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AN ULTRASTRUCTURAL STUDY OF MAST GRANULE FORMATION IN EMBRYONIC SKIN

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Abstract. Re-examination of human fetal skins at 20 to 26 weeks of age revealed that, besides (*a*) the four types of mast granules whose formation appeared predominantly to be associated with smooth endoplasmic reticulum and free ribosomes (referred to as the 1st mechanism) rather than the Golgi apparatus, a small number of granules possibly identified as mast granules appeared to be formed by the following mechanisms: (*b*) granules limited by a single membrane but displaying no internal membrane, presumed to be formed by the Golgi apparatus (referred to as the 2nd mechanism); (*c*) granules displaying neither limiting nor internal membrane, presumed to be formed by compact deposition of finely granular substances possibly coming from rough endoplasmic reticulum (referred to as the 3rd mechanism). A comparative study of mouse fetal skins at 15 to 19 days in utero revealed that granules presumably formed by the second mechanism were seen more frequently than those possibly formed by the first or third one and so suggested that the second mechanism might be predominant, in contrast to the human fetal skin.

In 1969 we carried out EM studies on the mast cell in human fetal skin (2) and there described four types of mast granules and the early stages of granule formation. We postulated a sequence in the formation of these types of mast granules and associated them with the smooth endoplasmic reticulum and free ribosomes rather than with the Golgi apparatus, an interpretation which differs from that advanced by Combs (1).

The present investigation on fetal human and mouse skin was made with special reference to the above difference. Mouse fetal skin was utilized in order to investigate whether or not its mast cells exhibit differences concerning mast granule formation, compared with those in human fetal skin.

MATERIALS AND METHODS

In the study of human fetal mast cells, the same skin samples (2) were used, which were obtained from freshly aborted human fetuses, 20 to 26 weeks of menstrual age. In the study of mouse fetal mast cells, the skin samples were removed from mouse fetuses 15 to 19 days in utero obtained by hysterectomy from the pregnant females of D–D strain which were experimentally mated, having been kept with males of the same strain in cages overnight.

These samples were fixed in 4 to 6.5% glutaraldehyde for 2 hours followed by postfixation in 1% osmium tetroxide for 1 hour. Both fixatives were buffered at pH 7.4 with the phosphate buffer of Millonig (5). After dehydration in graded ethanols, the materials were embedded in Epon 812 using Luft's method (4). The sections for electron microscopy were stained with 2% uranyl acetate and 0.4% lead citrate. Thick sections (about 1 μm) were stained with toluidine blue for examination under the light microscope.

RESULTS

Mast cells in human fetal skin

Re-examination of the skin from human fetuses 20 to 26 weeks of age revealed that Golgi vesicles or vacuoles containing a granule identical with Combs's progranule (1) were absent in most of the mast cells, though a few were present in some cells (Fig. 1). It also revealed that a small number of cells capable of being identified as mast cells in the buttock skin of a fetus 26 weeks of age presented a Golgi apparatus showing a morphology different from that of the cutaneous mast cells hitherto observed in human fetuses 20 to 26 weeks of age (Figs. 2 and 3). Their cytoplasm exhibited well developed Golgi vesicles and vacuoles, some of which contained electron-

