in den telenzephalen Strukturen über die der dienzephalen an. – Ein weiterer Unterschied zwischen den Stammganglien besteht darin, dass in den dienzephalen Nuclei subthalamicus und endopeduncularis die Fermentreaktionen mit dem Auftreten der Morphodifferenzierung positiv werden, wogegen in den telencephalen Kerngebieten die Chemodifferenzierung erst kurze Zeit nach Anlage der Gebiete beginnt. Dadurch ist hier das Fortschreiten der Differenzierung von frontolateral nach kaudomedial besonders gut zu verfolgen. In den dienzephalen Teilen der Stammganglien ist die Entwicklung von kaudomedial nach frontolateral gerichtet. Auch in diesem Zusammenhang erweist sich das Pallidum als dienzephaler Teil. – Besondere Bedeutung hat die Entwicklung um den 10. Lebenstag. Zu dieser Zeit beginnen sich die bleibenden Aktivitätsrelationen zwischen den verschiedenen Arealteilen zu entwickeln, z.B. beim Pallidum zwischen Internum und Externum. Die 2. Lebenswoche dürfte auch in funktioneller Hinsicht für die Entwicklung der Stammganglien bedeutungsvoll sein, da etwa ab 10. Lebenstag eine Koordination der extrapyramidal gesteuerten Bewegungen beobachtet wird[5].

Summary. In foetal rat stem ganglia originating from the diencephalon, chemodifferentiation and morphogenesis take place earlier than in those from the telencephalon. Between 3 and 10 days postnatally, enzyme activities of all regions largely level up; later, the structures from the telencephalon show higher activities. Exceptions are acid phosphatase and thiamine pyrophosphatase, whose activities are higher in the stem ganglia from the diencephalon than in those from the telencephalon. The globus pallidus behaves in every respect like the structures from the diencephalon.

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[5] Mit Unterstützung durch die Deutsche Forschungsgemeinschaft.

Melanocyte Precursor Cells in the Hair Follicle Germ During the Dormant Stage (Telogen)

The melanocytes responsible for hair pigmentation have long been characterized as being visible in the hair bulb at a certain stage of the hair cycle, namely Anagen III–IV, when they begin actively producing melanin granules. The origin of this new generation of melanocytes, which arises periodically in adult mammalian skin, has been in question because neither typical melanocytes nor recognizable precursor cells have been observed in the dormant stage (telogen)[1] of untreated follicles.

Observations of the hair follicle during telogen and early anagen have been confined either to thick paraffin sections viewed with the light microscope, or thin sections, highly enlarged, viewed with electron microscope. In the first case the technique is not sufficiently refined to diagnose certain cell types. In the second case, 2 problems arise: (1) The high degree of magnification with the consequent limitation of the field obscures the

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topography; and (2) the lack of serial sections makes it easy to overlook single special cells within the hair germ or early bulb.

We have used the light microscope to study mouse skin fixed in glutaraldehyde-osmium, embedded in epon, and stained with alkaline toluidine blue. Care was taken in orientation of the tissue both in embedding and cutting. Sections were cut at 1.5–2 μ, and serial sections of as many as 30 sections were often obtained[2]. Material consisted of the dorsal skin of 39 mice of several strains (C57 BL/Ch, BUB/Wi, C_3Hp/Wi, DBA-1/Ch, and DS/Ch), at various ages and stages of the hair cycle. Both spontaneous anagen, and anagen induced by plucking were observed. Hair germs in telogen and Anagen I were drawn or photographed in serial sections.

The easily recognizable melanocytes of Anagen III–IV are clustered about the upper end of the dermal papilla, intimately associated with the developing cortical cells[3] (Figure F). The melanocytes are large dendritic cells having a rounded nucleus and markedly chromophobic cytoplasm[4]. The ratio of nucleus to cytoplasm is low, and numerous pigment granules are present in the cytoplasm and dendrites. The pigment granules differ in the case of the various strains of mice (none being present in the amelanotic melanocytes of the albino, BUB[5]), but are easily differentiated from other possible cell inclusions by the black-brown color. In addition, the newly forming intracellular pigment granules are of various sizes (but less than 0.5 μ) and various intensities of blackness, and thus differ from occasional clumps of larger blacker melanin often present in the dermal papilla, left over from a previous hair cycle[6].

Cells in the same location, i.e. in contact with the upper part of the dermal papilla, and similar to the above in all respects except that they lack pigment and have a greater abundance of cytoplasmic organelles, are frequent and conspicuous in sections of follicles in Anagen III (Figures D and E), and earlier in Anagen II (Figure C). During this time these cells are often seen in division (Figure C).

