It is known that if both cuprous and cupric ions are present in solution, the radioactivity cannot be localized at either ion, regardless of which ion is originally tagged (*13*).

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## Thermodynamic Analysis of the Intracellular Osmotic Gradient Hypothesis of Active Water Transport


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The problem of transporting water from a solution of high osmolarity to one of low osmolarity has engaged the attention of biologic investigators for many years. The intracellular gradient hypothesis, and the mechanism for the maintenance of the gradient, formulated in detail by Franck and Mayer (*1*), seemed to us, on superficial examination, to be a reasonable working hypothesis. An alternative mechanism for the maintenance of osmotic gradients involving the flow of electric current through ion-selective circuits was considered (*2*). Detailed examination of the implications of such a system brought to light certain fundamental objections. It became apparent that such objections applied with equal validity to any intracellular osmotic gradient scheme.

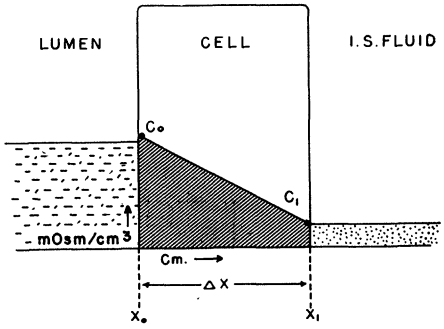


Fig. 1. Scheme of an intracellular osmotic gradient. The osmotic activity $C$ is plotted as a function of cell thickness $\Delta X$.

To illustrate the analysis, consider the application of the osmotic gradient hypothesis to the process of formation of a hypertonic urine. When (Fig. 1) the intracellular osmotic activity at the lumen side of the cell, $C_0$, is slightly higher than that of the lumen fluid, and the intracellular osmotic activity at the interstitial fluid side of the cell, $C_1$, is equal to that of the interstitial fluid, water could be transported from the lumen to the interstitial fluid. Consider the analysis applied to those cells concerned with active water transport, under conditions where the gradient is maintained but in which no water is being transported, that is, $C_0$ is equal to the osmotic activity of the lumen fluid and $C_1$ is equal to that of the interstitial fluid. The results of analyses applied to both a flat-sheet and a tubular arrangement of water-transporting cells were of a similar order of magnitude. For simplicity the flat-sheet arrangement of cells was chosen for presentation. The number of solute particles diffusing from the lumen side of the cell to the interstitial side must be equal to the number of solutes transported in the opposite direction by the mechanism maintaining the gradient. Under such steady-state conditions, the number of solute particles transported can be estimated from the integrated form of Fick's equation,

$$Q_0 = -D(C_1 - C_0)/\Delta X, \qquad (1)$$

where $Q_0$ is equal to the number of osmols per square centimeter per second diffusing from $X_0$ to $X_1$, $D$ is the diffusion constant, and $\Delta X$ is equal to the cell thickness. During the production of a hypertonic urine, reasonable values for the parameters in Eq. 1 are $C_0 = 1.5$ mOsm/cm$^3$, $C_1 = 0.3$ mOsm/cm$^3$, $\Delta X = 2 \times 10^{-3}$ cm, and $D = 2.0 \times 10^{-5}$ cm$^2$/sec. Upon substitution of these values in Eq. 1 and conversion of the units, it is found that $Q_0$ is equal to $4.3 \times 10^{-2}$ osmol/cm$^2$ hr.

The rate of change of free energy for the diffusion process may be evaluated by using the well-known formula

$$\delta(\Delta F)/\delta t = -Q_0 RT \ln(C_0/C_1), \qquad (2)$$

where $\Delta F$ is the change in free energy, $t$ the time, $R$ the gas constant, and $T$ the absolute temperature. Since diffusion is an irreversible process, the free-energy decrease of the diffusion process cannot be funneled back into the transporting mechanism. Substitution in Eq. 2 for a temperature of 37°C gives a value of $4.3 \times 10^{-2}$ kcal/cm$^2$ hr, which represents the minimum rate of expenditure of free energy for the uphill transport of the solutes.

To calculate the rate of change of free energy per unit volume, a specific gravity of 1.0 for cells was assumed. The minimum rate of expenditure of free energy that is required to maintain the gradient is then found to be 21,000 kcal/kg hr, which is approximately 1000 times the maximal rate for living cells.

The discrepancy becomes all the more apparent in considering the fact that a reasonable value for the efficiency of most biological mechanisms is about 20 to 30 percent.

