Studies on the crystalline lens.

XXI. Bidirectional carrier-mediated transport of lithium*, **

V. Everett Kinsey and Ian W. McLean

Movement of lithium both into and out of cultured rabbit lenses occurs by processes that obey Michaelis-Menten kinetics as well as by a nonsaturable process. The parameters describing the kinetics of transport are evaluated on the basis of a modified version of a pump-leak hypothesis that was shown previously to account for the fluxes of other alkali metal cations. Potassium is a potent competitive inhibitor of the carrier-mediated transport of lithium into the lens. Lithium weakly inhibits the influx of potassium by a noncompetitive process. The observation that lithium is a weak inhibitor of potassium transport (Ki = 70 mM) while having a moderate affinity for its carrier (Km = 4.0 mM) suggests that more than one site may be responsible for the transport of potassium and lithium into the lens. Active transport of lithium out of the lens does not appear to involve the sodium pump, since the rate of sodium efflux is unaffected by lithium. Both influx and efflux of lithium are inhibited by ouabain (10-5 M) but not by amiloride (10-4 M) or oxytocin (20 milliunits per milliliter). Lithium displaces proportionate amounts of sodium and potassium from intracellular fluid when lenses are cultured in the presence of this cation. The nonsaturable exchange of lithium is independent of observed differences in electric potential, indicating that lithium does not permeate the lenticular membranes by simple diffusion of lithium ions, but rather as a complex with either a free anion or a carrier within the membrane. The affinity of lithium for the carrier is essentially equal to that of cesium and much lower than that for rubidium, whereas the kd of both lithium and rubidium is much higher than that for cesium. These differences indicate that a common pathway cannot be responsible for the discrimination between cations by both the pump and the leak.

Key words: alkali metal cations, carrier systems, cation pumps, cation transport, diffusion, lens, lithium, lithium transport, membrane permeability, transport kinetics.

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**In conducting the research described in this report, utilizing animals, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.
Transport of potassium, rubidium, cesium, and more recently, thallium (Tl⁺), has been characterized on the basis of a mathematical model of a pump-leak system in which the quantity of a substance entering or leaving by carrier-mediated processes, located primarily in the epithelium, is balanced by diffusion of the substance in the opposite direction across the surface membranes. This was accomplished by fitting theoretical curves to experimental data showing movement of these ions into and out of lenses in the presence of various concentrations of each of the ions. Values of the parameters governing the pump led to the conclusion that all these cations are transported into the lens by the same carrier having a single active site, the order of preference for this site being Tl⁺ > K⁺ ~ Rb⁺ > Cs⁺. Values of the permeability coefficient of the lens for the ions suggest that diffusional exchange occurs at rates dependent on relative absorptive forces associated with the anionic field strength of the membranes rather than on frictional forces dependent on size of the ions in their hydrated states.

Movement of sodium into and out of the lens was also shown to take place according to postulates of the pump-leak system, but in a reverse direction from that for potassium, thus sodium enters the lens by diffusion and leaves primarily by a carrier-mediated system which, like that of the other cations studied, appears to be either identical or closely related to Na-K ATPase. There is no evidence for significant cell-mediated transport of sodium into the lens or of potassium or amino acids out of it.

The present paper is concerned with movement of lithium into and out of the lens. To the authors' knowledge, this subject has not been investigated previously. In some tissues lithium behaves like sodium, while in others it has a potassium-like action. For example, following systemic administration, the concentration of lithium is higher in kidney, muscle, and hair, and lower in liver and red blood cells than in serum, suggesting that, like sodium, it may be pumped out of some cells, and like potassium, pumped into others. The results of the present investigation will show that lithium movement in the lens is unique in that it is transported by carrier-mediated processes both into and out of the lens. Moreover, unlike other cations studied, the rate of diffusion is incompatible with a model that assumes it is affected by the electric gradient existing between the medium and the interior of the lens.

### Methods

#### Mathematical

The theoretical model of the pump-leak system used to evaluate rate constants of parameters for the fluxes of lithium is evaluated on the basis of a three-compartment system, as previously described for sodium. The capsule is considered to be in equilibrium with the medium after the first minute or two. The compartments are the lens capsule, which contains approximately 6 per cent of the lens H₂O, intracellular space (fibers), and medium.

