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THE ULTRASTRUCTURE OF THE SKIN OF HUMAN EMBRYOS

VII. *Formation of the Apocrine Gland*

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Abstract. The formation of the intrafollicular and intradermal apocrine duct in 15- to 18-week-old human embryos were studied in the axillary region using the electron microscope. The intrafollicular lumen was formed initially by an accumulation of variously-sized clear vesicles and intracellular dissolution of the cytoplasm, microvilli formation and pinching off of these villi. Larger cavities were produced by coalescence of the small ones. A continuous ductal space was produced by connection of intracellular spaces thus produced in neighboring cells. A small number of multivesiculated dense bodies were found in the luminal cells but neither keratohyaline granules nor keratinized cells were seen. The intradermal duct was formed by separation of the apposed luminal cells. Tight junction and terminal bar sealed the luminal end of the lateral junctions of the luminal cells. In contrast to the adult intradermal segment, abundant glycogen was not found in the basal cells.

As a continuation of a series of investigations on the ultrastructure of human embryos (3–6, 11, 12), this report deals with the embryonic development of the apocrine gland. The apocrine gland develops in many hair follicles of the axilla, umbilicus, anogenitalia, mammary areola, scalp and eyebrow. They may or may not develop in the hair follicles of other areas (14, 16, 17). After reaching a certain degree of maturation they undergo atrophy or may stay non-functional until puberty (14). In previous studies (11, 12), I found that the apocrine gland anlage was associated with some hairs of the scalp and the eyebrow as Pinkus reported (17). However, I chose axillary apocrine glands for this study because many more hair follicles developed an apocrine gland anlage in the axilla than in the scalp and eyebrow and because the axilla is the physiological site of their development and functioning. To the best of my knowledge, no previous report has been published on the embryonic development of this gland, although the adult apocrine duct (2, 7) and secretory segments (1, 8, 13, 15, 19, 20) were investigated by several workers using the electron microscope.

The main purpose of this study was to obtain more information about the mode of formation of the ductal lumen in embryonic, immature apocrine gland, both in the intrafollicular portion and the intradermal segment, and further to compare the data with those of the corresponding portions of the eccrine sweat gland. The immediate application of such information would be to the proper classification of various immature tumors of sweat gland origin into eccrine and apocrine types.

MATERIALS AND METHODS

Three Negroid embryos aborted between the 15th and 18th week of gestation (menstrual age) were used. The skin was removed from the axillary regions as soon as possible. Specimens were cut into 1 mm^3 pieces, fixed in 1% osmic acid in 1/10 M Veronal buffer. Dehydration was carried out through graded concentrations of ethanol and propylene oxide. Araldite was used for embedding.

Prior to thin-sectioning, 1 μ-thick sections were cut from each block and stained with 0.25% Azure-B on a hot plate. Only those glands which were cut longitudinally through their long axis and in which a definite connection with the follicle just above the sebaceous gland anlage was confirmed were utilized. This was necessary because many of the secondary hair germs emerged from the follicle-stratum germinativum junction and the eccrine sweat gland germs were also emerging from the stratum germinativum. In this study five apocrine glands satisfied these two criteria. Thin sections, 400–600 A, were cut serially through the entire thickness of the structure in

