic or cervical skin during light ether anesthesia on day 12 of pregnancy.

Bilateral ovariectomies were performed by a lumbar dorsal approach under ether anesthesia on day 14. Care was taken not to disturb the pellets.

Body weights were recorded daily beginning on day 12.

Two groups of mice were allowed to go to term and deliver their young (Groups A, B, Table I). All other pregnant mice were anesthetized with ether on day 19. The abdomens and uteri were opened. The numbers of live fetuses, dead fetuses, and empty implantation sites (*i.e.*, those from which abortions or

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