The rate of potassium exchange of the lens

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When net accumulation of radioactive potassium by the lens is followed, it is found that at least 85 per cent of lens potassium is available for exchange. A method is presented for determining the rate of potassium exchange across the lens. Rat, rabbit, and calf lenses show rates of 22, 6, and 3 per cent per hour, respectively. While the exchange rate is clearly related to the size of the lens, the flux of potassium across the lens is nearly equal for rabbit and calf lenses. Rat lenses, however, have a potassium flux nearly double that of the other two species.

The lens, in common with other tissues, maintains a potassium ion concentration which is many times higher than that of the fluid which nourishes it. Use of radioactive potassium and rubidium, which are handled similarly by the lens, has demonstrated that an active "pump" mechanism is able to maintain this high concentration despite appreciable permeability of the lens membranes to these cations.[1-3] The net accumulation of potassium or rubidium by the lens has been studied by measuring radioactive ion uptake[1, 2] and by following the restoration of potassium ion concentration after refrigeration or preincubation in calcium-deficient media.[3]

However, net accumulation of potassium by the lens occurs only when the rate of uptake by the tissue exceeds the rate of loss. At equilibrium, a dynamic balance between uptake and loss exists, so that the rates of transfer of potassium into and out of the lens are equal. The purpose of the present work is the determination of the rate of transfer, or exchange, of potassium into and out of the lens under equilibrium conditions. An equation has been derived which relates the initial net accumulation of radioactive potassium to the equilibrium exchange rate of nonradioactive potassium. The results of the application of this equation to the determination of potassium exchange rate in rat, rabbit, and calf lenses are reported. In addition, with the estimation of the size of the lenses, the potassium flux across the surface of each of these three species of lenses has been determined.

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Methods

Lenses from 100 gram male Sprague-Dawley rats, 1¼ pound albino rabbits, and young calves were used. The lenses were removed by a posterior approach to the globe. After removal of any adherent vitreous, the lenses were transferred on a wire loop to modified Merriam-Kinsey incubation tubes.

Rat lenses were incubated in 4 ml. of medium, while 10 ml. was used for rabbit and calf lenses. The composition of the incubation medium for rat lenses was: 0.462 mM. $MgSO_4$, 0.241 mM. Na_2HPO_4, 0.252 mM. KH_2PO_4, 3.33 mM. KCl, 23.1 mM. $NaHCO_3$, 0.840 mM. $KHCO_3$, 5.0 mM. glucose, 1.5 mM. $CaCl_2$, and additional NaCl to

bring the tonicity to 284 mOsm. The rabbit lens medium was identical to that used for rat lenses, except that it contained 6.0 mM. fructose in addition to glucose, and had an osmolarity of 292 mOsm. The calf lens incubation medium differed from that for the rat in having a $CaCl_2$ concentration of 2.3 mM., and an osmolarity of 302 mOsm. All media contained 0.05 μc per milliliter of ^{42}K, obtained from the Iso-Serve Corp., Cambridge, Mass. The pH of the media was maintained at 7.5 by continual equilibration with a 5 per cent CO_2 and 95 per cent O_2 atmosphere.

The incubations were carried out at 37°C. for 75, 150, and 300 minutes. At the conclusion of the incubation period, the lenses were removed from the culture tubes, blotted on filter paper, and weighed. Lens water was taken as 61.1 per cent of the wet weight of rat lenses and 67 per cent of the wet weight of rabbit and calf lenses. Those few lenses which were cloudy were not included in the data. The lenses were homogenized in potassium-free 10 per cent trichloroacetic acid, and aliquots of the deproteinized lens filtrate and final medium were taken for assay in a liquid scintillation counter.[4] Aliquots of the filtrate and medium were taken also for potassium analysis. This was done with an Advanced flame photometer, utilizing an internal lithium standard.

Surface area of the lenses was calculated by measuring the anterior-posterior and equatorial diameters of the lens with a micrometer. Lenses were chosen at random from each lot of animals or eyes used. The dimensions obtained were assumed to be those of an oblate spheroid, which closely approximates the configuration of the lens from all three species of animals.

The mathematical equations used for determining potassium exchange across the lens are similar to those developed by Raker, Taylor, Weller, and Hastings,[5] for erythrocytes. The modification necessary to adapt the red cell equations for the present work are presented in the Appendix.

