Electron Microscopic Studies on Histiocytes*

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Electron microscopic studies have been made on the ultrastructure of histiocytes of the subcutaneous connective tissue, in normal development of the cells and in various pathological conditions.

The histiocytes are morphologically characterized by predominance of the smooth components of the endoplasmic reticulum and abundance of cytoplasmic inclusion bodies. The cytoplasmic inclusion bodies observed in the present study can be divided into three types by their structure and origin. The first type called "H-granule (histiocyte granule)" in this study, is produced by accumulation of dense materials into the vesicles separated from the smooth-surfaced reticulum. The second type, "cytolysome", results from sequestration of the focal cytoplasmic degeneration. The third type, "phagosome", originates in the vacuoles containing phagocytized materials. All these heterogenous inclusion bodies are considered to be involved in the digestion of materials of both exogenous and endogenous origins.

The histiocytes in the loose connective tissue are identified precisely at the histological level by the intensive vital storage of acid dyes and the vigorous phagocytic activity. This method has been extensively employed by light microscopists for examining the morphology of histiocytes. However, there have been only few electron microscopic studies on the ultrastructure of these cells^{21-23, 28}), probably because of the difficulty in precisely identifying the cells at the submicroscopic level.

In our laboratory we have studied with the electron microscope the morphological changes of connective tissue cells due to various stimuli in the subcutaneous connective tissue. These experiments will be described in detail elsewhere. The present report describes the common feature of the histiocytes observed in various experimental conditions in an attempt for clarifying the cytological characteristic of the cells.

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MATERIALS AND METHODS

The materials presented here have been obtained from the subcutaneous connective tissues of normal embryonic and adult mice, of mice and guinea-pigs with healing wounds, of mice injected with cortisone or typhoid vaccine and of rabbits injected with protein silver, of guinea-pigs having scurvy and of mice with neoplastic growths induced by injection of 3:4 benzpyrene.

Some of the tissue were fixed in cold 1 or 2 per cent osmium tetroxide in veronal buffer (PH 7.2-7.9) for about 2 hours, and some others were fixed with potassium permanganate. After rapid dehydration in ethanol, they were embedded in a mixture of n-butyl methacrylate and styrene¹⁶⁾. Sections were cut on a Porter-Blum microtome or a JUM-4 type microtome and routinely stained with salts of heavy metals^{10,14,17)}.

Electron micrographs were taken with Hitachi electron microscopes HU-11 type and HU-9 type at magnifications ranging from 5,000 to 20,000.

RESULTS

The histiocytes vary in form, being round, oval, or more or less elongated and spindle-shaped. They are, however, easily identified by the predominance of vesicular components and various types of inclusion bodies in the cytoplasm. These structures distinguish them from fibroblasts, which are characterized by extensive development of the rough-surfaced endoplasmic reticulum^{11,13)}.

1) Endoplasmic reticulum

The histiocytes contain predominantly smooth-surfaced components of the endoplasmic reticulum. Their size, form and distribution appear to be influenced by the functional conditions and differentiation process of the cells. In immature cells, e.g. those in embryos, neoplastic growths, or early stage of wound healing, and in resting cells of the adult, these components are less abundant and uniform in size. They occur as apparently isolated vesicles of round or oval shape, scattered throughout the cytoplasm. The elongated forms are absent or very rare in all sections. These findings indicate that in these cells most of the components of the endoplasmic reticulum are actually vesicles of spherical shape. Some vesicles are in close contact with the cell membrane and others appear to have communication with the extracellular space. The cell membrane occasionally shows a number of infoldings, some of which appear to have a row of small vesicles attached to the inner end. These findings suggest that the vesicles at the periphery of the cytoplasm are derived from invagination of the cell membrane.

The smooth components of the endoplasmic reticulum promptly increase in number when the cell function is accelerated, for example, by injection of typhoid vaccine (Fig. 2). In such cases considerable changes are found in the morphology

Fig. 1. Schematic representation of submicroscopic structure exhibited by histiocyte in response to various stimuli. Part marked *A* represents immature and resting cells, showing predominance of small vesicles, probably of pinocytic origin. In actively functioning cells (marked *B*), the components of the smooth-surfaced endoplasmic reticulum may be implicated in forming the H-granules (*H*). Sequestration process of focal cytoplasmic degeneration is indicated at right (*Cy*). It is conjectured that multivesicular bodies (*Mv*) and some of "cytolysomes" are formed by similar processes. The part marked *C* shows phagocytosis, in which "phagosomes" (*Ph*) are derived from the cell membrane. Golgi complex (*G*) and filamentous structures (*F*) are shown in the area adjacent to the nucleus (*N*). The rough-surfaced endoplasmic reticulum (*Er*) is shown in the neighborhood of the mitochondria (*M*).

