Studies on the crystalline lens

XIII. Kinetics of potassium transport

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The rate of accumulation of ^{42}K in cultured rabbit lenses was measured and the coefficients for the parameters concerned with active transport into, and diffusion out of, the lens were evaluated using an analog computer to establish fits to a simple equation that described a pump-leak system. The turnover rate of potassium in lenses cultured under conditions essentially like those found in vivo was 4.5 to 5.0 per cent per hour, which corresponds to a flux of approximately 1.10 μmoles per lens per hour. The rate of accumulation of ^{42}K in cultured lenses decreased when concentration of nonlabeled potassium in the media was increased, suggesting that a carrier system responsible for active transport had become saturated and thus limited the capacity of the pump. A series of successive approximations, to compensate for the changed concentration of both labeled and nonlabeled potassium in the media during the culture experiments, was employed to establish the effect of concentration of potassium in the media on the velocity of the pump. The data from these experiments were found to obey Michaelis-Menten kinetics: the apparent constant, K_m, and maximum velocity, V_{max}, were 0.85 mmole per liter and 1.3 μmoles per lens per hour, respectively. With these fundamental constants which characterize the carrier system for active transport, a general pump-leak equation was devised. The equation was used to make separate calculations of the quantities of potassium that accumulated and that were actually pumped into lenses under conditions when the concentration of labeled and nonlabeled ion remained constant.

This study was designed to (1) determine the turnover rate of potassium in rabbit lenses, (2) evaluate the apparent Michaelis-Menten constant and maximum velocity of the carrier system responsible for its active transport, and (3) estimate the fraction of maximum capacity at which the potassium pump operates in vivo.

The lens appears to function like a pump-leak system, in which active transport across the epithelium is exactly balanced by outward diffusion, chiefly posteriorly, through the capsule; thus, the concentration of potassium in vivo remains constant.[1] When lenses are cultured in media that contain isotopically labeled potassium, the concentration of the isotope increases with time. In either case, equation 1 describes the process.

$$\frac{dC_l}{dt} = k_p C_m + k_d (C_m - C_l) \quad (1)$$

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k_p = transfer coefficient by active transport (pump), k_d = transfer coefficient by diffusion, and C_m and C_l = concentration of potassium in media and lens water, respectively. The coefficients of transfer have the dimension of reciprocal time; when multiplied by 100, they represent percentage per unit time, which in this study is percentage per hour.

When a batch technique, as used in this investigation, is employed for culturing lenses, the concentration of labeled potassium in the media decreases in proportion to the rate of uptake by the lens, and inversely with the volume of medium employed. The relationships are described by equations 2 to 4

$$-V_m \frac{dC_m}{dt} = V_l \frac{dC_l}{dt} \qquad (2)$$

thus

$$\frac{dC_m}{dt} = -\frac{V_l}{V_m} \frac{dC_l}{dt} \qquad (3)$$

integrating:

$$C_m = C_{m_{t=0}} - \frac{V_l}{V_m} C_l \qquad (4)$$

where V_m and V_l = volume of medium and lens water, respectively. Substituting for C_m in equation 1

$$\frac{dC_l}{dt} = k_p \left(C_{m_{t=0}} - \frac{V_l}{V_m} C_l\right) + k_d \left[\left(C_{m_{t=0}} - \frac{V_l}{V_m} C_l\right) - C_l\right] \qquad (5)$$

C_l = 0 for labeled potassium at time zero; C_l = 125 mmoles per liter[2] for total potassium at time zero.

The turnover rate is equal to k_d, and the velocity of the pump, in μmoles per hour, is equal to $k_p C_{m(total)} V_l$. Knowing the steady state ratio, $\frac{C_l}{C_m}$, the coefficients k_p and k_d can be evaluated for conditions prevailing in vivo by varying their values and solving the pump-leak equation until lines are produced which fit experimental data on rate of accumulation of ^{42}K in lenses cultured in a medium of essentially the same composition as aqueous humor.

The apparent Michaelis-Menten constant, K_m, the maximal velocity of the pump, V_{max}, and the efficiency of the pump in vivo can be determined from data showing the effect of different concentrations of potassium in the media on the velocity of the pump.

Methods

Lenses from albino rabbits weighing between 1.8 and 2.4 kilograms were cultured in 5 ml. of medium, by methods described elsewhere,[3, 4] and the rate of accumulation of ^{42}K* was measured. The average water content of the lenses was 65 per cent of the wet weight, and the average volume of water was 0.20 ml.; the medium KEI-4 contained 4.8 mmoles per liter of potassium.[1] When different concentrations of potassium were employed, equivalent amounts of sodium were either added or withheld to maintain the media isosmotic with the lens.