Results

The uptake of potassium expressed in terms of the ratio of counts per minute per kilogram lens water, divided by counts per minute per liter of medium (L/M ratio) is shown in Fig. 1 for rat, rabbit, and calf lens. The nonradioactive potassium L/M ratio at equilibrium is calculated by dividing the concentration of potassium in the lens water by the concentration in the medium. Since the concentration of potassium in lens water is about 130 mEq. per liter, and that in the medium is 5 mEq. per liter, the equilibrium ratio is approximately 26 : 1. A similar ratio would be expected when the radioactive ion achieves equilibrium between the lens and the medium. It can be seen from Fig. 1 that the radioactive ion is not in equilibrium between lens and medium at the end of 5 hours. In rat lens, an incubation period of even 24 hours results in a radioactive potassium L/M ratio of only 22, still short of the expected equilibrium value.

Another expression for the degree of equilibration of radioactive with nonradioactive potassium is given by comparing the specific activity of the potassium in the lens to that in the medium. Specific activity is defined as counts per minute per microequivalent of potassium. Such a comparison for the three species of lenses is given in Table I. The degree of equilibration achieved depends upon the length of incubation, and seems to be related to the size of the lens. The smaller rat lens approaches equilibrium mixing of the radioactive ion much faster than does the larger

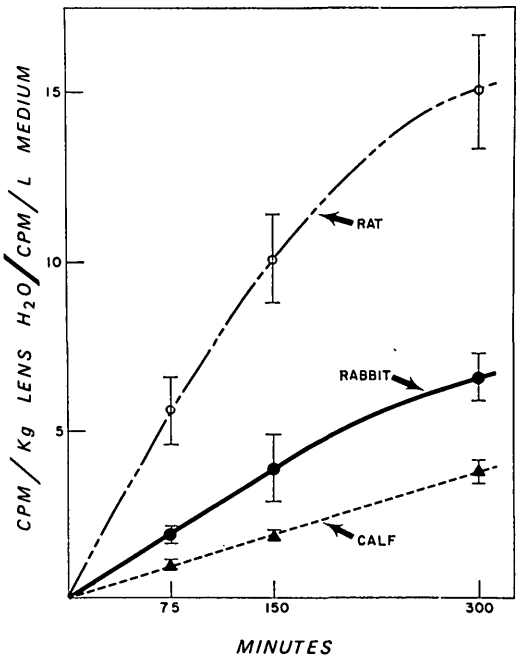

Fig. 1. Net accumulation of radioactive potassium by the lens. Each value shown represents at least 10 lenses. The values are bracketed by their standard deviations.

Table I. The equilibration of medium potassium with lens potassium

Time	*Rat*	*Rabbit*	*Calf*
1 min.	0.006 (4)*		
5 min.	0.020 (4)		
10 min.	0.031 (4)		
15 min.	0.046 (4)		
37 min.	0.125 (4)		
1¼ hr.	0.236 (15)	0.067 (11)	0.036 (16)
2½ hr.	0.371 (24)	0.132 (14)	0.063 (18)
5 hr.	0.538 (25)	0.229 (28)	0.124 (15)
10 hr.	0.720 (8)	0.379 (4)	
24 hr.	0.855 (4)	0.638 (4)	

The degree of equilibration is expressed in terms of the specific activity of the lens divided by the specific activity of the medium.
*Number of lenses.

rabbit lens, which in turn approaches equilibrium faster than the calf lens.

In the derivation of the potassium exchange equations presented in the Appendix, it is assumed that the total amount of potassium in the lens remains constant during the incubation period. To be certain that this assumption is valid, the weight and potassium concentration of the lens were determined before and after incubation by using pairs of lenses from rats and rabbits. One lens of each pair was removed from the animal, weighed, and analyzed for potassium. The second lens was analyzed after a 5 hour incubation period. No change in weight or potassium concentration was found for rat lenses; a 2 per cent loss of weight, but no change in potassium concentration was found for rabbit lenses. Similar data have been obtained using calf lenses,[6] where similar incubation was found to restore refrigerated lens weight and potassium concentration to values identical with those of unincubated controls.

