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# A Transmission Electron Microscopical Study of Dysplastic Naevi

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**In this study those features of naevi that fulfil the clinical and light microscopical criteria of dysplastic naevi have been further examined with transmission electron microscopy. The results have been compared with the structure of normal control skin and compound naevi. In dysplastic naevi most melanosomes were abnormal, with spherical melansomes, an incomplete inner structure and uneven melanin deposit, cigar-shaped melanosomes and macromelanosomes. The intraepidermal border between the nests of dysplastic naevi were uneven and the dysplastic melanocytes extended their cell bodies among surrounding keratinocytes with a tendency to invade the epidermis in an upward direction. These findings will serve as additional criteria for dysplastic naevi.** ***Key words: Ultrastructure; Melanocytes; Melanosomes.***

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Dysplastic naevi (DN) are considered to be potential histogenic precursors of cutaneous malignant melanoma. They appear in melanoma-prone families, the dysplastic naevus syndrome, and in the general population as sporadic, non-hereditary, dysplastic naevi (1). The diagnosis of DN is chiefly based on clinical and histopathological criteria (2, 3). However, the varying degree of melanocytic atypia in DN causes confusion and debate concerning the diagnosis of DN (2, 4, 5, 6). Recently, several studies on DN have been made using a variety of methods in addition to conventional light microscopy. A quantitative light microscopical study with dopa staining indicated that the number and mean diameter of the melanocytes in DN were similar to superficial spreading malignant melanoma (7). Histocompatibility locus antigens Class I and abnormalities in nuclear DNA which are found in tumour cells are present in melanocytes of DN (8). Abnormal melanosomes, which are characteristically seen in superficial spreading malignant melanoma and nodular melanoma, have also been reported to be present in DN (9).

While the diagnosis of DN depends on a combination of clinical and histological examination, there are only a few papers available (9–12) in which the ultramicromorphological structure of DN observed in transmission electron microscopy has been described. These papers are based on few cases and in only two papers is DN in sporadic non-heriditary cases described (9, 12).

In this study the features of naevi that fulfil the clinical and light microscopical criteria of DN have been further examined with transmission electron microscopy and the results have been compared with naevi, that have been characterized as banal compound naevi (CN) by the same pathologist.

## MATERIAL AND METHODS

The investigation included 13 patients, 4 women and 9 men, in the age range of 16–62 years. All naevi were photographed and clinically evaluated before excision. The excised naevi included in the study had clinical properties characteristic of DN. The naevi have been examined using light microscopy, the specimens having been stained with haematoxylin-eosin and the immunhistological staining protein S-100.

### *Transmission Electron Microscopy*

Punch biopsies were taken from a central portion of the naevus and from adjacent healthy skin (control). Each punch biopsy was divided into two portions, fixed in 4 % glutaraldehyde in cacodylate buffer, 0.1 M, pH 7.2 at 4°C for several hours, and washed in the same buffer. The specimens were postfixed in 2 % osmium tetroxide at 4°C for 1 hour, dehydrated in graded aceton, and embedded in Spurr. Subsequently 500 Å ultrathin sections were cut on a LKB ultramicrotome, collected on single slot grids coated with formvar, double-stained with uranyl acetate and lead citrate. From each patient 5 sections with 60–75 µm trimmed away between each thin section were observed under a JEM-100 S electron microscope at an accelerative voltage of 80 kV.

