Reports

Nature of Solvent Transfer in Osmosis

The phenomenon of osmosis appears to be looked upon with sharply differing points of view by many physiologists and others in related disciplines. The traditional view of workers in the field of capillary and glomerular permeability, as typified by the work of Starling and Landis and, in more recent years, by the work of Krogh, Ussing, and Jacobs holds that osmosis is a *mass* flow of the solvent through the "pores" of the barrier (membrane) arising by some obscure mechanism—usually not discussed—when a mole fraction difference of the solvent obtains by virtue of the presence of a macromolecule impermeable to the barrier. Another point of view has argued exclusively for the *diffusion* of the solvent—that is, a molecular-molecular random drift. This view has been most eloquently expressed in recent years by Chinard (*1*).

Although several workers (*2*) in recent years have demonstrated that osmotic transfer must be viewed as a mass flow, this work has been carried out on biological material and for this reason might appear to be ambiguous or inconclusive since more "complex" phenomena might be involved. The data presented in this report (*3*) have been obtained on a simple system to help focus attention on the fundamental process of osmotic transfer. It will be seen in the following discussion that two irreversible processes can take place, and usually do, to a varying degree. A simple binary system—namely, a solvent (water) and an uncharged macromolecule—is considered in conjunction with a typically inert barrier as used in osmometry of the Fuoss-Mead type (perforated stainless-steel disk covered with collodion) to which the macromolecule is *absolutely* impermeable.

The first task is to establish the nature of the solvent flux when a hydrostatic pressure difference is applied across the barrier. Direct observation on this barrier indicates a linear relationship between hydrostatic pressure and transfer of solvent, namely, 0.88×10^{-10} mole/sec, per dy/cm^2.

The diffusion component of flux can be evaluated by applying the general relationship (4)

$$\frac{dn}{dt} = -\frac{DA}{RT} C \frac{\Delta\mu}{\Delta X} \qquad (1)$$

where A is the effective area and D the self-diffusion coefficient of the solvent and μ is the chemical potential. This equation can be rewritten

$$\frac{dn}{dt} = -\frac{DA}{RT} \frac{\Delta P}{\Delta X} \qquad (2)$$

since

$$\Delta\mu = \overline{V}\Delta P$$

and

$$C\overline{V} = 1$$

The factor $DA/\Delta X$ can be evaluated, after observing the diffusion of H_2O^{18} across the same barrier, from the relation

$$\left(\frac{dn}{dt}\right)_{H_2O^{18}} = DA \frac{\Delta C_{H_2O^{18}}}{\Delta X} \qquad (3)$$

Integration of this, with the assumption of a linear gradient, yields the equation

$$\frac{DA}{\Delta X} = \frac{2.3V}{2\Delta t} \log \frac{C' + C'' - 2C''_t}{C' + C'' - 2C''_{t+\Delta t}} \qquad (4)$$

in which C′ and C″ (C′ > C″) are the initial concentrations of H_2O^{18}. A cell was constructed with equal volumes ($V = 6.5$ cm^3), and means for stirring were provided. Samples were taken at intervals of time Δt (15 min), with the first sample taken at time t several minutes after the cell had been filled. Analyses were made with a mass spectrometer. Typical data obtained for the initial concentrations $C' = 1.3$ percent and $C'' = 0.2$ percent are $C''_t = 0.28$ percent, $C''_{t+15} = 0.54$ percent, and $C''_{t+30} = 0.65$ percent. With these data, Eq. 4 gives $DA/\Delta X$ as 3×10^{-3}. Thus, by means of Eq. 2, the magnitude of flux per unit pressure difference is 0.12×10^{-12} mole/sec, per dy/cm^2. Comparison of this with the magnitude found experimentally shows that the diffusion component is about 1/730 of the total flux.

One is forced to conclude, therefore, that any pressure difference applied gives rise to a transfer of water which is predominantly nondiffusional in nature. It will be convenient to refer to this component as quasi-laminar since the flow lines in the barrier would be difficult to establish.

