Control of Chick Epidermis Differentiation by Rabbit Mammary Mesenchyme

Numerous studies have shown that the qualitative regional differentiation of embryonic epidermis depends on specific dermal stimuli. Thus, in bird embryonic epidermis, feathers are induced by dorsal dermis[1], preen gland by uropygial dermis[2] and scales by tarsometatarsal dermis[3]. Tissues other than chick dermis can, however, promote feather differentiation in chick ectoderm. Such heterotypic induction takes place when 5-day chick embryo corneal epithelium[4] or 6-day chick dorsal epidermis[5] interact with mouse dermis. Thus, chick epidermis is thought to be predetermined for feather formation which occurs when it is associated with any type of mesenchyme[6].

When rabbit mammary mesenchyme is used as a dermal support for chick epidermis differentiation, the results obtained are different. We have previously shown that in 12- and 13-day rabbit embryos, mammary mesoderm initiates the transformation of flank epidermis into mammary epithelium and subsequent appearance of mammary buds[7]. Recombinants of 6- and 7-day chick (or 6- to 8-day duck) embryonic epidermis and 12- to 14-day rabbit embryo mammary mesoderm, freed by trypsinization, were cultured in vitro for 1 week. They yielded spherical or elongated epithelial buds, displaying a thick basement membrane with a surrounding mesenchymal sheath[8]. Their shape was similar to that taken by rabbit mammary gland during its development, between 15th and 23rd day.

To obtain further morphogenesis of these buds, heterotypic associations were grafted onto the chorioallantoic membrane of 9-day chick embryos. Only 7 recombinants out of 39 grafted gave interesting results, after 10 or 11 days. The others were lost or necrotized. In 5 of these cases, the epithelial buds formed were slightly more developed than in organ culture and were surrounded by a well-developed mesenchymal sheath which was highly vascularized by the allantoic vessels. In each of the two latter grafts, heterotypic interactions between 6-day chick embryo epidermis and 13-day rabbit embryo mammary mesoderm resulted in fetal mammary gland-like structures (Figure 1), including a keratinizing primary bud and primary sprouts elongating in the mesenchyme. The number of primary sprouts (8) was the same as in the control fetal rabbit mammary gland. The primary bud and the sprouts were surrounded by a 5-cells-thick stromal capsule, differentiated by mesenchymal cells under epithelial influence.

Undoubtedly, these structures have been induced in chick epidermis by mammary mesenchyme. The fetal mammary gland does not display any feature specific to its own mammary condition, but its branching pattern does. However, from the beginning of its development, the mammary gland is characterized by its glycogen content which is located, during the sprouting phase, in the primary bud and at the tip of the sprouts. After PAS staining, the chimeric mammary gland-like structures exhibit a great amount of glycogen in the inner cells of the primary bud which undergo keratinization, and at the primary sprouts extremities (Figure 2). Control cultures have shown that at places where chick epidermis remained unaffected by mammary mesoderm, its cells did not synthesize glycogen and only the basement membrane reacted with PAS. Glycogen synthesis is thus induced in chick cells by mammary mesenchyme. At the same time, chick epithelial cell staining with hematoxylin, which is usually rather pale, becomes darker, suggesting some changes in the cytodifferentiation of these cells (a modification of their basophilia).

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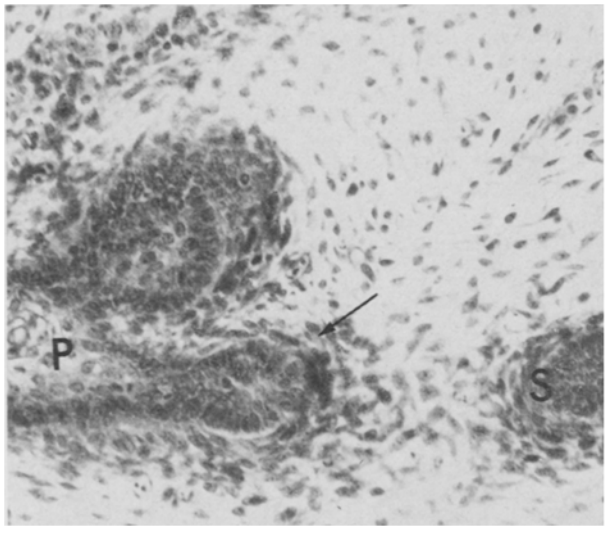

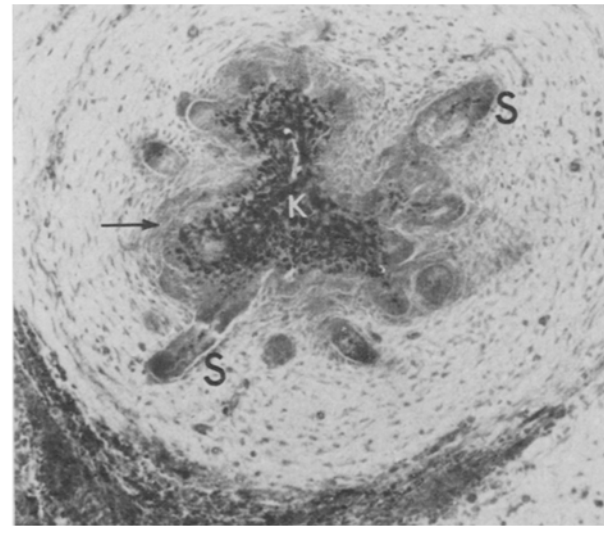

Fig. 1. A mammary gland-like structure induced by 13-day mammary mesenchyme in 6-day chick embryo epidermis, showing the primary bud (P), 2 secondary buds and a primary sprout (S). Notice the dark staining of chick epithelial cells and the well-developed connective sheath (arrow). Hematoxylin, eosin. × 230. Fig. 2. Localization of glycogen in another chimeric gland. Glycogen is abundant in keratinizing inner cells of the primary bud (K) and at the tip of the primary sprouts (S). The gland is circumscribed by the connective sheath (arrow). PAS, methyl green. × 130.