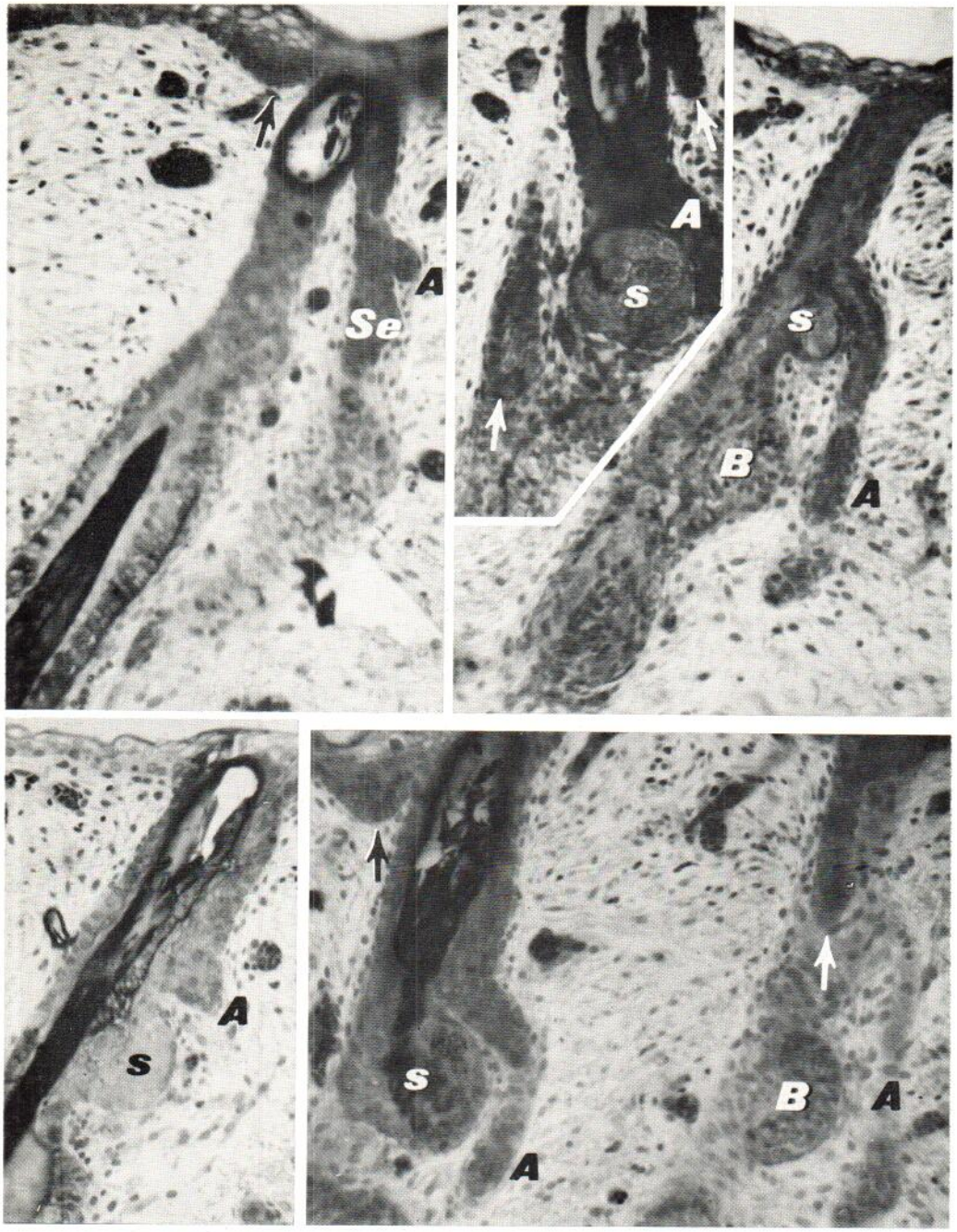

Fig. 1. Apocrine gland anlagen (A) are developing from primary as well as secondary (*Se*) epithelial germs at some distance from the epidermis, whereas the *secondary epithelial germs (arrows)* are budding very close to the primary germs and could easily be mistaken for the apocrine gland anlagen. *B*, bulge, *s*, sebaceous gland anlage. 18-week-old embryo, axilla. Thick sections cut from Araldite-embedded materials. × 340.

a Porter-Blum MT-2 Ultramicrotome. Sections were picked up on uncoated grids or on grids covered with a thin collodion membrane, stained with 1% uranyl acetate in 50% ethanol and, while half-dried, re-stained with Reynolds' lead citrate (18). Stained sections were examined with an Hitachi HU-11C high resolution electron microscope.

