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Transformation of Bovine Cells *in vitro* by Polyoma Virus, and the Properties of the Transformed Cells. (31963)

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Polyoma virus is capable of inducing tumors in a variety of species: Mouse, rat, hamster, guinea-pig, rabbit, ferret, and *Mastomys* (for references see 1,2). Transformation *in vitro* by polyoma virus has been demonstrated in cell cultures of mouse(3), hamster(3,4), and rat (4,5).

This report describes *in vitro* transformation of bovine embryonic lung cells by polyoma virus. The transformed cells are studied with regard to the presence of infectious polyoma virus, the presence of complement-fixing "tumor" antigen, and the susceptibility to various viruses. The characteristics of the polyoma-transformed cells are compared with those of SV40-transformed bovine cells derived from the same source.

Materials and methods. Cell cultures and media. Bovine cells for the transformation experiments were obtained from the lungs of an embryo. They were trypsinized and grown in monolayers in 4-oz prescription bottles. The medium consisted of Eagle's minimal essential medium (MEM) with 10% lamb serum. The cultures were split 1:1 or 1:2 by trypsinization twice weekly. Mouse cells were prepared from embryos using MEM with 10% calf serum as growth medium. Maintenance medium was MEM with 10% horse serum. BS-C-1 cells(6) were grown in MEM with 10% calf serum. The same medium with 2% calf serum was used for maintenance. Bovine cells to be used for virus titrations were prepared from kidneys of newborn calves using Hanks' salt solution with 0.5% lactalbumin hydrolysate and 10% calf serum as growth medium. The same medium, without serum, was used for maintenance.

Viruses. T polyoma virus (kindly supplied by Prof. Stoker, University of Glasgow) and a strain of SV40 isolated from a kidney culture of rhesus monkey were used.

The viral susceptibility of transformed cells was tested with the following viruses: Foot-and-mouth disease virus (FMDV) type C strain Detmold and its attenuated derivate; bovine enterovirus (BEV) strain V 100/60; infectious bovine rhinotracheitis virus (IBRV); pseudorabies virus (PRV) strain Z; parainfluenza virus type 3 (PIV-3) strain 23B; Newcastle disease virus (NDV) strain 205; and bovine viral diarrhoea virus (VDV) strains Oregon C24V and Ug59.

Virus assay. Titrations of infectivity were carried out in roller tube cultures. Serial 10-fold dilutions were inoculated in 0.1-ml amounts into each of 4 or 5 cultures. The titers were calculated according to Kärber and expressed as $\log_{10}$ units of TCD_{50}/0.1 ml. Polyoma virus was titrated in mouse cells, SV40 in BS-C-1 cells, and the other viruses in calf cells.

In tests for the presence of small amounts of polyoma virus and SV40, cultures were frozen and thawed, and the suspensions of cell debris were inoculated in 0.2-ml amounts into tube cultures. If the cultures did not show any changes characteristic of polyoma

or SV40 after 14 days, the medium was passed to new cultures and a further observation period of 14 days followed.

Complement fixation test. All tests were carried out by a microtechnique(7) using 2 exact units of guinea-pig complement and fixation overnight at 4°C. Antigen preparations consisted of 10% (v/v) suspensions of the cells in MEM. They were frozen and thawed 3 times prior to use. Sera were obtained from hamsters bearing polyoma- or SV40-induced tumors. (The polyoma-tumor serum was purchased from Flow Laboratories, Scotland; the SV40-tumor serum was kindly supplied by Dr. A. B. Sabin, Cincinnati, Ohio.)

Results. Transformation by polyoma virus. Cultures of the bovine embryonic lung cells were inoculated in the 8th passage with polyoma virus at a multiplicity of about 10^3 TCD_{50} per cell. The cultures were then subcultivated as described in *Methods*.

During the first 30 days after inoculation there were no differences in the appearance of inoculated and uninoculated cultures. There were regularly-growing cells of fibroblastic appearance (Fig. 1A). Mitotic figures were seldom seen in the confluent monolayer. Over the next 20 days the appearance of the inoculated cultures changed. The cells were often stellate or triangular and they grew at random, criss-crossing one another (Fig. 1B). Many mitotic figures were seen. During the following weeks these changes became more pronounced. By the 15th week the cultures consisted of several layers of cells having stellate or triangular shape and mitotic figures were abundant. The cultures showed an increased growth rate. The same changes were seen in all 6 cultures inoculated. No changes were observed in the uninoculated cultures.

