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## Studies on the crystalline lens

### XX. Influence of sodium substitutes on cation composition of intracellular fluid

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*This paper shows that significant and variable changes in cation composition of intracellular fluid of the lens occur when the concentration of Na<sup>+</sup> in media is isosmotically replaced by a number of frequently used Na<sup>+</sup> substitutes. The concentrations of sodium, potassium, and various substances (TEA, choline, Li, Tris, K, Cs, Rb, and sucrose) used to replace isosmotically 50 mmoles per liter of sodium in media were determined in intracellular fluid (fiber water) of rabbit lenses following 20 hours of culture. The sodium substitutes accumulate in the lens in concentrations varying from 7 mM. for TEA to over 100 mM. for potassium and rubidium. Total cation concentration is reduced by choline, increased by rubidium, cesium, and sucrose, and not significantly altered by other substitutes. Accumulation of organic cations and lithium is approximately balanced by loss of potassium, whereas accumulation of cesium and rubidium exceeds loss of potassium by approximately 20 mM., and loss of sodium and potassium combined by 15 mM. It is suggested that the observed changes in cation composition of intracellular fluid of the lens could affect transport processes independent from reduction in concentration of extracellular sodium.*

**Key words:** lens, sodium substitutes.

**I**n 1952, Christensen and co-workers<sup>1</sup> showed that replacement of sodium by potassium inhibited uptake of amino acids in red blood cells of the duck. Since then, increasing evidence has shown that sodium

may act as a driving force for transport of different classes of compounds in many tissues,<sup>2</sup> including amino acids in the lens.<sup>3-5</sup> The selection of a substitute for sodium in extracellular fluid has thus become an increasing problem to investigators who wish to determine the effect of sodium on transport of substances across cell membranes. Ideally, the substitute should be inert with respect to the transport system being studied and have little or no effect on physical properties of the incubation media such as osmotic pressure or ionic strength. It should not significantly alter the ionic composition of the tissue since effects produced by changes in the normal ionic concentrations in the intracellular

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**Table I.** Cation concentration in fiber water of lenses cultured 20 hours in KEI-4 medium in which 50 mmoles per liter of various sodium substitutes are used to replace an osmotic equivalent concentration of sodium

Cation substitute	No. lenses	Substitute	Na	$\Delta$ Na	K	$\Delta$ K	Cation	$\Delta$ Total	Water per cent wet weight
Control*	31	—	13 $\pm$ 3	0	139 $\pm$ 6	0	152	0	64.9 $\pm$ 0.1 (10)
TEA	7	7 $\pm$ 2	9 $\pm$ 2	-4	131 $\pm$ 8	-8	147	-5	64.8 $\pm$ 0.4 (4)
Choline	9	18 $\pm$ 3	6 $\pm$ 1	-7	117 $\pm$ 7	-22	141	-11	67.0 $\pm$ 0.6 (4)
Lithium	6	39 $\pm$ 2	8 $\pm$ 1	-5	101 $\pm$ 3	-38	148	-4	66.3 $\pm$ 0.9 (7)
Tris	7	45 $\pm$ 5.5	7 $\pm$ 3	-6	96 $\pm$ 8	-43	148	-4	66.3 $\pm$ 0.7 (4)
Potassium	8	147 $\pm$ 5	7 $\pm$ 1	-6	147 $\pm$ 5	+8	154	+2	66.7 $\pm$ 0.4 (4)
Cesium	14	94 $\pm$ 9	4 $\pm$ 1	-9	71 $\pm$ 7	-68	169	+17	67.5 $\pm$ 0.1 (10)
Rubidium	13	119 $\pm$ 5	11 $\pm$ 2	-2	38 $\pm$ 2	-101	168	+16	68.8 $\pm$ 0.7 (6)
Sucrose	8	9 $\pm$ 2	12 $\pm$ 1	-1	150 $\pm$ 3	+11	162	+10	64.0 $\pm$ 0.4 (7)

\*KEI-4 medium.

fluid could be mistakenly attributed to differences in sodium concentration in the extracellular fluid.

