Sterol synthesis by the ocular lens of the rat during postnatal development

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Abstract Great amounts of plasma membranes are formed during early postnatal development of the ocular lens as lens epithelial cells differentiate into fiber cells. Little information is available on the source of the lipids, and particularly cholesterol, required for formation of these plasma membranes. The present study measured the capacity of the lens of the rat to synthesize cholesterol during this dynamic period of growth. Incorporation by lens of ^3H_2O into total fatty acids was also examined. Absolute rates of cholesterol synthesis per whole lens were estimated in vitro from incorporation of ^3H from ^3H_2O into digitonide precipitable sterols (DPS) by intact lenses of 6- to 30-day old rats. Rates of cholesterol synthesis were calculated which were adequate to furnish from either 50–100% or 20–40% of the cholesterol required by the lens for growth, depending upon the animal's age and upon whether one considered NADPH to be generated by the pentose phosphate pathway or by oxidative enzymatic processes (NADPH from the pentose pathway is not labeled from ^3H_2O). Generation of the NADPH necessary for cholesterol synthesis principally by the pentose pathway would support the higher percent contribution of synthesis to the total growth requirement. The pentose pathway was clearly active in the young rat lens, since between 7.5 to 9.0 times more [1-^{14}C]glucose than [6-^{14}C]glucose was oxidized in vitro to $^{14}CO_2$ by 6- and 22-day old lenses. Incorporation of ^3H_2O into DPS decreases sharply after 2 weeks of age in spite of a constant rate of cholesterol accumulation by the lens. These results indicate that the ocular lens of the rat can furnish most if not all of its cholesterol requirements by synthesis de novo during the first 2 weeks of life, and imply a contribution from another source at older ages. Whether lipoproteins can supply cholesterol to the lens is still unclear, although neither HDL nor LDL altered the incorporation in vitro of [U-^{14}C]glucose into DPS by lens.—**Cenedella, R. J.** Sterol synthesis by the ocular lens of the rat during postnatal development. *J. Lipid Res.* 1982. **23:** 619–626.

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The vertebrate ocular lens is composed of two cell types, epithelial and fiber cells, encased in a collagenous basement membrane (the capsule). A single monolayer of epithelial cells covers the anterior surface of the lens; fiber cells, the predominant cell type, are arranged in onion skin fashion as multiple layers with younger cells overlaying older ones (1). In the lens' equatorial region, fiber cells arise from differentiated epithelial cells. This differentiation involves great cellular elongation with disappearance of subcellular organelles. In fact, the plasma membrane surface area per cell increases by 1000- to 1500-fold (2). Thus, it seems reasonable to assume that this terminal differentiation is accompanied by synthesis of large amounts of plasma membrane constituents. Indeed, rapid synthesis of the major intrinsic protein of fiber cell-plasma membrane occurs during differrentiation (3–5) along with synthesis of the crystallin (water-soluble) proteins. About 35% of the lens' wet weight is protein and about 90% of the protein is crystallin. As compared to proteins, much less information is available on the metabolism of the other major constituent of lens membranes; i.e., the lipids.

Essentially all of the lipid in the ocular lens is present in the fiber cell-plasma membrane (2). Phospholipids plus cholesterol appear to account for about 50% of the membrane's total mass, since the weight ratio of protein to total lipid in this membrane is 1 to 1.2 (5). The molar ratio of phospholipid to cholesterol is approximately one (6, 7). The human (8) and calf (9) lens in vitro and the embryonic chick lens both in vivo (10) and in vitro (11, 12) all rapidly incorporate ^{32}P into phospholipids and particularly into phosphatidylinositol. Phosphatidylinositol accounted for about 60% of the total ^{32}P incorporated by the human lens (8) and the embryonic chick lens (11). Furthermore, relative to the epithelial cell, the chick fiber cell synthesized phosphatidylinositol and other phospholipids more rapidly and degraded phosphatidylinositol much more slowly. This metabolic pattern would be consistent with the need to accumulate phospholipids for the increased membrane synthesis that occurs during formation and growth of the fiber cell.