The osmometer experiment is now to be considered. It is an experimental fact that the transfer of water through the barrier when a hydrostatic pressure difference is applied can be duplicated in the *absence* of a pressure difference by contaminating one volume of the solvent with a macromolecule—for example, dextran—to which the barrier is *absolutely impermeable*. The quantity, which is exactly equivalent to a given value of ΔP, is $(RT/\overline{V})$ in N_{H_2O} which reduces to RTc for small values of the concentration c of the macromolecule.

The fascinating nature of the mole fraction effect can be seen in that, while "intuitively" one might assume that this component of the chemical potential would give rise to a diffusion flux as prescribed by Eq. 1, the experimental fact observed is that the flux developed is predominantly nondiffusional but, as termed in a preceding paragraph, "quasi-laminar." This conclusion is inescapable since, in the osmometer experiment, we observed the equivalence of the mole fraction and the hydrostatic pressure variables and in the first experiment we established the nondiffusional nature of the flux due to hydrostatic pressure. Therefore, the mole fraction effect must also develop a nondiffusional flux. Thus, expressing this symmetrical relationship, we have

$$\frac{dn}{dt} = K f(A) [\Delta P - \pi]$$

where positive value of flux is defined from solution to solvent and ΔP is the excess hydrostatic pressure on solution over that of solvent, and π is the mole fraction term. The proportionality constant is some kind of "hydraulic conductivity" depending upon the nature of the flow process.

It should be emphasized that there is no kinetic theory in existence to explain the basis of the nondiffusional flux arising from the mole fraction effect. Unfortunately, the theory of liquids is inadequate at the present time for carry-

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ing out more than speculative analysis, but certainly, as suggested by Lars Onsager, there must be a momentum deficiency in the microdomain of the pore in the solution side of the barrier. That is, in a solid region of the barrier, the time average transfer of momentum is that prescribed by the hydrostatic pressure of the phase, but in the opening of the pore there is a deficiency since the momentum arising from the macromolecule is not transferred to the solvent species in the pore, being cut off by the finite size of the pore. Thus, within the pore and only within the pore, a gradient of pressure arises and quasi-laminar flow ensues from the solvent side to the solution.

Although the experimental observations in the osmometer experiment do not demonstrate the diffusion component of flux explicitly, it is reasonable to assume that this component is present:

$$\left(\frac{dn}{dt}\right)_{diff.} = \frac{-DA}{RT} C \frac{\Delta u}{\Delta X} \cdot$$

$$= \frac{DA}{RT} \frac{C}{\Delta X} [\bar{V}\Delta P + \Delta RT \ln N]$$

$$= \frac{DA}{RT} \frac{C\bar{V}}{\Delta X} [\Delta P + \frac{RT}{\bar{V}} \ln (1 - N_{H_2O})]$$

$$= \frac{DA}{RT} \frac{1}{\Delta X} [\Delta P - \pi]$$

Thus, the total flux is

$$\frac{dn}{dt} = \left[\frac{DA}{RT\Delta X} + K f(A)\right] [\Delta P - \pi]$$

The diffusion component would be all-important in a barrier whose "pores" have a cross-sectional area of the order of the solvent molecules such that only a molecular-molecular drift of the solvent could occur.

In conclusion, the point to be emphasized for workers in the field of membrane permeability is the fact that in osmotic transfer the chemical potential difference of the solvent can give rise to both a quasi-laminar flux and to a diffusion flux, the relative importance of the two components being dependent on the nature of the barrier. For most barriers, the predominant component is the quasi-laminar flux.

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References and Notes

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2. V. Koefoed-Johnson and H. H. Ussing, *Acta Physiol. Scand.* 28, 60 (1953); E. Zeuthen and D. M. Prescott, *ibid.* 28, 77 (1953); R. P. Durbin, H. Frank, A. K. Solomon, *J. Gen. Physiol.* 39, 535 (1956).
3. I am deeply indebted to my colleagues H. Morowitz, J. H. Wang, D. Hitchcock, G. Meschia, and Lars Onsager for their kind help with theoretical discussions and experimental details.
4. G. S. Hartley, *Phil. Mag.* 12, 473 (1931).

