tivity of ion transport mechanisms. The large size of the CP's and the orientation of the potential gradient perpendicular to the major axis of the neurons in the column indicate that these potentials must be generated across one or more of the faces of the two cell layers forming the body wall rather than by the neurons of the nerve net. Epithelia capable of producing electrical spikes are known in other hydrozoan coelenterates and may be of general occurence in this group (*10*). The only epithelia in higher animals known to give spike-like potential changes are the frog skin and toad bladder (*11*). In these the spikes occur only during a passage of current through the tissue and result from a transient decrease in the electrical conductance of the tissue. The concommitant increase in potential drop across the tissue during the current pulse produces the spike. The same explanation cannot account for the CP's of hydra, for these appear in the absence of imposed current, and, when initiated by current pulses, they can continue beyond the termination of the exciting current; the current source for the CP's must be within the body wall itself.

While these results indicate that CP's are generated by epithelial cells, they do not rule out the possibility that the epithelial responses are triggered by activity conducted along the nerve cells of the column. But epithelial conduction is known in coelenterates (*10*), and there are specialized junctions (septate desmosomes) between the epithelial cells of hydra (*12*), so it seems likely that conduction of the CP's in hydra is also an epithelial phenomenon. Thus, epithelial cells in hydra produce spike-like electrical potentials and can probably transmit these from one cell to the next, features which in higher animals are usually associated only with nerve and muscle cells.

ROBERT K. JOSEPHSON
Department of Biology, Western Reserve University, Cleveland, Ohio
MARTIN MACKLIN
Bioengineering Group, Case Institute of Technology, Cleveland, Ohio

References and Notes

1. T. L. Lentz and R. J. Barrnett, *Amer Zool.* **5**, 341 (1965); A. L. Burnett and N. A. Diehl, *J. Exp. Zool.* **157**, 217 (1964).
2. L. M. Passano and C. B. McCullough, *J. Exp. Biol.* **41**, 643 (1964); N. B. Rushforth and L. K. Brown, *Amer. Zool.* **4**, 307 (1964).
3. L. M. Passano and C. B. McCullough, *J. Exp. Biol.* **41**, 643 (1964).
4. N. B. Rushforth, *Amer. Zool.* **6**, 524 (1966).
5. R. K. Josephson, *J. Exp. Biol.*, in press.
6. L. M. Passano and C. B. McCullough, *ibid.* **42**, 205 (1965).
7. R. K. Josephson, *ibid.* **38**, 579 (1961); R. K. Josephson and G. O. Mackie, *ibid.* **43**, 293 (1965).
8. W. F. Loomis and H. M. Lenhoff, *J. Exp. Zool.* **132**, 555 (1956).
9. H. B. Steinbach, *Biol. Bull.* **124**, 322 (1963); S. J. Lilly, *J. Exp. Biol.* **32**, 423 (1955).
10. G. O. Mackie, *Amer. Zool.* **5**, 439 (1965).
11. A. Finkelstein, *J. Gen. Physiol.* **47**, 545 (1964).
12. R. L. Wood, *J. Biophys. Biochem. Cytol.* **6**, 343 (1959); T. L. Lentz and R. J. Barrnett, *J. Ultrastruct. Res.* **13**, 192 (1965).
13. Supported by NIH grant NB 06054 and a PHS special fellowship (GM28478) to M.M.

1 May 1967

Adenovirus Tumorigenesis: Role of the Viral Genome in Determining Tumor Morphology

Abstract. *Adenovirus type 12 transforms the fibroblastic BHK21 (baby hamster kidney) cell line into rounded or cuboidal cells that give rise in hamsters to undifferentiated small cell sarcomas indistinguishable from those induced in newborn hamsters by inoculation of the virus itself. In contrast, cells from this line transformed by polyoma virus retain their fibroblastic morphology and induce fibrosarcomas in hamsters. This suggests that the morphology of tumors induced by the adenovirus-transformed cells from this line may be determined by the viral genome and that such a mechanism may also explain the remarkably uniform microscopic appearance which seems to characterize tumors induced in hamsters by direct inoculation of adenovirus type 12.*

Macpherson and Stoker described a line of baby hamster kidney cells, BHK21, which is characterized by fibroblastic morphology and growth in monolayers in parallel arrays (*1*). Subcutaneous injection of these cells into adult hamsters results in local formation of tumors described as fibrosarcomas (*2*).