In contrast to the limited information on the phospholipid metabolism of the ocular lens, essentially nothing is known about the lens' sterol metabolism. This is surprising in view of the importance of cholesterol in

Abbreviations: DPS, digitonide precipitable sterols; DPM, disintegrations per min; HDL, high density lipoproteins; LDL, low density lipoproteins.

regulating membrane structure and function and of the special role of plasma membranes in maintaining lens transparency (13). In fact, changes in the relative composition and content of lens sterols could be the basis of cataracts produced by treatment with compounds that inhibit cholesterol biosynthesis. For example, the drugs triparanol (6, 14), 20,25-diazacholesterol (15), AY9944 (trans-1,4-bis(2-chlorobenzylaminomethyl)cyclohexane. 2 HCl) (16) and U18666A (3β-(2-diethylaminoethoxy)androst-5-en-17-one. HCl) (17, 18), agents known to inhibit cholesterol biosynthesis at late metabolic steps, can produce cataracts in rats and perhaps man. The present study examines the potential of the rat's ocular lens to synthesize sterols during the first month of postnatal life. This is a critical development period during which the lens attains about 50% of its adult size and accumulates much of the total lipid which it will eventually contain.

EXPERIMENTAL PROCEDURES

Animals and measurement of lens cholesterol

Pregnant Sprague-Dawley rats were purchased from Hilltop Lab Animals, Inc., Scottdale, PA and the time of birth of pups was recorded. Rats were then used between 6 and 30 days of age, a major developmental period for the ocular lens. The cholesterol content of lenses from 6-, 14-, 22- and 30-day old rats was determined on 5 to 13 separate groups of two or more pooled lenses at each age. Lenses were homogenized in chloroform–methanol 2:1 (v/v) as described before (17) and the cholesterol in the recovered total lipid was quantitated by gas–liquid chromatography using 5-α cholestane as internal standard (19).

Incubations in vitro with ^3H_2O

Short-term. Lenses (300–500 mg) from 10–25 rats of various ages were pooled onto filter paper (moistened with Krebs bicarbonate buffer) in a shallow dish kept at 37°C. Three separate pools of lenses (20–50 per pool) were prepared in each experiment and the individual pools were transferred to 25-ml Erlenmeyer flasks and weighed. Incubations were conducted according to Andersen and Dietschy (20). Two and one-half ml of Krebs bicarbonate buffer (pH 7.4) containing 15 μmol of D-glucose and 2.0 mCi of ^3H_2O (New England Nuclear Corp., Boston, MA, purchased at a specific activity of 1.0 mCi/g) were added to each lens pool, the flasks were sealed and then briefly gassed with 95% O_2–5% CO_2 via needles inserted through the stopper. The specific activity of the ^3H_2O in the incubation medium was calculated on the basis that each flask contained 2.0 mCi of ^3H_2O and 152.7 mmol of water (305.4 mg atoms of H of H_2O). This assumed that the medium (2.50 ml) was 98% water and that the lenses (avg 462 mg per flask) were 65% water (17). Also, the lens water was assumed to equilibrate rapidly with the medium water. Since the mg of lenses per flask varied, a slightly different specific activity value was calculated for each incubated pool of lenses. This value averaged 14,632 ± 153 dpm/μg atom of hydrogen of water. Two pools were incubated for 3 hr at 37°C with shaking (20 cycles per min) and the other for 3 hr at 0°C. The 0°C incubation was important to permit determination of non-specifically bound rather than incorporated ^3H_2O (20). The linearity of the incorporation of ^3H_2O into DPS and total fatty acids over the 3-hr incubation period was examined by incubating six pools of lenses (20 per pool) from 14-day old rats at 37°C and stopping the incubation after 1, 2, and 3 hr. Three identical pools of lenses were incubated at 0°C and extracted after 1, 2, and 3 hr of incubation. In all cases, the reactions were stopped by addition of 17 ml of methanol. The mixtures were transferred to large test tubes (20 × 2.5 cm) and homogenized for 30 sec using a Tekmar Tissumizer (20 cm). Thirty-four ml of chloroform was added and the homogenates were filtered through sintered glass funnels. The residues were recovered and rehomogenized in 10 ml of chloroform–methanol. The combined filtrates were washed with 0.2 vol of 0.12 M KCl. The recovered total lipids were then saponified in alcoholic KOH (19). Non-saponifiable lipids were extracted into hexane. The remaining aqueous phase was then acidified and the total fatty acids were extracted into hexane. DPS were prepared as described by Andersen and Dietschy (20). Count rates on all samples were measured for 50 min or to 1% error (σ) using a Beckman LS 7000 scintillation counter with external standardization. Counting efficiencies with tritium averaged about 45% and the maximum counting error was about 3% (for the background samples). Incorporation of ^3H of water into DPS was corrected for bound ^3H_2O, i.e., that incorporated at 0°C. Measurements were expressed as ng atoms of ^3H incorporated per lens per the 3-hr incubation.

