Synthesis of 3′-Phosphoadenosine-5′-phosphosulfate (PAPS) Increases during Corneal Development*

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The sulfate donor in glycosaminoglycan sulfation is 3′-phosphoadenosine-5′-phosphosulfate (PAPS). Ability of cell-free homogenates to synthesize PAPS from SO₃⁻ and ATP was investigated throughout embryonic development of the chicken cornea.

Corneas from embryos of different ages were homogenized, separated into supernatant and pelleted fractions, mixed with ATP and Mn⁺, and assayed separately for ability to synthesize, in sequence, ³²S[APS (3′-adenosine phosphosulfate), then ³⁵S]PAPS, and finally ³⁵S-labeled putative endogenous proteoglycan. Standard assays used optimal pH and concentrations of ATP and Mg++. Supernatant fractions of such corneal homogenates contained 73% of total ATP-sulfurylase and 97% of total APS-kinase activities. Both enzymes required ATP and Mg²⁺; Mn⁺ could substitute partially for Mg²⁺, but Ca²⁺ was inhibitory. Addition of hyaluronic acid, chondroitin 4-sulfate, or chondroitin 6-sulfate (0.1 to 2 mg/ml) had no effect on APS or PAPS biosynthesis.

Results from all embryonic ages suggested that: 1) the equilibrium concentration of ³²S[APS does not change during corneal development; 2) ability to form ³²S[APS increases 2½-fold between Day 8 and Day 16; and 3) the specific activity of ³⁵S-labeled putative endogenous proteoglycan in increasing in parallel with that of ³⁵S[APS, reaching a peak on Day 16. Days 8 to 16 includes the period during which the corneal stroma fibroblasts synthesize keratan sulfate and chondroitin sulfate of increased degree of sulfation and the period during which the cornea begins to become transparent. Degree of sulfation of corneal glycosaminoglycans, therefore, may be controlled by the availability of PAPS, rather than by changes in the specific activities of glycosaminoglycan sulfotransferases.

Keratan sulfate is the major glycosaminoglycan of vertebrate corneas (1) and is thought to be necessary for the orderly polymerization of the collagen fibrils that characterize the extracellular matrix of the cornea (2). The regular spacing and uniform, small diameter of corneal collagen fibrils is a requirement for corneal transparency (3).

The embryonic cornea is nontransparent during much of its early embryogenesis. It only becomes transparent late in development, an event which, in chicks at least, is under the control of thyroxine (4, 5). This latter period is characterized by a rapid and extensive dehydration and thinning of the corneal stroma (4, 5), as well as by the synthesis of keratan sulfate molecules that have remarkably higher apparent degrees of sulfation than those made during earlier stages of development (6). To begin to elucidate the mechanism controlling the degree of glycosaminoglycan sulfation, Hart (7) measured the specific activities of several specific glycosaminoglycan sulfotransferases of the cornea during development. However, this study revealed no increases in the activity of keratan sulfate sulfotransferase (or that for chondroitin sulfate) and led Hart to postulate that control of glycosaminoglycan sulfation in the cornea therefore might occur instead at the level of availability of the sulfate donor itself, PAPS.

In the present paper, we have tested this hypothesis by measuring the specific activities of ATP-sulfurylase and APS-kinase in cell-free homogenates under conditions where, as in intact cells, SO₃⁻ is coupled to ATP in sequence to generate APS and then PAPS, with the latter subsequently serving as sulfate donor for endogenous proteoglycans. These two enzymes have been studied extensively in other tissues (8–12), but to our knowledge have not been measured previously during corneal development.

EXPERIMENTAL PROCEDURES

Enzyme Preparation—Fertilized, White Leghorn chicken eggs were incubated at 38°C in a forced draft incubator. Corneas were dissected from embryos of ages from Day 8 to Day 20 (age also determined from Hamburger-Hamilton stages (13)). Corneas were removed to Saline G (pH 7.4) in plastic Petri dishes on ice. Under a dissecting microscope, whole corneas were trimmed free of limbus and scleral ossicles by using cylindrical stainless steel tubing with sharpened edges as uniform punches (perforating trephines). Inside diameter of punches was 1.8 mm for 8- and 10-day corneas, 2.1 mm for 12-, 14-, and 16-day, and 2.7 mm for 18- and 20-day corneas. Trimmed, whole corneas were blotted immediately to remove excess Saline (g and weighed (wet weight). Corneas then were transferred to a 2-ml Ten Broeck (Wheaton, Millville, NJ) glass homogenizer and sonicated under an ice bath by stroking the wall of the tube with the sonicator probe at 120 watts for 3 min (Branson, Plainville, NY). Supernatants were centrifuged at 25,000 × g for 25 min at 2°C. Supernatants were removed and placed in ice. Pellets were resuspended in 0.25 μl sucrose (volume equivalent to that for supernatants) and resuspended to form relatively homogeneous suspensions. Triplicate aliquots were removed for Lowry protein determination (15). All such supernatant and pelleted fractions were assayed immediately after preparation.