The criteria for a "perfect" sodium substitute are rigid and it is unlikely that such a substance exists. Some sodium substitutes, however, may possess advantages over others depending on the degree to which they affect transport directly or alter transport indirectly through disturbances in the normal ionic environment of the tissue. The present study is addressed to the latter aspect of the problem, viz., relative changes induced in concentration of ions in intracellular fluid of ocular lenses cultured in media in which about one third of the sodium has been replaced by various commonly used sodium substitutes. The author is unaware of any previous studies in which accumulation of sodium substitutes in the lens was determined in relation to displacement of normally present cations, but changes in concentration of sodium and potassium in lenses incubated in sodium-free media were measured by Cotlier and Beaty.<sup>3</sup>

### Methods

Lenses obtained from albino rabbits weighing approximately 2 kilograms were cultured by the method of Merriam and Kinsey<sup>6</sup> in 5 ml. of medium having the same composition as KEI-4,<sup>7, 8</sup> except that concentration of sodium was reduced from 145 to 95 mM. by replacing 50 mM. NaCl with an osmotic equivalent concentration of the following sodium substitutes: tetraethylammonium chloride (TEA), choline chloride, LiCl, pH

7.5, Tris-(hydroxymethyl)-aminomethane (Tris), CsCl, RbCl, KCl, and sucrose. Concentrations of pH 7.5 Tris and sucrose employed were 62 and 100 mM., respectively. The other substitutes were assumed to be equivalent osmotically to NaCl.

A significant fraction of substances found in the lens in concentrations below that in the aqueous, sodium for example, is contained in the porous capsule. Since the chief concern of the present investigation is to report on changes in cation distribution within ion-limiting membranes in the lens, most concentrations will be expressed in terms of water exclusive of that contained in the capsule, on the assumption that the concentration in the capsular water (6 per cent total water)<sup>9, 10</sup> is equal to that in the medium. The exact location of the remaining water is beyond the scope of this paper, but it is probably largely intracellular since the extracellular space separating adjacent lens fibers is only about 80 Å.<sup>11</sup> For the present purpose it will be considered to be present in the fibers. The relation between the concentration in total lens water to that exclusive of the capsule can be expressed as

$$V_L \cdot C_L = V_C \cdot C_C + V_F \cdot C_F$$

where  $V_L$ ,  $V_C$ , and  $V_F$  refer to volume of water in the whole lens, capsule, and fibers in that order. It follows that

$$C_F = 1.064 C_L - 0.064 C_M.$$

$C_M$  is concentration in the medium.

Following culture, lenses were blotted gently on filter paper moistened with media in which they were cultured, then weighed, homogenized in 2 ml. of 10 per cent trichloroacetic acid (TCA), and the sodium and potassium determined on supernatant fluid by flame photometry using an Hitachi Perkin-Elmer instrument. The concentrations of lithium and rubidium were determined using standards containing concentrations of all ions closely similar to those present in the lens extracts to compensate for interference. Concentrations of

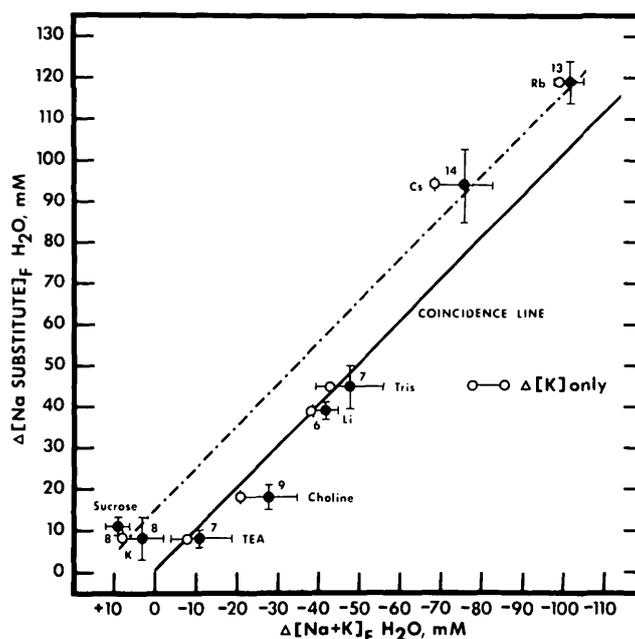


Fig. 1. Effect of osmotic equivalents of various sodium substitutes when used to replace 50 mmoles per liter of sodium in KEI-4 medium on the cation distribution in lenses cultured for 20 hours. Filled circles =  $\Delta$  [Na + K].

TEA, choline, pH 7.5 Tris, and sucrose were calculated from the ratio of concentrations of  $^{14}\text{C}$ -labeled compounds in the lens water/media determined in other lenses under identical experimental conditions. Approximately the same number of lenses was used for making these determinations as had been employed when analyses were performed for sodium and potassium. Radioactivity was determined with a scintillation counter. TCA in a concentration equal to that present in the lens extracts was added to media to compensate for possible quenching.