Long term. The pattern of incorporation by lens of ^3H from ^3H_2O into squalene and individual sterols was determined in lenses incubated for 24 hr using an organ-culture system. Lenses from 14- or 22-day old rats were pooled as described above. Five pools of lenses, each containing 400–500 mg (20–35 lenses), were prepared at both ages and suspended in 2.5 ml of the tissue culture medium used by Schenck, Fournier, and Patterson (21) to culture rat lens for 24–30 days. The medium was a modified TC 199 solution (Gibco and Hank's salts). The culture medium contained 0.8 mCi of ^3H_2O per ml and was sterilized by filtration prior to use. Three pools were incubated in sealed 25-ml Erlenmeyer flasks for 24 hr

at 37°C and two at 0°C. Total lipids were extracted as described above and subsequently fractionated by two different thin-layer chromatographic separations, the second using silica gel G prepared with $AgNO_3$, as we recently described in detail (22). The recovered individual lipid fractions (squalene, lanosterol, 7-dehydrocholesterol, desmosterol, cholesterol, and sterol esters) were separately assayed for ^3H content by liquid scintillation counting. After correction for non-specifically bound ^3H (determined from the 0°C incubation), the distribution of ^3H among the various fractions was calculated.

Calculation of rates of cholesterol synthesis from ^3H_2O incorporation

Rates of cholesterol synthesis were calculated on the assumption that the ^3H measured in the total DPS mainly represented [^3H]cholesterol. Experimental results showed this assumption to be correct. In order to relate the ng atoms of tritium incorporated into DPS to nmol of cholesterol synthesized, the number of tritium atoms incorporated per cholesterol molecule must be known. Twenty-two hydrogen atoms are incorporated per cholesterol molecule biosynthesized; 7 enter from the medium and 15 from NADPH (23). If the protons of the medium and of NADPH completely equilibrate with the ^3H of ^3H_2O, 0.81 tritium atoms would be incorporated per carbon atom of cholesterol (22 tritium atoms incorporated/27 carbon atoms in cholesterol) or 21.87 (0.81 × 27) tritium atoms would be incorporated per cholesterol molecule formed. This factor could then be used to estimate an absolute rate of cholesterol synthesis from the following equation: μg cholesterol synthesized per lens per day = (ng atom ^3H incorporated per lens per 3 hr × 8) ÷ 21.87 × 0.38664 μg cholesterol per nmol of cholesterol. This calculation would apply only if the lens' NADPH was generated totally by oxidative enzymatic reactions, since NADPH derived from the pentose pathway does not become labeled with ^3H from ^3H_2O (20). On the other hand, if the pentose pathway were the exclusive source of NADPH in the lens for sterol synthesis, only 7 tritium atoms would be incorporated per molecule of cholesterol synthesized; i.e., 0.26 tritium atoms would be incorporated per carbon atom of cholesterol. The rates of cholesterol synthesis were calculated for both extreme situations, i.e., F (factor) = 0.26 (all the NADPH generated by the shunt) and F = 0.81 (none of the NADPH generated by the shunt).