Standard Assay—The standard incubation mixture developed for use in this study contained the following: 60 μl of incubation buffer (0.02 M citrate, 0.02 M Na₂HPO₄ (pH 7) mixed together daily with an equal volume of 0.2 M Tris that had been adjusted to pH 7 with

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1 The abbreviations used are: PAPS, 3′-phosphoadenosine-5′-phosphosulfate; APS, 3′-adenosine phosphosulfate; GAG, glycosaminoglycan; PAP, 3′-phosphoadenosine-5′-phosphate; PP⁺, inorganic pyrophosphate.
glacial acetic acid) (14), 10 µl of 0.1 M MgCl₂, 10 µl of 0.05 M ATP (disodium, Sigma Chemical Co., St. Louis, MO; Catalogue No. 3127), 10 µl of distilled water containing 10 µCi of H₂³5SO₄ (carrier-free, ICN, Irvine, CA), and 10 µl of corneal homogenate fraction (supernatant or pellet suspension). Components were added to 1.5-ml plastic conical tubes and incubated at 37°C for 30 min. At the end of incubation, reactions were stopped by placing tubes in a boiling water bath for 3 min. Samples were then stored at −20°C until analyzed.

In ascertaining optimal conditions for the standard assay, duplicate samples were used for every reaction condition.

To determine the amount of radioactivity in APS, PAPS, and proteoglycan, the entire contents of each reaction tube (100 µl) were deproteinized in a single spot in the center of a 3-mm wide channel on Whatman No. 3MM chromatography paper in preparation for high voltage electrophoresis. Electrophoresis buffer in tanks and for wetting the paper consisted of 0.5 M citrate, 0.5 M Na₂HPO₄, (pH 7) mixed together daily with an equal volume of 0.1 M Tris that had been adjusted to pH 7 with glacial acetic acid (14). APS (Sigma), PAPS (P-L Biochemicals, Milwaukee, WI), and H₂³5SO₄ (ICN) were spotted as standards on adjoining channels with every set of experimental samples. Electrophoresis was performed at 14 V/cm (total = 1176 V) for 2 h, followed by drying at room temperature, and cutting the paper sheets into individual channels. Radioactivity was located on a Packard model 7201 radiochromatogram scanner. Nonradioactive APS and PAPS standards were located under short wavelength UV light. Under these standard electrophoresis conditions, putative endogenous proteoglycans remained at the origin; APS migrated 17 cm; and SO₄²⁻, 39 to 41 cm (in contrast to the behavior of putative endogenous proteoglycan, polysaccharides freed of core protein by proteolysis (e.g. chondroitin sulfate) or naturally free of protein (e.g. hyaluronic acid) migrated to a position between APS and PAPS). Only background levels of radioactivity were detected in regions between the origin, APS, PAPS, and SO₄²⁻. Radioactive areas were cut into 1-cm pieces, mixed with 0.5 ml of glass-distilled water, and 10 ml of ethanol/toluene scintillation fluid (16) and counted in a Packard Tri-Carb model 3320 liquid scintillation spectrometer. Counting efficiency under these conditions was 63% for ³⁵S.

Using the standard assay conditions described above, ATP-sulfurylase and APS-kinase specific activities were determined during corneal development by assaying supernatant and pelleted homogenate fractions prepared from corneas of seven different ages (50 corneas were pooled at each age). Ten replicate aliquots were assayed from each supernatant fraction at each age (five replicates were assayed from the corresponding pellet fraction). The entire experiment (7 ages x (10 + 5) replicates/age) was repeated four times. Within each experiment, variance of specific activity data was calculated according to the Taylor series approximation to the variance of a ratio (17).

Highly purified reference standard glycosaminoglycans (hyaluronic acid, chondroitin 4-sulfate, chondroitin 6-sulfate) were the generous gift of Dr. M. M. J. M. Mathews and J. A. Cifonelli, University of Chicago, IL. ATP (Equine, disodium salt) and pyrophosphatase (nucleotide pyrophosphatase, 0.0026 unit/mg of protein) were from Sigma.