The BHK21 cells can be transformed by polyoma virus (PV) and Rous sarcoma virus (RSV) (*1*, *3*). Transformation by these viruses is evident from loss of parallel orientation and from growth into multilayered piles of cells, but individual cells retain their fibroblastic appearance. Inoculation of animals with cells transformed by PV induces formation of tumors morphologically similar to fibrosarcomas initiated by untransformed BHK21 cells. In this respect, the tumors produced by the inoculation of BHK21 cells transformed by PV are also similar to those usually resulting from direct injection of PV into newborn hamsters (*4*). In mice, PV-induced tumors are highly variable in morphology (*5*); this circumstance makes it difficult to distinguish the relative roles of the viral genome and the target cell in determining the dominant cell type of the tumors.

Similarly, different lines of cells transformed by simian virus 40 (SV40) from various hamster tissues produced tumors of markedly different histological character in hamsters (*6*). These results pointed to the importance of target-cell determinants in morphology of tumors produced by SV40.

In contrast to both PV and SV40, adenovirus type 12 induces in newborn hamsters transplantable, undifferentiated small cell tumors of remarkable uniformity, regardless of the site of inoculation or of tumor growth (*7*). Moreover, cells cultured from such tumors (*8*) or neonatal hamster kidney cells transformed in vitro by adenovirus 12 (*9*) resemble those of the primary tumors. The histogenesis of the small undifferentiated malignant cells of adenovirus tumors has not been resolved; they may arise from specialized cells such as neuroepithelial elements associated with peripheral nerves (*10*). On the other hand, they may arise from cells that produce fibrosarcomas when transformed by PV or SV40, but the introduction of the adenovirus genome may determine the characteristic morphology. If the latter hypothesis were true, we would predict that transformation of a clonal line of BHK21 cells by adenovirus would enable these cells to produce tumors with morphology of the adenovirus type. Our findings are in accord with this prediction.

For use in our experiments, a homogeneously fibroblastic subline was established by two successive clonal isolations (*11*). To study transformation, we grew a preparation of adenovirus type 12, strain Huie, on human embryonic kidney (HEK) cells. The virus-infected cells were disrupted sonically and extracted with fluorocarbon. The

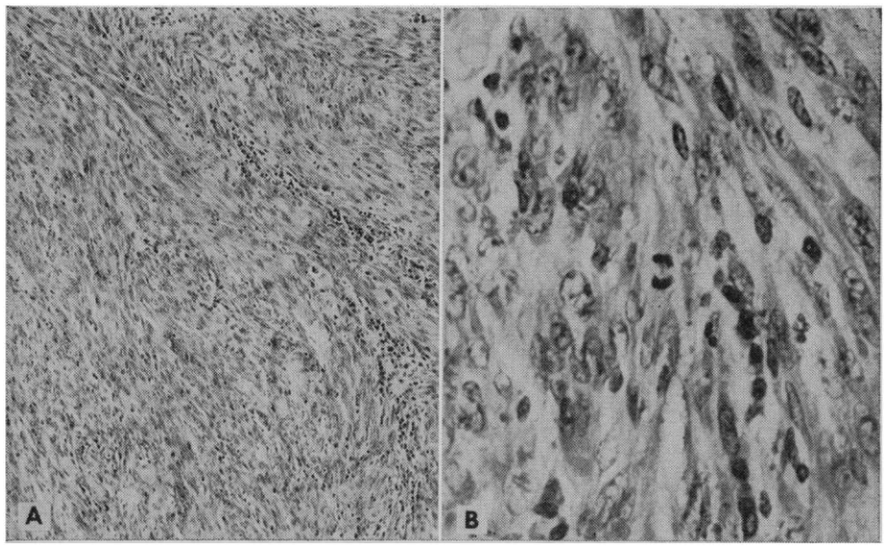

Fig. 1. (A) Section of tumor produced in hamster by injection of normal BHK21 cells. The tumor is a fibrosarcoma with interlacing bundles of elongated tumor cells. Hematoxylin and eosin stain, × 135. (B) Same as (A), × 690.

virus band was collected from centrifugation of a single CsCl density gradient. Plaque assays of this virus were performed on monolayers of HEK cells (*12*) modified by doubling of the amino acid and vitamin concentration and by addition of Eagle's nonessential amino acid mixture (*13*) and 10 percent fetal calf serum instead of the serum supplement described.