The terminology used in this report is the same as

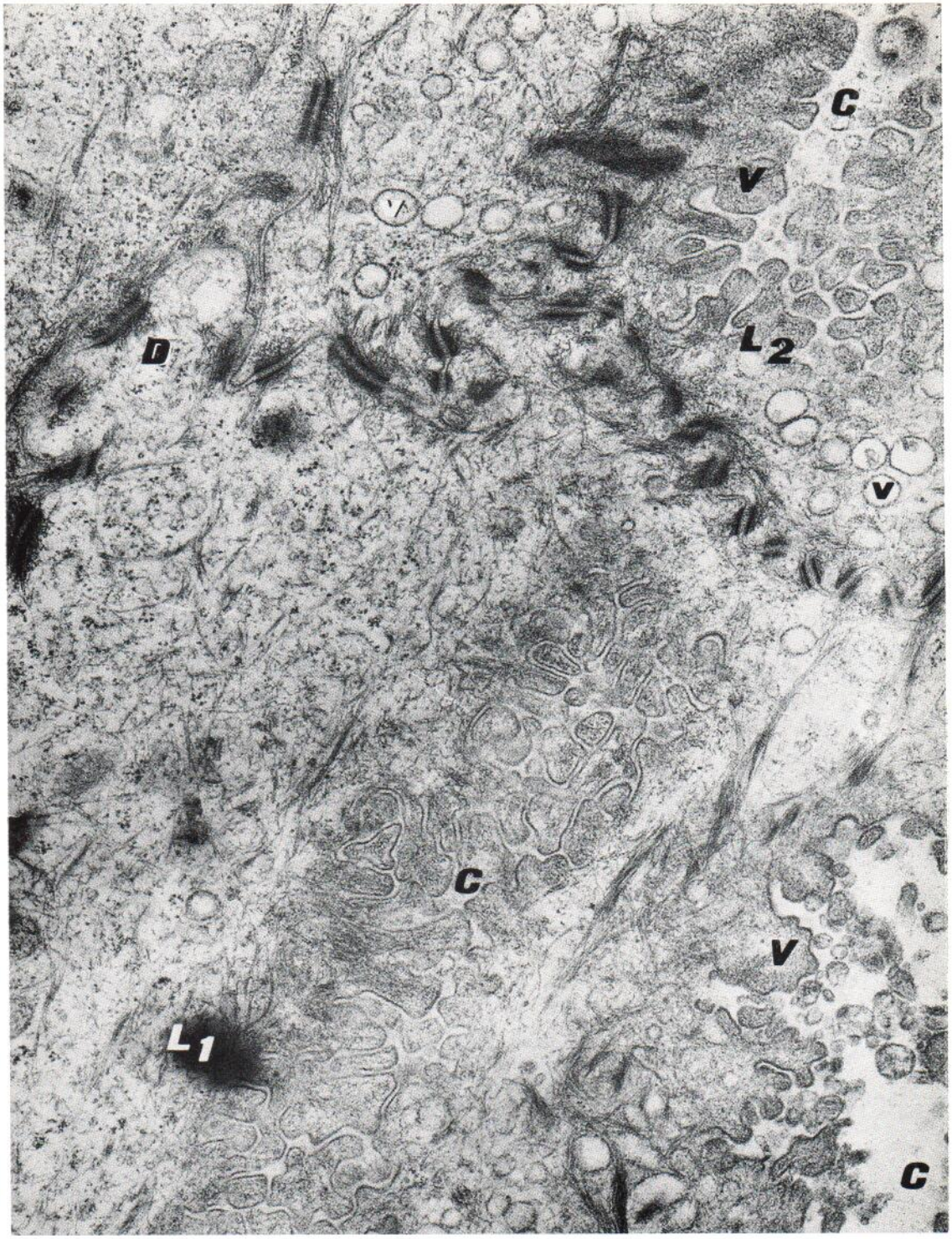

Fig. 2. Intrafollicular duct formation is taking place in two luminal cells (L_1, L_2) by the formation of intracytoplasmic cavities (*C*). Numerous clear vesicles (*v*) are seen near and in the cavities. Desmosomes (*D*) are well developed. *V*, microvilli. 18-week-old embryo, axilla. × 38,600.