RESULTS

The sequence of reactions by which inorganic sulfate is used to make APS and PAPS, and then is transferred to growing polysaccharide chains, involves the activities of ATP-sulfurylase, APS-kinase, and several specific glycosaminoglycan sulfotransferases. In order to be able to determine whether these activities varied during development of the embryonic cornea, we first determined the optimal conditions for the sequential biosynthesis of APS and PAPS. Previous work (7) already had demonstrated that the specific activity of endogenous corneal glycosaminoglycan sulfotransferase did not change substantially during corneal development, so we concentrated our study on ATP-sulfurylase and APS-kinase.

Optimal Conditions for Standard Assay—Both enzymes required Mg²⁺ for activity. Under standard assay conditions, in the absence of Mg²⁺, reaction rates were minimal (Fig. 1). Addition of MgCl₂ to the standard assay mixture stimulated activity only slightly above the particular enzyme tested. ATP, PAPS, proteoglycan, and SO₄²⁻, the total amount of incorporation in APS and PAPS was maximal for both compounds at 10 mM MgCl₂ (Fig. 1). Again using standard assay conditions, the optimal concentration of ATP was 5 mM for PAPS formation (Fig. 2). The peak of APS formation at 2 mM ATP shown in Fig. 2 did not appear in replicate experiments. Higher concentrations of ATP (20 to 40 mM) were not as inhibitory for APS synthesis as for that of PAPS (Fig. 2).

Both ATP-sulfurylase and APS-kinase showed highest activity in the range of pH 6.5 to 7.5 (Fig. 3, with pH 7 chosen as the optimum). The amount of ³⁵S incorporated into both APS and PAPS increased in a roughly linear manner (actually sigmoidal) as the volume of homogenate (supernatant fraction) was increased (Fig. 4). A volume of 10 µl was selected for use in the standard assay. Incorporation into both APS and PAPS also increased as the amount of H₂³5SO₄ was increased in the standard assay (Fig. 5).

The amount of ³⁵S incorporated in PAPS increased with incubation time, but that in APS did not (Fig. 6). This is consistent with the maintenance of a very low equilibrium concentration of APS: its constant synthesis from ATP and SO₄²⁻ by ATP-sulfurylase, its constant consumption by APS-kinase to make PAPS (with concomitant use of another ATP), and the known ability of APS to act as a feedback inhibitor of its own biosynthesis (12). Incorporation into PAPS was linear for approximately 60 min, then showed a plateau, and then increased again (Fig. 6). This same pattern was seen in four replicate experiments. In two of these experiments, the first linear phase lasted for 90 min. For the standard assay, we used a 30-min period of incubation, in the middle of the initial linear phase, for both PAPS and APS, and after APS had reached its equilibrium concentration. In three of the four experiments, the plateau period for incorporation into PAPS began after 90 min of incubation and lasted an average of 60 min. Following the plateau, a second period of incorporation into PAPS was seen. In three of the experiments, the slope of this second increase was approximately equal to that of the first. In all four experiments, the radioactivity incorporated in APS increased within 10 min to an equilibrium level which was maintained throughout the time when PAPS was undergoing its biphasic pattern of increase (Fig. 6). To test the possibility that the accumulation of radioactivity in PAPS reached a plateau after 90 min because of ATP depletion, in one experiment, we waited until the plateau was reached and then added additional ATP (5 mM). The results (not shown) indicated that the freshly added ATP had no effect. Doubling the initial ATP concentration from 5 to 10 mM would not have been expected to inhibit (or stimulate) significantly the incorporation into PAPS. The fact that no such stimulation occurred suggests that depletion of ATP did not account for the plateau in incorporation after the first 90 min of incubation (Fig. 6).

The data in Figs. 1 to 6 formed the basis for selecting the optimal conditions for the standard assay, as given under “Experimental Procedures.” All of these experiments were performed with homogenates from Day 14 corneas. Most of the total detectable enzyme activity was found in the supernatant fractions of the homogenates at this age, as well as at all other ages studied (see below): 73% of total ATP-sulfurylase was in the supernatant (range, 62 to 84%), as well as 97% of total APS-kinase (range, 94 to 98%).