The rate of change in concentration of lithium in intracellular water is described by Equation 1.

\[
\frac{d[L]_p}{dt} = K_{p_{in}} [L]_M - K_{p_{out}} [L]_F + P \beta ([L]_M \alpha - [L]_F)
\]

where \(K_{p_{in}}\) and \(K_{p_{out}}\) are transfer coefficients for active transport (pumps) across ion-restricting membranes. \(P\) is the permeability coefficient of these membranes for lithium, \(\alpha\) and \(\beta\) are coefficients that depend on the electric potential difference, \(E\), between the medium and the inside of the lens and are equal to

\[
\frac{\sigma (nFE/RT)}{1 - \sigma}
\]

respectively, under the assumption of a constant electric field. \(P\beta = Kd\), the transfer coefficient for diffusion, and \(n\), \(F\), \(R\), and \(T\) have their usual significance, where \(n\) bears the sign of the formal charge.

Change in concentration of lithium in the medium during the course of the experiment is described by Equation 2.

\[
\frac{d[L]_M}{dt} = - \frac{Vol_F}{Vol_M} \cdot \frac{d[L]_F}{dt}
\]

The transfer coefficient \(K_{p_{in}}\) for lithium will be shown to be dependent on concentration of both lithium (substrate) and potassium (competitive...
inhibitor) in the capsule, and is described by Equation 3.

$$K_{p, n} = \frac{V_{\text{out}, n} K_i}{V_{\text{OIF}} (K_{m, n} K_i + K_i [Li]_M + K_{m, n} [K]_M)}$$ (3)

The transfer coefficient $K_{\text{p, out}}$ is dependent on the intracellular concentration of lithium and is described by Equation 4.

$$K_{\text{p, out}} = \frac{V_{\text{max, out}}}{V_{\text{OIF}} (K_{m, \text{out}} + [Li]_P)}$$ (4)

The inhibitory effect of lithium on transport of potassium was determined indirectly using $^{86}$Rb instead of $^{42}$K because of the longer half-life of $^{86}$Rb. The results are believed to be applicable to potassium, since rubidium is transported by the same carrier as potassium and has an identical $K_m$.

Equation 5 describes the kinetics involved.

$$\frac{d[^{86}\text{Rb}]_L}{dt} = K_p [^{86}\text{Rb}]_L + \frac{P}{V_{\text{OIF}}} ([^{86}\text{Rb}]_L - [^{86}\text{Rb}]_M)$$ (5)

Equations 6 and 6a define $K_p$ for rubidium where lithium is considered to act as a non-competitive and competitive inhibitor, respectively. In both equations, potassium is assumed to be a competitive inhibitor with a value of $K_i$ equal to $K_m$ for rubidium. Accordingly, $K_i$ for potassium factors out and the equation reduces to:

$$K_p = \frac{V_{\text{max}} K_i}{V_{\text{OIF}} (K_m K_i + K_i [K]_M + K_m [Li]_M + [K]_M [Li]_M)}$$ (6)

$$K_p = \frac{V_{\text{max}} K_i}{V_{\text{OIF}} (K_m K_i + K_i [K]_M + K_m [Li]_M)}$$ (6a)

Change in concentration of $^{86}$Rb in the medium is described by Equation 7.

$$\frac{d[^{86}\text{Rb}]_M}{dt} = -\frac{V_{\text{OIF}}}{V_{\text{OIF}}} \cdot \frac{d[^{86}\text{Rb}]_L}{dt}$$ (7)

Bioelectric potentials of lenses cultured for 20 hours are shown as a function of concentration of lithium in the media (Fig. 1), and as a function of time when cultured in the presence of 100 mM lithium (Fig. 2) with and without $10^{-5}$ M ouabain. The potentials were determined at 37°C with glass microelectrodes by methods described elsewhere.\footnote{Confirmed experimentally for one level of lithium by determining rates of accumulation of $^{86}$Rb and $^{86}$Rb separately in the presence of 50 mM concentrations of lithium. Values for $C_1/C_0$ at 47 hours were 7.4 and 7.8 compared with 11.1 and 11.2 in the absence of lithium, all respectively.}

Mean values of $\alpha$ and $\beta$, calculated on the assumption that the time course for changes in lens potential for all concentrations of lithium is the same as that observed with 100 mM (Table I), were used in solving all equations.

