Lectin histochemistry in the developing oto-maxillo-facial primordia of the mouse embryo

S. LOURYAN, R. GLINEUR
Laboratoire d'Anatomie et Embryologie Humaines, Faculté de Médecine, Université Libre de Bruxelles

SUMMARY

The binding sites of several lectins (Con-A, SBA, WGA, PNA, RCA-I, UEA-I, DBA) were studied in the different tissues involved in mouse visceral cephalogenesis. As compared to various other lectins which have only a weak affinity for precartilaginous rudiments, PNA preceded by neuraminidase treatment shows a very strong fixation in the precartilaginous blastemata and their immediate environment. PNA receptors exist also on the enamel epithelium and mesenchymal sac. RCA affinity for blood vessels has also enabled detailed observations on the vascularization of the palatal shelves, particularly in area 3. Various other localizations have been described and correlated with other histochemical data.

KEY WORDS:

Mouse, embryo, lectins, head.

RÉSUMÉ

Les sites de fixation de différentes lectines (Con-A, SBA, WGA, PNA, RCA-I, UEA-I, DBA) ont été étudiés dans les différents tissus impliqués dans la céphalogenèse viscérale de la souris. En comparaison avec les autres lectines qui présentent une faible affinité pour les précartilages, l'étude de la fixation de la PNA précédée d'une pré-incubation à la neuraminidase montre une forte fixation sur les blastèmes précartilagineux et leur environnement immédiat. Des récepteurs à la PNA sont également relevés dans l'organe adamantin, alors que la RCA se fixe dans la zone périphérique du mésenchyme odontogène et sur le sac mésenchymateux. La fixation de la RCA sur la paroi des vaisseaux permet d'intéressantes observations relatives à la vascularisation des crêtes palatines, particulièrement dans la zone 3. De nombreuses autres localisations sont décrites et mises en corrélation avec d'autres données histochimiques.

MOTS-CLÉS:

Souris, embryon, lectines, tête.

INTRODUCTION

Lectins are animal or vegetal proteins of non-immune origin which bind selectively to terminal sugar residues of glycoconjugates and may therefore serve as useful probes to detect developmental changes in cell-surface or extracellular matrix macromolecules which contains carbohydrate-residues (Danguy *et al.*, 1988, Wacker, 1988). Changes in the binding pattern of various lectins have been demonstrated histochemically during the embryonic development of a number of organ systems and tissues in different species: limb buds (Aulthouse and Solursh, 1987; Hurle *et al.*, 1988; Milaire, 1991), central nervous system (Takahashi, 1988; Welim *et al.*, 1989; Griffith *et al.*, 1990), metanephros (Laitinen *et al.*, 1989), heart (Fazel *et al.*, 1989), biliary ducts (Shiojiri and Katayama, 1988), olfactory system (Plendl and Schmahl, 1988), lens (Webster and Uknis, 1987), bone marrow (Sorrell, 1988); tooth germs (Blottner and Lindner, 1987).

BIBLIOTECA DE LA UNIVERSIDAD

Other studies have demonstrated selective lectin binding patterns in very early embryos, some of them displaying changes corresponding to tissular and/or organ differentiation (Cook *et al.*, 1979: chick, Wu *et al.*, 1983: mouse, Slack, 1985: amphibian). Similar changes were found associated to modifications in the extracellular matrix of developing embryos (Thesleff *et al.*, 1979, 1981a,b, 1987, 1988: tooth germs; Xu *et al.*, 1990 chick primary palate; Brinkley and Morris-Wiman, 1984: secondary palate).

In the present study, the binding patterns of seven biotinylated lectins were examined in the oto-maxillo-facial primordia of 10- to 17-day mouse embryos and the results correlated with previous histochemical properties detected in the same rudiments.

MATERIAL AND METHODS

Pregnant NMRI mice were killed by cervical dislocation. Considering vaginal plug day as day O, embryos of 9 to 17 days were removed and fixed in Serra's medium (ethanol 95°: 6 vol., formalin: 3 vol., acetic acid: 1 vol.) or in Bouin with 1% acetic acid. The results obtained with both fixatives being identical, Serra's fixative was the most commonly used. The cephalic part of the embryos was sectioned and embedded separately in Paraplast (Technicon). Serial 10 μm horizontal or/and frontal sections were cut and stuck on slides with a water concentration solution of bovine serum albumin at 50 mg% (fraction 5, Sigma).

Lectin staining

Sections were deparaffinized in toluene, rehydrated and dipped in phosphate buffer saline (PBS) at pH 7.8.

The potential endogenous peroxidase was inhibited by 30 min incubation in 0.3% H_2O_2 (Perhydrit) in methanol. After a 20 min PBS wash, the sections were submitted to the blocking kit avidin-biotin (Vector) which blocks endogenous biotin, biotin receptors and the binding sites to avidin. In practice, the sections are treated for 15 min by a solution of avidin (3 drops/10 ml PBS), rinsed in PBS and immersed for 15 min. in a solution of biotin (3 drops/10 ml PBS).

The slides were then washed 3×5 min in PBS before being incubated 30 min in humid atmosphere in the presence of the different biotinylated lectin solutions (Vector); the lectin is extemporaneously diluted at a concentration of 10% in PBS. After two more 5 min washes in PBS, sections are submitted to the ABC reagent vectastain (Vector) containing avidin DH associated with horseradish peroxidase, then washed again 3×5 min in PBS. Revelation of peroxidase activity was performed by incubating the sections for 7 min in a 0.1% solution of diaminobenzidine in 0.02% H_2O_2. Slides are successively washed in tap and distilled water, counterstained wit Methyl green (0.075% at pH 4.7), dehydrated and mounted in Technicon medium.

Prior to PNA, RCA and DBA lectin staining, some alternated neighbouring sections were preincubated with *Clostridium perfringens* neuraminidase (Sigma Chemical Co, USA) at 1 unit/ml in 0.5 M acetate buffer at pH 5.3 for 60 min at 37° C. This treatment uncovers PNA receptors masked by terminal sialic acid. In order to verify lectin specificity, several preparations treated by PNA, RCA and SBA were pre-incubated with a solution of D-galactose at 0.3 M. Some WGA sections have been pre-incubated in N-acetylglucosamine at 0.3 M. Furthermore, sections at every embryonic stage have been treated by the entire staining procedure without lectin incubation.

RESULTS

1. *Concanavalin A (Con A)*

A diffuse staining was observed at all stages. In young embryos (10 to 12 days), the mesenchyme exhibits a uniform moderate staining as compared to vascular endothelia and several epithelia (stomodeal ectoderm, pharyngeal endoderm, nasal pit, otic vesicles, enamel organ) which are strongly positive. From day 13 onward, new binding sites characterize pretendinous blastemata, peripheral nerves and ganglia and precartilaginous anlagen, which show intra- and extracellular localizations.