Oxidation of [1-^{14}C]- vs. [6-^{14}Cl]glucose and incorporation into cholesterol

Pools of 20 to 30 lenses from 6 or 22 day old rats were collected as described earlier and incubated in triplicate for 3 hr at 37°C in 2.5 ml of Krebs-bicarbonate buffer (pH 7.4) containing 15 μCi of [1-^{14}C]glucose at a calculated specific activity of 0.86 mCi/mmol or 15 μCi of [6-^{14}C]glucose at 0.78 mCi/mmol. Incubations were also simultaneously conducted with [1-^{14}C]- or [6-^{14}C]glucose but in the absence of lenses in order to measure any spontaneous oxidation of glucose. Radiolabeled CO_2 was trapped in hyamine hydroxide as described by Awad (24) following acidification of the medium with H_2SO_4 and assayed for ^{14}C-content by liquid scintillation counting. Total lipids were extracted from the incubated lenses as described above. Aliquots of the total lipids were saponified and the DPS was recovered from the non-saponifiable fraction. The results were expressed as nmol of glucose oxidized to CO_2 or incorporated into DPS per lens per 3 hr. Both [1-^{14}C]- and [6-^{14}C]glucose yield [2-^{14}C]acetyl CoA without loss of radiolabel. Since 15 of the 18 carbon atoms of nine [2-^{14}C]acetyl CoAs reach cholesterol, the incorporation rates of [1-^{14}C]- or [6-^{14}C]glucose into cholesterol were multiplied by 1.2.

Isolation of lipoproteins

Lipoprotein fractions were recovered following ultracentrifugation of rat and human citrated plasma (20–25 ml). Plasma density was initially adjusted to 1.019 g/ml with a stock salt solution (25) and the plasma was centrifuged at 110,000 *g* for 24 hr (Beckman Model L ultracentrifuge). The upper 25% of the plasma volume was aspirated and discarded. The remaining infranatant fraction was recovered, adjusted to density 1.063 g/ml, and centrifuged as above. The lipoproteins in the supernatant (d 1.019–1.063 g/ml) were recovered and saved as the low density lipoprotein fraction (LDL). The infranatant from this spin was adjusted to density 1.23 g/ml and recentrifuged. The resulting lipoprotein fraction (d 1.063–1.23 g/ml) was recovered and saved as the high density lipoproteins (HDL). The LDL and HDL fractions were washed, respectively, with density 1.063 g/ml and 1.23 g/ml salt solutions and subsequently dialyzed against large volumes of isotonic saline. The cholesterol content of each fraction was measured by colorimetric assay (26). Rat HDL contained over 85% of the total cholesterol present in the combined HDL and LDL fractions. This agrees with the observation that in the rat HDL carries most of the plasma total cholesterol (27, 28).

Organ culture of lens with lipoproteins

Lenses collected from 22-day old rats were preincubated in groups of 10 in 2.5 ml of modified TC 199 medium (21) containing known concentrations of HDL (rat) or LDL (rat or human) cholesterol for 2, 22, or 24 hr in a National model 8241 CO_2-air humidifier. Fresh media, identical to that removed, was added following the preincubations and 10 μCi of [U-^{14}C]glucose (New England Nuclear Corp.) was added in 0.1 ml of

TABLE 1. Synthesis of cholesterol and fatty acids by the ocular lens during early postnatal development

Age (Days)	Cholesterol: ng Atoms ^3H Incorp/Lens/3 hr (Avg 2–4 Pools)[a,b]	Cholesterol: μg Cholesterol Synthesized/Lens/24 hr[c] F = 0.26[d]	Cholesterol: μg Cholesterol Synthesized/Lens/24 hr[c] F = 0.81[e]	Fatty Acid: ng Atoms ^3H Incorp/Lens/3 hr (Avg 2–4 Pools)[a,b]
6	1.81 (1.82, 1.80)	0.80	0.26	4.82 (4.41, 5.23)
14	1.63 ± 0.15 (n = 4)	0.72 ± 0.06	0.23 ± 0.02	4.44 ± 0.43 (n = 4)
22	0.77 (1.01, 0.52)	0.34	0.11	1.82 (2.30, 1.34)
30	0.89 (0.74, 1.03)	0.39	0.13	1.81 (1.47, 2.14)

[a] Separate pools of lenses (20–50 pool) from 6- to 30-day old rats were prepared and incubated for 3 hr at either 37°C or 0°C in 2.5 ml of Krebs-bicarbonate buffer containing 15 μmol of D-glucose and 2.0 mCi of ^3H_2O. The specific activity of the water was estimated for each incubated flask and averaged 14,632 ± 153 (SEM) dpm/μg atom of hydrogen of water. Total lipids were subsequently recovered, saponified, and the DPS was prepared. Total fatty acids were separately recovered and also assayed for ^3H content. Incorporation of tritium into DPS and total fatty acid at 37°C was correct for incorporation at 0°C. Values are the mean or mean ± SEM.

