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Cell-Culture Response to Fibroma Virus

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ABSTRACT

VERNA, JOHN E. (University of Minnesota, Minneapolis). Cell-culture response to fibroma virus. J. Bacteriol. **89**:524–528. 1965.—Three established rabbit cell lines (Minnesota CRP, RbK, and RbH) were infected with Shope's rabbit fibroma virus (Patuxent strain). The responses of the cell lines to virus infection differed from one another. Minn. CRP cells produced plaques (1 to 2 mm) on the 4th or 5th day which possessed characteristic cellular aggregates at the periphery of the plaque. Minn. RbH cells underwent degeneration with the elongation of cells and no aggregation formation. Minn. RbK cells responded with foci of cellular aggregates and no plaque formation. The resulting number of characteristic lesions in each cell line was related to virus dilution. The history of virus passage had no effect on the resulting lesion. All cells appeared to be equally sensitive to fibroma virus infection. Conclusive evidence for a viral-induced proliferative effect in RbK cells has not been found.

The in vitro response of rabbit cells to the Patuxent strain of Shope's fibroma virus has been described as a "proliferative" effect by Padgett, Moore, and Walker (1962). Primary and established rabbit cells infected with the same strain of fibroma virus have been described as undergoing a degenerative response under the conditions employed by Verna and Eylar (1962). More recently, Hinze, Padgett, and Walker (1964) concluded that the "proliferative response" was determined by the specific strain of fibroma virus employed. The purpose of this paper is to describe diverse responses to the Patuxent strain of fibroma virus by three established rabbit cell lines. These results indicate that the in vitro response to fibroma virus is determined to a great extent by the particular host cell used and possibly the cultural conditions also.

MATERIALS AND METHODS

Virus strain. The Patuxent strain of fibroma virus was the only strain employed in this investigation. Virus preparations were made as a 10% homogenate of fibroma rabbit tissue in Hank's balanced salt solution containing 1% gelatin (GBSS) and partially purified by differential centrifugation.

Virus which had been passed several times in rabbit cell cultures was also employed. These viral stocks were produced by harvesting infected cells when initial cytopathic effect was observed. Virus was released from cells by freezing and thawing, after which the cellular debris was removed by centrifugation.

Cell cultures. Of the three established rabbit cell lines used in this study, only the cottontail rabbit papilloma (Minnesota CRP) cell line has been previously reported (McLaren, Holland, and Syverton, 1959). The other two cell lines (Minn. RbK and Minn. RbH) were derived by serial passage of primary cultures of rabbit kidney and heart cells, respectively. Newborn domestic rabbits were used as a source of tissues for the primary cultures. These cell lines have gained an altered morphology from that of the primary cells but have retained the same range of viral susceptibility.

The CRP line was cultivated in a medium consisting of 20% rabbit serum in Hank's BSS containing 0.1% Difco Yeastolate ($RbS_{20}YE_{0.1}BSS$). Minn. RbK and RbH cell lines were cultivated in a medium consisting of 10% calf serum in Eagle's minimal essential medium ($CaS_{10}MEM$). Confluent cell monolayers were prepared in square glass bottles or plastic tissue culture flasks (Falcon #3012).

Virus inoculation. Cell monolayers, rinsed three times with BSS, were inoculated by adsorption for 2.5 to 3 hr with 0.1 ml of virus diluted in GBSS. The cells were then overlaid with fresh growth medium and incubated at 37 C. Cells were observed microscopically after the monolayer cultures were fixed with 10% formalin and stained with 0.01% crystal violet.

RESULTS

CRP cell response. When monolayer cultures of CRP cells were infected with approximately 100 plaque-forming units of fibroma virus, foci of