2. *Soybean agglutinin (SBA)*

Several epithelia were positive at all stages (surface and stomodeal ectoderm, endoderm, nasal pits and otic vesicles, salivary glands). A weak staining was observed on the surface of precartilaginous cells on day 13. Cartilaginous cells have shown intra- and extracellular fixation from day 13 onward whereas vascular endothelia showed a selective affinity for SBA between days 10 and 13 and later gradually loose that property. Myotubes become SBA – positive on day 17. Pretendinous rudiments, and temporo-mandibular joint disk were stained from day 13 onward (Fig.1).

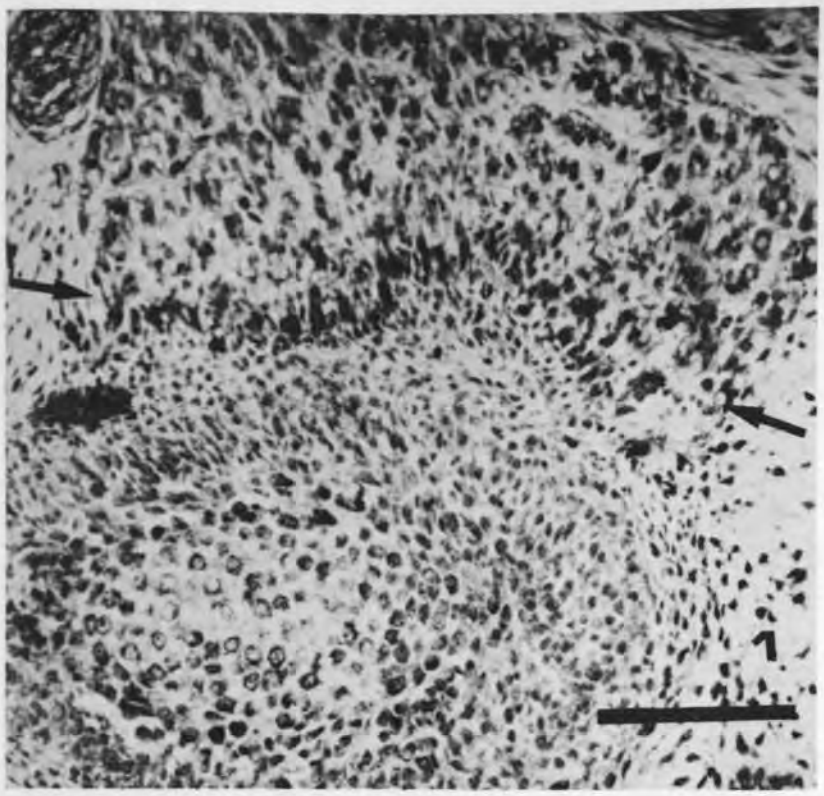

Fig. 1: SBA, day 16, selective staining of the rudiment of the lateral pterygoid muscle (arrows).
Fig. 1: SBA, 16 jours, fixation sur le rudiment du tendon du muscle ptérygoïdien latéral (flèches).

3. *Wheat germ agglutinin (WGA)*

The WGA binding sites appear to be relatively unspecific. A large majority of embryonic tissues are stained, both mesenchymal and epithelial in nature. WGA also binds to macrophage cells.

4. *Peanut agglutinin (PNA)*

Very selective PNA binding sites were observed. PNA binds strongly on epithelial cells, particularly to the tongue (Fig. 2) and enamel epithelium (Fig. 3) from day 13 onward. Between days 13 and 16, an intense staining was also observed in the dental mesenchyme. A very strong PNA-binding characterizes the salivary epithelium with a descrete regression on day 17. On days 12 and 13, pretreatment with neuraminidase reveals a marked extracellular staining in the preskeletogenic areas of the first two branchial arches as well as in their interconnecting tissue (Fig. 4 and 5). This binding site regresses after day 13; premuscular blastemata are free of binding (Fig. 13). This fixation is not strictly limited to precartilaginous areas (Fig. 4). PNA staining after neuraminidase treatment also demonstrates a very intense binding on vascular endothelia and macrophagic cells. Differentiating cartilaginous rudiments exhibit extra- and intracellular fixation from day 14 onward, with an intense binding around the cartilaginous axis, including the perichondrium.

A noticeable staining was observed between days 13 and 16 on the transient insertion of a part of the tensor tympan muscle on Meckel's cartilage at the level of the goniale bone attachment (Fig. 6).

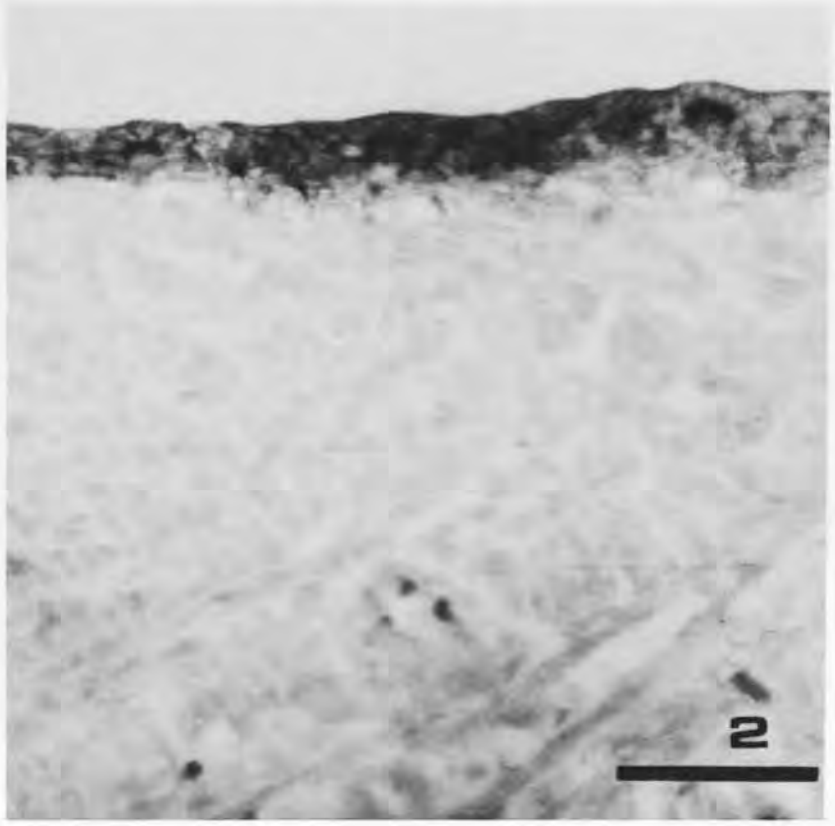

Fig. 2: PNA, day 16, intense staining of the tongue epithelium.
Fig. 2: PNA, 16 jours, fixation intense sur l'épithélium lingual.