[b] DPM ^3H incorporated per lens ÷ DPM (avg 14.6)/ng atom of ^3H of ^3H_2O.

[c] Cholesterol, μg synthesized per lens per 24 hr = (ng atom ^3H incorporated per 3 hr × 8) ÷ (0.26 × 27) or ÷ (0.81 × 27) × 0.38664 μg per nmol cholesterol.

[d] F = 0.26; μg atom ^3H incorporated/μg atom C (of cholesterol) assuming all NADPH comes from pentose phosphate pathway.

[e] F = 0.81; μg atom ^3H incorporated/μg atom C (of cholesterol) assuming all NADPH comes from oxidative enzymatic reactions.

water. The specific activity of the medium glucose was calculated to be 0.77 mCi/mmol. In one group of experiments, delipidated fetal calf serum, prepared according to Stein et al. (29) was included in the TC 199 medium. The flasks were sealed and incubated for an additional 3 hr at 37°C with shaking (20 cycles per min). The reactions were stopped by addition of 0.61 ml of 6 N H_2SO_4 and respiratory CO_2 was collected as described above. Following recovery and saponification of the total lens lipids, DPS were recovered as before and assayed for ^{14}C content by liquid scintillation counting. The results were expressed as nmol of glucose oxidized to CO_2 or incorporated into DPS per lens per 3 hr.

RESULTS

Andersen and Dietschy (20) recently demonstrated that use of ^{14}C-labeled compounds to measure sterol synthesis by extrahepatic tissues can result in great underestimations of the true synthetic rates. Rather than ^{14}C-labeled substrates, their work and that of others recommends use of ^3H_2O as the substrate of choice for measurement of cholesterol biosynthesis (20, 23, 30). The incorporation by lens of ^3H from ^3H_2O into DPS decreased from about 1.6–1.8 ng atoms of tritium per lens for the 3 hr incubation at 6 and 14 days of age to about 0.8–0.9 ng atoms at 22 and 30 days of age (**Table 1**). Between two to three times more ^3H_2O was incorporated into total fatty acids per lens than into DPS (Table 1). Incorporation into fatty acids also decreased with age in parallel to the decrease in DPS. If the incorporation had been expressed per g of lens (wet weight), the decrease between 6 and 22–30 days of age would have been greatly magnified.

The rates of incorporation of ^3H_2O into cholesterol were converted to rates of synthesis and expressed as μg of cholesterol synthesized per lens per 24 hr. This calculation assumes that the rate of incorporation of ^3H_2O into DPS is linear over the 3-hr incubation. The rates of incorporation of ^3H_2O into both DPS and total fatty acid was essentially linear for the first 2 hr of incubation followed by some decrease during the third hour (**Fig. 1**). Thus, the calculated rates of cholesterol synthesis per lens per 24 hr presented in Table 1 could be slightly lower than the true in vitro rates. When the synthetic rates were calculated on the basis of all of the lens NADPH being generated by the pentose pathway (F = 0.26), between 0.7 μg to 0.8 μg of cholesterol would have been synthesized per day per lens at 6 and 14 days of age as compared to between 0.3 μg to 0.4 μg per day at 22 and 30 days of age. The estimated rates of synthesis would be only about one-third of these values if calculated on the basis of all of the lens' NADPH being generated by oxidative enzymatic reactions (F = 0.81). Between 6 and 30 days of age the cholesterol content per lens increased at a linear rate from 6.4 to 22.6 μg (**Fig. 2**). This corresponds to an increase of 0.68 μg of cholesterol per lens per day.

