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A comparative study on the effects of 5-fluorouracil on glycosaminoglycan synthesis during palate development in quail and hamster

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Summary. A comparative study was undertaken to investigate the effects of 5-fluorouracil (FU) on glycosaminoglycans (GAG) synthesis during morphogenesis of the secondary palate in birds (where, unlike mammals, palate morphogenesis begins in a horizontal direction *ad initium* and lacks mammalian-type shelf reorientation) and mammal. Previous studies have shown that FU induces cleft palate in both birds and mammals. Air sacs of quail eggs were injected with 100 µg FU in 0.1 ml saline or 0.1 ml saline only. Hamsters were given intramuscular injection of 81 mg/kg FU in 1 ml saline or 1 ml saline only. Total GAG synthesis was measured by incorporation of ^{3}H-glucosamine. Sulfated and non-sulfated GAGs were identified by Alcian Blue histochemistry combined with the use of GAG-degrading enzymes. The results indicated that a continuous synthesis of GAG at a steady rate was associated with normal palate morphogenesis in both quail and hamster. The amount of GAG synthesized in hamster palate was four-fold higher than in quail palate. In contrast to the developing hamster palate where the predominant GAG was hyaluronate, the major GAGs present during quail palate development were sulfated and were concentrated on the nasal side. FU treatment did not affect the rate of GAG synthesis in the developing palate of quail. In contrast, FU administration altered the rates of GAG synthesis, and affected hyaluronate accumulation, during palate morphogenesis in hamster. Following an analysis of data of the present study and those from literature, it was suggested that, in contrast to the proposed role of hyaluronate in mammalian shelf reorientation, the sulfated GAGs may contribute to shelf volume and may be associated with tissue differentiation in both hamster and quail. Also, critical differences in timings, rates, and types of macromolecular synthesis between quail and hamster may be related to the evolutionarily diverse morphogenesis of palate seen in birds and mammals.

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Introduction

The development of secondary palate differs among various vertebrates. Although the initial appearance of palatal shelves in a vertical or a horizontal direction seems to be Class-specific (for recent review see Shah et al., 1990) reorientation of palatal shelves is known to occur only in mammals, and closure of palate in mammals and alligator (Greene and Pratt, 1976; Ferguson, 1981; Shah, 1984).

During the past 30 years, evidence has accumulated in the literature indicating that the synthesis of glycosaminoglycans (GAG), a component of the extracellular matrix (ECM), plays an important role in the reorientation of mammalian palatal shelves (Larsson, 1962; Jacobs, 1964; Pratt et al., 1973; Jacobson and Shah, 1981; Brinkley and Vickerman, 1982; Brinkley and Morris-Wiman, 1984; Turley et al., 1985). Specifically, it is suggested that an accumulation of non-sulfated GAG, hyaluronate, would be a component of the local milieu which would facilitate the migration of mesenchymal cells during the shelf reorientation (Lassard et al., 1974; Shah, 1979; Brinkley and Morris-Wiman, 1984). *In vitro* studies have also supported *in vivo* observations on the putative involvement of GAG during palatal development (Greene et al., 1982).

An avian experimental system could be potentially useful in analyzing the putative role(s) of various ECM molecules in vertebrate palate morphogenesis. This is because the avian palatal shelves develop horizontally *ad initium* and lack the mammalian-type reorientation stage of palate morphogenesis (Shah and Crawford, 1980). Recently, GAG accumulation was measured biochemically in the developing palate of chick by Forman et al. (1991) who observed that sulfated GAG formed the major species in the developing palate of chick.

5-fluorouracil (FU) is a growth suppressive agent

which causes malformations of limb, eye, beak, tail, brain, gut and palate in birds and mammals (Karnofsky et al., 1958; Dagg, 1959; Ruddick and Runner, 1974; Shah and Mackay, 1978). Administration of FU in pregnant hamster leads to cleft palate in the fetus by delaying reorientation of the palatal shelves from a vertical to a horizontal plane (Shah and Wong, 1980). The delay was associated with damage to palatal cells, suppression of collagen synthesis and inhibition in the expansion of the shelf area (Shah et al., 1984, 1989b; Benkhaial et al., 1993). In quail, FU induced cleft palate was associated with supression of collagen synthesis (Benkhaial et al., 1993). Whether FU treatment affects GAG synthesis in any developing system, including palate is, not known. Since perturbation of various ECM molecules have been associated with abnormal palate development, in the present study, we have examined hitherto unavailable information of the effects of FU on the synthesis of GAG during the secondary palate development in both hamster and quail.