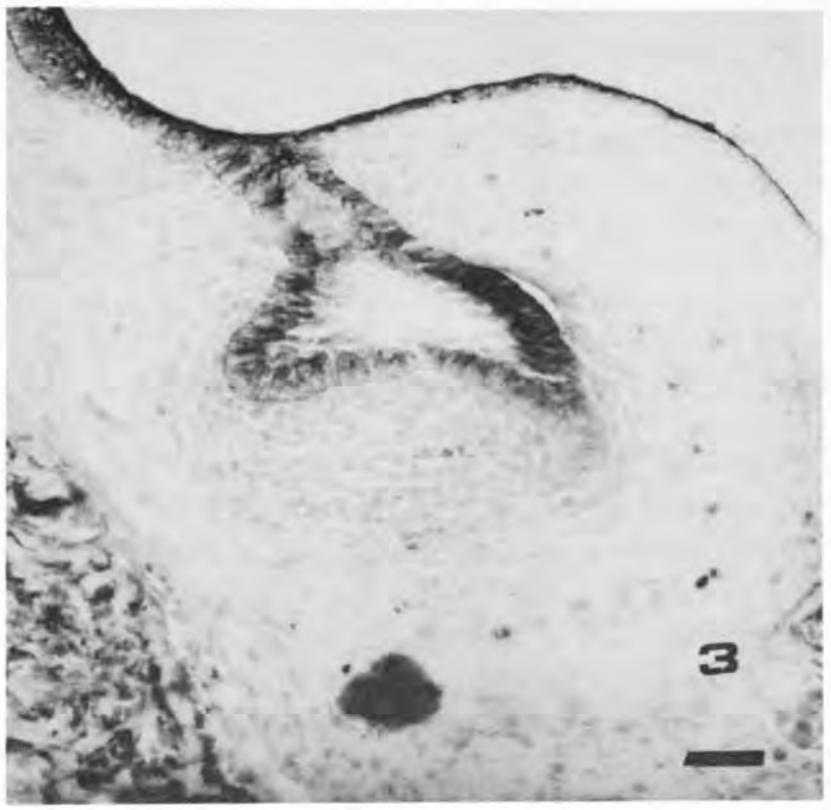

Fig. 3: PNA, day 13, strong staining of the enamel epithelium.
Fig. 3: PNA, 13 jours, forte fixation sur l'épithélium adamantin.

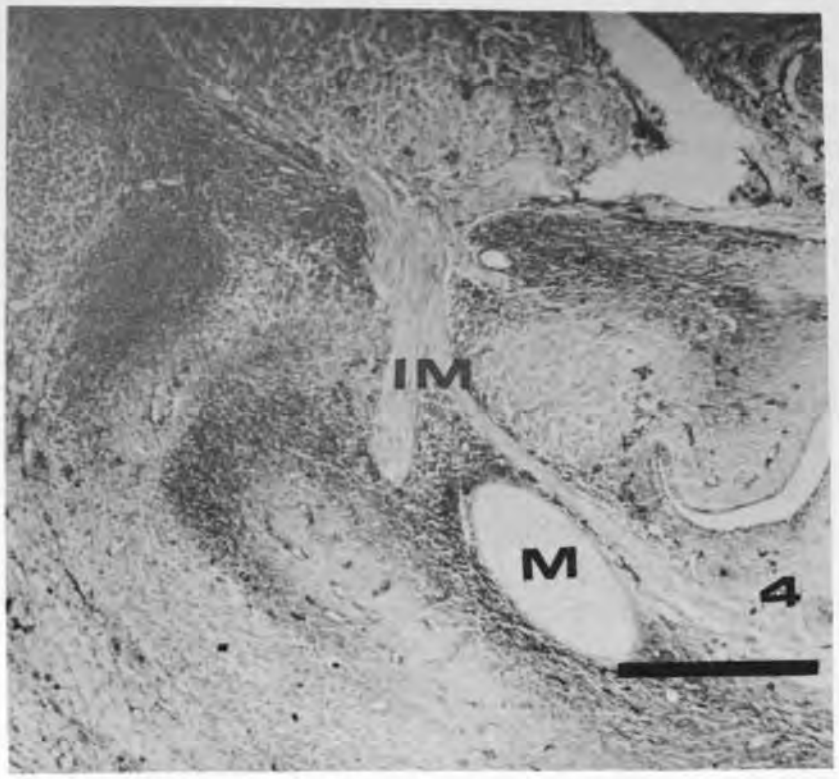

Fig. 4: PNA preceded by preincubation with neuraminidase, day 13. Intense staining of preskelettic tissue (M: Meckel's cartilage, IM: inferior maxillary nerve).
Fig. 4: PNA précédée par une pré-incubation à la neuraminidase, 13 jours, fixation intense sur les tissus présquelettiques (M: cartilage de Meckel, IM: nerf maxillaire inférieur).

Other tendinous rudiments display moderate binding including the prospective fibrocartilage of the temporo-mandibular joint from day 15 onward.

5. *Ricinus communis agglutinin (RCA)*

At all stages considered, a very strong affinity for RCA was observed on blood vessels, including those of the central nervous system. Mesenchymal staining did not show signficant specificity in 10- to 12-day embryos, except in precartilaginous rudiments which exhibit a moderate intracellular binding (Fig. 7). Positive blood vessels surround the avascular precartilaginous blastemata. From day 13 on, cartilaginous anlagen like branchial cartilages or otic capsule show intra- and extracellular binding affinities similar to those of other lectins. At day 13, the dental papilla is surrounded by a crown of weakly stained blood vessels. At days 13 to 15, a pluristratifed group of positive cells appears around the papilla (Fig. 8 and 9) and the RCA staining persists until day 16 in the mesenchymal sac. In the palatal shelves, subepithelial mesenchymal cells are strongly positive in area 3 and a uniform staining also characterizes the entire epithelium (Fig. 10).

Nerve fibers from the pterygopalatine ganglion are also positive and could be traced along positive blood vessels up to area 3 (Fig. 11).

Pretendinous rudiments stain intensely and are particularly well visible in the soft palate resulting from palatal shelves fusion.

6. *Ulex europaeus agglutinin (UEA)*

The mesenchyme appears moderately stained. On day 12, a discrete UEA staining of precartilaginous cells was observed in the branchial precartilages. Later on, UEA binds to the intra- and extracellular compartments of differentiating cartilages as well as to the perichondrium, whereas pretendinous blastemata and the temporo-mandibular joint disk become positive on day 16.