Oxidation of both [1-^{14}C]- and [6-^{14}C]glucose by the lens clearly increased with age while incorporation into DPS did not significantly change (**Table 2**). Lens me-

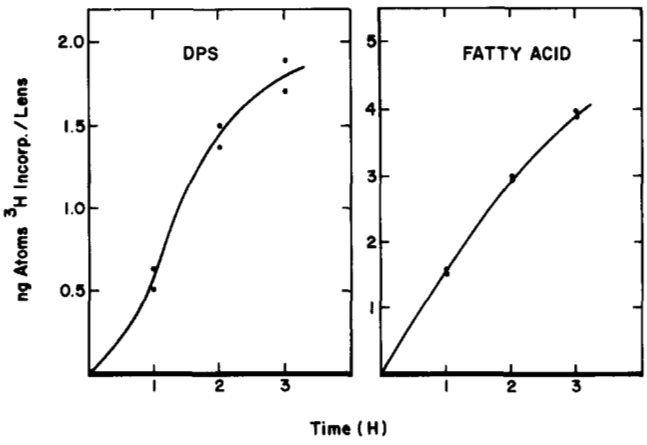

Fig. 1. Incorporation of ^3H_2O into DPS and total fatty acids versus duration of incubation. Each point represents the incorporation by a separate pool of 20 lenses from 14-day old rats. Reaction was initiated by addition of 2.50 ml of Krebs-bicarbonate buffer containing 2.0 mCi of ^3H_2O to each pool. The specific activity of the medium water, which took into consideration the water content of the lens, was calculated to be 15,254 dpm per μg atom of hydrogen of water. The incorporation at each time was corrected for incorporation at 0°C. The ^{3}H recovered in DPS and fatty acid from lenses incubated at 0°C was constant over the 3-hr period.

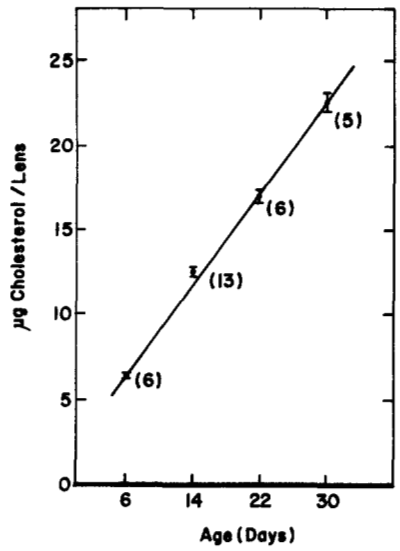

Fig. 2. Free cholesterol content of lens from rats of various ages. Essentially all of the cholesterol in the lens is free or unesterified. Values are mean ± SEM. for the indicated number of samples (numbers in parentheses). Each sample consisted of a minimum of two pooled lenses.

tabolism of [1-^{14}C]glucose versus [6-^{14}C]glucose was estimated primarily to provide an index of pentose phosphate pathway activity at various ages. The ratio of oxidation of [1-^{14}C]- to [6-^{14}C]glucose to $^{14}CO_2$ at 6 days of age was about 7.5 and about 9.0 at 22 days of age (Table 2). However, no differences were seen at either age in the incorporation of [1-^{14}C]- versus [6-^{14}C]glucose into cholesterol. One would anticipate that the specific activity of the [^{14}C]acetyl CoA formed from metabolism of [1-^{14}C]- and [6-^{14}C]glucose to be similar, since oxidation of the number one carbon of glucose represents only a minor pathway of glucose metabolism even by the lens. Kinoshita and Wachtl (31) observed that 80% of both [1-^{14}C]- and [6-^{14}C]glucose metabolized by the rabbit lens in vitro was recovered as [^{14}C]lactate, even though 40 times more [1-^{14}C]glucose than [6-^{14}C]glucose was oxidized to $^{14}CO_2$.

As mentioned earlier, estimation of cholesterol synthesis from incorporation of radiolabeled substrates into DPS assumes that cholesterol is the main labeled sterol in the DPS. In 14- and 22-day old lenses, cholesterol accounted for between 50 and 60% of the tritium recovered from the total sterol fraction (data not shown). Lanosterol accounted for essentially all of the remaining label. Recent observations indicate that lanosterol is only partially and often poorly precipitated by digitonin.[1] Thus, it would appear justified to assume that incorporation of ^{3}H from ^3H_2O into DPS by the lens largely represents incorporation into cholesterol.