Accumulation of ^{42}K is expressed in terms of the ratio of concentration of labeled potassium in the lens water to that present in the media at the beginning of culture $\left(\frac{C_l}{C_m}\right)$. In some experiments the concentration of total potassium in the media was determined after lenses had been cultured for 24 hours; analyses were performed by flame photometry.

An analog computer† was used for evaluating the coefficients and for making other calculations to be described later in this paper. When selecting values for k_p and k_d, the boundary condition, i.e., ratio $\frac{C_l}{C_m}$ at steady state for labeled potassium, was assumed to be the same as for the nonlabeled ion, viz., 25, as established by Harris[2]; thus the

*Supplied by Oak Ridge National Laboratory, Oak Ridge, Tenn. At the time of shipment, which took one day in transit, the specific activity of the ^{42}K was over 2,000 mc. per gram. After arrival, all experiments were completed within two days; hence, only a negligible quantity of nonlabeled ion was added to the media containing the labeled ion.

†Manufactured by Applied Dynamics Co., Ann Arbor, Mich.

ratio $\frac{k_p}{k_d} = \left[\frac{C_l - C_m}{C_m}\right]$ or 24.* The ratio $\frac{V_l}{V_m} =$ 0.04, i.e., $\left(\frac{0.2 \text{ ml.}}{5.0 \text{ ml.}}\right)$

Results

The filled circles in Fig. 1 show accumulation of ^{42}K by lenses cultured for different periods of time in KEI-4 medium, as indicated by the legend on the right ordinate. The ascending and descending lines were drawn by the analog computer by solving equation 5 for the values of k_p and k_d shown on the chart.

Most of the data lie close to the lines generated by solving equation 5 for values of $k_d = 0.045$ to 0.050 hour^{-1} and $k_p = 1.08$ to 1.20 hour^{-1}. Thus, the turnover rate of potassium in rabbit lenses weighing approximately 300 mg. is 4.5 to 5.0 per cent per hour ($t_{1/2}$ = 14 to 15 hours), and the velocity of the pump is 1.04 to 1.15 μmoles per lens per hour.†

The implicit assumption that concentration of nonradioactive potassium in both lens and media remains constant during the culture period used in the preceding experiments was verified experimentally by determining the concentration of potassium in the media, before and after culture. No difference in concentration was detected in the six samples examined.

Fig. 2 shows the accumulation of ^{42}K in lenses cultured for 24 hours in media which had an initial concentration that varied between 0 and 20 mmoles per liter. The quantity of labeled ion in the lens decreases significantly as the concentration of total potassium in the media increases, indicating that the pump has a limited capacity for actively transporting potassium into the lens.

To evaluate K_m and V_{max} of the carrier system presumably responsible for the pump, the effect of concentration on the coefficient of transport, k_p, was determined.

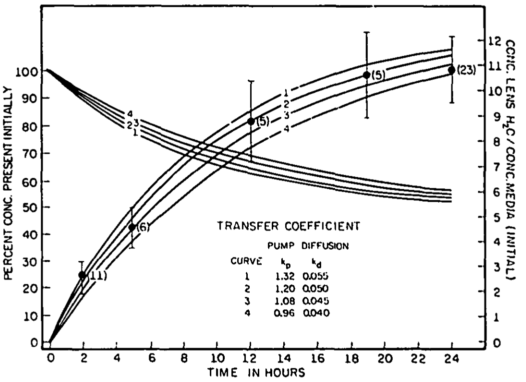

Fig. 1. Rate of accumulation of ^{42}K by lenses cultured in medium (KEI-4) (filled circles). Ascending lines show theoretical rate of accumulation based on solution of a pump-leak equation. The values of parameters employed are indicated on the graph. Descending lines show corresponding concentration of ^{42}K in the media in percentage of that present initially. Number of lenses shown in parentheses; vertical bar indicates one standard deviation.

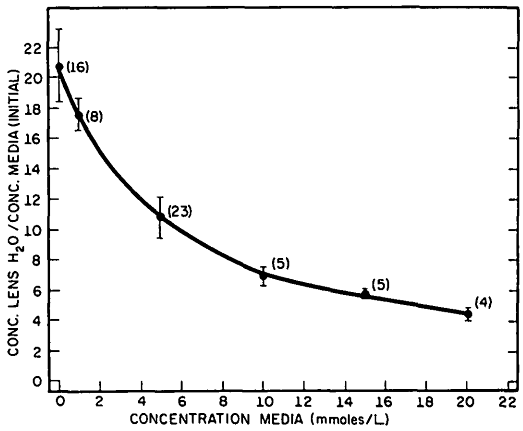

Fig. 2. Effect of concentration of potassium on accumulation of ^{42}K in lenses cultured for 24 hours. Number of lenses shown in parentheses; vertical bar indicates one standard deviation.