of the smooth components; the changes are represented by occurrence of oval vesicles and large vacuoles, and frequently of elongated tubules which show branchings and anastomoses, suggesting formation of a tubular reticulum in tridimensional disposition (Fig. 3). Frequently the tubules display bleb-like protrusions, presumably on the way of becoming isolated vesicles (Fig. 3). In some places the tubules assume the form of strings of vesicles by successive swellings. In addition, there are found relatively vast cisternae of irregular shapes bounded by poorly defined membranes and having a few fenestrations. A tubular protrusion from the edge of the cisternae suggests existence of communication between the cisternae and tubular reticulum.

The small vesicles, tubules and cisternae all have light and homogeneous contents, but the larger vesicles are apparently empty.

These morphological variations in the smooth-surfaced components are

found more frequently in the central region than in the marginal zone of the cells.

The rough-surfaced elements of the endoplasmic reticulum are less abundant and occur in the form of anastomosing tubules in the central region of the cell. It is noted that they are frequently located near mitochondria (Fig. 2). Occasionally portions of the reticulum lack the RNP particles attached to the membrane and exhibit smooth surfaces.

2) Golgi complex

The typical Golgi complex is composed of large vacuoles, flattened lamellar membranes and small vesicles. However, in actively proliferating cells, e.g. in response to typhoid vaccine, the Golgi zone is replaced by numerous small vesicles and broad, membrane-bounded cisternae which have a sponge-like appearance because of numerous fenestrations. As the fenestrations become wider, the cisterna appears as a closely packed tubular reticulum. Examination of a large number of electron micrographs suggests that the tubular reticulum is reoriented into parallel array by the lossening of anastomosis and eventually becomes part of the Golgi lamellar membrane. The local dilatation of the tubules and cisternae forms the Golgi vacuoles. These continuous membrane systems are surrounded by clusters of numerous vesicular components, which appear to be separated from the edge of the tubules and cisternae (Fig. 4).

When the vesicular components assume a diffuse form, it is often difficult to ascertain whether the vesicles belong to the Golgi components or to the smooth-surfaced variety of endoplasmic reticulum. Some of the electron micrographs suggest existence of structural communication between the Golgi lamellae and tubules of the smooth-surfaced endoplasmic reticulum (Fig. 4).

3) Cytoplasmic inclusion bodies

The histiocytes contain cytoplasmic inclusion bodies of different sizes and forms. They have been variously called "dense bodies"⁴, "cytosomes"²⁵, "lysosomes"^{6,19}, etc. The most characteristic bodies in histiocytes is similar in appearance to the lysosomes observed in hepatic⁹ and other cells^{7,15}.

The author has named them "histiocyte granules (H-granules)", because histiocytes are distinguished from other mesenchymal cells by the abundance of these granules. The typical H-granule is spherical or oval in shape and 0.2 to 0.5 μ in diameter, and contains dense matrix surrounded by a single membrane. It is noteworthy that the electron-transparent zone, about 180 Å wide, is found invariably between the central matrix and limiting membrane (Figs. 5-7).

The granules vary in number and size depending on the functional activity of the cells; they are larger and more numerous in actively functioning cells, especially in the central region of the cytoplasm, than in immature or resting cells. In active cells the granules vary much in size and diversified in shape, some being

almost circular and others more or less elongated, but they can be always identified by the presence of the characteristic electron-transparent zone between the limiting membrane and the matrix.

A small amount of dense material is occasionally observed within the vesicles or short tubules of the endoplasmic reticulum (Fig. 6). This is interpreted as H-granules in their initial stage of formation. The dense material so far has never been found within either the flattened Golgi membranes or the Golgi vesicles. Moreover, there is no evidence that the granules are located preferentially in the Golgi zone. The granules are located close to the Golgi zone in some cells, but some distance from it in others.

The content of H-graule is usually homogeneous or finely granular. Frequently, however, various kinds of structural changes occur in the content, as will be mentioned below. The changes in the content are almost always accompanied by increase in size of the granules, but the characteristic electron-transparent zone remains persistently as a narrow shell inside the granules (Figs. 8-10).

Certain granules contain particles of high electron density within the matrix. The initial deposition of the particles occurs inside the electron-transparent zone. In some instances the granules are filled with particles of varying sizes and shapes. Lamellar structures are only occasionally observed in the polygonal particles (Fig. 8). The structures consist of several "lines" running parallel to one another, with free ends protruding beyond the edge of the particles into the granule matrix.

