# Distribution of endogenous β-galactoside-specific lectin, fibronectin and type I and III collagens during dermal condensation in chick embryos

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## SUMMARY

The distribution of endogenous β-galactoside-specific lectin, fibronectin, type I and III collagens was studied by the indirect immunofluorescence method during the formation of dermal condensation in the feathered region (dorsal skin) of a chick embryo. Endogenous β-galactoside specific lectin was concentrated in the condensed dermal region, coinciding with the formation of condensation of dermal cells. It was also detected in epidermal placodes. Fibronectin was weakly stained in dermis prior to the formation of dermal condensation but not in epidermis. Condensation of dermal cells resulted in the formation of thicker fibrils of fibronectin in the condensed region. Distribution of type I collagen was found to be very different from that of endogenous β-galactoside-specific lectin and fibronectin. Type I collagen in dermis decreased along with the formation of dermal condensation. Epidermis had no type I collagen. Type III collagen was not found in dorsal skin. Relationship found between these distribution patterns and dermal condensation in the embryonic chick skin is discussed.

## INTRODUCTION

Mesenchymal condensations among a variety of embryonic organs (kidney, pancreas, lung, salivary glands, etc.) are indispensable steps for their organogenesis. In the development of skin derivatives, such as the feather, scale and hair, mesenchymal condensation takes place as a condensation of dermal cells. The mechanism behind the formation of dermal condensation has not yet been elucidated fully. The dermal cells of the dorsal skin of a chick embryo are tightly packed in the area of condensation, where an individual feather emerges, as compared with those of non-condensed areas (Wessells, 1965). Although this high density of dermal cells found locally in the feather papilla may arise, at least in part, through cell proliferation (Wessells, 1965), cell migration also could be involved significantly (Stuart & Moscona, 1967; Stuart, Garber &

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Moscona, 1973). The reason why these dermal cells settle and aggregate themselves into a dermal papilla, however, has not yet been studied. The settlement and aggregation of dermal cells can depend on the adhesiveness inherent to individual dermal cells. Though the characteristics of intercellular adhesion *in vivo* have not been hitherto examined, cell surface as well as intercellular materials are thought to play a major role in cell adhesion.

Developmentally regulated endogenous lectins, found in a variety of embryonic organs, can be a potential candidate for cellular adhesion occurring in embryonic development. Previous study has shown that the activity of endogenous $\beta$-galactoside-specific lectin increased during dermal condensation, in the dorsal skin of a chick embryo where feathers were formed (Kitamura, 1980a). The results obtained suggested a possibility that endogenous $\beta$-galactoside-specific lectin found in the dorsal skin play a role in the formation of dermal condensation. This report first deals with the distribution of the endogenous lectin in the development of dermal condensation.

The contribution of collagenous elements in the development of feather germs of a chick embryo has been described by many investigators (Stuart & Moscona, 1967; Wessells & Evans, 1968; Ede, Hinchliffe & Mees, 1971; Stuart et al. 1973). Dermal cells which participate in the formation of dermal condensation have been thought to migrate along collagenous tracts (Stuart & Moscona, 1967; Stuart et al. 1973). However the role of collagens during the phase in which the dermal cells aggregate themselves into a dermal papilla has not yet been elucidated. The distribution of two types of collagens in the development of dermal condensation will be described and their distribution patterns will be compared with those of fibronectin.

Fibronectin, known as a cell surface protein, is a major component of connective tissue matrix and basement membrane (Linder, Vaheri, Ruoslahti & Wartiovaara, 1975; Stenman & Vaheri, 1978). A role has been proposed for fibronectin in cell–cell and cell–matrix interactions which lead to morphogenesis (Kurkinen et al. 1979; Critchley, England, Wakely & Hynes, 1979; Dessau, von der Mark, von der Mark & Fischer, 1980). The results in the present study show that, with the advancement of morphogenesis, significant changes take place among the distribution patterns of the above-mentioned three molecules, the endogenous lectin, collagens and fibronectin. A part of this report was presented at the 13th Annual Meeting of the Japanese Society of Developmental Biologists (Kitamura, 1980b).

MATERIALS AND METHODS

*Biological.* Fertilized eggs of White Leghorn chicken were obtained from a local hatchery and incubated at 37 °C until use.

*Histologic techniques.* For immunofluorescence staining, specimens of the embryos were processed as recommended by Sainte-Marie (1962). Dorsal skin
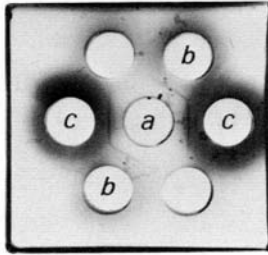
Fig. 1. Double gel diffusion of immune gamma globulin, purified endogenous lectin and skin extract. The contents of the wells are as follows. (*a*) Immune gamma globulin; (*b*) purified endogenous lectin; (*c*) concentrated extract from dorsal skin of 10-day-old embryos. Non-immune gamma globulin shows no reaction with this antigen.

was dissected out from embryos at various stages and transferred onto a Millipore filter. The tissue samples were fixed with 3·5% formaldehyde diluted with phosphate-buffered saline (PBS) for 45 min at room temperature. They were dehydrated in a graded buthanol-ethanol series and embedded in Paraplast (Scherwood Medical Industries). They were serially sectioned at 5 $\mu$m. Deparaffinization was achieved by two consecutive baths in cold xylene. Xylene was removed by a graded ethanol series. Deparaffinized sections were washed in PBS and Millipore filter was removed from specimens.