Transformation by SV40. Bovine lung cells derived from the same embryo as those infected with polyoma virus were also inoculated in the 8th passage with SV40 at a multiplicity of about 10^3 TCD_{50} per cell. The cultures were then treated in the same way as those inoculated with polyoma virus.

The inoculated cultures were transformed by the 44th to the 84th day after inoculation. The morphological appearance (Fig. 1C) was the same as previously described for bovine lung cells transformed by SV40(8).

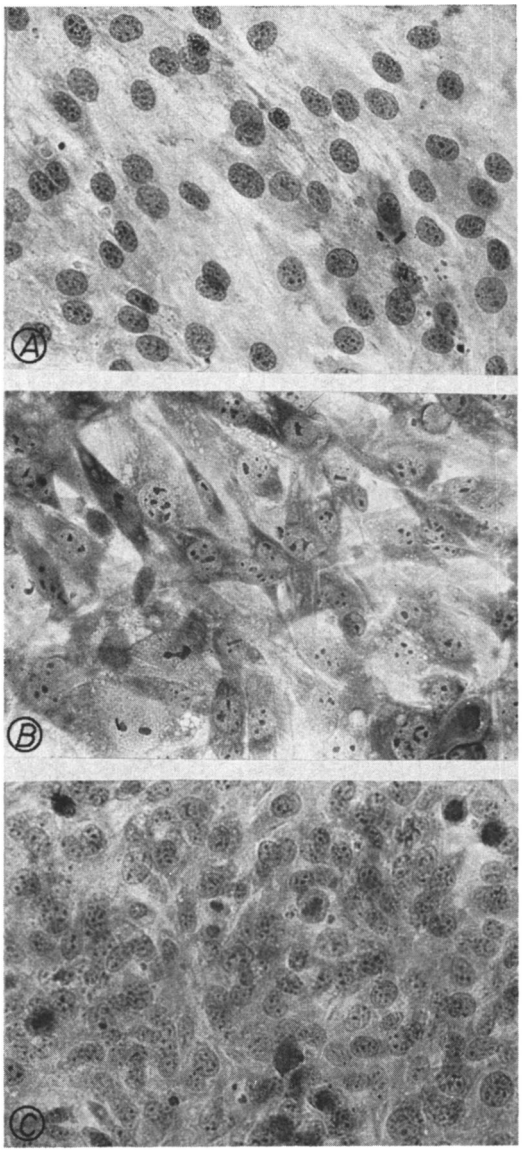

FIG. 1. Cultures of bovine embryonic lung cells. May-Gruenwald-Giemsa. × 250. A. Normal cells; B, Polyoma-transformed cells; C, SV40-transformed cells.

Presence of infectious virus in the transformed cells. As shown in Fig. 2, the culture fluids contained virus only during the first weeks after the infection. The infectivity found decreased gradually.

All 6 lines of the polyoma-transformed cells and 2 lines of the SV40-transformed cells

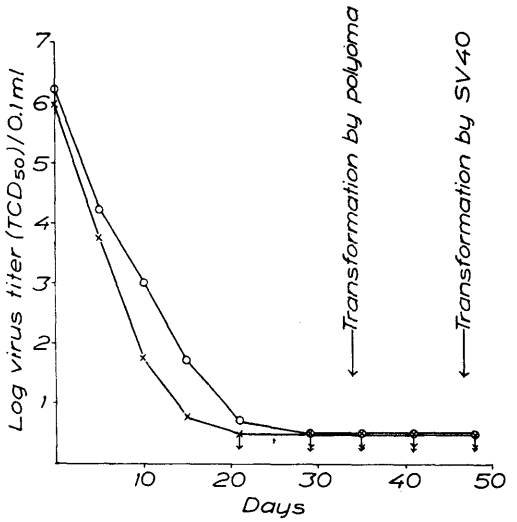

FIG. 2. Titers of polyoma (x) and SV40 (o) in cultures of bovine embryonic lung cells at different intervals after inoculation.

were tested for infectivity. Five passages of each line were studied. No virus was found in the polyoma-transformed lines whereas the SV40-transformed lines yielded virus in minute amounts.

Presence of complement-fixing "tumor" antigen in the transformed cells. The polyoma-transformed cells were tested for CF antigen at different passage levels. The serum which was obtained from hamsters bearing polyoma-induced tumors was used in a dilution of 1:40.