Incubation of lenses with high concentrations of either HDL or LDL cholesterol (0.8–1.0 mM) resulted in no consistent changes in incorporation of [U-^{14}C]glucose into DPS regardless of whether the lenses were preincubated for 2, 22, or 24 hr with these lipoproteins (**Table 3** and **Table 4**). Preincubation of lenses for 1 day prior to incubation with [U-^{14}C]glucose resulted in about a 70% decrease in both oxidation and incorporation of radiolabeled into DPS during a subsequent 3-hr incubation (Table 3).

TABLE 2. Metabolism of glucose by the lens: oxidation to CO_2 and incorporation into cholesterol[a]

Lens Age (Days)	Glucose Isotope	nmol Glucose Oxidized or Incorp/Lens/3 hr	
		CO_2	DPS
6	1-^{14}C	4.31 ± 0.25	0.242 ± 0.041
6	6-^{14}C	0.56 ± 0.07	0.233 ± 0.026
22	1-^{14}C	8.65 ± 0.17[b]	0.291 ± 0.010
22	6-^{14}C	0.96 ± 0.10[b]	0.272 ± 0.010

[a] Twenty to thirty lenses from 6- or 22-day old rats were incubated in triplicate for 3 hr at 37°C in 2.50 ml of Krebs-bicarbonate buffer containing 15 μCi of either [1-^{14}C]glucose or [6-^{14}C]glucose at a specific activity of 0.86 mCi/mmol and 0.78 mCi/mmol, respectively. Radiolabeled CO_2 was trapped in hyamine hydroxide and radiolabeled cholesterol was isolated from the saponified lens lipids as the digitonide. Values are means ± SEM.

[b] P(t) of difference from corresponding 6-day oxidation rates is <0.05 (Student's t test).

[1] Cenedella, R. J. Results in press (*Lipids*).

TABLE 3. Effect of lipoprotein cholesterol on incorporation of [U-^{14}C]glucose into CO_2 and DPS by the rat lens—experiment A

System	mM Cholesterol	nmol Incorporated per Lens per 3 hr[a] 2-hr Preincubation CO_2	2-hr Preincubation DPS	24-hr Preincubation CO_2	24-hr Preincubation DPS
Control	0	6.28	0.131	2.08	0.037
Rat HDL	0.82	6.11	0.122	1.55	0.038
Rat LDL	0.19	4.22	0.096	1.52	0.035

[a] Duplicate pools of 10 lenses from 22-day old rats were preincubated for either 2 or 24 hr in 2.5 ml of TC199 medium (minus fetal calf serum) containing no cholesterol (Control), 0.82 mM cholesterol in rat HDL, or 0.19 mM cholesterol in rat LDL. Fresh media, identical to that removed, was added plus 10 μCi of [U-^{14}C]glucose (sp act of medium glucose = 0.77 mCi/mmol) in 0.1 ml of water and the lenses were incubated 3 additional hours with collection of expired CO_2.

DISCUSSION

Culp et al. (32) reported that label from intraocularly injected [^{14}C]acetate was incorporated into cholesterol of the young (47–55 day old) rabbit lens. Cholesterol accounted for about 14% of the total ^{14}C label recovered from the lens lipids. Unfortunately, the source of this lens [^{14}C]cholesterol is unclear from their results, since it would have arisen from synthesis de novo in the lens itself or from uptake of [^{14}C]cholesterol that was synthesized in other ocular tissues or even extraocular tissues and then contributed to the aqueous humor. Cholesterol is apparently present in ocular fluids inasmuch as Schmut and Zirm (33) reported that human aqueous humor contains high density lipoproteins. Also, as demonstrated by Andersen and Dietschy (20), large and highly variable errors are inherent in estimating sterol synthesis in vitro from ^{14}C-labeled substrates such as [^{14}C]acetate, and these errors may even be greater if such substrates are used to measure synthesis in vivo (31).

The first few weeks of postnatal life is a dynamic period for development of the rat lens. Between 6 and 30 days of age the cholesterol content of the individual lens more than tripled from about 6.4 to 22.6 μg/lens (about 40% of the total cholesterol content of an adult lens). Since the ocular lens does not shed cells or secrete substances, this observed increase should describe the true cholesterol requirement of the lens during this growth period. The calculated rates of synthesis of cholesterol, measured from incorporation of tritium of ^3H_2O, vary considerably depending upon the significance assigned to the pentose pathway in generating NADPH; NADPH so formed does not become labeled from ^3H_2O (20). If all the lens' NADPH came from the pentose pathway, the calculated rates of cholesterol synthesis would have been adequate to furnish about 100% of the len's requirement prior to 2 weeks of age and about 50% at the later ages. If all of the NADPH came from oxidative enzymatic reactions, synthesis de novo could have supplied only 40 to 20% of the total need.

