Sialoconjugates and development of the tail bud

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Summary
Using lectin histochemistry, we have previously shown that there are alterations in the distribution of glycoconjugates in the tail bud of chick embryos that parallel the developmental sequence of the caudal axis. If glycoconjugates or the cells bearing them play a role in caudal axial development, then, restriction of their availability by binding with lectins would be expected to produce abnormalities of caudal development. In the present study, we treated embryos at various stages of tail bud development by microinjection with a variety of lectins. Administration of WGA by sub-blastodermal injection resulted in high incidences of secondary neural tube and notochordal abnormalities in lectin-treated embryos. The incidence of malformations was dependent upon both the dose of WGA received and the stage of development at the time of treatment. Using an anti-WGA antibody, we have also shown binding of the lectin in regions where defects were found. The lectin WGA binds to the sialic acid residues of glycoconjugates and to N-acetylgalcosamine. Treatment of embryos with Limulus polyphemus lectin (LPL), which also binds to sialic acid, produced results similar to those of WGA. Treatments using lectins with other sugar-binding specificities, including succinylated WGA (with N-acetylgalcosamine specificity only) produced defects that differed from those produced by WGA and LPL, and only with the administration of much higher doses. The results suggest that glycoconjugates in general and sialoconjugates in particular, or the cells carrying them, may have a role in caudal axial development.

Key words: chick embryos, sialic acid, secondary neuraxis, tail bud development.

Introduction
In early avian embryos, the axial structures consist of the neural tube, notochord and primitive gut. Holmdahl (1925a, b, c) has described the development of these structures in two phases. The germ layers of classical embryology develop during 'primary body development', and these in turn give rise to the axial structures of the anterior body. During 'secondary body development', the axial structures of the posterior body develop from the indifferent cell mass of the tail bud.

The development of the neural tube from the tail bud by secondary neurulation has been well documented (Criley, 1969; Jelinek et al. 1969; Klika and Jelinek, 1969; Schoenwolf, 1977, 1978, 1979; Schoenwolf and DeLongo, 1980). During secondary neurulation, the caudal portion of the neural tube develops by a process involving the aggregation of mesenchymal cells of the tail bud to form a solid medullary cord; the cavitation of the medullary cord to form a neurocoele; and the simultaneous mesenchymal-epithelial transformation of medullary cord cells into the neuroepithelial cells of the secondary neural tube (Schoenwolf, 1979; Schoenwolf and DeLongo, 1980). Secondary neurulation in avians is further complicated by the existence of a well-defined overlap zone of neurulation in the future lumbosacral region (approximately somites 28–34) (Costanzo et al. 1982) where both primary and secondary neurulation occur simultaneously. Dorsally within this zone, the neural tube is formed by primary neurulation, from the neuroectodermal folds of the posterior neuropore. Ventrally, the medullary cord tapers cranially, and gives rise to the neural tube by secondary neurulation.

We have previously found a changing pattern of lectin binding during the development of the secondary neuraxis in chickens (Griffith and Wiley, 1989a) and in mice (Griffith and Wiley, 1989b). Lectins are proteins and glycoproteins of non-immune origin that recognize and bind in an antibody-like fashion to specific sugar residues of glycoproteins or glycolipids. The changes in the binding of wheat germ agglutinin (WGA), which is specific for sialic (N-acetyleneuraminic) acid and N-acetylgalcosamine (GlcNAc) residues, appeared to follow a distinctive and developmentally regulated pattern in chick caudal axial development. However, very little binding was observed with the lectin succinylated WGA (sWGA). Since sWGA has only GlcNAc specificity, the changing pattern of WGA binding appeared to be due to changes in the sialic acid moieties.

The objective of this study was to further investigate the correlation between the presence of sialoconjugates and the development of the caudal axis. To achieve this, WGA was administered to chick embryos at various
stages during tail bud development in order to perturb the normal configuration of sialic-acid-containing glycoconjugates present during this process.

Materials and methods

Fertile White Leghorn eggs (Glen Fenelon Farms, Stroud, Ontario) were kept in a forced draft, humidified incubator at 38°C until stages 11 to 16, according to the criteria of Hamburger and Hamilton (1951), were reached.

