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Replacement of the Larval Basement Lamella by Adult-Type Basement Membrane in Anuran Skin during Metamorphosis[1]

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INTRODUCTION

Ever since Gudernatsch (1912, 1914) proved that the mammalian thyroid gland contains an "*agens*" which will hasten metamorphosis when fed to amphibian larvae, biologists have been investigating the mechanisms whereby the thyroid participates in controlling vertebrate development. Reviews by Allen (1938), Lynn and Wachowski (1951), Etkin (1955), and Kollros (1959) should be consulted for excellent discussions of earlier literature on amphibian metamorphosis. Our concern in this paper will be the normal changes that transform the thin larval dermis of the frog *Rana pipiens* into the thickened dermis of the adult. In particular, we will follow the fate of the larval basement lamella (Kemp, 1959, 1960) and the development of the basement membrane of adult skin.

The basement lamella in the tail of well-differentiated larvae of *Amblystoma punctatum*, *A. opacum*, *Rana pipiens*, and *Xenopus laevis* (Weiss and Ferris, 1954a,b), and in the tail of a 25-mm tadpole of *Rana sylvatica* (Kemp, 1959) contains 20 ± 2 orthogonally aligned layers of collagenous filaments embedded in ground substance. Fibrillogenesis of these filaments proceeds extracellularly in the larva, beginning in *Rana pipiens* at Shumway stage 18. As the larva grows, does the basement lamella reach a constant thickness, or does it continue to thicken throughout larval life (Weiss and Ferris, 1954b)? Evidently deposition of new layers continues in the head region of *Rana pipiens*, for 32 aligned layers could be counted in the head skin of a 36-mm tadpole (Kemp, 1959).

What structural changes occur in the dermis of amphibian skin

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during metamorphosis? Weiss and Ferris (1954b) have already reported that mesenchyme cells invade the basement lamella early in metamorphosis. They suggested that migration of cells between the plies of the basement lamella may be "aided by some lytic secretion (hyaluronidase?)." Figure 8 in their publication illustrates mesenchymal cells already in the membrane at stage XI (Taylor and Kollros, 1946). Stearner's (1946) valuable study on differentiation of pigment in the skin of *Amblystoma tigrinum, Amblystoma maculatum*, and *Triturus torosus* provides an accurate picture of the microscopically discernible events in the metamorphosing skin of urodeles. She observed that a 24-mm larva of *A. tigrinum* had a thin "collagenous" dermis underlain by subdermal melanophores and "unpigmented cells." What she calls the dermis at this early stage is evidently the fibrous membrane that we call the basement lamella (Weiss and Ferris, 1954a,b) or the basal membrane (Salpeter and Singer, 1959). She observed that subdermal unpigmented cells later move upward into "the fibrous layer and extend laterally between the fibers." Melanophores likewise move from a subdermal position into the fibrous layer. Migration of cells into the dermis is accompanied by "a decrease in number of subdermal cells," and "by an increase in thickness of the dermis and a looser arrangement of the fibers." Skin glands develop and appear "to push the collagenous fiber layer down before them" as they grow into the dermis. In the advanced larva the dermis comes to consist of three layers: "a thin dense layer of connective tissue fibers" adjacent to the epidermis (outer compact layer of dermis, Dawson, 1920), a middle layer of loose connective tissue (stratum spongiosum, Lindemann, 1929; Helff and Stark, 1941; intermediate spongy layer of dermis, Dawson, 1920), and a deep layer "of densely arranged connective tissue fibers" and a few embedded unpigmented cells (stratum compactum, Lindeman, 1929; inner compact layer of dermis, Dawson, 1920). Speidel (1926) includes the loose connective tissue immediately subjacent to the stratum compactum as part of the dermis or "cutis."

MATERIALS AND METHODS

Larvae of *Rana pipiens* were raised from eggs ovulated and fertilized by Rugh's (1934) technique. Except for weekends, animals were fed daily on boiled spinach. They were grown in 12 × 18-inch enamel pans containing about 1 inch of water previously conditioned

by bubbling air through tap water for at least a day. Probably because of the crowding effect reported by a number of workers, including Rose (1960), larvae in the same pan often grew at different rates after they began to feed. The larger tadpoles in a pan appeared to inhibit growth of smaller ones, although the smaller larvae would usually grow well when isolated in another pan. After forelimbs appeared and tails began to shorten, larvae were moved to a special dish where they could complete metamorphosis without danger of drowning. This dish contained small stones and was tilted so that at one end the emerging frog could be out of water. Metamorphosis was completed in the laboratory in 3–4 months. Animals in stages of metamorphosis from III to XXV, classified according to Taylor and Kollros (1946), were selected for histological examination.

Fragments to be fixed for thin sectioning were excised either from the tail or from the posterodorsal part of the head after removal of the lower jaw. Pieces were dropped directly into ice-cold 1% osmic acid containing 0.09 *M* sucrose and buffered with acetate-veronal to pH 7.4–7.5. Fixation was generally continued for 1–2 hours. After rapid dehydration, fragments were infiltrated through three changes of a mixture of 40% methyl methacrylate and 60% butyl methacrylate. They were embedded in catalyzed, prepolymerized plastic in No. 00 gelatin capsules. Completion of polymerization was aided by heating in an oven at about 55° C. Tips of the hardened blocks containing the tissue were frequently cut off, trimmed, and remounted with hard wax so that sections could be cut normal to the surface of the skin. Sections were cut with a Porter-Blum microtome and observed with an RCA EMU 3E electron microscope. Micrographs were taken at initial magnifications up to 5600 and enlarged photographically. Pieces of tissue from head, trunk, and tail were fixed in Bouin's fluid for paraffin-embedding and routine histological study.

