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The changes in lectin activity during the development of embryonic chick skin

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SUMMARY

Changes in lectin activity during development of embryonic chick skin were studied. In the dorsal skin of the chick embryo in which feathers were formed, lectin activity first increased, during the period of dermal condensation, and then it decreased during the development of feathers. A similar change in lectin activity was also found in the anterior shank skin, the prospective scale region of the chick embryo. The embryonic cornea, in which no mesenchymal condensation took place, had lectin activity and did not show any developmental changes in lectin activity. Apteria regions of the dorsal skin, experimentally formed by treatment with hydrocortisone, gave low lectin activity. The lectin found in the embryonic skin showed specificity for lactose. The relationship found between lectin activity and dermal condensation in the embryonic chick skin is discussed.

INTRODUCTION

In recent years lectins have been found in a wide variety of animal organs. The lectin studied in most detail is the hepatic lectin which participates in the clearance of glycoproteins from plasma (Ashwell & Morell, 1977). Some lectins have been reported to mediate incorporation of glycoproteins into lysosomes and macrophages (Neufeld, Lim & Shapiro, 1975; Kaplan, Fisher & Sly, 1978; Stahl, Rodman, Miller & Schlesinger, 1978; Kawasaki, Etoh & Yamashina, 1978).

Lectins have also been found in various embryonic chick tissues, such as pectoral muscle (Nowak, Haywood & Barondes, 1976), liver, brain, heart (Kobiler & Barondes, 1977), retina and spinal cord (Eisenbarth, Ruffolo, Jr., Walsh & Nirenberg, 1978). These embryonic lectins have two common characteristics. Firstly, they are developmentally regulated. Secondly, they have a high specificity for lactose and thiodigalactoside. Their roles in development, however, have not been well defined as yet.

Mesenchymal condensations commonly occur in embryonic organs (kidney, pancreas, lung, salivary gland, etc.) and their morphogenetic roles have been demonstrated (Sengel, 1971), although the mechanism of formation of these condensations has not been elucidated. In the developing skin, mesenchymal

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condensation takes place as condensation of dermal cells. Dermal condensations in embryonic skin results in feather formation in the dorsal skin and in scale formation in the anterior shank skin. Within the dermal condensation in the prospective feathered region, for instance, the cellular density increases rapidly from 2·60 nuclei/1000 μm^3 to 5·52 nuclei/1000 μm^3 (Wessells, 1965). No conclusive answer has yet been given whether the dermal condensation arises through differential mitosis or cell migration (Sengel, 1976).

The present study was initiated in order to analyse the formation of the dermal condensation from the standpoint of intercellular adhesion. In this report, I will show that the lectin activity found in extracts of embryonic chick skin changes during skin development, demonstrating that the formation of the dermal condensation is accompanied by a high lectin activity. The embryonic skin lectin is inhibited specifically by lactose.

MATERIALS AND METHODS

Fertilized white Leghorn chicken eggs were obtained from a local hatchery and incubated at 37 °C until use. The dorsal skin, cornea and anterior shank skin at various stages were rapidly dissected out from embryos. Lectins from embryonic tissues were extracted according to the method of Nowak, Kobiler, Roel & Barondes (1977). Embryonic tissues were homogenized for 4 min in a Vertis homogenizer at 4 °C in nine volumes of MEPBS containing 0·3 M lactose (MEPBS: 75 mM NaCl, 75 mM Na_2HPO_4, 75 mM KH_2PO_4, 4 mM β-mercaptoethanol and 2 mM ethylenediaminetetraacetic acid, pH 7·2). The homogenate was centrifuged at 100000 *g* for 1 h. The supernatant was dialysed against MEPBS to assay lectin activity.

Lectin activity was assayed with Pronase P (Kaken-Kagaku)-treated, glutaraldehyde-fixed rabbit erythrocytes according to the method of Nowak, *et al.* (1976). Lectin activity was determined in a microtiter U plate (Limbro), using serial two-fold dilutions of the extract in MEPBS. Protein was determined by a modification of the method of Lowry, Rosebrough, Farr & Randall (1951) using crystalline bovine serum albumin as a standard. Results of the lectin assay are expressed as units per mg of protein in the extract added to the incubation medium. One unit is defined as the highest dilution of the extract which caused the agglutination of erythrocytes. A single batch of glutaraldehyde-fixed erythrocytes was used for all assays to reduce variability in assay conditions.