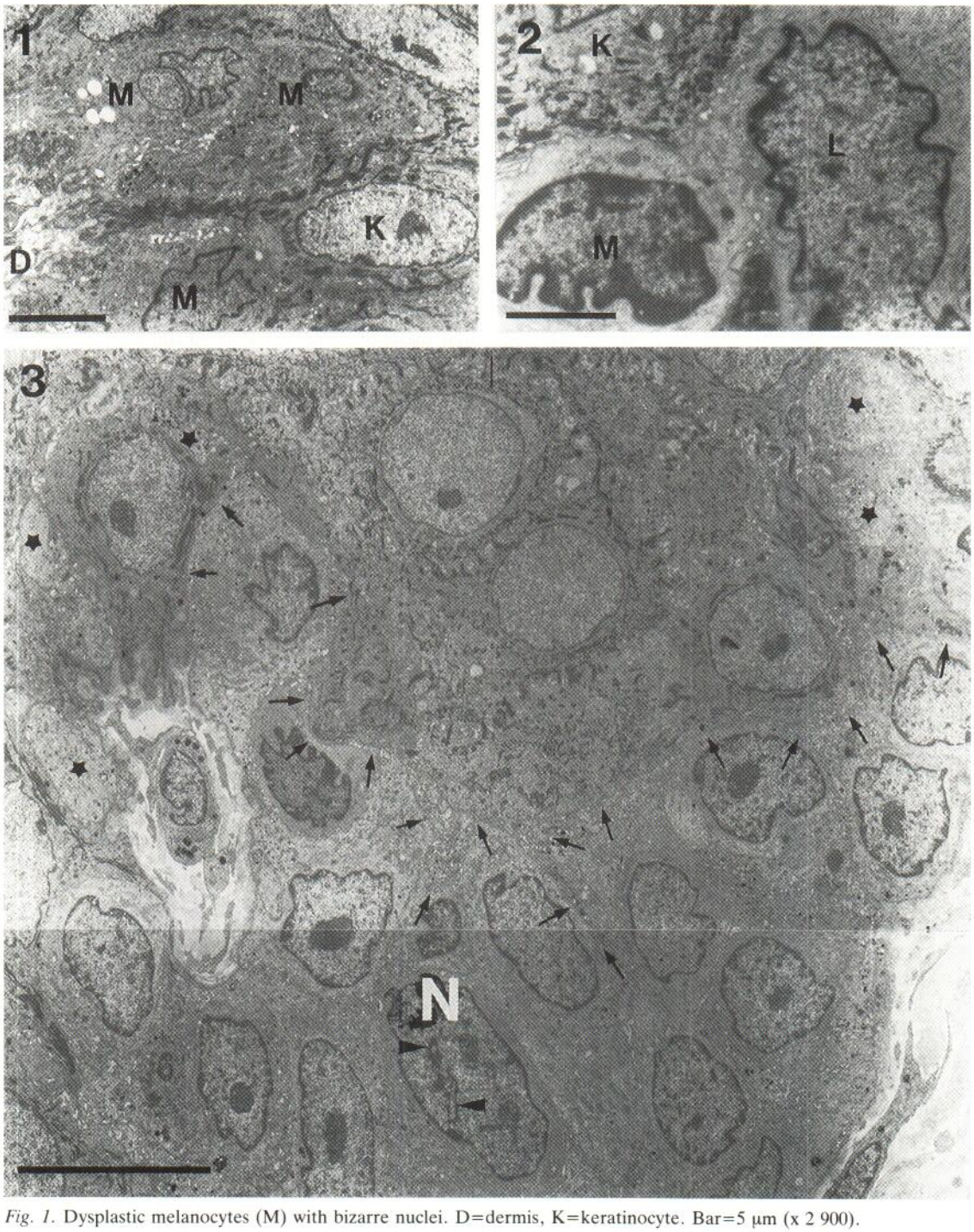



*Fig. 1.* Dysplastic melanocytes (M) with bizarre nuclei. D=dermis, K=keratinocyte. Bar=5 μm (x 2 900).

*Fig. 2.* A melanocyte (M) in a dysplastic naevus in close contact with a Langerhans cell (L) in the midportion of the epidermis. K= Keratinocyte. Bar=2 μm (x 8 400).