Both *in vivo* and *in vitro* morphogenesis of quail and hamster palate have been described earlier (Shah and Chaudhry, 1974; Kiso et al., 1984; Shah et al., 1985a,b; Burdett and Shah, 1988; Shah and Cheng, 1988). The details are not repeated for the sake of brevity.

Materials and methods

Random bred, wild type Japanese quail, *Coturnix japonica*, were obtained from the Quail Genetic Stock Center, University of British Columbia. They were incubated in an environment of 37.5 °C and 39% relative humidity.

The air sac in each egg was injected with either 100 µg FU (Sigma Chemicals, batch #2649-240) in 0.1 ml saline or 0.1 ml saline on day 4 of incubation. A group of eggs were also incubated to serve as an uninjected control. This dose-time regimen provided the most appropriate combination to increase the gap between palatal shelves, i.e., «cleft palate», in all the embryos with no embryotoxic effect (Benkhaial et al., 1993). A similar dose-time regimen for «cleft palate» induction by FU was also reported for chick embryos by Karnofsky et al. (1958) and Ruddick and Runner (1974). Under sterile conditions, the untreated and saline-treated (control) and FU-treated embryos were harvested at 24 hours intervals between days 5-9 of incubation. The developmental stage of each quail embryo was determined according to the method described by Hamburger and Hamilton (1951) for the chick embryo which, in the previous studies, showed a high correlation between the age of quail and the stage of its development (Graham and Meier, 1975; Shah et al., 1985a).

Male and female Golden Syrian hamsters were caged singly and kept in a controlled environment of 50±5% humidity, 22±2 °C temperature and alternate light and dark cycle (6AM to 6PM). The animals were allowed to eat and drink *ad libitum*. Females weighing at least 85 grams were mated with males between 7AM and 9AM, and the midpoint of the mating period, 8AM, was considered the beginning of day zero of gestation.

On day 11:00 of gestation, the animals were given an intramuscular injection of either 81mg/kg FU or 1 ml saline (Shah and Mackay, 1978). For controls, embryos from 3-5 litters were obtained at 6 hour intervals between days 11:00 and 12:00 of gestation and then at two hour intervals until day 12:04. Drug treated embryos were similarly obtained until day 13:00 of gestation (Fig. 2).

For measurement of GAG synthesis, quail embryonic secondary palates were dissected between days 5-9 of incubation and grouped according to the HH stage. An average of 6-8 palates were incubated for each experiment in a growth medium containing 1.8 ml Dulbecco's Modified Eagle Medium (DMEM), 0.2 ml of fetal calf serum (10%), and 0.04 ml ^{3}H-glucosamine HCl to a final concentration of 1.0 mCi/ml (specific activity 30 Ci/mmol; NEN Research) for 6-8 hours at 37 °C in an atmosphere of 5% CO_2 and 95% air. The incubation period of 6-8 hours was chosen because the *in vitro* uptake of ^{3}H-glucosamine peaks at this time (Derby and Pintar, 1978). The fetal hamster palates were isolated in pools of 4 to 6 for each experiment and incubated in the same conditions as the quail palates.

Following incubation, the palates were washed three times each in fresh DMEM to remove any unbound ^{3}H-glucosamine. Subsequently, the processing of tissue for GAG analysis was conducted according to the method described by Turley et al. (1985). Briefly, the washed palates were homogenized by sonication (Sonic Dismembrator, Model 300, Fisher Scientific) in order to liberate GAGs and proteins (including proteoglycan core proteins), and digested in 1.0 ml of 0.2M Tris HCl, 0.02% sodium azide and 1.0 mg/ml pepsin (Sigma Chemicals) at pH 8.0 for 24 hours at 56 °C. The pepsin digestion was terminated by heating the sample at 100 °C for 5 minutes to denature the enzyme. Protein was precipitated from the digested sample with 0.5 ml 50% (by weight) trichloroacetic acid (TCA) at 0-4 °C for 30 minutes. The samples were then centrifuged at 2000 g for 10 minutes at 0-4 °C. The supernatant and precipitate were separated. Three aliquots of 100 µl supernatant were dried on Whatman's microfiber glass filters. The filters were then immersed in 3.0 ml aqueous scintillation counting solution (Amersham) and counted in a Philips PW 4700 Liquid Scintillation counter. The TCA extracted precipitate was dissolved in 1.5 ml 1.0N NaOH for total protein determination by the Biuret method (Gornall et al., 1948). Each experiment was repeated 3-7 times. The data were analyzed by Student t-test, or ANOVA as appropriate, at the 5% significance level.