As shown in Table I, the titers varied between 1:2 and 1:16. No positive reactions were found with antigens prepared from the normal cells or the SV40-transformed cells (controls). Antigen preparations from the latter cells reacted with serum from hamsters bearing SV40-induced tumors. In tests using this serum and antigens from the polyoma-transformed or normal cells no positive reactions were found.

TABLE I. Titers of Complement-Fixing (CF) Antigen in the Polyoma-Transformed Cells.

Passage No.	Days after transformation	Reciprocal titer of CF antigen
59	154	4
65	175	2
71	196	8
79	224	16
85	245	4
Controls		<2

Viral susceptibility of the transformed cells. The polyoma-transformed cells were compared with SV40-transformed cells as well as uninoculated control cells with regard to the susceptibility to various viruses. The cells were grown in plastic dishes and used at the same passage levels, *i.e.*, from the 45th to the 74th passage. At this stage the cultures were subcultured 21 to 42 times after the establishment of transformation. A polyoma-transformed culture contained an average of 3×10^6 cells, a SV40-transformed culture 4×10^6, and a normal culture 2×10^6 cells. One line of each cell type was used in these experiments.

Foot-and-mouth disease virus (FMDV) and bovine enterovirus (BEV) were inoculated in amounts of 10^2 TCD_{50}, infectious bovine rhinotracheitis virus (IBRV), pseudorabies virus (PRV), parainfluenza virus type 3 (PIV-3), Newcastle disease virus (NDV), and bovine viral diarrhoea virus (VDV) in amounts of 10^3 TCD_{50}. At intervals after inoculation samples were taken from the culture fluids and titrated as described in *Methods*.

As seen in Fig. 3, the wild strain of type C of FMDV multiplied to much higher titers in the polyoma-transformed cells than in the SV40-transformed and normal cells. Cytopathic effect was seen only in the polyoma-transformed cultures. As to the attenuated strain high titers and cytopathic effect were obtained much earlier in the polyoma-transformed cells than in the other cells (Fig. 3).

As also seen in Fig. 3, BEV and PRV multiplied to about the same titers in the polyoma-transformed cells and the normal cells whereas lower titers were obtained in the SV40-transformed cells. In the latter cells the cytopathic effect started later than in the other cells.

IBRV, PIV-3, NDV, and VDV multiplied to about the same titers in the different types of cells and there were no differences as regards the cytopathic effect with the exception of that caused by VDV. Both strains of this virus showed a stronger effect in the SV40-transformed cultures than in the polyoma-transformed and the normal cultures.

Discussion. This study shows that bovine cell cultures inoculated with polyoma virus

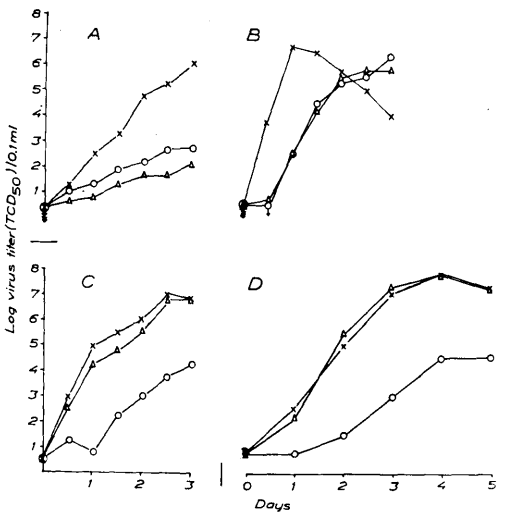

FIG. 3. Growth of a wild (A) and an attenuated strain (B) of type C of FMDV, BEV (C), and PRV (D) in polyoma-transformed (x), SV40-transformed (o), and normal (△) bovine lung cells.

change morphologically and that the transformed cells differ in certain respects from SV40-transformed and normal cells derived from the same source. It may be asked whether the morphological changes were really caused by polyoma virus. The changes resembled closely the transformation of mouse, hamster, and rat cells induced by polyoma virus (for references, see 9). This similarity, the presence of the complement-fixing polyoma "tumor" antigen, and the absence of changes in the control cultures constitute strong evidence that the alterations obtained were caused by polyoma virus. The changes seen in the SV40-infected cultures were very similar to those described earlier for SV40-transformed cells of bovine and other origin (for references, see 9, 10). The SV40-transformed cultures contained the SV40 "tumor" antigen. This fact and the morphological appearance leave little doubt that the alterations obtained were really induced by SV40.