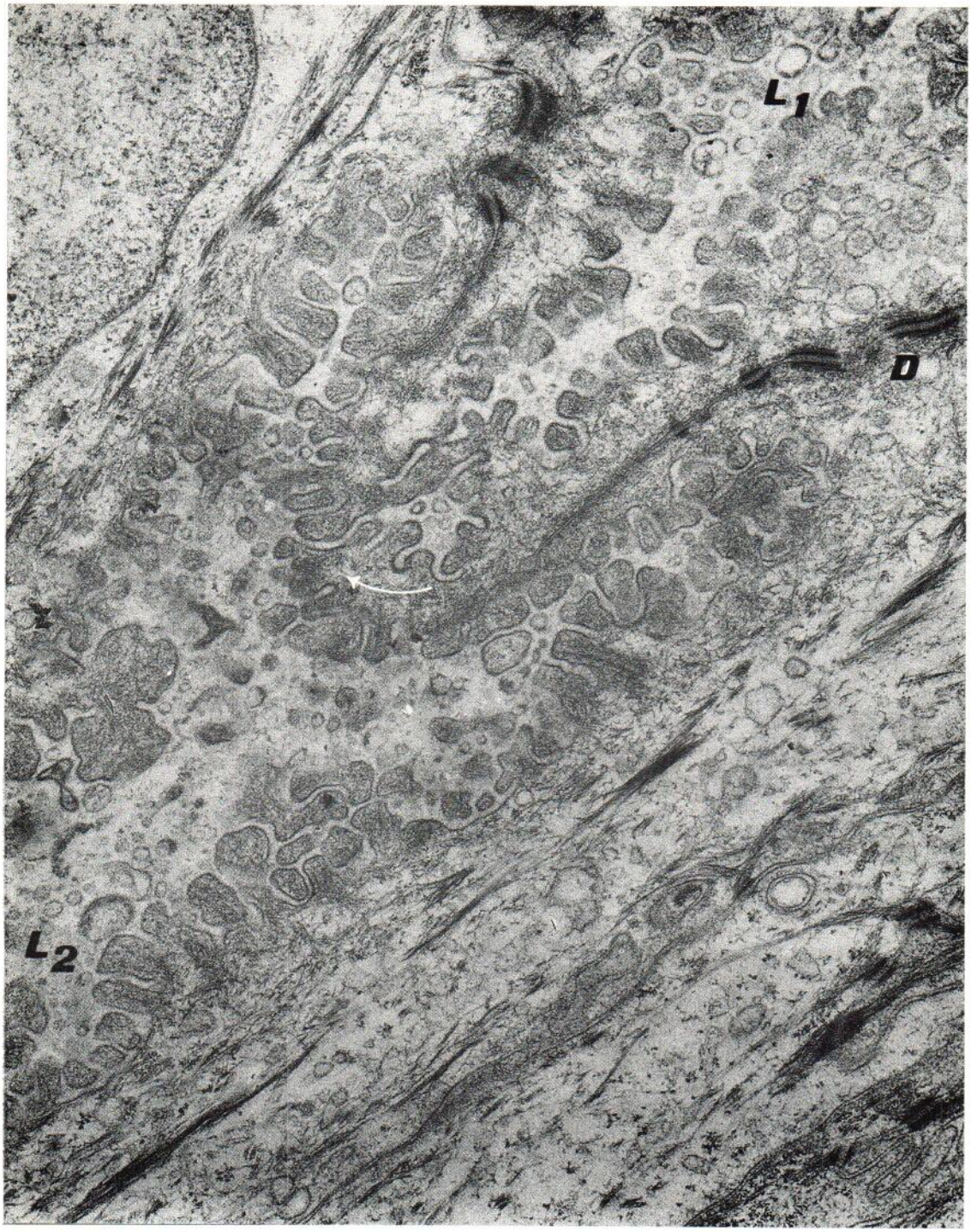

Fig. 3. Intrafollicular duct. Enlarged intracytoplasmic lumina of two neighboring cells (L_1, L_2) are about to merge at the arrow. Well-developed desmosomes (*D*) are seen. 18-week-old embryo, axilla. × 38,600.

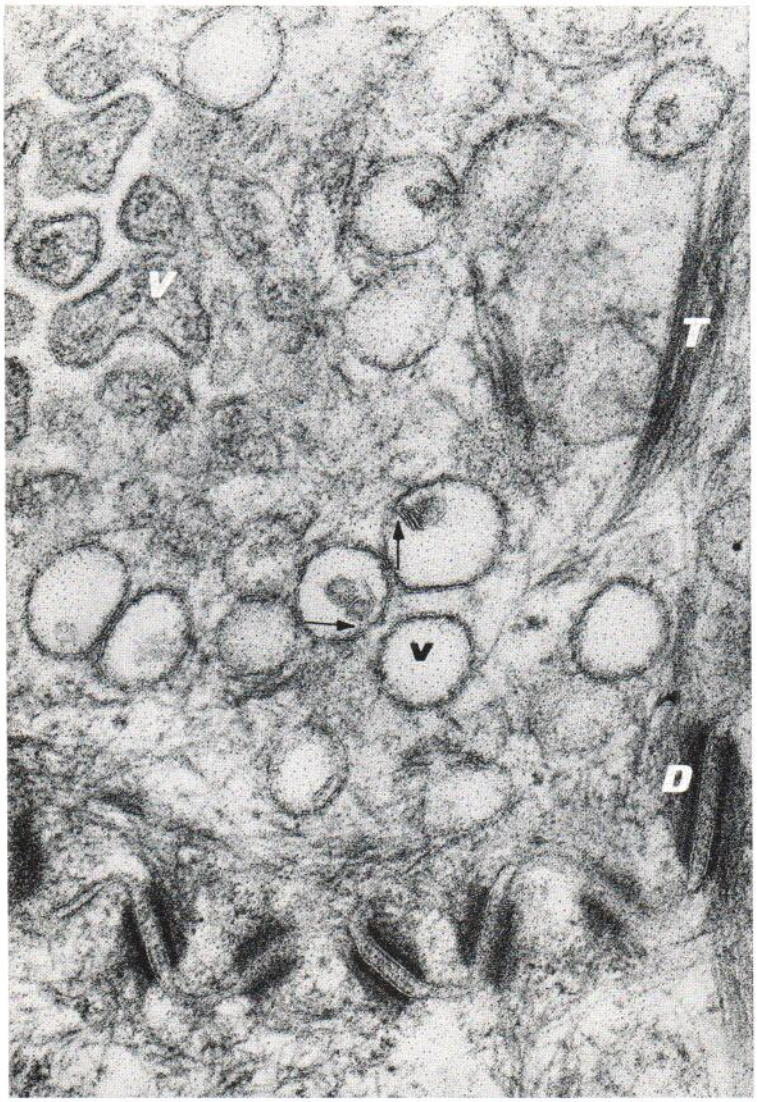

Fig. 4. Intrafollicular duct. Enlargement of the area labeled with a black *v* in Fig. 2. Vesicles (*v*) and the microvilli of the liminal border *(V)* are delimited by a trilaminar membrane. Some vesicles contain smaller vesicles or trilaminar membrane often contiguous with the wall of the vesicle (*arrows*). *T*, tonofilaments. 18-week-old embryo, axilla. × 100,000.

that used originally by Stöhr (21) and Felix Pinkus (16) as translated into English by Herman Pinkus (17), as well as that used by Herman Pinkus (17).

RESULTS

In the axilla of 15- to 18-week-old embryos, the apocrine gland anlage was formed just above the sebaceous gland anlage as a small swellling of the outer surface of the follicle. The developmental stage of the follicle was late hair peg to bulbous peg stage. The apocrine gland anlage developed at some distance from the basal layer of the epidermis and never merged with it (Fig. 1). Thus, it could easily be distinguished from the secondary epithelial germ which often grew as a club-shaped structure from the junction of the primary epithelial germ with the epidermis (Fig. 1). As the anlage grew, it first projected into the perifollicular mesenchyme at right angles to the long axis of the follicle and then bent itself downward as if it embraced the upper margin of the sebaceous gland anlage (Fig. 1). It continued to descend until the level of the bulge was reached (Fig. 1). In this study serial sections did not reveal any further growth.