For histochemical characterization of sulfated and non-sulfated GAG components in the extracellular matrix of the developing palate, 3-4 control and FU-treated quail and hamster embryonic heads of different ages were fixed in Carnoy's fixative supplemented with 0.5% cetylpyridinium chloride at 0-4 °C for 18-24 hours.

The tissues were dehydrated in ethanol, cleared in xylene, embedded in paraffin and sectioned at 6 μm. The quail tissue was sectioned in the anterior fourth of the developing secondary palate because in quail palate morphogenesis begins in this region (Shah et al., 1985a). Hamster sections were similarly taken from the middle third of the secondary palate where all stages of morphogenesis begins (Shah and Travill, 1976). The deparaffinized sections were incubated at 37 °C with either *streptomyces hyaluronidase* (75 units/ml in 0.1M phosphate buffer at pH 5.0; Sigma Chemicals, St. Louis, Cat. # H-1136, Lot # 70H0612), bovine testicular hyaluronidase (200 units/ml in 0.1M phosphate buffer at pH 5.5; Sigma Chemicals, St. Louis, Cat. # H-2001, Lot # 24C-3290) or 0.1M phosphate buffer (pH 5.5) as described by Yamada (1971). They were then stained with Alcian Blue 8GX at pH 1.0 or pH 2.5 and counterstained with Nuclear Fast Red. Distribution of sulfated and non-sulfated GAGs was observed during quail and hamster palate morphogenesis as described in Tables 1 and 2. *Steptomyces hyaluronidase* digests hyaluronate whereas testicular hyaluronidase degrades chondroitin, chondroitin sulfate A and C and hyaluronate. At pH 1.0, only sulfated GAGs are stained whereas at pH 2.5, both sulfated and non-sulfated GAGs are stained. For recording alcian blue staining intensity, Copp and Wilson's (1981) method was slightly modified as follows: strong (+++), moderate (++), weak (+), absent (-).

Results

Measurement of glycosaminoglycan synthesis

Quail

The data presented in Figure 1 show that in untreated palates the GAG synthesis, as measured by ^{3}H-glucosamine incorporation, showed a 35% decline between days 5 (HH stages 25-26) and 6 (HH stages 27-28) of incubation ($P< 0.05$). One may, however, note that in quail the palatal primordia appears as intraoral extensions of the maxillary processes on day 5 of incubation (see Figs. 1, 2 in Shah et al., 1985a). At this stage, it is technically difficult to isolate the palatal bulges for experimental manipulation. Hence, in the present study, part of the maxillary processes were dissected along with the palatal bulges which may account for a high incorporation of ^{3}H-glucosamine on day 5. During the next four days, i.e., between days 6-9 of incubation (HH stages 31-37) the rate of incorporation remained unchanged.

Following saline or FU injection the rate of ^{3}H-glucosamine incoporation remained comparable to the untreated palates ($P< 0.05$). When the rates of GAG synthesis were compared between various groups of palates, i.e., untreated, saline-treated and FU-treated, on day 5 of incubation (Fig. 1) ^{3}H-glucosamine incorporation in FU-treated palates was higher than in the untreated palates ($P< 0.05$). However, there were no