*Purification and antibody preparation of endogenous $\beta$-galactoside-specific lectin.* Endogenous $\beta$-galactoside-specific lectin (abbreviation: endogenous lectin) from 10-day-old embryos was purified by affinity chromatography on lactoside-derivatized Sepharose by a modification of the method of Nowak, Kobiler, Roel & Barondes (1977). Extracts were prepared through homogenization of the acetone powder of whole embryos, in MEPBS containing 0·3 M lactose (MEPBS: 75 mM-NaCl, 75 mM-Na$_2$HPO$_4$, 75 mM-KH$_2$PO$_4$, 4 mM $\beta$-mercaptoethanol and 2 mM ethylenediaminetetraacetic acid, pH 7·2). The extract was centrifuged at 33 500 g for 30 min and the supernatant was dialyzed against MEPBS. The dialysate was slowly added to the *p*-aminophenyl-$\alpha$-D-lactoside-Sepharose column. The column was washed successively with MEPBS, then with 0·3 M sucrose in MEPBS until no further protein was leaving the column. The endogenous lectin was then eluted with 0·3 M lactose in MEPBS. This procedure yielded highly purified material which gave a single band (apparent mol. wt. 16 500 daltons) in polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate and 2-mercaptoethanol. Antibody against the endogenous lectin was prepared in rabbits according to the method of Beyer, Tokuyasu & Barondes (1979). Anti IgG fraction was prepared by precipitation method in 33% saturated ammonium sulphate. By gel diffusion, the purified endogenous lectin and concentrated extract of the dorsal skin of 10-day-old embryos gave a single precipitin band with this anti IgG (see Fig. 1). When, in immunofluorescence of the embryonic chick skin specimens, the anti IgG was

substituted either with normal rabbit IgG or with the anti IgG pretreated with the purified endogenous lectin (25 µg/ml), no fluorescence was detected.

*Purification and antibody preparation of fibronectin.* Fibronectin was purified from human serum as described by Engvall, Ruoslahti & Miller (1978) using gelatin insolubilized on Sepharose. The purity of the purified fibronectin was controlled with sodium dodecyl sulphate-polyacrylamide gel electrophoresis, in which a single band was detected (Stenman, Wartiovaara & Vaheri, 1977). Rabbits were immunized by intramuscular injection of 25 µg of fibronectin in Freund's complete adjuvant and the injections were repeated four times at a weekly interval. Serum containing antibody was harvested 10 days after the final injection and anti IgG fraction was obtained. The anti IgG gave a single precipitin line against the purified antigen and the extract of embryonic chick fibroblasts. In control experiments, the anti IgG was substituted with normal rabbit IgG, with the anti IgG pretreated with purified plasma fibronectin (60 µg/ml) or with PBS.

*Purification and antibody preparation of type I and III collagens.* Type I collagen was purified from cranial bones of 17-day-old chick embryos and type III collagen from the back skin of a new-born calf (von der Mark, von der Mark & Gay, 1976). Antibodies against type I and III collagens were prepared in rabbits as described by Becker, Nowack, Gay & Timpl (1976). Their specificity was checked by immunoadsorption (Nowack *et al.* 1976). Each antibody was not inhibited by another type of collagen in passive haemagglutination. The theoretical possibility that fibronectin and collagenous proteins would cross-react immunologically has been ruled out by passive haemagglutination.

*Immunofluorescence staining.* Deparaffinized specimens were separately treated for 30 min at room temperature with the four different antibodies diluted in PBS. Washing with PBS for 5 min was repeated three times, and fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit gamma globulin, diluted 1:20 in PBS, was applied to the sections for 30 min at room temperature. After three washings for 5 min, each with PBS, the stained sample was mounted using buffered glycerol.

*Fluorescent microscopy.* Stained sections were examined under a Zeiss standard microscope with the IV FI epi-fluorescence condenser (Carl Zeiss, Oberkochen, Germany) and a super-pressure mercury lamp (HBO 50 W). For photography, Kodak TRI-X Pan film was used. Fields were first photographed with fluorescence microscopy, then the same field was photographed with Nomarski differential interference contrast microscopy.

RESULTS

1. *Distribution of the endogenous lectin, fibronectin and type I and III collagens during dermal condensation*

For the sake of a description to follow, an account of normal development of dorsal skin will be presented, though it was given by several investigators (Wessells, 1965; Ede *et al.* 1971; Sengel, 1971; Stuart *et al.* 1973; Sengel, 1976). In the dorsal skin of a 6-day-old embryo, the earliest stage examined, epidermis consists of the primary periderm and basal layer and dermis is loosely packed by dermal cells (Fig. 2*a*). In a 7-day-old embryo, epidermal placodes are formed at the positions of presumptive feathers, which are characterized by vertical elongation of epidermal cells (Fig. 2*b*). Dermal cells beneath each epidermal placode become condensed (Fig. 2*b*). In an 8-day-old embryo, the dermal condensations are now very distinct and by 9 days, the outgrowth of feather papilla is found to be formed (Figs. 2*c*, *d*).

(*a*) *The endogenous lectin*. Little or no endogenous lectin was detected in the dermis of a 6-day-old embryo, although the epidermis was stained with anti-endogenous lectin (Fig. 2*e*). As dermal cells condensed in a 7-day-old embryo, the endogenous lectin was preferentially found in the condensed region of dermal cells (Fig. 2*f*). Epidermis was also stained with anti-endogenous lectin (Fig. 2*f*). As the region of dermal condensation was enlarged, the condensed dermal cells were strongly stained, whereas non-condensed dermal cells had only a very faint fluorescence on their cell bodies (Fig. 2*g*). Such a tendency became more conspicuous in the dermal condensations of a 9-day-old embryo (Fig. 2*h*).