### Table I. Mean values for $\alpha$ and $\beta$ for 20 hour culture period

<table>
<thead>
<tr>
<th>[Li] mM</th>
<th>$\alpha$</th>
<th>$\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.8</td>
<td>0.40</td>
</tr>
<tr>
<td>5</td>
<td>4.6</td>
<td>0.41</td>
</tr>
<tr>
<td>10</td>
<td>4.5</td>
<td>0.42</td>
</tr>
<tr>
<td>20</td>
<td>4.4</td>
<td>0.43</td>
</tr>
<tr>
<td>50</td>
<td>4.1</td>
<td>0.45</td>
</tr>
<tr>
<td>100</td>
<td>3.8</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Knowledge of the concentration of potassium in the medium is needed in order to solve equations describing movement of both lithium and rubidium. This was provided by solving equations which describe movement of potassium between the lens and medium, simultaneously with Equations 5 to 7 by the method of successive approximations as employed previously.\footnote{Values for the unknown parameters in equations 1 to 4 were also determined by successive approximation by first obtaining a rough estimate of the value of $P$ for lithium. It was assumed that the tangent of the initial slope of a curve showing rate of accumulation of 20 mM lithium in lenses cultured in the presence of $10^{-4}$ M ouabain is a reasonable measure of permeability, since ouabain at this concentration was found to suppress most of the carrier-mediated transport. Equations 1 to 4 were then solved for fluxes of lithium using this value of $P$ (0.10 hr$^{-1}$) and different values for the unknown parameters involving the pumps. In this manner, curves were generated that showed rates of influx and efflux of lithium when present in various concentrations both intra- and extracellularly. A set of values was found that would produce fits to the influx data within one standard deviation, but not to the efflux data. Another set, using a different value of $P$, provided similarly good fits to the efflux data, but the curves generated by this set no longer agreed with those for accumulation of lithium. No single set of values could be found that would fit both fluxes simultaneously, indicating that the theoretical model, described by Equation 1, does not correctly predict rates of diffusional exchange of lithium in the lens.}

A search for the source of the deficiency of the model revealed that the value, $P'$, for the rate of diffusional influx which had been found to give the best fit to data for lithium accumulation, was identical with that which had been observed to fit the efflux data, $P$. This observation suggests
that the rate of diffusion is not affected by the
electric gradient. To test this possibility, and also
to employ a model that more accurately describes
diffusional flux of lithium when evaluating the
parameters governing active transport systems, all
theoretical curves shown in the figures were
calculated on the basis of a modified mathematical
model which assumes that $\alpha$ and $\beta$ are both equal
to 1.0.

Rates of movement of potassium and rubidium,
however, were calculated from values of $\alpha$ and $\beta$
based on observed potential differences, since they
were found previously to be predicted accurately
by a theoretical model which assumes that dif-
fusional exchange depends on the electric gra-
dient.\(^1\) It should be noted that none of the con-
clusions of the present study would be altered
appreciably had the calculations been based on
the assumption that movement of potassium and
rubidium, like lithium, is also unaffected by
charge.

A digital computer using an iterative procedure
was employed in making all calculations.

Experimental. Lenses were removed posteriorly
from enucleated eyes of young adult albino rabbits
(2 kilograms) that were killed by injecting air
into the heart. Zonules were severed close to the
ciliary body as described elsewhere.\(^1\) Lenses were
transferred to tubes where they were cultured
aseptically by the method of Merriam and Kinsey\(^8\)
in 5 ml. of medium having a composition similar
to that of aqueous humor (KEI-4).\(^8\) In the
absence of a usable radioisotope of lithium ($^{6}$Li
has a half-life < 1 second), nonlabeled lithium,
as LiCl, was employed and substituted for equiv-
alent concentrations of NaCl. Following culture,
lenses were blotted gently on filter paper moistened
with medium, weighed, and homogenized in 10
per cent trichloracetic acid (TCA). Concentration
of lithium, and in some experiments, sodium and
potassium, was determined in the supernatant
fluid by flame photometry using an Hitachi Perkin-
Elmer instrument. Accumulation of lithium in the
lens is expressed as a ratio of concentration in lens
water (65 per cent of wet weight) to that in the
medium at beginning of culture.