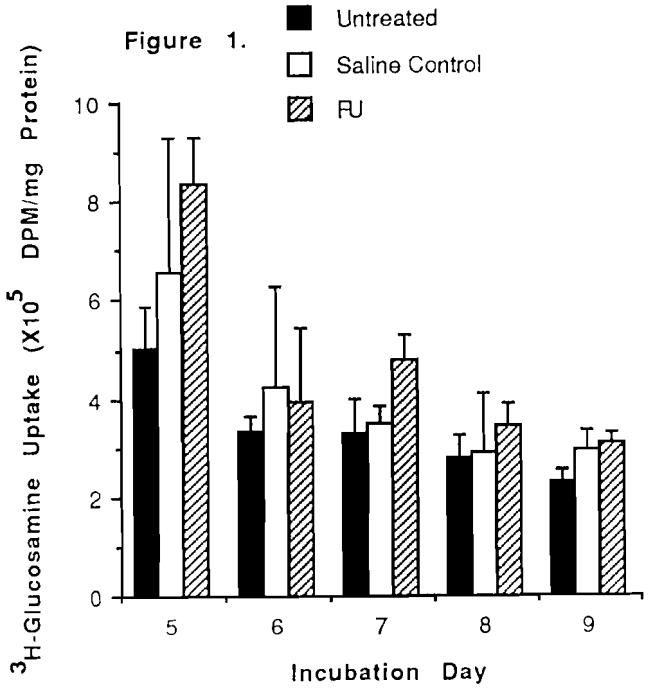


Fig. 1. Glycosaminoglycan synthesis in the untreated, and saline- and FU-treated developing quail secondary palate.

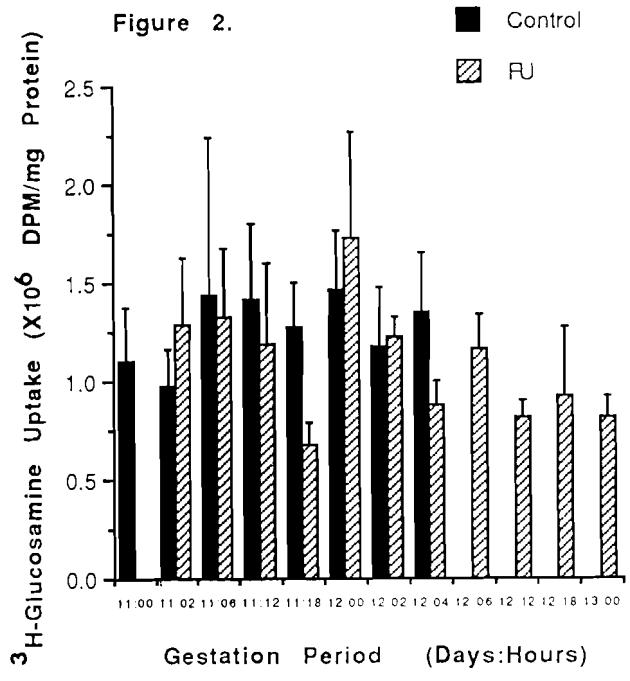


Fig. 2. Glycosaminoglycan synthesis in the control and FU-treated developing hamster secondary palate.

differences in the rates of GAG synthesis between untreated and saline-treated, or saline-treated and FU-treated palates on day 5 of incubation ($P < 0.05$). No inter-group differences in the rates of GAG synthesis were present on days 6-9 of incubation (Fig. 1).

