Table 1, the relative amounts of both AH^+ and BH^+ increase by a factor of about 2 as the *p*H is changed from 8.90 to 7.05.

The electric charge of the guanidinium group should not change over the *p*H range used in this study. This implies an essential role for the hemilactal-lactone part of the molecule. Since there are two cationic forms in solution, it is difficult to determine which is the active form. There are a number of possible mechanisms to explain the interactions of both cations with receptor sites.

First, there is the possibility that the absence of the electronegative charge decreases the electrostatic repulsion by negative sites of the membrane surface (Fig. 3A). This mechanism applies equally to both cations.

Second, the protonic hydrogen of BH^+ may serve to form a hydrogen bond between the tetrodotoxin molecule and the receptor site (Fig. 3B). Since tetrodotoxin is now thought to act on the outside of the cell membrane (*8*), the receptor is most probably a protein. However, hydrogen bonding may also occur with phosphate groups. Since surfaces of proteins themselves are sensitive to *p*H change, we must not overlook the possibility that the potential binding sites are affected by the *p*H changes. This might happen without changes in any of the electrophysiological parameters that we measured.

A third possibility would be that the tetrodotoxin first binds to a negative receptor site by the guanidinium group. If then a sterically suitably situated hydroxyl group on the receptor could react to form an intermolecular hemilactal configuration preferentially to the intramolecular reaction leading to BH^+, a secondary anchoring would take place between AH^+ and the receptor (Fig. 3C).

In conclusion, whatever the mechanism, this study supports the statements of Narahashi *et al.* (*9*) that more than the guanidinium group is involved in determining the potency of tetrodotoxin. More specifically, we have shown that the lactone-hemilactal configuration of the tetrodotoxin molecule is also important.

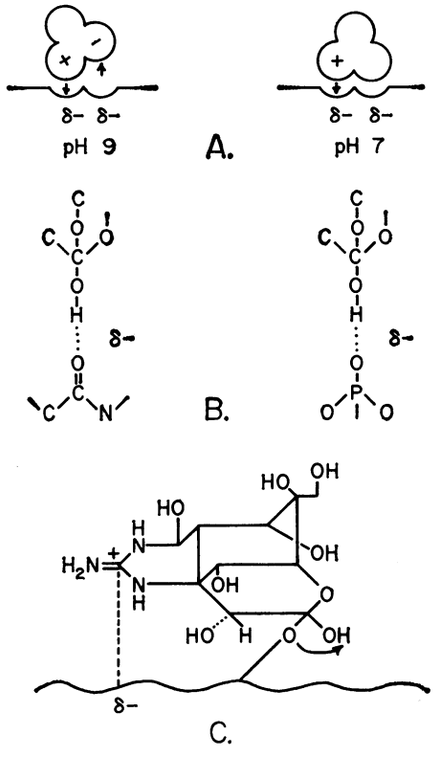

Fig. 3. (A) Models of the molecular interaction of tetrodotoxin with a receptor site on the outer layer of an excitable membrane. The zwitterion is partially repelled by electronegative regions of the cell membrane. The cationic form has less repulsion, thus resulting in a higher probability of proper steric interaction with the receptor site. (B) The structural formulas show possible interactions of the hemilactal part of BH^+ with an electronegative oxygen of a peptide chain, or an electronegative oxygen of a phosphate group by hydrogen bonding. (C) Postulated interaction of tetrodotoxin with a receptor site by electrostatic attraction and secondary intermolecular hemilactal formation.

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Crystallization of a Sulfate-Binding Protein (Permease) from Salmonella typhimurium

Abstract. *Crystallization is reported of a protein that is a component of an active transport system for sulfate into* Salmonella typhimurium. *This appears to be the component that is specific for binding the substrate.*

Isolation of a protein with high specific affinity for a single sulfate anion has been reported (*1*). This protein was pure by the usual physical-chemical criteria. Its crystallization is reported here.

Purified protein (4 mg lyophilized from distilled water) was taken up in 0.55 ml of 1.8 m*M* potassium phosphate buffer, *p*H 7.0, at 24°C, and then 1.0 ml of 2-methyl-2,4-pentanediol (*2*) was added to produce a turbid suspension. Distilled water was added (0.3 ml) until the turbidity nearly disappeared. A few seed crystals, which were obtained by a similar procedure after 50 days at 4°C, were added and the tube was stoppered and kept at 4°C. Crystals were observed after 7 days (Fig. 1); the largest were more than 0.1 mm long. They were quite birefringent in the polarizing microscope.

The crystals were collected by centrifugation, washed with cold 80 percent

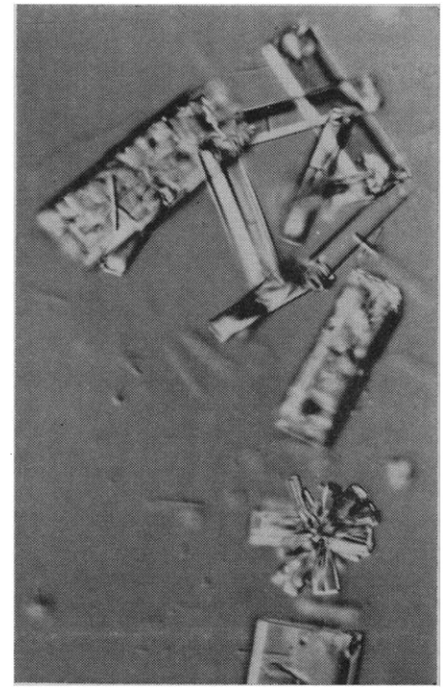

Fig. 1. Crystals of sulfate-binding protein. Nomarski optics were used. The largest crystal shown was 0.18 mm long.

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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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