Efflux of lithium from lens fibers was found
to occur exponentially with time and was estimated
by preloading lenses for 20 hours in media con-
taining 5 mM potassium and different concentra-
tions of lithium, following which they were cul-
tured for additional periods up to 24 hours in a
lithium-free medium (KEI-4). The amounts of
lithium in the medium and lens were then deter-
mined from which the percentage of lithium lost
was calculated.

Rate of accumulation of $^{86}$Rb in lenses was
measured by assaying for radioactivity present in
TCA extracts of lenses using an end-window flow-
gas counter. The ratios of concentration of $^{86}$Rb,
in the presence of various concentrations of lithium. The methods employed for both procedures are described in detail elsewhere.1

The ratio of concentration of lithium, lens/media, under steady-state conditions was obtained by culturing lenses for 20 hours in medium containing 20 mM lithium; fresh media containing lithium in lower concentrations were then substituted and lenses were cultured for an additional 24 hours. In this way, the ratio of concentration at the start of the second period of culture could be adjusted by trial and error to approximate that prevailing at steady-state.

Results

Results of experiments showing movement of lithium into and out of lenses under varying circumstances are presented in Figs. 3 to 6. All lines are calculated with a digital computer programmed to solve Equations 1 to 4 which describe accumulation and net efflux of lithium using methods of successive approximation until theoretical curves are generated that fit the data obtained experimentally. The values for the parameters that provide fits shown in the figures are summarized in Table II.

Rate of accumulation of lithium in lenses cultured in medium containing 20 mM lithium and 5 mM potassium, and rate of efflux from lenses preloaded with lithium to a concentration of approximately 20 mmoles per liter are shown by the solid lines of Fig. 3. Broken lines in the figure
show the inhibiting effect of $10^{-5} \text{ M}$ ouabain on the rates of accumulation and efflux of lithium under otherwise similar conditions.

Accumulation of lithium in lenses cultured for 20 hours as a function of concentration of lithium in the media with potassium (5 mM) and without added potassium, is shown in Fig. 4 by the solid lines composed of filled and open circles, respectively. The broken line shows the added effect of $10^{-5} \text{ M}$ ouabain on accumulation of lithium in the presence of 5 mM potassium. The effect of various concentrations of potassium on the accumulation of lithium when present in the medium in a concentration of 5 mmoles per liter for a 20-hour period, is shown in Fig. 5. The results indicate that transport of lithium into the lens takes place by a process that can be saturated by both lithium and potassium. Agreement of the theoretical curves with the experimental data indicates that lithium transport obeys Michaelis-Menten kinetics and that potassium acts as a competitive inhibitor for the carrier system involved.

The percentage of lithium lost from lenses after 24 hours is decreased significantly when concentration of intracellular lithium is increased (Fig. 6, solid line), and is further reduced when ouabain ($10^{-5} \text{ M}$) is added to the media, as shown by the broken line. These results indicate that lithium is transported out of the lens by a process that is also carrier-mediated and sensitive to ouabain.

The data in Fig. 7 show the inhibitory effect of lithium on accumulation of rubidium with and without inclusion of 5 mmoles per liter of potassium in the media (open and filled circles, respectively). The solid lines are calculated on the basis of a noncompetitive model, Equations 5 and 6, with a $K_i$ for lithium equal to 70 mM, whereas the broken lines are calculated on the assumption that inhibition is competitive, Equations 5 and 6a, with a $K_i$ equal to $K_m$ for lithium, namely, 4.0 mM. Only the curves corresponding to the noncompetitive model fit the experimental observations. It was not possible to fit both sets of data with any value of $K_i$ for lithium when calculations were based on a model involving competitive inhibition.*

Lithium displaces proportionate quantities of sodium and potassium from the

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*Values of $P$ for rubidium used to solve Equation 5 for accumulation of $^{86}$Rb were determined experimentally from measurements of the rate of efflux of $^{86}$Rb and found to increase from 0.06 to 0.10 hr$^{-1}$ with concentration of lithium from 0 to 50 mM in the presence of 5 mM potassium, and from 0.03 to 0.08 hr$^{-1}$ in the absence of added potassium. Similarly, $P$ for potassium also increased with the concentration of lithium in the medium, a factor which was taken into account when calculating the change in concentration of potassium in the medium. Values of $K_m$ and $V_{max}$ employed for calculating rubidium accumulation were 1.0 and 1.1, respectively. The value for $V_{max}$ is slightly, but probably not significantly, higher than that found several years ago which provided fit to experimental data on rubidium transport.
intracellular fluid of lenses cultured for 20 hours in the presence of varying concentrations of this cation, while the total cation concentration remains approximately constant (Fig. 8).