Monolayers of BHK21 cells were infected with gradient-purified adenovirus 12 at a calculated multiplicity of 25 plaque-forming units per cell. The infected cells were removed from the dishes 24 hours later, and approximately 10^5 cells per plate were seeded into soft agar medium (*14*). Fresh nutrient agar was added at 7-day intervals. After 21 days, 500 to 1000 small, but grossly visible, colonies were present. Several colonies, distinguished by their relatively larger size, were isolated and subcultured in liquid medium. All colonies plated were mixtures of normal-appearing fibroblasts and rounded or cuboidal cells with very little cytoplasm. The latter cells produced the adenovirus-12-specific tumor (T) antigen, as determined by reaction in immunofluorescence tests with serums collected from hamsters with adenovirus tumors. In contrast, T antigen was never detected in any of the fibroblastic cells in these mixed cultures or in any of several morphologically unchanged cell lines derived from BHK21 cells infected with adenovirus 12 (*15*). No infectious adenovirus 12 could be recovered from the transformed cells either by the plating of the sonically disrupted samples on HEK cells or by mixed cultivation of intact cells with HEK cells. The transformed cells were subjected to two consecutive clonal isolations under liquid medium before inoculation into hamsters.

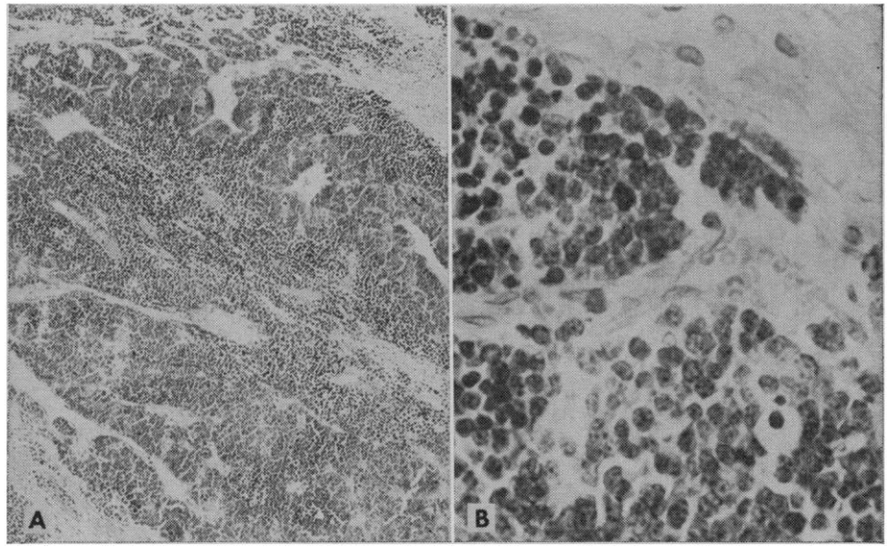

Fig. 2. (A) Tumor produced in hamster by injection of BHK21 cells transformed by adenovirus 12. Tumor is composed of sheets of small undifferentiated round cells similar to those in tumors produced by inoculation of adenovirus 12 into newborn hamsters. Hematoxylin and eosin, × 135. (B) Same as (A), × 690.

Hamsters (3 weeks old) were inoculated subcutaneously with either normal BHK21 or cloned transformed cells. In animals inoculated with 10^4 transformed cells, tumors were first seen at 7 weeks. These were removed for histological study 10 weeks after inoculation, when they were about 3 cm in diameter, freely movable, well encapsulated, and friable. Extensive hemorrhagic areas were seen on the surface. When allowed to grow, the tumors rapidly developed extensive areas of necrosis. Tumors produced by the inoculation of 10^2 normal BHK cells were first visible at 4 weeks and attained a size of 4 to 9 cm in diameter by the time of removal at 10 weeks. These tumors were very firm and without associated hemorrhage or striking evidence of necrosis (*16*).