Wheat germ agglutinin treatment

(1) Dose response to WGA

Embryos at HH stages 13 to early 14 (tail bud anlagen stage) were used in this study. Each egg was windowed and a subblastodermal injection of wheat germ agglutinin (WGA; Vector Labs) dissolved in a 0.9% saline solution was administered under the tail bud. Based on reports of concanavalin A (ConA) neural teratogenicity (Lee, 1976; Lee et al. 1976a,b), doses of 10, 20, 25, 30, 40 and 50 μg WGA in a total volume of 5 μl per embryo were tested. The eggs were then sealed with Sellotape and reincubated for another 48 h.

(2) Stage response to WGA

4 groups of embryos were used in this portion of the study. The embryos were at HH stages 11–12, 13–early 14 (tail bud anlagen), mid–late 14 (early tail bud stage), and 15–16 (late tail bud). Embryos were injected sub-blastodermally with 25 μg WGA in 5 μl saline solution, after which the eggs were resealed and reincubated for approximately 48 h.

Controls

(1) Windowed and saline-injected embryos

In order to eliminate the possibility that malformations observed in the embryos were due to mechanical damage during the processes of windowing or injection, embryos were either windowed only, or windowed and injected with 5 μl of saline. 40 windowed and 40 saline-injected embryos were included in the dose–response study, and 60 saline-injected embryos were prepared for the stage response study.

(2) Heat-inactivated WGA

In order to relate the induction of malformations to the biochemical activity of the lectin, a solution of WGA (25 μg per embryo, the optimal dosage for induction of malformations) was boiled for 20 min and then allowed to cool to room temperature before injection. 40 embryos were used in this group.

(3) WGA and N-acetylglucosamine

GlcNAc was used as a competing hapten to show the binding specificity of WGA since this sugar has been shown to inhibit agglutination of cells by WGA far better than other sugar residues (Le Vine et al. 1972; Allen et al. 1973) and is able to competitively displace bound N-acetyllulinic acid residues (Roth, 1978; Reutter et al. 1982). Moreover, in previous studies, we have shown that in the presence of the hapten, binding of WGA to tail-bud-derived tissues was reduced (Griffith and Wiley, 1989a,b). In this study, 2 mM GlcNAc (Sigma) was added to the WGA (25 μg/embryo) and allowed to react for at least 2 h prior to use. 40 embryos were used for each lectin and sugar mixture. An additional 40 embryos were injected with 2 mM GlcNAc only.

(4) Binding by non-sialic-acid-specific lectins

In order to determine if the defects produced by WGA were specific to this particular lectin, or non-specific results of lectin-induced embryotoxicity, embryos were treated with either ConA or Lens culinaris agglutinin (LCA). These are both lectins, which bind to either α-mannose or α-glucose residues. Two batches of 40 embryos were treated with 100 μg per embryo of either lectin, a dose that produced malformations in a preliminary study. As a further control for the specificity of WGA-induced defects, an additional 40 embryos were treated with doses of 200 μg per embryo of PNA, a lectin that is specific for galactose (gal) and Galβ1–3GalNAc residues.

Analysis of lectin effects

(1) Gross assessment for external malformations

The proportion of survivors recovered after the 48 h reincubation period was noted and embryos were fixed in Carnoy's fluid. The embryos were then examined for gross caudal axial defects before processing for histological examination.

(2) Histological examination for internal malformations

The tissue at the level of, and caudal to, the hindlimb buds was routinely processed for wax embedding. Transverse serial sections of 6 μm thickness were made for each sample and stained with haematoxylin and eosin (H and E). For windowed and saline-treated controls, the embryos with external malformations and 10 'normal' embryos chosen at random were sectioned for histological examination.