Results of observations designed to estimate the steady-state concentration of lithium in the lens are shown in Fig. 9. Lenses were first cultured for 20 hours in medium containing 20 mM lithium, and then in media containing lithium in reduced concentrations, as indicated on the graph. Values for the ratios, $C_L/C_M$, at the start of the second culture period are calculated on the assumption that the intracellular concentration is 20 mmoles per liter (Fig. 3). The solid lines are visual fits and the broken lines are extrapolations to presumed steady-state conditions, estimated to be 2.55 when lithium is present in the medium in a concentration of 8 mM. The corresponding ratio for intracellular concentration, $C_L/C_M$, is 2.65. The steady-state ratio will vary for other concentrations, since rates of influx and efflux of lithium are both concentration dependent.

**Discussion**

The results of this investigation show that lithium is transported into and out of rabbit lenses by carrier-mediated processes (pumps) that obey Michaelis-Menten kinetics, as well as by a non-saturable process. The parameters that describe the kinetics involved have been evaluated by fitting theoretical curves, calculated on the basis of a modified version of the pump-leak hypothesis, to experimental data showing lithium fluxes under a variety of conditions. Despite the numerous factors controlling lithium fluxes the computed curves, in almost all instances, agree with the data within one standard deviation. Moreover, when values for the parameters representing both of the pumps and the leak are employed to calculate the steady-state ratio of lithium between intracellular fluid and medium at a particular concentration, 8 mM, the resultant value is in approximate agreement with that found experimentally, namely, 2.8 vs. 2.65. This difference may be due in part to lens damage resulting from the prolonged period of culture required in making the measurements.

Previous studies of the active influx of potassium, rubidium, cesium, and thallium show that the affinity of the carrier, $K_m$, for each ion is equal to the $K_i$ for each ion inhibiting transport of each of the other ions. This was interpreted to mean that all the ions compete for a single site on the carrier. The present study shows that the carrier responsible for movement of lithium into the lens is inhibited competitively by potassium, and that the value of $K_i$ is equal to its $K_m$ (1.1 mM), which suggests that lithium may also be transported by the same carrier as the other cations. However, observations of the effect of lithium on accumulation of rubidium (Fig. 7), which is assumed to be identical with potassium, do not support this contention. They indicate, rather, that lithium is a poor and
probably noncompetitive inhibitor for potassium with a Ki of 70 mM. If lithium and potassium were transported by the same site, the values of Ki and Km for lithium would be equal, i.e., 4 mM, and inhibition would be competitive, which is inconsistent with the results as shown in Fig. 7. Thus, either multiple sites on a single carrier or different carriers appear to be responsible for transport of potassium and lithium into the lens.

Results of the present study do not indicate which alternative is correct, but the following observations lend credence to the first possibility. Lithium is known to substitute for potassium in the activation of Na-K ATPase, the presumed carrier of the other alkali metal cations, as well as thallium. The rank order of Michaelis-Menten constants for activation of this enzyme is the same as the Km values for transport coefficients of these cations into the lens, Tl⁺ < K⁺ ≈ Rb⁺ < Cs⁺ < Li⁺. Moreover, lithium transport, like that of other cations, is depressed by ouabain which inhibits Na-K ATPase. Evidence that the ATPase molecule in the erythrocyte has at least two potassium sites is supplied by the observation that two ions of potassium and three ions of sodium are transported in erythrocytes for each molecule of ATP consumed. Although ATP consumption in the lens relative to potassium transport has not been measured, the ratio of potassium transported to that of sodium is also 2:3.

Further evidence that two sites on a carrier molecule are involved in cation transport, including lithium, in erythrocytes is provided by Sachs and Welt. They showed that rates of active potassium influx, observed with varying concentrations of potassium, rubidium, cesium, and lithium in the external media, can be described by a model in which cations must be present simultaneously at two sites on a carrier. These investigators suggested that rubidium, cesium, and lithium are transported into erythrocytes by competing for these sites which may be identical with the two potassium sites on the Na-K ATPase molecule.