By the time the tip of the gland passed the sebaceous gland anlage, the ductal lumen was regularly noticed in the intradermal portion by use

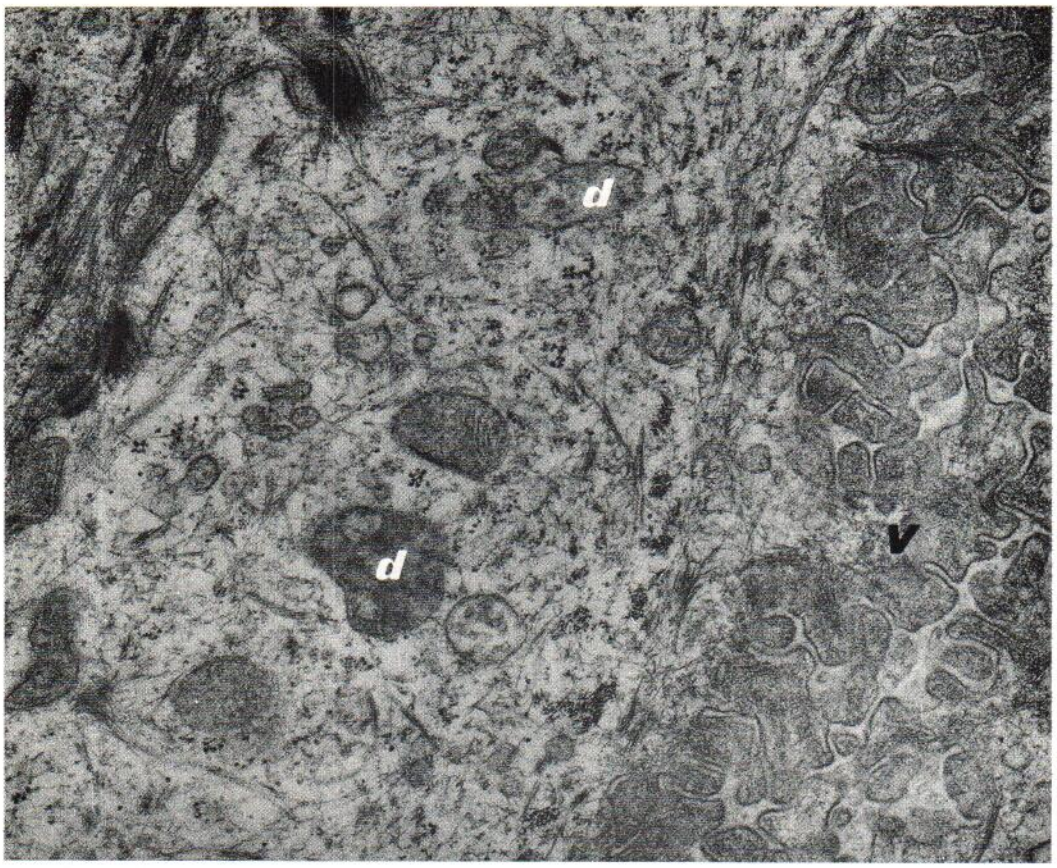

Fig. 5. Intrafollicular duct. Multivesiculated dense bodies (*d*) are seen in the periluminal cytoplasm. *V*, luminal microvilli. 18-week-old embryo, axilla. × 38,600.

of the electron microscope. The intrafollicular portion of the duct was found only in two relatively elongated glands.

I. *Intrafollicular Duct.* One or two foci of lumen formation were observed in 400–600 Å thick sections within the mass of the cells composing the intrafollicular portion of the gland. These cells were connected to each other with a large number of desmosomes and contained a small number of tonofilaments. Individual desmosomes were relatively well-developed in comparison with the rest of the follicular cells. The amount of glycogen present was small.

The first sign of lumen formation was the appearance of numerous clear vesicles of various sizes (500–1,000 Å) within the cytoplasm of the future luminal cells. Simultaneously, multiple small cavities were produced in the area where these vesicles were aggregated. These cavities were lined with numerous microvilli (Fig. 2). The villi pinched off into the cavities continuously and thus the size of the individual cavities increased. Several small cavities coalesced to form a large intracellular lumen. Similar lumina produced in the neighboring cells merged together to form a larger space and eventually a continuous ductal lumen (Fig. 3).

The vesicles seen in the lumen-producing cells were delimited with a unit membrane (Fig. 4). They occasionally contained small bodies which were also membrane-bound (Fig. 4). Only a small number of multivesiculated dense bodies, i.e., lysosomes identical to those found in the embryonic intraepidermal eccrine duct formation (3), were observed in the lumen-forming cells (Fig. 5).