Treatment of the chick embryo with hydrocortisone was carried out essentially following the method of Sengel and Zvüst (1968). A dose of 0·1 mg hydrocortisone phosphate (dissolved in 0·1 ml phosphate-buffered saline) per embryo was dropped onto the chorionic membrane of 5½-day-old embryos through a window made in the shell. Dorsal skin was dissected when the embryo had reached 10 days of incubation. The dorsal skin of hydrocortisone-treated embryos was separated into the pteryla and apteria regions under a dissecting

Table 1. *Effect of reagents on extraction of lectin*

Addition to MEPBS	Lactin Activity (units/mg protein)
None	130
Triton X-100 0·5%	180
Nonidet P-40 0·5%	140
Sucrose 0·1 M	153
Lactose 0·1 M	350
Lactose 0·3 M	550

Dorsal skin from 10-day-old chick embryos was homogenized in nine volumes of MEPBS containing the indicated concentration of reagents. The homogenates were centrifuged at 100000*g* for 1 h and the supernatant was dialysed against MEPBS before determination of lectin activity and protein. Lectin activity was measured with Pronase P-treated fixed rabbit erythrocytes. Lectin activity is expressed by units divided by milligrams of protein in each extract.

microscope. These regions of the dorsal skin were separately homogenized and served for the assay of lectin activities.

Histological procedures were as follows. Tissue samples were fixed in freshly prepared Bouin's solution overnight at room temperature. Material was dehydrated in a graded butanol–ethanol series and embedded in Paraplast (Sherwood Medical Industries). Tissue samples were serially sectioned at 5 μm and stained according to a modified method of Mallory's trichrome staining using fuchsin, aniline blue and orange G (Everett & William, 1973).

RESULTS

1. *Effect of reagents on initial extraction of lectin*

Extracts of the dorsal skin made with MEPBS showed only low lectin activity (Table 1). To extract the lectin more efficiently from the dorsal skin, the effect of several reagents on the initial extraction of lectin was examined on the dorsal skin of a 10-day-old embryo. Addition of detergents, such as Triton X-100 and Nonidet P-40 to the homogenizing medium, did not improve the degree of lectin extraction as compared with that by MEPBS alone. Addition of sucrose to the homogenizing medium did not enhance the lectin activity. Addition of 0·1 M lactose increased the activity of the lectin in the extracts about three-fold as compared with that in the extracts made in the absence of the reagent. Raising the lactose concentration to 0·3 M further increased the activity of the lectin extracted. Therefore 0·3 M lactose was added to the homogenizing medium in the following series of experiments.

2. *Lectin activity from embryonic dorsal skin*

The change of lectin activity on extracts of dorsal skin during feather development is shown in Fig. 1. The lectin activity of the extract of day 4 was

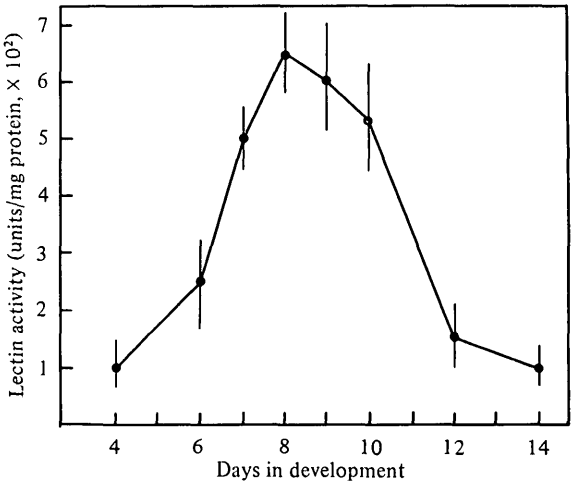

Fig. 1. Lectin activities in extracts from embryonic chick dorsal skin at various stages of development, as measured with Pronase P-treated fixed erythrocytes. Lectin activity is expressed as units divided by milligrams of protein in each extract. Each point is the mean (±S.E.M.) of separate determinations made with three to five different extracts.

about 100 units/mg protein. On day 6, although the dermal condensation was still not conspicuous in the dorsal skin when compared to that on day 8 (Figs. 2*b* and *c*; Wessells, 1965), the lectin activity began to increase. Thereafter, as the dermal condensation advanced (Figs. 2*c* and *d*), the lectin activity rapidly increased and on day 8 it reached a level five or six times that on day 4. After day 10, there was a decline in lectin activity though the reason is not obvious at present. On day 14, lectin activity fell to its basic level of day 4 (approximately 100 to 150 units/mg protein). These results suggest that formation of the dermal condensation is accompanied by developmental alteration of lectin activity.