These results demonstrate an instructive effect exerted by mammary mesenchyme on chick epidermis differentiation. Heterotypic interactions between the tissues result in a mammary-like morphogenesis which chick epidermal cells undergo. At first, a spherical epithelial bud develops, which becomes elongated before the sprouting of the secondary buds while its inner cells keratinize and synthesize glycogen. Since mesenchymal cells organize themselves into a connective sheath around the sprouts, inductive interactions occurring in the recombinants are similar to those taking place during normal mammary development. Mammary mesenchyme thus controls not only the branching pattern of the structures differentiated by chick epidermis, but also cell cytodifferentiation and metabolism. How mammary mesenchyme can influence chick epidermal cells has not yet been explained. We must conclude that chick cells possess some surface properties allowing them to interact with mammary mesenchyme, and the genetic information necessary for the building up of mammary-like structures. Experimental mammary-like morphogenesis takes place faster than normal mammary gland development in vivo (12 days instead of 16): the resting phase is therefore reduced with chick epithelium.

These results may be compared with those of KOLLAR[9]: 4- or 5-day chick embryonic mandibular epithelium interacts with the dental papilla of 15-day mouse embryo tooth germs, whose cells differentiate as odontoblasts while matrix material, characteristic of tooth development, forms a deposit. A genetic compatibility between chick and mammalian tissues is perhaps involved in these heterotypic interrelationships.

The competence of chick epidermal cells to react with mammary mesenchyme suggests that they can be influenced by an induce rable to promote a morphogenesis which does not normally occur. Experiments are underway to investigate if these mammary-like structures could be stimulated to secrete proteins under hormonal influence.

Résumé. Le mésenchyme mammaire d'embryon de lapin de 13 jours induit dans l'épiderme embryonnaire de poulet de 6 jours, la différenciation de structures ressemblant à des glandes mammaires fœtales, après 2 jours de culture in vitro et 10 jours de greffe sur la membrane chorio-allantoïdienne d'embryon de poulet. La répartition du glycogène, synthétisé par les cellules épithéliales de poulet sous l'influence du mésenchyme mammaire est identique à celle des glandes témoins, ce qui signifie que la ramification des bourgeons épithéliaux et le métabolisme des cellules épidermiques sont également sous le contrôle du mésenchyme mammaire.

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Phosphorylase in the Adult Rat Testis: The Use of Dextran in Histochemical Studies

Phosphorylase has been demonstrated histochemically in the adult rat testis using TAKEUCHI's technique[1-3]. In agreement with other authors[4] in a series of investigations using this technique, we were unable to confirm this finding[5]. More recently, however, modifications have been made to the original technique of TAKEUCHI[6-11], one of the most interesting of which is the use of dextran, first introduced by MEIJER[10,12]. The aim of the present investigation was to study the effect of dextran on the histochemical demonstration of phosphorylase in the adult rat testis.

Materials and methods. 30 adult rats (Wistar) were used in these experiments. Biopsy specimens of testicular tissue were immediately frozen at −70 °C on dry ice and absolute ethyl alcohol. 12 µm sections cut on the cryostat were incubated in a medium containing 0.2 *M* acetate buffer pH 5.6, glucose-1-phosphate, NaF and EDTA for the histochemical demonstration of active phosphorylase. The same incubation medium, with the addition of 20 mg AMP, was employed for the demonstration of inactive phosphorylase. Other sections were incubated in the same medium to which dextran (500,000 mol.wt.) had been added in the ratio 2 g/25 cm³ medium. The sections incubated at 37 °C for 1 h and washed in 40 % ethyl alcohol were fixed in absolute ethyl alcohol and stained by the Schiff-dimedone method[13]. Control sections were incubated in a medium without substrate.

Results. When dextran is used in the incubation medium, the reaction product of active phosphorylase is very faint and is visible as fine granules localized inside the tubules in close proximity to the tubular wall. The tubular wall itself and the interstitium also show a positive reaction. With dextran the reaction product of inactive phosphorylase is much stronger and appears as well-stained granules distributed throughout the tubule, being more evident close to the tubular wall and in the peripheral zone of the preparation (Figures 1 and 2). Also in this case, the reaction is strongly positive along the tubular wall and the interstitium. Sections incubated in the medium without dextran show no reaction.

Discussion. Phosphorylase appears to be histochemically detectable with the use of dextran and is visible in the form of fine granules inside the seminiferous tubule. In a previous series of investigations, in contrast to other authors[1-3], we were unable to detect phosphorylase histochemically in the adult rat testis. Our present research confirms this earlier finding. It is therefore feasible to hypothesize that dextran, a predominantly 1:6 linked glucose polymer, is able to serve as glucosyl-acceptor(primer) for the demonstration of phosphorylase activity[10,12]. This addition of dextran to the incubation medium thus appears to be important since it makes

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