Myo-inositol: Active transport by the crystalline lens

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$^{14}$C myo-inositol was actively transported into rabbit crystalline lenses incubated in Tyrode’s medium. The lens/media accumulation ratios of $^{14}$C myo-inositol reached values of 10 to 12 after 24 hr., in vitro incubations. The active transport of $^{14}$C myo-inositol into the lens was markedly decreased by metabolic inhibitors of glycolysis and oxidative phosphorylation, and by ouabain, or by an absence of Na$^+$ ions in the incubation media. Kinetic studies revealed a $K_m$ of 0.07 mM. per liter for myo-inositol transport into lens. The efflux of $^{14}$C myo-inositol from preloaded lenses was slow, but it increased by iodoacetate. Gas chromatography determinations of myo-inositol in the lens and intraocular fluids were as follows (averages in mM. per kilogram of water): lens, 8835; anterior chamber aqueous humor, 135.1; posterior chamber aqueous humor, 93.2; vitreous humor, 235.5; and serum, 40.9. The studies support the concept of active transport and slow efflux of myo-inositol into the lens, as well as the presence of transport mechanisms specific for cyclic alcohols in tissues.

Key words: inositol, crystalline lens, transport mechanisms, ocular fluids, Tyrode’s medium, radioactivity, chromatography, rabbit.

Inositol (myo-inositol, meso-inositol) is widely distributed in animal tissues, plants, and microorganisms, in free and phosphorylated forms. In the bovine crystalline lens, free myo-inositol represents more than 99 per cent of the total tissue inositol in concentrations of 14 to 20 mM. per kilogram of wet lens. Myo-inositol averages for adult sheep, rabbit, and human lenses are 30, 7, and 14 mM. per kilogram of lens, respectively.1-3

Relatively few attempts have been made to explain the reasons for such high levels of myo-inositol in the lens. Kinoshita and his co-workers29 found that calf lenses incubated with $^{14}$C$\alpha$ glucose contained a minimal amount of radioactivity in the inositol fraction; the main bulk of radioactivity remained as glucose, or was present in sorbitol or fructose. These experiments indicate that a slow myo-inositol synthesis from glucose takes place in the lens. Another possibility is that an active transport system for myo-inositol, similar to those existing in kidney4 and Erhlich ascites cells,5 is functioning in the lens. This report deals with such a system, including active transport and slow efflux of myo-inositol from the rabbit crystalline lens.
Material and methods

Preparation of tissue. Albino rabbits (Haskins Rabbitry, Creve Coeur, Mo.), weighing 2 to 3 kg, were used. They were killed by intracardiac air injections. The lenses were obtained by enucleation and opening of the eye at the posterior pole. Immediately after removal, the lenses were carefully transferred, with a Teflon-coated loop, to the incubation vessels.

Incubations and sampling

Transport into lens. Lenses (320 to 360 mg) were incubated in 2 or 10 ml of Tyrode's medium (pH 7.4), containing 14C myo-inositol (20 μM per milliliter), in Kjeldhal round-bottom flasks placed in a Magni-Whirl, metabolic shaker at 37°C. The Tyrode's solution contained (in mM per liter) NaCl, 137; KCl, 2.68; CaCl2 • 2H2O, 2.04; MgCl2 • 6H2O, 0.49; NaH2PO4, 0.416; NaHCO3, 11.9; and D-glucose, 5.50. It was gassed before incubations with a mixture of 95 per cent O2 and 5 per cent CO2. Two control flasks without tissue were incubated simultaneously. Short-term incubations (4 hr.) took place in 2 ml of medium; longer incubations (8, 12, and 24 hr.), in 10 ml of media. A maximum of 1 μM of medium glucose per lens per hour was utilized, amounting to 35 per cent of the total glucose in the flask with 2 ml of incubation media (4 hr.) and 43 per cent of the incubation glucose in the flask with 10 ml of incubation medium (24 hr. incubations).