Water content was estimated on separate groups of cultured lenses by placing them in tared test tubes and drying them at room temperature to a constant dry weight (48 hours) under high vacuum. Lenses remained clear in all media employed, although in other experiments higher concentrations of choline and sucrose caused lens opacity.

## Results

Concentrations of various sodium substitutes in lens fiber water after 20 hours of culture are shown in Table I. Concentrations of sodium, potassium, and total cations, along with differences from control lenses cultured in KEI-4 for a similar period of time are also shown. All concentrations are calculated on the basis of water

content for each group as shown in the last column.

Sodium substitutes accumulate in the fiber water at the expense of sodium and potassium in concentrations varying from 7 mM. for TEA to over 100 mM. for potassium and rubidium. All cations, except TEA, cause moderate increases in water content, whereas sucrose reduces it by 1 per cent. The total cation content is decreased slightly by all organic cations and lithium, and increased by the other metal cations and sucrose. The only changes in concentration of total cations having probable statistical significance are those associated with choline, rubidium, cesium, and sucrose.

The relation between accumulation of sodium substitutes and loss of sodium plus potassium is shown graphically in Fig. 1. The increase in concentration of organic cations and lithium (filled circles) is approximately balanced by loss of sodium and potassium, i.e., roughly corresponds to the solid coincidence line. The correspondence is improved when only the loss potassium is considered (open circles).

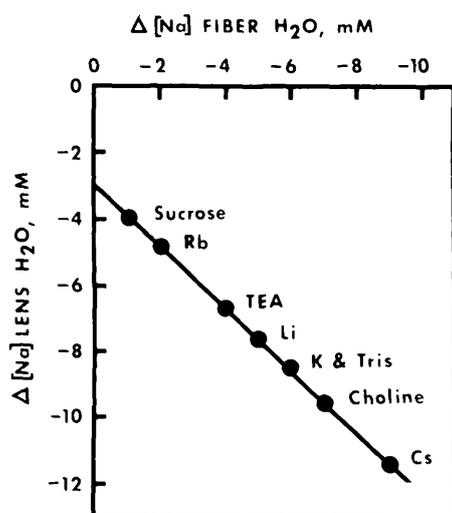


Fig. 2. Changes in sodium concentration in lens and fiber water following 20 hours of culture in media in which 50 mmoles per liter of sodium is replaced with osmotic equivalents of various substances.

In other words, these sodium substitutes appear to replace potassium on approximately a one for one basis. The increase in concentration of cesium, rubidium, and sucrose, shown by the filled circles, visually fitted by the broken line, exceeds the loss of sodium and potassium by about 15 mM., a difference which is increased by an additional 5 mM. (open circles) if loss of sodium is not taken into consideration.

In contrast to potassium, no obvious proportionality exists between loss of sodium and accumulation of the sodium substitutes. Nevertheless, the amount of sodium displaced in all but two instances, sucrose and rubidium, represents a substantial proportion (half) of the total amount of sodium present in the fiber water. The rank order of the cation substitutes with respect to changes produced in concentration of sodium in both fiber water and whole lens water is shown in Fig. 2.

Despite accumulation of large quantities of both cesium and rubidium the amount of sodium displaced was much greater with cesium. The difference is highly significant ( $P < 0.001$ ). The observation was confirmed by determining steady-state values

for  $^{22}\text{Na}$  of lenses cultured in the presence of 50 mM. cesium or rubidium, thus avoiding the use of photometric analysis by flame photometry.

In an effort to determine whether differences in ion distribution are related to the mode of transport of the substitute into the lens, accumulation of  $^{14}\text{C}$ -labeled organic cations and lithium (5 mM.) was determined in lenses cultured for 20 hours in three different media: (1) KEI-4, (2) KEI-4 without potassium, and (3) KEI-4 in which nonlabeled cation is substituted for 50 mM. of sodium. The results expressed as the ratio of concentration in fiber water to that present in media are shown by the bar graphs of Fig. 3 along with similar data for the radioisotopes of alkali metal cations taken in part from a previous paper.<sup>12</sup> Accumulation of tracer TEA and Tris is unaffected by the presence of either potassium or the nonlabeled compound. Accumulation of labeled choline is also not affected significantly by lack of potassium, but is reduced almost half by the presence of 50 mM. nonlabeled choline. Accumulation of lithium, however, and all the alkali metal cations is increased by the absence of potassium and is reduced significantly by a concentration of 50 mM. of nonlabeled ion.

