with that of a mature male, being practically devoid of vane on the inner side and having very little on the outer side. It shed these after approximately 1½ years, and thereafter resembled other young lyrebirds. One of its vaned retrices began to undergo filamentation in 1962 and by December of that year filamentation was well advanced. It retained that feather until September 1964, by which time it had acquired its first pair of new medians. Figure 6 shows its state of development at the end of May 1964. The increase in length and width of the filamentary feather as compared with the plain feathers may clearly be seen. Visual observations and photographic records establish the fact that this feather had grown considerably since December 1962. At the time of writing (November 1964) this bird had shed its lyrates and all but two of the plain feathers, as well as the solitary filamentary and the medians shown in Fig. 6; it is now growing new filamentaries, lyrates, and medians. Among these the two plain feathers are conspicuous.

It is not yet known whether the barbs and barbules which disappear during the transformation of a plain feather are actually lost by the breaking of their attachments to the rachis and barbs, respectively, or whether they are reabsorbed. At any rate, the whole process of the development of the tail feathers must be under the control of some complicated hormone system which has yet to be investigated.

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Successive Transformations of an Established Cell Line by Polyoma Virus and SV40

Abstract. *Two different oncogenic viruses, polyoma and SV40, are capable of transforming mouse cell line 3T3. The properties of the transformed cells produced by the two viruses are in some ways similar, but in other ways they are specific for the infecting virus. This fact permits testing whether a cell line transformed by the one oncogenic virus is still susceptible to the tranforming action of the second virus. Two different clonally isolated polyoma-transformed lines when infected with SV40 give rise to cells with properties characteristic of SV40-transformed cells. The frequency of transformation, however, is considerably reduced compared to that of the parent cell line, 3T3.*

The established mouse fibroblast line, 3T3, which is strongly contact-inhibited in culture, is susceptible to transformation by two different oncogenic viruses, polyoma and SV40 (*1*). In each case the transformed cells are characterized by their ability to grow readily over one another under conditions where the untransformed cells remain strictly confined to a monolayer. This loss of contact inhibition of cell division is a stable property, passed on to all the progeny cells. The transformed cells produced by the two viruses may be distinguished from each other; SV40-transformed 3T3 cells are able to grow to a considerably higher saturation density (up to 15 × 10^5 cells/cm²) than polyoma transformed 3T3 cells (2 to 4 × 10^5 cells/cm²) and therefore produce much denser colonies when inoculated sparsely (*2*). The transformed cells produced by the two viruses may also be distinguished from each other on the basis of detailed colonial morphology (*1*). Because of these characteristic differences it is now possible to demonstrate SV40 transformation of a cell line already transformed by polyoma virus.

Two polyoma-transformed cell lines were used. One, PY-3T3-31, is a polyoma virus transformed line that has been cloned twice and has a saturation density (2 × 10^5 cells/cm²) which is in the low range for polyoma-transformed cell lines. The line was produced by infection of a stationary phase culture of 3T3 with polyoma virus in the presence of 100 μg of 5-iodo-2′-deoxyuridine (IDUR) per milliliter. The transformation frequency under these conditions is not reduced and may in fact be increased (*3*). The other line was PY-3T3-11, a clonal isolate of a line transformed in the absence of IDUR, and having a saturation density of 4 × 10^5 cells/cm². Whereas many polyoma-transformed 3T3 cultures continue for a long time to show a cytopathic effect and release high titers of virus, these two clones no longer did so. Both clones were maintained in culture by transfers of 1:1000 dilutions of subconfluent cultures.

Cultures of PY-3T3-31 in exponential growth were exposed for 3 hours to 0.5 ml of a stock of SV40 strain 776 (*4*) containing $10^{8.2}$ tissue culture infective doses per milliliter (*5*). The next day the cells were plated, as were uninfected cells, at 100 to 40,000 cells per plate. Two weeks after plating, the cells were fixed and stained. By this time the larger inocula of both the control and infected cells had grown to saturation density, and the cultures appeared as fairly homogeneous layers of interlacing cells, a few cells thick. However, the cultures infected with SV40 also contained colonies of tightly packed epithelioid cells that showed a consid-

Table 1. Transformation frequency of polyoma-transformed cell lines after infection with SV40.

Plating efficiency* (%)		Cells per plate	Total cells plated (× 10^{-3})	Transformed colonies		Transformation frequency† (%)
Control	Infected			Control	Infected	
			Cell line 3T3			
44	67	100	4.0	0	73	2.7
44	67	1000	50	0	781	2.3
			Cell line PY-3T3-31			
43	34	100	2.4	0	0	—
43	34	4000	132	0	13	0.03
43	34	40,000	240	0	18	0.02
			Cell line PY-3T3-11			
48	56	100	1.2	0	0	—
48	56	12,000	72	0	22	0.05

* Plates inoculated with 100 cells and fixed 10 days later. Average of six or more plates.
† $\frac{\text{Transformed colonies}}{\text{Cells plated}} \times 100 \times \frac{100}{\text{plating efficiency}}$.

erably greater degree of multilayered growth. These colonies stained very darkly and could be recognized with the unaided eye against the lighter-staining background cells (Figs. 1*A* and 1*B*). When cultures containing one or more of these dense colonies were replated and the cells fixed before a confluent layer was formed, two distinct kinds of colonies were obtained. One is typical of PY-3T3-31 (Fig. 1*C*, left); the other, seen only in plates infected with SV40 (Fig. 1*C*, right), is much denser and has sharper borders. Such SV40-transformed colonies when recloned gave rise only to dense colonies with sharp borders, and cell lines derived from such clones were able to grow to a saturation density greater than 10^6 cells per cm^2.