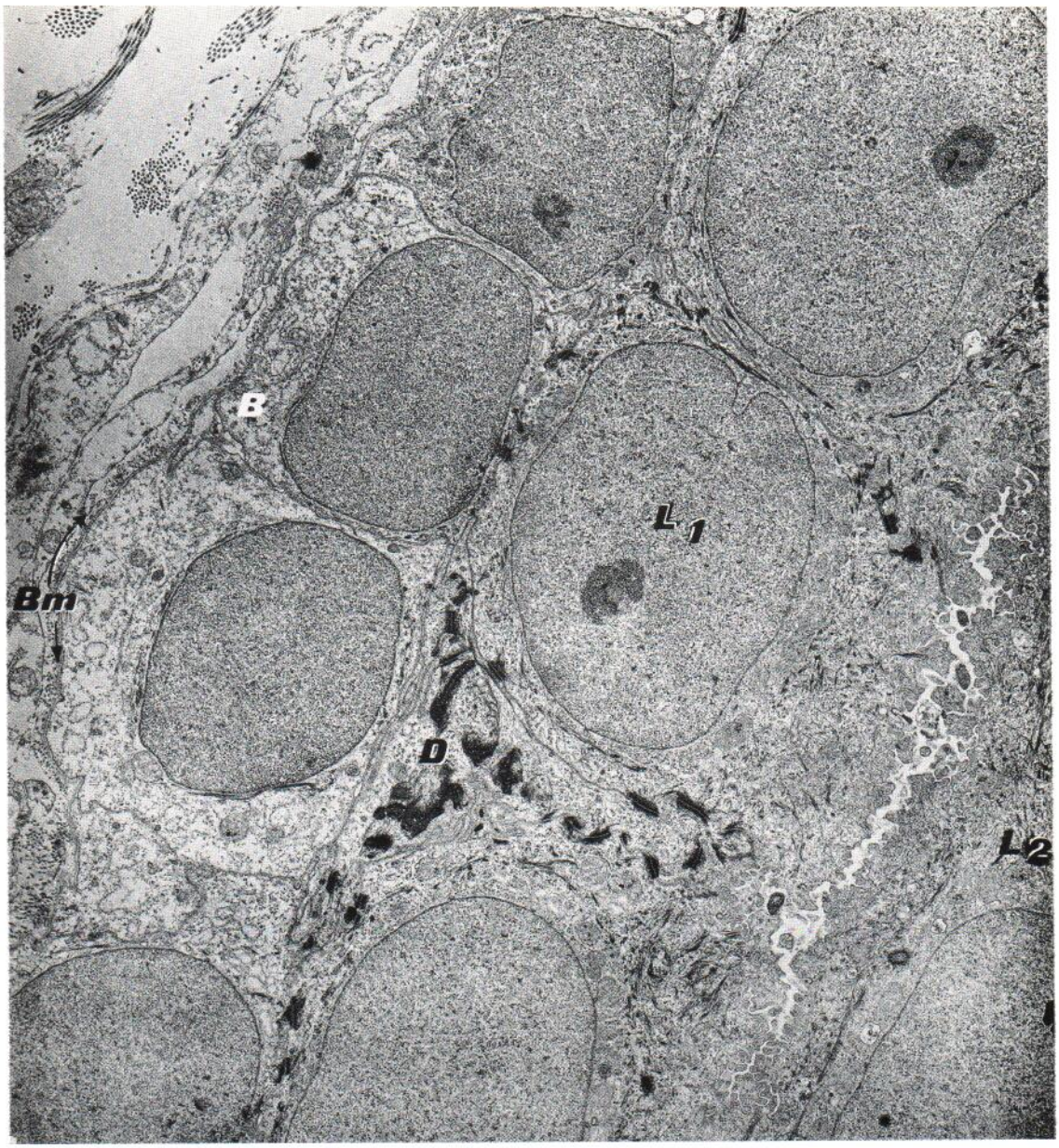

Fig. 6. Intradermal duct formation is seen as the result of a rather clear-cut separation of the contact between two apposed luminal cells (L_1, L_2). The lateral border of the neighboring luminal cells is sealed by well-developed desmosomes (*D*), but intricate invagination of the plasma membranes is not seen. The peripherally located basal cells (*B*) do not contain glycogen. *Bm*, basement membrane. 18-week-old embryo, axilla. × 8,300.

Neither keratohyaline granules nor keratinized cells were seen. No connection of the duct with the infundibular part of the hair canal was observed.

II. *Intradermal Duct.* The extrafollicular portion was composed of two central rows of apposing cells and one layer of the peripheral (basal) cells (Fig. 6). These central cells, the future luminal cells, were also connected with a number of well-developed desmosomes (Fig. 6) in contrast to the poorly developed desmosomal contact in the peripheral cells and the rest of the follicle.