(*b*) *Fibronectin*. Fibronectin was weakly stained in the entire region of dermis of a 6-day-old embryo but not in the epidermis (Fig. 3*a*). As dermal cells condensed, in a 7-day-old embryo, fibronectin began to concentrate (Fig. 3*b*). Basement membrane located on the condensed dermal cells also showed staining of fibronectin but epidermis had no fibronectin (Fig. 3*b*). The intensity of fluorescence by fibronectin had increased evenly throughout the dermal condensation as it enlarged (Fig. 3*c*). The fixation and staining procedure with anti-fibronectin used in this study has been known to stain cell surface-associated fibrillar fibronectin (Stenman *et al.* 1977). It was shown from an observation on frontal sections, cut in parallel with the body surface, that the fibronectin-specific fluorescence was more intensive in the fibrils which were found among the condensed dermal cells, than those in dermis surrounding condensations (Figs. 4*a*, *b*). Unlike the previous embryonic stages, the staining was not uniform within the region of dermal condensation of a 9-day-old embryo (Fig. 3*d*). The 'core' and 'periphery' of dermal condensation showed stronger staining by anti-fibronectin than the remaining regions of dermal condensation (Fig. 3*d*). While the basement membrane located on the 'periphery' of dermal condensation had an intensive staining by anti-fibronectin, the basement membrane in the top region of dermal condensation was stained weakly (Fig. 3*d*).
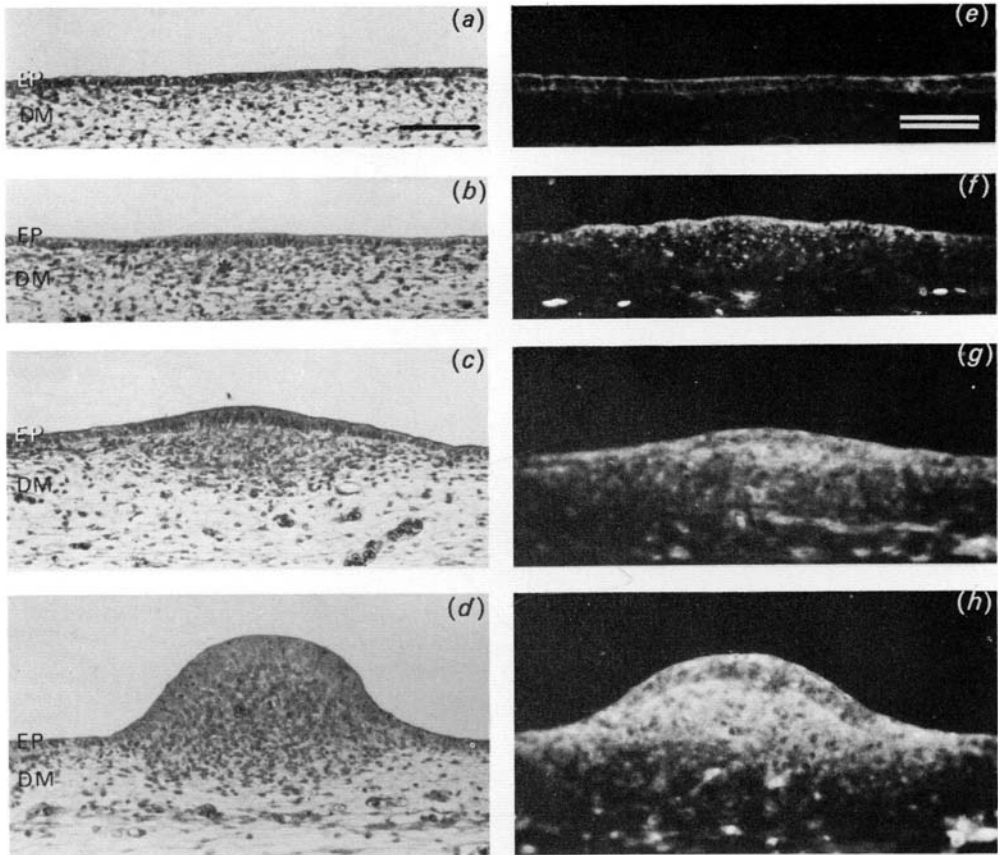
Fig. 2. (a–d) Development of dermal condensation in feathered regions of dorsal skin. Transverse sections stained with a modified method of Mallory's trichrome staining (Everett & William, 1973). (a) 6-day-old embryo; no dermal condensation. (b) 7-day-old embryo; a slight dermal condensation is indicated by (*). (c) 8-day-old embryo; definitive dermal condensation. (d) 9-day-old embryo. Bar = 67 μm. EP, epidermis; DM, dermis.

(e–h) Indirect immunofluorescence staining for endogenous lectin. Transverse sections. (e) 6-day-old embryo. Almost no endogenous lectin is detected in the dermis, whereas epidermis is stained with anti-endogenous lectin. (f) 7-day-old embryo. The presence of endogenous lectin is shown by an intense staining in the region of dermal condensation as opposed to the very faint staining seen in the noncondensed region. Epidermis is stained with anti-endogenous lectin. (g) 8-day-old embryo. (h) 9-day-old embryo. In both figures (g) and (h), the regions of dermal condensation and the epidermis are intensely stained with anti-endogenous lectin. Bar = 56 μm.