Other granules show concentric myeloid structure in their matrix (Fig. 9). They appear initially as dark lines with free ends inside the electron-transparent zone. As the lines develop from the periphery towards the center of the granules, they form a concentric closed system.

The matrix of still other granules shows vesicles with a homogeneous content of moderate density (Fig. 10). Some of the grnaules contain the myeloid structures, dense particles and/or vesicles.

Cytoplasmic inclusion bodies of another type have a different origin and morphology from the H-granules and are called "cytolysomes"²⁰, being considered to be produced by sequestration of the cytoplasmic area undergoing lytic processes in physiological or pathological conditions. In the present study these bodies are observed most often in the later stage of cell proliferation and in response to cortisone (Figs. 11-13). They are composed of portions of the cytoplasm enveloped by a single membrane and show, in the simplest type, a homogeneous or slightly granular matrix, sometimes containing a few vesicles. An early stage of this process is probably represented by the body in Fig. 11, which shows a small area of the cytoplasm partially enclosed by a membrane apparently

newly formed at its periphery. When the bodies contain a large number of small vesicles, they have a strong resemblance of the multivesicular bodies reported to be found in many different kinds of cells^{1,3,18,24}). The multivesicular bodies are encountered also in actively proliferating histiocytes, particularly in the neighborhood of the Golgi complex. In cortisone injection, however, a large number of these bodies are found throughout the cytoplasm. Occasionally the myeloid structures are observed within them (Fig. 13).

Other inclusion bodies show more complicated contents, such as vacuoles, tubules, granules and fragmentous membranes (Fig. 12). On the basis of appearance, the contents seem to be derived from the cytoplasmic components, although the identification of the components is difficult in many cases. Inclusions of this type are, in general, larger and more irregularly shaped than H-granules.

With increase in such inclusions, numerous lipid deposits frequently occur in the cytoplasmic matrix, suggesting progressive degeneration of the cells. When the degeneration process extends to a larger portion of the cytoplasm, the cell membrane sometimes shows complicated invaginations, suggesting the cell membrane contributes to sequestration of the damaged area of the cytoplasm.

Finally, inclusion bodies of still another type are produced by a phagocytic process and called "phagosomes"²⁶). Injected protein silver particles are found frequently in large inpocketings of the cell membrane and within the intracellular vacuoles probably derived from invaginations of the cell membrane. The phagocytic vacuoles frequently contain an amorphous material of low density (Fig. 14). In some instances lamellar structures appear at the periphery of the vacuole matrix and develop towards the center to form concentric myeloid structures, with a small central space containing silver particles. When the vacuoles are filled with ingested silver particles, their limiting membrane may be ruptured and the particles extruded into the cytoplasmic matrix. In the later stage of phagocytosis some of the inclusions contain a variety of cytoplasmic components, including vesicular elements, fragments of membranes and occasionally altered mitochondria. Concentric myeloid structures are also observed. These inclusions may or may not contain silver particles. No silver particles, however, has been seen within the H-granules.

4) Other cytoplasmic components

Mitochondria with typical structure are found preferentially in the central area of cytoplasm. In degenerating cells such as resulting from cortisone injection, they appear to swell and part of the limiting double membrane is converted into a single membrane.

Filamentous structures are observed in only a few cells (Fig. 2). They are invariably located at the perinuclear region and are composed of bundles of fine

filaments arranged in roughly parallel arrays, as described by Petris *et al.*⁵⁾

DISCUSSION

The present study on the morphology of histiocytes in physiological and pathological conditions confirmed the views expressed in a previous report¹¹⁾ that the histiocytes are characterized by predominance of smooth components of the endoplasmic reticulum. A number of electron micrographs indicate that these components are the organelles most responsive to the activity and growth of cells. In immature cells and resting cells most of them are isolated vesicles of spherical shape, whereas in actively functioning cells they appear as part of a more or less continuous reticular system, especially in the central region of the cytoplasm. It is beyond dispute that most of the vesicles in the marginal region of the cytoplasm are of pinocytic or phagocytic origin. It seems, however, very unlikely that all the vesicles in the central region result from such processes, because some of them appear to be pinched off from the continuous membrane systems. The functional significance of the vesiculation of the membrane systems is unknown. A possible interpretation is that this process may be related to segregation and digestion of metabolic products. This interpretation is supported by the finding that the material, which are probably involved in the digestive process, accumulate within the vesicles, as will be discussed below.

