Submicroscopic Structure of Cell Necrobiosis of Yoshida Sarcoma as Revealed by Electron Microscopy

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SUMMARY

The submicroscopic structure of the normal and degenerating cells of the Yoshida sarcoma has been studied in thin cell sections by electron microscopy. The significance of the homogeneous or granular substance appearing in the vesicles in the endoplasm of normal Yoshida sarcoma cells has been discussed in relation to current concepts of function of cell organelles. The first step of degeneration is replacement of the nucleus peripherally by grouped vesicles, vacuoles, and mitochondria.

The lipomatosis has occurred in the area where the mitochondrial cristae degenerate to a more or less homogeneous mass, but the limiting membranes often remain their double-layered structure. At the beginning of lipomatosis, a homogeneous lipide droplet of moderate density is characteristically surrounded by the osmiophilic myelin figures derived from any of the numerous intracytoplasmic lipoprotein systems. As the lipide droplets are accumulated, they become denser and denser, and their irregular shape transforms gradually to a round profile which demonstrates no dense myelin figures.

The cell necrobiosis begins with isolation of the nuclear membrane following pyknosis. The double-layered structure of the nuclear membrane isolated is similar to that of the smooth-surfaced endoplasmic reticulum. Characteristic patterns of the karyorrhexis and karyolysis have been demonstrated in the necrobiotic cell; they are due to a pathological specialization of the cell organelles in their vacuolization resulting in a discontinuance of their normal function.

Electron microscopy has made cytologists aware of a number of the structural features of cells which thus far have been poorly resolved. Electron microscopy has further made it possible to observe simultaneously all the features involved in a cell. This has naturally led to inquiries into the function of the structures revealed. As a result, it has become possible to study their comparative morphology in different stages of cell function.

Particular interest centers around Yoshida sarcoma cells because of the possibility that higher resolution will uncover units or systems of structures closely related to the cell necrobiosis. Herefore, several reports have appeared on electron microscopy of ascites tumor cells, including articles by Selby et al. (19, 20), Friedlaender et al. (4, 5), Friedlaender and Moore (3), Yasuzumi and Higashizawa (23), Adams and Prince (1), Niklowitz (14), and Yasuzumi and Sugihara (24) on Ehrlich ascites tumor cells; Wesel and Bernhard (21) on Ehrlich ascites tumor cells and Yoshida sarcoma cells; and Yasuzumi and Sugihara (25) on Ehrlich ascites tumor cells, Yoshida sarcoma cells, and human cancerous ascites cells. However, not all the stages of the cell necrobiosis of Yoshida sarcoma have been reported.

Excellent light microscope observations on cell necrobiosis have, however, been described in a summary statement by Lepeschkin (11). He states that cell necrobiosis is accompanied by lipomatosis, vacuolization, pyknosis, karyorrhexis, karyolysis, etc. Classical studies on cell necrobiosis indicate that, while the light microscope has in many instances enabled one to lay down a broad structural plan, there are doubtless many features which will be elucidated by much higher resolution. The present electron microscopic study coincides in many respects with the early optical observations. It also helps to trace the presently unclarified structural details of the nuclei and cell organelles subject to changes during the degeneration.
MATERIALS AND METHODS

The ascites fluid from rats bearing Yoshida sarcoma cells of an inoculation age of 4–5 days were used in the present study. Approximately 0.5 ml of ascites fluid was removed by capillary pipette from the peritoneal cavity. The fixation was carried out at 10° C. for 30 minutes in 2 ml of 1 per cent osmium tetroxide adjusted with veronal-acetate buffer to pH 7.3. Thereafter, without being washed in distilled water, the specimen was dehydrated rapidly in a graded series of ethyl alcohols, impregnated with a mixture of 80 per cent n-butyl methacrylate and 20 per cent methyl methacrylate, and finally embedded in the same resin by polymerization with 2,6-dichlorobenzoyl peroxide at 46° C. Sections, ~200Å in thickness, were cut with a Shimadzu microtome and mounted on grids coated with formvar. They were examined, without removal of the embedding plastic, in an Akashi electron microscope, model TRS-50, and micrographed at magnifications ranging from 2,000 to 30,000. Higher magnifications were obtained by photographic enlargement of the electron micrographs.