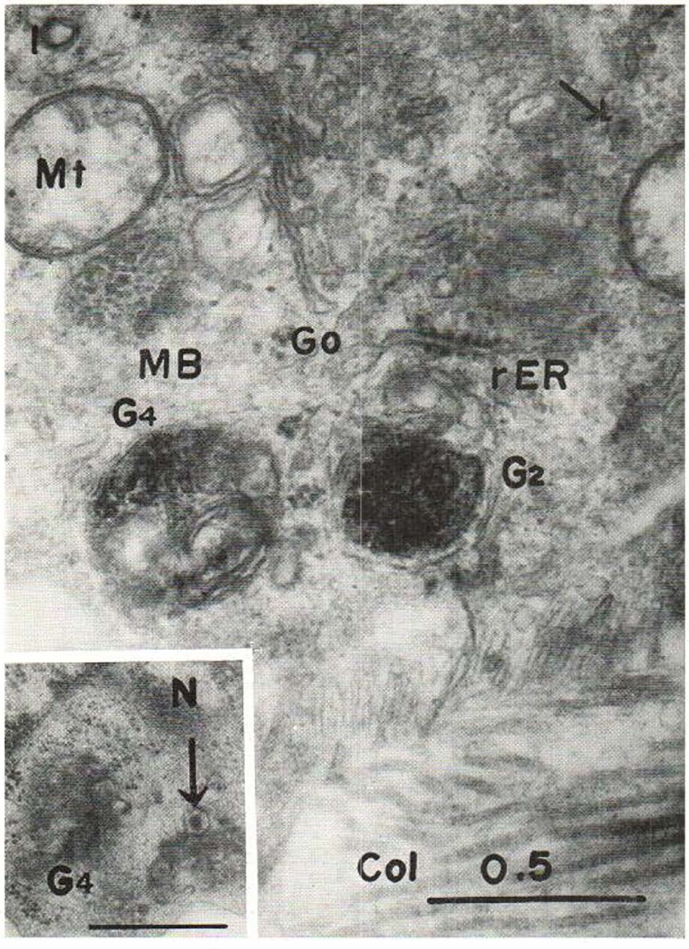

Fig. 1. The cytoplasm of a mast cell in human fetal limb skin at 26 weeks. *rER*, rough endoplasmic reticulum; *Mt*, mitochondria; G_2, a mast granule of type 2; G_4, a mast granule of type 4; MB, presumable multivesicular body present around Golgi apparatus (*Go*); Arrow indicates a granule, approximately 50 μm in diameter, seemingly identical with Combs's progranule; *Col*, Collagen fibers. × 57 000.

Inset: Portion of a mast cell in the human fetal limb skin at 20 weeks. Arrow indicates a vacuole containing a granule, approximately 100 μm in diameter, seemingly identical with Combs's progranule. *G4*, a type 4 mast granule; *N*, nucleus. × 19 000.

dense small granules very similar to Combs's progranule (Fig. 2 A). In the vicinity of these granules, there were many electron-dense large round granules which were limited by a single membrane and presumed to be formed by enlargement of the former within Golgi vacuoles.

In the neighbourhood of these cells were found some cells whose cytoplasm contained many granules which exhibited no internal membrane, thereby differing from the mast granules described previously (Fig. 2 B). These cytoplasmic granules were limited by a single membrane and divisible into three types with respect to their contents, (1) a granule containing homogeneously electron-dense substances and seemingly identical with those seen in the cytoplasm of the above cells, (2) a granule containing filamentous or homogenous substances of a low electron density, and (3) a granule containing a packed mass of "threads" of a medium electron density, often separated by a narrow electron-lucent space from the limiting membrane. The ultrastructure of these granules appeared very similar to that of a type of mast granule commonly seen in human adult skin mast cells. These cells (Fig. 2 B) were presumed to be of the same type as that shown in Fig. 2 A and Fig. 3.

The rough endoplasmic reticulum in most mast cells of human fetal skin was not so well developed. But occasional mast cells displayed conspicuously well developed cisternae (Fig. 4). As seen in Fig. 4, the rough endoplasmic reticulum is arranged in concentric lamellae, and encloses an ovoid granule with an appearance different from that of the granules formed by either of the two mechanisms mentioned above. It has a comparatively low and homogeneous electron density and does not exhibit limiting membrane, internal