OBSERVATIONS

The Larval Basement Lamella

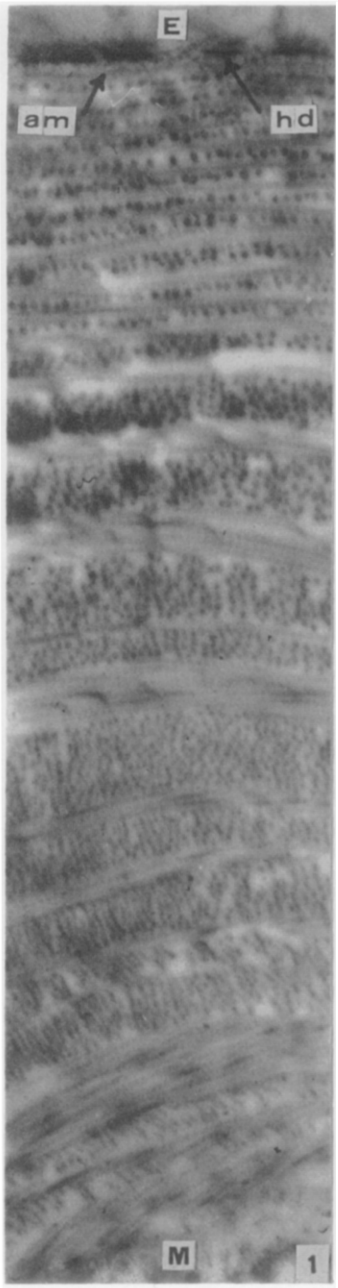
Further information on the question of constancy of thickness of the larval basement lamella is provided by counting the layers of filaments in the basement lamellas from head and tail regions of the 75-mm tadpole illustrated in Figs. 1 and 2. Since there were 46 layers in the head and about 32 layers in the tail of this specimen, one can

conclude first that the basement lamella of the head does continue to thicken by adding new layers during growth beyond the 36-mm length of the tadpole mentioned in the Introduction. Secondly, one can conclude that the basement lamella in the skin of the tail does not keep pace with cephalic basement lamella in its rate of growth. Examination of ordinary histological sections leads one to realize that the basement lamella does vary in thickness in different regions of the larval body at any given stage. For example, the skin in the region of the future dermal plicae of the head develops more rapidly than in other parts of the head (Helff and Stark, 1941). The basement lamella over the muscular part of the tail appears to be thicker than the lamella over the tail fins, but exact comparative counts of layers in these different regions have not been made.

In Fig. 1 observe that layers closest to the epidermis are composed of single rows of filaments. Layers of the lower three-fourths of the membrane appear to contain multiple rows. The apparent greater thickness of deeper layers was also observed by Weiss and Ferris (1954b). They urged caution in interpreting the significance of this gradient in apparent thickness of layers of the basement lamella, for differential compression or local folding of the membrane could be accountable. Some of the deeper layers in the basement lamella of Fig. 1 look exactly as one would expect if single-rowed layers had been cut obliquely. Other layers, however, look as though they were composed of several parallel rows of filaments cut in cross section. Deeper layers of the membrane, which are added after feeding begins at Shumway stage 25 (Kemp, 1959), may actually be deposited in the form of multiple rows of filaments before the mechanism controlling orthogonality alternates the direction of alignment. Such a multirowed pattern of deposition might result from the increased proliferative activity of fibroblasts in the older larva as compared to the younger.

FIG. 1. Basement lamella of head skin of 75-mm tadpole with well developed hind legs. Forty-six layers of collagenous filaments can be counted between the adepidermal membrane (*am*) and a mesenchyme cell (*M*). Border of basal epidermal cell (*E*) bears dense "bobbins" or half desmosomes (*hd*). Magnification: × 19,405.

FIG. 2. Basement lamella of tail skin of same 75-mm tadpole with head skin illustrated in Fig. 1. About 32 layers of collagenous filaments span the distance between epidermal cell (*E*) and mesenchyme cell (*M*). Magnification: × 21,670.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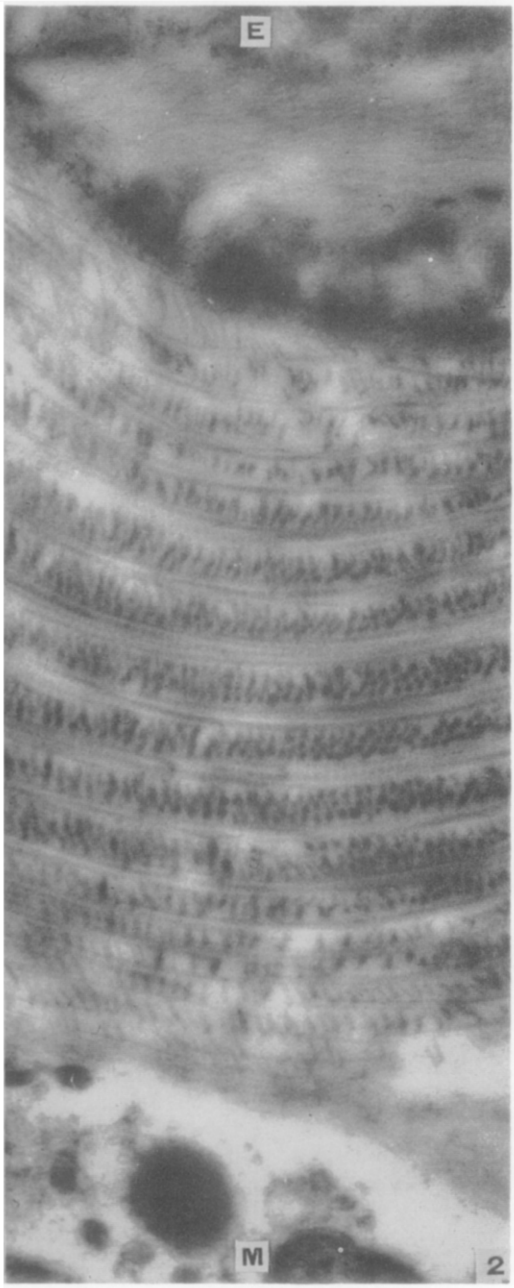
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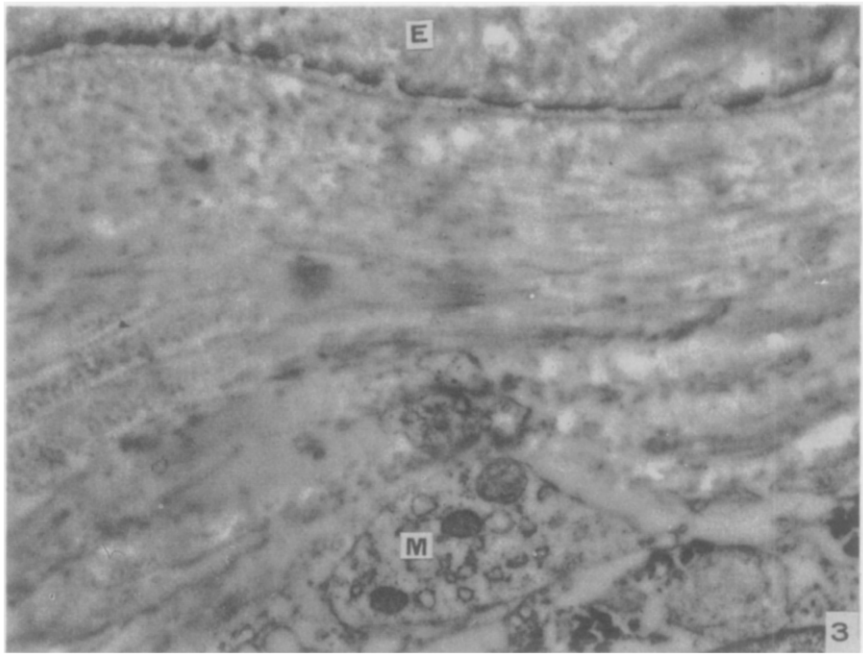
Externally the basement lamella is limited by the thin adepidermal membrane and separated from the epidermis by the adepidermal space (Salpeter and Singer, 1959, 1960). Along the border of the epidermis are the "bobbins" (Weiss and Ferris, 1954a,b), which are considered to be homologous to the "half prickles" described by Charles and Smiddy (1957) for human skin. They may also be designated half desmosomes (Selby, 1957). The peripheral cytoplasm of the basal epidermal cells contains filaments (Weiss and Ferris, 1954a,b) or microfibrils (Kemp, 1959) which may properly be called tonofilaments (Selby, 1955). Bundles of tonofilaments undoubtedly correspond to what earlier cytologists (Weed, 1934) called the "figures of Eberth." On its internal side the larval basement lamella is bordered by two kinds of mesenchymal cells, one containing abundant endoplasmic reticular elements and the other characteristically containing vesicles. These two types, tentatively identified as fibroblasts and as melanoblasts or melanophores, are illustrated at later stages in Figs. 7 and 8.