3. *Lectin activities in other skin systems*

To elucidate more clearly the nature of the increase in lectin activity at the time when the dermal condensation occurred, change of lectin activities in other skin systems was examined. The skin derivatives examined were embryonic cornea, embryonic anterior shank skin and hydrocortisone-treated dorsal skin.

(*a*) *Embryonic cornea*

In embryonic cornea, a skin derivative, there was little change of lectin activity from day 5 to 13 and activity remained low, at the basal level found in the dorsal skin of day 4 (Fig. 3). The stroma of the cornea at this stage did not show any condensation but was invaded by mesenchymal cells from the limbic region. The mesenchymal cells appeared to be distributed equally in all regions of the stroma (Nuttall, 1976). In clear contrast to the dorsal skin, where dermal

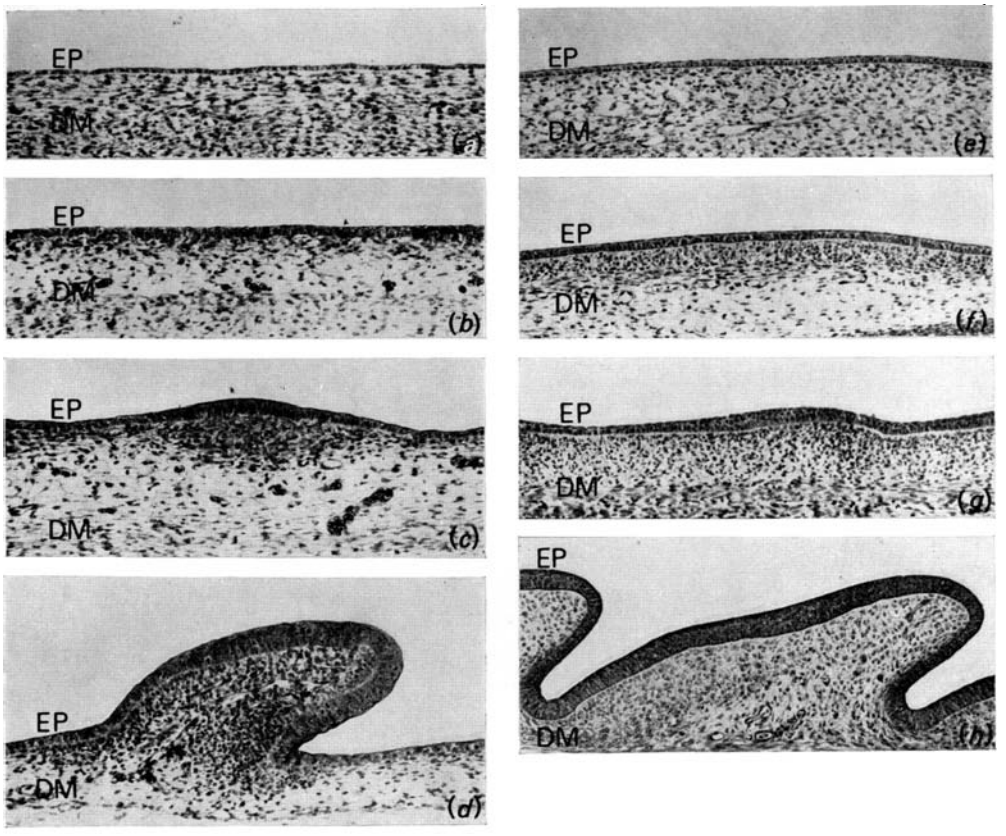

Fig. 2. Development of feathers in the dorsal skin (*a–d*), and scales in the anterior shank skin (*e–h*). (*a*) 5-day-old embryo; no dermal condensation. (*b*) 6-day-old embryo; a slight dermal condensation. (*c*) 8-day-old embryo; definitive dermal condensation. (*d*) 10-day-old embryo. (*e*) 7-day-old embryo; no dermal condensation. (*f*) 8-day-old embryo; a slight dermal condensation. (*g*) 9-day-old embryo; definitive dermal condensation. (*h*) 11-day-old embryo. × 110. EP, epidermis; DM, dermis.

condensation took place when the feather developed, no increase in lectin activity was noted in the cornea stroma where no stromal condensation occurred.