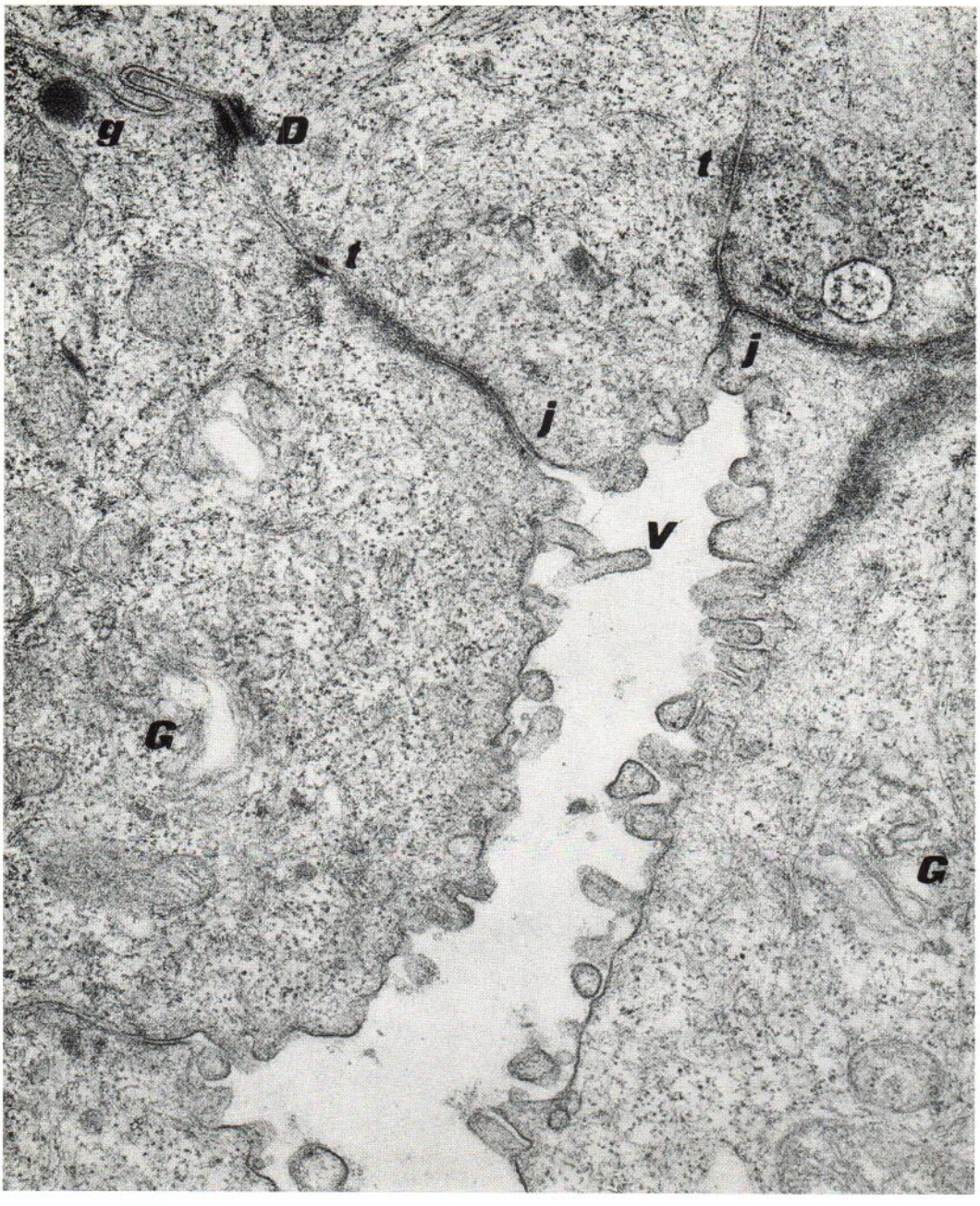

Fig. 7. The secretory type of luminal cell contains well-developed Golgi apparatus (*G*), occasional dense (secretory?) granules (*g*) and sparsely distributed, rather tall luminal microvilli (*V*). No periluminal band of tonofilaments (see Fig. 8) is present. Desmosomes (*D*), terminal bars (*t*) and tight junctions (*j*) seal the luminal end of the lateral junction of the luminal cells. From the round terminal end of a gland. 18-week-old embryo, axilla. × 37,000.

The first sign of the lumen formation was a separation of the desmosomal contact between two or three apposed central cells. A clear-cut separation between the two rows of the central cells soon ensued over the entire length of the intradermal part of the gland. Along the border of the

