Table 1. Virus cultures of tears and saliva of 35 normal human volunteers. In addition to those shown, among those patients with previous lesions 15 showed no lesions during our study, and no positive cultures were obtained; among those with no previous lesions, six showed no lesions, and no positive cultures were obtained.

Clinical lesions		Herpes cultures		
Type	Time	Right eye	Left eye	Saliva
		Previous herpetic lesions		
Lip	Day 14	Day 14, 18		
Lip	Day 1			
None				Day 18, 20
None		Day 8	Day 8, 18	Day 8, 13
Lip	Day 19			Day 20
Conjunctivitis (left eye)	Day 5		Day 5	
Lip	Day 4			Day 20
None		Day 9		
Lip	Day 20			
		No previous lesions		
None			Day 10	
None				Day 10
None		Day 9		Day 9
None		Day 9, 17		Day 18, 19

film and in the saliva could be detected. Daily virus cultures were obtained 5 days a week for 20 days (Table 1). Of 11 people without a history of herpes, there were eight positive cultures (four from saliva and four from eyes). These were from four individuals, none of whom had symptomatic lesions during the period of observation. A group of 24 people with a history of recurrent labial herpes were also studied. In this group, there were 13 positive cultures (six from saliva and seven from eyes) with visible lesions in eight individuals. The total proportion of positive cultures is similar to that found in other studies of random populations, but documents the repeated virus release by individuals over a period of time. In addition to the positive virus cultures, three fever blisters developed in individuals in whom virus was not detected for the duration of the fever blister.

Regardless of negative clinical history, more than 90 percent of adults have been exposed to herpes, although most are not aware of any lesions produced by the virus (*7*). In fact, the four patients in our study with virus release but no previous lesions had neutralizing antibodies to the virus at the start of the study. Virus has also been noted by others in the secretions of adults and children without lesions as well as those with lesions (*7*). Therefore, the clinical presence of lesions in some individuals may be a function of local susceptibility to the frequent contact with virus. This susceptibility, rather than differences in exposure, may determine the presence of disease.

The frequent occurrence of herpesvirus in tears and saliva in the absence of lesions makes it unnecessary to consider virus latent in the affected tissues as a cause of recurrent disease. On the contrary, we must explain the rare occurrence of lesions in tissues frequently bathed by virus, and the susceptibility or resistance to this virus.

Our techniques of virus culture are relatively insensitive. Less than 0.05 ml of fluid on a cotton swab is immersed into the fluid bathing a tissue culture. This is but a tiny sample of the estimated 1000 to 1500 ml of saliva and 1 to 2 ml of tears produced each day. In into the fluid bathing a tissue culture. techniques, the relatively frequent isolation of virus from these fluids points to the possibility that chronic virus multiplication in structures such as the lacrimal and salivary glands, rather than latency, may be responsible for at least some recurrent herpes; true latency of herpesvirus has never been demonstrated.

HERBERT E. KAUFMAN
DAVID C. BROWN
EMILY MALONEY ELLISON
Department of Ophthalmology, University of Florida, Gainesville 32601

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27 April 1967

Transepithelial Potentials in Hydra

Abstract. *There is a maintained electrical potential of 15 to 40 millivolts across the two epithelial layers forming the body wall of* Hydra, *the inside of the animal being positive. Negative-going (depolarizing) spikes are recorded spontaneously and sometimes in response to depolarizing current pulses. These spikes usually overshoot the zero potential level. The large size of the spikes and the orientation of the potential difference across the body wall indicate that this electrical activity is epithelial rather than nervous in origin.*

The column of the coelenterate *Hydra* is a hollow cylinder composed of two concentric epithelial layers, the outer one termed the epidermis and the inner one, the gastrodermis. The two layers are separated by a thin, acellular mesoglea, and each is essentially one cell in thickness. Both epithelia are composed principally of epithelial cells which have contractile bases lying against the mesoglea. The contractile elements of the epidermis are longitudinal; those of the gastrodermis are

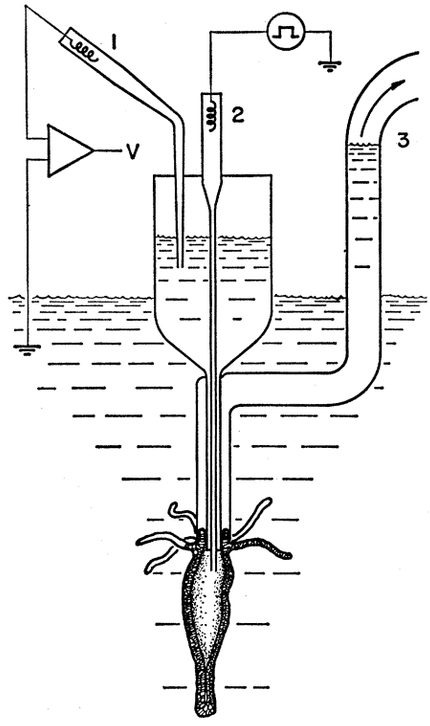

Fig. 1. The holder used to measure transepithelial potentials in *Hydra* and to pass current through the body wall. Potentials were measured between electrode 1 and a ground electrode in the bathing solution. Electrode 2 was used to pass current. The animal was held in place by slight suction on tube 3.

