side, the membrane reaches "breaking point" at *p*H 7.7 to 7.8 (Fig. 2D). Under the same conditions, without Ca^{2+} present, the breaking point is reached at *p*H 8.5 (Fig. 2D).

When the same amount of Ca^{2+} is present on both sides (starting aqueous solution, 100 m*M* NaCl, 1 m*M* $CaCl_2$, *p*H 7.0), the membranes can withstand considerably wider changes of *p*H. The breaking point is reached only at *p*H 10.5 and 1.5 with inside *p*H held at 7.0 (Fig. 2D). With the same salt solution as above, chelation of Ca^{2+} only outside by EDTA makes the membranes unstable at *p*H 7.5 to 8.0, which is a much lower *p*H compared to when Ca^{2+} is present on both sides.

Our results suggest that bilayers composed of PS are very unstable under conditions of an asymmetric distribution of charges or divalent cations bound to the fixed negative charges. Each molecule of PS carries one net negative charge at *p*H 6 to 8, more than one negative charge at *p*H above 8, and less than one negative charge to neutral between *p*H 6 to 2 (*3*). It is apparent (Fig. 2) that difference in ionization of one charge or less per PS molecule between the two sides of the bilayer produces instability and breaking. Asymmetric Ca^{2+} binding at neutral or alkaline *p*H, where one equivalent of Ca^{2+} is bound per PS molecule (*10*), also produces instability manifested as lowering of d-c resistance and breaking. Previous experiments with PS monolayers have shown that considerable changes in surface tension occur after addition of Ca^{2+} or changes of *p*H in the aqueous phase (*3*).

It seems reasonable to suggest that the instability of PS membranes described here is due to the difference in surface energy between the two opposing sides of the bilayer. Calculations of energy differences of the two surfaces resulting from asymmetric distribution of charges (*11*) suggest that it is possible that, under these conditions, molecules or clusters of molecules will "invert" from one side to the other. In doing so they would increase the permeability of the membranes which under extreme conditions reach a breaking point. Moreover, the formation of polymeric assemblies of PS with Ca^{2+} (*3*) may introduce an additional entropy factor which would facilitate the "inversion" phenomenon.

Some of the properties of axon membrane in relation to Ca^{2+} and *p*H (*12*) suggest that the molecular mechanism studied here might be relevant in understanding certain aspects of the nerve excitation process. Recent evidence on the existence of fixed negative charges on the axon membrane (*13*) and the birefringence changes observed during stimulation of nerve (*14*) give some support to the suggestion presented here involving molecular reorientation of specific phospholipid molecules as a response to asymmetric distribution of fixed charges and counter-ions. This suggestion is also easily reconciled with some existing theories on the mechanism of action potential (*15*).

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16. Supported by grant DRG-1001 from Damon Runyon Memorial Fund and grant 68698 from the American Heart Association. D.P. is an established investigator of the American Heart Association, supported in part by Cattaraugus County Heart Chapter (New York). Technical assistance by Mrs. C. Whaley.
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12 February 1969; revised 2 April 1969

Neoplastic Transformation in vitro of Hamster Lens Epithelium by Simian Virus 40

Abstract. *Hamster lens epithelium infected with simian virus 40 underwent transformation in vitro and produced tumors when injected into homologous hosts. Undisturbed lens epithelium in man and experimental animals has not been observed to undergo neoplastic change. The virus-induced tumors contained undifferentiated cells that were either polygonal or spindle-shaped. Their origin from lens epithelium seems certain since it is possible to isolate this unique structure free of connective tissue and blood vessels.*

The potentialities of the continuously proliferating lens epithelium for malignant growth have been recognized. Mann in 1947 (*1*) reported epithelial tumors occurring in mice after the subcutaneous implantation of lenses admixed with methylcholanthrene. Von Sallmann *et al.* (*2*) observed that the lens epithelium of rainbow trout maintained on a thioacetamide diet showed invasive proliferation having the characteristics of neoplastic growth. We now demonstrate that lens epithelium infected with simian virus 40 (SV40) undergoes malignant transformation.

The lens epithelium constitutes a population of a single cell type. It can be isolated without contamination by any other tissue elements as the lens is surrounded by a noncellular laminated capsule. The epithelial cells lie beneath the capsule and form a single layer covering the anterior surface of the lens. Near the equator the cells elongate and differentiate to form lens fibers. Their nuclei are no longer capable of division, and the fibers ultimately become anucleate. This pure, well-defined cell population is a useful model for studying viral neoplastic transformation, and particularly for observing the role of the viral genome in determining tumor morphology.