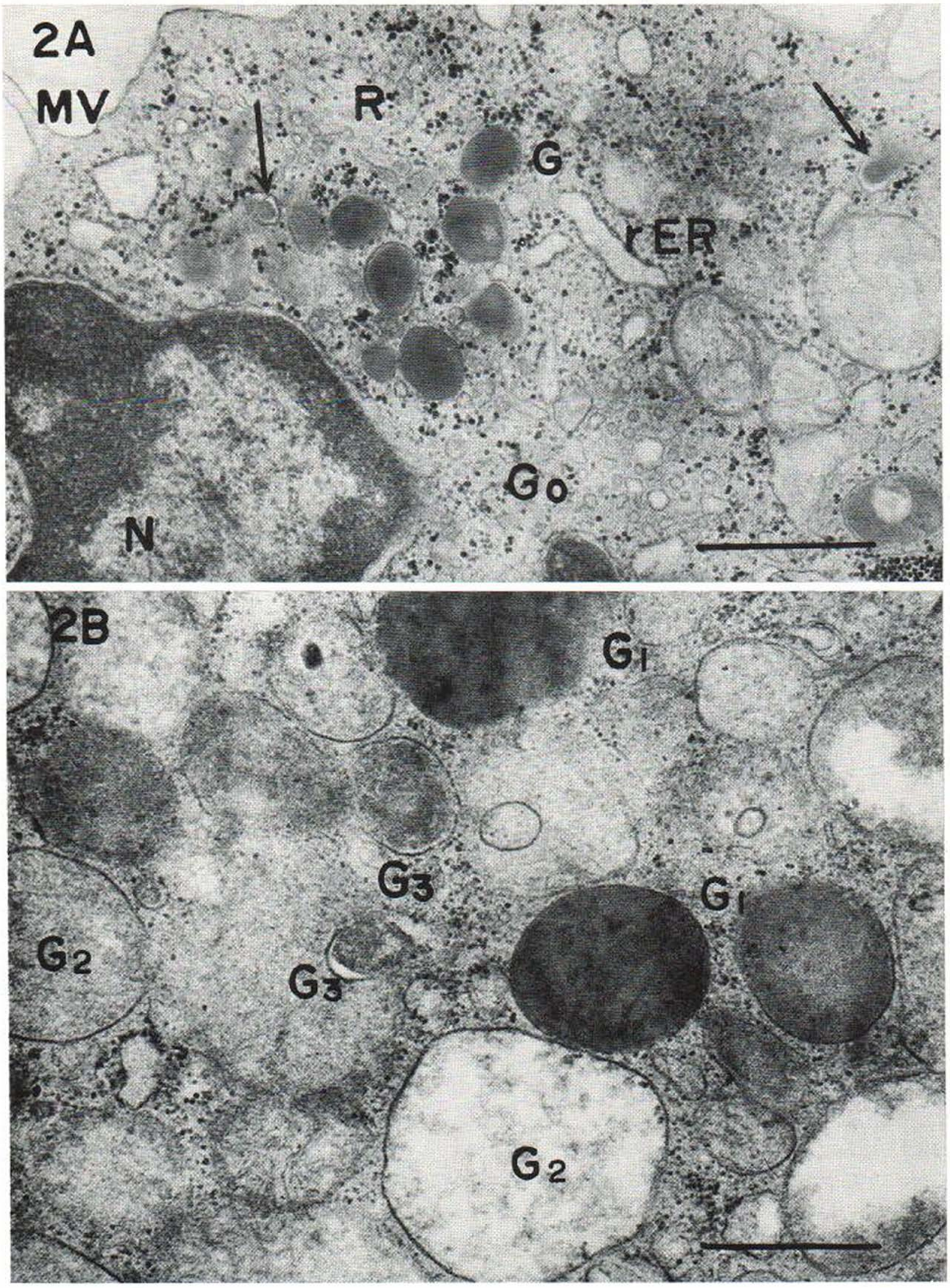

Fig. 2. (A) Portion of a cell possibly identified as mast cell found in human fetal buttock skin at 26 weeks. *N*, nucleus; *MV*, micro-villi; *Go*, well-developed Golgi vesicles and vacuoles some of which contain a granule very similar to Combs's progranule (arrows); *G*, many electron-dense granules which are located around Golgi apparatus (*Go*) being limited by a single membrane, and so presumed to be formed by the enlargement of the above granules (arrows) by the second mechanism (Fig. 6); *rER*, rough endoplasmic reticulum; *R*, numerous free ribosomes intermingled with a comparatively small number of probable glycogen granules. × 23 000.