(*b*) *Embryonic anterior shank skin*

While the dorsal skin of the chick embryo forms feathers, the anterior shank skin forms scales following dermal condensation, which is initiated on day 8 (Figs. 2*e–h*). This observation is supported by analysis of cell proliferation kinetics in the early stages of scale development (Tanaka, 1979). As shown in Fig. 4, the pattern of lectin activity in the extract of anterior shank skin was surprisingly similar to that found in dorsal skin. Coinciding with the period when the formation of the dermal condensation advanced, a rapid increase of lectin activity was observed in anterior shank skin (Figs. 2*g* and *h*). Change in lectin

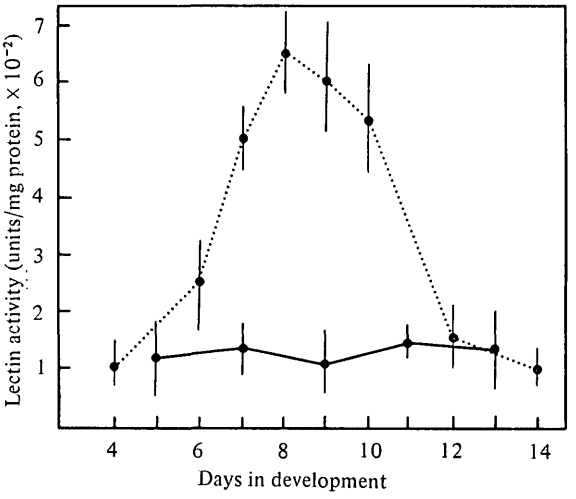

Fig. 3. Lectin activities in extracts from embryonic chick cornea at various stages of development, as measured with Pronase P-treated fixed erythrocytes. Lectin activity is expressed as units divided by milligrams of protein in each extract. Each point is the mean (± S.E.M.) of separate determinations made with three to five different extracts. ●—●, Cornea; ● ··· ●, dorsal skin.

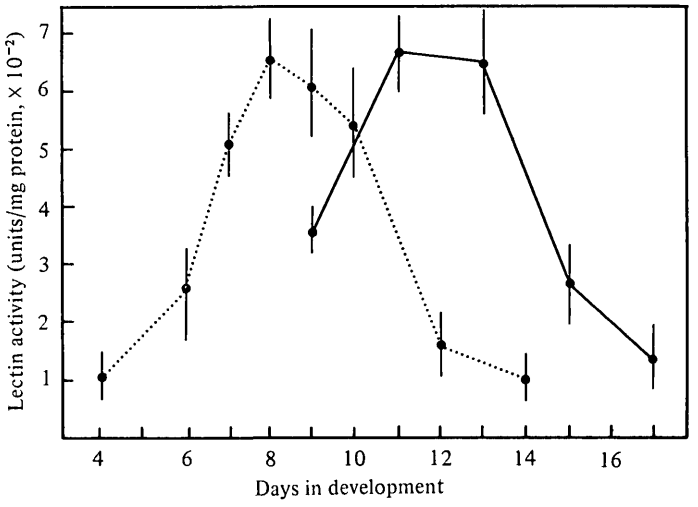

Fig. 4. Lectin activities in extracts from embryonic chick anterior shank skin at various stages of development, as measured with Pronase P-treated fixed erythrocytes. Lectin activity is expressed as units divided by milligrams of protein in each extract. Each point is the mean (± S.E.M.) of separate determinations made with three to five different extracts. ●—●, Anterior shank skin; ● ··· ●, dorsal skin.