Localization of lectin binding

Embryos at HH stages 13 to early 14 were used as these were at the optimal stages for induction of malformations. Embryos were recovered 6, 12 or 24 h after treatment and fixed in 4% paraformaldehyde in a 0.1 M cacodylate buffer, pH 7.2–7.4 overnight at 4°C. They were then washed in the cacodylate buffer, dehydrated through a graded ethanol series, cleared in xylene and embedded in paraffin wax. 4% polyvinylpyrrolidone (PVP) (M, 40 000) was added to the paraformaldehyde fixative, wash buffer and the ethanol series to protect the tissues against osmotic changes and to help retain antigenicity (Kuhlmann and Krishan, 1981). Embryos recovered 48 h after treatment were fixed in Bouin's fluid and routinely processed for wax embedding.

To visualize tissue-bound WGA, serial sections (6 μm) of the caudal regions of embryos were rehydrated, and placed in 1% hydrogen peroxide in methanol for 15 min to quench endogenous peroxidase activity, just prior to staining with antibody to WGA. After quenching, the sections were incubated in 0.1 M phosphate-buffered saline (PBS) containing 4% foetal calf serum (PBS/FCS) to minimize non-specific binding. The sections were then treated with a 0.01 mg ml⁻¹ solution of biotinylated anti-WGA antibody (Vector Labs) for 1 h, after which excess antibody was washed off with PBS/FCS. The sections were then incubated in a solution of avidin–horseradish peroxidase (avidin–HRP; Vectastain ABC Kit) for 30 min. Unbound avidin–HRP was rinsed off with PBS/FCS and the slides washed in 0.1 M Tris–HCl buffer, pH 7.6 for 30 min. Section-bound HRP-complexed anti-WGA was visualized by subsequent reaction with a freshly prepared solution of 0.05% diaminobenzidine (DAB) and 0.03% hydrogen peroxide in the Tris–HCl buffer for 5 to 10 min. Finally, the sections were washed in Tris–HCl for 15 to 20 min, dehydrated, cleared and mounted in Permount.

Embryos exposed to 200 μg of PNA for 48 h were also...
processed for binding studies using an anti-PNA antibody to determine if there was any binding of this lectin to tail bud tissues at the dosage administered. Corresponding saline-injected controls were also prepared. 6 to 10 embryos were used in each group.

**Sialic acid versus N-acetylglucosamine**

Since WGA is known to recognize both sialic acid and GlcNAc residues, it was of interest to resolve whether sialic acid or GlcNAc was related to the induction of malformations. This was accomplished by the use of 2 lectins, *Limulus polyphemus* lectin (LPL) and sWGA. The former recognizes only sialic acid (N-acetyleneuraminic acid) while the latter is specific for GlcNAc residues. A dose of 25 µg per embryo of LPL (Sigma) was given to 40 embryos at stages 13–early 14. Another 40 embryos were treated with 100 µg sWGA (Vector Labs). These dosages were chosen since they produced the optimal combination of malformation together with a relatively low frequency of mortality in preliminary studies. The embryos were then allowed to develop for another 48 h.

**Statistical analysis**

The effect of increasing doses of WGA on survival and teratogenicity was analyzed by linear regression. The survival rate and proportions of affected embryos obtained by treatment of embryos at different developmental stages were analyzed by Chi-square proportional analysis of a 2×2 contingency table, with a correction for continuity (Zar, 1984). Where comparisons were made among more than 2 groups, a Tukey multiple comparison test was used to analyze arcsin transformed data of percentages of abnormal embryos (Zar, 1984). Statistical significance was set at P<0.05.

**Results**

**Controls**

(1) Windowed and saline-injected embryos

There were no significant differences in either the survival rate or incidence of malformation between the 40 windowed embryos and the 40 windowed and saline-injected embryos (P>0.05) (Table 1). The survival of the embryos in each of the different age groups was excellent and the percentage of malformed embryos was low. Fig. 1 shows sections through a typical 'normal' embryo.

(2) Heat-inactivated WGA

There were no significant statistical differences (P>0.05) in the survival rates or in the occurrence of defects between the embryos treated with heat-inactivated WGA and those that were injected with saline only (Table 1).