(B) Many cytoplasmic granules (G_1, G_2, G_3) contained in a cell possibly identified as mast cell present in the vicinity of the cell shown in Fig. 2 A. G_1, granules displaying an almost identical structure with that of the granule (*G*) shown in Fig. 2 A, in that they are limited by a single membrane, contain homogenous, electron-dense substances but display no internal lamellae; G_2, granules limited by a single membrane containing various amounts of filamentous to homogenous substances of a low electron density; G_3, granules limited by a single membrane containing a packed mass of threads of medium electron density and being separated by a narrow electron-lucent space from their limiting membranes. × 23 000.

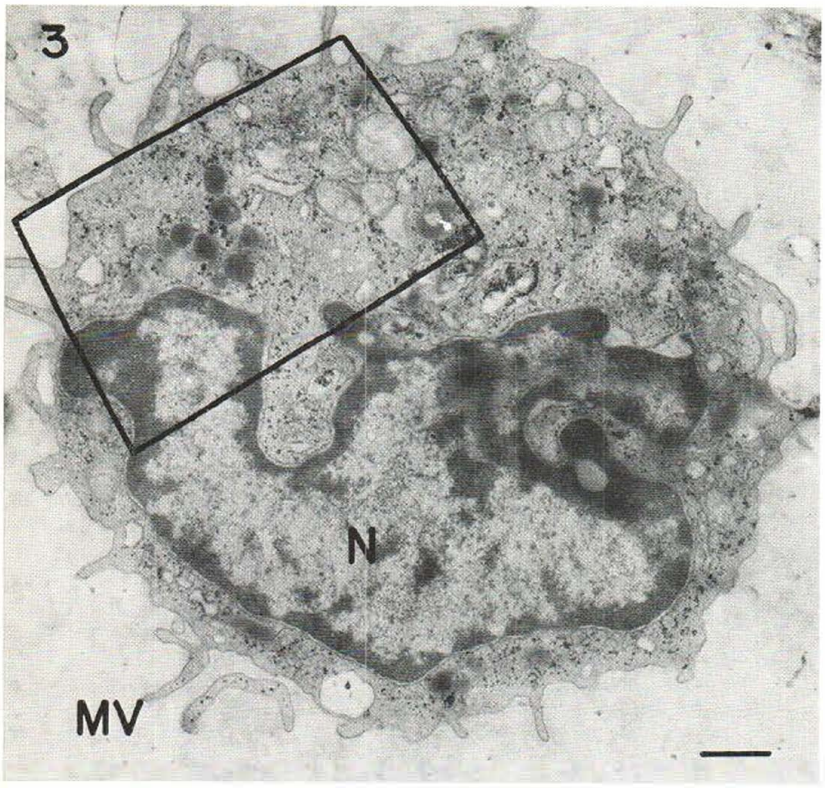

Fig. 3. Plan view of the cell shown in Fig. 2 A. *MV*, numerous microvilli protruding from the plasma membrane and nucleus with deep indentations (*N*) characteristic of the mast cell. × 9 200.

lamellae, or coarsely granular substances. In the vicinity of this granule, several mast granules of type 1 (*G* in the inset of Fig. 4) are recognizable.

Mast cells in the mice fetal skin

Electron microscopic investigation of the skin of mouse fetuses 15 to 19 days in utero revealed that, in contrast to human fetal skin, the cells having granules, consisting of membranous, coarsely granular and finely granular components, and presumed to be formed by the same mechanism as the first one in human fetal skin, were recognizable, though definitely small in number (upper inset of Fig. 5). The mechanism of the formation of the granule shown in the lower inset of Fig. 5 is still uncertain. It may be interpreted as a specialized form of granule of type 2 formed by the first mechanism, since it displays internal membranes consisting of a unit-membrane structure and exhibiting fingerprint-like concentric lamellae.

