THE BASEMENT LAMELLA OF AMPHIBIAN SKIN

Its Reconstruction after Wounding*

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Plates 89 and 90

The investigations dealt with in the following preliminary report form part of a larger experimental program on morphogenesis at submicroscopic levels. The basement lamella under the epidermis of amphibian larvae offers unique opportunities for such research: It is a non-cellular sheet containing units of supramolecular order of great regularity, which, in turn, are arrayed in an orderly fabric separating the epidermal cell layer from the underlying connective tissue. It thus lends itself readily to an experimental analysis of the manner in which the cell types on either side contribute to its formation and characteristic architecture. After experiencing some technical difficulties in the study of its primary development in the embryo, we turned to its secondary reformation in the healing of skin wounds in later larval stages.

A description of the submicroscopic structure of the basement lamella, as revealed in ultrathin sections of osmium-fixed preparations, has been given previously (Weiss and Ferris (1)). Here is a brief recapitulation of its main features in urodele larvae. The basement lamella is continuous with the underside of the epidermal layer whose cells are cemented to it by an intermediary film of about 600 Angström thickness containing a single layer of characteristic spherical granules of *ca.* 500 A diameter. Along their undersides bordering on this film, the epidermal cells are dotted with bobbin-shaped, strongly osmiophilic bodies, which might be assumed to serve as "adhesive discs." The basement lamella itself is laminated (Fig. 1). Each layer, measuring about 2000 to 2500 A in thickness, consists of a ground substance, electronoptically homogeneous, in which cylindrical fibers are embedded in loose packing. These fibers run parallel to one another in the plane of the lamella. They are cylindrical with a diameter of around 500 A. In the axial direction, they show the rhythmic banding characteristic of collagen, with a period averaging about 500 to 520 A, and with the corresponding segments of all fibers of a given layer laterally in register. The most striking feature is the fact that the orientation of the fibers changes abruptly from layer to layer, those of one layer running perpendicularly to those of the next adjoining layers. This can be clearly seen

* Supported by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

in Fig. 1, which shows a section in a plane nearly parallel to the course of the fibers in one set of layers (showing long stretches of fibers in profile), hence, only the cross-sections of fibers (circular dots) in the alternate layers. Due to this arrangement, the basement lamella, when viewed from the surface, appears as a pattern of orthogonal lines (see Rosin (2)). There are, on an average, about twenty such layers stacked up to a total depth of approximately 4 micra in midlarval stages. Except for their parallel alignment, the fibers are grouped at random. Most fibers appear to be endless; that is, continuous throughout a given layer. Sporadically, one also encounters a small bundle of fibers running crosswise through the membrane.

This description of the basement lamella pertains to the skin of certain body regions only, especially the trunk. The basement lamella of the balancer (Harrison (3)), for instance, is composed of a mat of thinner fibers, lacking uniform orientation and lamination. These regional distinctions will be dealt with on a future occasion. The present paper refers to the laminated basement lamella of flank skin only. For further details, consult our original article.

An orderly fabric of this kind raises important morphogenetic questions far beyond the ordinary problems of fibrogenesis; for, besides the sources of the molecular constituents, there are now at issue the causes for their compounding into cylindrical cables of a set size range ($\sim$500 A), for the arrangement of these cables in parallel, for their lateral correspondence, for the definite depth of each layer, for the perpendicular orientation of the fibers of adjoining layers, and for the rather constant thickness of the assembled plate ($\sim$20 layers or $\sim$4 micra). These questions relate not so much to problems of chemical composition as such, as to problems of orderly assemblage, alignment, and arrangement of units—in other words, to problems of bioarchitecture and biotechnology. Answers would presuppose concrete information on just how such a tissue fabric is being built up. The following observations contain some of the relevant data.