Other Influences on the Standard Assay—When Ca²⁺ (2 to 20 mM) was added to the standard assay mixture from which MgCl₂ was omitted, incorporation into APS remained at the low level which typified Mg²⁺ absence (see Fig. 4), whereas incorporation into PAPS was decreased to approximately one-half of its nonstimulated level. When Ca²⁺ (2 to 20 mM) was added to the standard assay mixture in the presence of optimal Mg²⁺ (10 mM), Ca²⁺ inhibited incorporation into both APS
Changes in Enzyme Activity during Corneal Development—Corneas from chicks of different embryonic ages were homogenized and analyzed in the standard assay described under “Experimental Procedures.” Results of three of the four experiments completed are shown in detail in Fig. 7. The ability to form PAPS rises from Day 8 and reaches a peak at Days 16 to 18. However, the equilibrium concentration of APS, assayed in the very same tubes, remains constant during development. The data of Fig. 7 are shown to indicate the degree of variability between experiments and the magnitude of the standard errors calculated for each experiment. The data for all four experiments are summarized in Fig. 8 (solid circles) and indicate that the ability to form PAPS rises about 2½-fold between Day 8 and Day 16, whereas the equilibrium concentration of APS remains constant throughout this period.

The specific activity of material remaining at the origin after electrophoresis (putative endogenous proteoglycan) also remains constant (Fig. 8, solid circles). However, all data shown in Fig. 7 and in Fig. 8 (solid circles) were derived from assays of the supernatant fractions of corneal homogenates. Although we found the majority of both ATP-sulfurylase and APS-kinase activities in such supernatant fractions (73% and

**Fig. 1 (left).** Effect of Mg**2+** concentration on **35**SO**4** incorporation into PAPS and APS. Corneas from Day 14 chick embryos were homogenized and centrifuged; supernatant fractions were incubated with **35**SO**4** under conditions of the standard assay described under “Experimental Procedures,” except that Mg**2+** concentration was varied. Arrow indicates that 10 mM Mg**2+** was chosen as optimal for use in our standard assay at other embryonic ages. Error bars indicate range of measurements. ●—●, PAPS; ○—○, APS.

**Fig. 2 (left center).** Effect of ATP concentration on **35**SO**4** incorporation into PAPS and APS. Same legend as Fig. 1, except that ATP concentration was varied, with 5 mM chosen as optimal for the standard assay. Error bars indicate range of measurements. ●—●, PAPS; ○—○, APS.

**Fig. 3 (right center).** Effect of pH on **35**SO**4** incorporation into PAPS and APS. Same legend as Fig. 1, except that pH was varied, with pH 7 chosen as optimal for the standard assay. Error bars indicate range of measurements. ●—●, PAPS; ○—○, APS.

**Fig. 4 (right).** Effect of amount of homogenate on **35**SO**4** incorporation into PAPS and APS. Same legend as Fig. 1, except that amount of homogenate (supernatant fraction) was varied, with 10 µl chosen for use in the standard assay. Error bars indicate range of measurements. ●—●, PAPS; ○—○, APS.

**Fig. 5 (left).** Effect of amount of **35**SO**4** on **35**SO**4** incorporation into PAPS and APS. Same legend as Fig. 1, except that amount of **35**SO**4** was varied, with 10 µCi chosen for use in the standard assay. Error bars indicate range of measurements. ●—●, PAPS; ○—○, APS.

**Fig. 6 (right).** Effect of incubation time on **35**SO**4** incorporation into PAPS and APS. Same legend as Fig. 1, except that incubation time at 37°C was varied, with 30 min chosen for use in the standard assay. Error bars indicate range of measurements. ●—●, PAPS; ○—○, APS.

and PAPS to the same extent (40% of controls at 5 mM Ca**2+**). When the same experiments were performed with Mn**2+** (2 to 20 mM) in the absence of Mg**2+**, the presence of 10 mM Mn**2+** stimulated incorporation into APS one-half as much as did optimal Mg**2+** (10 mM). When Mn**2+** (2 to 20 mM) was tested in the presence of optimal Mg**2+** (10 mM), 2 mM Mn**2+** stimulated incorporation very slightly into both APS and PAPS, but was steadily inhibitory at higher concentrations. Thus, Ca**2+** could not substitute for Mg**2+** in the assay, whereas Mn**2+** could to some degree, as in yeast (8). Both Ca**2+** and Mn**2+** inhibited the stimulatory effect of the optimal Mg**2+** concentration when they were present at concentrations higher than 2 mM.