(c) *Type I collagen.* In a 6-day-old embryo, staining of type I collagen was very intense throughout the dermis as compared with that of the endogenous lectin and fibronectin (Fig. 3e). Type I collagen was not, however, detected in the epidermis (Fig. 3e). In a 7-day-old embryo, the distribution of type I collagen had changed considerably. Making a clear contrast with the endogenous lectin and fibronectin, the intensity of staining of type I collagen decreased in
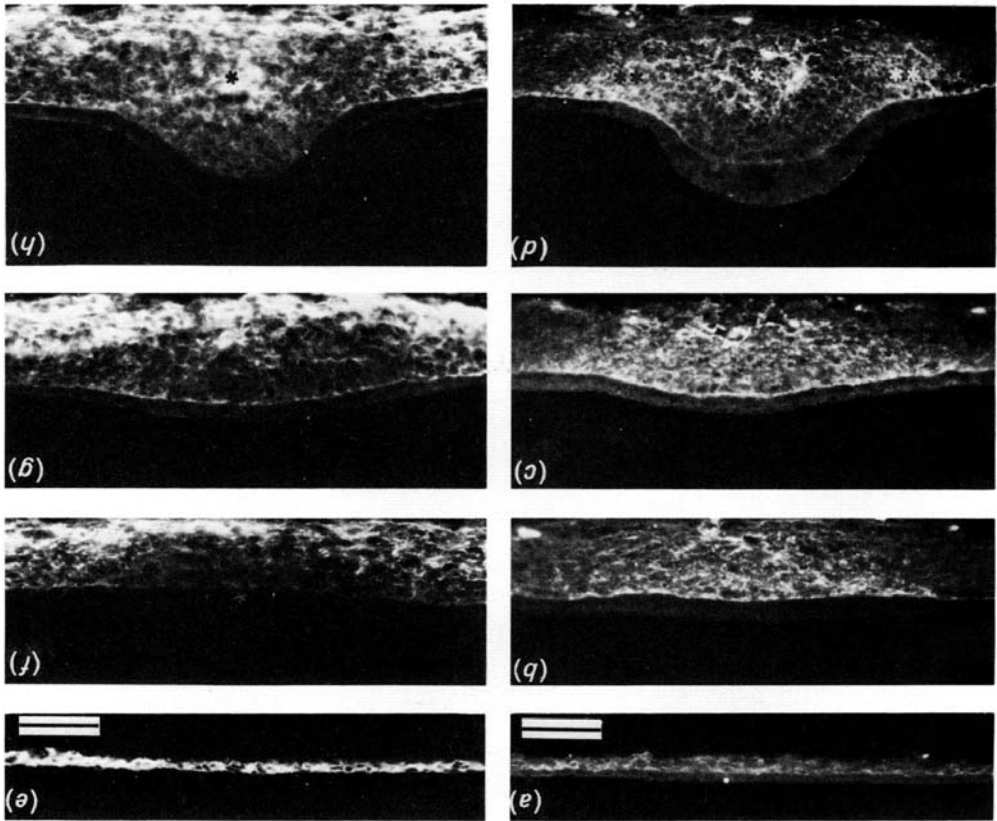
Fig. 3. (a–d) Indirect immunofluorescence staining for fibronectin. Transverse sections. (a) 6-day-old embryo. Fibronectin is weakly stained in the dermis but not in the epidermis. (b) 7-day-old embryo. Strong staining with anti-fibronectin is seen in the region of dermal condensation. Basement membrane located above the condensed dermal cells shows the presence of fibronectin. Epidermis is not stained with anti-fibronectin. (c) 8-day-old embryo. (d) 9-day-old embryo. The 'core' (*) and 'periphery' (**) of dermal condensation are stained more strongly than the remaining regions of dermal condensation. The basement membrane located above the periphery of dermal condensation is stained strongly with anti-fibronectin, whereas the basement membrane in the projected portion of dermal condensation shows weak staining. Bar = 56 $\mu$m.

(e–h) Indirect immunofluorescence staining for type I collagen. Transverse sections. (e) 6-day-old embryo. Intensive staining with anti-type I collagen is seen in the dermis but not in the epidermis which is hardly visible. (f) 7-day-old embryo. Staining with anti-type I collagen decreases remarkably in the region of dermal condensation.The region surrounding dermal condensation and the lower dermis are stained intensively with anti-type I collagen. Type I collagen is not found in the epidermis which is hardly visible. (g) 8-day-old embryo. (h) 9-day-old embryo. The 'core' region (*) of dermal condensation is stained with anti-type I collagen, whereas type I collagen is stained very weakly in the remaining portions of dermal condensation. Bar = 56 $\mu$m.
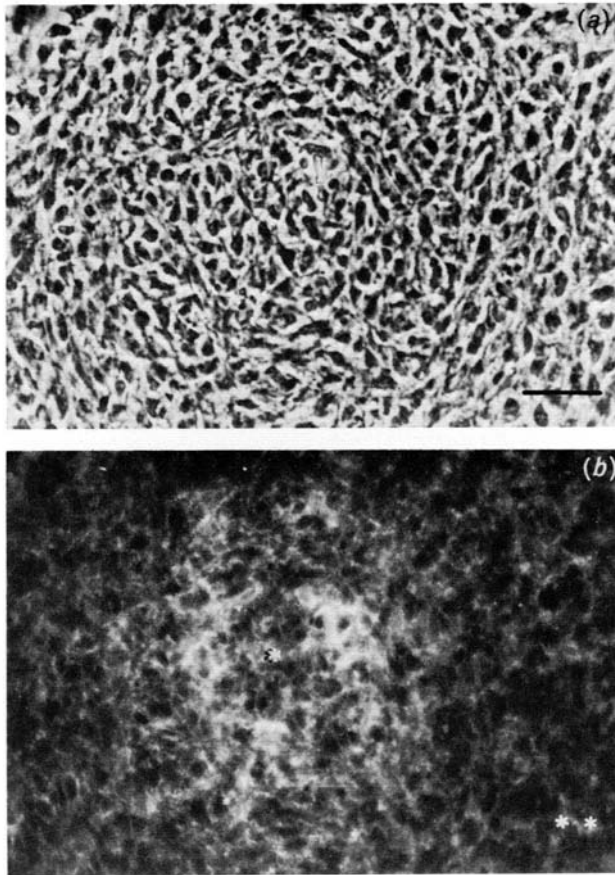
Fig. 4. Phase-contrast (a) and immunofluorescence (b) micrographs of a frontal section of dorsal skin of an 8-day-old embryo, stained by indirect immunofluor-escence for fibronectin. Fibrils found in the region (*) of dermal condensation are more strongly stained than those in the region (**) of surrounding dermal condensa-tion. Bar = 25 μm.