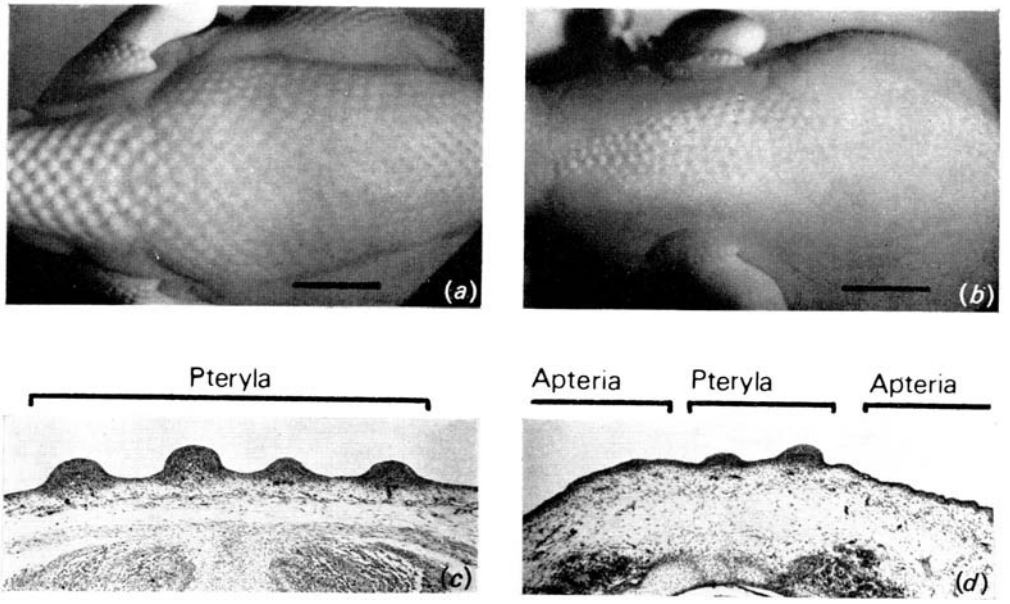

Fig. 5. Development of feather germs in the dorsal skin of normal and hydrocortisone-treated embryos. (*a*) The dorsal view of a 10-day-old normal embryo. Bar = 2 mm. (*b*) The dorsal view of a 10-day-old hydrocortisone-treated embryo. Bar = 2 mm. (*c*) Cross section of the dorsal skin of a normal embryo. ×35. (*d*) Cross section of the dorsal skin of a hydrocortisone-treated embryo. ×35.

activity in the dorsal skin preceded by 2 to 3 days that found in the anterior shank skin. The temporal difference found in lectin activity between feather and scale formation, corresponds with the observation that while dermal condensation in the dorsal skin starts at about day 6, in the anterior shank it occurs at about day 8 (Figs. 2*b* and *f*). After reaching maximal activity, decline in the lectin activity was found after day 13 in the anterior shank skin.

(*c*) *Dorsal skin treated with hydrocortisone*

Suppression of feather formation has been known to be induced by hydrocortisone treatment (Moscona & Karnofsky, 1960; Sengel & Zvüst, 1968). Figures 5*a* and *b* show the dorsal regions of normal and hydrocortisone-treated 10-day-old embryos. Hydrocortisone-treated embryos had only a few small feather germs in the spinal region compared with the numerous large feather germs formed in the dorsal skin of a normal embryo (normal pteryla, Fig. 5(*c*). While in the spinal region of the dorsal skin of hydrocortisone-treated embryos the formation of feather germs was partially inhibited (experimental pteryla), in the lateral region the formation of feather germs was completely inhibited (experimental apteria) (Fig. 5*d*). The lectin activities found in three regions, normal pteryla, experimental pteryla and experimental apteria, are shown in Table 2. The lectin activity in experimental pteryla was three fifths of the lectin

Table 2. *Lectin activities of hydrocortisone-treated embryonic skin*

Area	Lectin activity (units/mg protein)
Normal Pteryla	550±98
Experimental Pteryla	304±52
Experimental Apteria	98±35

Each extract was tested with Pronase P-treated erythrocytes. Lectin activity is expressed as units divided by milligrams of protein in each extract. Results are the average of determinations on three separate extracts.