(3) WGA and N-acetylglucosamine

Neither GlcNAc treatment, nor treatment with WGA in the presence of GlcNAc had a significant effect (P>0.05) on 48 h survival or the incidence of caudal malformations (Table 1). The difference between the lectin-sugar group and those treated with only the lectin, however, was statistically significant (P<0.05). The results of this control study showed that the addition of competing sugar residues was able to reduce both the mortality rate and the incidence of caudal axial defects.

When examined histologically, the malformations were found to be associated with oedematous blebs and haematomas. 1 embryo exposed to the GlcNAc–WGA combination showed a notochordal–neural tube fusion defect, and another had an accessory neural tube lumen in the tail (defects common to WGA-treated embryos).

(4) Binding by non-sialic acid-specific lectins

Administration of ConA and LCA produced non-specific but severe defects at teratogenic doses (100 µg). These doses were 5 times higher than the WGA dose (20 µg) required to produce a statistically significant increase in the incidence of malformed embryos. The proportions of embryos with the different defects observed are shown in Fig. 2. These defects ranged from developmental dysplasia and necrosis of tail bud-derived structures (Fig. 3A,B,D) to the total absence of a tail and axial structures in the lumbosacral region (Fig. 3C). The incidence and severity of the defects were higher in ConA-treated embryos than in LCA-treated embryos.

Despite doses 10 times higher than those needed to produce malformations in WGA-treated embryos, PNA had no significant effect on the frequency of malformation (P>0.05) (Fig. 2). Localization of PNA binding using an antibody to PNA showed that the lectin had access to the tail bud tissues at this dosage.

**Dose response to WGA**

A summary of the effects of increasing dosages of WGA on the survival and malformation incidence is given in Fig. 4. The rate of survival showed a highly significant (P<0.0001) linear decrease with increasing doses of WGA. The incidence of malformation increased with increasing doses of the lectin, but there was no significant linear relationship (P>0.05).

A spectrum of gross external malformations was observed (Fig. 5). However, the severity of the defects...
Fig. 1. Transverse sections through the caudal region of a control embryo, comparing the portion of neural tube derived from secondary neurulation with one that formed by a combination of primary and secondary neurulation. (A) Tail, neural tube formed by secondary neurulation (n). c, notochord; g, developing ganglion; s, somites. (B) Lumbosacral region, showing neural tube formed dorsally, by primary neurulation, and ventrally, by secondary neurulation. Bars. 100 μm.

Fig. 2. Histograms showing the proportion (%) of surviving embryos with the various types of defects produced by nonsialic acid-specific lectins, PNA, ConA and LCA. * Ourenteric outgrowths are projections of tissue into the gut, which contain combinations of neural tube, notochord and/or somites. The notochord segments in such outgrowths were continuous with the notochord of the trunk, while the neural tube segments were isolated.

was not dose related. The abnormalities most frequently observed were total or partial caudal regression, and shortened tails. Dysplasia of the hind limb buds and scoliosis in the lower trunk were also frequent occurrences.

Examination of serial sections of the caudal regions of embryos showed that the spectra of caudal regression observed in the gross specimens corresponded to different degrees of disruption of the secondary neuraxis. In severe caudal regression, only a tail stump with little or no organized structure was present. In cases of partial regression, abnormalities ranged from the presence of rudiments of caudal axial structures (Fig. 6A) to disrupted and disordered but morphologically identifiable structures. Abnormal neural tube–notochord relationships such as the fusion of the notochord to the ventral portion of the neural tube (Fig. 6B) or even replacement of regions of the ventral neural tube by notochord fragments (Fig. 6C) were characteristic of the defects induced by WGA. Accessory neural tube lumina (Fig. 6C,D) were also common malformations induced by WGA. Where an accessory lumen was present in the lumbosacral region (the area of overlap between primary and secondary neurulation), the abnormal relationship between notochord and neural tube was always with the more ventrally placed neural tube (Fig. 6C). In addition, accessory fragments of notochord were found within or fused to the wall of the tail gut (Fig. 6C). Ourenteric outgrowths containing a combination of neural tube, notochord and/or somites were also found (Fig. 6E). Approximately 2% of embryos had open posterior neuropores.