Flank wounds were made by small incisions through the entire thickness of the skin, including the basement lamella, allowing the wound edges to gape slightly. Previous experiments from our laboratory (Chiakulas (4); Lash (5)) had revealed the course of events following such a lesion, as studied in both living and fixed specimens under the light microscope: A wave of mobilization proceeds from the wound edge by which the epidermal cells of the adjacent skin district are progressively detached from the basement lamella and move in the general direction of the defect, which has become quickly covered by a coagulum of exudate and blood. By the concentric advance of the epidermal front (at an average rate of 120 micra per hour), the wound is covered in a matter of hours, whereupon the cells come to a halt. Cell growth and multiplition have no part in this primary closure. The basement lamella, by contrast, remains rigid and passive, its wound edges sharply outlined in their prior positions. It takes several weeks for the missing patch of basement lamella to be reformed along the underside of the epidermal wound covering.

To study this course of events electronmicroscopically, the wound areas were fixed in veronal-buffered osmium tetroxide at intervals of from 1 hour to 28 days, embedded in plastic, sectioned (at *ca.* 0.1 micron) transversely or tangentially, placed on formvar-coated or carbon film grids (Watson (6)), and studied under our RCA-EMU-3 instrument. All dimensions will be given here in approximate values, detailed histograms of size distribution to be furnished in a subsequent paper.

The specimens taken during the first few hours have revealed interesting details on the mode of detachment of the epidermal cells (further intimating an adhesive role of the bobbins) and on their mode of migration. These facts will not concern us here, save for one point: As the cells become detached, the double-contoured film of about 600 A thickness, intermediary between the basement lamella and the inner surface of the epidermis (illustrated in Fig. 1 of Weiss and Ferris (7)), peels off, leaving its spherical granules freely exposed in the denuded surface of the basement lamella. In turn, as the emigrated epidermal cells settle down over the wound coagulum, a similar narrow space of hyaline substance, about 600 A wide, soon appears between them and their substratum, and spherical granules, loosely spaced, can shortly be recognized again in it. The presence of this film, preceding by many days the first traces of fibers in the new basement lamella, is important evidence against the assumption that the epidermal cell contributes formed fiber components to the underlying basement lamella; for such contributions, if resolvable by the electron microscope, would have to be seen in their transit across this space.

The epidermal cells settling over the wound show at first a rather jagged contour, some vacuoles near their surfaces, and a ruffled disarray of the characteristic furry cytoplasm described in an earlier paper (Weiss and Ferris (7)). The underlying substratum of wound exudate is, except for sporadic signs of cell debris, structureless. In line with the insignificance of true inflammatory reactions in these forms, phagocytes are infrequent. Within a few days, spindle-shaped mesenchyme cells, "fibroblasts," appear at the lesion in greater numbers. Their cytoplasm (Fig. 2) is densely filled with endoplasmic reticulum in the form of distended "cisternae" (Palade and Porter (8)), the walls of which are often dotted with the granules described in these positions in highly active cells. In favorable instances, one recognizes a fine striation in these walls, appearing on closer study as the outline of columnar elements. This fact, taken together with repeated indications in our pictures that terminal parts of cisternae perforate the cell surface and are pinched off into the surrounding medium, where their outlines are still vaguely retained (see Fig. 2, at arrows), and noting further that the first true fibrous filaments of the basement lamella can be seen crowded about such outlined spaces in the matrix, at least suggests that fibrous precursors may be discharged from the producing fibroblasts in such packages, rather than in molecular dispersion. This is presented here merely as a possibility, which will require much further evidence. Besides the

fibroblasts, one regularly encounters bodies with cell-shaped outlines, filled for the most part with clear vacuoles of up to half a micron in diameter, which are separated by narrow partitions containing granules and sometimes mitochondrion-like bodies. Their nature and function remain to be explored.