*The curve would rise to a value of $\frac{C_l}{C_m} = 25$, under steady state conditions, if the concentration of ^{42}K in the media remained constant from the outset.

†The possibility that the diffusion rate of potassium inside the unstirred lens is so low that it is rate-limiting was investigated by employing partial differential equations to solve equation 5 in a manner analogous to that used by Kinsey and Reddy[5] for studies of aqueous humor dynamics. The lens was assumed to consist of three compartments, and the diffusion rate for potassium, inside the lens, was determined experimentally from the rate of movement of ^{42}K and used in making the calculations. No significant difference in the value of the coefficients was required to produce a fit to the experimental data (Fig. 1).

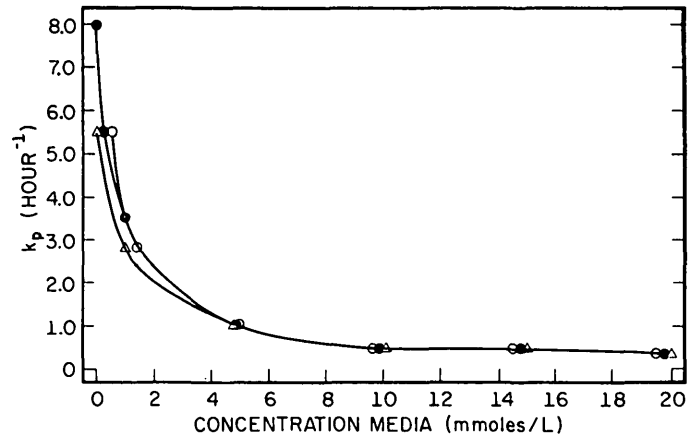

Fig. 3. Values for transfer coefficient by active transport (pump) which will produce a fit to the experimental data of Fig. 2; uncorrected, triangles; adjusted for average concentration of nonlabeled potassium present in the media during culture, open circles; true relation, filled circles (see text).

A series of successive approximations was used to establish the true relation between velocity of the pump and concentration of labeled and nonlabeled potassium in the media. In all of the calculations it was assumed that the coefficient of diffusion remained constant at 4.5 per cent per hour,* and was unaffected by concentration.

Assuming that no change in concentration of nonlabeled potassium in the media took place during 24 hours of culture, values for k_p were selected which, when used to solve equation 5, produced a fit to the experimental data on accumulation of ^{42}K (Fig. 2). The values, shown by the triangles in Fig. 3, are plotted as a function of the concentration of total potassium present initially in the media.

The change in concentration of total (labeled and nonlabeled) potassium in the media during the period of culture was then calculated by solving equations 1 and 4 simultaneously, using the values for k_p obtained previously. The results, shown in Fig. 4, are plotted as differences between the initial concentration and that predicted from the calculation. When the concentration of total potassium in the media differs significantly from the level in the aqueous, i.e., approximately 5 mmoles per liter, the net gain or loss alters the concentration appreciably.

The validity of the calculation, and also to some extent the assumption that k_d is essentially unaffected by changes in concentration in the media, were tested experimentally following 24 hours of culture by analyzing samples of media which had initial concentrations of potassium of 0 or 1 mmole per liter. In five instances the average concentration was 0.80 and 1.3 mmoles per liter, respectively, which agrees well with values predicted from the calculations, viz., 0.85 and 1.6 mmoles per liter.

New values of k_p were then determined, again by obtaining fits to data showing measured rates of uptake of ^{42}K (Fig. 2), but this time the concentration of total potassium in the media over the 24 hour time course of the experiment was assumed to be the average of the calculated concentration rather than the level present initially. In Fig. 3 the curve drawn through open circles shows the new values. The difference between the curves composed of triangles and those made up of open circles represents the effect of partially

*This value was selected because it corresponds closest to data showing the ratio C_l/C_m at 24 hours (Fig. 1).

compensating for the change in concentration of total potassium in the media, which occurred during the course of the experiment.

The last of the successive approximations was performed by programming k_p as a function of C_m, rather than average C_m, and solving equation 5 in the form of equation 5a at the bottom of the page.