Before and after incubations, 20 μL samples of media were obtained and plated in planchets. Following incubations the lenses were weighed on a Roller-Smith balance, transferred to Kjeldhal round-bottom flasks, and media. Rabbit lenses were weighed, homogenized in 2 ml of 15 per cent TCA, and centrifuged at 2,500 r.p.m. The supernatant solutions were treated with trioctylamine to remove TCA, and lyophilized to a volume of approximately 400 μL. Then, 5 μL of the concentrated supernatants and of the final incubation media, and 5 μL samples containing 1 mg per milliliter of D-glucose, myo-inositol, D-glucuronic acid, D-fructose, and D-sorbitol were placed at the origin of Whatman No. 4 paper strips. All samples were run overnight in ethyl acetate-pyridine-water (Vv: 120:50:40) in the ascending direction, using the “pad technique.” To further separate myo-inositol and D-glucuronic acid, lyophilized lens supernatants and standards were run in phenol-NH4OH (200:1) for 16 hr. by ascending chromatography. Chromatograms were developed with AgNO3 as described by Smith.5 Rf values were calculated relative to glucose (Rf = 100). The paper strips were sliced along each millimeter of their length, and counted directly for 14C radioactivity in a Nuclear Chicago gas-flow counter.

Distribution of 14C myo-inositol in the lens. Nine lenses, incubated as described above for 1, 2, or 3 hr. in Tyrode's medium containing 14C myo-inositol, were dissected in the following fashion: the lenses were frozen in dry ice, and trephined in the anterior-posterior direction with a 6 mm. cork core. This central core was divided by sectioning with a razor blade into five, approximately equal parts from the anterior to the posterior segment. The most anterior area, or No. 1, included the epithelium of the lens. Each section was immediately weighed and processed in a fashion similar to that used for the entire lens. The equatorial, or peripheral, ring of lens outside the trephined area was processed simultaneously any 14C myo-inositol) and incubated for 4 hr. in the shaking bath at 37°C. At hourly intervals, 20 μL samples from the media were obtained, plated in planchets, and counted as described above. At the end of the incubations, the lenses were weighed, homogenized in 15 per cent trichloracetic acid (TCA), and processed as lenses for transport studies. The results were calculated as 14C myo-inositol effluxed from the lens, a percentage of the total in the lens at each timed interval:

\[\text{Eff. lens} = \frac{\text{Eff. media}}{\text{Acc. lens} + \text{Eff. media}} \times 100.\]

Efflux from lens. Lenses were incubated in a fashion similar to that described above with 14C myo-inositol for 18 to 22 hr. The lenses were then gently transferred to Kjeldhal round-bottom flasks containing 2 ml of Tyrode's medium (without any 14C myo-inositol) and incubated for 4 hr. in the shaking bath at 37°C. At hourly intervals, 20 μL samples from the media were obtained, plated in planchets, and counted as described above. At the end of the incubations, the lenses were weighed, homogenized in 15 per cent trichloracetic acid (TCA), and processed as lenses for transport studies. The results were calculated as 14C myo-inositol effluxed from the lens, a percentage of the total in the lens at each timed interval:

\[\text{Eff. lens} = \frac{\text{Eff. media}}{\text{Acc. lens} + \text{Eff. media}} \times 100.\]
with the other areas of the lens. The results were expressed in ratios of $^{14}$C myo-inositol in lens water of each segment of $^{14}$C myo-inositol in medium.