Progranules and mast granules, on the other hand, were often encountered, whose internal structures seemed almost identical with those of mast granules described by Combs (1), being contained inside a membrane-limited vacuole displaying neither boundary nor internal membrane and so presumed to be formed by enlargement of the progranule (*G* in Fig. 5, G_1 in the middle inset). Granules consisting of finely granular substances and displaying neither limiting membrane nor internal lamellae were suspected to occur. The occurrence might, however, be very rare, as in the human fetal skin (arrow in the middle inset of Fig. 5).

From these findings it was presumed that in mouse fetal skin the occurrence of the second mechanism of mast granule formation seemed to be more frequent than the first one, and the possible occurrence of the third one could not be discounted.

DISCUSSION

In our previous EM studies (2) on the mast cell in human fetal skin at 20 to 26 weeks of age, we reported the four types of mast granules with

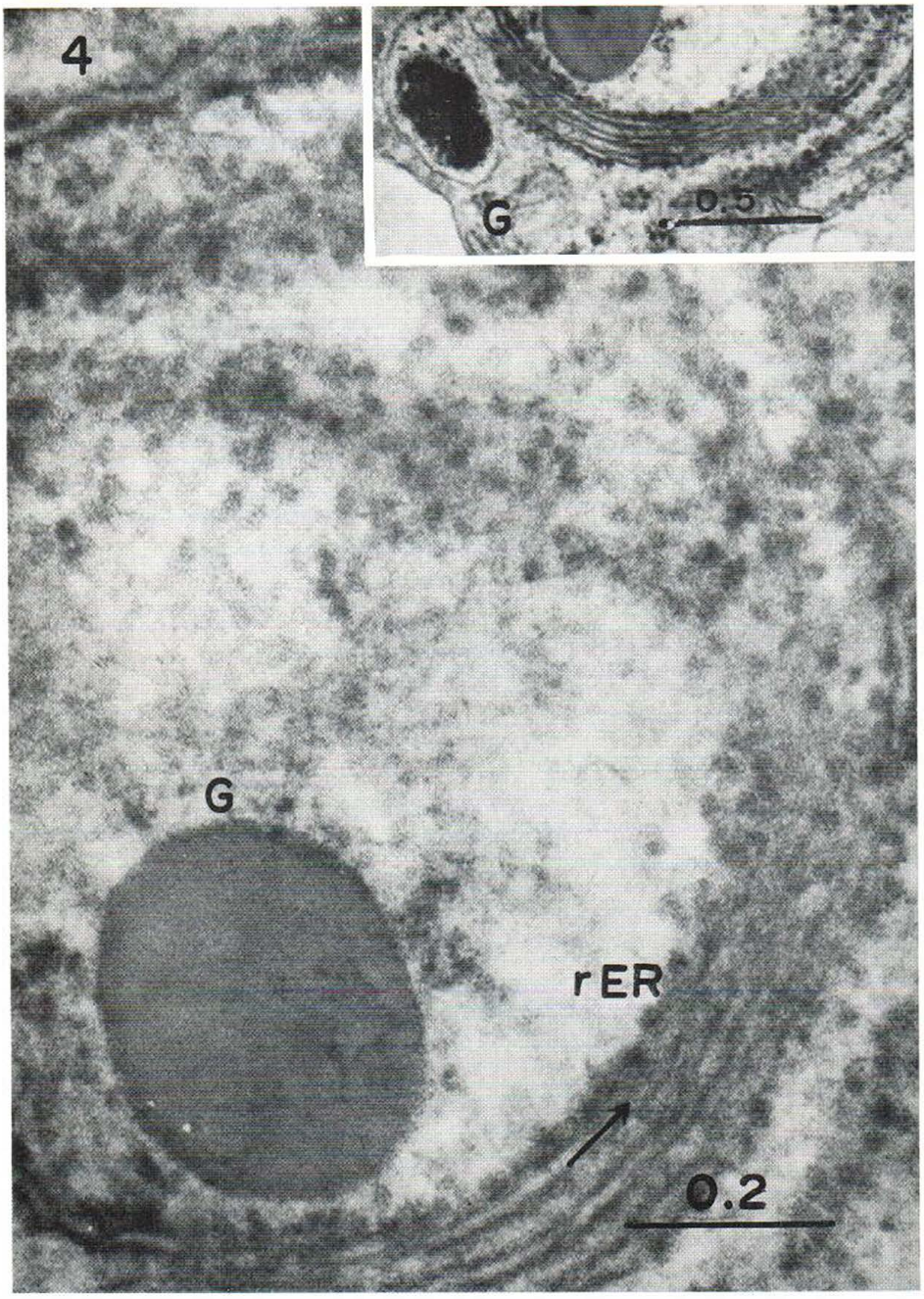

Fig. 4. Lamellate rough endoplasmic reticulum enclosing an ovoid granule displaying neither limiting nor internal membrane found in a mast cell of human fetal limb skin at 26 weeks. *G*, the granule appears packed with finely granular substances with electron density similar to that of the substances (arrow) contained inside the cisternae of the surrounding rough endoplasmic reticulum (*rER*) and so suggestive of being formed by the compact deposition of the latter. × 115 000.

Inset: Type 1 mast granule (*G*) present outside of the lamellate rough endoplasmic reticulum in the cell shown in Fig. 4. × 46 000.