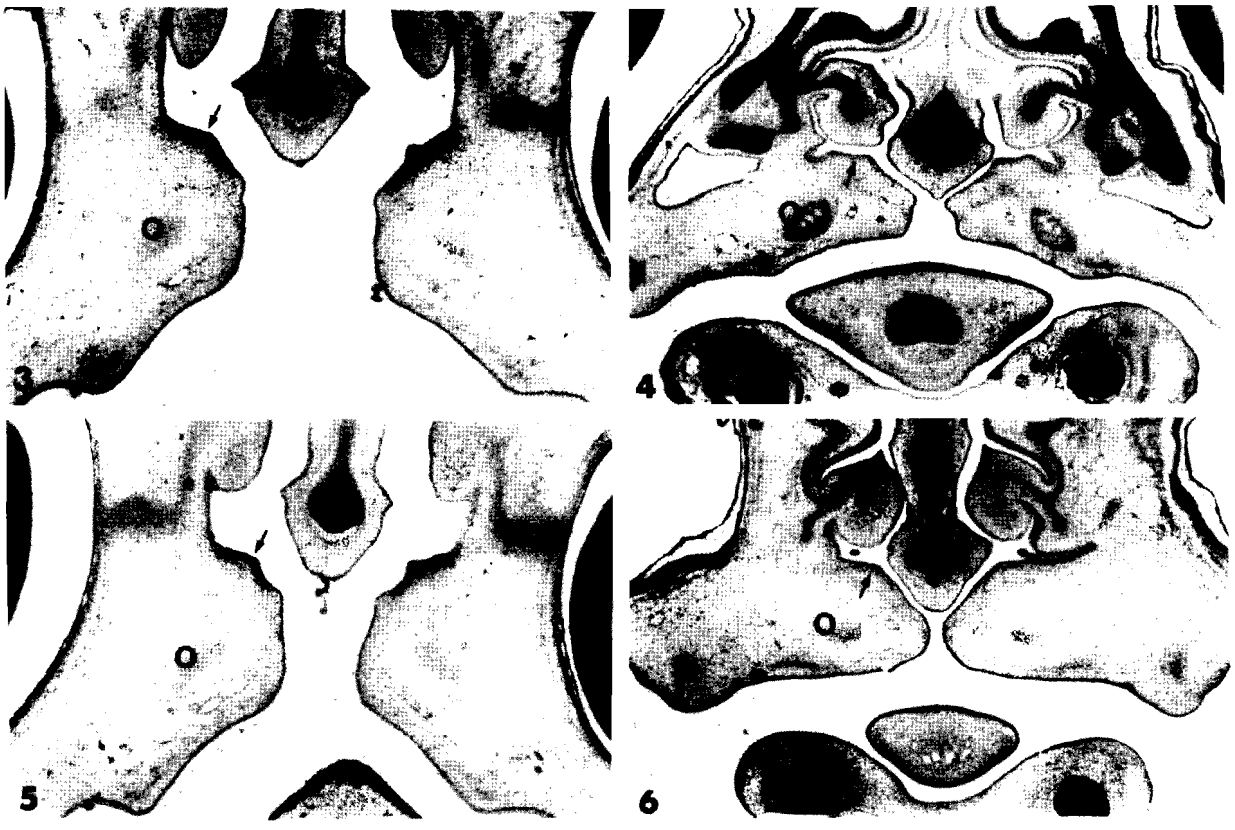
Hamster

The results of GAG synthesis from control and FU-treated hamster palates, at different times during gestation, are outlined in Figure 2. In control palates, the rate of GAG synthesis remained steady throughout the

Table 1. Summary of Alcian Blue staining of the developing secondary palate of quail.

INCUBATION PERIOD (DAYS)	NASAL						MEDIAL EDGE						ORAL					
	pH 1.0			pH 2.5			pH 1.0			pH 2.5			pH 1.0			pH 2.5		
	B	SH	TH	B	SH	TH	B	SH	TH	B	SH	TH	B	SH	TH	B	SH	TH
Saline-treated																		
5	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
6	++	++	-	++	++	-	+	+	-	+	+	-	+	+	-	+	+	-
7	++	++	-	++	++	-	+	+	-	+	+	-	+	+	-	+	+	-
8	+++	++	-	++	++	-	+	+	-	+	+	-	+	+	-	+	+	-
9	+++	+++	-	+++	+++	-	++	++	-	++	++	-	++	++	-	++	++	-
FU-treated																		
5	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
6	++	++	-	++	++	-	+	+	-	+	+	-	+	+	-	+	+	-
7	++	++	-	++	++	-	+	+	-	+	+	-	+	+	-	+	+	-
8	++	++	-	++	++	-	+	+	-	+	+	-	+	+	-	+	+	-
9	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-

B: buffer; SH: streptomyces hyaluronidase; TH: testicular hyaluronidase. Intensity of staining: +++, strong; ++, moderate; +, weak; -, absent.



Figs. 3-6. Frontal sections of developing quail secondary palate showing the presence of sulfated GAGs in the nasal region (arrows). Alcian Blue (pH 2.5) and Nuclear Fast Red stain. O: site of osteogenesis. x 32. **3.** Saline-treated. Day 6 of incubation. **4.** Saline-treated. Day 9 of incubation. **5.** FU-treated. Day 6 of incubation. **6.** FU-treated. Day 9 of incubation.

Table 2. Summary of Alcian Blue staining of the developing secondary palate of hamster.