**Myo-inositol in ocular fluids and lens.** Samples from ocular fluids, lens, and plasma were obtained from five albino rabbits weighing 2 kilograms each. The animals were restrained with a rabbit wrangler, the eyes gently proptosed, and samples of posterior chamber and anterior chamber aqueous humor withdrawn into a calibrated pipette. Vitreous humor was aspirated with a syringe following aqueous sampling. Blood was obtained by cardiac puncture, and the serum was separated by centrifugation. These samples and the lenses were frozen immediately in dry ice, they were then chromatographed by the method of Sweeney and his colleagues as follows: a 50 μL portion of amhydrous pyridine–hexamethylidisilazane–trimethyl-chlorosilane (17:2:1, by volume) was added to the freeze-dried samples of the tissue extracts; these were kept at room temperature for 48 hr. before chromatography. Then, 1 μL portions of the pyridine extracts were injected into a Hewlett-Packard F and M model 810 gas chromatograph with a 10 ml. Hamilton syringe fitted with a Chaney adapter. Chromatography was carried out on 15 per cent ethylene glycol succinate (EGS) on 80 to 100 mesh Car-Chrom P (Applied Science Laboratories, State College, Pa.) in a 12 ft. by 0.25 in. glass tube at temperatures between 150° and 160° C. The carrier gas was helium with a flow of 100 ml per minute; a flame ionization detector was used. Measurements were obtained from the gas chromatographic record by comparing the peak heights of individual sugars in tissue extracts with the heights of peaks from the corresponding sugars in standards run under identical conditions. Linear correlation between peak height and weight of sugar derivatives was obtained from the gas myo-inositol by the method of Sherman and Stewart.

**Chemicals.** $^{14}$C myo-inositol was obtained from Nuclear Chicago, Des Plaines, Ill.; sodium cyanide and D-fructose from Mallinckrodt Chemicals, St. Louis, Mo.; 2,4-dinitrophenol from Fisher Scientific, St. Louis, Mo.; sodium iodoacetate, ouabian, D-sorbitol, D-galactitol, D-xylitol, and myo-inositol from Sigma Chemical Co., St. Louis, Mo.; and D-galacturonic acid from Nutritional Biochemicals, Cleveland, Ohio.

**Saturation experiments and kinetic constants.** In saturation experiments, cold myo-inositol in concentrations ranging from 10 to 900 μM per liter was added to Tyrode’s medium containing $^{14}$C myo-inositol, as shown in the abscissa of Fig. 4. The conditions of incubation and volume were identical to those described above. Calculations of the total myo-inositol transported into the lens were obtained from the saturation experiments. The total net myo-inositol transported per time unit was found as: μM myo-inositol per lens per hour = lens/medium $^{14}$C myo-inositol accumulation ratio × lens water × concentration of myo-inositol in the medium. As a small variation in water content occurred among lenses, this calculation was done on an individual basis. The values for total myo-inositol transported into the lens were plotted as a Lineweaver-Burk plot. The Michaelis-Menten constant ($K_m$) for myo-inositol (dissociation constant of the substrate-carrier complex) and the maximum velocity ($V_{max}$) of myo-inositol transport into the lens were found.

**Ca**++-free or Na**+-**free media. $Ca^{++}$-free Tyrode's media were prepared without CaCl among the components of the media. Na**+-**free media were prepared as previously described substituting equimolar concentrations of sucrose for NaCl.

**Results**

**Accumulation of $^{14}$C myo-inositol by the lens.** Rabbit lenses accumulated $^{14}$C myo-inositol against the concentration gradient when incubated in vitro (Fig. 1). The lens/medium accumulation ratios were found to be $2.31 ± 0.32$ at 4 hours ($n = 36$); $4.30 ± 0.58$ at 8 hours ($n = 4$); $5.54 ± 0.72$ at 12 hours ($n = 4$); $9.30 ± 0.98$ at 19 hours ($n = 8$); and $12.60 ± 1.2$ at 24 hours ($n = 4$). As shown in Fig. 1, the active transport of $^{14}$C myo-inositol was linear with time, and at 24 hr. had not reached saturation.

These tissue/media accumulation ratios reflected only in part the gradient against which $^{14}$C myo-inositol was taken up by the lens, as the rabbit lenses contained 5 to 7 mM per liter of myo-inositol, while the concentrations of $^{14}$C myo-inositol in the medium were 3 to 5 μM per liter. In studies on kidney transport of myo-inositol, tissue slices were preincubated in medium to decrease the tissue levels of inositol; this also served to increase the accumulation ratios of labeled myo-inositol. Attempts to decrease the endogenous levels of myo-inositol in the lens were made, but preincubation in Tyrode's medium for 2 to 24 hr. at $37^\circ$ or $4^\circ$ C. did not result in significant losses of lens myo-inositol.