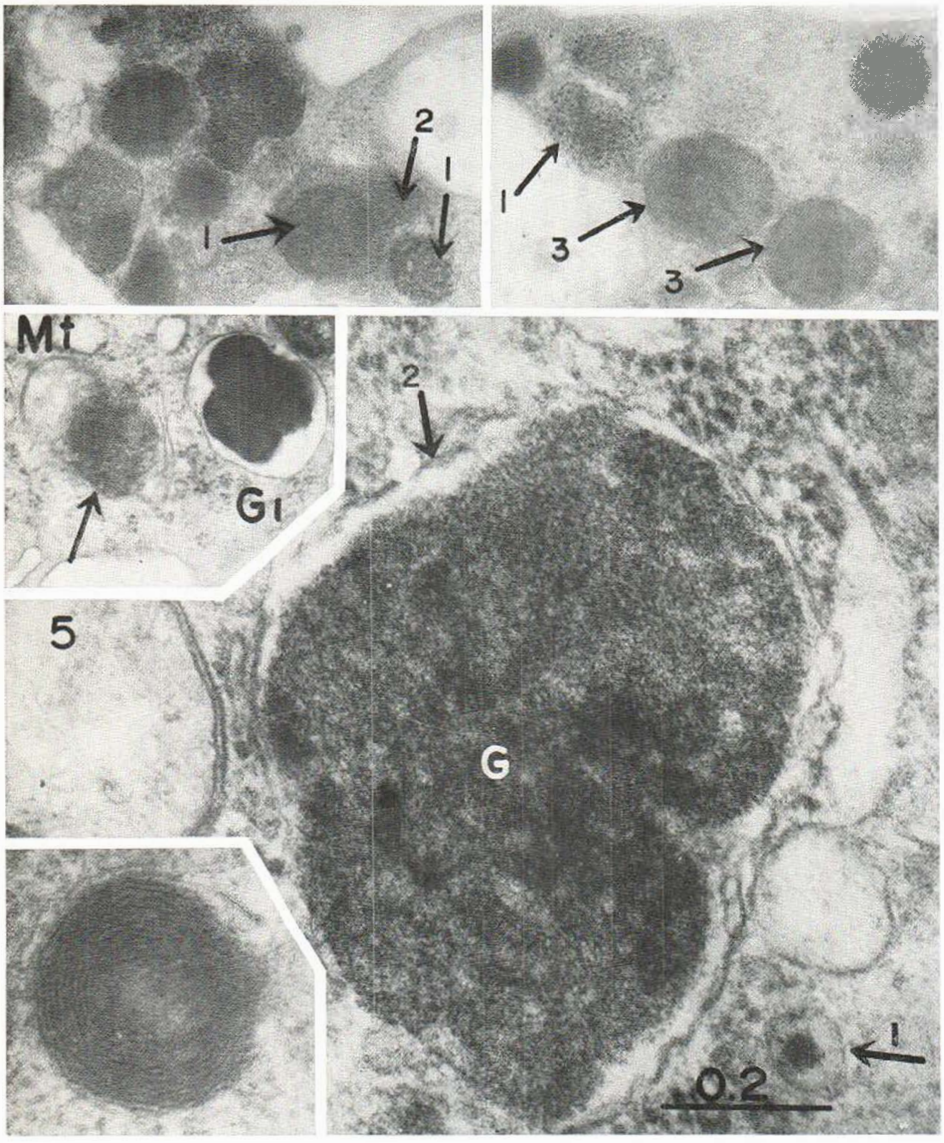

Fig. 5. Mast granule in a mast cell of mouse fetal limb skin at 19 days in utero. *G*, a mast granule contained in a membrane-limited vacuole (arrow 2), and whose internal structure seems identical with that of rat cutaneous mast granule described by Combs, consisting of electron-dense finely granular substances and displaying neither boundary nor internal membrane. Arrow 1 indicates a progranule, approximately 60 μm in diameter. × 115 000.

Inset (top): Mouse fetal skin mast granules at 15 days in utero. The granules consist of coarsely granular (arrows 1), finely granular (arrow 2) and membranous component (arrows 3) and seem identical in internal structure with the granules formed by the first mechanism (Fig. 6) in human fetal skin. × 34 500.

Inset (middle): Arrow indicates a structure consisting of finely granular substances of a low electron density and displaying neither limiting membrane nor internal lamellae and so suggestive as representing an early stage of mast granule formation possibly by the third mechanism or by the first (Fig. 6). G_1, type 1 mast granule formed by the first mechanism (Fig. 6) in mouse fetal skin at 19 days in utero; *Mt*, mitochondria. × 23 000.

Inset (bottom): A mouse fetal skin mast granule at 19 days in utero displaying internal membranes which consist of a unit-membrane structure exhibiting a finger-print-like pattern. × 69 000.