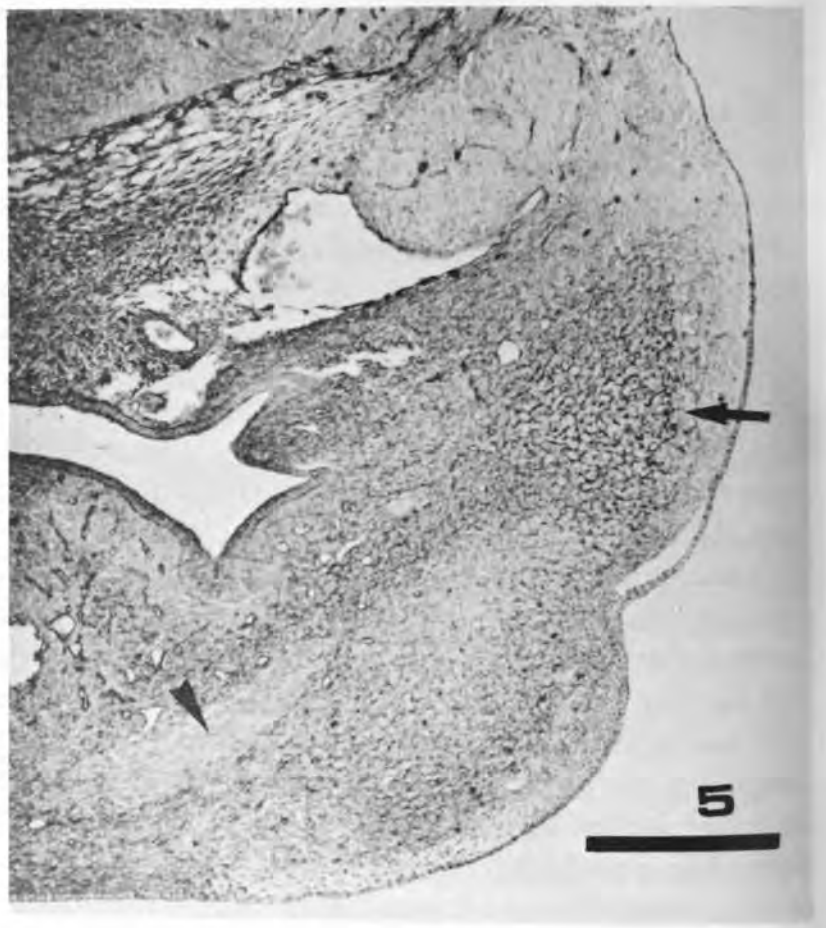

Fig. 5: PNA preceded by neuraminidase, day 12. Widespread PNA-positive area at the site of precartilaginous branchial rudiments (arrow). The premuscular area is free of lectin binding (arrowhead).
Fig. 5: PNA précédée par une pré-incubation à la neuraminidase, 12 jours. Zone étendue PNA-positive dans le site correspondant aux ébauches précartilagineuses branchiales (flèche). La zone prémusculaire ne montre aucune fixation (tête de flèche).

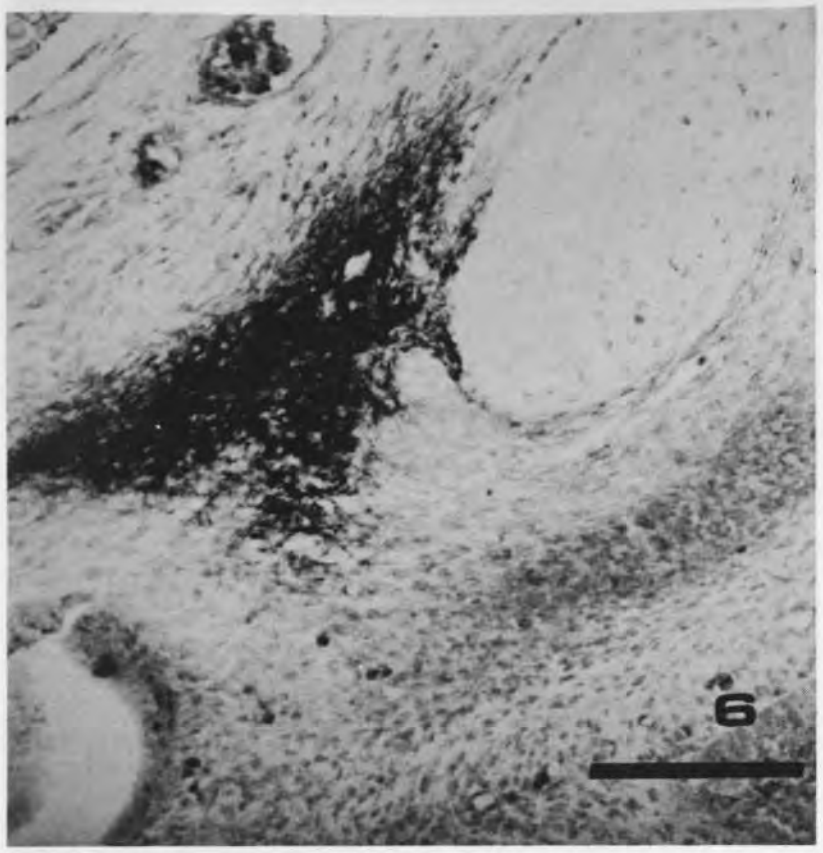

Fig. 6: PNA, day 16, selective intense staining of the tensor tympani prospective tendon at the level of its transient attachment to the Meckel's cartilage.
Fig. 6: PNA, 16 jours, fixation intense et sélective dans le futur tendon du muscle du marteau au niveau de son union transitoire au cartilage de Meckel.

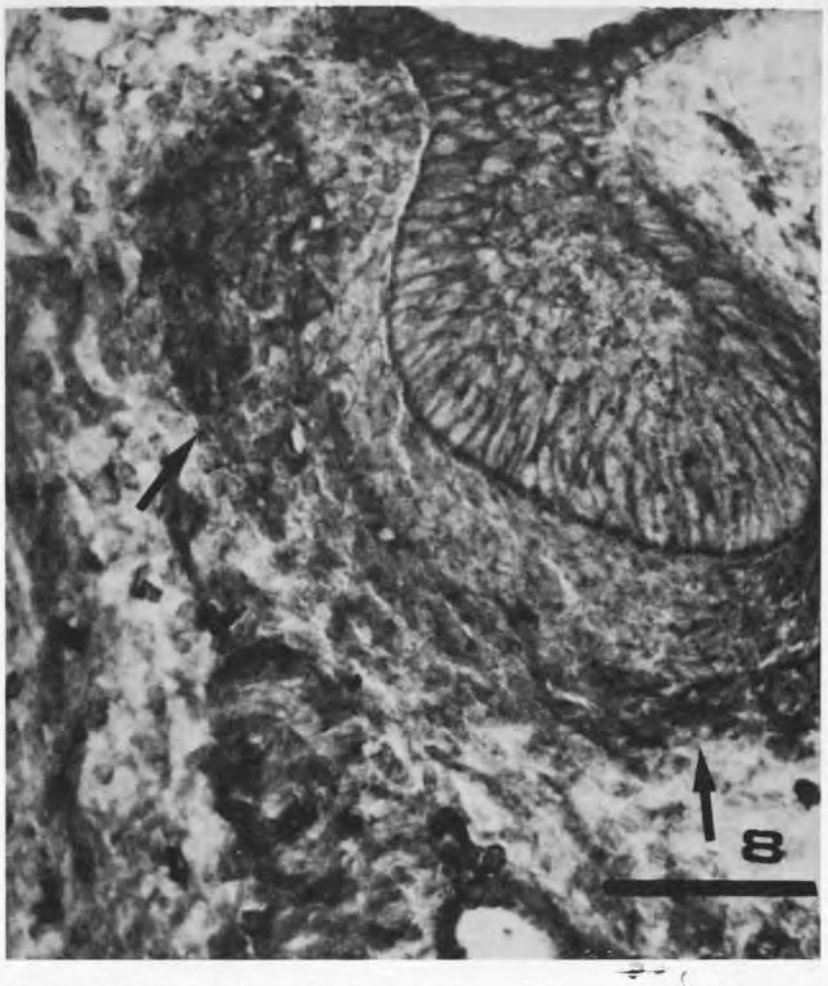

Fig. 8: RCA, day 13, selective staining of the periodontal mesenchyme (arrows).
Fig. 8: RCA, 13 jours, fixation sélective sur le mésenchyme périodontal (flèches).