Evidently, the wound coagulum forms a primordial barrier between the epidermal covering and the fibroblasts; but, being structureless, its fate and gradual transformation into the ground substance of the future basement lamella cannot well be traced electronmicroscopically. Within the 1st week after wounding, filaments begin to appear in this layer. They have rather uniform diameters of 150 to 200 A, a faint periodic structure along the fiber axis of about the mature segmental length, and course singly or in small groups in random directions. They are irregularly spaced, widely apart, occupying (according to a gross calculation for a 12 day specimen) no more than one thousandth of the space of the matrix in which they are embedded. They thus form a very loosely matted feltwork without any trace of layering. Whether they have been issued bodily from their cells of origin (see above) or have been precipitated out and assembled *in loco* from smaller precursor units, with or without the aid of epidermal catalysts, remains undetermined. These early filaments are dispersed throughout the space between epidermal and fibroblast layers without showing, at first, any consistent density gradient of distribution; if anything, there is a tendency to greater concentration toward the epidermal side. In view of the high fluid content of this space during the early days, one might question, however, whether the fixed preparation portrays the natural disposition reliably. The cut edge of the old basement lamella remains clearly identifiable by the abrupt termination of the laminated fiber fabric. For a short distance from the edges, the laminae flare slightly and the orientation of the old fibers is upset, presumably due to dissolution of cementing ground substance. The fibers, themselves, however, have retained their integrity and dimensions. Consequently, if one takes a picture near the wound edge, one can clearly distinguish old fibers of 500 A diameter and, intermingled with them, clusters of newly formed fibers of the smaller size class (Fig. 3, at arrows), with relatively fewer fibers of intermediate size range. However, the exact determination of the fiber size spectrum is still under way and will be reported on a later occasion.

In the former wound area proper, one finds exclusively fibers of the small size and of random orientation well into the 2nd week. This is illustrated by Fig. 4, which shows the reforming basement lamella at its epidermal border (in slightly oblique section, which obscures the interposed granulated border film; as one can see, the epidermal cell has already restored a heavily osmiophilic set of bobbins along its underside).

The new basement lamella forms a smooth and continuous lining of the epidermal cell sheet, while it bears no configurational relation to the scattered fibro-

blasts with which it is only in partial contact. Thus, it is evidently the epidermal underside which serves as foundation for the architectural development of the basement lamella, although not as a major source of its building materials. And it is actually from this epidermal surface that the next phase of structural elaboration—the orientation and layering process—proceeds.

Close inspection of Fig. 4 shows that while the fibers in the deeper regions are still in random orientations, those near the epidermal border are beginning to manifest a preferential alignment parallel to that border. One also recognizes the incipient emergence of orthogonality in short fragments of fibers hit obliquely by the section, and oriented at right angles to the former set. For a more demonstrative illustration, however, we turn to another section of this same specimen in which the plane of sectioning has hit the epidermal underside more tangentially, instead of crosswise (Fig. 5). The sliced-off cap of the epidermal cell shows the bobbins in surface view, next the granules of the intermediary film, and further peripherally, the basement lamella with two systems of parallel fibers intersecting orthogonally. (That the angle of intersection is less than 90° is due to distortion, as both fiber systems are inclined relative to the plane of sectioning.) The picture reveals several important facts: It shows that orthogonality and layering appear in conjunction; that the ordering spreads from the epidermal border over the rest of the fiber population, progressively aligning the latter into orientations conforming to the foundation layers; that during this phase of incipient organization, the fibers still are of the original small size order (<200 A) and that they are spaced at rather regular intervals, which significantly are of the order of about 500 A. The significance of this spacing lies in the fact that it corresponds approximately to (*a*) the segmental period of the fibers, (*b*) the diameter of the mature fibers, and (*c*) the distance of the granules in the intermediary film, which, as one readily notes, for instance, in Fig. 5, are arrayed in a regular grid. A formal, and perhaps causal, relation between these granules and the pattern of the fabric of the basement lamella is thus intimated. Point (*a*) deserves notice in connection with our earlier suggestion (Weiss and Ferris (1), p. 538) that orthogonality may be due to the fact that the laterally aligned segments of one ply of fibers might serve as attachment points—cross-ties, as it were—for the homologous segments of the next ply, in which case the distance between neighboring fibers would, of course, have to be the same as that of the segmental period.