Cellular Invasion of the Basement Lamella

Larval growth in length continued, in the specimens measured for this study, up to a maximum of 90 mm by stage XIX and began to decline as the tail started to shorten at about stage XX. Invasion of the basement lamella by mesenchymal cells was first detected in the head at stage XI when the five digits of the hind limbs were just beginning to elongate. Mesenchymal cells beneath the basement lamella were found to be just starting to extend protoplasmic processes outward into the basement lamella at this stage. Figure 3 illustrates early invasion of the basement lamella in the head of a larva at stage XII.

FIG. 3. A mesenchyme cell (*M*), tentatively identified as a fibroblast, has started to migrate upward into the basement lamella of the head skin of a 55-mm tadpole at stage XII. Epidermis (*E*) above basement lamella. Vagueness of layered pattern in lamella of this specimen is probably due in part to artifacts induced during fixation or further processing of the tissue. Magnification: × 15,215.

FIG. 4. Mesenchyme cell (*M*) of the head skin of an 83-mm tadpole at stage XIV has spread out horizontally between layers of the basement lamella and is now completely embedded within the lamella. Cisternae of endoplasmic reticulum (*er*) are elongated in the direction of elongation of the cell. Epidermal cells (*E*) are above basement lamella. Magnification: × 11,255.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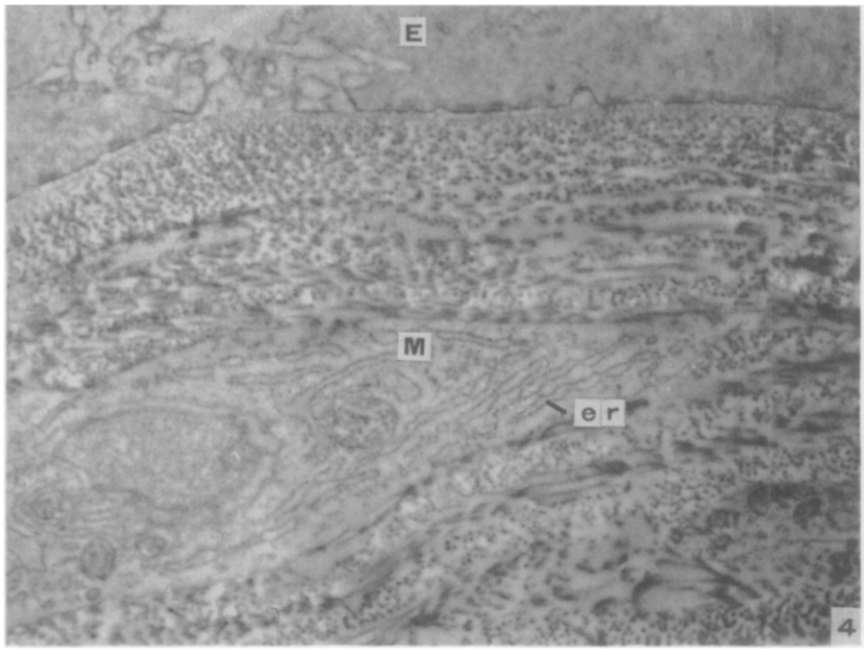
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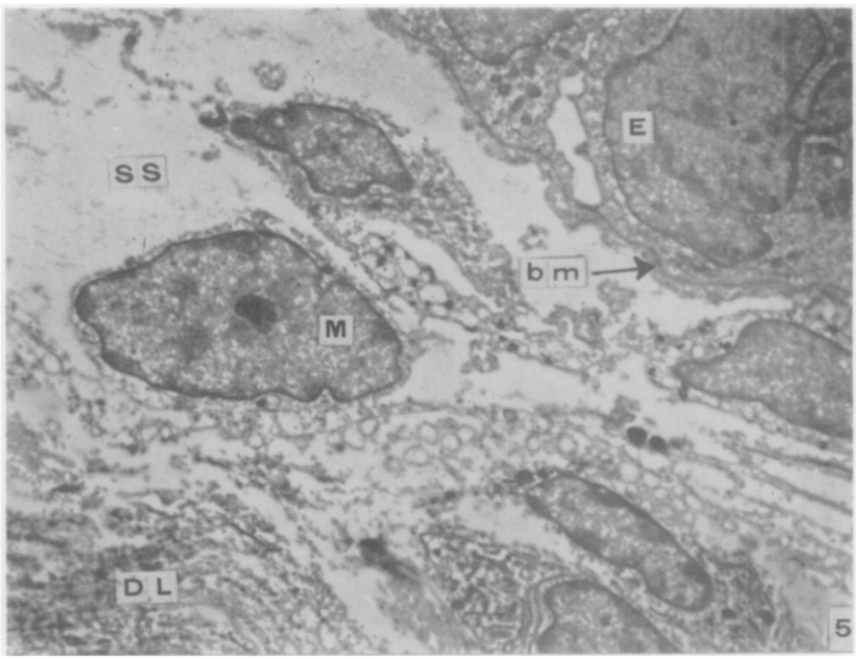
Endoplasmic reticulum of invading mesenchymal cells proliferates so that one observes abundant cisternae in the cytoplasm when cell migration begins. Cell processes at first move outward toward the epidermis (Fig. 3), but some of them then shift their direction 90 degrees and move along cleavage planes between layers of the basement lamella. After pathways have been opened by the pioneering cell processes, the main bodies of mesenchymal cells move into the basement lamella and may elongate in directions paralleling the underside of the epidermis (Fig. 4). Mesenchymal cells were detected up close to or against the adepidermal membrane beneath the epidermis by stage XIV. As a result of incorporation of the mesenchymal cells, the basement lamella becomes markedly increased in thickness. How the cells digest their way through the basement lamella can only be surmised. Filaments are obviously moved apart and possibly also depolymerized to make room for cells. Presumably some lytic secretion (Weiss and Ferris, 1954b) breaks down the supporting framework of the ground substance between filaments. The changes described above for the head do not occur in the tail. Invasion of the basement lamella by mesenchymal cells has been detected only in the head and trunk.