Stage response

The different stage groups observed were HH stages 11–12, 13–early 14, mid–late 14, and 15–16. The survival and incidence of caudal axial malformations among these different lectin-treated groups is illustrated in Fig. 7. The 2 younger groups of embryos (HH stages 11–12; 13–early 14) were highly susceptible to the effects of WGA. However, there appeared to be a threshold in development at HH stage 14, with regards to the responsiveness of the tissues to the lectin treat-
Fig. 3. Transverse sections through the lumbosacral regions of embryos treated with the D-glucose- and D-mannose-binding lectins, ConA and LCA. (A) ConA-treated. The neural tube (n) is poorly developed, distended and the ventral portion has ruptured. The disproportionately large notochord (c) is attached to the gut (gt). (B) ConA-treated, showing dysplasia of caudal structures. Accessory neural tube lumina are present and the neural tube contains many pyknotic cells. Somites (s) are disrupted, distended and displaced. Segments of somites are fused to the neural tube or closely appose it. g, ganglion. (C) ConA-treated, showing the total absence of caudal axial structures. h, hindlimb bud. (D) LCA-treated, showing an apparent unaffected primary (pn) portion of the neural tube, while the secondarily-derived portion (sn) is disrupted. An accessory notochord segment (c) is present. Bars, 100 μm.

There was an association between the stage of development of the embryos at the time of WGA treatment and the severity of the defects produced. A higher incidence of total and partial caudal regression was found in the younger embryos (stages 11–early 14). Other caudally located malformations encountered.
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Fig. 4. The effect of increasing doses of WGA on the survival and incidence of induced malformations in chick embryos recovered 48 h after exposure to the teratogen.

![Graph showing survival rates and malformations vs doses](image)

Fig. 5. Chick embryos recovered 48 h after treatment, showing the spectrum of malformations produced by WGA. (A) Control. (B–D) WGA-treated embryos, showing increasing severity of caudal regression (shortening of the tail) with dysplasia of the hindlimb buds. Embryo (D) also shows scoliosis of the lower trunk. Bar, 25 mm.

were open posterior neuropores, especially in the stage 11–12 embryos, and hind limb bud dysplasia (Fig. 5).

Histological examination of the caudal regions of stage 11–12 treated embryos showed varying degrees of disruption of tail structures resembling those at stages 13 to early 14 (Fig. 6). Totally structureless tail stumps were observed in severe cases of caudal regression. In less severe cases, defects in the tail ranged from absent neural tubes and/or notochords to rearrangements in the positions of various tail structures. For example, the most common malformation induced by WGA was fusion or incorporation of the notochord into the ventral neural tube at the lumbar sacral segments of the embryo (as illustrated for stages 13–early 14; Fig. 6B,C). Frequently, accessory fragments of notochord were present in sections (see Fig. 6C,E), representing branches of the notochord from a common stem. Such fragments were often closely associated with the tail gut wall. Open posterior neuropores (myelo-
schisis) were also encountered in the younger embryos (9%).

In the older stage 15–16 embryos, the defects encountered were less severe. The most frequently observed defect was the malpositioning of the dorsal root ganglia. These were usually fused to each other and located ventral to the neural tube (Fig. 6F). In such cases, the notochords were displaced.

**Binding of WGA to tail buds**

Binding of the lectin to the tail bud was identified with an antibody to WGA. By 6 h after treatment, in all WGA-treated embryos, the lectin was bound to the gut, while, in some of the embryos, WGA binding was also found in the paraxial mesoderm and tail bud, especially in the ventral portion of the medullary cord. Later in development, WGA binding was localized in the ventral regions of the cavitating medullary cord and developing notochord. In the saline controls, there was no anti-WGA staining.

In WGA-exposed embryos that showed no morphological malformations at 48 h after treatment, binding of anti-WGA was mainly to the gut and associated blood vessels. However, in malformed embryos, heavy binding was found in the regions of the defects, for example, at the region of fusion of the notochord to the ventral neural tube (Fig. 8B). In saline controls, there was no anti-WGA binding (Fig. 8A). These results demonstrate the presence of WGA in the affected tissues. They also suggest that the lectin had failed to bind to susceptible tissues in embryos that appeared to be unaffected by the treatment.