**$^{14}$C myo-inositol in lens supernatants and media.** By paper chromatography in ethyl...
acetate–pyridine–water, myo-inositol spots (R_f = 28) separated from D-glucuronic acid (R_f = 12), sorbitol (R_f = 84), D-glucose (R_f = 100), and D-fructose (R_f = 120). After precipitation with TCA, the \(^{14}\text{C}\) radioactivity of aliquots of lens supernatants and final incubation media was distributed in the chromatograms as shown in Fig. 2. By this procedure, as well as by chromatography in phenol-ammonia–water, 100 per cent of the \(^{14}\text{C}\) counts in the supernatants and media fractions were found in myo-inositol, indicating that no further metabolism of the cyclic alcohol had taken place after entering the lens, and that no other \(^{14}\text{C}\) labeled compounds had diffused into the media.

Protein and lipoprotein in the TCA precipitates were washed twice with water, plated, and counted for \(^{14}\text{C}\) radioactivity. Only 0.6 to 0.9 per cent of the total \(^{14}\text{C}\) counts in the lens were found in the precipitates. Of the \(^{14}\text{C}\) myo-inositol found in the lens, 99 per cent or more was in the TCA supernatants as free myo-inositol.

The possibility that a \(^{14}\text{C}\) myo-inositol bond to protein or lipid was broken by the vigorous TCA extraction was explored by extracting single lenses with 6 ml. of chloroform-methanol (2:1). The precipitate, or
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2.5

0.5

102
137
171

Fig. 3. The 14C myo-inositol accumulation ratios of rabbit lenses incubated in Tyrode's media containing various Na+ ion concentrations. To preserve osmolarity, wherever Na+ ions were omitted, equimolar concentrations of sucrose were added to the media. Each point represents the mean of four lenses, incubated for 4 hr. as described in the section on methods and materials.

Metabolic and Na+ ions requirements.
The active transport of 14C myo-inositol into the lens was dependent on both glycolytic and oxidative phosphorylation. In 4 hr. experiments the addition of the following inhibitors resulted in the following 14C myo-inositol lens/media accumulation ratios as percentages of control lenses: iodoacetate (1 mM. per liter), 10 per cent; cyanide (1 mM. per liter), 73 per cent; 2,4-dinitrophenol (1 mM. per liter), 50 per cent; and ouabain (0.1 mM. per liter), 40 per cent. In 19 hr. experiments the effects of these inhibitors were augmented. The incubation of lenses in glucose-free media did not alter the 14C myo-inositol accumulation ratios compared to controls in the four hour experiments but decreased those of lenses incubated for 19 hr. to 50 per cent of controls. Thus, in the absence of glucose, the endogenous lens substrates appeared to provide the necessary energy for myo-inositol transport in short-term incubations.

The absence of Ca++ resulted in 14C myo-inositol lens/media accumulation ratios of 38 per cent of controls. The removal of Na+ ions from the media resulted in decreases in the transport of 14C myo-inositol into the lens (Fig. 3), proportional to the concentration of Na+ in the media.

Kinetics of myo-inositol transport into lens. The addition of nonlabeled myo-inositol to the media resulted in saturation of the transport system and decreased 14C myo-inositol accumulation ratios (Fig. 4). From the values of total myo-inositol transported into the lens at various external concentrations, the maximum velocity (Vmax) and half-saturation concentration (Km) were calculated. The Vmax was 28 μM per kilogram lens water per hour, and the Km = 62 μM per liter. At lower Na+-media concentrations (Na+ = 68 mEq. per liter or Na+ = 34 mEq. per liter), the total myo-inositol transported into lens decreased (Fig. 5) with an increase in the Km to 350 μM per liter but without appreciable change in the Vmax (Fig. 6). These findings were suggestive of a common transport system for inositol and Na+ ions, each sharing similar carriers, as has been previously postulated for the Na+-linked transport of myo-inositol by Ehrlich cells. It is currently accepted that a majority of the active transport systems for carbohydrates, amino acids, and other organic compounds in various tissues, including lens, depend on the concentration of Na+ ions in the media.