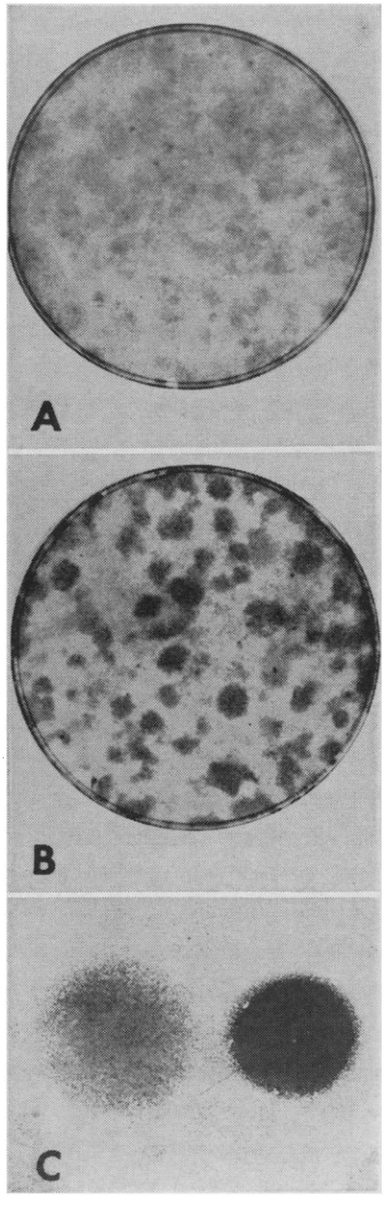

Fig. 1. Morphology of PY-3T3-31 cultures transformed by SV40. *A*, Saturation density PY-3T3-31, 12 days after inoculation of 1000 cells. *B*, Colonies transformed by SV40 12 days after inoculation of 1000 cells from a virus-infected culture. These colonies can be easily distinguished against the background of PY-3T3-31. They are very numerous, as the parent culture was allowed to remain at high density for several days after infection, in order to increase selectively the number of SV40-transformed cells. *C*, Discrete colonies from an SV40-infected PY-3T3-31 culture, 10 days after plating 100 cells. The dense colony on the right is SV40-transformed. All cultures were fixed with formalin and stained with hematoxylin.

The frequency of transformation of PY-3T3-31 cells by SV40 can be measured quantitatively in the same manner as described for transformation of 3T3 cells by SV40 (*3*, *6*). With SV40 at a titer that gives transformation frequencies of from 2 to 3 percent with 3T3 or clonal lines derived from 3T3 (*7*), we obtained transformation frequencies of PY-3T3-31 of from 0.02 to 0.03 percent (Table 1). Plates of PY-3T3-31 inoculated with 100 cells and read before confluence was reached showed no SV40 colonies out of a total of 2400 counted, a value consistent with a greatly reduced transformation frequency of PY-3T3-31. Similar values were obtained with line PY-3T3-11.

The production of different types of polyoma-transformed cells, some that form thin colonies and have a relatively low saturation density and others that form thick colonies and have a high saturation density, has been described by Vogt and Dulbecco (*8*) and Stanners, Till, and Siminovitch (*9*) using hamster cells and Weisberg (*10*) using mouse cells. While some of their clonally isolated lines of "thin" polyoma-transformed cells gave rise spontaneously during serial cultivation to "thick" colonies (*8*, *9*), PY-3T3-31 and PY-3T3-11 do not appear to contain thick colony-forming cells, at least under our culture conditions.

There is now evidence that the viral genome, or some portion of it, persists in transformed cells even in cases where the intact virus can no longer be demonstrated (*11*). Since both polyoma- and SV40-transformed cells contain new and specific cellular antigens (*12*), the doubly transformed cells described here might be expected to contain both antigens. If this is so, the presence of new genetic material from one oncogenic virus does not fully prevent the cell from being transformed again by a second virus, though it may be responsible for the greatly reduced susceptibility to SV40 transformation of the polyoma lines as compared with 3T3.

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Transplantation Immunity of Gestational Origin in Infant Rats

Abstract. *Comparison of the survival times of homografts of BN skin on 3-day-old Lewis rats born of mothers of the same isogenic strain with those of BN grafts on infant Lewis hosts that had developed in an F_1 (Lewis × BN) hybrid, gave no evidence of maternally induced tolerance as a result of development in an antigenically alien environment. On the contrary, the significantly shorter median survival time of the grafts on the hybrid-derived Lewis group suggests that sensitization had occurred as a consequence of natural exposure during gestation to small numbers of maternal cells.*

Inoculation of fetal or infant mammals with living homologous tissue cells may induce tolerance of foreign transplantation antigens. The degree to which the animals are rendered unresponsive depends upon the species, their genetic disparity, the timing and route of inoculation, the dosage of cells, and other variables (*1*).

Consequently, in outbred popula-