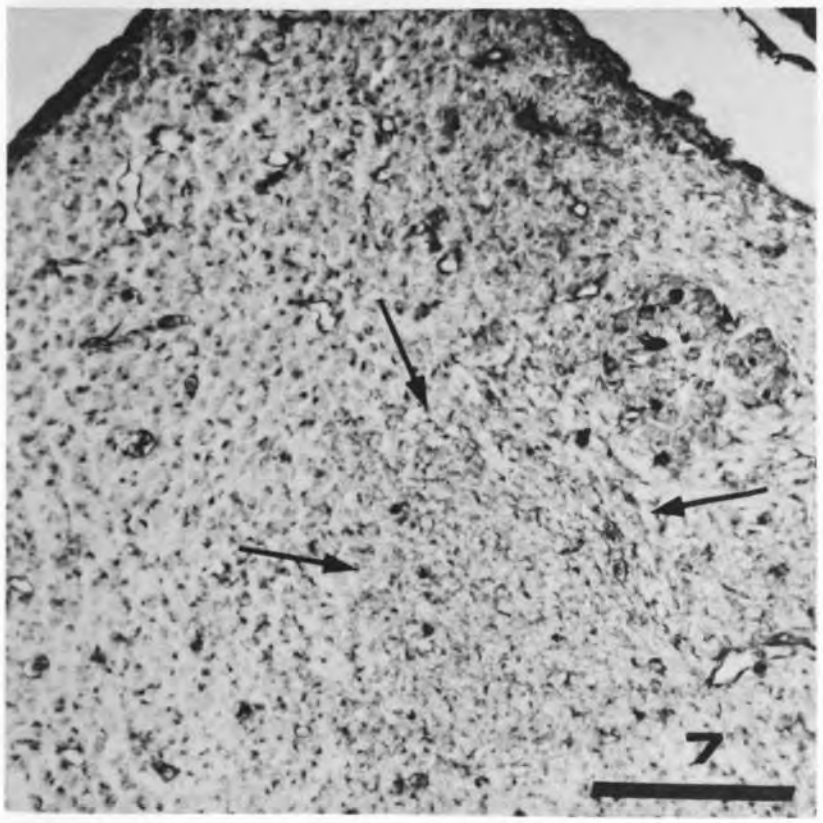

Fig. 7: RCA, day 11, discrete intra- and extracellular staining of the precartilaginous anlage of the first visceral arch (arrows).
Fig. 7: RCA, 11 jours, fixation discrète intra- et extracellulaire sur l'ébauche du premier précartilage branchial (flèches).

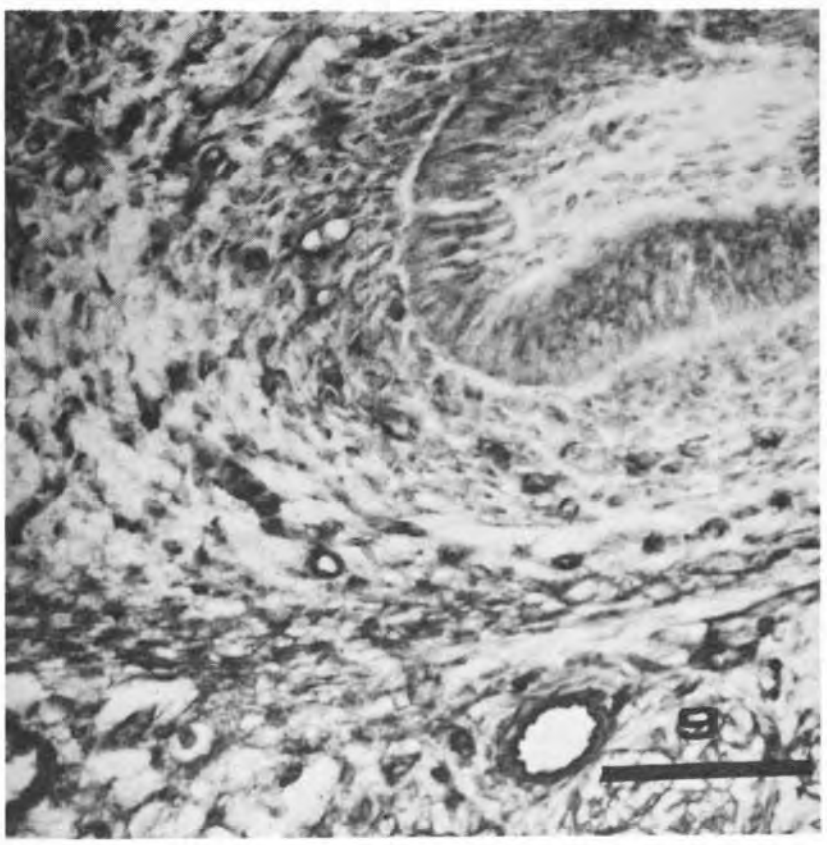

Fig. 9: Id. RCA-positive blood vessels around odontogenic mesenchyme.
Fig. 9: Idem. Des vaisseaux RCA-positifs s'observent autour du mésenchyme odontogène.

7. Dolichos biflorus agglutinin (DBA)

In young embryos, a weak DBA binding affinity is expressed mainly in epithelial tissues of both ecto- and endodermal origin. On day 13, a faint staining appears in the precartilaginous anlagen and a moderate binding affinity appears in the mesenchyme with a pattern similar to that of RCA binding sites in tooth germs. Cartilaginous staining is the same as with other lectins but DBA also binds to the perichondrium. If it is preceded by neuraminidase treatment, DBA staining provides a very selective vizualisation of the developing blood vessels and salivary epithelium (Fig. 12).

Table I
Utilized lectins and their sugar affinities

Con-A	*concanavalia ensiformis*	α-D mannose
SBA	*glycine max*	α-D galactose
WGA	*tritium vulgare*	N-acetylglucosamine
PNA	*arachis hypogea*	Galactosyl- B-1,3-N Acetylgalactosamine D-galactose
RCA-I	*ricinus communis*	β-D galactose
UEA-I	*ulex europaeus*	α-L fucose
DBA	*dolichos biflorus*	β-D-galactose N-acetylgalactosamine