GESTATION AGE (Day:Hour)	NASAL						MEDIAL EDGE						ORAL					
	pH 1.0			pH 2.5			pH 1.0			pH 2.5			pH 1.0			pH 2.5		
	B	SH	TH	B	SH	TH	B	SH	TH	B	SH	TH	B	SH	TH	B	SH	TH
Saline-treated																		
11:00	+	+	-	++	++	-	+	+	-	++	++	-	+	+	-	+	+	-
11:06	+	+	-	++	++	-	+	+	-	++	++	-	+	+	-	+	+	-
11:12	+	+	-	+++	++	-	+	+	-	+++	++	-	+	+	-	+	+	-
11:18	+	+	-	+++	++	-	+	+	-	+++	++	-	+	+	-	+	+	-
12:00	+	+	-	+++	++	-	+	+	-	+++	++	-	+	+	-	+	+	-
12:02	+	+	-	+++	++	-	+	+	-	+++	++	-	+	+	-	+	+	-
12:04	+	+	-	+++	++	-	+	+	-	+++	++	-	+	+	-	+	+	-
FU-treated																		
11:02	+	+	-	++	++	-	+	+	-	++	++	-	+	+	-	++	++	-
11:06	+	+	-	++	++	-	+	+	-	++	++	-	+	+	-	++	++	-
11:12	+	+	-	++	+	-	+	+	-	++	+	-	+	+	-	++	++	-
11:18	+	+	-	++	+	-	+	+	-	++	+	-	+	+	-	++	+	-
12:00	+	+	-	++	+	-	+	+	-	++	+	-	+	+	-	++	+	-
12:02	+	+	-	++	+	-	+	+	-	++	+	-	+	+	-	++	+	-
12:04	+	+	-	++	+	-	+	+	-	++	+	-	+	+	-	++	+	-
12:06	+	+	-	++	+	-	+	+	-	++	+	-	+	+	-	++	+	-
12:12	+	+	-	+++	++	-	+	+	-	+++	++	-	+	+	-	+++	++	-
12:18	+	+	-	+++	++	-	+	+	-	+++	++	-	+	+	-	+++	++	-
13:00	+	+	-	+++	++	-	+	+	-	+++	++	-	+	+	-	+++	++	-

B: buffer; SH: streptomyces hyaluronidase; TH: testicular hyaluronidase. Intensity of staining: +++, strong; ++, moderate; +, weak; -, absent.

period of palatogenesis.

Following FU treatment, the rate of GAG synthesis in the developing palate remained unchanged during the initial 12 hours. However, in comparison to controls, it was reduced by approximately 50% in the drug treated palates on day 11:18 of gestation ($P< 0.05$). Subsequently, although on day 12:00 of gestation GAG synthesis recovered to control level, it dropped significantly during the next four hours ($P< 0.05$) and remained low thereafter.

Alcian blue histochemistry

Quail

The data on alcian blue staining in the developing secondary palate of quail are summarized in Table 1. On day 5 of incubation, a small amount of GAG was detected in the nasal region, but not in the medial edge and oral regions of the developing secondary palate of saline-treated quail. The staining activity was completely eliminated following exposure to testicular hyaluronidase whereas after exposure to *streptomyces hyaluronidase* the staining intensity of alcian blue remained unchanged. Also, in the buffer control group at pH 2.5 all GAGs were stained. Hence, the GAGs present in the developing palate on day 5 of incubation are interpreted as that of a sulfated variety. Between days 6 and 8 of incubation, the activity of sulfated GAGs were moderate in the nasal region and weak in the medial edge and oral regions (Fig. 3). On day 9 of incubation, the activity of sulfated GAGs increased in all regions of the palate. It was strong in the nasal region and moderate in the medial edge and oral regions (Fig. 4). Throughout the period of observations in the present study, *Streptomyces hyaluronidase* treatment, indicative of presence of hyaluronate, did not affect alcian blue staining. This would suggest that hyaluronate is not a significant component of the GAGs during the normal morphogenesis of the secondary palate in quail.

Following FU treatment between days 5 and 8 of incubation, activity of sulfated GAGs in different regions of the palates resembled its control counterparts (Fig. 5). On day 9, however, in comparison to controls, the GAG activity, characterized by alcian blue staining was weak in all regions of the developing secondary palate (Fig. 6). In addition, like controls, between days 5-9, hyaluronate was absent in FU-treated quail palates.

Hamster

The data on alcian blue staining in the developing hamster palate are summarized in Table 2. In control palates, between days 11:00 and 12:04 of gestation, GAG activity was present in all regions of the developing palate. The oral region showed a weak staining intensity with alcian blue at both pH 1.0 and 2.5 which was obliterated by testicular hyaluronidase but not by *Streptomyces hyaluronidase* suggesting the presence of only sulfated GAG in the oral region. *Streptomyces hyaluronidase* partially obliterated alcian blue staining between days 11:12 and 12:04 of gestation in both the nasal and medial edge regions indicating the presence of non-sulfated GAG.