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Aggressive Behavior in Castrated Starlings

Androgens have long been known to affect the aggressive behavior of birds and mammals. Experiments conducted during the last two decades have shown that animals of various species ceased aggressive behavior when they were castrated, or rose in social rank when they were given injections of testosterone. This paper (*1*) reports the maintenance of aggressive behavior in castrated starlings and the failure of testosterone to affect their social rank.

The methods consisted of observing castrated starlings (*Sturnus vulgaris*) that were living in a large room (14 by 16 feet). Eleven birds were bilaterally castrated on 20 Dec. 1956, when the testes were still in the regressed winter condition but were starting to increase. The birds were painted on the tail with bright colors for individual identification. These birds maintained fighting and singing behavior for a month. A conventional diagram describing the social rank was prepared. In most cases the relative position was clear, but in some cases the birds may have been tied for position, and in other cases no contests were observed.

On 15 Jan. a series of injections of graded doses of testosterone was begun, to determine the effect of testosterone on the seminal vesicle (*2*). The dosage was not known to the observer. The rank of the individuals did not change during a period of 10 days. The birds that were injected with control material remained in their rank. The birds that received the highest amounts of testosterone were sixth and ninth in rank even at the end of the 10-day period. It was suspected that three of the birds might have some testicular tissue because their bills remained yellow. These birds ranked first, third, and eighth and, on autopsy, were found to have some tissue.

Because these results were the gleanings from another experiment, a program was specifically planned. Five birds were castrated 2 Feb. and were observed until 11 Mar. A rank was obvious, and song continued vigorously. Injections of testosterone (begun 11 Mar.) at various dosages had no effect on rank. On autopsy, on 21 Mar., one bird (second in rank) had 25 mg of testicular issue, but the top-ranking bird had none.

These results demonstrate that castrated male starlings maintain a rank, as do normal birds. Since the aggressiveness of these adults might be the result of learning, experiments with young birds are planned. However, the aggressiveness might result from androgen from another source. But the threshold of response would have to be below the level that controls bill color and growth of seminal vesicles because castrated birds have black bills and minute seminal vesicles. Furthermore, the fact that injections of large amounts of testosterone did not alter rank indicates that androgens are not involved. The aggressiveness might be responsive to another hormone, such as a hypophyseal hormone, since Witschi (*2*) concluded that, in some birds, plumage changes are controlled by gonadotropins. This possibility is being explored.

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References and Notes

1. This work was conducted under a grant from the National Institute of Mental Health.
2. A description of this work is in preparation.
3. E. Witschi, *Mem. Soc. Endocrinol.* 49, 149 (1955).

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Nature of Fluorophore Localizing in Tetracycline-Treated Mouse Tumor

It has been previously observed that certain chemical agents such as fluorescein (*1*) and hematoporphyrin (*2*), when administered parenterally to tumor-bearing animals, tend to localize in the tumor tissue. This phenomenon finds limited clinical applications in the localization and diagnosis of neoplastic diseases (*3*). The fluorophore in the tumor tissue was usually assumed to be the unchanged compound administered, without, however, inquiry being made into its exact chemical nature.

Recently, Rall *et al.* (*4*) reported that, in animals bearing transplantable tumors, localized fluorescence was noted in the bones and the tumor tissue after treatment with any of the tetracyclines. The discovery aroused considerable interest in that a variety of animal tumors as well as a few human neoplasms exhibited this behavior. In addition, the localized fluorescence persisted as long as the animals survived (*5*).

In view of the sustained interest in the problem and the obvious chemotherapeutic possibilities implied, an effort has been made in our laboratory to study the chemistry of the fluorophore in the tetracycline-treated mouse tumor. In this report, evidence is presented to show that the localized fluorescence is attributable to unchanged tetracycline which, however, probably does not exist as such in the tumor tissue, but rather as a loose complex bound with a peptide which is one of the normal constituents of mouse sarcoma tissue.

CAF_1 mice weighing 20 to 24 g with 6-day-old sarcoma S-37 were injected in-