The transfer coefficient k_p is now a variable, dependent upon the total concentration of potassium in the media, $C_{m(total)}$, as shown by the line composed of open circles in Fig. 3. A function generator in the analog computer was used to simulate the line electronically, and small corrections, Δk_p, were added or subtracted to k_p until fits to the experimental data of Fig. 2 were again obtained. The values for k_p computed in this manner show the true effect of concentration of potassium in the media on the transfer coefficient of the pump, since they incorporate all corrections required to take into account changes in concentration of both labeled and nonlabeled potassium in the media during the 24 hour period of culture. The values are also plotted in Fig. 3 (filled circles) and were used to calculate the velocity of the pump in terms of the amount of potassium actively transported into the lens, per hour, as a function of concentration in the media (Fig. 5). The rate at which potassium is pumped into the lens increases almost linearly with concentration up to approximately 1 mmole per liter, and then more slowly as the active transport system becomes saturated.

The data relating velocity of the pump to concentration in the media, as shown in Fig. 5, were treated in a manner described by Lineweaver and Burk,[6] and the results are plotted in Fig. 6. The straight-line relationship between the reciprocal of the concentration in the media and that of

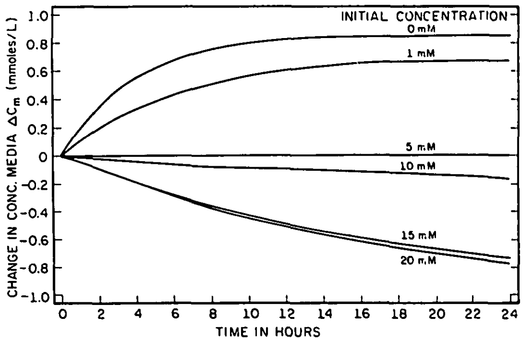

Fig. 4. Calculated change in concentration of total potassium in media during culture caused by net gain or loss from the lens.

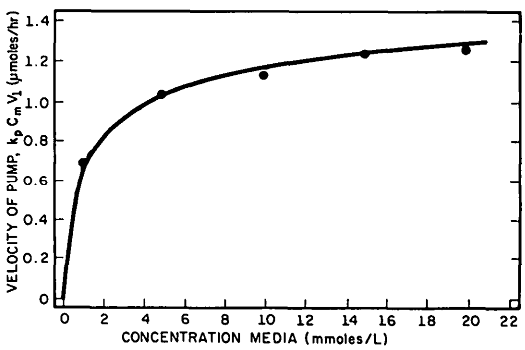

Fig. 5. Effect of concentration of total potassium on velocity of pump.

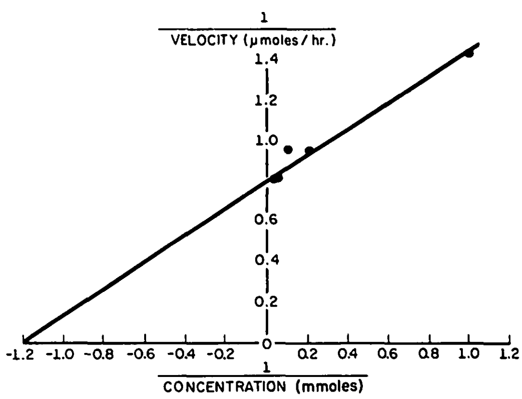

Fig. 6. Lineweaver-Burk plot of data shown in Fig. 5. The apparent Michaelis-Menten constant K_m = 0.95 mmole per liter and V_{max} = 1.3 µmoles per lens per hour.

$$\frac{dC_l}{dt} = \left[\left(f\,C_{m(total)} + \Delta k_p\right)\left(C'_{mt=0} - \frac{V_l}{V_m} C_l\right)\right] + k_d\left[\left(C_{mt=0} - \frac{V_l}{V_m} C_l\right) - C_l\right] \quad (5a)$$

the velocity of the pump is in agreement with Michaelis-Menten kinetics. The apparent Michaelis-Menten constant of the system, K_m, is 0.85 mmole per liter; V_{max} is 1.3 μmoles per lens per hour.

The velocity of the pump in vivo, where the concentration of potassium in the media bathing the lens is approximately 5 mmoles per liter, is about 1.1 μmoles per hour; thus, the pump must operate at approximately 85 per cent capacity.

Discussion

In this study the changes in concentration of labeled and nonlabeled potassium during culture are compensated for by mathematical treatment, which requires a computer. If the experiments could have been performed under conditions where these changes did not occur, the calculation would be less complex. Two methods are possible: use of a much larger volume of media, or a perfusion system in which the flow rate is relatively rapid. But both of these procedures require more elaborate apparatus than was used in the present investigation, and also further development before they can be practical for studying lenses in numbers required to evaluate the different parameters involved with reasonable accuracy.