The pentose pathway clearly is active in the young rat lens, since the ratio of oxidation of [1-^{14}C]glucose to [6-^{14}C]glucose was 7.5 to 1 and 9.0 to 1 at 6 and 22 days of age, respectively. The lens of the mature rabbit was reported to oxidize [1-^{14}C]- and [6-^{14}C]glucose at a ratio of 40 to 1 (31). The low level of incorporation of radiolabeled glucose as compared with ^3H_2O into DPS is consistent with the finding of Andersen and Dietschy (20) that in many tissues the rates of cholesterol synthesis measured in vitro with [^{14}C]glucose were only 2 to 88% of the true rates measured with ^3H_2O. Although the ratio of oxidation of [1-^{14}C]- to [6-^{14}C]glucose does not quantitatively describe the fraction of the total NADPH that is generated by shunt activity in the rat lens, it does suggest a major contribution by the pentose pathway in the rat lenses studied. Thus, the factor of 0.26 ng atoms of ^{3}H incorporated per carbon atom of cholesterol used to calculate cholesterol synthesis is probably closer to the correct factor than 0.81, the value that assumes that all of the NADPH is formed by oxidative processes.

Although the results of the present study do not permit a precise determination of the capacity of the developing lens to satisfy its cholesterol requirements by synthesis de novo, they do indicate the likelihood that the lens can furnish a large fraction if not all of its cholesterol needs by this mechanism at early ages. The results are consistent with the presence of an essentially fixed population of cells sythesizing the cholesterol required by the lens for formation of fiber cell-plasma membranes. The rates of cholesterol synthesis measured from ^3H_2O assessed the capacity of the lens to synthesize cholesterol in vitro and do not necessarily exclude other sources from supplying cholesterol to the lens in vivo. In fact, the apparent decrease in the rates of incorporation of ^3H_2O into DPS

TABLE 4. Effect of lipoprotein cholesterol on incorporation of [U-^{14}C]glucose into CO_2 and DPS by the rat lens—experiment B

System	mM Cholesterol	nmol Incorporated per Lens per 3 hr[a] 22-hr Preincubation CO_2	22-hr Preincubation DPS
Control	0[b]	2.16 ± 0.21[c]	0.028 ± 0.001
Rat HDL	1.0	2.05 ± 0.22	0.023 ± 0.004
Human HDL	1.0	2.60 ± 0.16	0.031 ± 0.004

[a] Lenses were incubated as in experiment A except in triplicate and the medium (which contained 2.5% delipidated fetal calf serum) was changed after 10 hr of preincubation in addition to after 22 hr.

[b] The cholesterol content of delipidated fetal calf serum was 23 μg/ml versus 301 μg/ml for the whole serum. Thus, 2.5% delipidated fetal calf serum should have yielded a medium cholesterol concentration of 0.0014 mM.

[c] Values are the mean ± 1 SEM.

after 2 weeks of age, without a decrease in the rate of cholesterol accumulation by the lens, suggests a source in addition to synthesis de novo for lens cholesterol, at least at these older ages. Both high and low density lipoproteins can markedly influence sterol synthesis in many tissues and contribute cholesterol to these tissues (27, 34). The reported presence of high density lipoprotein in human aqueous humor (33) suggests this as a potential source of lens cholesterol. The observation that neither rat HDL nor LDL nor human LDL decreased incorporation of [U-^{14}C]glucose into DPS by rat lens in vitro indicates that these lipoproteins do not regulate sterol synthesis in this tissue. However, it is yet possible that lipoproteins could be taken up by the lens and contribute cholesterol to it without affecting the activity of 3-hydroxy-3-methylglutaryl-CoA reductase (EC 1.1.1.34), the rate-limiting enzyme in cholesterol synthesis. Future measurements on the uptake and metabolism of ^{125}I-labeled lipoproteins might provide information on this possibility.

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