Table 1. Properties of the crystals.

Property	Original protein	Crystals
Total protein (mg)	4.0	2.0
$A_{280\ m\mu}$*	1.4	1.6
$A_{280\ m\mu}/A_{260\ m\mu}$	2.0	2.0
SO_4^{2-} bound (mμmole)*	1.9	2.5

* Per milligram of protein.

ethanol and dissolved in phosphate buffer. The yield of crystals was 50 percent of the added protein. Protein recovery (*3*), optical absorbancy, and sulfate binding activity (*4*) (Table 1) indicated that the crystals are composed of the binding protein.

Poorly shaped crystals were obtained from 80-percent-saturated ammonium sulfate by a similar procedure.

Evidence has been presented that this protein is part of the sulfate-active-transport system of *Salmonella typhimurium* (*4*), other work supports this conclusion. More recently similar proteins or soluble materials have been reported which are thought to be involved in transport of galactosides (*5*), amino acids (*6*), or galactose (*7*). They are located near the bacterial surface, as indicated by their ready release by osmotic shock (*8*) and, in the case of the sulfate binder, by sulfate binding to intact bacteria incapable of transporting sulfate (*9*). Unlike these substances, other specific surface-binding sites for galactosides (*10*) and proline (*11*) are firmly attached to the bacteria. Also, protein made under the control of the Lac Y (galactoside permease) gene has been observed by chromatography (*12*). These results support the conclusion that the sulfate-binding protein is a specific part (permease) of the sulfate-transport system (*13*).

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14. I thank Miss K. Watanabe for assistance. Photographs were taken by L. H. Bernstein in the laboratory of Dr. L. I. Rebhun with a Zeiss Ultraphot microscope equipped with Nomarski and polarizing optics. Aided by grant AI-04409 from USPHS.

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Recurrent Herpes in the Rabbit and Man

Abstract. *Herpesvirus was present in secretory glands and frequently in tears of rabbits with recurrent herpetic keratitis even in the absence of corneal lesions. In normal people, herpesvirus could be cultured from saliva and tears. Chronic virus multiplication in structures such as the lacrimal and salivary glands, rather than latency, may cause recurrent herpetic disease.*

It has generally been assumed that herpesvirus remains latent within infected tissue and is reactivated, in some way, to produce recurrent disease (*1*). Attempts at unmasking such virus, however, have failed (*2*). Laibson and Kibrick, and subsequently Nesburn *et al.*, described animal models for the study of recurrent herpetic ocular infection in rabbits (*3*). The accessibility of the ocular tissues and the possibility of examining the eye with the slit-lamp microscope, which can detect minimum tissue damage, make this a good system for the study of pathogenesis of recurrent herpetic infection.

Although primary attacks of herpetic keratitis can be managed with antiviral drugs (*4*), the overall rate of recurrence of this disease in man is 25 percent, and patients with more than one attack have a 43 percent chance of having an additional episode within 2 years (*5*). These recurrences gradually cause additional corneal damage and may result in blindness. We studied the pathogenesis of recurrent corneal and labial herpes simplex infection in rabbits and in humans.

Bilateral corneal infections were established in albino rabbits with Rhodanus strain herpesvirus (*6*). The initial infection spontaneously subsided in 12 to 14 days; daily observation for recurrent disease was begun 25 days after infection and continued through 95 days. In all cases, the initial lesions had healed well before the beginning of our study. Daily virus cultures of the precorneal tear film were taken on cotton swabs and inoculated into cultured human amnion tissue immediately or after storage at −65°C. Care was taken to be certain that no corneal damage was done in the process of culturing, and corneas were carefully studied each day with the slit-lamp microscope before and after culturing. This confirmed the absence of corneal damage from the culture procedure. Thirty-five rabbits were initially infected but 20 died during the acute phase of the disease, so that 15 were continued in our study. Throughout this study, four uninfected control animals were kept with the infected animals; all the animals were handled similarly, and cultures were taken from all to check for possible contamination. None was found.

Every animal manifested at least three episodes of spontaneous virus release without any specific provocation. Although 60 percent of the rabbits had at least one spontaneous corneal ulcer, 43.8 percent of these had positive cultures before any microscopically detectable corneal lesion was visible. If any single positive culture or any consecutive group of positive cultures is considered a single episode and if negative cultures are required for at least 2 days between episodes, there were 73 episodes of virus shedding detectable by culture. Sixteen recurrent corneal ulcers were detected; the average corneal ulcer lasted 3.1 days, whereas the average duration of virus positivity per episode lasted 2.1 days.

The remarkably frequent finding of virus in the precorneal film in the absence of detectable lesions in the cornea, and the frequent appearance of virus in the precorneal film before any detectable corneal ulcer, suggested that the initial herpetic infection produced a generalized infection of the periocular structures such as the conjunctiva or lacrimal gland, and that continued virus shedding from these structures, rather than virus latent in the cornea, might be responsible for the corneal infection. Of ten animals studied, virus was found in three lacrimal glands and one Harder's gland.

We then studied normal healthy humans to determine whether recurrent virus shedding in the precorneal tear

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.

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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

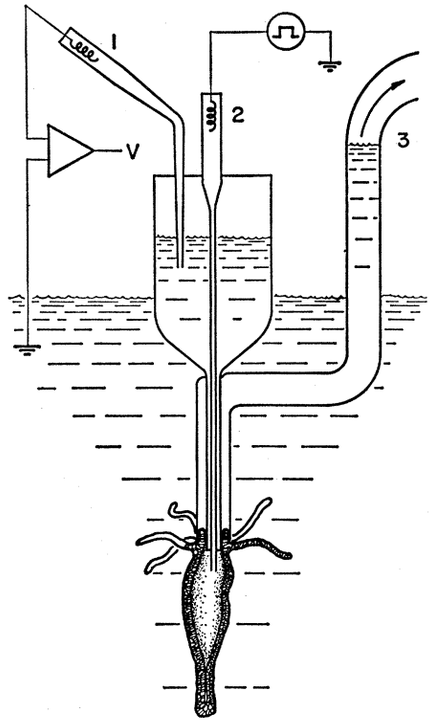

Fig. 1. The holder used to measure transepithelial potentials in *Hydra* and to pass current through the body wall. Potentials were measured between electrode 1 and a ground electrode in the bathing solution. Electrode 2 was used to pass current. The animal was held in place by slight suction on tube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