Abundance of the H-granules is another important characteristic of the histiocyte. In size and number, however, the granules exhibit considerable varieties, which may be regarded as an expression of different degrees of cell function. Similar bodies have been found in other cells^{7,9,15)} and considered to be "lysosomes" or "lysosome derivatives". Essner and Novikoff⁹⁾ have reported that the lysosomes in hepatoma cells are produced by dilatation and separation of portions of the Golgi cisternae in which secretory products accumulate. The present study, however, shows some evidences supporting the view that the H-granules may be formed by accumulation of dense materials within the smooth components of the endoplasmic reticulum. Firstly, as shown in Fig. 6, the dense material is found within the small vesicle of the endoplasmic reticulum. From its appearance, the material is interpreted as initial accumulation of the H-granule matrix. Secondly, in the early stage of the development, some of the granules frequently show elongated or rod-like shape with terminal swelling (Fig. 7). The profiles appear to correspond with those of the components of smooth-surfaced endoplasmic reticulum. Thirdly, the H-granules are found most often in the central region of the cytoplasm, where the smooth components of the endoplasmic reticulum proliferate vigorously.

H-granules are only occasionally observed close to the Golgi complex. In such cases it is difficult to tell precisely whether the granules originate from the vesicles belonging to the endoplasmic reticulum or from the Golgi vesicles. It

villi of the visual cell's border and have a honeycomb-like structure. Recently, Eakin and Westfall¹⁷⁾ described that the *Stiftchensaum* is formed by infoldings of the cell membrane to form a maze of long, irregular tubules, in opposition to the information of Satir, and that the distal part of these tubules is an undulating tubule of relatively uniform bore (10 m μ), whereas the proximal segment is frequently swollen.

In the present study it has been proved that the *Stiftchensaum* is composed of tightly packed microvilli of the visual cell. The author supposes that the distal segment described in their report corresponds to the intermicrovillus space described in the present study and the proximal to the tubular compartment underlying the microvilli respectively. As the author has mentioned previously, the walls of the microvilli have not yet been observed to be continuous with the walls of the latter. In the micrographs of Eakin and Westfall the direct continuation of the distal segment with the proximal one is not demonstrated anywhere. For these reasons the author dares to say that their description is incorrect in this respect.

The existence of intracellular filamentous structures arouses one's interest, although it is not sure that they are identical with the neurofilaments.

Especially noteworthy is the presence of a bundle of banded fibrils enveloped by the Golgi complex. Its periodicity is generally common to the rootlets of cilia or flagella of other tissues of Amphioxus. Thus the existence of the intracellular banded fibril and of the flagellum in the intracellular cavity between the pigment cell and the visual cell strongly suggests that the visual cell is flagellated and lends support to the idea of the ependymal origin of this visual cell, although the photoreceptor structure is not the derivative of the flagellum. The pigment cell is also suggested to be ciliated on the luminal side, not on the visual cell side. Therefore, the flagellum found in the intercellular cavity between the visual cell and the pigment cell seems to belong more likely to the former rather than to the latter.

In mollusks the cephalopods often contain axial filaments in the axes of the rod-shaped visual cell¹²⁾. In the electron microscope investigation of the proximal retina of Pecten¹⁾, the axial filament has been seen with a banded appearance in the sense cell which possesses the rhabdomere as photoreceptor structure. Moreover, it has been proved that the distal sense cell appendage of Pecten is composed of concentric lamellae, each of which is continuous with a ciliary stalk and basal body³²⁾.

The existence of abundant glycogen granules in the basal part of the cell makes a feature of the visual cell of the *Becheraugen* to distinguish it from the cone of the vertebrate lateral eye, in which glycogen is proved in the paraboloid³³⁾. The paraboloid is usually a solid or semi-solid body distal or apical to the nucleus, which is just the reverse of the *Becheraugen*. Rods may have paraboloids, but only when they have had a particular history³⁴⁾.

tion of focal cytoplasmic damage, presumably intended for protecting further spread of the degenerative process. All these processes contribute to the formation of cytoplasmic inclusion bodies which are possibly related to digestion of the material segregated within them. It is to be noted that these heterogeneous cytoplasmic inclusions frequently show common structures in the later stage of their development. This suggests that similar chemical changes occur in the digestive process in the inclusions.

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Fig. 14.

