out either component nor can an osmotic pressure be exerted on the membrane by either component. We imagine that solution A is forced across the membrane; according to the continuity equation (*2*) for gases which has been applied to solutions (*3*), under steady-state flow the relation between the total solute flow J_s and the volume flow J_v is

$$J_s = \frac{J_v (C_s^B \, e^{-J_v \Delta x/D} - C_s^A)}{(e^{-J_v \Delta x/D} - 1)} \quad (1)$$

Here flows J_s and J_v are positive when their direction is from left (side A) to right (side B), and both flows are measured with respect to the available pore area; Δx is the thickness of the membrane (or length of the pores); D is the diffusion coefficient for the solute; and C_s^A and C_s^B are the solute concentrations in compartments A and B, respectively. Overman and Miller (*4*) obtained data across a simple capillary which support the validity of this equation. Garby (*5*) showed that Eq. 1 adequately predicts J_s for membranes used in his work; for our ideal membrane we believe that its validity cannot be disputed (*6*).

According to the irreversible thermodynamic approach (*1*), the solute flow J_s for the above process across an open membrane (measured with respect to the available pore area for consistency with J_s as defined in Eq. 1) is given by

$$J_s = J_v \, \bar{C}_s + \omega \, \Delta \pi \quad (2)$$

Here $\bar{C}_s$ is an average concentration defined by

$$\bar{C}_s = (C_s^A - C_s^B)/\ln(C_s^A/C_s^B)$$

and

$$\Delta \pi = RT(C_s^A - C_s^B)$$

where R is the gas constant, T is the absolute temperature, and ω is the coefficient of solute permeability at zero volume flow

$$\omega = (J_s/\Delta\pi)_{J_v = 0} \quad (3)$$

Yet if we interpret Eqs. 1 and 2 as accurately representing the same process, ω must be *dependent* on J_v in a rather complicated way (*7*); thus for values of J_v that are large as compared with $D/\Delta x$, ω is directly proportional to J_v

$$\omega = J_v (C_s^A - \bar{C}_s)/\Delta\pi \quad (4)$$

This strong dependence of ω on J_v invalidates its use as a parameter descriptive of properties intrinsic to the membrane, except for the case where $J_v = 0$, when ω becomes constant (Eq. 3) and thus is useful as a diffusive permeability coefficient. The usefulness of Eq. 2 is thus limited to systems where (i) $J_v = 0$, so that J_s represents purely diffusive flow, as given by $\omega\Delta\pi$; and (ii) $\Delta\pi = 0$ (implying that $\bar{C}_s = C_s^A = C_s^B$), so that J_s represents purely convective flow, as given by $J_v C_s^A$.

Other investigators (*8*) have discussed practical limitations in certain applications of irreversible thermodynamics. We have pointed out (*9*) difficulties associated with an attempt to apply Onsager's reciprocal relation to convective-diffusive processes. Onsager's relation is useful in treating certain membrane processes such as the interaction of solute flows in ternary diffusion systems. It is, nevertheless, our opinion that interaction between convective and diffusive flows cannot be treated in an analogous fashion because they do not interact in the same sense as the solute flows in ternary diffusion systems.

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6. At high flows Eq. 1 reduces to the form
$$J_s = J_v C_s^A$$
The fact that at high flows diffusion does not merely contribute a negligibly small fraction to solute flux but actually approaches zero may seem surprising. However, since random molecular motions have small but finite time requirements, at high bulk flow velocities sufficient time will not be available for appreciable diffusive flow to occur across the membrane.
7. Equating Eqs. 1 and 2 and solving for ω, we obtain
$$\omega = \frac{J_v}{\Delta\pi}\left(\frac{C_s^B e^{-J_v\Delta x/D} - C_s^A}{e^{-J_v\Delta x/D} - 1} - \bar{C}_s\right)$$
or more explicitly in terms of concentrations
$$\omega = \frac{J_v}{RT}\left[\left(\frac{1}{C_s^A - C_s^B}\right)\left(\frac{C_s^B e^{-J_v\Delta x/D} - C_s^A}{e^{-J_v\Delta x/D} - 1}\right) - \frac{1}{\ln(C_s^A/C_s^B)}\right]$$
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4 December 1968

Isolation of Western Equine Encephalomyelitis Virus from an Opossum

Abstract. *The first isolation of an arbovirus (western equine encephalomyelitis virus) from an opossum in the United States is reported.*

The principal epidemic cycle for western equine encephalomyelitis virus (WEE) involves wild birds and mosquitoes. The virus has been isolated from only a few mammalian species, but surveys for antibodies and laboratory experiments indicate that a wider range of susceptible mammals exists (*1–3*).

An opossum *Didelphis marsupialis* was caught in September 1968, in Butte County, California. A portion of the brain, preserved in glycerol saline, was tested for rabies by the fluorescent antibody technique and the results were negative. Anaerobic and aerobic cultures for bacteria were negative. Fluorescent antibody (FA) tests for plague and tularemia organisms were also negative.

Suckling mice inoculated intracerebrally and intraperitoneally with a 5 percent suspension of the opossum brain died 2 days later; WEE in the mouse brains was detected by FA staining with serum from hamsters immunized against WEE. The standard intraperitoneal neutralization test in suckling mice with serum from rabbits immunized against WEE was also positive. The virus was reisolated, and the titer by intracerebral inoculation in suckling mice was determined to be $10^{-3.0}$ per 0.02 ml of 5 percent opossum brain suspension. Direct FA staining of impression smears from the opossum brain with WEE-immune hamster serum showed a few single or small groups of infected cells.

Opossums have rarely been included in surveys for WEE antibodies, although antibodies against group B or California group arboviruses have been reported in the United States or Canada (*1*, *3*). A few attempts to infect opossums experimentally with WEE virus suggested that they are relatively resistant (*4*).

