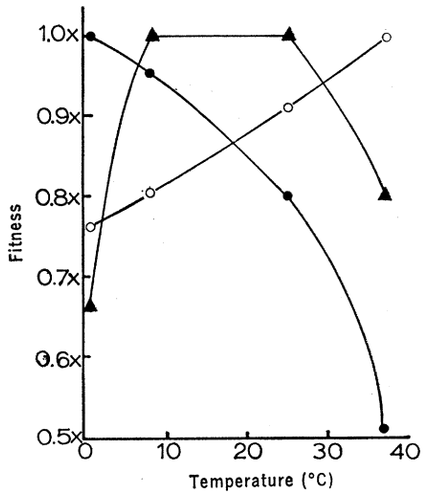

Fig. 2. Relationship between temperature-dependent *Es-I* activities of genotypes and "fitness." ○—○, *Es-I*$^{a/a}$; ▲—▲, *Es-I*$^{a/b}$; ●—●, *Es-I*$^{b/b}$.

fered from that at higher temperatures (Fig. 1B). Populations with *Es-I*^{b} exhibited activity much greater than at 37°C —nearly ten times that of either *Es-I*^{a} or the heterozygote. The observed activity of *Es-I*^{b} is not only greater than that observed for the heterozygote at 37°C, but nearly equal to *Es-I*^{a} at the higher temperature. Activity of the heterozygote was intermediate at upper limits of the tested temperature range, but highest at intermediate temperatures (Fig. 2).

The different temperature optimums of the three genotypes illustrate a functional basis for the observed geographic distribution of the two alleles. The most frequent allele in populations of *C. clarkii* at southern extremes of the range, *Es-I*^{a}, has a higher activity at higher temperatures. The more frequent allele in northern populations, *Es-I*^{b}, has the highest activity at 0°C.

If maximum enzymatic activity is related to maximum genotype fitness at the *Es-I* locus, one can demonstrate a relationship between the temperature dependency of the three genotypes and a selection model explaining the clinal distribution of esterase alleles within these fish populations. Maximum observed activity is equated with "fitness" relative to the slope in change of observed activity over the tested temperature range (Fig. 2). The "fitness" scale so expressed has no absolute meaning, but is merely a means of obtaining relative values for the three genotypes as a function of the slope of individual plots.

In a genetic system where two alleles are selectively maintained by heterozygote advantage, a stable equilibrium exists:

$$\hat{p} = S_2/S_1 + S_2$$

where S_1 is the coefficient of selection against one homozygote (*Es-I*$^{a/a}$), S_2 is the coefficient of selection against the other homozygote (*Es-I*$^{b/b}$), and p is the frequency of the allele represented by the *Es-I*$^{a/a}$ homozygote (*4*).

Arrangement of the temperature-dependent activity profiles of the three genotypes in the manner described above permits the derivation of "fitness" values (x) for each of the genotypes at any specific temperature. Since $S = 1 - W$ (in this case $W = x$), the equilibrium condition $\hat{p}$ can be predicted. As an example, at 10°C maximum activity is exhibited by the heterozygote ($x = 1.0$), whereas *Es-I*^{b} activity is lower ($x = 0.95$; $S_2 = 1 - x$; $S_2 = 0.05$), and *Es-I*^{a} activity is the lowest ($x = 0.81$; $S_1 = 1 - x$; $S_1 = 0.19$). Hence, $\hat{p} = 0.05/0.19 + 0.05 = 0.21$. In other words, an environmental selection temperature of 10°C will produce, by the above scheme, an equilibrium condition where the frequency of the *Es-I*^{a} allele will equal 0.21 (within individuals of that generation). At temperatures lower than approximately 7°C and higher than 30°C a condition of disequilibrium will exist which results in the fixation of *Es-I*^{b} or *Es-I*^{a}, respectively (Fig. 2). Where S_1 equals S_2 (slightly less than 20°C), $\hat{p}$ will equal 0.50.

The functional characteristics of the enzymes demonstrate a somewhat predictable situation: variant allelic products differing in adaptive properties. It is striking, however, that the two enzymes exhibit such markedly different temperature optimums. It seems reasonable to postulate similar functional characteristics for all other heterotically maintained two-allele polymorphisms, although the component of selection need not be temperature, and the functional allelic differences need not be so great. These characteristics would depend on the magnitude of variation in the particular environmental component as well as on the tolerance of the genetic products to the variation.

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Diffusive and Convective Flow Across Membranes: Irreversible Thermodynamic Approach

Abstract. *The experimentally verified hydrodynamic approach to a description of diffusive and convective flow of solute across mutual membrane pathways is compared with the phenomenological equation resulting from the application of irreversible thermodynamics. Inherent nonlinearities in this equation severely, if not absolutely, limit its usefulness.*

In the study of transport across membranes one must frequently find a suitable expression for solute flow when convection (bulk flow of solution) and diffusion are occurring simultaneously. In the classical approach to the problem, one assumes idealized structures for the pores in the membrane and solves the continuity equation after making appropriate mathematical approximations. In another approach developed by Kedem and Katchalsky (*1*) the theory of irreversible thermodynamics is used to obtain a relatively simple linear flow equation for the combined processes. We here examine the consistency of the two approaches for an idealized membrane.

The system to be considered consists of solution *A* in compartment *A* on the left side of the membrane and solution *B* in compartment *B* on the right side. Each solution is dilute, infinitely well stirred, and confined on its side of the membrane by a movable piston. The membrane is homoporous, consisting entirely of right cylindrical pores all having the same radius. The membrane is open to the solute and solvent; that is, it cannot selectively filter

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