PROSTAGLANDIN-B₁, EMBRYONIC SKIN, AND THE DERMO-EPIDERMAL JUNCTION

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INTRODUCTION

In previous studies, prostaglandins have been applied to organ cultures of dorsal skin from the chick embryo (11, 12). When prostaglandin-B₁ (PGB₁) is added to the culture medium in which skin pieces are explanted at a time when feather organ loci are present, the latter fail to develop during incubation but the skin continues to grow and mature. Control skins demonstrate normal feather development during 5–6 days of incubation.

We are continuing the effort to explain this effect, especially through examination of the tissues by electron microscopy. In the course of these studies we have observed an unusual defect in the dermo-epidermal junction. Throughout the entire PGB₁-treated skin there are numerous areas in which the dermo-epidermal junction is discontinuous. This effect is attributed directly to treatment of the tissue with PGB₁. It is repeatable and has been found as early as the second day of incubation of the cultured skin. In some cases these areas of disjunction can be followed by serial section and are seen to lead into complete perforations of the epidermis, through which mesenchymal cells migrate or proliferate and coat the free surface layer (Fig. 1).

MATERIALS AND METHODS

Dorsal skin of the chick embryo was removed and placed in organ culture according to procedures previously described (11). The culture medium for treated skins contained 50 μ g/ml of crystallized PGB₁. The cultures were fixed at daily intervals for 5 days in cold 1% glutaraldehyde buffered with cacodylate and CaCl₂. After a cacodylate buffer wash, they were postfixed in 1% osmium tetroxide buffered with cacodylate and CaCl₂. The tonicity of both fixatives and the buffer wash was adjusted to 300 mosmols. Dehydration was accomplished through a graded series of ethanol followed by embedment in Epon 812. Sections were cut on a diamond knife from 600 A to 800 A, doubly stained in lead citrate and uranyl acetate, and examined in an RCA EMU 3G electron microscope.

An equivalent number of control tissues and PGB₁-treated tissues were examined. In addition, because of the possibility that the disjunctions and perforations arose from mechanical probing of the tissue with the dissecting needles during preparation of the skin for culture, a series of control tissues were deliberately and repeatedly stabbed with a No. 11 beading needle. These tissues were cultured, processed, and examined as above.

RESULTS

Fig. 2 shows a gap in the dermo-epidermal junction. When breaks could be traced through serial

All figures depict areas of dorsal skin of the chick embryo grown in organ culture from 3 to 5 days in the presence of $50 \mu\text{g}/\text{ml}$ of PGB_1 .

FIGURE 1 A photomicrograph of a thick plastic section of dorsal skin showing a complete perforation of the epidermis (*E*). Mesenchymal cells (*M*) have formed a plaque atop. $\times 1200$.

sectioning, it was commonly found that the periphery of a break contained a thickened basal lamina (Fig. 3). In some cases, isolated sections of the basal lamina can be found across the break (Fig. 4). Also, in other instances, cells can be seen wedged in-between the space of the break (Fig. 5). Examination of serial sections suggests that these cells are essentially spherical and lack filopodia, which would ordinarily indicate motility.

In the case of the control skins which were deliberately stabbed in order to produce the breaks mechanically, no such disjunctions were found. For the period up to 5 days of incubation, the epidermis and basement membrane were observed to be intact. It should be similarly noted that the senior author, who over a period of several years has been examining cultured skin prepared in the usual way

with beading needles, has never observed breaks in the dermo-epidermal junction such as reported here.

DISCUSSION

Several reports in the literature have demonstrated discontinuities, or breaks, of the dermo-epidermal junction. In some cases, the basal epidermal membrane and basal lamina have appeared fused and granular (10). However, an observation such as this might be accounted for by tangential sectioning through this area. In other cases, small papillary processes of the basal epidermal cells have been observed to penetrate into the space of the mesenchyme through a small gap in the basal lamina (2, 5). In still others, complete separations of the basal lamina have been accompanied by

FIGURE 2 A disjunctive area of the dermo-epidermal junction. Basal lamina (*B*) is separated by intimate contact between epidermal (*E*) and mesenchymal (*M*) cells. $\times 13,200$.