Following FU treatment, between days 11:06 and 13:00 of gestation, the sulfated GAG remained unaffected in all three regions of the developing palate (Table 2). Unlike controls, however, between days 11:12 and 12:12 of gestation, the non-sulfated GAG in treated palates showed reduced accumulation in both the nasal and medial edge regions as evidenced by the *Streptomyces hyaluronidase* digestion of alcian blue staining at pH 2.5. Subsequently, during the next 12 hours, i.e., between days 12:12 and 13:00 of gestation when FU-treated shelf reoriented, non-sulfated GAG accumulation increased to a level comparable to that seen during palate shelf reorientation in control embryos.

Discussion

Two factors contributes to the spatial growth of vertebrate palatal shelves: cell proliferation and synthesis of ECM. The results of the present study show that, although morphogenesis of the secondary palate in birds and mammals differs, a continuous synthesis of GAG was an important component of ECM during palatogenesis in both quail and hamster. As palate development progressed in quail (between days 6-9 of incubation) and hamster (between days 11:00 and 12:04 of gestation) the overall rate of GAG synthesis remained steady. A continuous accumulation of GAG during mammalian palatogenesis was also observed in other studies (Larsson, 1962; Jacobs, 1964; Nanda, 1971; Pratt et al., 1973; Jacobson and Shah, 1981; Brinkley and Vickerman, 1982; Brinkley and Morris-Wiman, 1984). The rate of synthesis of GAG, however, was approximately four-fold higher in hamster palate than in quail palate during the period of morphogenesis. Also, during the period of palate morphogenesis, the rates of DNA synthesis and cell proliferation progressively declined in quail (Hehn and Shah, 1993; Shah and Cheng, 1993) but remained steady in hamster from day 11:00 of gestation onward (Shah et al., 1989a,b). Clearly, during *in vivo* growth of palate in both mammals and birds, synthesis of both DNA (indicative of cell proliferation) and total GAG did not parallel each other, a phenomenon also demontrated in *in vitro* studies of palatal (Greene et al., 1982; Sasaki and Kurisu, 1983; Yoshikawa et al., 1987) as well as in other developing and regenerating systems (Janners and Searls, 1970; Solursh et al., 1981a,b; Bryant and Gardiner, 1987).

The Alcian Blue staining data of the present study demonstrated that the ECM of quail palate mesenchyme shows a region-specific distribution during development. Specifically, the mesenchyme on the nasal side of developing quail palate had more sulfated GAGs than the oral and medial edge regions. Recently, Forman et al. (1991) have also observed a presence of sulfated and non-sulfated (hyaluronate) GAG in the developing palate of chick. However, hyaluronate was absent in developing quail palate. This presence/absence of hyaluronate could be related to subsequent osteogenesis of chick, but not quail palate. Further, these observations of avian palate development were in sharp contrast to the data obtained from mammalian palatal shelves in which both hyaluronate and sulfated GAGs are uniformly distributed in the developing palatal shelves (Larsson, 1962; Jacobs, 1964; Ferguson, 1978; Jacobson and Shah, 1981; Brinkley and Morris-Wiman, 1984; present study). Although precise functional roles for each GAG molecule has not yet emerged in the literature, an overwhelming number of studies have suggested that hyaluronate creates tissue spaces for cell migration and/or proliferation (Solursh and Morris, 1977; Toole, 1982; Pauli et al., 1983; Burk, 1985; Tucker, 1986; Van Hoof et al., 1986; Docherty et al., 1989; Jackson et al., 1991), whereas sulfated GAGs promotes cell differentiation (see review in Manasek, 1975; Toole, 1982; Becchetti et al., 1984; Kuettner and Kimura, 1985; Caplan, 1986; Jahoda et al., 1987; Jackson et al., 1991). It is possible that various GAGs may have different functional roles during mammalian and avian palatogenesis. In mammalian palate, the hyaluronate rich ECM, due to its hydrophilic properties, creates tissue spaces to presumably facilitate cell migration during reorientation (Lassard et al., 1974; Shah, 1979; Brinkley and Morris-Wiman, 1984). On the other hand, in the developing quail palate (no reorientation), the major cellular activities appear to be related to growth of the palatal shelf and tissue differentiation (Shah et al., 1985a). Since, in both hamster (between days 11:00 and 12:04 of gestation; also in other mammals as well) and quail (between days 5 and 9 of incubation) (1) the proliferative activity of mesenchymal cells does not seem to correspond to the expansion of shelf area (volume, size; Brinkley and Morris-Wiman, 1984; Burdett et al., 1988; Shah et al., 1989a,b,c; Hehn and Shah, 1993), and (2) there are regional differences in the distribution of sulfated GAG in the developing palate (present study), it would be reasonable to suggest that, in contrast to the putative function of hyaluronate in mammalian shelf reorientation, the possible role for the accumulation of sulfated GAG during both the hamster and quail palate morphogenesis could be: a) to contribute to the volume of the growing shelf, and b) to associate with tissue differentiation. Earlier, Larsson (1962) and Jacobs (1964) noted that an increased production of sulfated GAG may cause shelf reorientation in mammal. However, a strong accumulation of sulfated GAG in birds (Forman et al., 1991; present study) may also suggest that the sulfated GAG could play a role in aspects other than causing the shelf to reorient.