Surveys for antibodies indicate that at least 30 arboviruses can infect marsupial species, including members of group A which are closely related to WEE (*1*). At least ten arboviruses have been isolated from marsupials in Central and South America.

A small percentage of *Culex tarsalis* mosquitoes, the principal vector for WEE virus in the western United States, has been shown to feed on oppossums

in areas of Colorado (*5*) and California (*6*). Squirrels dying of WEE virus infection were found during 1968 in the same area as was the opossum. It thus seems possible that the opossum was infected by mosquito bite, or by eating an infected animal. Our findings indicate that there was active infection of the brain and not merely an incidental viremia.

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2 December 1968; revised 7 January 1969 ■

Generalized Gangliosidosis: Impaired Cleavage of Galactose from a Mucopolysaccharide and a Glycoprotein

Abstract. *We have demonstrated a tenfold (or greater) reduction of the cleavage of galactose from a mucopolysaccharide and a glycoprotein in generalized gangliosidosis, a lysosomal β-galactosidase deficiency disease. The bony abnormalities in this disease may be due to defective catabolism of mucopolysaccharides in connective tissue.*

Generalized gangliosidosis is an inborn error of metabolism involving the accumulation of ganglioside GM_1 in brain (*1*, *2*) and viscera (*1*). The disease is characterized by (i) progressive cerebral degeneration leading to death within the first 2 years of life; (ii) accumulation of glycolipid in neurons and of glycolipid and mucopolysaccharide in hepatic, splenic, and other histiocytes, and in renal glomerular epithelium; and (iii) the presence of skeletal abnormalities resembling those seen in Hurler's syndrome (*3*). Okada and O'Brien (*4*) have demonstrated a 10- to 30-fold deficiency of a β-galactosidase (*p*H 5.0), an enzyme that cleaves the terminal galactose from the stored ganglioside, GM_1—{galactosyl-(1 → 3)-*N*-acetyl-galactosaminyl-(1 → 4)-[(2 → 3)-*N*-acetylneuraminyl]-galactosyl-(1 → 4)-glucosyl-(1 → 1)-[2-*N*-acyl]-sphingosine}. Generalized gangliosidosis can be classified as a glycosphingolipid hydrolase deficiency, the ganglioside accumulation resulting from an impairment of catabolism.

Mucopolysaccharides also accumulate in the viscera in generalized gangliosidosis (*5*). One of these has (*6*) a structure similar to that of keratan sulfate; it contains nearly equimolar proportions of glucosamine and galactose with much smaller proportions of other sugars. The mechanisms leading to the storage of the mucopolysaccharide is unknown. The possibility exists that β-galactosidase participates in the degradation both of ganglioside GM_1 and of mucopolysaccharides containing galactose and that accumulation of the latter may also be due to an impairment in catabolism.

To test this possibility, we isolated the "keratan sulfate-like" mucopolysaccharide from the liver of a patient who died at 2 years of age from generalized gangliosidosis. The isolation procedure (*6*) involved extraction of the tissue with a mixture of chloroform and methanol (2:1), digestion of the solvent-insoluble residue with papain for 24 hours at 60°C, and isolation of mucopolysaccharides by precipitation as their sodium salts with 80 percent ethanol. The preparation was then digested with α-amylase for 2 hours at 37°C and precipitated from 80 percent ethanol. The precipitate was then dissolved in water, dialyzed, and lyophilized.

The isolated mucopolysaccharide contained galactose and hexosamine in nearly equimolar amounts (galactose : hexosamine 0.9). This was determined by hydrolyzing the compound in 1*N* HCl at 100°C for 18 hours, and, after purification on a charcoal column (*7*), estimating galactose by galactose oxidase assay (*8*). Hexosamine was determined by the method of Boas (*9*). The hexosamine was identified as glucosamine by chromatography (*10*). Only small amounts of galactosamine were present. The intact mucopolysaccharide migrated in a way similar to keratan sulfate, with an R_F of 0.8 in a silica-gel thin-layer chromatography system (*11*). The stored mucopolysaccharide stained pink with the orcinol sulfuric acid spray, also characteristic of keratan sulfate (*11*). It was resistant to degradation by testicular hyaluronidase.

A portion of this mucopolysaccharide was then incubated in the presence of β-galactosidase partially purified from equal amounts of liver tissue from normal patients and that from generalized gangliosidosis. The protein and water contents of the patient's tissue were nearly identical to normal. The enzyme was purified (*12*); the particulate enzymes and free galactose present in the tissue were removed, and the enzyme was obtained in soluble form. Small losses of β-galactosidase activity occurred in preparing the enzyme from the normal and diseased livers, but

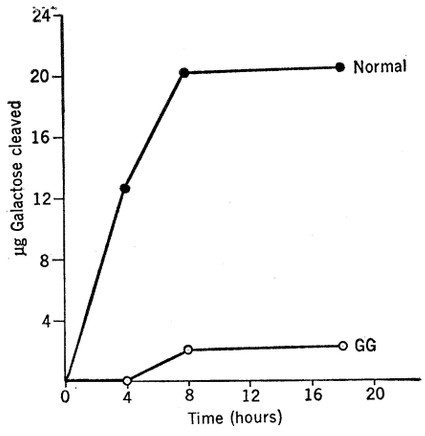

Fig. 1. Cleavage of galactose from 1.5 mg of "keratan sulfate" from the liver of a patient with generalized gangliosidosis. β-Galactosidase was prepared from 35 mg of liver from a normal patient or from a patient with generalized gangliosidosis (*GG*). The enzyme and the mucopolysaccharide were incubated over an 18-hour period in acetate buffer (0.1*M*), *p*H 5.0, at 37°C, and the galactose released was determined by galactose oxidase assay.