Addition of hyaluronic acid, chondroitin 6-sulfate, or chondroitin 4-sulfate (0.1 to 2 mg/ml) to the standard assay mixture had no effect on the incorporation into either APS or PAPS. To determine whether more rapid removal of pyrophosphate (one of the products, along with APS, of ATP sulfurylase) would markedly stimulate incorporation into APS and PAPS (10, 12), we added 1 to 2 units of exogenous pyrophosphatase to the standard assay mixture. Instead of stimulation, we observed an inhibition of incorporation into both APS and PAPS to 10 to 20% of control levels, a result which we cannot explain.

When nonradioactive PAPS (0.1 to 5 A**260** units/tube) was added to the standard assay mixture, incorporation into APS was not affected, as expected, but incorporation into PAPS fell to 20 to 40% of control levels, an effect we have not seen reported previously. When nonradioactive APS (0.1 to 5 mM) was added to the standard assay mixture, incorporation of **35**SO**4** into APS fell to 25% of control levels (at 0.1 mM), as expected because of feedback inhibition of ATP-sulfurylase (12), but then increased steadily to 1½ times higher than control levels (at 5 mM). Incorporation into PAPS, however, fell to 15% of control levels (at 0.1 mM) and remained there at all higher concentrations, presumably because the APS pool was of lower specific activity (9, 21).
Changes in the specific activity of PAPS and APS synthesized by homogenates (supernatant fractions) of corneas of different embryonic ages. Corneas were dissected from embryos of several embryonic ages, homogenized, and centrifuged, as described under "Experimental Procedures." Supernatant fractions were incubated according to conditions of the standard assay described. Results of three of four complete experiments are shown. Each point is the average of ten replicate aliquots assayed from each supernatant fraction prepared at each age in each experiment. Lowry protein assays were performed on the same fractions. Error bars indicate standard deviations, where they are larger than the data point. •, PAPS; ○, APS.

DISCUSSION

Data presented in this paper suggest that PAPS becomes more available in corneal cells during embryonic development, and that, as predicted previously (7), this results in the production of glycosaminoglycans of higher degrees of sulfation. These events occur during the same period of development when the embryonic cornea is becoming transparent, suggesting that the biochemical modifications and the morphogenetic process may be linked.

Enzyme Assay—A sequence of two enzymic reactions is required to generate PAPS, the active sulfate donor during biosynthesis of sulfated glycosaminoglycans. Their kinetics has been discussed previously (8–12). Our results suggest that the enzyme system detected in the embryonic cornea, which synthesizes PAPS under our standard conditions, behaves in a manner consistent with earlier studies of ATP-sulfurylase and APS-kinase in a variety of organisms: (a) The latter two enzyme activities were found predominantly in the supernatant fractions, whereas glycosaminoglycan sulfotransferase activity was mainly in the pellet fraction (7, 8, 14, 18, 19). (b) Levels of radioactivity detected were highest in PAPS, lower in APS (20), and still lower in material remaining at the origin (putative proteoglycan) (Fig. 8), consistent with the expected very low equilibrium concentrations of APS (9, 21), accumulation of PAPS (to concentrations as high as 1 mM (22)), and participation of PAPS in sulfation of endogenous proteoglycans (7). (c) Synthesis of both APS and PAPS was ATP-
dependent, with an optimum at 5 mM, similar to results from mouse mast cells (23) and hen oviduct (14). (d) Synthesis of both APS and PAPS was Mg\textsuperscript{2+}-dependent, with an optimum at 10 mM, similar to results from rat retina and liver (18). (e) Optimum pH was 6.5 to 7.5, a range generally lower than that seen in yeast (9) and rat liver (19), but similar to that of mouse mast cells (23). (f) The amount of radioactivity incorporated into APS and PAPS increased reasonably linearly with increases in microcuries of H\textsubscript{2}\text{35}SO\textsubscript{4} added, but increased in a slightly sigmoidal manner as enzyme concentration was increased, a pattern seen previously and ascribed to an effect of enzyme concentration on the apparent equilibrium constant for APS (9). (g) The amount of APS detected reached an equilibrium level after approximately 10 min and remained there for at least 4 h of incubation, a pattern identical with that seen by Robbins and Lipmann in yeast (9). PAPS, on the other hand, accumulated with time in a linear manner for approximately 60 min, a time period somewhat longer than that seen in some other studies (19, 20, 23). The sigmoidal pattern of PAPS accumulation observed over a 4-h period has been seen in more muted form in rat retina (18). (h) Although sulfate activation is saturated at 20 mM (8), addition of non-radioactive sulfate (1 to 40 mM) to our standard assay mixture caused only a sharp inhibition of incorporation, a pattern identical with that seen in mouse mast cells (23) and presumably due to synthesis of APS and PAPS of lower specific activity even though greater total quantities of both products may have been synthesized.