RESULTS

Normal Yoshida sarcoma cells.—To facilitate description of the cell necrobiosis, brief reference will be made to features characterizing normal Yoshida sarcoma cells. Sections of a typical example are shown in Figures 1 and 2. Some part of the endoplasm in the normal Yoshida sarcoma cell consists of an aggregation of single, membrane-limited vesicles with varying size and varying electron density. One or more spherical or irregularly shaped granules of different sizes and different density are occasionally found within the vesicles. Some vesicles are occupied with a denser substance than the cytoplasmic matrix. The mitochondria with a few mitochondrial cristae appear around the vesicles. Many of the mitochondria are swollen, measuring about 1.0–1.6 μ in diameter, and the enveloping double membrane is disintegrated at several places where the vesicles are closely applied to the mitochondrial surface (Fig. 1).

In another part of the endoplasm the mitochondria are randomly disposed and of cylindrical form, with mitochondrial cristae arranged in a more or less parallel pattern. Bodies interpreted as destroyed mitochondria are occasionally observed; they tend to be smaller than normal, and their limiting membranes and cristae become disintegrated. An oval-shaped profile is observed, which is characterized by the presence of a great deal of the heterogeneous material of intermediate density, being enveloped in the discontinuous double membranes. The endoplasmic reticulum in a form of strings of vesicles in a circular or an irregular shape is seen to be disposed close to the nucleus (Fig. 2).

The nuclei consist of the heterogeneous or finely granular substance (Figs. 1 and 2). The granules are more densely congregated in certain regions, particularly near the nuclear membrane. Occasionally, short strings of granules are 220–300Å in diameter (Fig. 2). The nucleus is enveloped in the double-structured nuclear membrane, measuring from 300 to 600Å in total thickness (Fig. 1).

Lipide droplets appearing in the degenerating Yoshida sarcoma cell.—As a sign of cell necrobiosis, dense lipide droplets appear in the cytoplasm, which has a heterogeneous character due to the presence of abundant fine granules, fine vesicles, degenerating mitochondria, and fibrous elements (Figs. 3 and 4). The fibrous elements have been frequently observed in the cytoplasm of Yoshida sarcoma cells. The same structure has been reported by Wesel and Bernhard (21), and Yasuzumi and Sugihara (28). In some instances, the fine granules are of rather uniform appearance throughout the cytoplasm, but in other cells they appear in a group showing a dense appearance. The degenerated mitochondria are devoid of their internal ridges and appear to be homogeneous; but their limiting membranes often remain, keeping their double-layered structure. A homogeneous lipide droplet of moderate density appears inside the area surrounded by the osmiophilic myelin figures. As the lipide droplets are accumulated, they become denser and denser, and their irregular shape transforms gradually to a round profile in which the dense membranes have never been observed (Figs. 3, 4).

Nuclei of the degenerating Yoshida sarcoma cells.—In an early stage of degeneration, the nucleus displaced to the periphery of the cell shows an oval-shaped profile in section. The double-layered, more or less undulating membrane demarcates the nucleus from its surrounding homogeneously granular cytoplasm. The chromatic substance is moderately osmiophilic and granular, the granules being packed more closely in the peripheral zone. It is interesting to note that the nuclear membrane partially disintegrates and the karyoplasm communicates through pores with the cytoplasmic matrix (Fig. 5). As the degeneration proceeds, the nucleus becomes pyknotic and consists of a closely packed chromatic substance which appears to be granular or finely coiled fibrilar. The nuclear membrane tends to be isolated from the nuclear surface, which demonstrates the same structure to
the endoplasmic reticulum appearing in the juxtanuclear region (Fig. 6).

In a more advanced stage of degeneration, an interesting pattern has been obtained (Fig. 7), in which the nuclear membrane is found to be completely isolated from the nucleus by 85–200 mμ. Thus, the nucleus is directly surrounded by a narrow space of the cytoplasm, which shows a finely granular appearance. The karyoplasm appears finely reticular, being composed of coiled fibrils. The isolated nuclear membrane appears double-layered, being composed of two dense layers (each about 100 A thick) separated by an electron-lucent interspace (60–170 A wide). The nuclear membrane demonstrates a structure similar to that of the endoplasmic reticulum which is found running parallel with the nuclear membrane or connected with the nuclear membrane. The endoplasmic reticulum appears to be connected with the degenerating mitochondria.