Detachment of Larval Basement Membrane

After the mesenchyme cells have reached the underside of the epidermis, the next major change comes when the basement lamella actually loses its attachment to the epidermis. A space develops between basement lamella and the adepidermal membrane below the epidermis, and mesenchyme cells move into it. Two major changes

FIG. 5. In a 60-mm tadpole at stage XVI the basement lamella, which now may be called the dermal lamella (outer edge seen at *DL*), has separated from the epidermis (*E*), and mesenchyme cells (*M*) are accumulating between dermal lamella and epidermis in the newly formed stratum spongiosum (*SS*). No intact layers of the larval basement lamella remain in contact with the epidermis, but the thin anlage of the basement membrane (*bm*) which would develop into the adult type can be seen just below the adepidermal membrane. Magnification: × 7125.

FIG. 6. View of the full thickness of the dermal lamella (*DL*) in the head skin of a 65-mm tadpole at stage XVI. Mesenchyme cells (*M*), probably fibroblasts, are embedded in the membrane. Upper edge of the lamella bordering on the stratum spongiosum is in upper right corner of the micrograph, lower edge at lower left corner. Magnification: × 6025.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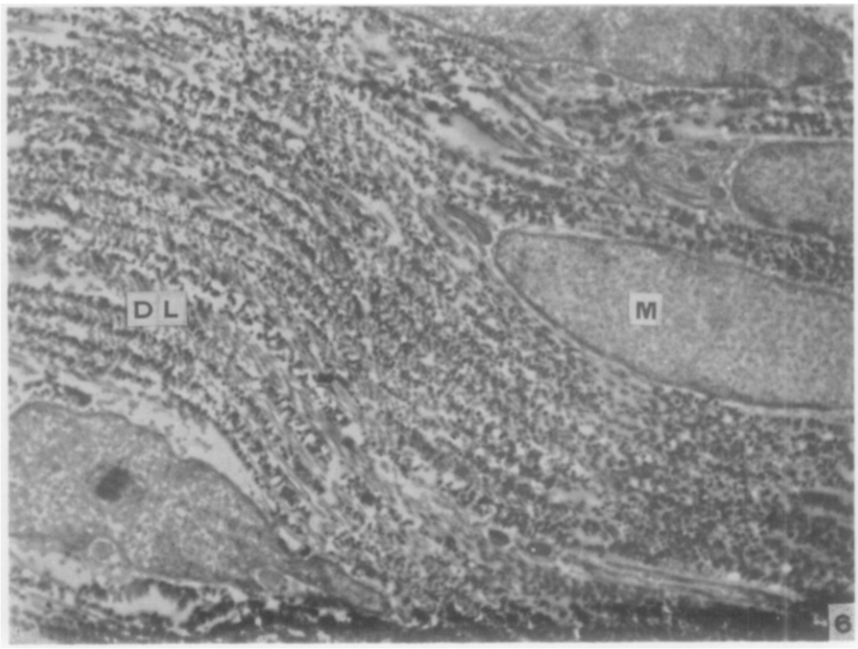
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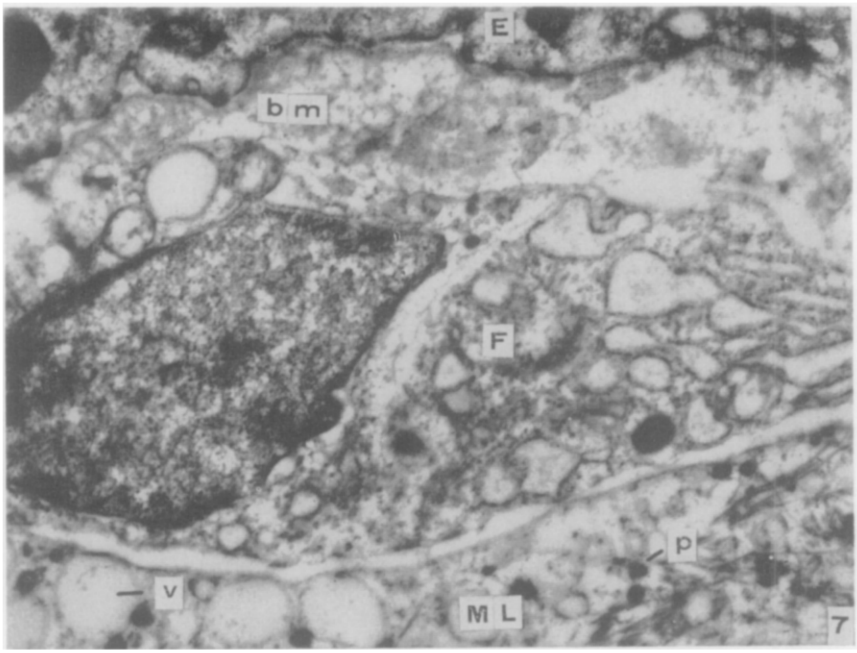
result from development of this space between basement lamella and epidermis. One is that there is now room for the outgrowth of skin glands which begin to proliferate at about stage XIV (Kollros and Kaltenbach, 1952). The other change is that a population of connective tissue cells builds up around the skin glands to form the stratum spongiosum (Fig. 5). The larval basement lamella comes to lie farther and farther separated from the epidermis as skin glands grow. In its new position detached from the epidermis (Fig. 6) the basement lamella, still containing some embedded mesenchyme cells, becomes the stratum compactum. I propose that it also be called the "dermal lamella" to indicate its origin from the larval basement lamella. Whether the dermal lamella continues to add new layers after its separation from the epidermis has not been ascertained.