Fragments of each type of tumor were fixed in 10 percent buffered formalin. Histologic sections were stained with hematoxylin and eosin, Masson's trichrome, and Hortega-Foot reticulum stain. Histologically, tumors produced by the untransformed BHK21 cells were fibrosarcomas composed of interlacing bundles of elongated cells (Fig. 1) with abundant reticulum and moderate amounts of collagen. The tumors produced by the BHK21 cells transformed by adenovirus 12 were composed of sheets of small hyperchromatic cuboidal cells with small amounts of poorly defined cytoplasm and no collagen or reticulum (Fig. 2). These tumors were indistinguishable from those produced in hamsters by inoculation of oncogenic adenoviruses (*7*).

Several of the tumors produced by BHK21 cells transformed by adenovirus 12 were again established in cul-

ture by trypsinization of the tumor tissue. The resulting cultures exhibited colonial and cellular morphology identical with that of the original transformed, inoculated cells; they still produced T antigen.

Apparently, the adenovirus 12 genome, when interacting with a homogeneously fibroblastic BHK21 cell line, is capable of determining the morphology of the resulting transformed cells as well as the characteristic morphology of tumors induced by these cells. Of course, the extent to which one may generalize on the basis of these results is limited, without similar studies on pure strains of cells from a number of diverse tissues. Because the BHK21 cells used were clonally purified, we avoided the ambiguities of the malignant change in vivo or in mixed cell populations (for example, hamster embryo cultures). The demonstration of the virus-specific nature of the morphological alterations accompanying transformation by an adenovirus further defines the participation of this viral genome in oncogenesis.

WILLIAM A. STROHL
Department of Microbiology,
Rutgers Medical School,
New Brunswick, New Jersey 08903
ALAN S. RABSON
Pathologic Anatomy Branch, National Cancer Institute, Bethesda, Maryland
HARRIET ROUSE
Department of Microbiology,
Rutgers Medical School

References and Notes

1. I. Macpherson and M. Stoker, *Virology* **16**, 147 (1962).
2. V. Defendi, J. Lehman, P. Kraemer, *ibid.* **19**, 592 (1963).
3. I. Macpherson, in *Malignant Transformation by Viruses*, W. H. Kirsten, Ed. (Springer-Verlag, New York, 1966), p. 1.
4. B. E. Eddy, S. E. Stewart, R. Young, G. B. Mider, *J. Nat. Cancer Inst.* **20**, 747 (1958).
5. M. F. Stanton, S. E. Stewart, B. E. Eddy, R. H. Blackwell, *ibid.* **23**, 1441 (1959).
6. P. H. Black, W. P. Rowe, H. L. Cooper, *Proc. Nat. Acad. Sci. U.S.* **50**, 847 (1963); G. T. Diamandopoulos and J. F. Enders, *Amer. J. Pathol.* **49**, 397 (1966).
7. J. J. Trentin, Y. Yabe, G. Taylor, *Science* **137**, 835 (1962); R. L. Kirschstein, A. S. Rabson, F. J. Paul, E. A. Peters, *Cancer Res.* **26**, 1361 (1966).
8. W. A. Strohl, H. C. Rouse, R. W. Schlesinger, *Virology* **21**, 513 (1963).
9. W. D. McBride and A. Weiner, *Proc. Soc. Exp. Biol. Med.* **115**, 870 (1964).
10. K. Ogawa, A. Tsutsumi, K. Iwata, Y. Fujii, M. Ohmuri, K. Taguchi, Y. Yabe, *Gann* **57**, 43 (1966).
11. The BHK21/13 cells were obtained from Dr. H. Koprowski, Wistar Institute, Philadelphia.
12. H. C. Rouse, V. H. Bonifas, R. W. Schlesinger, *Virology* **20**, 357 (1963).
13. H. Eagle, *Science* **130**, 432 (1959).
14. I. Macpherson and L. Montagnier, *Virology* **23**, 291 (1964).
15. W. A. Strohl, H. Rouse, R. W. Schlesinger, in preparation.
16. The uninfected BHK21 cells produced tumors more rapidly and with smaller inocula than did the transformed cells. This possibly results from a greater incompatibility of transplantation of the transformed cells due to additional adenovirus-12-specific antigens present in these cells.
17. We thank Dr. R. W. Schlesinger for helpful discussions. Supported by NIH grant CA-08851, formerly AI-06011.