Lenses were obtained from the eyes of six 4-week-old Syrian hamsters. The capsules with attached epithelium were aseptically removed by microscopic dissection, cut into explants of approximately 0.5 mm^2, and used to establish cultures in six 2-ounce prescription bottles (*3*). All cultures were incubated at 37°C in medium No. 199 with 20 percent fetal bovine serum. The fluid was changed three times weekly. On the

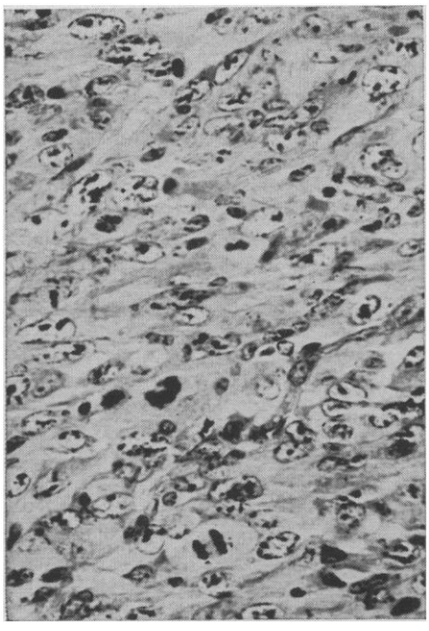

Fig. 1. Tumor developing in hamster after injection of SV40-transformed lens epithelium (hematoxylin and eosin; × 400).

third day of culture, when cells began to migrate from the explants, three bottles were infected with $10^{7.9}$ tissue culture infective doses, 50 percent effective ($TCID_{50}$) of SV40. The other three bottles were kept as virus-free controls. Infected cultures underwent transformation as evidenced by sustained rapid growth, development of an acid *p*H soon after addition of fresh media, and formation of multiple layers and clumps of cells. Immunofluorescence studies of the transformed lens epithelium demonstrated the presence of SV40 "T" antigen. Control cultures grew poorly and contained decreasing numbers of viable cells. No control cultures transformed spontaneously.

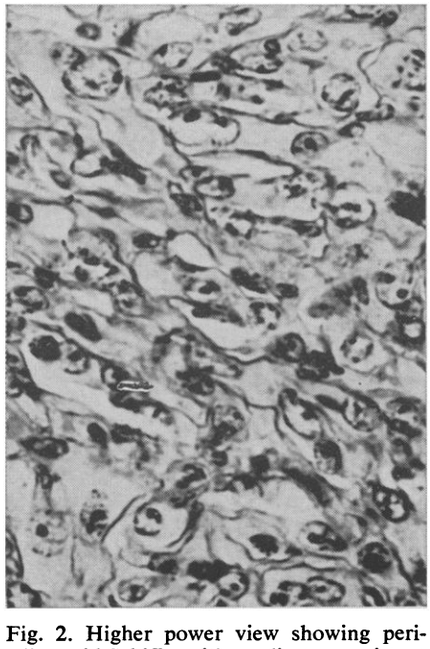

Fig. 2. Higher power view showing periodic acid-Schiff-positive, diastase-resistant intercellular substance (× 640).

The infected cells were subcultured to three 32-ounce bottles 40 days after infection. A confluent cell layer was present in each bottle 10 days later, and the cells were then scraped from the glass and suspended in 1 ml of medium. Approximately 10^8 cells were injected subcutaneously into each of three irradiated (400 r) 4-week-old hamsters. Similar animals inoculated subcutaneously with 10^6 to 10^7 $TCID_{50}$ of SV40 did not demonstrate tumors during a 6-month observation period. Within 3 weeks after injection of the transformed cells, the hamsters developed palpable neoplasms at the injection sites. On gross examination the tumors were firm and white with small areas of central necrosis.

The cells in the tumors were spindle and epithelioid types (Fig. 1). Both had either round or elongated vesicular nuclei, clumped chromatin, and lightly staining, tapering, eosinophilic cytoplasm. Mitoses were numerous, and multinucleate giant cells were observed in moderate numbers. The tumors were morphologically similar to SV40-induced tumors of retina, choroid, and iris (*3*). There appeared to be more basement membrane-like material in the stroma than was seen in tumors derived from other tissues (Fig. 2). The tumors, however, contained no structures resembling lens fibers.

Portions of the initial tumors were injected into other hamsters, and transplantable tumor lines were easily established. Neoplasms were carried through six generations without alteration in morphology or spontaneous regression of the tumors.

The relative roles that the viral genome and the target cell play in determining the dominant cell type of virus-induced tumors have not been resolved. The virus-specific nature of the morphological alterations accompanying transformation by adenoviruses, particularly adenovirus type 12, has been well demonstrated (*4*). In contrast SV40 and other papovaviruses give rise to tumors with a wider variety of cell types (*5*). The majority of SV40 tumors have sarcoma-like growth and are composed of large, pleomorphic cells interspersed with characteristic giant cells (*6*). They are similar to the tumors we have derived from transformed lens epithelium.

It has been suggested that the various neoplastic responses of SV40 are due to differences in the nature of the target cell (*7*). The common pleomorphic sarcoma-like SV40 tumor would accordingly be expected to arise from primitive, undifferentiated cells which retain only a limited capacity to differentiate after transformation. In our experiment cultures of lens epithelium—which normally develops in vivo into specialized cells—were transformed into anaplastic tumor cells. Tumors of similar appearance have been derived from hamster pineal gland and prostate and retain endocrine function (*8*). The production of tumors with a similar histological appearance from a variety of different cell types by SV40 suggests that the SV40 genome is important in determining morphology.

This study demonstrates that a cell type with no apparent malignant potential under natural circumstances can undergo neoplastic transformation after oncogenic virus infection in vitro. This is consistent with the results of earlier studies on the effect of chemical carcinogens on lens epithelium (*1*, *2*).

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13 March 1969