The coefficient of active transport, k_p, can be defined in terms of the constant, K_m, which is a characteristic of the carrier system thought to be involved in the action of the pump. By analogy with the equation describing Michaelis-Menten kinetics, where the velocity of decomposition of substrate, S, is equal to $\frac{V_{max}\,(S)}{K_m + (S)}$, the velocity of the pump $(k_p C_m V_1)$ is

$$\frac{V_{max}\ C_m}{K_m + C_m}$$

thus

$$k_p = \frac{V_{max}}{(K_m + C_m)\ V_1} \qquad (6)$$

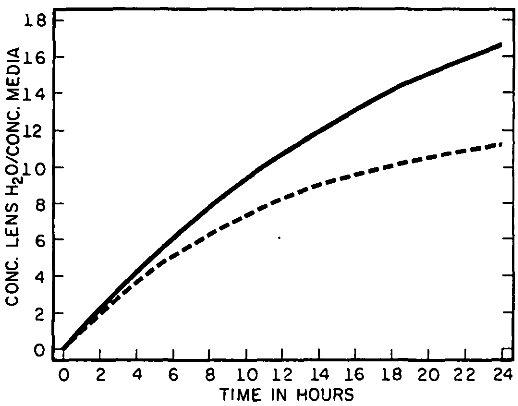

Fig. 7. Rate of accumulation of ^{42}K in a lens cultured in media similar in composition to aqueous humor. Solid line, calculated on basis of constant concentration of labeled potassium in media. Broken line, calculated on basis of initial concentration of labeled potassium in media.

and equation 1, describing the pump-leak system of the lens, can be written in the more general form:

$$\frac{dC_1}{dt} = \frac{V_{max}}{(K_m + C_m)\ V_1}\ C_m + k_d(C_m - C_1) \quad (7)^*$$

Since all the parameters of the pump-leak system have now been evaluated under conditions independent of changes in concentration of both labeled and nonlabeled potassium in the culture media, the true time course of the accumulation of ^{42}K, for any concentration, can be obtained from the solution of equation 7. For example, the rate of accumulation of ^{42}K in a medium having a concentration of nonlabeled potassium equal to that of the aqueous humor, viz., 5 mmoles per liter, has been calculated, and is shown in comparison with that obtained under the experimental conditions employed in this investigation

*It would appear profitable to take advantage of some of the elegant mathematical treatment available on the subject of enzyme inhibition[7] and explore the possibilities of modifying equation 7 so that it would describe the effect of competitive or noncompetitive inhibitors on accumulation of various substances in the lens.

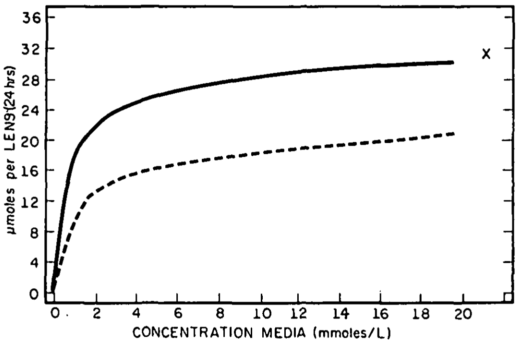

Fig. 8. Quantity of potassium pumped into lens (solid line) compared with that accumulated (broken line) during 24 hours of culture. The maximal quantity which can be actively transported during this period is shown by the *X*.

where the concentration of the tracer in the media decreases during culture (Fig. 7). Values of the coefficients and constants employed in performing the calculation were: $k_d = 0.045$ hour^{-1}, $V_{max} = 1.3$ μmoles per hour, and $K_m = 0.85$ mmole per liter.

Fig. 8 shows that the quantity of tracer potassium accumulated by the lens at any level of saturation of the pump is significantly less than the amount pumped into it during the same 24 hour period. The results, which for convenience are expressed in absolute units,* were obtained by solving equation 7 with and without the leakage term. The difference between the amount accumulated and that pumped into the lens is appreciable and essentially constant, illustrating the necessity for calculating Michaelis-Menten constants from experimental observations on saturation of a pump, rather than accumulation.

Much of the data showing saturation of the carrier system responsible for transporting potassium was obtained in collaboration with Dr. D. V. N. Reddy as were also some of the data showing the accumulation of ^{42}K in cultured lenses. Emily Vivian and Joan Glowacki provided technical assistance.

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*This amounts to assuming that all of the potassium in the media is labeled.