

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 ISHERWOOD⁸ 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. MASSON⁹ used this word to describe dendritic cells which eventually produce pigment. CHASE and SMITH¹⁰ 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-rays¹².

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 cells¹³.

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.

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⁷ W. MONTAGNA, F. HU and L. GIACOMETTI, in *Ultrastructure of Normal and Abnormal Skin* (Ed. A. S. ZELICKSEN; Lea and Febiger, Philadelphia 1967), p. 16.

⁸ W. C. QUEVEDO JR. and J. E. ISHERWOOD, *Proc. Soc. exp. Biol. Med.* 99, 748 (1958).

⁹ P. MASSON, *Spec. Publs N.Y. Acad. Sci.* 4, 15 (1948).

¹⁰ H. B. CHASE and V. W. SMITH, *Zoologica* 35 (pt. 1), 24 (1950).

¹¹ T. B. FITZPATRICK, W. C. QUEVEDO JR., A. L. LEVENE, V. J. MCGOVERN, Y. MISHIMA and A. G. OETTL, *Science* 152, 88 (1966).

¹² C. S. POTTEN and A. HOWARD, Paper delivered at the 16th Ann. Rad. Res. Meet., Houston, Texas, April 1968.

¹³ Research supported by USPHS Grants Nos. FR-07085-02 (Sub No. 7) and CA-00592-17.

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 aortas¹. 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 solution². 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 DDSA³.

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 endothelium⁴ and in epidermis⁵ have been observed and reported on by other investigators. These authors suggested a relationship between the crystalline

¹ C. N. SUN and J. J. GHIDONI, in *Proceedings Electron Microscopy Society of America 26th Annual Meeting* (Ed. C. J. ARCEAUX; Claitor's Publishing Division, Baton Rouge, La. 1968), p. 172.

² D. D. SABATINI, K. BENSCH and R. J. BARNETT, *J. Cell Biol.* 17, 19 (1963).

³ R. A. ERLANDSON, *J. Cell Biol.* 22, 704 (1964).

⁴ M. J. FINEGOLD, *Lab. Invest.* 16, 912 (1967).

⁵ S. ROSEN and C. C. TISHER, *Lab. Invest.* 18, 240 (1968).

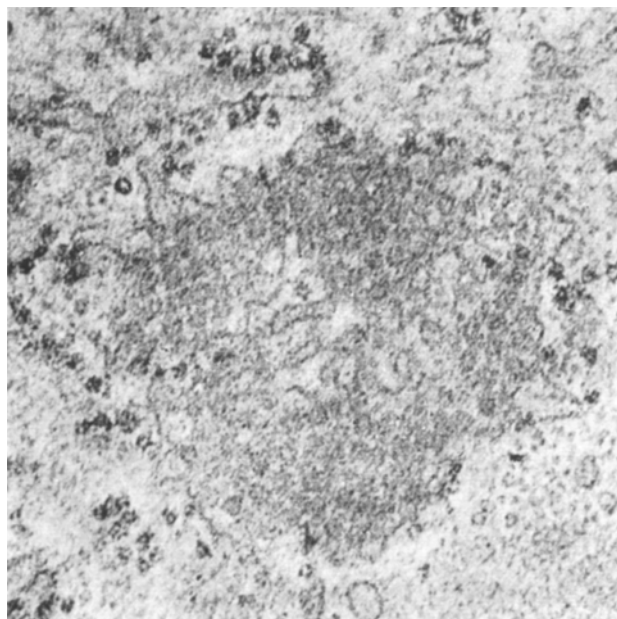


Fig. 1. Portion of endothelial cell from canine aorta. Membrane-bound cytoplasmic structure contains loosely packed microtubules which measure approximately 230 Å in diameter. Most microtubules are seen in cross section, but some are sectioned obliquely. Uranyl acetate, lead citrate. $\times 120,000$.

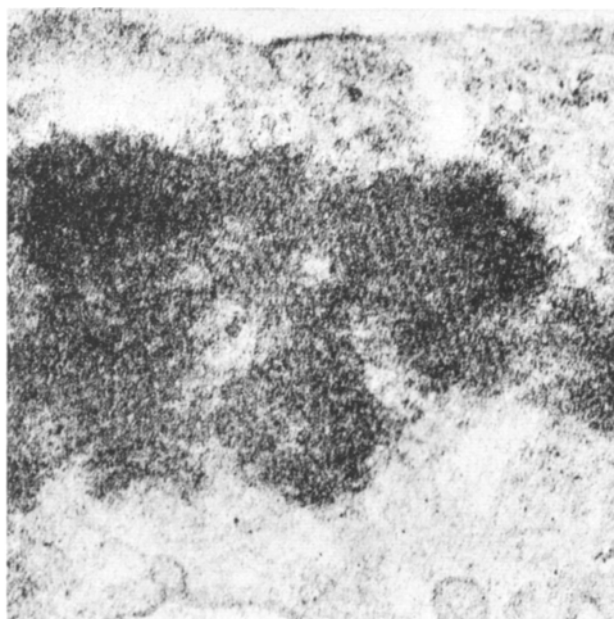


Fig. 2. Portion of endothelial cell from canine aorta. Cytoplasmic structure contains crystalloid material with a prominent period of 130–180 Å in one direction. The bounding membrane of this structure is frequently obscured because of its juxtaposition to the electron-dense crystalloid. Uranyl acetate, lead citrate. $\times 120,000$.

structures and free cytoplasmic microtubules. Similarly, a possible relationship of the microtubule-containing structures we are reporting to both free cytoplasmic microtubules and to the crystalline structures exists.

A third type of structure which we observed, the tubulated body, has been previously described in endothelium of rat lung⁶. This information may well be associated with the other 2 structures described in this communication and with cytoplasmic microtubules in general.

The available data do not allow for definitive interpretation of the morphologic observations, and the function of these apparently related structures remains problematic⁷.

Zusammenfassung. Es wurden 2 Typen von membran-gebundenen Zytoplasmastrukturen im vorliegenden Endothel von normalen Hundearten gefunden. Teilweise

handelt es sich um rauhe parallele Mikrotubuli (240 Å) und um kristallines Material mit einer Periode von 130–180 Å. Die funktionelle Korrelation von Mikrotubuli und den kristallinen Strukturen ist noch unklar.

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⁶ E. R. WEIBEL and G. E. PALADE, *J. Cell Biol.* 23, 101 (1964).

⁷ Supported by USPHS Grants Nos. HE-05435 and HE-11277.

Antigenic Components of Germinated Spores of *Bacillus megaterium*

Our previous studies have demonstrated that the vegetative forms of *Bacillus megaterium* possess at least 2 antigens in common with its spore form¹.

The present work is concerned with the relation between antigenic components of mature spore, germinated spore and vegetative forms of *B. megaterium*.

B. megaterium, Paris strain, was grown on a medium consisting of: Difco peptone, 0.1%; Difco meat extract, 0.3%; Difco yeast extract, 0.3%; manganese sulfate, 0.01%; agar, 1.5%; and 1000 ml of water. Vegetative

cells (V) were harvested after 7 h of incubation and were washed 5 times with distilled water. Mature spores (MS) were harvested after 7 days of incubation and were washed 15 times in distilled water. For preparation of germinated spores, washed mature spores were suspended in 0.02 M phosphate buffer, pH 7.5, to an OD of 450 nm

¹ P. MASTROENI, A. NACCI and A. ROCCA, *J. Bact.* 94, 2073 (1967).