Development of Adult Basement Membrane

Immediately after separation of the basement (dermal) lamella from epidermis at about stage XIV or XV, only a few granules or short filaments embedded in ground substance constitute the precursor of a new epidermal basement membrane. Whether these elements are left behind from disassembled components of part of the larval basement lamella or whether they come from materials newly secreted by fibroblasts is not known at present. As the various components are fabricated into a new membrane, however, it seems

FIG. 7. The basement membrane (*bm*) in a 55-mm tadpole with both pairs of legs and with tail partly resorbed, approximately at stage XXI, appears to consist largely of granules embedded in ground substance. Fine filaments can sometimes be observed in the basement membrane of specimens at this stage. Epidermal cell (*E*) along upper edge of micrograph shows wavy border. Cell just beneath basement membrane, probably a fibroblast (*F*), contains abundant endoplasmic reticular elements. Cell at bottom of micrograph, identified as a young melanophore (*ML*), contains numerous vesicles (*v*) and some pigment granules (*p*). Magnification: × 13,960.

FIG. 8. Basement membrane (*bm*) in a tadpole shortened to 38 mm, approximately at stage XXII, appears to consist of granules and occasionally fine filaments (*f*) embedded in ground substance. Adepidermal membrane (*am*) follows folds in border of epidermis (*E*). Mitochondria (*m*) are abundant in cytoplasm of basal epidermal cells. Microfibrils and half desmosomes characteristic of larval basal epidermal cells have largely disappeared. In the stratum spongiosum beneath the basement membrane are a fibroblast (*F*) and below it a melanophore (*ML*) containing vesicles and a few pigment granules. Magnification: × 16,070.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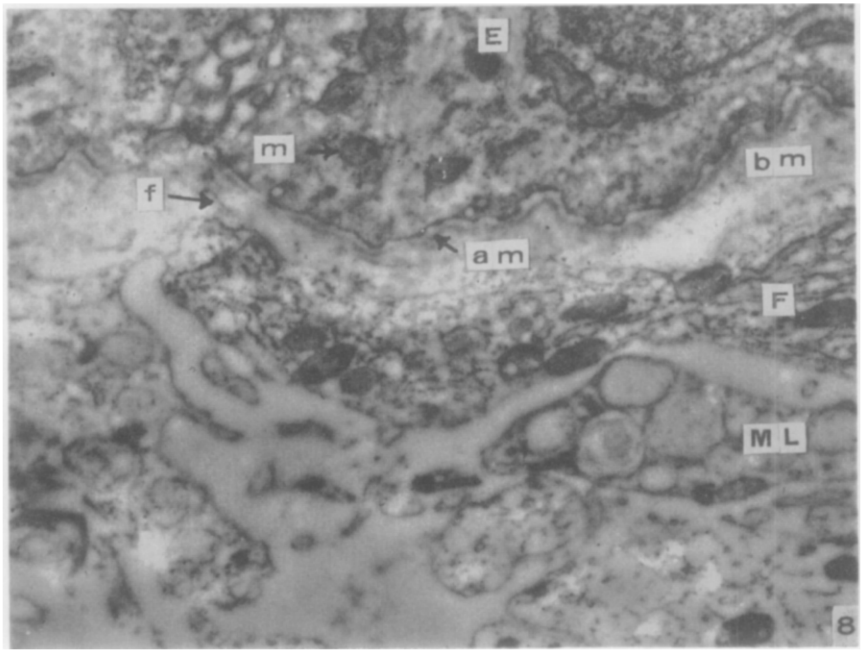
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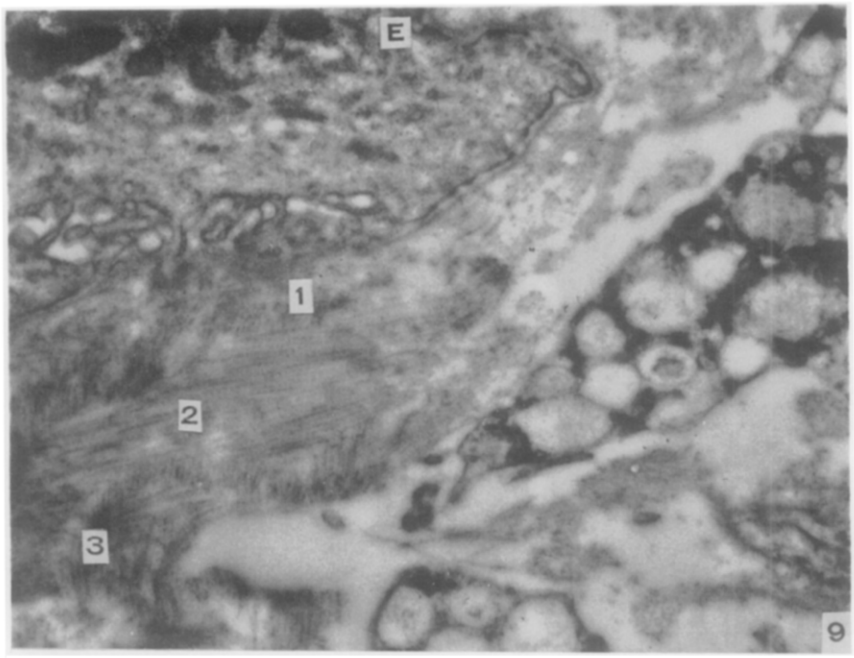
probable that fibroblasts do contribute new supplies of raw materials. They probably secrete tropocollagen units (Gross, 1956) and possibly also the mucopolysaccharides of the ground substance (Fitton-Jackson, 1956).

In a report to the American Association of Anatomists (Kemp, 1960), changes in the basement membrane after the onset of tail shortening were described. These changes occurred chiefly between stages XX and XXV after forelimbs had emerged. A larva which had shortened to 55 mm, approximately at stage XXI (Fig. 7), had a basement membrane containing numerous granular bodies and a few fine filaments. A larva of about stage XXII (Fig. 8), which had shortened to 38 mm, had a similar relatively unpolymerized membrane which contained granular constituents and some fine filaments.