The initial step in the calculation of the potassium exchange of the lens is to determine the slope of the straight line which results when the logarithm of the difference between specific activity of potassium of medium and lens is plotted as a function of time. Examples of such graphs are shown in Figs. 2, 3, and 4 for rat, rabbit, and calf lenses. In Fig 2, the slope, m, of the line connecting the points is –0.0970.

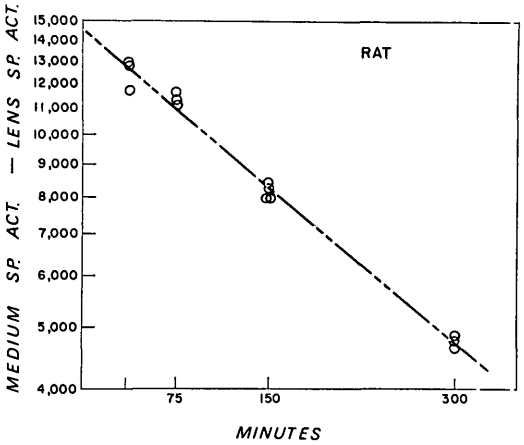

Fig. 2. The logarithm of the difference between *medium specific activity* and *lens specific activity*, as a function of time, for rat lens incubation. The difference of medium specific activity and lens specific activity is plotted on the logarithmic scale. Each value represents a single lens and its medium.

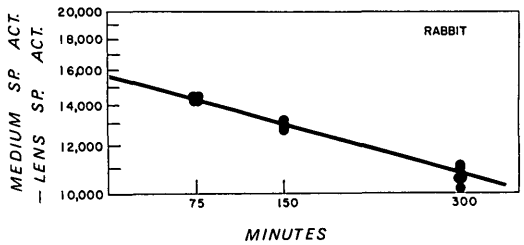

Fig. 3. The logarithm of the difference between *medium specific activity* and *lens specific activity*, as a function of time, for rabbit lens incubation.

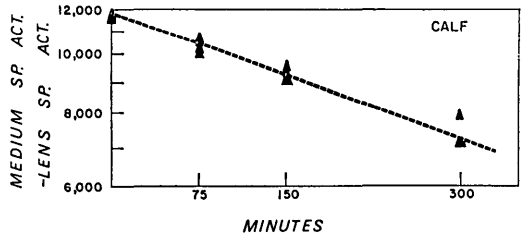

Fig. 4. The logarithm of the difference between *medium specific activity* and *lens specific activity*, as a function of time, for calf lens incubation.

The fraction of lens potassium transferred to the medium per hour is defined as k_2. The value for k_2 can be determined by knowing m, the lens potassium concentration, the amount of lens water, the medium potassium concentration, and the

volume of medium. The final equation which relates these quantities is derived in the Appendix, and is:

$$k_2 = \frac{-2.3\ m}{1 + \left(\frac{[K^+]_{lens}\ (Vol.)_{lens\ H_2O}}{[K^+]_{med.}\ (Vol.)_{med.}}\right)}$$

For the group of rat lenses shown in Fig. 2, $k_2 = 0.204$. In this case, 20.4 per cent of the lens potassium is transferred to the medium each hour.

It is apparent that k_1, the fraction of medium potassium transferred to the lens per hour, can also be obtained from the relationship:

$$2.3\ m = -(k_1 + k_2).$$

In the present example, $k_1 = 0.019$, so that 1.9 per cent of the potassium in the medium is transferred to the lens per hour. The value of k_1 is less useful than that for k_2, however, since the amount of potassium in the medium is dependent upon the medium concentration and the volume. On the other hand, k_2 is not sensitive to changes in the volume of medium or the medium potassium concentration, but depends upon the relatively fixed quantity of lens potassium.

The results obtained from several experiments are shown in Table II. Each value for potassium exchange represents a single experiment, utilizing 12 to 16 lenses. The average fraction of rat lens potassium transferred to the medium per hour is 0.22, or 22 per cent. At this rate, one half of the potassium ions present in the lens at the beginning of the incubation are exchanged with the medium in about 3 hours, since for a first order process $t_{1/2} = \frac{0.693}{k}$. In absolute quantity, 0.4 μeq of potassium moves from the lens to the medium each hour. Since the lens is in equilibrium with the medium, an identical quantity moves from the medium to the lens each hour.