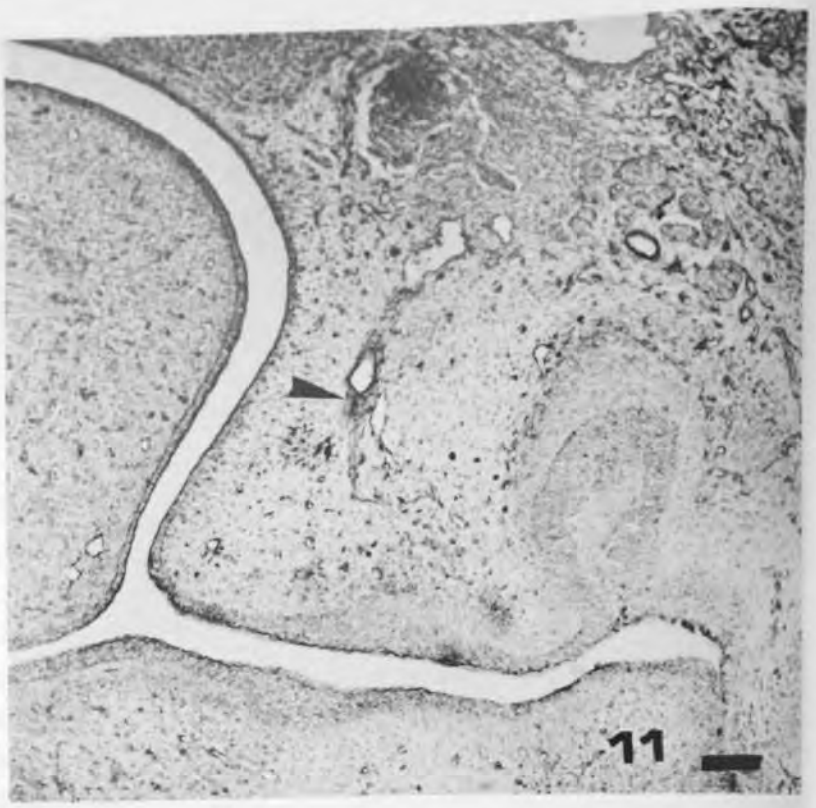

Fig. 11: Id. Selective staining of the pterygopalatine nerve fibers and associated blood vessels on their way to the area 3 of the palatal shelve (arrowhead).
Fig. 11: Idem. Fixation sur les fibres en provenance du ganglion ptérygo-palatin ainsi que sur les vaisseaux sanguins associés se dirigeant vers la zone 3 de la crête palatine (tête de flèche).

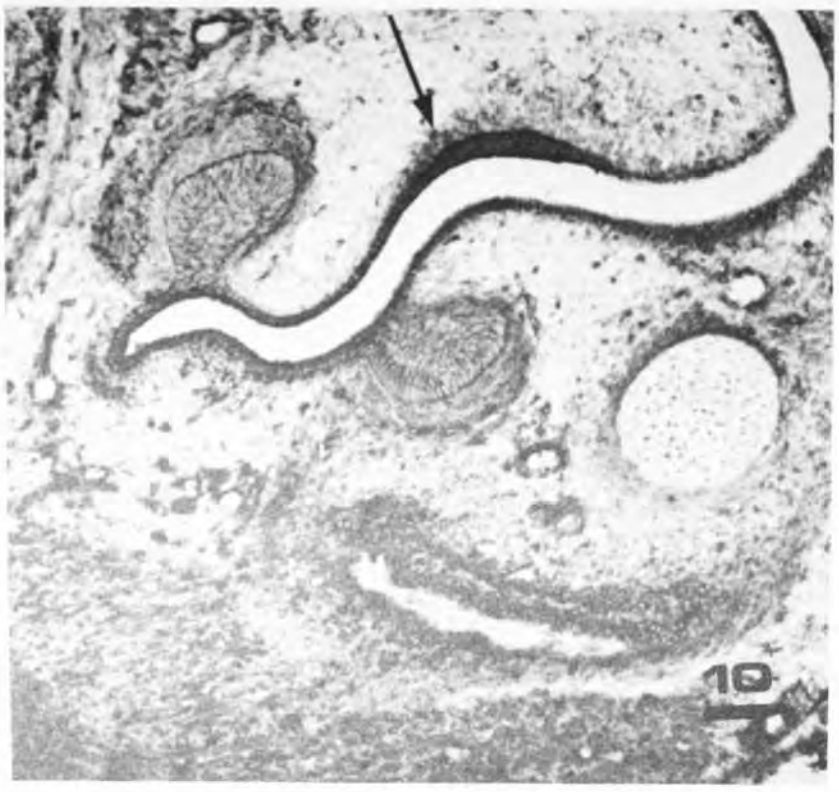

Fig. 10: Id. Selective staining of the mesenchyme subjacent to the palatal shelve epithelium in area 3 (arrow).
Fig. 10: Idem. Fixation sélective sur le mésenchyme sous-jacent à l'épithélium de la crête palatine dans la zone 3 (flèche).

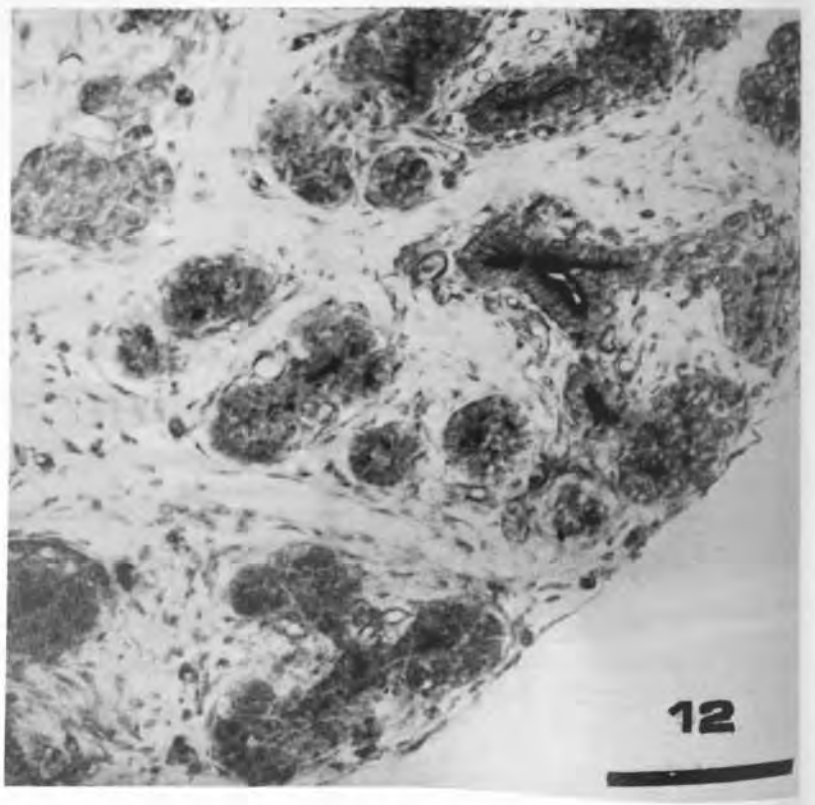

Fig. 12: DBA, day 16, intense staining of the canalar epithelium in the submandibulary gland.
Bar = 100 μm.
Fig. 12: DBA, 16 jours, fixation intense sur l'épithélium canalaire de la glande sub-mandibulaire.
Echelle: 100 μm.