the region of dermal condensation (Fig. 3f). The region surrounding the con-densation and the lower dermis, however, showed an intensive staining for type I collagen (Fig. 3f). Epidermis had no type I collagen (Fig. 3f). Along with the growth of dermal condensation, the fluorescence intensity of type I collagen had decreased evenly throughout the condensed region of dermal cells (Fig. 3g). It was shown, from the observations on frontal sections, that the fluorescence specific for type I collagen was less intense in fibres among the condensed dermal cells than that among the region surrounding the condensation (Fig. 5f). In the dorsal skin of a 9-day-old embryo, a change in the distribution pattern of type I collagen within dermal condensation was found (Fig. 3h), as in the case of fibronectin (Fig. 3d). The 'core' of the region of dermal condensation had a very strong staining of anti-type I collagen whereas other regions of dermal

condensation remained to be stained weakly (Fig. 3*h*). This result was also accorded by the observation of frontal sections stained by anti-type I collagen (Fig. 5*h*).

(*d*) *Type III collagen.* No type III collagen was found both in the epidermis and dermis of the dorsal skin of 6- to 9-day-old embryos.

2. *Orientation of dermal cells and distribution of type I collagen in the formation of dermal condensation*

In order to study a possible relationship between the orientation of dermal cells and the distribution pattern of type I collagen at the formation of dermal condensation, frontal sections were used as follows. Dermal cells of a 6-day-old embryo showed a random arrangement without showing any orientation before dermal condensation took place and the distribution of type I collagen was also found to be random, making a network in dermis at 6 days (Figs. 5*a, b*). At the early stage of the formation of dermal condensation, 7 days, dermal cells in the condensed region were oriented running towards a neighbouring condensation (Fig. 5*c*). Collagen fibres, stained weakly, were also oriented, thus matching the oriented arrangement of condensed dermal cells (Fig. 5*d*, compare with Fig. 5*c*). In an 8-day-old embryo, the condensed region of dermal cells consisted of two parts; the 'core' where dermal cells were closely packed and the 'periphery' where dermal cells were arranged to encircle the 'core' (Fig. 5*e*). Collagen fibres in the condensed region, stained very faintly, had no orderly arrangement (Fig. 5*f*). In a 9-day-old embryo, the condensed region of dermal cells retained the same dual organization found in an 8-day-old embryo (Fig. 5*g*). Collagen, however, showed a quite different orientation of fibres in the 'core' and in the 'periphery'. In the 'core', collagen fibres were stained very strongly and had no regular arrangement (Figs. 3*h*, 5*h*). In the 'periphery', collagen fibres were stained weakly and showed an arrangement of radiated pattern (Figs. 3*h*, 5*h*).