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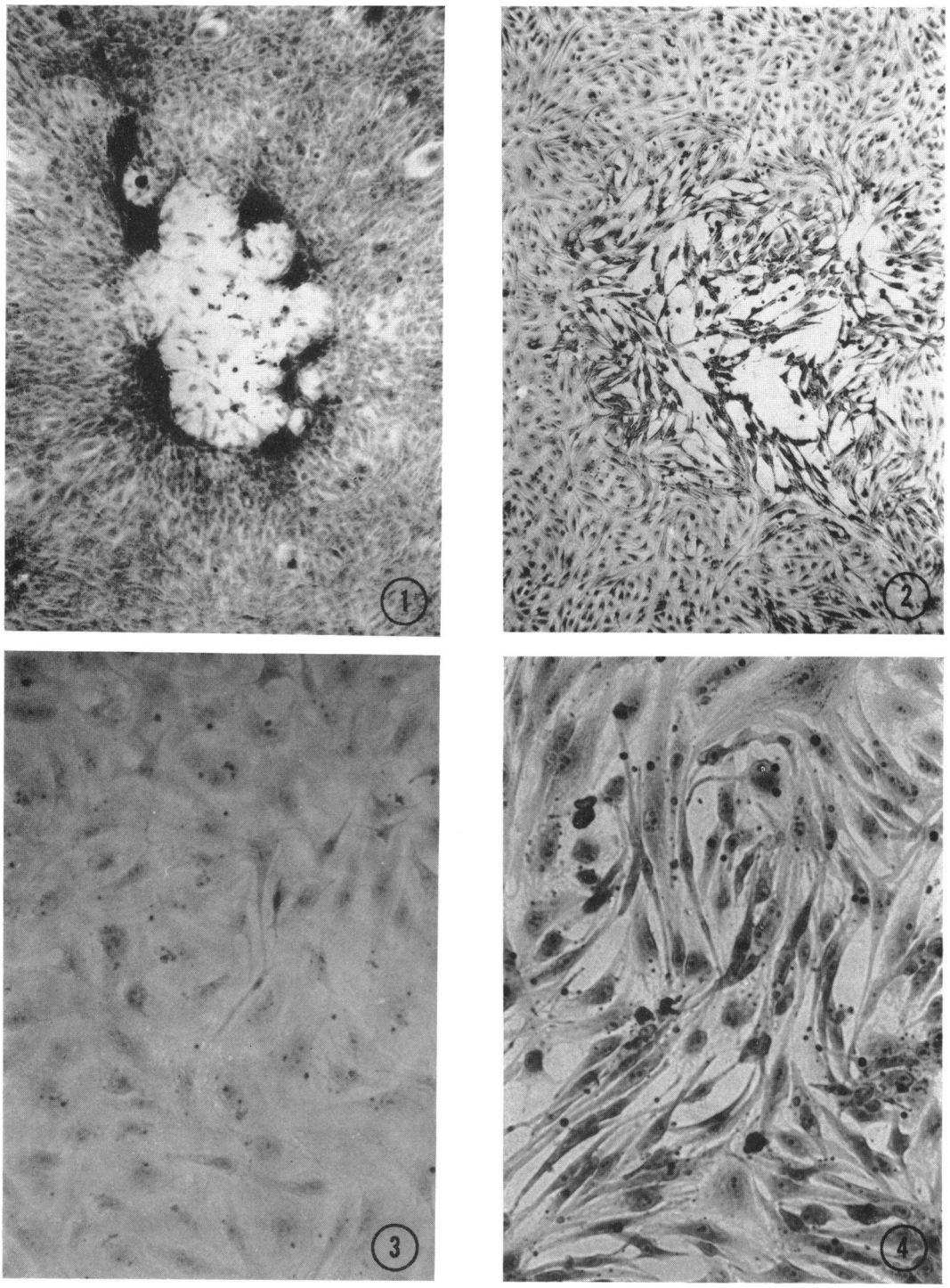

FIG. 1. *Minn. CRP cell monolayer with a fibroma virus area of cytopathology. 150 ×.*
FIG. 2. *Minn. RbH cell monolayer with an area of fibroma virus cytopathology. 150 ×.*
FIG. 3. *Minn. RbH normal cell line. 400 ×.*
FIG. 4. *Minn. RbH cells infected with fibroma virus. 400 ×.*