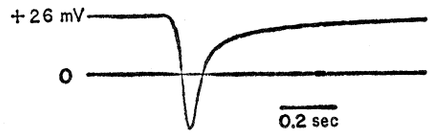

Fig. 2. A spontaneous contraction pulse.

circular. Nerve cells, which are most concentrated near the base of the tentacles and in the basal disc, form a network of fine fibers which runs among the muscular processes in the epidermal cell layer (*1*). Shortening of the hydra's column, brought about by contraction of the longitudinal musculature, occurs spontaneously and periodically (*2*). Each column contraction is preceded by an electrical pulse (contraction pulse, CP) easily recorded with external electrodes on the column (*3*). Column contractions and the associated CP's occur both as single events and in bursts (*4*). The CP's are rather large, up to several tens of millivolts as recorded with external electrodes, and are conducted up or down the column at about 5 cm/sec (*5*). Since the responding musculature is epidermal, it has been suggested that the column contractions are coordinated by the epidermal nerve net (*6*). The large size of the CP's in hydra and of similar potentials in other hydroids (*7*) has been perplexing for it does not seem likely that potentials of this magnitude could arise from the activity of fine, dispersed neurons in a nerve net. In some early experiments, we probed the body wall of the hydra with microelectrodes in an attempt to identify the origin of CP's. We found that there is a resting potential across the body wall and that CP's result from a large, transient change in this resting potential. Our experiments were begun to characterize this transepithelial electrical activity.

The animals used were *Hydra oligactis* grown in a culture solution containing $1.5 \times 10^{-3}M$ $CaCl_2$, $1.2 \times 10^{-3}M$ $NaHCO_3$, and $1.2 \times 10^{-4}M$ sodium ethylenediamine tetraacetate (*8*). These animals were starved for 24 hours before being used. Measuring the transepithelial potential with an electrode through the body wall was unsatisfactory because the column tore against or pulled away from the electrode during contraction. Consequently the holder shown in Fig. 1 was designed for measuring the potential between the inside of the column and the outer bathing solution. The holder consists of two concentric glass tubes, the inner one about 0.4 mm in external tip diameter and the outer one about 0.7 mm in internal tip diameter. The inner tube passes through the wall of the outer one and then expands to form a small cup. The inner tube and cup are filled with culture solution before the hydra is put on the holder. The inner tube, which projects slightly beyond the outer one, is forced into the mouth of a hydra, and the animal is held in place by slight suction applied to the outer tube. Putting a hydra on the holder does not seem to damage the animal, and when it is subsequently released it is normal in appearance and behavior. Transepithelial potentials are measured between the cup, which is in electrical contact with the inside of the animal, and the outer bathing solution with silver-silver chloride electrodes contacting the solution through $2M$ KCl-agar salt bridges. In some experiments, an electrode similar to the recording electrodes was inserted into the hydra's column through the cup and used to pass current between the inside of the column and the bathing solution. All experiments were done at 20° to 23°C.

In most animals the inside of the column is electrically positive with respect to the bathing solution. Typically, the transepithelial potential is 5 to 15 mv immediately after the animal is mounted, and it rises after the first 10 to 20 minutes of recording to reach 15 to 40 mv. The transepithelial potential is not stable and may drift considerably during long-term recording.

The CP's occur spontaneously at a somewhat higher frequency and more often in bursts in mounted animals than in unrestrained ones. Those recorded from inside the hydra are large, negative-going deflections which usually overshoot the zero potential level so that the inside of the animal becomes transiently negative (Fig. 2). The displacement phase of the spikes begins rather abruptly, and the peak is reached in 50 to 90 msec. The rate of potential return is at first rapid but becomes progressively slower, and full recovery may take several seconds. If there is a refractory period, it must be considerably shorter than the duration of the CP, for one CP can arise out of the initial portion of the return phase of the preceding one, and spike peaks may be separated by as little as 160 msec. The size of the CP's and the maximum negative potential reached are both variable and may be different for successive CP's of a burst.

In two animals it has been possible to consistently initiate CP's by passing inward current steps through the column, depolarizing it by 40 to 60 mv. In more than 20 animals, however, CP's were not initiated by current pulses of either polarity even when these displaced the transepithelial potential by as much as 100 mv. Even in the electrically excitable animals the CP threshold, as judged by the transepithelial potential at their onset, was not constant but varied from current pulse to current pulse (Fig. 3). The reasons for the variability of the resting potential and of the CP size and excitability are not yet known.

The electrical resistance of the body wall of hydra, measured with long-duration current pulses or low-frequency alternating current is approximately 163 kohm (man of 23 animals, S.D. = 77 kohm).

The sodium and potassium concentrations of hydra tissue are considerably greater than those in the surrounding medium, an indication that hydra has some means for accumulating ions (*9*). The transepithelial resting potential may result from the ac-

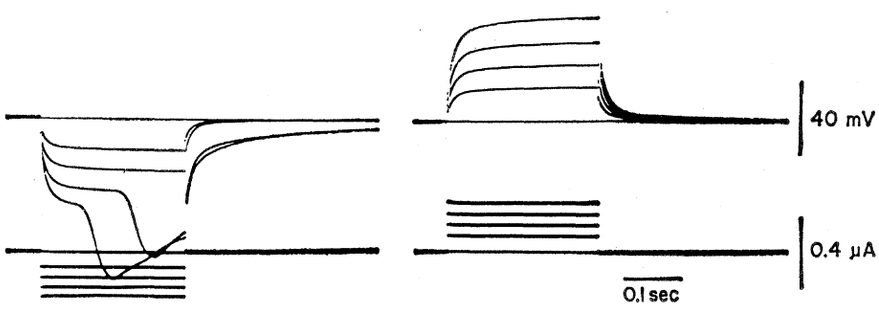

Fig. 3. Transepithelial potentials during passage of depolarizing current (left) and hyperpolarizing current (right) through the body wall.

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

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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