Polyoma virus has been reported to have a remarkably broad host range as regards tumor formation *in vivo*, but a more limited range to induce transformation *in vitro*. SV40, on the other hand, has a broad host range as regards transformation *in vitro* whereas only two species have been found to react with tumors after SV40 infection *in vivo* (for references, see 9, 10). In earlier studies on the effect of SV40 and polyoma virus on bovine cells(8), it was found that only SV40 was capable of inducing transformation. It is conceivable that the substitution of lamb for calf serum or the more frequent passages have influenced the ability of polyoma virus to induce transformation *in vitro*.

The titers of polyoma virus as well as those of SV40 decreased gradually after infection. Attempts to isolate infectious polyoma virus from the polyoma-transformed cells were negative whereas SV40 was recovered in minute amounts in the SV40-transformed cells. It seems therefore probable that the bovine cells are capable of propagating SV40. No conclusions can be drawn from the present study whether or not polyoma virus multiplies in bovine cells.

The polyoma-transformed cells contained an antigen reacting in the complement fixation test with serum from hamsters bearing polyoma-induced tumors. Such an antigen has been described for other cells transformed *in vitro* as well as for tumor cells induced *in vivo* by polyoma virus(11). A similar antigen was found in the SV40-transformed bovine cells by using serum from hamsters bearing SV40-induced tumors.

The polyoma-transformed cells were capable of propagating a wild strain of type C of FMDV to high titers whereas in the SV40-transformed and normal cultures low titers were obtained. As regards the susceptibility of the three types of cells to BEV and PRV, the SV40-transformed cells produced lower titers in comparison with the other cells. The explanation of the altered response of the transformed cells to certain viruses cannot be given. It was previously reported(12) that a line of BHK21 cells transformed by polyoma virus was insusceptible to certain strains of FMDV including the wild strain of type C used in this work. The insusceptibility of the transformed cells was found to be due to an inability of the cells to adsorb virus. In the work of Van der Noordaa *et al*(13), an increased resistance to herpes simplex virus of hamster and human cells transformed by SV40 was demonstrated but no explanation

to the altered susceptibility by differences in adsorption or penetration of virus or in production of interferon was offered.

Summary. Cell cultures of bovine embryonic lung were found to undergo morphological changes after inoculation with polyoma virus. The changes were characteristic for polyoma transformation with cells having stellate or triangular shape and lying at random, crisscrossing one another.

Cell lines of rapidly-growing transformed cells were obtained. Attempts to isolate infectious virus from the lines were negative. The lines contained "tumor" antigen as demonstrated by complement fixation tests using serum from hamsters bearing polyoma-induced tumors.

A comparison of the viral susceptibility between the polyoma-transformed cells and SV40-transformed and normal cells derived from the same source revealed certain differences. Whereas the polyoma-transformed cells showed fairly high susceptibility to a wild strain of type C of foot-and-mouth disease virus (FMDV) the SV40-transformed and normal cells were less susceptible. The SV40-transformed cells showed a decreased susceptibility to bovine enterovirus (BEV) and pseudorabies virus (PRV) in comparison with the polyoma-transformed and normal cells.

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Influence of Environmental Temperature on Resistance to Endotoxin. (31964)

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It has been reported that mice stressed by an environmental temperature of 37°C, when compared with non-stressed mice at 22° or 23°C, exhibit an increased mortality to Gram-negative bacteria(1) or their extracts(2), including endotoxin(3). It is not known, however, if mortality varies with environmental temperature within a temperate and non-stressful range. The present report describes such variation within the range 18° to 33°C in mice injected with endotoxin.*

* This work was presented at the meeting of the Federation of American Societies for Experimental Biology: Fed. Proc., 1967, v26, 583.

Materials and methods. Constant temperature rooms. Well-ventilated rooms, about 4.5 × 3 × 2 m^3, were each kept at a constant temperature, ± 1°C, by means of an adjustable heating unit, an adjustable cooling unit, and a thermostat. Each room was kept at one of the following temperatures: 18°, 22°, 27.5°, 31.5°, and 33°C. (It has been reported that in environments below 18°C, non-treated mice develop subnormal body temperatures, while in environments above 33°C, they develop fever(4). Consequently, we did not make observations either below 18° or above 33°C. Furthermore, we have observed