Table 3. *Concentrations (mM) of saccharides for half maximal inhibition of the activities of lectins in dorsal and anterior shank skin*

Saccharides	Dorsal skin (10d)	Anterior shank skin (11d)
Lactose	0·29	0·20
Melibiose	25·0	25·0
D-Galactose	25·0	12·5
Methyl α-D-galactoside	6·25	6·25
Methyl β-D-galactoside	25·0	25·0
N-Acetyl β-D-galactosamine	12·5	6·25
N-Acetyl β-D-glucosamine	> 50	> 50
D-Mannose	> 50	> 50
L-Fucose	> 50	> 50

A range of saccharide concentrations was tested against a constant concentration of each extract. The saccharide concentration that inhibited lectin activity by 50% was determined. Results are the average of determinations on separate extracts.

activity in normal pteryla. Furthermore, lectin activity in experimental apteria was found to be only one fifth of that of normal pteryla, and corresponded to the basal activity in the dorsal skin of day-4 controls where no feather germs had yet been formed.

Results obtained in different skin systems clearly demonstrate that the increase of lectin activities correlates with the occurrence of dermal condensation.

4. *Effect of saccharides on lectin activities of dorsal and anterior shank skin*

Table 3 summarizes the concentrations of various saccharides which caused half maximal inhibition of the lectin activities of dorsal and anterior shank skin. Lactose was the most potent inhibitor of both lectins, suggesting that the active sites of the lectins in the skin have a relatively high affinity for galactose in this glycosidic linkage. The other saccharides were much less potent. The dorsal and anterior shank skin lectins closely resembled one another, when judged by their inhibition by various saccharides.

DISCUSSION

In the present study the lectin activities of extracts of the dorsal and anterior shank skin were found to increase when the dermal condensation began to occur. It should be noticed that lectin activity began to rise before the dermal condensation in the dorsal skin became conspicuously recognizable by histological means. On the other hand the lectin activity remained low (the basal activity of the dorsal skin of day 4) throughout the development of cornea, in which no mesenchymal condensation occurred. These results suggest the possibility that the embryonic skin lectin participates in the formation of the dermal condensation. This was further supported by the hydrocortisone experiment. When the dermal condensation in the dorsal skin is fully suppressed by hydrocortisone treatment, the lectin activity in the apteria region of the dorsal skin was found to be one fifth that of normal dorsal skin, and corresponded to the basal activity in the dorsal skin on day 4.

After reaching maximal activities, decline in lectin activities was found in both the dorsal and anterior shank skin. Measurement of total lectin activity in a constant number of scale ridges (20 scale ridges) showed that total activity also gradually decreased after day 13 (data not shown). The increase and subsequent decrease of both specific and total lectin activities are characteristic for the early morphogenetic phase of feathers and scales.

In the present study, whole skin, without separation of epidermal and dermal elements, was used for the determination of lectin activity. In a small series of experiments, in which the anterior shank skin of a 13-day-old embryo was separated into the epidermal and dermal layers by EDTA-treatment, lectin activity characteristic of whole embryonic skin was almost completely recovered from the dermal layer (specific activity: 680 ± 121 units/mg protein). Although the lectin activity of the epidermal layer could not be measured reliably because of the insufficient quantity of epidermal cells available, the increase of lectin activity in the whole embryonic skin at the time of dermal condensation, reflects at least the increase found in the dermal layer.

The dermal cells are tightly packed in the area of condensation as compared with the non-condensation area (Wessells, 1965); high mutual adhesion among dermal cells is thus expected within the dermal condensation. Though the mechanism of intercellular adhesion *in vivo* is not understood, endogenous lectin is one of the likely candidates for the molecule responsible for intercellular adhesion. In cellular slime molds, for instance, the lectins called 'discoidin' and 'pallidin', specific for galactose, appear at about the time when individual cells seek each other out to aggregate into an organized mass (Simpson, Rosen & Barondes, 1974; Barondes & Rosen, 1976). The embryonic skin lectin might also participate in intercellular adhesion *in vivo* at the formation of the dermal condensation. As an initial step to test the possibility that the observed changes of lectin activity are actually an integral part of the mechanism of the dermal

condensation, the distribution of the embryonic skin lectin during the process of the dermal condensation is currently being examined by histochemical methods.

Embryonic lectin found in the skin in the present study was specific for lactose. The majority of lectins so far found, have been reported to be specific for lactose and thiodigalactoside (Nowak, *et al.* 1976; Kobiler & Barondes, 1977; Eisenbarth, *et al.* 1978). At present, however, it is not possible to say that these lectins are identical when judged by the results obtained by the inhibition experiment in which mono- and di-saccharides are used. It has not yet been shown, *in vivo*, which molecules these lectins may recognize. According to the exploratory experiment, the skin lectin described in this report is inhibited by the glycopeptide of collagen type prepared from the digest of dorsal skin by pronase. Stuart & Moscona (1967) reported that the dermal cells might slide toward the center of the mass along a lattice of collagen fibers. It is a testable possibility that dermal cells may bind to collagen through the embryonic skin lectin.