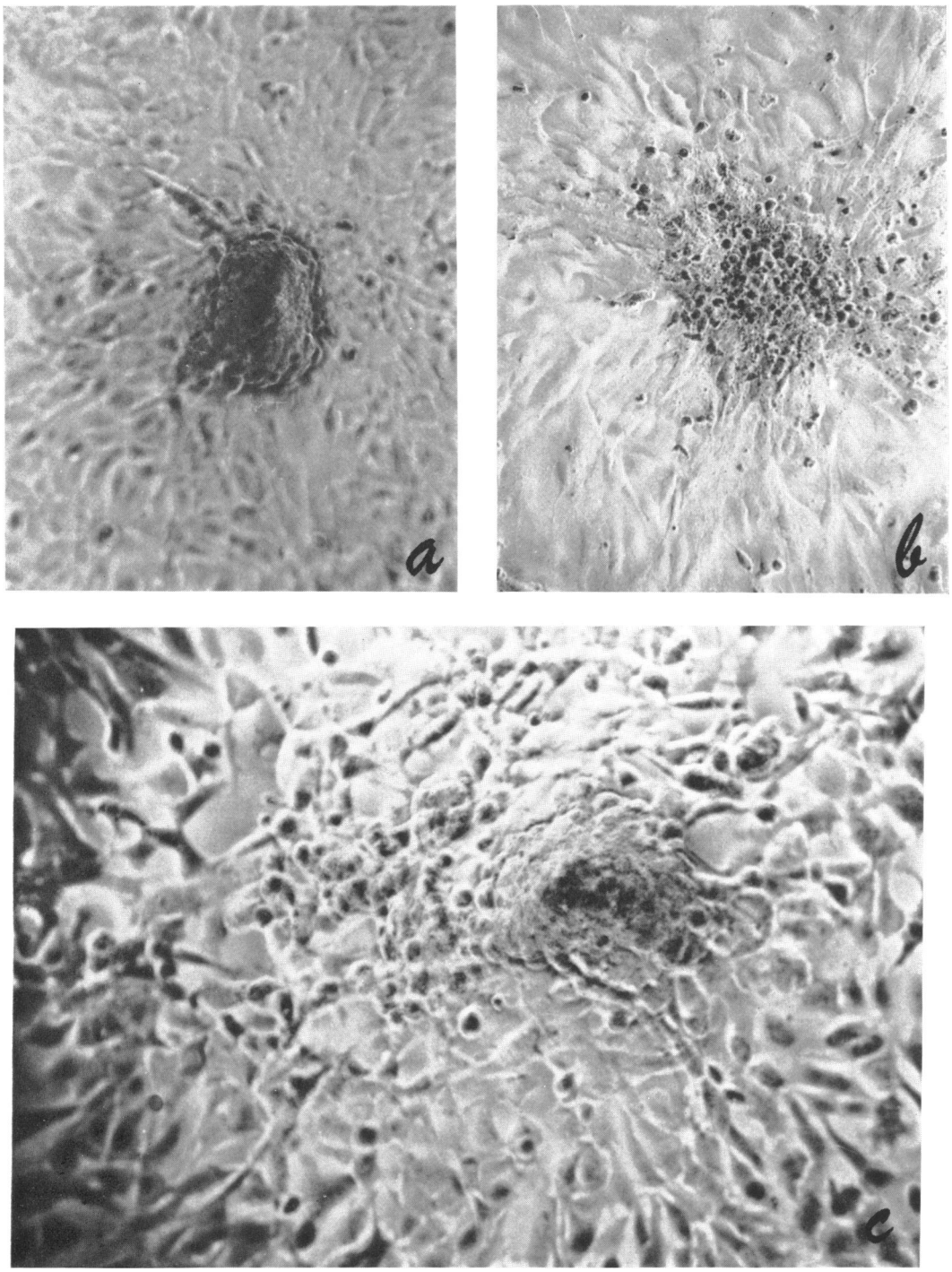

FIG. 5. *Minn. RbK cell monolayer with a fibroma virus foci of cytopathology* (*a and b, 400 ×; c, 800 ×*).

cellular changes were first observed on the 3rd or 4th day after inoculation. An initial rounding of cells with an increase in cytoplasmic density which progressed to an apparent accumulation or aggregation several cell layers thick was observed. This apparent aggregation of affected cells resulted in spaces in the monolayer with rounded cells accumulated at the periphery (Fig. 1). The space or "plaque" contained a few normal-appearing cells. The plaques reached a maximal size of 1 to 2 mm on the 5th or 6th day.

Inoculation of a monolayer of CRP cells with a concentration of virus sufficient to infect most cells resulted in a confluent cytopathic effect, as described above. Small aggregates of cells were found throughout the surface, with the remainder of the area occupied by a few isolated, normal-looking cells.

RbII cell response. The infection of RbH cells with rabbit fibroma virus resulted in a cytopathic response which was different from that of other rabbit cells seen in this laboratory. When RbH cells were inoculated with approximately 100 plaque-forming units of fibroma virus, cells did not form aggregates, as described for CRP cells. Infected RbH cells appeared elongated and separated from each other (Fig. 4). The cytoplasm of these cells stained more intensely than that of normal cells. Microscopically, the area within the infected foci appeared sparsely populated with spindle-shaped cells (Fig. 2). However, because of the greater staining intensity of these infected cells than that of normal cells, the foci of cytopathology were easily seen macroscopically. The appearance of such an infected monolayer was one of the distinct darkly stained foci in a field of lightly stained normal cells. The foci reached a maximum of 1 to 2 mm in diameter. With continued incubation, these infected areas became less populated and appeared as "diffuse" or "net-like" plaques.

RbK cell response. Infection of RbK cell monolayers with a low concentration of fibroma virus so as to give discrete foci of cytopathology resulted in a third response which was distinct from that described for the previous two cell lines. The initial foci were similar to that formed in CRP cell lines. The cells in the foci became spherical with an increase in cytoplasmic density and appeared crowded in comparison to the remainder of the monolayer. By the 4th or 5th day, aggregates of cells were macroscopically visible and were surrounded by a somewhat complete monolayer of cells (Fig. 5). The dark-staining, multicell layer aggregate did not lead to the development of spaces in the monolayer with time. Continued observation for 20 days failed to show any significant change in appearance. The aggregates were frequently stellate in shape with portions of cells extending to join cells in monolayer. The normal-looking cells in the immediate vicinity of the cell aggregate stained relatively lighter than the normal cells in the rest of the cell monolayer.