For the rabbit lens, 6 per cent of the lens potassium moves to the medium each hour, or 0.9 μeq per hour. Calf lens potassium exchanges with the medium at a rate of 3 per cent per hour, or 2.4 μeq per hour. One half of the ions present at the beginning of the incubation are transferred to the medium in about 23 hours.

When the potassium exchange, in microequivalents per hour, is related to the wet weight of the lens, or to the lens water, the exchange rate for rat lens is about 8

Table II. Potassium exchange of the lens

Potassium exchange (%/hour)	*Lens weight (mg.)*	*Lens water weight (mg.)*	*Surface area (mm.²)*	*Potassium exchanged (μeq/hr./lens)*	*Potassium exchanged (mμeq/mg. lens/hr.)*	*Potassium exchanged (mμeq/mg. lens water/hr.)*	*Potassium flux (mμeq/mm.²/hr.)*
Rat							
18.9	22.7	13.9	33.1	0.344	15.2	24.8	10.4
22.6	23.4	14.3	37.6	0.418	17.9	29.2	11.1
22.9	21.2	13.0	33.4	0.394	18.6	30.3	11.8
18.7	21.8	13.3	34.3	0.325	14.9	24.4	9.5
Rabbit							
6.7	154	103	131	0.923	6.0	9.0	7.0
5.5	177	118	137	0.945	5.3	8.0	6.9
5.7	151	110	137	0.771	5.1	7.1	5.6
Calf							
3.1	926	621	471	2.42	2.6	3.9	5.1
3.6	865	580	475	2.66	3.1	4.6	5.6
2.7	925	619	488	2.23	2.4	3.6	4.6
3.3	853	573	445	2.49	2.9	4.3	5.6

The methods used to calculate potassium exchange, amount of lens water, and surface area are given in the text. Each value for potassium exchange is obtained using 12 to 16 lenses.

times that for calf, and about 3 times the rate found for rabbit lenses.

The last column of Table II shows the potassium flux which is calculated for the three species of lenses. Flux is defined as quantity of potassium transferred per unit of time per unit of surface area. With the surface areas shown in the table, the potassium flux across rat lens is found to be about 11 mμeq per square millimeter per hour. The flux across rabbit lens is 6.5 mμeq per square millimeter per hour, only slightly higher than the 5.2 mμeq per square millimeter per hour, found for calf lens.

Discussion

Despite the rapid exchange in the rat lens, equilibration of radioactive potassium across the lens is not complete even after 24 hours of incubation. Since the radioactivity level in the lens is still increasing at the end of 24 hours, at least 85 per cent of the lens potassium is available for exchange with medium potassium. The possibility that some lens potassium is unavailable for exchange exists, however, and has not been ruled out by the present studies.

The rate of potassium exchange is constant for the three species of lenses for at least 5 hours of incubation. If it were not, the plot of the logarithm of the specific activity of the medium minus the specific activity of the lens as a function of time would not be a straight line, and at least two values of m could be determined, each of which would yield a different exchange rate. Since this is not the case, the exchange determined for this initial 5 hour period probably involves potassium which is uniformly accessible to the exchange process.

The determination of the L/M ratio is a useful method for following the uptake of a radioactive substance. However, the amount of radioactive substance in the medium must be large compared to the amount in the lens, in order to avoid a significant difference between the initial and final amounts of radioactivity available to the lens for exchange. This problem has been explored in detail for potassium.[7] Another shortcoming of the L/M ratio is that the rate of exchange and the flux of the nonradioactive substance are not obtained readily from the data. By combining such data with determinations of the nonradioactive potassium concentration in the lens and medium, however, the exchange rate can be calculated.

From the results obtained from rat, rabbit, and calf lenses, it is clear that the fraction of lens potassium exchanged with the medium per hour decreases as the size of the potassium pool in the lens increases. It might be expected, because of this, that rat lens would reflect an alteration in its environment considerably faster than calf and rabbit lenses. This may account in part for the relatively rapid loss of clarity which occurs in some rat lenses during incubation. Rabbit and calf lenses are comparatively sluggish, and may not reflect medium imbalances so quickly.