Within the 3rd week, the layering process advances in depth and is now readily discernible even in transverse sections such as that shown in Fig. 6, which represents a cross-section through the entire thickness of the new basement lamella, with the epidermal cell on top and a fibroblast below. About nine of the future twenty layers have formed, the fibers of their orthogonal systems showing alternately in (longitudinal) profile and (circular) cross-sections. These fibers measure about 500 A in diameter, hence, have attained

mature size. The outer strata are well organized, rather densely settled with fibers, and of relatively uniform width, while the deeper layers are still imperfectly defined, in some places just barely indicated, in others wholly missing. The lower half of the basement lamella, adjoining the fibroblast layer, consists merely of ground substance with a random array of fibers embedded in it without signs of lamination. The gradual progress of structural order has thus been directly visualized.

One of the most important features of this stage is, however, that here the fibers appear suddenly in their mature size range. Just how these mature fibers replace primordial smaller ones, whether the former become simply compacted by aggregation of the latter or arise essentially *de novo* by a sudden dissolution and reprecipitation in new shape of the latter, is the subject of further investigations which are now under way.

In the described manner, a new basement lamella of normal architecture is eventually restored in the area of the wound. Its layers link up with the layers of the old basement lamella surrounding the lesion, which immediately suggests numerous experiments regarding possible ordering influences emanating from the wound border as contrasted with organizing influences solely from the over- and underlying cell layers. Since many more experiments to establish the substantial as well as architectural contributions of epidermal and mesenchymal layers, respectively, are in progress, we wish to refrain, for the time being, from any interpretation of the described facts and merely present the record, pure and simple, of the sequence of events by which a highly ordered architecture is built up gradually from submicroscopic units, which in themselves are of supramolecular order. We wish to place in the foreground of attention the phenomenon and the problems it raises, rather than any spurious explanation, for which our data are too fragmentary—and, perhaps, our concepts too immature.

SUMMARY

The basement lamella under the epidermis of amphibian larvae shows a submicroscopic architecture of remarkable geometric regularity: It consists of about twenty layers of ground substance in which cylindrical fibers (presumably collagenous) of about 500 Angström diameter are embedded parallel to one another, but with the fiber directions alternating by 90° from layer to layer. The repair of this membrane after wounding was studied electronmicroscopically in ultrathin sections. The sequence of events is as follows: (1) Epidermal cells cover the wound exudate by migration. (2) Rather uniform fibers of small size (<200 A) appear in the space between the epidermal underside and the subjacent fibroblasts; these fibers are sparse and oriented at random. (3) Proceeding from the epidermal surface downward, a wave of organization spreads over this primitive fiber tangle, resulting in the fibers becoming (*a*) straightened; (*b*) oriented; (*c*) packed into the characteristic layered structure; and (*d*) brought up into the 500 A diameter class.