In the early stage of the formation of dermal condensation at 7 days, dermal cells between neighbouring dermal condensations formed a distinct lattice arrangement (Fig. 6*a*), as reported by other investigators (Wessells & Evans, 1968; Ede *et al.* 1971; Stuart *et al.* 1973; Sengel, 1976). In such lattice arrangements, the orientation of fibres of type I collagen was found to be identical with that of the dermal cell (Fig. 6*b*). The quantity of type I collagen deposited in the lattice region was much more abundant than that in the core region of dermal condensation and less than that in the outer regions occupying the outside of dermal condensation and the lattice portion.
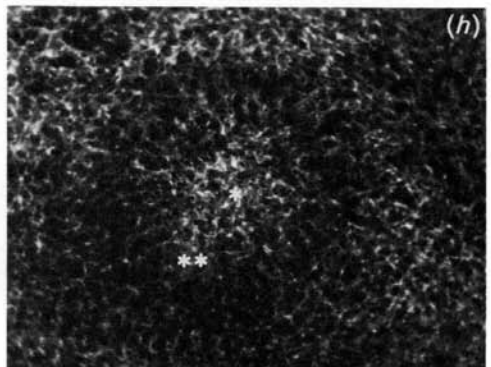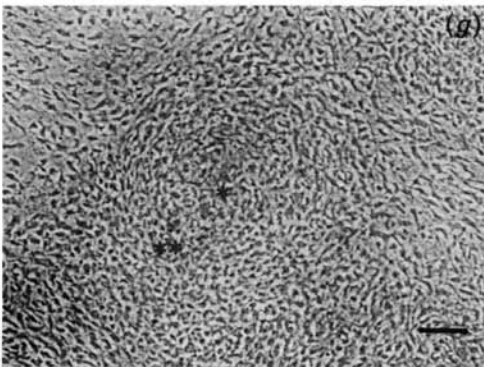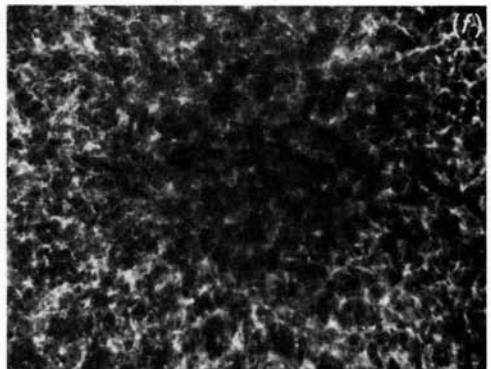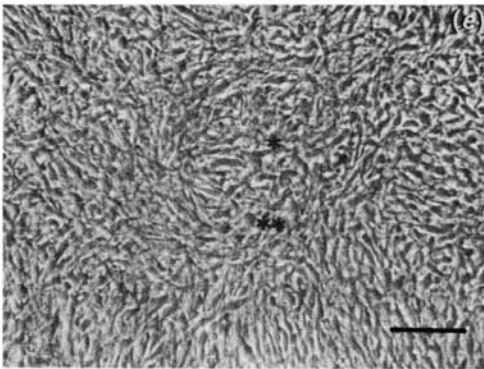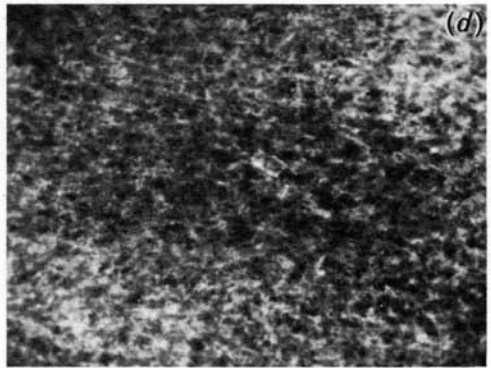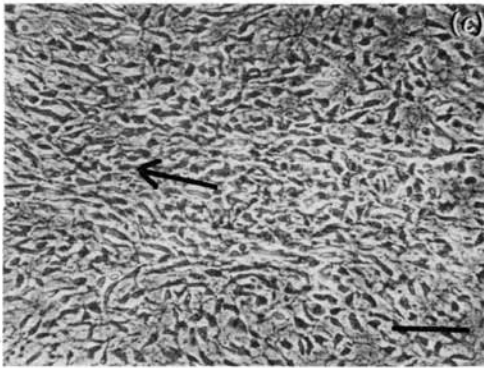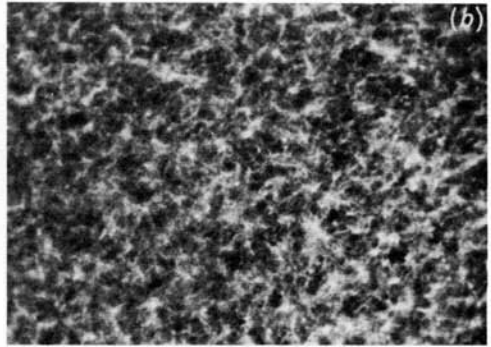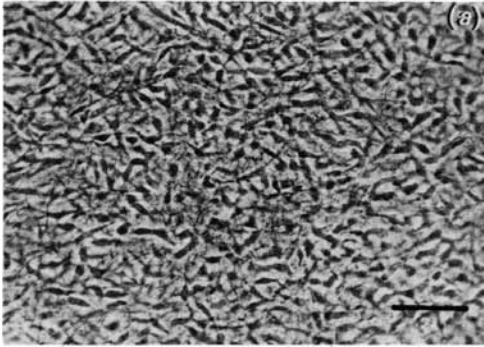
## DISCUSSION

Thus far, development of the dermal condensation has been studied mainly from the standpoints of cell proliferation (Wessells, 1965) and migration (Stuart & Moscona, 1967; Stuart *et al.* 1973). Little emphasis, however, has been placed on molecular approaches to elucidate the mutual adhesion among dermal cells during the process of dermal condensation. The present study was initiated with an intent to obtain precise information with respect to the adhesive mechanism of condensed dermal cells. Endogenous lectins, fibronectin, various types of collagens and proteoglycans are thought to be potential candidates playing significant roles for causally related cellular adhesion *in vivo*. The present study has shown that significant changes took place in the distribution patterns of the endogenous lectin, fibronectin and type I collagen during the formation of dermal condensation. As dermal condensation in the feather papilla proceeded, the endogenous lectin and fibronectin were concentrated in the condensed region, whereas type I collagen decreased.

The activity of endogenous lectin of embryonic chick skin increased, co-inciding with the initiation and advancement of dermal condensation (Kita-mura, 1980a). Whether endogenous lectin is located in the region of dermal condensation or not was left as an important question to be answered. This study has demonstrated that the condensed region of dermal cells had much more endogenous lectin when compared with the non-condensed region of dermis (Figs. 2*f*, *g*, *h*). The restricted distribution of the endogenous lectin in the region of dermal condensation, as shown in this report, and its high specific activity in the dorsal skin possessing dermal condensation, as described in the previous study (Kitamura, 1980a), may strongly suggest the possibility that the endogenous lectin is actually involved in the condensation mechanism of dermal cells. In the region of dermal condensation of a 9-day-old embryo, staining with anti-endogenous lectin among the condensed dermal cells was not uniform