DISCUSSION

The results presented here need to be correlated with lectin binding sites described in developing tissues of other animal species. High lectin binding in cellular and pericellular structures was considered to be an expression of high concentrations of oligosaccharides involved in the metabolism of glycosaminoglycans (GAGS). In addition, intracellular lectin binding in cartilaginous tissue is most probably related to the involvement of the Golgi apparatus in the intracellular turnover of synthetized GAGS' although different conclusions were reported in this respect by Mallinger *et al.* (1986).

In the chick embryo, Aulthouse and Solursh (1987) provided evidence that PNA can be considered as a precartilaginous blastema marker, a conclusion confirmed by Hurle *et al.* (1988) on the same material. In the present study, the PNA staining observed after neuraminidase treatment in the branchial arches of mouse embryos appears to be mainly extracellular and is not strictly limited to the precartilaginous area. In young amphibian embryos, Slack (1989) observed that a PNA receptor masked by sialic acid is present throughout the extracellular matrix. It is therefore possible that PNA fixation after neuraminidase treatent in the branchial arch mesenchyme is associated with extracellular matrix deposition in and around preskeletal blastemata. RCA- positive blood vessels surrounding precartilaginous areas were also observed in developing limb buds by Milaire (1990); this investigator suggests that extracellular matrix deposition might somehow be related to the gradual fading of the vascular network in chondrogenic areas. Changes in RCA binding are very interesting at early stages of tooth development. The formation of an early crown-shaped vascular bed provides a clear cut delineation of the young dental pulp anlage. The very strong and specific binding of the dental sac and periodontal mesenchyme provides further evidence of the genesis of an early difference between the prospective dental pulp and surrounding tissues. A very nice correlation can be made with the immunohistochemical localization of the cell-surface proteoglycans (Thesleff *et al.*, 1988), which show a similar pattern. A selective concentration of laminin was also demonstrated by Thesleff *et al.* (1981a) in the mesenchymal dental sac as well as in the basement membrane. These histochemical features seem to be the expression of several epithelial-mesenchymal interactions involved in dental germ formation as described in details by Slavkin *et al.* (1969, 1972a,b, 1976, 1988). Fixation of PNA on dental mesenchyme in early odontogenesis is in good agreement with the data reported by Blottner and Lindner (1987). It is most likely that changes in lectin binding properties detected in dental primordia are the expression of important modifications in the composition of the extracellular matrix, as described by Thesleff *et al.* (1979, 1981a,b, 1987, 1988) and Hurmerinta *et al.* (1986).

The very selective affinity of RCA for all blood vessels was very useful in the demonstration of the vascular network which invades the palatal shelves mesenchyme in parallel with nerve fibers originating from the pterygopalatine ganglion. The accompanying Schwann cells are similarly RCA positive and both vessels and fibers converge near the subectodermal area 3 which itself exhibits an intense RCA binding both in the mesectoderm and in the ectoderm. These histochemical transient properties appear very interesting in regard to theories which suggest the involvement of nervous factors in the mechanism of palatal shelves elevation (Babiarz *et al.*, 1975, Meyer Kuhn *et al.*, 1980, Zimmerman and Wee, 1984, Widelec, 1986, Lauder and Zimmerman, 1988), a process in which extracellular matrix deposition is also important (Brinkley and Morris-Wiman, 1984). Cells of area 3 are known to possess specific morphological features especially as regards their spatial orientation (Meyer-Kuhn *et al.*, 1980) and also some selective enzymatic properties like the ATPase activity (Babiarz et al., 1975). An increase of B-D galactose on the surface of these cells as revealed by RCA bindig could be considered as a sign of regional differentiation.

The presence of similar lectin binding affinities on pretendious rudiments and on the developing temporo-mandibular disk contributes to support Symon's view (1952) according to which the disk is a part of the lateral ptherygoid muscle tendon. The early delineation of the branchial premuscular blastema, which is free of PNA binding, offers a nice correlation with the alkaline phosphatase activity of premuscular branchial anlagen recently demonstrated in mouse embryos (Louryan, 1990).

From the morphological standpoint, the demonstration of a preskeletal interconnecting blastema between the first two branchial arches of 12-day embryos provides new evidence for the existence of an early anlage including the material for the handle of the malleus and long crus of the incus (Louryan, 1986, 1988, 1989).

In conclusion, the study of lectin binding patterns in visceral head rudiments reveals interesting transient

histochemical properties of developing tissues; it will probably be of great help in disentangling the complex interactions between the various tissues involved in normal and abnormal cephalogenesis.

Research supported by the grant GB 1611CB2380 of the Fonds National de la Recherche Scientifique, Brussels, Belgium.

ACKNOWLEDGEMENTS

We thank Prof. A. Danguy for initiation to lectin histochemistry, Prof. J. Milaire for reviewing the manuscript, Mrs. A. Werry-Huet for technical assistance and MM. A. Demeire and M. Verheyden for photographic documentation.

REFERENCES

Aulthouse, A.L., Solursh, M. — The detection of a precartilage blastema specific marker. *Devel. Biol., 120:* 377-384, 1987.

Babiarz, B.S., Allenspach, A.L., Zimmermann, E.F. — Ultrastructural evidences of contractile systems in mouse palate prior to rotation. *Devel. Biol., 97:* 32-44, 1975.

Blottner, D., Lindner, E. — Light-microscopic studies on spatial and temporal binding of the lectins concanavalin A, wheatgerm agglutinin and peanut agglutinin in early rat odontogenesis. *Arch. oral. biol., 32:* 35-42, 1987.

Brinkley, L.L., Morris-Wiman, J. — The role of extracellular matrices in palatal shelf closure. In Moscona A.A., Monroy, A. (eds): Palate development: normal and abnormal cellular and molecular aspects. Orlando, Academic Press, pp. 17-36, 1984.

Cook, G.M.W., Zalik, S.E., Milos, N., Scott, V. — A lectin which binds specifically to B-D galactoside groups is present at the earliest stages of chick embryo development. *J. Cell. Sci., 38:* 293-304, 1979.

Danguy, A., Kiss, R., Pasteels, J.L. — Lectins in histochemistry. A survey. *Biol. Struct. Morph., 1:* 93-106, 1988.

Fazel, A.R., Sumida, H., Schulte, B.A., Thompson, R.P. — Lectin histochemistry of the embryonic heart: fucose-specific lectin binding sites in developing rats and chicks. *Am. J. Anat., 189:* 76-84, 1989.

Griffith, C.M., Wiley, M.J. — Distribution of cell surface glycoconjugates during secondary neurulation in the chick embryo. *Anat. Rec., 226:* 81-90, 1990.

Hurle, J.M., Ros, M.A., Hinchliffe, J.R.. — Spatial and temporal changes in the pattern of glycoxylation of the developing chick limb bud tissue components as revealed by fluorescent conjugated lectin probes. *Cell differ, 24:* 149-158, 1988.

Hurmerintai, K., Kuusela, R., Thesleff, I. — The cellular origin of fibronectin in the basement membrane zone of developing tooth. *J. Embryol exp. Morph., 95:* 73-80, 1986.

Laitinen, L., Lehtonen, E., Virtanen, I. — Differential expression of galactose and N-acetylgalactosamine residues during fetal development and postnatal maturation of rat glomeruli as revealed with lectin conjugates. *Anat. rec., 223:* 311-321, 1989.