*Fig. 3.* An intraepidermal nest (N) of a dysplastic naevus. Note the uneven demarcation between the nest and the surrounding keratinocytes *(arrows)*. Pseudoinclusions of cytoplasm within the nuclei are observed *(arrow heads)*. D=dermis, stars=dendrites of dysplastic melanocytes. Bar=10 μm (x 2 900).

## RESULTS

### *Light microscopy*

In the LM investigation 2 naevi were diagnosed as CN and the remaining 11 had histopathological properties characteristic of DN. The immunohistological staining with S-100 did not add any further information or increase the possibility of distinguishing CN from DN.

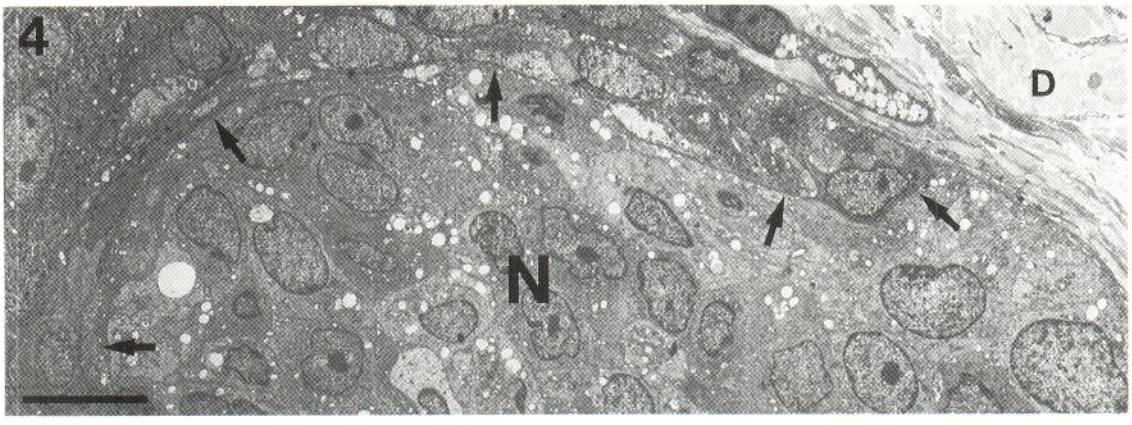
### *Electron microscopy*

*Controls.* Melanocytes with rather large nucleus and narrow cytoplasm were observed at regular intervals in the basal layer of the epidermis just above the basal lamina. In the cytoplasm few cell organells were observed. In several melanocytes, mitochondria with ruptured cristae were seen. Vacuoles were common in the melanocytes. Small single melanosomes could also be seen in the melanocytes, mainly distributed to the cell periphery. Most melanocytes had no nucleoli in their nucleus. Overview of the melanocytes showed small dendrites. In the surrounding basal keratinocytes, rather small round melanosome complexes, consisting of small ellipse formed melanosomes were seen. No specific distribution in the keratinocytes could be observed. The basal membrane was intact.

*Naevi.* The dysplastic melanocytes had an ellipsoid like form and the nuclei were oval or indented with a diameter of 8~10 μm x 5~7 μm (Figs. 1–3). The chromatine was mellow in the central part of the nucleus and dense clumping chromatine was localized to the nuclear membrane. The nucleoli of these atypical melanocytes were extremely prominent, reminiscent of a folded rope. Occasionally melanocytes with pseudoinclusions of cytoplasm within the nuclei or two or more nuclei were encountered. Elongated, oval mitochondria were abundant and slightly long, straight rough endoplasmatic reticulum and polyribosomes, rather than monoribosomes, were present in the cytoplasm. Cytoplasmic filaments were also present in the cell bodies and dendrites. Dendrites with melanosomes and microvilli were well developed and more prominent in the nests. Active Golgi apparatuses with many small vesicles, probably secondary lysosomes related to the melanogenesis, were always encountered. In contrast to CN (Fig. 4), indented nuclei, pseudoinclusions of cytoplasm within the nuclei and dendrites seemed to be more prominent in DN.