In Anagen I and in telogen they were not so immediately observable, and serial sections of entire hair germs had to be obtained and studied. In these cases it was always possible to find 1 or 2 clear dendritic cells per germ, touching the basement membrane between the germ and dermal papilla (Figures A and B). During telogen these cells are smaller, with fewer cytoplasmic organelles. The nucleus may be irregularly notched, and nuclear chromatin is inconspicuous.

Many other clear dendritic cells are present in the capsule and outer root sheath of the telogen hair follicle, as well as in the epidermis[7]. The production of pigment in Anagen III–IV, however, seems to be strictly limited

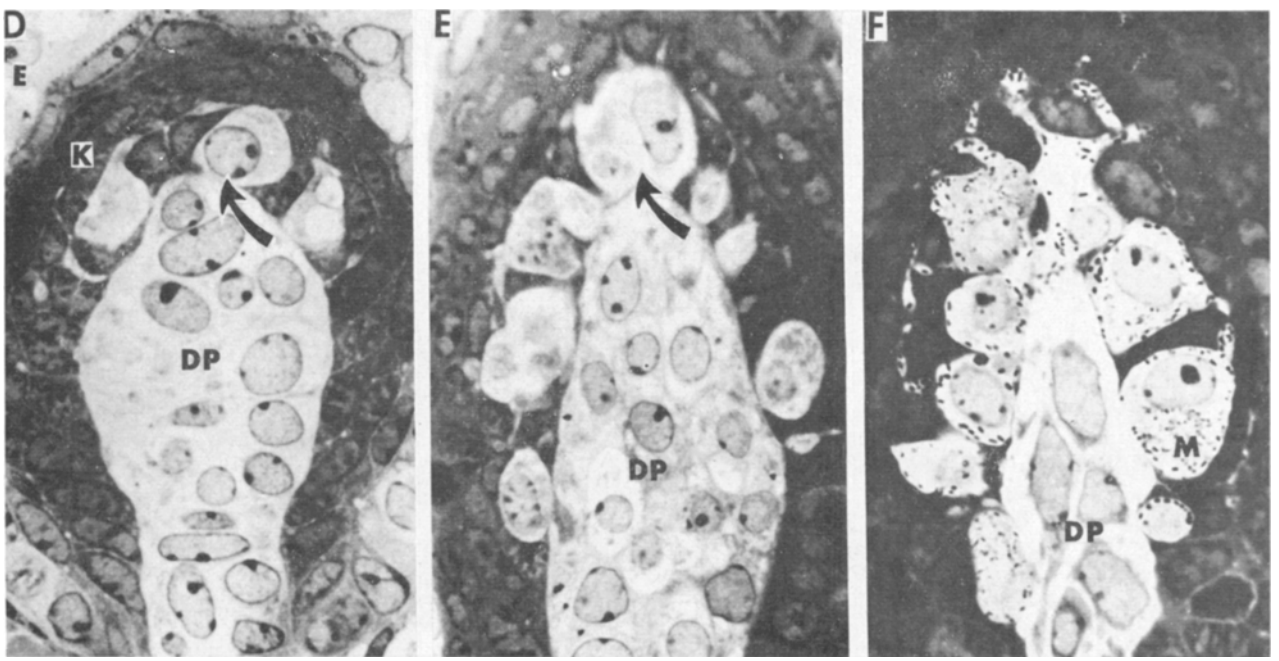
[2] We thank Mrs. MARGARET DUNN for careful and intelligent technical assistance.

[3] N. A. BARNICOT and M. S. C. BIRBECK, in *The Biology of Hair Growth* (Ed. W. MONTAGNA and R. A. ELLIS; Academic Press, New York 1958), p. 241.

[4] W. C. QUEVEDO JR. and H. B. CHASE, Anat. Rec. *129*, 87 (1957).

[5] W. K. SILVERS, Anat. Rec. *130*, 135 (1958).

[6] H. B. CHASE, in *The Biology of Hair Growth* (Ed. W. MONTAGNA and R. A. ELLIS; Academic Press, New York 1958), p. 234.



Melanocyte precursor cells (melanoblasts) of hair follicles in telogen and early spontaneous anagen. (A) Telogen (resting stage). Small dendritic clear cell (melanoblast) is in the germ, touching the basement membrane between germ and dermal papilla. (B) Anagen I. Melanoblast is centrally located in the germ, immediately adjacent to the dermal papilla. The nucleus is larger and rounder, and the nuclear chromatin is more conspicuous than in the resting stage. Pale outer cells of the germ (E) are presumptive external sheath cells. Dark compressed inner cells (K) are presumptive keratinizing cells of inner sheath and cortex. (C) Anagen II. The germ is beginning to engulf the dermal papilla. Central melanoblast has just divided. (D) Anagen III, early. Three melanoblasts are at the upper end of the dermal papilla, which is now surrounded by the growing follicle. (E) Anagen III, late. Cytoplasmic organelles of melanoblasts are very evident at this stage, just before pigment is produced. (F) Anagen III–IV. Pigment granules are now visible in the melanocytes (M) clustered around the upper end of the dermal papilla. Dermal papilla cells are markedly changed in appearance, with irregular nuclei and 'shredded' cytoplasm. – Magnification, indicated in B, is the same throughout. Arrows indicate melanoblasts, not all of which are designated. B, basement membrane; C, capsule of club hair; DP, dermal papilla; E, presumptive external sheath cells; G, hair germ; K, presumptive keratinizing cells; M, melanocyte.