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EXPLANATION OF PLATES

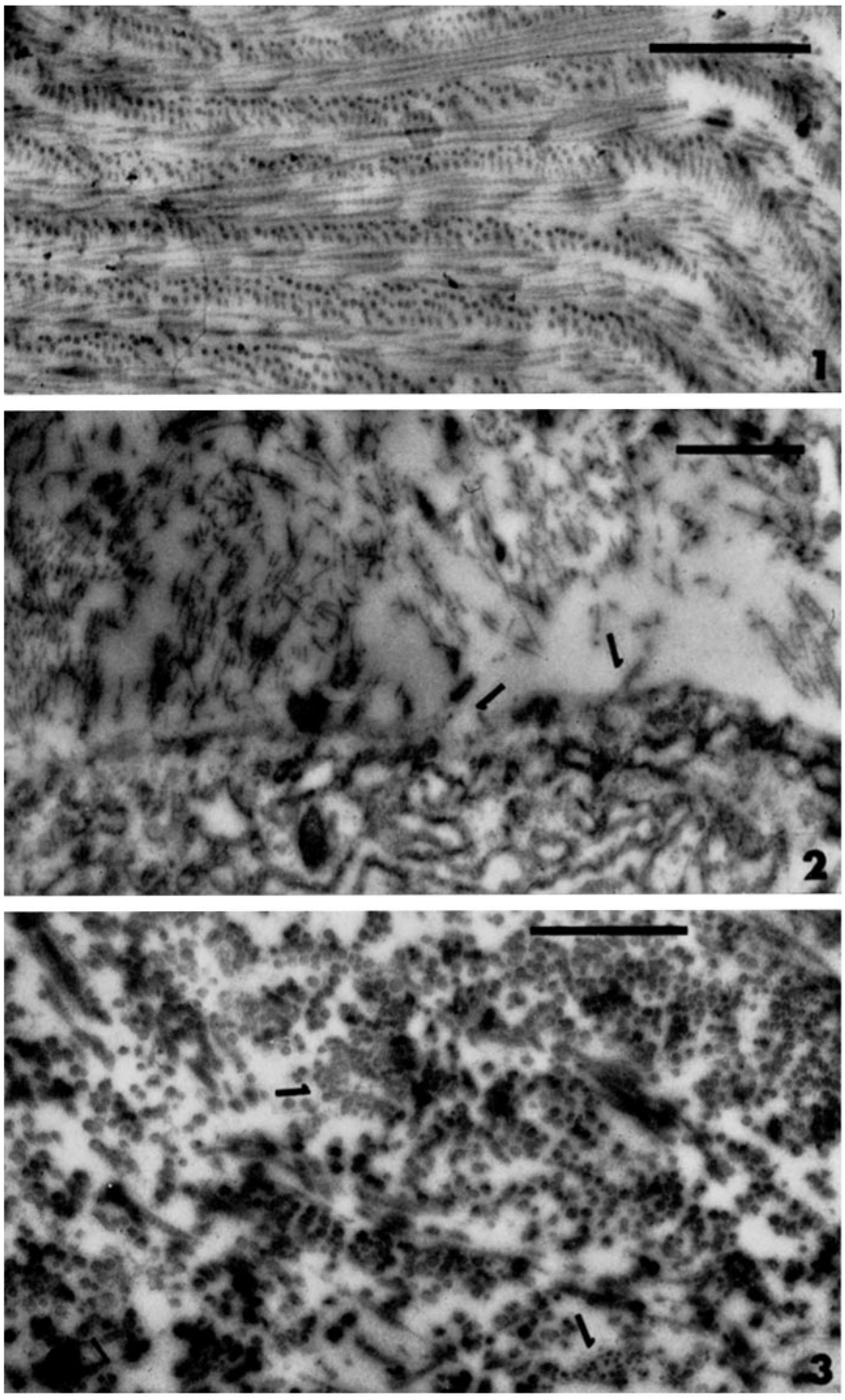
Scale unit in all figures is 1 micron.

PLATE 89

FIG. 1. Cross-sectional sample of subepidermal basement lamella of *Ambystoma opacum* larva, (30 mm. length), showing the laminar fiber systems alternatingly in profiles and cross-sections. × 22,600.

FIG. 2. Fiber mat in ground substance of 12 day wound. Fibroblast occupies lower part of picture. Note protrusions and points of perforation of cell surface at arrows. × 18,000.

FIG. 3. Old fibers and new fibers (arrows) at edge of 8 day wound. × 22,000.