Although the fraction of lens potassium exchanged per hour decreases with increased lens size, the absolute quantity of potassium exchanged increases with larger lenses. This increased exchange is not directly proportional to the increased weight of the lens, however. Since this is the case, much of the bulk of larger lenses must be relatively inactive in the exchange process.

By expressing potassium exchange in terms of flux across the surface of the lens, the differences among the exchange activities of the three species of lenses are decreased. Although the flux across rat lens is nearly double that for rabbit lens, the rabbit lens flux is nearly equal to that found for calf.

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Appendix

The equations used by Raker, Taylor, Weller, and Hastings[5] for calculating potassium exchange in red cells have been modified for use with lenses. As in red cell incubation, the potassium is considered to be located in two separate compartments, in this case, the lens and the medium which surrounds it. The quantity of potassium in the medium is termed A, and that in the lens, B.

An energy-requiring process probably is responsible for the transport of potassium into the lens, while passive diffusion accounts for an equal loss, under equilibrium conditions. Regardless of the mechanism of the exchange, if x moles of A go to B in time, t, the rate of change of the quantity (in moles) of A is given by:

$-dA/dt = k_1 (A-x)$, where k_1 = the fraction of A transferred to B per unit time. Also,

$-dB/dt = k_2 (B + x)$, where k_2 = the fraction of B transferred to A per unit time.

$$dx/dt = k_1 (A-x) - k_2 (B + x) \quad (1)$$

At equilibrium, $dx/dt = O$, and

$$k_1 (A-x) = k_2 (B + x) = D \quad (2)$$

In the incubation system, if the change in the amount of nonradioactive potassium inside or outside the lens is O, equation (2) describes the exchange. Therefore,

$(A - x) = [K^+]_{med.\ HOH} (Vol.)_{med.}$

and

$(B + x) = [K^+]_{lens\ HOH} (Vol.)_{lens\ HOH}$.

Substituting these quantities into (2),

$$k_1[K^+]_{med.} (Vol.)_{med.} = k_2[K^+]_{lens} (Vol.)_{lens\ HOH} = D \quad (3)$$

For the radioactive tracer, however, $dx/dt \neq O$, and (1) must be integrated and solved, yielding:

$$\ln \frac{1}{k_1 (A - x) - k_2 (B + x)} = (k_1 + k_2)t - C.$$

At $t = O$, $x = O$, so that $-C$ is given by:

$$\ln \frac{1}{(k_1A - k_2B)} = - C.$$

The solution at any time t is:

$$\ln \frac{k_1A - k_2B}{k_1 (A - x) - k_2 (B + x)} = (k_1 + k_2)t. \quad (4)$$

For the radioactive ion, $(A - x)$ equals the amount of ^{42}K in the medium at time t. The radioactivity is expressed in terms of counts per minute. If the specific activity is expressed as counts per minute per microequivalent K^+, the total ^{42}K in the medium at any time t is:

$(A - x) = [K^+]_{med.} (Vol.)_{med.}$ (specific activity $_{med.}$), and that in the lens is:

$(B + x) = [K^+]_{lens} (Vol.)_{lens\ HOH}$ specific activity $_{lens.}$)

Substituting into (4), since

$D = k_1 [K+]_{med.} (Vol.)_{med.\ HOH} = k_2 [K^+]_{lens} (Vol.)_{lens\ HOH}$,

$$- \ln \frac{k_1A - k_2B}{D} + \ln (\text{sp. act. med.} - \text{sp. act. lens}) = (k_1 + k_2)t. \quad (5)$$

Since the first term of (5) is a constant, the slope of the plot ln (sp. act. med. - sp. act. lens) vs. time is equal to $-(k_1 + k_2)$. Changing to $\log_{10}$,

$$2.3\ m = -(k_1 + k_2). \quad (6)$$

With equations (3) and (6), the proportion of the lens potassium transferred to the medium per hour can be calculated, with knowledge of the medium and lens potassium concentrations, the amount of lens water, the volume of the incubation medium, and the slope of the plot of the logarithm of the difference of the medium and lens specific activities vs. time. The final equation used is obtained by combining (3) and (6),

$$k_2 = \frac{-2.3\ m}{1 + \left(\frac{[K^+]_{lens} (Vol.)_{lens\ HOH}}{[K^+]_{med.} (Vol.)_{med.}} \right)}.$$