**Sialic acid versus N-acetylglucosamine**

WGA binds to residues of both GlcNAc and sialic acid. In order to determine which of these residues might be more closely associated with WGA effects on tail bud development, the effects of LPL and sWGA were compared with those of WGA. LPL has a specificity for only sialic acid residues, while sWGA is specific for only GlcNAc. The survival rate was 53% in WGA-treated embryos, 98% in LPL-treated embryos and 95% in sWGA-treated embryos.

In embryos that were treated with LPL, the total incidence of caudal axial malformations in surviving embryos was similar to that induced by WGA (46%), while in sWGA-treated embryos, only 29% of survivors showed abnormalities.

The malformations observed in LPL-treated embryos, both at the gross and histological levels, were fairly similar to those previously described for the WGA-treated embryos (Figs 9A, 10). LPL did not differ significantly from WGA (P>0.05) in its ability to induce abnormal notochord–neural tube and notochord–tail gut relationships, or the presence of accessory neurocoels. However, the neural tube–notochord fusion defects induced by LPL were in general less severe than those of WGA-treated embryos. Unlike the WGA-treated embryos, LPL-treated embryos that had caudal axial malformations also had oedematous blebs in
Fig. 6. Sections through embryos treated with WGA, and recovered 48 h later, showing the spectrum of malformations induced by the lectin. (A) Transverse section through the tail, showing the presence of a rudimentary secondary neural tube (n) and disorganized somites (s). The next 3 sections show the types of abnormal neural tube–notochord relationships induced by WGA. (B) Fusion of the notochord (c) to the split ventral portion of the secondary neural tube in the tail. (C) Section through the lumbosacral region. The lumina of the primary (pn) and secondary (sn) portions of the neural tube have not coalesced. An accessory notochord is present. One of the notochord segments replaces a portion of the secondary neural tube, while the other is fused to the gut (gt). (D) Section through the tail of a WGA-treated embryo, with two neural tubes. (E) Lumbosacral section from a WGA-treated embryo. An oureneric outgrowth (o) containing a neural tube, notochord and somite is seen within the gut. (F) Section through the tail of an embryo treated with WGA at stages 15–16, showing fused ganglia located under the neural tube. See Fig. 1A for control comparison. Bars, 100 μm.
Fig. 7. The effects of WGA on the survival rate and incidence of malformations of embryos treated with the lectin at different stages in caudal axial development. * Significant difference from saline controls (P<0.05; Chi-square test).

Fig. 8. Transverse sections through embryos that have been stained with an antibody to WGA. (A) Saline-treated control. n, neural tube; c, notochord. (B) Embryo injected with WGA. Dark spots represent areas of lectin binding. These were found in areas where defects occurred. The abnormal fusion of an accessory fragment of notochord (c) to the neural tube is indicated by the arrow. gt, gut; Bar, 100 μm.

Discussion

In this study, we have demonstrated that binding of the lectin WGA to surface glycoconjugates of the tail bud of chick embryos results in the disruption of the normal morphogenesis of the caudal axis. Treatment of chick embryos by sub-blastodermal injection of WGA induced a variety of defects including; the total disruption of tail development, abnormal relationships between the notochord and the neural tube or tail gut, the presence of accessory branches of the notochord and the presence of accessory secondary neural tube lumina. Immunohistochemistry of sections from treated embryos demonstrated WGA-binding in the affected areas.

The results also suggest that there is a stage-dependent response of embryos to WGA treatment. There was a threshold stage (the late tail bud anlagen to early tail bud stage; HH stage 14) in development where younger embryos, but not the older ones, were highly responsive to the lectin treatment. The decreasing responsiveness of the developing tail bud to WGA, and the decrease in WGA-binding with age (Griffith and Wiley, 1989a) suggests either a decrease in binding sites for the lectin, or progressive inaccessibility to those sites.