In 9 cases, a vast majority of melanosomes in the dysplastic melanocytes, were spherical in shape (Figs. 5–8). Some of these melanosomes exhibited an almost complete lack of specific inner structures. Others consisted of an amorphous material or incomplete lamellae. Different types of aberrant melanin deposits were seen, such as C-shape, ring-shape or eye-shape. Thus, abnormal melanosomes were spherical in shape with an incomplete inner structure and uneven melanin deposits. In one case of DN the melanosomes were almost cigar-shaped with large variations in size (Fig. 9). The center of some melanosomes was empty and a fibrillar or lamellar inner structure became visible. Macromelanosomes and melanosome complexes were also present in dysplastic melanocytes (Fig. 10). Macromelanosomes did not seem to be bounded by membranes but were enveloped by cytoplasmic filaments and contained numerous electron lucent granules. Most melanosomes in CN, on the other hand, were normal, ellipsoid melanosomes with even melanin deposits and a fillamentous or periodic lamellar structure (Fig. 11).

A large number of melanocytes with atypical properties were seen among basal keratinocytes, the suprabasal layer and the midportion of epidermis. These dysplastic melanocytes in the basal layer of the epidermis, which were distributed in solitary units, were frequently positioned in a back-to-back fashion, without intercellular junctions or intermingling thin cytoplasmic processes of keratinocytes (Fig. 1). The dysplastic melanocytes of the midportion of the epidermis were solitary in most cases, but in a few cases were in close contact with Langerhans' cells (Fig. 2). In CN we could observe a few melanocytes positioned in a back-to-back fashion, but the main impression was that CN melanocytes were positioned as solitary units in the basal layer of the epidermis. Melanocytes were observed in intraepidermal nests of DN (Fig. 3). These melanocytes situated on the epidermal side of intraepidermal nests extended their cell bodies and dendrites between the surrounding keratinocytes and the demarcation of the apical portion