Corneal Development—Chicken corneas are opaque through most of their early development, but then become transparent during a relatively short, final period of embryogenesis. The mechanism by which this occurs is not clear. In the chick, the onset of transparency appears to require thyroxine (4) and to involve extensive dehydration and thinning of the cornea, as well as changes in electrolyte content (5), distributions of intramembranous particles (24), and synthesis of glycosaminoglycans of higher apparent degrees of sulfation than those made previously (6). Despite these findings, the primary mechanisms responsible for initiating transparency are unknown.

Although it was once believed that synthesis of the corneal-specific glycosaminoglycan, keratan sulfate-I, began at a time coincident with the onset of transparency, refined assays for this polysaccharide revealed that its rapid synthesis begins at least 8 days before transparency begins (6). However, that study disclosed that the apparent degree of sulfation of keratan sulfate-I increased markedly when transparency began (6). This change could not be attributed to an increase in the specific activity of keratan sulfate-I sulfotransferase, or that of other glycosaminoglycan sulfotransferases (7). These results therefore led Hart (7) to postulate that the degree of sulfation of corneal glycosaminoglycans might be increased by an increase in the availability of PAPS.

Our data clearly suggest that during embryonic development corneal homogenates become more active in their ability to synthesize PAPS. Specific activity of PAPS rises during the period preceding the time when transparency begins (Day 14), continues to rise to a peak at a time when the transparency increase is half-completed (Days 16 to 18) (25, 26), and then falls during the period when adult levels of transparency are attained (Days 18 to 20) (25, 26). This 2½-fold increase in the specific activity of PAPS during development is seen when supernatant fractions of corneal homogenates are assayed. Because 97% of such PAPS biosynthetic ability is detected in the supernatant fractions, we feel that the rise in specific activity seen in these fractions is an accurate reflection of total corneal PAPS biosynthesis and availability during development. The supernatant fractions also actively synthesized APS (which was used to make the PAPS) and contained 73% of the total APS-synthetic activity. However, no changes were noted during development in the specific activity of APS made by supernatant fractions, perhaps reflecting the maintenance of a constant equilibrium concentration of APS. About 40% of the total activity for transferring \(^{35}S\) from available \(^{35}S\)PAPS to endogenous proteoglycan (i.e. glycosaminoglycan sulfotransferases) was also found in the supernatant fractions, but the specific activity of such proteoglycan, like that of APS, remained constant during development. However, when the pelleted fractions were assayed, i.e. the fractions where, in the absence of detergents, most of the glycosaminoglycan sulfotransferase activity was expected (7) and detected, the specific activity of the endogenous proteoglycans increased in a pattern which mimicked the increase in the availability of PAPS. Because previously these same sulfotransferase enzymes, when solubilized by detergents, displayed no increases in specific activity during development (7), we think that the increased specific activity of the endogenous proteoglycans detected here arises from increased availability of PAPS, even in the pelleted fraction of the homogenates, rather than from increased activity of the nonsolubilized sulfotransferases. Finally, it may be important to note that the standard assay used here only measures the capacity for PAPS synthesis in vitro; the extent to which such capacity reflects actual PAPS levels in vivo has not yet been determined.

If indeed PAPS becomes more available within corneal cells during development, there are at least two ways by which this could result in an increase in the apparent degree of sulfation of corneal glycosaminoglycans. First, the frequency of sulfate groups added to polysaccharide chains then being synthesized might simply increase and yield a uniform spectrum of chains having a gradually higher degree of sulfation (27). Alternatively, sulfation might occur in an “all-or-none” fashion, as recently described (28, 29), with higher proportions of fully sulfated chains being made when intracellular concentrations of PAPS rose above some threshold level. The latter alternative raises the possibility that greater availability of PAPS could quickly result in the appearance of a very different type of polysaccharide chain, a pattern resembling what happens in the cornea after Day 14 (6). Initiation of synthesis of a highly sulfated type of keratan sulfate-I proteoglycan by corneal fibroblasts (30), perhaps by an “all-or-none” sulfation mechanism, might have a rapid effect on the water-binding properties of the extracellular matrix, mode of collagen fibril polymerization, and electrolyte milieu of the cornea.

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