The maximum rate of active transport of lithium out of the lens is much less than that in the opposite direction (V_max =

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**Fig. 8. Cation distribution in intracellular fluid of lenses after 20 hours of culture in media containing various concentrations of lithium.**

**Fig. 9. Estimation of steady-state concentration of lithium in lenses cultured for 20 hours in media containing 20 mM lithium, after which they were cultured for 24 hours in fresh media containing concentrations of lithium shown on the face of the graph.**
0.065 vs. 0.50 μmoles per lens hr.\(^{-1}\), and when the pump is operating at maximal capacity it has approximately \(\frac{1}{50}\) the rate of active transport of sodium out of the lens under physiologic conditions.\(^4\) In other tissues, too, lithium is either pumped out relatively slowly\(^{10-18}\) or not at all.\(^{19-20}\) Keynes and Swan\(^{18}\) concluded from observations on frog muscle fibers that lithium is actively transported by the same mechanism as sodium, but only about \(\frac{1}{10}\) to \(\frac{1}{25}\) as rapidly.

While lithium appears to share the active transport system responsible for influx of potassium into the lens, it is improbable that active transport of lithium out of the lens involves the sodium pump. Thus, the rate of efflux of sodium, like that in barnacle muscle fibers,\(^{17}\) is unaffected by intracellular concentrations as high as 50 mmoles per liter of lithium,\(^{1'}1\) and the concentration of sodium in the lens decreases rather than increases with increasing concentrations of intracellular lithium (Fig. 8). The theoretical model shown previously to describe cation fluxes in the lens\(^1', 2, 4, 6\) assumes that passive diffusion of these ions is dependent both on the chemical and electric gradient. However, the rate constants for nonsaturable components of influx and efflux of lithium, \(K_{dx}\) and \(K_d\), are equal, indicating that they are unaffected by the electric gradient, and thus the fluxes are not the result of simple diffusion of positively charged lithium ions. These observations suggest that lithium permeates the ion-restricting membranes of the lens as a complex with either a free anion or a carrier within the membrane.

Wieth\(^{22}\) postulated that lithium could permeate the plasma membrane of erythrocytes as LiCO\(_3\)^-. He based this conjecture on the observation that passive accumulation of lithium occurred more rapidly from a medium containing a high concentration of bicarbonate than from one with a low concentration. If this hypothesis were to apply to the lens, both LiCO\(_3\)^- and Li\(^+\) would have to permeate the ion-restricting membranes, since permeation of LiCO\(_3\)^- by itself would also be affected by the electric gradient. The alternate possibility that lithium may penetrate as dissociated LiHCO\(_3\) appears unlikely, since so far as we can ascertain, LiHCO\(_3\), at least as a solid, does not exist.

According to Glynn,\(^{23}\) passive efflux of potassium from the erythrocyte also appears to take place in part by a pathway other than simple diffusion. Contrary to what was postulated earlier for the lens,\(^1\) it may be that the nonsaturable component of potassium movement, like that of lithium, is also more complicated than simple diffusion.

None of the effects on ion fluxes appears to result from gross metabolic changes in the lens, since glucose consumption of lenses cultured for 20 hours in media containing either 20 or 100 mmoles per liter of lithium is indistinguishable from normal. Ouabain (10\(^{-5}\) M) inhibits active transport of lithium, both into and out of the lens, by approximately 70 per cent. This effect was also noted with other alkali metal cations\(^1\) and with thallium,\(^4\) and is identical in amount with that observed for efflux of sodium.\(^4\) Neither amiloride (10\(^{-5}\) M) nor oxytocin (20 mUnits per milliliter) has an appreciable effect on movement of lithium in either direction.

This study of the kinetics of lithium transport in rabbit lenses completes an investigation designed to characterize transport of all the alkali metal cations,\(^1, 4\) and thallium (Tl\(^+\)) which, in certain respects, behaves like the Group 1 metals. Ion fluxes have been described quantitatively in terms of the carrier-mediated mechanisms responsible for active transport (pump), and nonsaturable processes concerned with passive diffusion (leak). Values for rate coefficients of all the cations investigated are summarized in Table III, and values for the apparent Michaelis-Menten constants and maximum velocity of the carriers responsible for active transport are shown in Table IV.