of the nests were indented or uneven (Fig. 3). The basal parts of the nests were densely surrounded by keratinocytes and basement membrane. The surface facing the basal lamina was smooth and uniform, resembling the intraepidermal CN nests. There were no junctions between dysplastic melanocytes, between dysplastic melanocytes and keratinocytes and no filamentous structure in the intercellular spaces between the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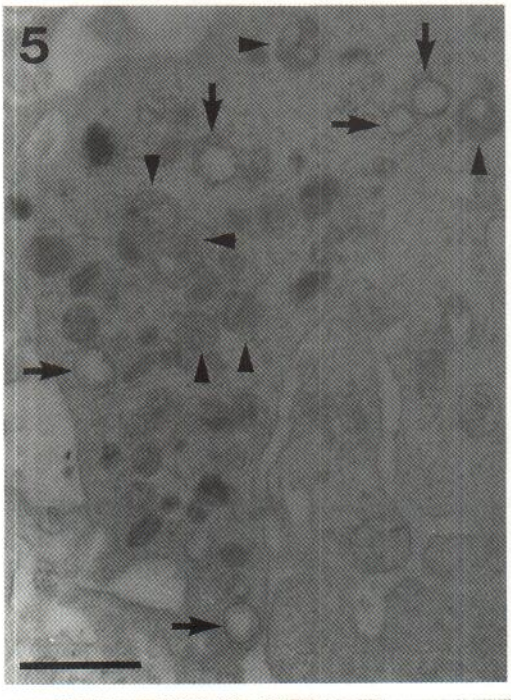
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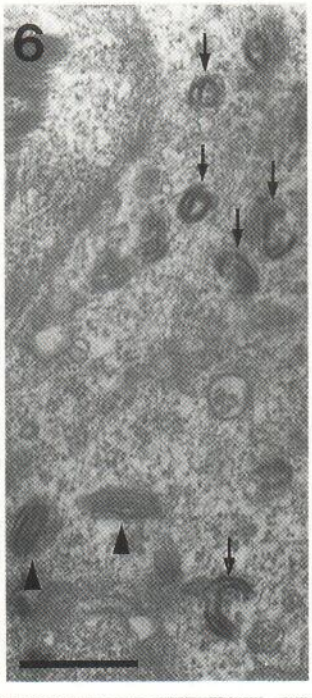
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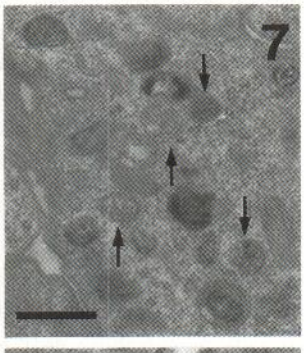
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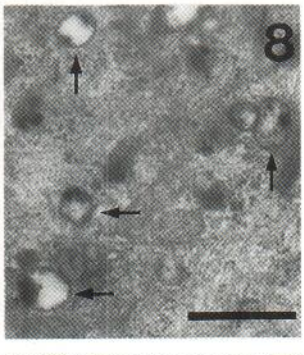
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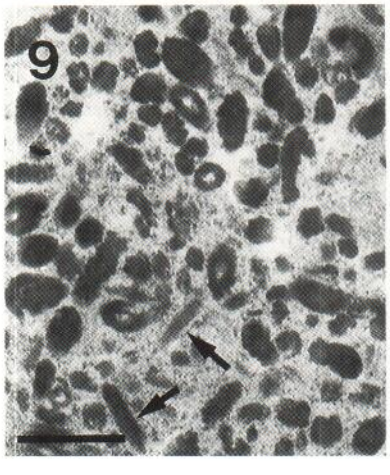
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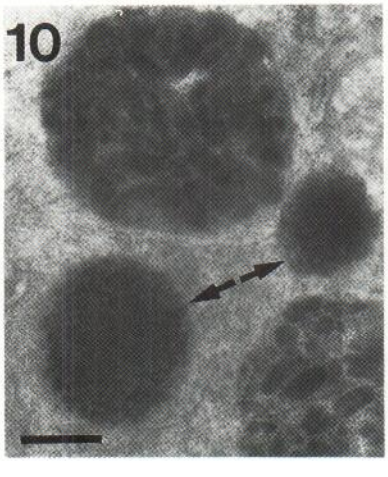
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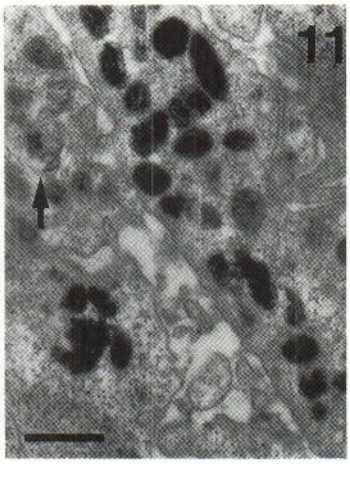
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nests and the surrounding keratinocytes. However, semidesmosome-like junctions were observed between dysplastic melanocytes and the basal lamina. In contrast, intraepidermal nests of CN were ball-shaped, demarcated by the surrounding keratinocytes. No CN naevus cells were seen to project their cell bodies or dendrites between the keratinocytes (Fig. 4).

The naevus cells in the dermis were aggregated into small clusters by thin strands of connective tissue in the same manner as was the case for benign compound naevi. No difference in micromorphological appearance between the nevus cells in the dermal part of DN and CN could be detected. However, in DN, an inflammatory infiltration consisting mainly of lymphocytes was more pronounced than was the case in CN. Also mast cells and melanin-laden macrophages were frequently present in the dermal portion of the dysplastic naevi, as were lymphocytes in contact with solitary melanosomes, which were enveloped by discontinuous basement membrane.