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Fig. 5. Phase-contrast (*a*, *c*, *e* and *g*) and immunofluorescence (*b*, *d*, *f* and *h*) micro-graphs of frontal sections of the dorsal skin of 6- to 9-day-old embryos, stained by indirect immunofluorescence for type I collagen. (*a*) Dermal cells in a 6-day-old embryo are arranged without any orientation. (*b*) Dense type I collagen is distri-buted at random. (*c*) Dermal cells in early dermal condensation of a 7-day-old embryo are condensed and oriented towards a neighbouring dermal condensation (the orientation is indicated by an arrow). (*d*) Collagen fibres in dermal condensation are very weakly stained. The orientation of fibres matches the direction of condensed dermal cells. (*e*) Dermal condensation of an 8-day-old embryo consists of two parts: the 'core' (*) where dermal cells are closely packed and the 'periphery' (**) where dermal cells are arranged to encircle the 'core'. (*f*) Few collagen fibres are found in the region of dermal condensation, consisted of two parts, and show no orderly arrangement. (*g*) Cellular arrangement in dermal condensation of a 9-day-old embryo is similar to that of an 8-day-old embryo. (*h*) The distribution of collagen fibres is different from that of an 8-day-old embryo. Collagen fibres in the 'core' are stained strongly and show no regular arrangement, whereas collagen fibres in the 'periphery' are stained weakly and show a radiated arrangement. Bar = 45 $\mu$m.
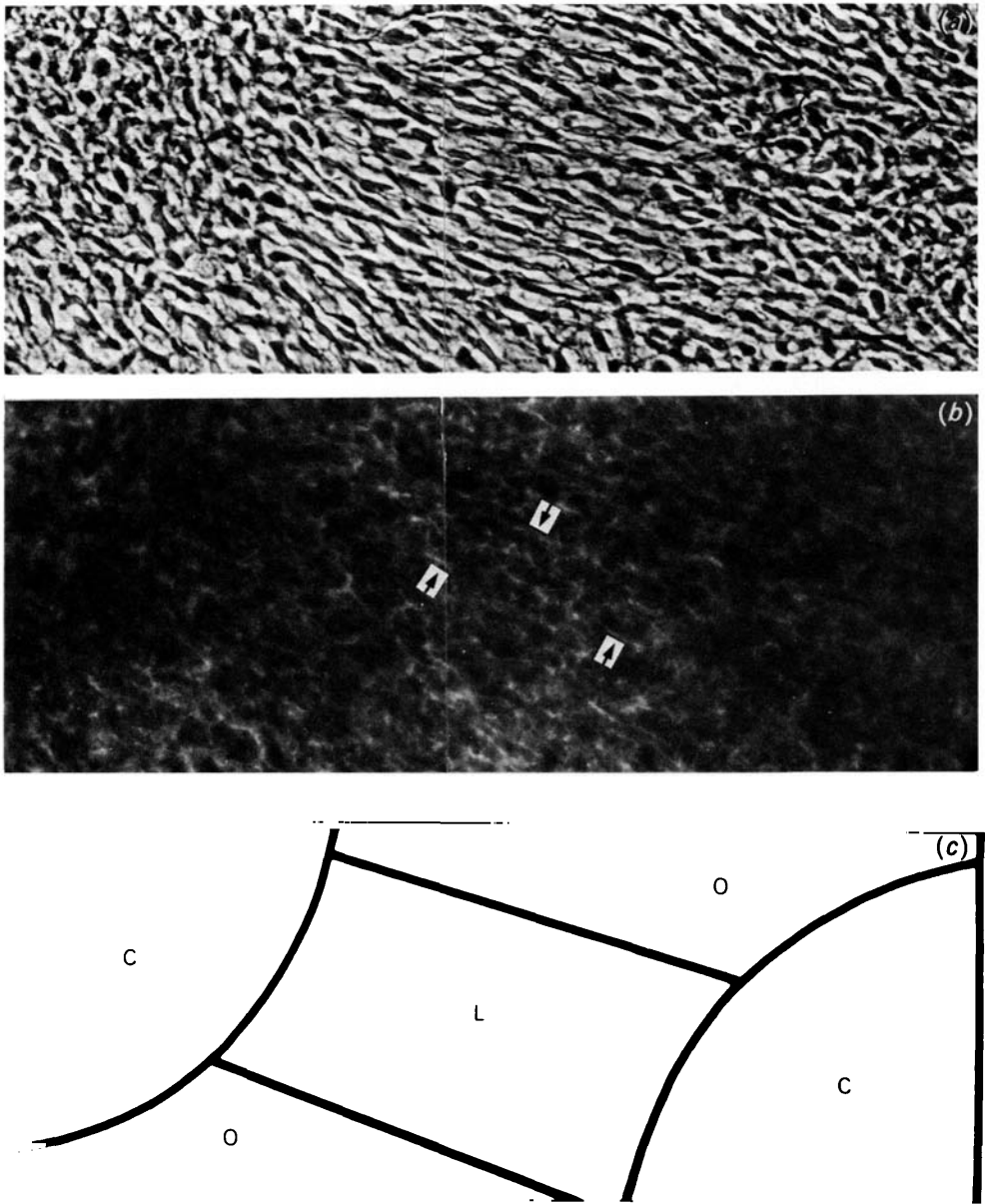
Fig. 6. Phase-contrast (*a*) and immunofluorescence (*b*) micrographs and schematic representation (*c*) of a frontal section of the dorsal skin of a 7-day-old embryo, stained by indirect immunofluorescence for type I collagen. Three regions are found in the dorsal skin of a 7-day-old embryo: the region of dermal condensation, 'C', the lattice region characterized by lattice arrangement of dermal cells oriented between two neighbouring dermal condensations, 'L', and the outer region outside of dermal condensations and the lattice region, 'O'. Collagen fibres in 'L' (indicated by arrows) are distributed matching the oriented arrangement of dermal cells between neighbouring dermal condensations. They are stained more strongly than those in 'C' and more weakly than those in 'O'. Bar = 26 $\mu$m.

(Fig. 2*h*). The possibility that endogenous lectin is located either on the cell surface or in the intercellular spaces of condensed dermal cells has been raised by preliminary experiments using an immunophotomicroscopic method. Since this method, however, does not allow one to determine the cellular location of endogenous lectin with a satisfying degree of certainty, the strict cellular locality of the endogenous lectin in the dermal cells is being currently examined by means of immunoelectron microscopy.