Distribution of 14C myo-inositol in lens.
The distribution of 14C myo-inositol in the lenses after 1 and 2 hr. of incubation is shown in Fig. 7. The equatorial area and the anterior part of the lens containing the epithelium showed much higher accumulation ratios of the cyclic alcohol than other areas of the lens. These findings indicated that the active transport system for myo-inositol took place mainly through the
epithelium of the lens, a characteristic of other active transport systems of the lens such as those for $^{86}$Rb, $^{42}$K, or $^{14}$C α-aminoisobutyric acid.\textsuperscript{10,12}

**Ages of lenses.** The $^{14}$C myo-inositol lens/media accumulation ratios by rabbit lenses decreased with the age of the rabbits. The accumulation ratio values at 4 hr. for rabbit lenses of various ages were:

- 5 days old (52 mg.\textsuperscript{*}), 3.58;
- 15 days old (97 mg.), 2.74;
- 30 days old (140 mg.), 2.46;
- 3 months old (329 mg.), 1.87;
- 6 months old (475 mg.), 1.50; and
- 1 year old (545 mg.), 0.902 (Fig. 8).

\textsuperscript{*}Average weight of the rabbit lenses.
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**Efflux of $^{14}\text{C}$ myo-inositol from lens.** The efflux of $^{14}\text{C}$ myo-inositol proceeded at a maximum rate of 4 per cent of the total in the lens. As seen in Fig. 9, these findings agree with those of van Heyningen, who found only traces (0.06 mg.) of lens inositol in the media after 5 hr. incubations in a Krebs-phosphate-Ringer media containing glucose. The lens efflux of $^{11}\text{C}$ myo-inositol was not significantly modified by additions to the efflux media of nonlabeled inositol in various concentrations up to 30 mM per liter, 1 mM ouabain, or into Ca$^{++}$- or Na$^{+-}$ free Tyrode's medium.

The addition of 1 mM sodium iodoacetate to the efflux media resulted in increased effluxes of $^{13}\text{C}$ myo-inositol as shown in Fig. 9. This effect of iodoacetate on $^{13}\text{C}$ myo-inositol efflux from the lens could result from a decreased uptake (reconcentration) by the tissue as the initial 30 minute rate of efflux was not affected.

**Discussion**

At steady state, the concentrations of myo-inositol in the lens exceed many times...
that in the surrounding fluids. Average ratios of myo-inositol lens water to posterior chamber aqueous humor are 95; the lens water/vitreous humor ratios are 37 (see Table 1). Thus the active transport, or “pump,” for myo-inositol must be operative in vivo. In addition, in rabbits, myo-inositol concentrations in aqueous and vitreous humor are above plasma levels. Either active transport of myo-inositol occurs into intraocular fluids, or these high concentrations represent myo-inositol that has leaked from the lens and accumulated in the aqueous and vitreous humor compartments of the eye. In human cerebrospinal fluid, Bunuel and Bunuel found a threefold level of myo-inositol as compared to plasma levels.

The “pump” and “leak” concepts, proposed as the means of active transport of K⁺, Rb⁺, or amino acids into the lens, may be equally applicable to the active transport and efflux of myo-inositol. The lens accumulation ratios for 14C myo-inositol in the experiments followed for 24 hr. indicate that the actual transport rate was linear and slow, and that, presumably, saturation had not been reached at that time. Saturation would be reached after longer incubation periods in vitro, and when the tissue/media ratios approach those found in vivo between the lens and its surrounding fluids.