Lauder, J., Zimmerman, E.F. — Sites of serotonin uptake in epithelia of the developing mouse palate, oral cavity and face: possible role in morphogenesis. *J. Craniof. genet. dev. biol., 8:* 265-276, 1988.

Louryan, S. — Morphogenèse des osselets de l'oreille moyenne chez l'embryon de souris. I. Aspects morphologiques. *Arch. Biol.* (Bruxelles), *97:* 317-337, 1986.

Louryan, S. — Morphogenèse des osselets de l'oreille moyenne chez l'embryon de souris. II. Etude de la chondrogenèse. *Arch. Biol.* (Bruxelles), *99:* 453-463, 1988.

Louryan, S. — Développement des ébauches squelettiques du complexe mandibulo-otique chez *Mabuia megalura* (Lacertilia: scincidae). *Ann. Soc. roy. Zool. Belg., 119:* 49-59, 1989.

Louryan, S. — Morphogenèse des ébauches musculaires branchiales chez l'embryon de souris: corrélations avec les observations recueillies chez le poulet, chez *Mabuia megalura (Lacertilia: scincidae)* et chez *Scyllium canicula (chondrichtyes: selachii). Eur. Arch. Biol., 101:* 65-75, 1990.

Mallinger, R., Geleff, S., Bock, P. — Histochemistry of glycosaminoglycans in cartilage ground substance. *Histochem., 85:* 121-127, 1986.

Meyer Kuhn, E., Babiarz, B.S., Lessard, J.L., Zimmerman, E.F. — Palate morphogenesis. I. Immunological and ultrastructural analyses of mouse palate. *Teratol., 21:* 209-223, 1980.

Milaire, J. — Lectin histochemistry in normal and abnormal limb morphogenesis in the mouse. *Prog. Histochem. Cytochem.*, to be published.

Plendl, J., Schmahl, W. — *Dolichos biflorus* agglutinin: a marker of the developing olfactory system in the NMRI-mouse strain. *Anat. Embryol., 177:* 459-464, 1988.

Shiojiri, N., Katayama, H. — Development of *Dolichos biflorus* agglutinin (DBA) binding sites in the bile duct of the embryonic mouse liver. *Anat. Embryol. 178:* 15-20, 1988.

Slack, J.M.W. — Peanut lectin receptors in the early amphibian embryo: regional marker's for the study of embryonic induction. *Cell, 41:* 237-247, 1985.

Slavkin, H.C., Bringas, P., Camaron, J., Lebaron, R., Bavetta, L.A. — Epithelial and mesenchymal cell interactions with extracellular matrix material *in vitro. J. Embryol. exp. Morph., 23:* 395-405, 1969.

Slavkin, H.C., Bringas, P.J., Croissant, R., Bavetta, C.A. — Epithelial mesenchymal interactions during odontogenesis. II. Intercellular matrix vesicles. *Mech. Age Dev., 1:* 139-161, 1972.

Slavkin, H.C., Croissant, R., Bringas, P.Jr — Epithelial mesenchymal interaction during odontogenesis. III. A simple method for the isolation of matrix vesicles. *J. Cell. Biol., 53:* 841-849, 1972.

Slavkin, H.C., Bringas, P.Jr — Epithelial-mesenchyme interactions during odontogenesis. IV. Morphological evidence for direct heterotypic cell-cell contacts. *Devel. Biol., 50:* 428-442, 1976.

Slavkin, H.C., Mac Douglas, M., Zeichner-David, M., Oliver, P., Nakamura, M., Snead, M.L. — Molecular determinants of cranial neural crest-derived odontogenic ectomesenchyme during dentinogenesis. *Am. J. Med. Gen. suppl., 4:* 8-22, 1988.

Sorrell, J.M. — Ultrastructural localization of peanut lectin binding to extravascular white blood cells in the bone marrow of embryonic chicks. *Cell. tissue Res., 251:* 301-305, 1988.

Symons, N.B.B. — The development of the human mandibular joint. *J. Anat., 86:* 326-332, 1952.

Takahashi, H. — Changes in peanut lectin binding sites on the neurectoderm during neural tube formation in the bantam chick embryo. *Anat. Embryo, 178:* 353-358, 1988.

Thesleff, I., Stenman, S., Vaheri, A., Timpl, R. — Changes in the matrix proteins fibronectin and collagen during differentiation of mouse tooth germ. *Devel. Biol., 70:* 116-126, 1979.

Thesleff, I., Barrach, H.J., Froidart, J.M., Vaheri, A., Pratt, R.M., Martin, G.R. — Changes in the distribution of type IV collagen, laminin, proteoglycan and fibronectin during mouse tooth development. *Devel. Biol., 81:* 182-192, 1981.

Thesleff, I., Hurmerinta, K. — Tissue interactions in tooth development. *Differentiation, 18:* 75-88, 1981.

Thesleff, I., Mackie, E., Vainio, S., Chiquet-Ehrismann, R. — Changes in the distribution of tenascin during tooth development. *Development, 101:* 289-296, 1987.

Thesleff, I., Jackanen, M., Vainio, S., Bernfield, M. — Cell surface proteoglycan expression correlates with epithelial-mesenchymal interaction during tooth morphogenesis. *Devel. Biol., 129:* 565-572, 1988.

Wacker, R.A. — The use of lectins in histology and histopathology. A review. In Bog-Hansen T.C., Freed D.L.J. (eds): Lectins: biology, biochemistry, Clinical biochemistry vol. 6. St Louis Sigma, 591-600, 1988.

Webster, E.H., Uknis, M.E. — Transient appearance of and regional differences in apical cell surface materials during early morphogenesis of the chicken lens. *Histochem. J., 19:* 203-209, 1987.

Welim, H.B., Thies, M., Herken, R. — Appearance of lectin-binding sites during vascularization of the primordium of the central nervous system in 10 to 12-day-old mouse embryos, 1989. *Cell. Tissue Res., 255:* 627-630.

Widelec, J. — Histochemical changes observed during the development of the secondary palate of mouse embryos. *Arch. Biol., 97*suppl.*:* 132, 1986.

Wu, T.C., Wan, Y.J., Damjanov, I. — Fluorescein conjugated *Bandeiraea simplicifolia* lectin and trophoblastic differentiation in the mouse embryo. *Differentiation, 24:* 55-59, 1983.

Xu, Z., Parker, S.B., Minkoff, R. — Distribution of type IV collagen, laminin and fibronectin during maxillary process formation in the chick embryo. *Am. J. Anat., 187:* 232-246, 1990.

Zimmerman, E.F., Wee, E.L. — Role of neurotransmitters in palate development. In Moscona A.A., Monroy. A. (eds): Palate development: normal and abnormal cellular and molecular aspects. Orlando, Academic Press, pp. 37-63, 1984.

Address for correspondence: S. Louryan, Laboratoire d'Anatomie et Embryologie Humaines (C.P. 619), Faculté de Médecine, Université Libre de Bruxelles, bât. G 1, 808 route de Lennik, 1070 Bruxelles, Belgium.