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REFERENCES

Ashwell, G. & Morell, A. G. (1977). Membrane glycoproteins and recognition phenomena. *Trend. Biochem.* **2**, 76–78.

Barondes, S. H. & Rosen, S. D. (1976). Cell surface carbohydrate-binding proteins: Role in cell recognition. In *Neuronal Recognition* (ed. S. H. Barondes), pp. 331–356. New York: Plenum Press.

Eisenbarth, G. S., Ruffolo, Jr., R. R., Walsh, F. S. & Nirenberg, M. (1978). Lactose sensitive lectin of chick retina and spinal cord. *Biochem. biophys. Res. Commun.* **83**, 1246–1252.

Everett, M. M. & William, A. M. (1973). Adaptation of Mallory's trichrome stain to embryonic and fetal material. *Stain Technology* **48**, 5–8.

Kaplan, A., Fisher, D. & Sly, W. S. (1978). Correlation of structural features of phosphomannans with their ability to inhibit pinocytosis of human β-glucronidase by human fibroblast. *J. biol. Chem.* **253**, 647–650.

Kawasaki, T., Etoh, R. & Yamashina, I. (1978). Isolation and characterization of a mannan binding protein from rabbit liver. *Biochem. biophys. Res. Commun.* **81**, 1018–1024.

Kobiler, D. & Barondes, S. H. (1977). Lectin activity from embryonic chick brain, heart and liver. Changes with development. *Devl Biol.* **60**, 326–330.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *J. biol. Chem.* **193**, 265–275.

Moscona M. H. & Karnofsky D. A. (1960). Cortisone induced modifications in the development of the chick embryo. *Endocrinology*, **66**, 533–549.

Neufeld, E. F., Lim, T. W. & Shapiro, L. J. (1975). Inherited disorders of lysosomal metabolism. *A. Rev. Biochem.* **44**, 357–376.

Nowak, T. P., Haywood, P. L. & Barondes, S. H. (1976). Developmentally regulated lectin in embryonic chick muscle and a myogenic cell line. *Biochem. biophys. Res. Commun.* **68**, 650–657.

Nowak, T. P., Kobiler, D., Roel, L. E. & Barondes, S. H. (1977). Developmentally regulated lectin from embryonic chick pectral muscle. *J. biol. Chem.* **252**, 6026–6030.

NUTTALL, R. P. (1976). DNA synthesis during the development of the chick cornea. *J. exp. Zool.* **198**, 193–208.

SENGEL, P. (1971). The organogenesis and arrangement of cutaneous appendages in birds. In *Advances in Morphogenesis.* (ed. M. Abercrombie, J. Brachet & T. T. King), pp. 181–230. New York: Academic Press.

SENGEL, P. (1976). *Morphogenesis of Skin.* London: Cambridge University Press.

SENGEL, P. & ZVÜST, B. (1968). Malformations du plumage obtenues par l'injection d'hydrocortisone á l'embryon de Poulet. *C. r. hebd. Séanc. Acad. Sci., Paris* **267**, 1304–1307.

SIMPSON, D. L., ROSEN, S. D. & BARONDES, S. H. (1974). Discoidin: A developmentally-regulated carbohydrate-binding protein from Dictyostelium discoideum, purification and characterization. *Biochemistry.* **13**, 3487–3493.

STAHL, P. D., RODMAN, J. S., MILLER, M. J. & SCHLESINGER, P. H. (1978). Evidence for receptor-mediated binding of glycoproteins, glycoconjugates, and lysosomal glycosidases by alveolar macrophages. *Proc. natn. Acad. Sci., U.S.A.* **75**, 1399–1403.

STUART, E. S. & MOSCONA, A. A. (1967). Embryonic morphogenesis: role of fibrous lattice in the development of feathers and feather patterns. *Science* **157**, 947–948.

TANAKA, S. (1979). Unpublished data.

WESSELLS, N. K. (1965). Morphology and proliferation during early feather development. *Devl Biol.* **12**, 131–153.

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