FIGURE 3 Periphery of break shown in Fig. 2. Arrow indicates approximate point where serial sectioning demonstrates site of dermo-epidermal gap. Basal lamina (*B*) of basement membrane. Epidermis (*E*). Mesenchyme (*M*). $\times 13,200$.

FIGURE 4 An isolated, membrane-bound section of basal lamina (*B*) is seen within and above the break in the dermo-epidermal junction (*J*). Mesenchyme (*M*). Epidermis (*E*) $\times 13,600$.

FIGURE 5 A section of a cell (*M*), presumably from the mesenchyme, is wedged within the area of disjunction. Isolated sections of the basal lamina (*B*) lie above the continuous portions of the dermo-epidermal junction (*J*). Epidermis (*E*). $\times 9200$.

penetration of large processes of the basal epidermal cells into the underlying mesenchyme (1, 3, 4, 6, 7, 9, 13, 15). These processes are commonly seen to be devoid of cellular organelles.

However, in none of these reported cases have mesenchymal or dermal cells, or the processes thereof, been shown to penetrate into the epidermis.

The fact that, in many sections, isolated portions of the basal lamina can be observed, which, when followed serially are found to fuse together, lead us to believe that, in these cases, the periphery of the gap in the basal lamina must appear somewhat scalloped. Perhaps, then, the wider or more expansive breaks have been formed from fusion of several distinct holes. Further, in cross-section study, the isolated portions of basal lamina lie above the continuous portion. This suggests displacement from below, perhaps as a result of mesenchymal cells pushing upward in their advance through the epidermis. What relationship this geometric configuration might have with the mode of action of PGB_1 within the skin, or specifically on the basal lamina, remains to be understood.

The question arises, can these breaks be analogous to skin lesions involving ulceration? Hansen et al. (8) demonstrated disruption of the basement membrane of skin of dogs fed a diet deficient in essential fatty acids. These areas were traced to complete perforations of the epidermis.¹ In a recent report by Menton (14), who studied adult mice deprived of essential fatty acids in the diet, no defects in the basement membrane were cited. However, he did report that he observed wide intercellular spaces in the Malpighian layer of the epidermis, and eventual loss of hair. It is interesting to note that the prostaglandins are related to the essential fatty acids, in that some of the latter serve as the precursors for the synthesis of the former.

It cannot be determined at this time whether the disjunction phenomenon is directly related to the failure of the down feather to develop when skin is treated with PGB_1 . If this were the case, one might expect to see disjunct areas strategically located in the treated skin, e.g., in presumptive feather sites. Such a possibility must still be considered.

Whether or not PGB_1 -induced breaks in the dermo-epidermal junction are peculiar to embry-

onic skin remains to be demonstrated. However, it would be of special interest to attempt to duplicate the effect in human adult skin. If this were possible, a metabolic explanation for the development of certain skin lesions might be closer at hand.

SUMMARY

The down feather organ derived from the dorsal skin of the chick embryo fails to develop from presumptive sites when treated in vitro with prostaglandin- B_1 (PGB_1). Examination of the cultured tissues by electron microscopy reveals many breaks or gaps in the dermo-epidermal junction. In some cases, these areas of disjunction are continuous with complete perforations of the epidermis. Mesenchymal cells fill the perforated area and form plaques on the dorsal surface of the skin.

It has yet to be determined whether or not this phenomenon is directly related to the morphogenetic block of the feather organ produced by PGB_1 .

This work was supported by a grant from the Department of Health, Education, and Welfare, National Institute of Health, 5-R01 AM 13530-01.

PGB_1 was supplied through the courtesy of Dr. John Pike, The Upjohn Company.

Received for publication 17 March 1970, and in revised form 27 April 1970.

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