**Table I. Concentration and distribution ratios of myo-inositol in lens and intraocular fluids of rabbit eyes**

<table>
<thead>
<tr>
<th>Samples*</th>
<th>Myo-inositol amoles/Kg. of fresh tissue or amoles/Kg. of water (± S.D.)</th>
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</thead>
<tbody>
<tr>
<td>Lens</td>
<td>5890 ± 208</td>
</tr>
<tr>
<td>Lens water</td>
<td>8833 ± 312</td>
</tr>
<tr>
<td>Anterior chamber (AC) aqueous humor</td>
<td>136.1 ± 25.1</td>
</tr>
<tr>
<td>Posterior chamber (PC) aqueous humor</td>
<td>93.2 ± 8.2</td>
</tr>
<tr>
<td>Vitreous humor</td>
<td>235.4 ± 28.5</td>
</tr>
<tr>
<td>Serum</td>
<td>40.9 ± 6.9</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Distribution ratios</th>
</tr>
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<tbody>
<tr>
<td>Lens water:PC aqueous</td>
</tr>
<tr>
<td>Lens water:AC aqueous</td>
</tr>
<tr>
<td>PC aqueous:serum</td>
</tr>
<tr>
<td>Lens water:vitreous humor</td>
</tr>
<tr>
<td>Vitreous humor:serum</td>
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<tr>
<td>AC aqueous:serum</td>
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*Average ± S.D. of five individual samples.

For such studies, systems for prolonged lens cultures, like those developed by Kinsey, must be used. The slow initial velocity for myo-inositol transport is also reflected in the calculated 

V_max of 28 μM per kilogram of lens water per hour, a value considerably lower than the V_max for accumulation of 86Rb or 14C α-AIB by the lens of 10.5 and 2 mM. per kilogram of lens water per hour respectively. The affinities of the transport "carriers" for myo-inositol, however, are higher than those of other compounds actively transported into lens. The K_m for myo-inositol is 62 μM per kilogram of lens water, that of Rb is 2.3 mM. per kilogram of lens water, and that of α-AIB is 2.5 mM. per kilogram of lens water. These kinetics actually favor the active uptake of myo-inositol by the lens in physiologic conditions as the concentration in the posterior chamber is 93 μM per kilogram of water, a value slightly above the K_m for transport into lens.

In the lens, the efflux of 14C myo-inositol into media is slow, at a maximum rate of 4 per cent of the total in the lens. The efflux is not increased by 1 mM. ouabain.
nor 10 to 30 mM. “cold” myo-inositol in the media. According to Heinz, the addition of high concentrations of a nonlabeled substance to the efflux media, while measuring the runout from the tissue of such a compound in the labeled isotope form, is a measure of (1) exchange-diffusion (carrier-mediated diffusion out of a tissue), and (2) saturation on the outer side of the membrane blocking re-entry of the isotope into the tissue. The difficulties in demonstrating outside saturation, or exchange-diffusion, for lens $^{14}$C myo-inositol could represent the different affinities of the carriers for influx and efflux. Helmreich and Kipnis’ nomenclature for these influx and efflux carrier affinities is $K_i$ and $K_s$, respectively. They have proposed that exchange-diffusion is more likely to occur in a system where the $K_i$ or $K_m$, in the inner surface of the membrane is of similar magnitude to that of the $K_s$, or $K_m$, in the outer surface of the membrane. When $K_s > K_i$, the affinities of the carrier for efflux are considerably smaller than the affinities for active uptake; consequently a slow efflux takes place.

If that were the case with $^{14}$C myo-inositol in the lens, increasing the $K_i$ by lowering the Na+ concentration in the media should increase the efflux of myo-inositol. However, increases of the $K_i$ for myo-inositol active transport from 62 to 350 M. per liter by lowering the Na+ concentration in the media from 149 mEq. per liter to 34 mEq. per liter did not affect the efflux rate during a 5 hr. efflux period. Thus, the mechanisms for efflux of myo-inositol from the lens do not appear to be explained on the basis of “carriers,” nor kinetic constants for uptake or efflux. It might be possible that unidirectional carriers, transporting myo-inositol into the lens, are the only operational carriers. The relative impermeability of the lens membranes to linear sugar alcohols has been pointed out previously by Kinoshita and co-workers, and by Firie and van Heyningen. These authors found high accumulations in the lens of sugar alcohols, such as galactitol or sorbitol, produced through metabolism of high levels of galactose or glucose, respectively. The main differences between these linear alcohols and the cyclic alcohol, myo-inositol, is that only the latter is actively transported into the lens.