This analysis renders untenable the Franck-Mayer hypothesis for the maintenance of the osmotic gradient. However, it does not rigorously exclude an osmotic gradient hypothesis for the transport of water if (i) the water-transporting cells have a metabolic rate more than 1000 times greater than the maximal value reported for mammalian tissues, (ii) a gradient-maintaining mechanism can be found that would be capable of funneling energy from a high fraction of the total cellular mass to a few milligrams of water-transporting mass, (iii) there were cells of inordinate thickness ($\Delta X > 6$ cm), or (iv) there existed in cells small solute particles of high osmotic activity with a much smaller diffusion constant than that recorded for the largest protein molecule. The analysis will be presented in detail elsewhere (*3*).

### References and Notes


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## A Study of Leucine Biosynthesis in *Torulopsis utilis*


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As part of a study of biosynthetic mechanisms, the formation of the branched chain amino acids, valine, isoleucine, and leucine in *Torulopsis utilis* is under investigation using isotope tracer methodology. Data are presented in this paper (*1*) that indicate that the leucine carbon chain consists of an acetyl group attached to the isobutyryl moiety of valine. In previous studies (*2*), results of experiments were reported in which *T. utilis* was grown on glucose as the principal carbon source in the presence of variously $C^{14}$-labeled acetates and lactates. Valines and isoleucines were isolated from the yeast cells, they were degraded by chemical procedures, and the individual carbon atoms were assayed for radioactivity. Based on these findings, mechanisms for the biosynthesis of these amino acids have been proposed (*2*).

Using a degradation procedure similar to that employed for valine and isoleucine, $C^{14}$-distribution patterns were obtained for the leucines isolated from the same experiments; these are given in Table 1. Acetate carboxyl carbon was exclusively and abundantly present in the carboxyl carbon of leucine, and the acetate methyl carbon appeared overwhelmingly in the leucine α carbon. Lactate carbon 1 was incorporated to a negligible extent, hence, degradation was not conducted. Large amounts of lactate carbons 2 and 3 also appeared in the respective carboxyl and α carbons of leucine, a result anticipated on the basis of the ready conversion of lactate carbons 2 and 3 to acetate. However, lactate carbon 2 was also incorporated readily and equally in leucine carbons 3 and 4, and lactate carbon 3 also appeared in leucine carbons 5,5′. The similarity in the distribution of all three lactate carbons in leucine carbons 3 to 5,5′ to that observed previously in carbons 2 to 4,4′ of valine (*2*), shown at the right of the table, leaves little doubt of the common origin of the isobutyryl moieties of both amino acids. Gilvarg and Bloch (*3*) and Ehrensvard *et al.* (*4*) also found that acetate carboxyl and methyl carbons were incorporated into leucine biosynthesized by yeast. Adelberg (*5*) reported learning, in a private communication from Ehrensvaard, that yeast grown on acetate as the sole carbon source yielded valine and leucine with the same isotope distribution in their isobutyryl moieties. Abelson, (*6*) recently showed that pyruvate, α-ketoisovalerate and L-valine all lowered the specific activities of leucines synthesized by *Escherichia coli* from uniformly labeled glucose. He suggested that these substances are intermediates of leucine biosynthesis, and further suggested that α-ketoisovalerate combines with acetate to yield the

Table 1. Pattern of $C^{14}$ distribution in leucine and valine. Values are based on standard dosage of 100 μc administered.

| Substrate | Specific activity of leucine | Percentage of total activity in leucine carbon | | | | | Specific activity of valine | Percentage of total activity in valine carbon | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5,5′ $(CH_3)_2$ | 4 CH | 3 $CH_2$ | 2 $CHNH_2$ | 1 COOH | | 4,4′ $(CH_3)_2$ | 3 CH | 2 $CHNH_2$ | 1 COOH |
| Acetate-1-$C^{14}$ | 14,040 | 0 | 0 | 0 | 1 | 99 | | | | | |
| Acetate-2-$C^{14}$ | 21,380 | 3 | 1 | 2 | 89 | 2 | | | | | |
| Lactate-1-$C^{14}$ | 156* | | | | | | 12,080 | 0 | 0 | 1 | 99 |
| Lactate-2-$C^{14}$ | 25,630 | 2 | 32 | 33 | 1 | 31 | 20,670 | 1 | 47 | 49 | 3 |
| Lactate-3-$C^{14}$ | 21,540 | 59 | 1 | 2 | 37 | 1 | 14,730 | 91 | 4 | 4 | 1 |

* This leucine sample was not degraded.