The parameters for all cations, except those relating to lithium, were evaluated by fitting theoretical curves to experimental
observations on the assumption that the rate of passive diffusion depends on the electric as well as the chemical gradients. To the extent that these rates may, like lithium, be independent of the bioelectric potential, the values for $K_p$ for those ions that are actively transported into the lens would be higher than indicated in Table III. For instance, $K_p$ for potassium would increase from 0.82 to 0.98 hr.$^{-1}$ if passive influx of potassium was not affected by the electric gradient. On the other hand, the value of $K_p$ for sodium, which ion is actively pumped out of the lens, would be reduced from 0.58 to 0.55 hr.$^{-1}$ if diffusional exchange was independent of the electric gradient.

Values for the rate coefficients concerned with active transport of rubidium, cesium, and thallium were derived from data obtained from lenses cultured in a physiologic medium (KE1-4) containing only trace quantities of cations other than sodium and potassium. Values for the lithium pumps were also calculated, using Equations 3 and 4, on the assumption that the concentration of lithium is essentially zero in both the medium and lens; potassium was considered to inhibit lithium transport only in an inward direction.

The rank order of the carrier in selecting between thallium, potassium, rubidium, cesium, and sodium, as measured by $K_p$ for the in-pump, and the rank order of permeability of the ion-restricting membrane, as measured by $K_d$, are identical (Table III). However, the affinity of lithium for the carrier, assumed to be $1/K_m$, is essentially equal to that of cesium, and much lower than that for rubidium, whereas the $K_d$ of both lithium and rubidium is much higher than that for cesium (Table IV). These differences suggest that the same process is not responsible for the selectivity of both the in-pump and the leak, at least where lithium is concerned.

Eisenmann has proposed that the selectivity of the biological membranes controlling both active and passive transport is dependent on the interaction of Coulomb forces between cations in aqueous solution and anionic groups within the membrane. This model predicts that the transport of lithium by both the in-pump and the leak, relative to the other cations, would be lower than that observed in the lens. Diamond and Wright in a review article, observed that biological systems frequently discriminate against lithium less sharply than predicted by the model based on Coulomb forces. Ling has proposed a model which includes non-Coulomb effects as well as those due to Coulomb forces, which might provide an explanation for the more rapid movement found for lithium in the present experiments. An alternative explanation for the rapid exchange of lithium is that there are additional pathways that favor transport of lithium. This

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**Table III. Coefficients of active transport and diffusion for alkali cations and Tl$^+$ under approximately physiologic conditions**

<table>
<thead>
<tr>
<th>Cation</th>
<th>$K_p$ (hr.$^{-1}$)</th>
<th>$K_d$ (hr.$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium</td>
<td>0.11</td>
<td>0.023</td>
</tr>
<tr>
<td>Sodium</td>
<td>nil</td>
<td>0.0095</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.58</td>
<td>0.039</td>
</tr>
<tr>
<td>Rubidium</td>
<td>0.82</td>
<td>0.052</td>
</tr>
<tr>
<td>Cesium</td>
<td>0.27</td>
<td>0.010</td>
</tr>
<tr>
<td>Thallium</td>
<td>2.25</td>
<td>0.105</td>
</tr>
</tbody>
</table>

Rank order, in-pump $Tl > K \approx Rb > Cs > Li > Na$.
Rank order, leak $Tl > K > Rb \approx Li > Cs \approx Na$.

*These values are based on the volume of lens water. Values would be increased by 6 per cent if based on volume of fiber water only.

**Table IV. Apparent Michaelis-Menten constants and $V_{max}$ of the carrier for active transport of alkali cations and Tl$^+$**

<table>
<thead>
<tr>
<th>Saturating ion</th>
<th>$K_m$ (mM)</th>
<th>$V_{max}$ (nmoles/hr./lens)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium</td>
<td>4.0</td>
<td>0.50</td>
</tr>
<tr>
<td>Sodium</td>
<td>1.5</td>
<td>0.065</td>
</tr>
<tr>
<td>Potassium</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Rubidium</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Cesium</td>
<td>3.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Thallium</td>
<td>0.15</td>
<td>0.375</td>
</tr>
</tbody>
</table>

Rank order, $K_m$ influx $Tl < K \approx Rb < Cs < Li < Na$. 

Rb < Cs < Li < Na.
hypothesis, as discussed earlier, would be consistent with the observations that the nonsaturable component of lithium transport is independent of the membrane potential and the active component involves multiple sites.

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REFERENCES