Hamster and quail palate reacted differently to FU treatment. Administration of FU did not affect total GAG synthesis in the developing palate of quail. This is in contrast to inhibition, followed by recovery, of GAG synthesis prior to and during the hamster palatal shelf reorientation. Qualitative analysis with Alcian Blue staining revealed that, in comparison to controls, FU-treated quail palates showed a reduction in sulfated GAGs on day 9 of incubation. Clearly, the effect of FU

on GAG is manifested late in the morphogenesis of quail secondary palate and may reflect a post-hoc phenomena in a cascade of events during a cleft formation. However, FU-treated hamster palate showed a reduced non-sulfated GAG (hyaluronate) between days 11:12 and 12:12 of gestation. There are no reports in literature analyzing the effects of FU on GAG synthesis in any developing system; hence, results of the present study are difficult to compare with literature. On the basis of the data of the present study, however, one may suggest that FU treatment does not appear to induce cleft palate in quail through interference with the GAG component of the ECM but it does appear to do so in hamster.

A question still remains: How would FU induce cleft palate in quail and hamster? In a series of earlier studies (Shah et al., 1984, 1989b; Benkhaial et al., 1991, 1993) it was shown that FU treatment delays the reorientation of hamster shelf by damaging the palatal cells, suppressing collagen synthesis, and inhibiting expansion of the shelf area (volume, size) by day 11:18 of gestation. The reorientation was accomplished only after restoration of these features to normal levels by day 12:12 of gestation. The observations of the present study on hyaluronate inhibition and recovery, in hamster, are consistent with these earlier observations on effects of FU on palatogenesis (references cited above). Indeed, inhibition of ECM synthesis by various other teratogens have been claimed to eventually delay the shelf reorientation (Jacobs, 1964; Nanda, 1971; Ferguson, 1978; Jacobson and Shah, 1981; Brinkley and Morris-Wiman, 1984). This analysis would further reinforce the proposition made in the literature that an optimum accumulation of specific ECM molecules may be closely associated with palatal shelf reorientation. In addition, FU also affects collagen synthesis in the developing palate of both quail and hamster (Benkhaial et al., 1991, 1993) and protein synthesis following *in vitro* FU exposure of rat palate (Abbott et al., 1993). Earlier, Heidelberger et al. (1984), Lewin et al. (1987) and Sandborg and Siegel (1990) have indicated that changes in protein synthesis may be reflective of alterations in RNA metabolism: a proposition that needs to be clarified in the developing palate of both hamster and quail. Nevertheless, the results of both the normal and FU-treated development of palate from the present study, along with the data from literature, further suggest that critical differences in timings, rates and types of macromolecular synthesis may be related to the evolutionarily diverse morphogenesis of palate seen in these two classes of vertebrates.

In summary, the foregoing Results and Discussion indicate that GAGs are important components of the developing secondary palate of quail and hamster. The rate of total GAG synthesis, although four-fold higher in hamster than in quail, remained steady with progressive morphogenesis of palate in both classes of vertebrates. In quail, the main GAGs were of the sulfated variety whereas in hamster both the sulfated as well as non-sulfated varieties accumulated during development. FU treatment does not induce cleft palate in quail through interference with GAG synthesis whereas in hamster it affected GAG synthesis, specifically the accumulation of hyaluronate, indicating that FU-induced alterations in hyaluronate accumulation may be a part of a cascade of events leading to cleft palate formation in hamster.

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