Lectin activity in the epidermis could not be measured reliably due to the fact that an insufficient quantity of epidermal cells was available (Kitamura, 1980*a*). The endogenous lectin was found in the epidermis prior to the formation of epidermal placodes, in contrast to fibronectin and type I collagen (Fig. 2*e*). The endogenous lectin was concentrated in epidermal placodes (Fig. 2*f*), but its definitive roles remain to be clarified.

Fibronectin, a major matrix glycoprotein, has been detected in the primitive mesenchyme (Linder *et al.* 1975; Stenman & Vaheri, 1978). Loss of fibronectin has been observed during the process of terminal differentiation of mesenchyme, such as bone and cartilage formation (Linder *et al.* 1975; Dessau, Sasse, Timpl & von der Mark, 1978; Hassell, Pennypacker, Yamada & Pratt, 1978; Dessau *et al.* 1980). Furthermore, the maturation and condensation of cornea stroma of chick embryos were accompanied with a parallel disappearance of fibronectin (Kurkinen *et al.* 1979). In the dorsal skin of a chick embryo, fibronectin was already detected in the dermis prior to the formation of dermal condensation (Fig. 3*a*). With the development of dermal condensation, it was concentrated in the condensed region of dermal cells (Fig. 3*b*). Fibronectin in the condensed region appeared in the form of fibrils, thicker than those localized in the region surrounding the condensation (Fig. 4*b*). Fibrils formed by fibronectin, however, were not arranged with a regular orientation in contrast to the fibres formed by type I collagen (compare Fig. 4*b* with Figs. 5*d*, *f*, *h*). Such an observation may suggest that fibronectin contributes to the process of the cellular adhesion among neighbouring dermal cells rather than to the process of spatial arrangement and migration of dermal cells – the role said to be played by collagens (Stuart & Moscona, 1967; Stuart, *et al.* 1973).

The idea that collagen serves as a non-specific supportive substance in inter-cellular matrices has proved to be an over-simplified one. It has now become clear that there are several different types of collagens, and each is associated with a particular array of cell types (Miller, 1976; Gay & Miller, 1978; Bornstein & Sage, 1980). Type III collagen occurs ubiquitously in the skin of calf and human (Nowack *et al.* 1976). Interestingly, type III collagen was not found in the dermis of embryonic chick skin as shown in the present study. Lack of type III collagen has also been reported in the cornea stroma of chick embryos (von der Mark, von der Mark, Timpl & Trelstad, 1977). It is conceivable that embryonic chick fibroblasts do not synthesize type III collagen but, principally, type I collagen *in vivo*, whereas fibroblasts isolated from chick embryos synthe-

size both type I and III collagens when cultured *in vitro* (Conrad, Dessau & von der Mark, 1980). The role of type I collagen, therefore, in the formation of dermal condensation becomes a more important one than has been considered before.

Absence of collagens in the dorsal skin of 6- to 7-day-old embryos was reported (Ede *et al.* 1971; Stuart *et al.* 1973), but the present study has shown that type I collagen, its presence shown by the immunofluorescence method, was predominantly found in the dorsal skin before the condensation of dermal cells was initiated (Fig. 3*e*). In the development of feather papillae, collagen has been thought to serve mainly as a substratum for the migration of elongated dermal cells (Stuart & Moscona, 1967; Stuart *et al.* 1973). The present study also showed the presence of a parallel alignment of fibres specific to type I collagen between neighbouring dermal condensations (Figs. 6*a*, *b*). Only a few studies with respect to collagens in the condensed region of dermal cells are available at present. Ede and coworkers (1971) have shown histologically that collagen was laid down in the lower layers of dense dermis in the condensations, whereas Overton & Collins (1976) could not find any modification at the ultrastructural level in the fibre network corresponding to the expected area of feather germs.

Present study showed that initiation of the condensation of dermal cells precisely coincided with the decrease of type I collagen (Figs. 3*e*, *f*). Furthermore, the growth of dermal condensation was closely connected with the rearrangement of dermal cells and type I collagen fibres (Figs. 5*a–h*). A question may be raised here, whether the decrease of type I collagen found by the immunofluorescence method was caused by masking of type I collagen fibres with other molecules, such as fibronectin or hyaluronate. To examine this possibility, sections of dorsal skin of 7- and 8-day-old embryos were pretreated with trypsin or hyaluronidase and examined by the immunofluorescence method. Such enzymatic treatments resulted in the amplification of the intensity of the fluorescence specific to type I collagen, as already shown in the cartilage collagen (von der Mark *et al.* 1976), but no increase in the fluorescence of condensed region of dermal cells was noted. Therefore, this observation may support the idea that there are few type I collagen fibres *per se* in the condensed region.

Replacement of the greater part of type I collagen fibres with cell-adhesive fibres, such as fibres composed of fibronectin, is thought to be indispensable for the mutual adhesion among dermal cells. Following such a replacement, the network is thought to be reconstructed by a very small number of type I collagen fibres for dermal cells to rearrange themselves for the formation of dermal condensation. A further study is in progress to examine whether the decrease of collagen in the condensed region could be caused by either the digestion of collagen secreted or the suppression of collagen synthesis. It is interesting that fibronectin and type I collagen were not codistributed in the condensed region of dermal cells, whereas fibronectin has been known to be bound to type I, II, III and IV collagens with high affinity (Dessau, Adelman,

Timpl & Martin, 1978) and fibronectin and type I collagen are reported to be codistributed in the vitreous body, lens capsule, secondary stroma, sclera (Kurkinen *et al.* 1979) and the limb bud of a chick embryo, prior to the formation of cartilage blastema (Dessau *et al.* 1980). Molecular relationship between fibronectin and type I collagen in dermal condensation remains still to be clarified.

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