28 April 1967

Homology of Retractile Filaments of Vampire Squid

Abstract. *The axial filament nerves of* Vampyroteuthis infernalis *are joined to two separate parts of the brain. One branch of the nerve, apparently efferent, arises from the anterior portion of the middle subesophageal mass, and the other, apparently afferent, enters the ventral magnocellular lobe. Since this innervation is entirely different from that of the arms, the filaments can no longer be considered homologous with the arms. The validity and primitive nature of the order Vampyromorpha, therefore, needs to be reexamined.*

Vampyroteuthis infernalis, a primitive, deep-sea cephalopod, was first described by Chun in 1903 (*1*) and placed in its own family, the Vampyroteuthidae, within the Octopoda. In 1929, Robson (*2*) elevated the group to subordinal ranking on the basis of increased knowledge of its peculiar anatomical features. Of particular importance was the discovery of a pair of retractile filaments situated between the first and second (dorsal) pairs of arms. Joubin (*3*) suspected that these filaments were related to the decapod tentacles, although they occupied a different position on the brachial crown (the two decapod tentacles represent the modified fourth pair of arms). Robson (*4*), however, who had been able to follow the axial filament nerve past the brachial lobe in a macerated specimen, was hesitant to accept the homology with the arms and suggested, ". . . it is not altogether impossible that they [the filaments] may be secondary pedal outgrowths."

Pickford, after preliminary anatomical investigations on *Vampyroteuthis* (*5*, *6*), concluded that the filaments were homologous with the arms. Her evidence was based chiefly on the similarities of the venous drainage of the arms and filaments, and on the apparent origin of the axial filament nerves from the brachial lobe. She indicated, however, that the origin of the filament nerves was not definitely settled.

As a result of her investigations, Pickford erected a new order for *Vampyroteuthis* and, as a primary character for the establishment of the Vampyromorpha, stated (*6*), ". . . there are five pairs of arms, the second dorsal pair being modified to form retractile filaments." In addition, the structure of the pen, the attachment of the fins to the shell-sac, and the free position of the spermatophore glands within the mantle cavity led Pickford to conclude that the order had a primitive origin.

In 1964, Donovan (*7*), accepting Pickford's views that *Vampyroteuthis* has ten arms, found it necessary to assume that the order had separated, ". . . very early from the decapod stem before the fourth pair of arms had become specialized as tentacles in the ancestors of the squids and cuttlefish." Therefore, the arrangement of the brachial crown both confirmed and demanded a primitive position for the order.

The nature of the retractile filaments is clearly of primary importance in determining the phylogenetic position of this animal. During studies on the anatomy of *Vampyroteuthis*, it has become apparent that the filaments are not homologous with the arms. Evidence to support this view is derived primarily from the anatomical relationships of the axial nerves of the filaments.

Each filament nerve is situated between the axial nerves of the first and second arms. The arm nerves send most of their fibers into the anterior subesophageal mass (brachial lobe) (Fig. 1). Each filament nerve, consisting of two separate bundles of fibers, by-passes this lobe completely. The smaller bundle, probably consisting of efferent fibers, passes dorsal to or occasionally through the brachio-palliovisceral connective and enters the middle subesophageal mass. The fibers then plunge diagonally toward the anterior-median area of the lobe where the tract loses its identity. The larger bundle, apparently with afferent fibers, passes ventral to or sometimes through the brachio-palliovisceral connective and enters a lateral side of a large oval lobe which is situated on the ventral surface of the middle subesophageal mass. This lobe is a single median