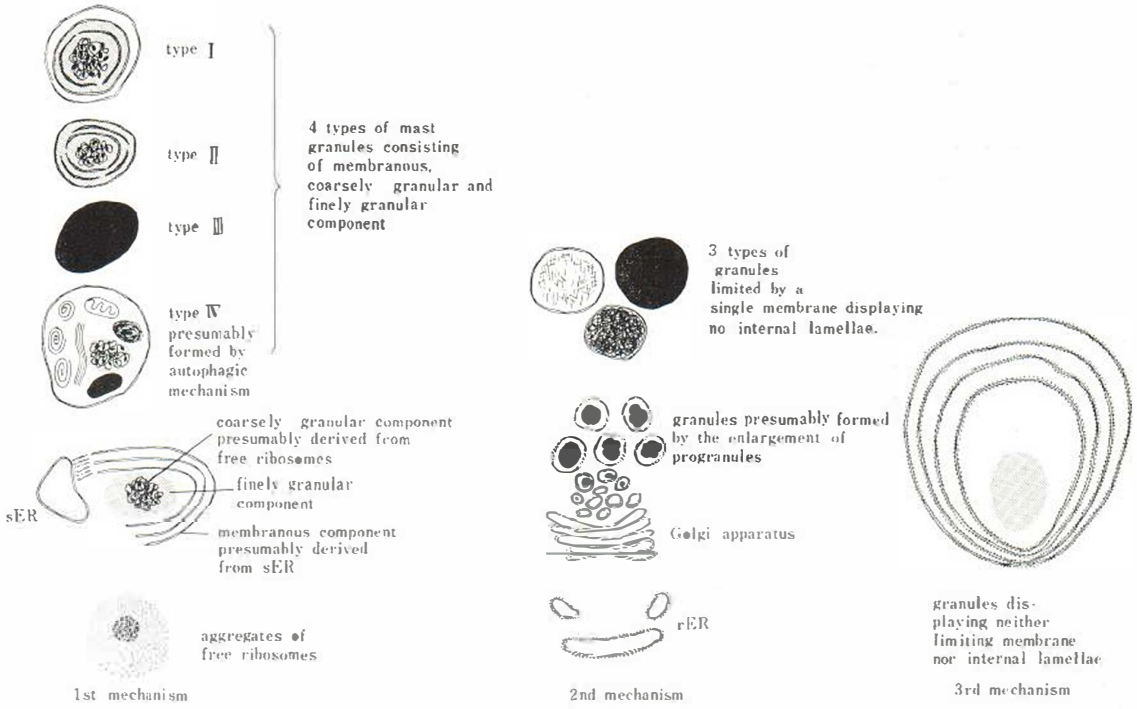

Fig. 6. Three mechanisms possibly associated with the formation of mast granules in human fetal skin.

internal structures consisting of membranous, coarsely granular and finely granular component and early stages of granule formation. We postulated a sequence predominantly associated with the smooth endoplasmic reticulum and free ribosomes rather than with the Golgi apparatus in formation of these four types of mast granules (the first mechanism in Fig. 6).

In the previous study, no Golgi vesicles or vacuoles containing a granule identical with the progranule described by Combs (1) were found in the mast cells. However, at the present re-examination, a few Golgi vacuoles containing a granule very similar to Combs's progranule were detected in a small number of mast cells in the limb skin of human fetuses of 20 and 26 weeks of age (Fig. 1), although no granules displaying internal structures identical with those of the mature granules described by Combs were found. The re-examination also revealed in the buttock skin of a fetus, 26 weeks of age, a small number of cells that might possibly be identified as mast cells. The identification could be made on the following grounds: (1) some of them contained electron-dense small granules very similar to Combs's progranule, and large round granules limited by a single membrane, possibly formed by enlargement of the former (Fig. 2 A); (2) other cells present were presumed to be of the same type as the former, since they contained many cytoplasmic granules whose internal structures were similar to those of the mast granule type commonly seen in human post-partum skin, although they differed from ordinary mast granules, namely they did not show the various stages of maturation present in mast granules (Fig. 2 B).

From these, it seemed impossible to discount the possibility that besides the granules formed by the first mechanism, there might also be a small number of mast granules which were initially formed as progranules and matured within the Golgi apparatus.