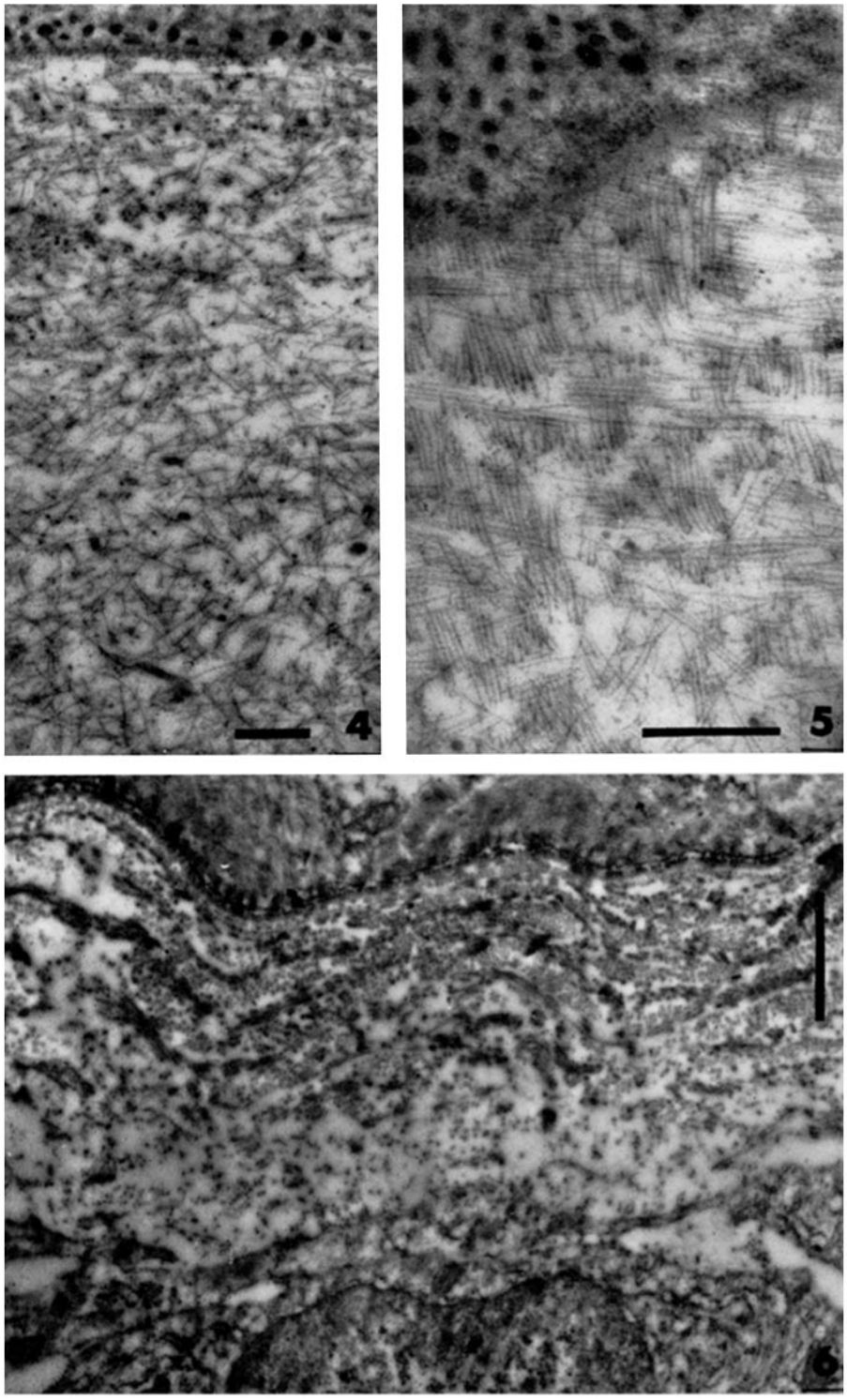
(Weiss and Ferris: Basement lamella of amphibian skin)

PLATE 90

FIG. 4. Fiber mat near epidermal side (epidermal cell with bobbins on top) in 11 day wound. × 11,000.

FIG. 5. Near tangential section of same preparation as that in Fig. 4. Moving from left upper to right lower corner, observe succession of following structures: Bobbins, grid of granules in intermediary film, denser precipitate in outer surface of basement lamella, small fiber systems in orthogonal arrangement, unoriented fibers. × 19,500.

FIG. 6. Section through whole thickness of partially reconstructed basement lamella in 28 day wound. Proceeding from top to bottom, note: Epidermal cell with bobbins, intermediary film with granules, completed orthogonal layers of fibers, incomplete layers, ground substance with unoriented fiber content, thin cytoplasmic process of one fibroblast, cytoplasm and nucleus of another fibroblast. × 18,000.



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