## DISCUSSION

The most obvious ultramicromorphological difference between CN and DN observed in this study was the abnormalities seen in DN melanosomes. The spherical melanosomes with incomplete inner structure and uneven melanin deposit agree with the findings of Takahashi et al. (9, 12). Wassilew et al. (11) described changes similar to those demonstrated in Figs. 8 and 9 in what they called familial multiple naevogene melanomas. These changes in melanosome structure have also been reported from electron microscopic investigations of non-hereditary malignant melanoma (13–16). Moreover, cigar-shaped melanosomes observed in one case are similar to melanosomes previously described as being present in the malignant naevocytoma (13) and Type A melanoma (14). In contrast, most melanosomes in CN were ellipsoid in shape with a periodic inner structure and even melanin deposits and did not differ from the melanosomes observed in the control specimens. Thus, the changes observed in DN melanosomes, could suggest a profound difference in the character of the melanocytes in DN as compared to normal skin and other forms of benign naevi, indicating that the melanocytes in sporadic DN also represents an intermediate cell-line with premalignant properties. In addition, we can agree with the suggestion of Takahashi et al. (12) that the detailed structural characterzation of abnormal melanosomes is a new adjunct for histopathological diagnosis of DN.

We observed macromelanosomes in only one case of DN. Findings of macromelanosomes have been demonstrated in cases of dysplastic naevus syndrome (17), nevocellular naevi, lentigo simplex and malignant melanoma (18). Thus, macromelanosomes cannot be considered to be a specific ultrastructure of DN.

One observation, that has not to the best of our knowledge been reported previously, is that the borders of the intraepidermal DN nests were indented. These nests consisted of atypical melanocytes that extended their cell bodies between the surrounding keratinocytes. In contrast, the demarcation of intraepidermal CN nests was smooth and uniform. The cells of these nests did not extend their cell

*Fig. 4.* Note the smooth round demarcation *(arrows)* between the the intraepidermal nest (N) of the compound naevus and the surrounding keratinocytes. D=dermis. Bar=10 μm (x 1 600).

*Fig. 5.* Detail from an atypical melanocyte in a dysplastic naevus. Spherical melanosomes with an almost complete lack of specific inner structure *(arrows)* or an amorphous material *(arrow heads)*. Bar=0.5 μm (x 32 800).

*Fig. 6.* Spherical melanosomes with uneven melanin desposit; C-shape or ring-shape *(arrows)*. Normal melanosomes with fibrillar and periodic structures are present *(arrow heads)*. Bar=0.5 μm (x 30 900).

*Fig. 7.* Spherical melanosomes with incomplete lamellae *(arrows)*. Bar=0.5 μm (x 22 300).

*Fig. 8.* Spherical melanosomes with uneven melanin deposit, eye shape (arrows). Bar=0.5 μm (x 28 700).

*Fig. 9.* Cigar-shaped melanosomes. The center of some melanosomes are electron lucent and some melanosomes have fibrillar structures *(arrows)*. Bar = 0.5 μm (x 28 600).

*Fig. 10.* Macromelanosomes *(arrows)* and melanosome complexes. Bar=0.5 μm (x 22 000).

*Fig. 11.* Ellipsoid and round normal melanosomes in compound naevus cells with different degrees of melanization. Note the presence of a few round melanosomes with uneven melanin deposit *(arrow)*. Bar=0.5 μm (x 21 800).

bodies between surrounding keratinocytes. This growing pattern of melanocytes in intraepidermal DN nests seems to exhibit similarities to the invasive growth of the malignant melanocytes in all layers of epidermis and into the dermis observed in malignant melanoma (15). Furthermore, the results of immunohistochemical and cytophotometrical analysis suggest that atypical melanocytes in DN possess premalignant properties (8). In addition to the abnormal melanosomes, the indented growing pattern of intraepidermal melanocytic nests, will in our opinion serve as an additional criterion for DN.

## ACKNOWLEDGMENTS

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