## Discussion

It is noteworthy that some loss of sodium occurs in the lens fibers with all of the cations tested, thus indicating that none of them competes effectively for the pump that actively extrudes sodium from the lens. This observation suggests that the sodium transport system is much more specific than that for potassium which is inhibited by all of the alkali metal cations.<sup>12</sup>

Identical osmotic equivalents of the sodium substitutes result in widely varied changes in the distribution of sodium and potassium, which are only partly dependent on the extent to which the substitutes accumulate in the lens. Thus, on the one hand, the organic cations and lithium substitute for almost equivalent concentrations

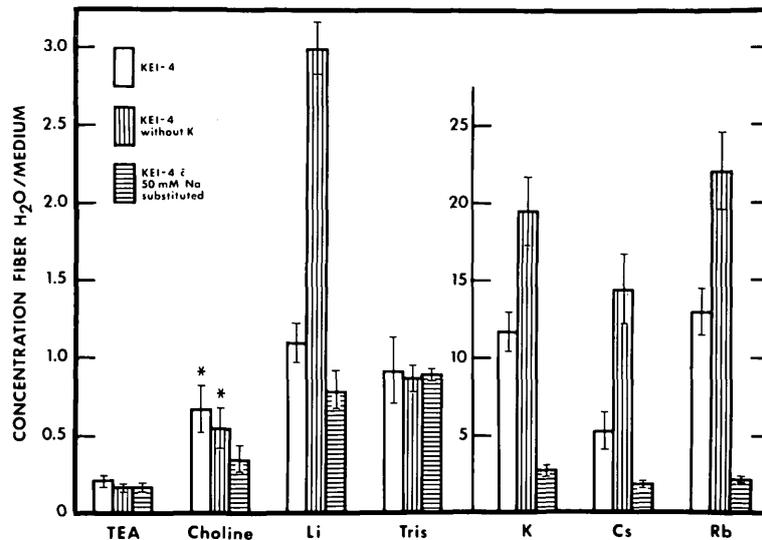


Fig. 3. Accumulation of  $^{14}\text{C}$ -labeled organic cations and radioisotopes of alkali metal cations in lenses cultured for 20 hours in control medium (KEI-4), KEI-4 without potassium, and KEI-4 in which osmotic equivalents of nonlabeled cations were substituted for 50 mmoles per liter of sodium. Media contains 5 mM. lithium in absence of a usable radioisotope.

of potassium, and in addition replace some sodium which, in the case of choline, amounts to over half of that present in the fiber water. On the other hand, rubidium and cesium displace approximately 25 mM. less than an equivalent concentration of potassium, and 15 mM. less than sodium and potassium combined, resulting in a net increase of about 10 per cent in total cation concentration. This occurs even though rubidium and cesium are known to inhibit the active transport of potassium and so might be expected to decrease potassium concentration in the lens by reducing re-entry.<sup>12</sup> Sucrose, for reasons that are not apparent, results in a net gain of potassium without an appreciable loss of sodium.

Results of experiments showing the influence of potassium on accumulation of the two groups of ion substitutes (Fig. 3) may provide an explanation for the mechanism by which such substitutes affect the level of potassium in the lens. Thus, organic ions enter the lens by processes unrelated to those responsible for the transport of potassium and replace it on approximately a one for one basis. Rubidium and cesium, however, whose influx is dependent on the

concentration of potassium in the media, share the mediated carrier system that actively transports potassium into the lens. This enables them to replace more than equivalent quantities of potassium and maintain appreciable concentration gradients between lens and media, the magnitude of which must be determined by the relative values of  $K_i$  for the potassium transport system and the permeability of the lens to leakage of each ion.<sup>12</sup>

Lithium accumulation is also depressed by potassium, which suggests that it too is transported by the potassium pump, but unlike rubidium and cesium, each equivalent of lithium replaces only one equivalent of potassium. Results of recent studies on the kinetics of lithium movement in the lens provide possible reasons for the difference in behavior of the ions.<sup>13</sup> First, the affinity of lithium for the potassium carrier system is much lower than that of rubidium and cesium; secondly, lithium, like sodium, is actively transported out of the lens, thus tending to limit the rise in lithium concentration.

By whatever mode of action, substitution of osmotic equivalent concentrations of

various substances results in appreciable and significantly different changes in ionic composition of cultured rabbit lenses and might, therefore, influence transport of substances independent of reduction in sodium concentration in media as observed for  $\alpha$ -AIB,<sup>14</sup> taurine,<sup>5</sup> and myoinositol.<sup>15</sup>

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