Although it is known that myo-inositol is an essential requirement for replication of such yeasts as Schizosaccharomyces pombe, Kloeckera brevis and S. carlsbergens, the cellular function of this cyclic alcohol in higher vertebrates has remained largely undefined. It appears now that myo-inositol is an essential requirement for the growth of normal and cancer cells in culture, and for the preservation of the transport of amino acids by cells cultured in vitro. Only that part of the transport system for $\alpha$-AIB, serine, and glycine dependent on extracellular Na+ ions (or the Na+-K+ ATPase) is absent from inositol-deficient cells. A similar function can be tentatively assigned to myo-inositol in lens, as lenses of animals fed high galactose diets that have lost 70 to 80 per cent of the free amino acids of the lens have markedly decreased levels of myo-inositol (3 per cent of control lenses). Lembach and Charalampous have considered, among other things, the effects of myo-inositol on cell membranes as playing either a structural role in the formation of the membrane or an intermediate role in the phosphatidic acid cycle of Hokin and Hokin. This concept would be applicable to tissues such as kidney, brain, or liver where a high percentage of injected $^{32}$P myo-inositol is incorporated into phospholipids and cell membranes. However, it does not appear valid for the lens as, in agreement with previous investigators, we failed to demonstrate a significant binding of myo-inositol to lipids or proteins of the lens membranes. It is likely that the functions of free inositol are altogether different from those of bound myo-inositol.

The adult rabbit lens does not appear to metabolize any significant amounts of myo-inositol. Radioactive myo-inositol recovered
from the lens supernatants after 4 hr. of incubation accounted for 98 per cent, or more, of the radioactivity missing from the media. In the incubation experiments with 5-day-old lenses, however, there was a discrepancy between $^{14}$C myo-inositol radioactivity in media and lens water, with 30 per cent of the media counts remaining unaccounted for. The possibility of myo-inositol conversion to CO$_2$ through various routes, such as described in intact rats, must be explored in newborn rabbit lenses.

In rat kidney, myo-inositol is metabolized to D-glucuronic acid, and kinetic studies of such an enzyme system indicate a high $K_m$ for inositol, $2.21 \times 10^{-2}$ M per liter. It thus appears doubtful that the kidney enzyme system has a major biological significance in vivo. Free myo-inositol in the kidney is actively taken by the tubules and accumulated against the concentration gradient in vitro. The alterations of these transport systems result in excessive urinary inositol in diabetes mellitus, or following glucose loads in man.

The conversion of glucose to myo-inositol through cyclization was first suggested by Fischer, and found in testicular tissue. On the other hand, myo-inositol formation through condensation of 2 and 4 carbon fragments of glucose has led Charalampous and Lyras to postulate an alternative hypothesis for glucose conversion to myo-inositol by yeast and tissues. The rates of $^{14}$C glucose conversion to myo-inositol are not remarkably high. Brain slices incubated in Krebs-Ringer with 2 mM D-glucose form myo-inositol at a rate of 10 $\mu$M per kilogram of wet brain per hour. Assuming a similar rate of myo-inositol formation for rabbit lenses, metabolizing at a rate of 3 mM glucose per kilogram lens per hour, only 0.3 per cent of lens glucose would be converted to myo-inositol. Kinoshita and his associates found minimal inositol labeling (65 to 83 c.p.m. per micromole), following calf lens incubations for 24 hr. with 10 mM per liter of $[^{14}$C$_6$] glucose with specific activity of 1,050 c.p.m. per mole. Most of the radioactivity was found in lens glucose, lens sorbitol, and lens fructose. The reverse reaction, namely, the formation of glucose, or glucose intermediates, from myo-inositol, may take place but, from the results of the present experiments, does not appear of major significance in the normal lens. Whether lenses deprived of glucose utilize endogenous inositol to any significant extent remains to be determined.

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