Polymerization of new filaments in the basement membrane was in progress in a young frog which had just completed metamorphosis and had shortened to 20 mm at stage XXV (Fig. 9). The membrane at this stage contains filaments that appear to be aligned chiefly in two directions. Filaments within the bundle at *2*, Fig. 9, are all parallel with nodes in register, while filaments at *1* and *3* run at about 70 degrees from the direction of those at *2*. This near-orthogonality may represent a distortion of an actual orthogonal orientation, or it may mean that orthogonal alignment is not usually achieved in the morphogenesis of the adult type of basement membrane. Certainly the larval lamellar fabric of orthogonally aligned flat layers is not duplicated in the adult membrane. The abundant filaments present in the new basement membrane probably result chiefly from new polymerization of precursor units rather than from persistence of

FIG. 9. Basement membrane of a 20-mm young frog at stage XXV, which has just completed metamorphosis. Filaments are now abundant and appear to be aligned predominantly in two directions, either vertically in the plane of the micrograph as at *1* and *3* or horizontally as at *2*. Alignment in the two directions is not exactly orthogonal; for filaments in the vertical orientation run at about 70 degrees from those in the horizontal orientation. Border of epidermis (*E*) is folded. Magnification: × 14,445.

FIG. 10. Dermoepidermal junction of skin from adult frog showing two multistranded cables in basement membrane at *1* and *2* with filaments aligned perpendicular to the plane of section. Elsewhere filaments may run at right angles (*3*) to those in *1* and *2*, or they may run in other directions as at *4*. Border of epidermal cells (*E*) is highly folded. Melanophore (*ML*) abuts against under side of basement membrane. Magnification: × 8090.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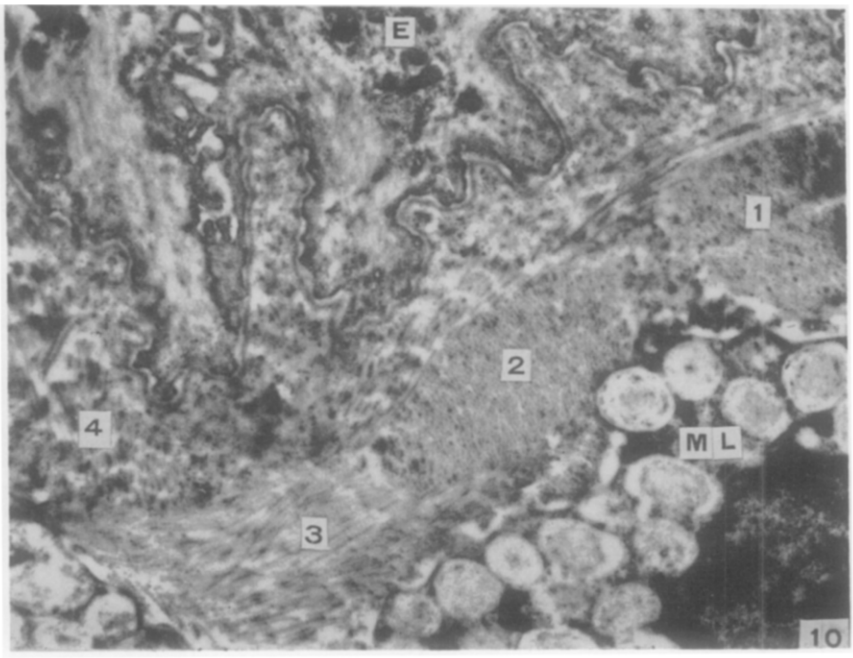
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filaments that might have been left behind as the basement (dermal) lamella separated from epidermis.

The morphology of the basement membrane of a full-grown adult frog (Fig. 10) is similar to that of the newly metamorphosed frog, although bundles of filaments appear to be aligned in more than two directions. The zone of the membrane just below the folded border of the epidermis looks less fibrillar than the rest of the membrane in Fig. 10. This appearance may be an artifact or may indicate that the zone contains some granular, unpolymerized units. Folding of the dermal border of the basal epidermal cells begun during metamorphosis (Figs. 7, 8, 9) has progressed further in the adult. Perhaps the greater surface exposed to the dermis by this folded border is important for the considerable transport of metabolites that must occur for maintenance of the thickened adult epidermis. Basal cells of the Malpighian layer of the adult skin have a submicroscopic morphology suggestive of active metabolism. Endoplasmic reticular membranes and mitochondria are abundant throughout the cytoplasm. The conspicuous bundles of tonofilaments so characteristic of basal epidermal cells in the larva are absent from the adult basal cells. Metamorphic changes within the epidermis are being investigated further.

DISCUSSION

From the work of Etkin (1950) it is known that the skin of anurans becomes responsive to thyroxine about the time when the operculum starts to develop (Shumway stage 23). Etkin (1935, 1955) believes that the normal sequence of events in metamorphosis is controlled by the gradual rise and eventual decline in the level of thyroid hormone circulating in the blood. If one exposes a larva to a high external concentration (Etkin, 1935) or to a high local dose (Kollros and Kaltenbach, 1952), certain structures may respond at abnormal rates. For example, the tail may shorten precociously. The effects of the natural thyroid hormones (Shellabarger and Brown, 1959) or of their analogs (Money *et al.*, 1958) on the submicroscopic structure of the integument have yet to be determined.

In any consideration of the development of the basement lamella, we are confronted with the problem of fibrillogenesis. Electron microscopic observations of the present and previous investigations (Weiss and Ferris, 1954b, 1956; Kemp, 1959) make it likely that

mesenchymal cells contribute the structural units which are somehow assembled into the collagenous filaments of the basement lamella. Tissue culture experiments in several laboratories prove that fibroblasts (Robbins *et al.*, 1955; Porter and Pappas, 1959) or osteoblasts (Fitton-Jackson and Smith, 1957) can produce collagen fibers *in vitro*. The exact mechanism of development of collagen fibrils is not known, but the observations of Porter and Pappas (1959) provide evidence that unit fibrils first form at the surface of fibroblasts and then grow by accretion of units of monomeric collagen secreted into the extracellular environment. The role of the epidermis in development of the larval basement lamella is not clear. Weiss and Ferris (1954b) consider that the epidermis has some organizing influence on the alignment of collagen filaments that underlie it. Porter and Pappas (1959) suggest that material for fiber development in the basement membrane "seems to be organized and contributed by the basal cells of the epidermis."