Infection of all the RbK cells in the monolayer resulted in the formation of many small aggregates throughout the area, with a few apparently unaffected cells remaining between the aggregates.

Relationship of virus dilution to number of foci. When virus dilutions of fibroma virus were inoculated onto monolayers of CRP, RbK, and RbH cells, the number of characteristic lesions formed in the respective cells was found to be proportional to the virus dilutions.

The results of a virus titration in the three cell lines, as shown in Table 1, indicated their comparative sensitivities to virus infectivity.

Virus isolation from different cell lines. Fibroma virus passed in cultures of RbK were used to inoculate RbH cells, and virus passed in RbH cells was used to inoculate RbK cells. In all cases, the resulting type of cytopathology was characteristic for the cell line employed for viral assay.

The source or passage history of the virus appeared to have no effect on the type of cytopathology. Virus which had been passed only in rabbits induced the same type of lesions as did virus which had been passed several times in cultures of rabbit cells.

The cytopathology induced by the Lausanne strain of myxoma virus in these cell lines has been found to parallel that described for the Patuxent strain of fibroma virus (Verna, *unpublished data*). The cellular responses of myxoma

TABLE 1. *Comparative assays of fibroma virus (Patuxent) in three rabbit cell lines*[a]

Minn. CRP[b]	Minn. RbH[c]	Minn. RbK[d]
5×10^5	6×10^5	2×10^5
3×10^5	2×10^5	6×10^5
5×10^5	3×10^5	3×10^5
2×10^5	—	5×10^5

[a] Results indicated in plaque-forming units per 0.1 ml.

[b] Minn. CRP = cottontail rabbit papilloma cell line.

[c] Minn. RbH = domestic rabbit heart cell line.

[d] Minn. RbK = domestic rabbit kidney cell line.

virus appeared 2 to 3 days sooner and were more extensive than those of the fibroma virus.

DISCUSSION

The important role that the cell plays in the in vitro response to fibroma virus is indicated by these results. The same strain of fibroma virus infecting each of three rabbit cell lines exhibited three distinct types of cellular response. The plaque versus "pock" response of primary rabbit and rat cell cultures to the Patuxent strain of fibroma virus (Israeli and Sachs, 1964) gives further evidence of the importance of the host cell to the resulting pathological lesion. The explanation for this varied cytopathology must await further study.

One possibility of a factor affecting the response of the cells might be their physiological characteristic which may be related to the growth potential and virus-synthesizing ability. As previously noted (Verna and Eylar, 1962), a modification in serum or other ingredients of the medium altered the extent of cytopathology induced by fibroma virus in CRP cells. The presence of an inhibitor in agar was found to retard plaque formation by fibroma virus in primary rabbit kidney cells (Israeli and Sachs, 1964).

A second factor, which could be considered the cause of the diverse cellular response, is the particular "monolayer architecture" or physical relationship of one cell to another in the cell monolayer. The extent of cytoplasmic continuity between cells might affect the cell-to-cell passage of the virus. In addition, the type of cell morphology present (fibroblastic or epithelial-like) might be of importance. Clonal-derived lines of these rabbit cells have not been tested.

The use of the word "proliferative" in connection with the in vitro cellular response to fibroma virus would appear premature. The response of RbH and possibly CRP cells certainly indicates a degenerative process. The mechanism by which RbK cells accumulate to form characteristic multilayered lesions is currently under investigation. The absence of mitotic figures and the presence of a relatively sparse area surrounding the cellular aggregate might suggest that these cells are drawn together, rather than accumulated, by an enhanced multiplication at the site. No evidence for cellular division of fibroma virus-infected cells has been found. Cultivation of infected cells has failed, and infection of all cells in monolayer results in detachment from the glass surface and loss of most cells.

Further study is needed to determine of what value these models are in the understanding of the proliferative response induced by fibroma virus in vivo.

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ADDENDUM IN PROOF

In a recent publication (Hinze and Walker, J. Bacteriol. **88**:1185, 1964), rabbit-kidney cells infected with fibroma virus were reported to undergo multiplication. Similar experiments were conducted in our laboratory with Minnesota RbK cells infected with Shope fibroma virus. Cells from infected cultures were kept under optimal nutritional conditions, and were enumerated with a Coulter Counter. Results of several experiments failed to give evidence of cellular replication with the RbK cell line.

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