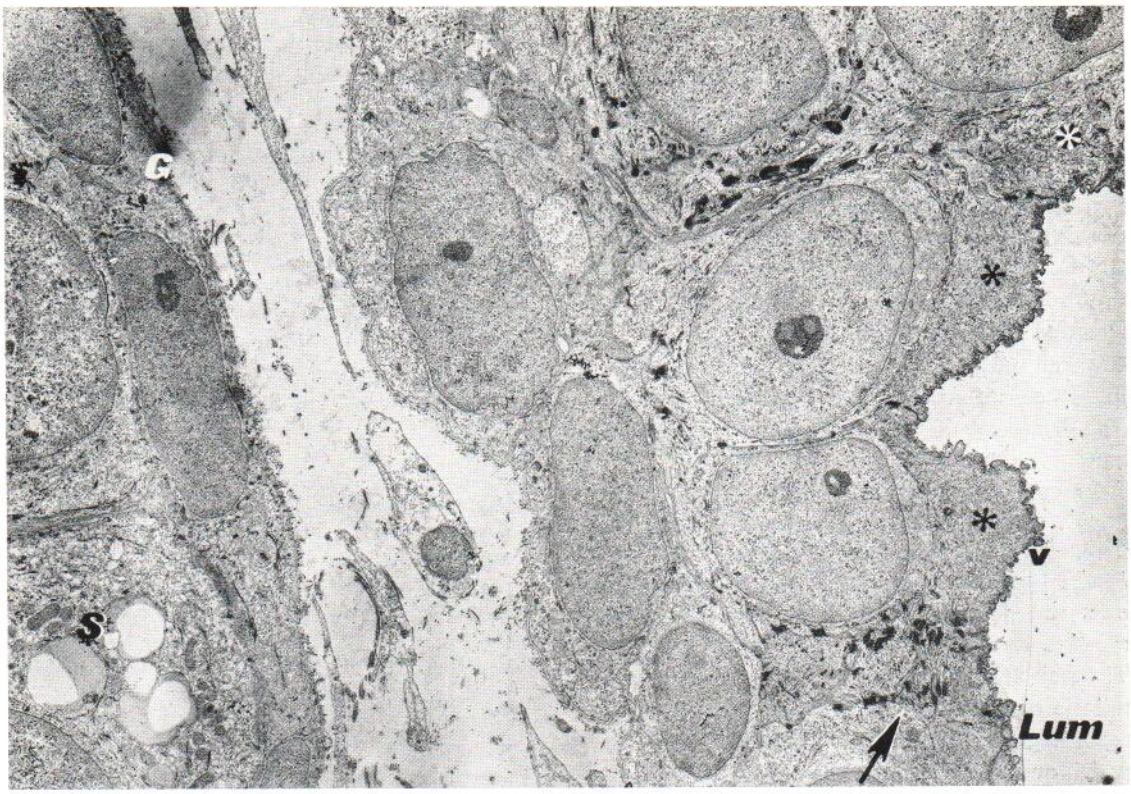

Fig. 8. A large lumen (Lum) is surrounded by the villous border (*v*) of luminal cells in which a periluminal band of tonofilaments (*) is well developed. The lateral borders of the luminal cells are intricately invaginating into each other and sealed with a number of desmosomes (*arrow*). *G* germinative cell of the sebaceous gland; *S*, sebaceous cell. 18-week-old embryo, axilla. × 4,100.

separated plasma membranes, a number of microvilli were formed and lined the new duct (Fig. 6). Tight junction and terminal bar usually sealed the luminal end of the cell-to-cell contact between neighboring luminal cells (Fig. 7). Convoluted infolding of the lateral plasma membranes of the luminal cells and the periluminal band of tonofilaments, as was observed in the adult duct (2, 7), were poorly developed (Fig. 6), except for fairly developed glands in which a large open lumen was formed (Fig. 8). Accumulation of neither vesicles nor multivesiculated dense bodies was seen in the luminal cells. In the glands studied, no secretory segment was developed. However, some luminal cells toward the round, terminal end of the gland contained a moderately developed Golgi apparatus, a few dense granules and rather sparse but long luminal villi (Fig. 7), thus exhibiting some similarity to the adult apocrine secretory cells (1, 8, 13, 15).

DISCUSSION

The method of formation of the intrafollicular duct in the embryonic apocrine gland was found to be similar to that employed by the intraepidermal eccrine sweat duct during early embryonic life (3). In both instances the process begins with small intracytoplasmic cavity formation and a complete duct is produced by enlargement and coalescence of such cavities. On the other hand, the intradermal part of the apocrine and eccrine ducts is formed by a separation of cell-to-cell contact.

In syringoma (9) and eccrine poroma (10), for example, the presence of multivesiculated dense bodies (lysosomes) and intracytoplasmic cavity formation were found and this similarity of the lumen formation to the eccrine intraepidermal lumen formation was used as one of the criteria to classify these tumors into the eccrine type. From the above it is apparent that the criterion

of intracellular cavity formation cannot be used as freely as before because a similar process was found in embryonic apocrine intrafollicular duct formation. However, if one stresses differences such as the predominance of clear vesicles instead of multivesiculated dense bodies and the lack of keratohyaline granules and complete keratinization in both embryonic and adult (2) apocrine intrafollicular luminal cells, one may still say that there are some differences between eccrine and apocrine intra-epithelial lumen formation and further that the predominance of these organelles in the cells forming the eccrine intra-epidermal duct helps classification of the so-called eccrine type of tumours.

In comparison with the adult apocrine duct (2, 7), the early embryonic duct lacks the periluminal tonofilament band, intricate convolutions of the lateral plasma membranes of the luminal cells and glycogen in the peripheral basal cells, most probably due to the immaturity of the cells. The lack of glycogen may be related to the active mitosis of the cells. Even in the adult structure, according to Charles (2), the intrafollicular duct which opens into the keratinized infundibular portion of the hair canal does not undergo keratinization. Such tumors as syringoma, in which not only numerous multivesiculated dense bodies but also keratinization of the luminal cells occur, may, therefore, still be considered to be of eccrine type even without considering histochemical similarities to the eccrine sweat duct (9, 10, 22). In this study the number and the stage of development investigated were limited and it is felt that further study, though very difficult, would be necessary to establish absolute criteria for the immature apocrine intrafollicular type of lumen formation.

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