There are at least two different types of mesenchyme cells in the subcutaneous connective tissue of the anuran larva. One type, which is judged to be a melanoblast or melanophore, is usually packed with vesicles and may contain pigment granules. In its larval position below the basement lamella, it is probably what Elias (1939, 1942; Elias and Shapiro, 1957) classifies as a subcutaneous melanophore. Elias's drawing of adepidermal nonpigmented melanophores found in tadpoles of a semialbino strain of *Bombinator* (Elias, 1939) closely resembles the electron microscopic appearance of this first type of mesenchyme cell. The second type of connective tissue cell, probably a fibroblast, is the kind that first invades and later becomes embedded in the basement lamella (Fig. 4). It is distinguished by elaborate development of endoplasmic reticular cisternae (cf. Fig. 23, Porter and Pappas, 1959). This type of cell is probably the source of the tropocollagen units (Gross, 1956) presumed to be produced by fibroblasts. Robbins and associates (1955) call attention to certain acidophilic granules in the cytoplasm of cultured fibroblasts. From the evidence that these granules are greatly diminished in cultures exposed to anticollagen serum, they suggest that a precursor of collagen may be formed in them.

In addition to its possible role in synthesizing tropocollagen, the supposed fibroblast seems to digest its way through the larval basement lamella, probably by means of a lytic enzyme (Weiss and

Ferris, 1954b). A number of hydrolases are known to be associated with the microsome fraction, some of them possibly more specifically in the lysosomes, of mammalian tissues (Hogeboom *et al.*, 1957). Perhaps the abundant endoplasmic reticulum, chief source of the microsome fraction, within cells invading the basement lamella is a morphological indication that they are synthesizing lytic enzymes as well as monomeric collagen units.

Weiss' discussion (Weiss and Ferris, 1954b; Weiss, 1956) remains the best speculation on the problem of orthogonal alignment of the layers of the larval basement lamella. It is evident from the study reported here, however, that the factors creating the larval pattern of orthogonal layers do not control development of the adult basement membrane. One difference between larva and adult is that the larval epidermis has a smooth inner border, probably reflecting the firm cortex of its basal cells, while the adult epidermis has a folded border, possibly indicative of greater cortical plasticity in its basal cells. The differing geometrical relationships between mesenchyme cells and the underside of the epidermis in larva and adult may provide a clue to their differing basement membranes. Mesenchyme cells aggregate against the inner side of the developing larval membrane and come to form a fairly tight layer there, whereas connective tissue cells of the stratum spongiosum of the metamorphosing or adult frog are more loosely arranged and probably move about more freely. Other factors that may influence the pattern of the basement membrane include rate of delivery of precursor units by fibroblasts, rate of polymerization of these units, and the intrinsic or extrinsic influences affecting orientation of collagen filaments. The role of the ground substance in membranogenesis remains obscure, although Hall (1959) refers to earlier work indicating that treatment of collagen "with reagents specific for polysaccharides indicates that at the intact-fiber level polysaccharide is associated with the collagen in such an intimate fashion as to affect its physical stabilization."

After separation of the larval basement lamella from epidermis, it is possible that part of the membrane persists in the form of disassembled units of collagen and ground substance within the subepidermal extracellular space. Repolymerization of these units could initiate membranogenesis of the adult type of basement membrane. Probably the bulk of the filaments in the adult basement membrane,

however, are derived from new tropocollagen units delivered by active fibroblasts and added to the existing framework of the membrane. Reassembly of dissociated units may possibly occur also within the dermal lamella after it separates from epidermis. If thickening of this membrane (stratum compactum) occurs during growth to adulthood, however, it presumably proceeds by assembly from precursor units newly synthesized. To distinguish between reassembly of old depolymerized units and initial assembly of units newly delivered to intercellular space, either for construction of the adult basement membrane or growth of the dermal lamella, will be difficult.

SUMMARY

The basement lamella in the head of a 75-mm tadpole of *Rana pipiens* contained 46 orthogonally aligned layers, while the basement lamella over the muscular part of the proximal end of the tail in the same animal had about 32 layers.

Invasion of the basement lamella by subdermal mesenchyme cells was first detected in the head skin of a tadpole at Taylor-Kollros stage XI. Invading cells, which appear to be fibroblasts, move upward toward the epidermis, then may spread horizontally between layers of the basement lamella. Mesenchyme cells are abundant within the membrane by stage XIV. Invasion of the basement membrane does not occur in the tail.

At about stage XIV or XV, the basement lamella of head skin becomes detached from the epidermis. Connective tissue cells, principally fibroblasts and melanophores, then move into the subepidermal space and epidermal glands grow into it. The layer thus created is the stratum spongiosum. The basement lamella, which may now be called the dermal lamella, becomes the inner layer of the dermis, the stratum compactum.

A new basement membrane develops under the epidermis after the larval lamella becomes detached. At stages during resorption of the tail, the basement membrane in the head consists only of granules or short filaments embedded in ground substance. Filaments are again abundant in the membrane by stage XXV when metamorphosis is complete.

The adult frog retains the type of basement membrane developing at stage XXV. Filaments are grouped into bundles that appear to

run in several directions beneath the highly folded border of basal epidermal cells. The regular layered pattern of the larva is not re-established.

I wish to thank Dr. Paul A. Weiss for inspiring this investigation, which was actually a continuation of work started in his laboratory. I am also indebted to Dr. Jerry J. Kollros for helpful suggestions. My research assistant Mrs. Elizabeth A. Gibbons has shown extraordinary skill in preparing tissues for electron microscopy, and my assistant Miss Marilyn A. Cortright has rendered valuable service in preparing slides for light microscopy.

REFERENCES

ALLEN, B. M. (1938). The endocrine control of amphibian metamorphosis. *Biol. Revs. Cambridge Phil. Soc.* **13,** 1–19.

CHARLES, R., and SMIDDY, F. G. (1957). The tonofibrils of the human epidermis. *J. Invest. Dermatol.* **29,** 327–338.

DAWSON, A. B. (1920). The integument of *Necturus maculosus*. *J. Morphol.* **34,** 487–589.

ELIAS, H. (1939). Die adepidermalen Melanophoren der Discoglossiden, ein Beispiel für den phylogenetischen Funktionswechsel eines Organs, seinen Erstaz in der früheren Funktion durch ein neues Organ und sein schliessliches Verschwinden. *Z. Zellforsch. u. mikroskop. Anat. Abt. A* **29,** 448–461.

ELIAS, H. (1942). Chromatophores as evidence of phylogenetic evolution. *Am. Naturalist* **76,** 405–414.

ELIAS, H., and SHAPIRO, J. (1957). Histology of the skin of some toads and frogs. *Am. Museum Novitates No.* **1819,** 1–27.

ETKIN, W. (1935). The mechanisms of anuran metamorphosis. I. Thyroxine concentration and the metamorphic pattern. *J. Exptl. Zool.* **71,** 317–340.

ETKIN, W. (1950). The acquisition of thyroxine-sensitivity by tadpole tissues. *Anat. Record* **108,** 541.