The significance of the ovoid granule shown in Fig. 4 is still uncertain. It differs from the granule formed by either of the two mechanisms mentioned above, in that it does not have limiting membrane, internal lamellae, or coarsely granular component and seems to be of a lipid nature, displaying a comparatively low and homogenous

electron density. The finding that it is enclosed by conspicuously developed rough endoplasmic reticulum arranged in a lamellar form seems to suggest that it might be formed by the compact deposition of finely granular substances of a comparatively low electron density possibly deriving from the surrounding rough endoplasmic reticulum (the third mechanism in Fig. 6). This type of granule might subsequently become a subgranule of the compound form of mast granule after being engulfed in the membranous activity above mentioned. Kobayasi & Asboe-Hansen (3), in a study on urticaria pigmentosa, reported that the cytoplasm of mast cells contained lucent bands arranged in strata presumably derived from rough endoplasmic reticulum. They postulated that the mast cells containing these specialized structures might be in the regranulation phase. The appearance of rough endoplasmic reticulum observed in the present study is somewhat similar to that of the lucent bands described by Kobayasi & Asboe-Hansen, and seems to suggest that the cell in Fig. 4 might be in a regranulation phase.

In the investigation of fetal skin of mice 15 to 19 days in utero, mast granules presumed to be formed by enlargement of the progranule within the Golgi apparatus by the second mechanism were seen more frequently than those possibly formed by the first or the third one. From these results, it seems likely that in the formation of mast granules in mouse fetal skin, the second mechanism might be predominant in contrast to the case of human fetal skin.

ACKNOWLEDGEMENT

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