Of the variety of malformations induced by WGA, the defect that was most characteristic of WGA treatment was the fusion of segments of the notochord to the ventral portion of the neural tube, or in more severe cases, the replacement of the ventral neural tube with a notochordal segment. Control embryos treated with non-sialic-acid-binding lectins, ConA, LCA, PNA and...
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Fig. 9. A comparison of the most common defects produced by the lectins LPL and sWGA. (A) Section through an embryo treated with LPL, showing a disrupted neural tube (n) with multiple lumina. The main lumen is open (arrow), as the embryo also has an open neural tube defect. Two notochord (c) fragments are present, one being fused to the neural tube. g, ganglion; s, somite. (B) Section through a sWGA-treated embryo with a large ourenteric outgrowth (o) through the roof of the gut (gt). The outgrowth has a well-formed neural tube (n), notochord (c) and somites (s) of its own. The main neural tube shows an overgrowth at the dorsal end. Bars, 100 μm.

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Fig. 10. Comparisons of the various types of malformations induced by the lectins WGA, LPL and sWGA. * Significant difference (P<0.05; Tukey multiple comparison test) from WGA.

sWGA showed that the malformations induced by WGA were specific to that lectin and not a result of generalized embryotoxicity. While defects apparently specific to WGA such as notochord–neural tube fusions and replacements were not induced by these non-sialic-acid-binding lectins, nevertheless, they also had profound effects on the development of the caudal axis. This is not surprising, in view of the importance of glycoconjugates in cellular interactions during development (Damjanov, 1987; Janzer, 1986; McLone and Knepper, 1986; Moscona, 1974; Muramatsu, 1988a, b). In fact, PNA was the only non-sialic-acid-specific lectin that failed to induce malformations of the caudal axis, although the lectin was shown to be bound to tail-bud-derived tissues by the binding of anti-PNA antibody.

The malformations induced by WGA-binding appeared to be primarily in the associations of developing organs (e.g. secondary neural tube with primary neural tube and/or secondary neural tube with notochord), rather than in the associations of cells within organs. This suggests that WGA exerted its effects on the tail bud very early on in development, prior to organogenesis of the tail bud structures. The WGA effect was likely on whole populations of cells, possibly those sharing similar surface glycoconjugates, or bound by common extracellular glycoconjugate-containing matrices.

From results of extirpation experiments, transplants using labelled heterografts, and chick–quail xenografts, Schoenwolf (Schoenwolf, 1977, 1978; Schoenwolf et al. 1985) has postulated that, in chicks, the segment of notochord that underlies the secondary neural tube is a caudal extension of the notochord of the trunk. Gajovic et al. (1989), however, have argued that both the tail gut and associated notochord are derivatives of a common precursor within the tail bud. The abnormal relationships between the neural tube and notochord observed in our study also suggest a close relationship between the development of the tail gut, secondary neural tube and the underlying segment of the notochord, since the disruption of the tail bud with lectins produced defects in these axial structures. Our observations, therefore, appear to support the hypothesis of a common precursor of the secondary neural
tube, tail gut and the intervening segment of the notochord.

WGA binds to both sialic acid and GlcNAc residues (Peters et al. 1979; Roth, 1978; Reuter et al. 1982). The results of treatment with LPL and sWGA, however, suggest that the effect of WGA on the development of the neural tube and notochord resides with its specificity for sialic acid. LPL, which is specific only for sialic acid, produced a pattern of defects similar to that of WGA. The pattern of defects induced by sWGA, which is specific for GlcNAc, differed significantly from that induced by LPL or WGA. The greater teratogenicity of WGA in comparison to LPL suggests a potentiating effect from the simultaneous disruption of GlcNAc residues. There are several possible mechanisms by which WGA and LPL may have exerted their effects: (1) WGA and LPL may have immobilized cells that have sialic acid on their surface, preventing them from undergoing normal morphogenetic movements; (2) the lectins may have caused 'capping' or aggregation of their respective specific receptor-bearing molecules on the surfaces of these cells, thereby hindering receptor-mediated cell–cell interaction; or (3) by steric hindrance, the lectins may have prevented the binding of another unrelated molecule more essential to the process of differentiation and organogenesis. Nevertheless, the results of this study demonstrate a correlation between lectin binding to sialoconjugates and the abnormal development of the avian tail bud.

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