Fig. 2. Central region of a histiocyte 2 weeks after injection of typhoid vaccine. There are found numerous vesicular components of various sizes (Vs), some of which are aligned in a row (r). Rough-surfaced endoplasmic reticulum (Er) are located preferentially near mitochondria (M). H-granules show round, oval (H_1) or elongated (H_2) profiles. Filamentous structures (F) and part of the Golgi complex (G) can be seen at the perinuclear zone. N, nucleus. Osmium tetroxide fixed; lead hydroxide staining. $\times 40,000$.

Fig. 3. Central region of a histiocyte 2 weeks after injection of typhoid vaccine. Tubular reticulum (T) is seen in addition to numerous vesicles of varying sizes (Vs). Small vesicles appear to be pinched off from local swellings of the reticulum (arrows). Round and elongated oval granules with dense matrix (H) are identified with the H-granules. N, nucleus. Osmium tetroxide fixed; lead hydroxide staining. $\times 40,000$.

Fig. 4. The Golgi complex in a histiocyte 1 week after injection of typhoid vaccine, showing the continuity between the Golgi membranes (Gm) and smooth-surfaced endoplasmic reticulum (Es). The part indicated by T suggests the conversion of the fenestrated cisterna (F) into the Golgi membranes arranged in a parallel array. Another Golgi membranes (G) with typical orientation can be seen close to the nucleus (N). M, obliquely sectioned mitochondria. Potassium permanganate fixed. $\times 50,000$.

Fig. 5. Typical H-granules in a histiocyte from a 5-day-old wound. Note the narrow electron-transparent zone between the dense matrix and limiting membrane. Osmium tetroxide fixed; potassium permanganate staining. $\times 45,000$.

Fig. 6. Part of a histiocyte 2 weeks after injection of typhoid vaccine. Arrow indicates dense material within a small vesicle of the endoplasmic reticulum, interpreted as initial accumulation of the H-granule matrix. M, mitochondrion. Osmium tetroxide fixed; lead hydroxide staining. $\times 55,000$.

Fig. 7. Part of a histiocyte from a 5-day-old scorbutic wound. The H-granules vary in form, being round, oval or elongated. Their profiles appear to correspond to those of the smooth-surfaced endoplasmic reticulum. Osmium tetroxide fixed; lead monoxide staining. $\times 50,000$.

Fig. 8. H-granules of a histiocyte 1 week after injection of typhoid vaccine. The matrix of the granules contains various amounts of particles with high density (H_1 - H_3). Note the electron-transparent zone remaining at the periphery of the large granule (arrows). Osmium tetroxide fixed; lead hydroxide staining. $\times 30,000$. Inset: Higher magnification of a particle marked P, showing lamellar structures aligned at right angles to the long axis of the particle. $\times 75,000$.

Fig. 9. The myeloid structures in the H-granules of a histiocyte 2 weeks after injection of typhoid vaccine. The structures develop from the periphery towards the center of the granules as indicated by arrows. A large granule contains additionally dense particles (P) and vesicles (V). Osmium tetroxide fixed; lead monoxide staining. $\times 35,000$.

Fig. 10. H-granules of a histiocyte 1 week after injection of typhoid vaccine. A granule (H_1) contains several vesicles with homogeneous content of moderate density. The matrix of other granules (H_2) shows small amount of fine particles. Osmium tetroxide fixed; lead hydroxide staining. $\times 35,000$.

Fig. 11. Portion of a histiocyte 2 weeks after injection of typhoid vaccine. A body marked B is partially enclosed by a membrane as pointed by arrow, suggesting an initial process of sequestration of part of the cytoplasm. Osmium tetroxide fixed; lead hydroxide staining. $\times 55,000$.

Fig. 12. A large inclusion body ("cytolysome") in a histiocyte from the same material as in the preceding micrograph. It contains vacuoles (Va), vesicles (Vs) dense materials (D) and fine particles (P). (A dense mass pointed by arrow is possibly a contamination during staining procedure). H, H-granules. Osmium tetroxide fixed; lead monoxide staining. $\times 35,000$.

Fig. 13. Inclusion bodies seen in a histiocyte from a 5-day-old wound. Cortisone was injected at the time of injury. An inclusion body (Mv) has a strong resemblance to the multivesicular body. Some of the bodies (A) contain several myeloid structures. Another body (B) is filled with tightly packed lamellar structures. Osmium tetroxide fixed; lead hydroxide staining. $\times 35,000$.

Fig. 14. Inclusion bodies in a histiocyte 30 hours after injection of protein silver. A large number of silver particles (P) are seen in the homogeneous matrix of the bodies. M, mitochondria. N, nucleus. Osmium tetroxide fixed; lead hydroxide staining. $\times 30,000$.