ETKIN, W. (1955). Metamorphosis. *In* "Analysis of Development" (B. H. Willier, P. A. Weiss, and V. Hamburger, eds.), Sect. XII. Saunders, Philadelphia, Pennsylvania.

FITTON-JACKSON, S. (1956). The morphogenesis of avian tendon. *Proc. Roy. Soc.* **B144,** 556–572.

FITTON-JACKSON, S., and SMITH, R. H. (1957). Studies on the biosynthesis of collagen. I. The growth of fowl osteoblasts and the formation of collagen in tissue culture. *J. Biophys. Biochem. Cytol.* **3,** 897–912.

GROSS, J. (1956). The behavior of collagen units as a model in morphogenesis. *J. Biophys. Biochem. Cytol. Suppl.* **2,** 261–274.

GUDERNATSCH, J. F. (1912). Feeding experiments on tadpoles. I. The influence of specific organs given as food on growth and differentiation. A contribution to the knowledge of organs with internal secretion. *Arch. Entwicklungsmech. Organ.* **35,** 457–483.

GUDERNATSCH, J. F. (1914). Feeding experiments on tadpoles. II. A further

contribution to the knowledge of organs with internal secretion. *Am. J. Anat.* **15,** 431–480.

HALL, D. A. (1959). The fibrous component of connective tissue with special reference to the elastic fiber. *Intern. Rev. Cytol.* **8,** 211–251.

HELFF, O. M., and STARK, W. (1941). Studies on amphibian metamorphosis. XVIII. The development of structures in the dermal plicae of *Rana sylvatica*. *J. Morphol.* **68,** 303–327.

HOGEBOOM, G. H., KUFF, E. L., and SCHNEIDER, W. C. (1957). Recent approaches to the cytochemical study of mammalian tissues. *Intern. Rev. Cytol.* **6,** 425–467.

KEMP, N. E. (1959). Development of the basement lamella of larval anuran skin. *Develop. Biol.* **1,** 459–476.

KEMP, N. E. (1960). Fate of larval basement lamella in the skin of *Rana pipiens* during metamorphosis. *Anat. Record* **136,** 222.

KOLLROS, J. J. (1959). Thyroid gland function in developing cold-blooded vertebrates. *In* "Comparative Endrocrinology" (A. Gorbman, ed.), pp. 340–350. Wiley, New York.

KOLLROS, J. J., and KALTENBACH, J. C. (1952). Local metamorphosis of larval skin in *Rana pipiens*. *Physiol. Zoöl.* **25,** 163–170.

LINDEMAN, V. F. (1929). Integumentary pigmentation in the frog, *Rana pipiens*, during metamorphosis, with especial reference to tail-skin histolysis. *Physiol. Zoöl.* **2,** 255–268.

LYNN, W. G., and WACHOWSKI, H. (1951). The thyroid gland and its functions in cold-blooded vertebrates. *Quart. Rev. Biol.* **26,** 123–168.

MONEY, W. L., MELTZER, R. L., YOUNG, J., and RAWSON, R. W. (1958). The effect of change in chemical structure of some thyroxine analogues on the metamorphosis of *Rana pipiens* tadpoles. *Endocrinology* **63,** 20–28.

PORTER, K. E., and PAPPAS, G. D. (1959). Collagen formation by fibroblasts of the chick embryo dermis. *J. Biophys. Biochem. Cytol.* **5,** 153–166.

ROBBINS, W. C., WATSON, R. F., PAPPAS, G. D., and PORTER, K. R. (1955). Some effects of anti-collagen serum on collagen formation in tissue culture: a preliminary report. *J. Biophys. Biochem. Cytol.* **1,** 381–384.

ROSE, S. M. (1960). A feedback mechanism of growth control in tadpoles. *Ecology* **41,** 188–199.

RUGH, R. (1934). Induced ovulation and artificial fertilization in the frog. *Biol. Bull.* **66,** 22–29.

SALPETER, M. M., and SINGER, M. (1959). The fine structure of the adepidermal reticulum in the basal membrane of the skin of the newt, *Triturus*. *J. Biophys. Biochem. Cytol.* **6,** 35–40.

SALPETER, M. M., and SINGER, M. (1960). Differentiation of the submicroscopic adepidermal membrane during limb regeneration in adult *Triturus*, including a note on the use of the term basement membrane. *Anat. Record* **136,** 27–40.

SELBY, C. C. (1955). An electron microscope study of the epidermis of mammalian skin in thin sections. I. Dermo-epidermal junction and basal cell layer. *J. Biophys. Biochem. Cytol.* **1,** 429–444.

SELBY, C. C. (1957). An electron microscope study of thin sections of human

skin. II. Superficial cell layers of footpad epidermis. *J. Invest. Dermatol.* **29,** 131–149.

SHELLABARGER, C. J., and BROWN, J. R. (1959). The biosynthesis of thyroxine and 3:5:3′-triiodothyronine in larval and adult toads. *J. Endocrinol.* **18,** 98–101.

SPEIDEL, C. C. (1926). Studies of hyperthyroidism. IV. The behavior of the epidermal mitochondria and the pigment in frog tadpoles under conditions of thyroid accelerated metamorphosis and of regeneration following wound infliction. *J. Morphol. and Physiol.* **43,** 57–79.

STEARNER, S. P. (1946). Pigmentation studies in salamanders, with especial reference to the changes at metamorphosis. *Physiol. Zoöl.* **19,** 375–404.

TAYLOR, A. C., and KOLLROS, J. J. (1946). Stages in the normal development of Rana pipiens larvae. *Anat. Record* **94,** 7–24.

WEED, I. G. (1934). Cytological studies of the epidermis of *Rana pipiens* and *Rana clamitans* tadpoles, with special reference to the figures of Eberth. *J. Morphol.* **56,** 213–229.

WEISS, P. (1956). The compounding of complex macromolecular and cellular units into tissue fabrics. *Proc. Natl. Acad. Sci. U. S.* **42,** 819–830.

WEISS, P., and FERRIS, W. (1954a). Electronmicrograms of larval amphibian epiderms. *Exptl. Cell Research* **6,** 546–549.

WEISS, P., and FERRIS, W. (1954b). Electron-microscopic study of the texture of the basement membrane of larval amphibian skin. *Proc. Natl. Acad. Sci. U. S.* **40,** 528–540.

WEISS, P., and FERRIS, W. (1956). The basement lamella of amphibian skin. Its reconstruction after wounding. *J. Biophys. Biochem. Cytol.* **2,** 275–282.