to the clear dendritic cells of the hair germ which are immediately adjacent to the dermal papilla. Our conclusion that these cells are in fact the precursor cells of the melanocytes of later anagen, is based upon their specific location, their peculiar morphology, the temporal continuity of their presence, and also on default. No cells from outside the follicle have been seen migrating into the core of the hair germ during telogen or early spontaneous anagen. Our findings indirectly confirm the report of QUEVEDO and ISHERWOOD[8] that dopa-reactive dendritic melanocytes may be seen in the germ of telogen follicles following treatment of the skin with low doses of X-ray.

The potential melanocytes described in this communication should properly be called melanoblasts. MASSON[9] used this word to describe dendritic cells which eventually produce pigment. CHASE and SMITH[10] used the same term to describe, in newly invaginating follicles of young mice, cells which later produce pigment. More recently the term melanoblast was defined by a number of other workers in the field of pigment research as 'a cell which serves at all stages of the life cycle as the precursor of the melanocyte'[11].

The smallest telogen follicles (zigzags) contain the fewest melanoblasts, probably only 1 per single germ. This bears out the prediction made recently by POTTEN, based on calculations derived from a quantitative study of depigmentation produced by split-doses of X-rays[12].

The telogen melanoblast of the adult mouse, which we have described here, undergoes a transformation from a small non-dividing cell type to a large mitotically active cell type every time the anagen phase of the hair cycle commences. By the same token, it must change back to the dormant cell type when telogen is reestablished. This transformation is predictable, in so far as the hair cycle schedule is predictable, and thus the entire system seems peculiarly suitable for studying the general phenomenon of dormant or latent cells[13].

Zusammenfassung. Der Ursprung der Melanozyten, die in der Haarwurzel während jedes Haarzyklus erscheinen, ist bisher unbekannt gewesen. Helle dendritische Zellen im ruhenden Haarkeim, welche an der Haarpapilla liegen, die sich während der frühen Anagen teilen und während Anagen III–IV Farbkörperchen hervorbringen, sind augenscheinlich die Vorläufer der Haarmelanozyten.

A. F. SILVER, H. B. CHASE and C. S. POTTEN

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Membrane-Bound Microtubular and Crystalline Structures in Endothelial Cells of Normal Canine Aorta

Previously, we reported on the ultrastructure of complex vesicles, microtubules, and cytoplasmic filaments in endothelial cells of normal canine aortas[1]. Recent observations of endothelium from such aortas have revealed 2 morphologic types of unusual membrane-bound structures containing either microtubules or crystalline material. This is a preliminary description of the ultrastructure of these cytoplasmic inclusions.

Abdominal aortas of adult mongrel dogs were perfused and distended with a solution of 5% glutaraldehyde in cacodylate buffer for 15 min. They were then cut into longitudinal strips about 1 mm wide and fixed for 2–4 h in fresh 5% glutaraldehyde solution[2]. After prolonged washing in buffer, the tissue was postfixed for 1 h in 2% osmic acid buffered with cacodylate. After dehydration, the tissue was embedded in a mixture of Maraglas D.E.R. 732 and DDSA[3].

The first type of structure, containing loosely aggregated microtubules, has not been previously described. These cytoplasmic structures were bounded by serpiginous membranes and they measured up to 0.8 μ in greatest dimension (Figure 1). The microtubules were approximately 230 Å in diameter and were usually arranged in loose parallel array, although many were randomly situated. The most microtubules noted in a single structure was 240.

The second type of structure contained a crystalline material. This material appeared to be bounded by a membrane, but adjacent to the dense crystalline material it was frequently difficult to define. These structures were generally round and the larger ones appeared nodular as if formed by a confluence of several small ones (Figure 2). The crystalline material had a period of 130–180 Å in one direction; periodicity at right angles to the major period has not been resolved. In the larger, seemingly nodular, structures the period lines run at different angles in different areas, which supports the interpretation of an aggregation of smaller structures.

Similar crystalline cytoplasmic inclusions in pulmonary and glomerular endothelium[4] and in epidermis[5] have been observed and reported on by other investigators. These authors suggested a relationship between the crystalline

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