Finally, the karyorrhexis occurs in the nucleus, showing at least three nuclear fragments. The reticular structure of the karyoplasm becomes looser and less osmiophilic than that in earlier stages. The isolated nuclear membrane appears free in the ectoplasm or attached to the nuclear fragments. The karyolysis occurs, furthermore, in the nuclear fragments: fibrillar or granular elements come out from the nuclear fragments into the cytoplasm, and they appear sporadically as clusters (Fig. 8).

Cell organelles in the degenerating Yoshida sarcoma cells.—In the cytoplasm opposite to the nucleus, vacuoles appear as clear space within the cytoplasmic matrix, and they show definite limiting membranes. Some vacuoles seem to be related to the endoplasmic reticulum and others to the mitochondria. It should be mentioned that degenerating mitochondria vary considerably in size from 0.6 μ in diameter to less than 0.1 μ in diameter, the latter being too small to be clearly resolved with the light or phase-contrast microscope. The degenerating mitochondria are also characterized by several features. Some are occupied with a low or dense substance. Others appear entirely depleted. The more or less homogeneous mass of moderate density, having no limiting membrane, is also the degenerating mitochondrial material. The elongated endoplasmic reticulum can be observed in small amount (Fig. 5).

As degeneration proceeds, there is a marked increase in granularity throughout the cytoplasm. A sign of degeneration of the cytoplasm is evidenced by the disappearance of particulate background material (Figs. 6–8). The existence of two types of degenerating mitochondria has been found (Fig. 7)—namely, one type which transforms to a homogeneous mass of intermediate density, being devoid of mitochondrial cristae, the other type degenerating to a vacuole with electron-lucent contents. It is interesting that the mitochondria, degenerated to more or less homogeneous masses of moderate density, are found in the area surrounded by the elongated endoplasmic reticulum, which frequently appears connected with the degenerating mitochondria (Fig. 7).

In the degenerating stage in which the nucleus breaks up to many fragments, a great number of vacuoles of different sizes and different forms, enveloped by single or double membranes, appear in a group. The endoplasmic reticulum is found near the nuclear fragments, which may be a remnant of the nuclear membrane. The mitochondria, provided with typical internal ridges or matrix, disappear completely in this stage (Fig. 8).

DISCUSSION

Some part of the endoplasm in the Yoshida sarcoma cell consists of an aggregation of single membrane-limited vesicles of varying size and varying density. One or more spherical or irregularly shaped granules are often found within the vesicles. These findings are similar to the results obtained in the previous report (25) in which no discussion has been made as to the significance of the substance within the vesicles. The mitochondria appearing around the vesicles are swollen, and their limiting membranes are disintegrated in places where we have seen real evidence of continuity between the mitochondrial matrix and the cytoplasmic vesicles. In this circumstance, it seems reasonable to consider that the components in the vesicles are associated with the secretion.

Dalton and Felix (2) have revealed that the vacuoles of the Golgi complex in the mouse pancreas are bounded by membranes which are approximately 80 A thick, at least twice as thick as the membranes of the endoplasmic reticulum of the same cell. In the course of study of the spermatogenesis of the pond snail, Yasuzumi et al. (26) have demonstrated that the dense walls of the flattened saccules in the Golgi complex have a porous structure which is different from that of the endoplasmic reticulum in the spermatid. In the present study in low magnification, it is difficult to differentiate these two cell organelles, but it is possible that the endoplasm observed is composed of a compound structure of these two organelles.

In the rat liver tumor cells, Howatson and Ham (7) have showed degenerating mitochondria which contained a great deal of granular material arranged in irregular clumps. In the cytoplasm of
rapidly growing uninfected HeLa cells, Harford et al. (6) have found numerous globoid bodies which are assumed to have been transformed from the mitochondria. Yasuzumi and Sugihara (25) have demonstrated a spindle-like body in the Yoshida sarcoma cell; its origin seems to be related to the mitochondria. A peculiar body observed in the present study is suspected to have a closer relation to the endoplasmic reticulum than to the mitochondria, because the limiting membrane of the body consists of an array of vesicles in a circular or irregular shape, and some material released from the body shows a vesicular appearance of the endoplasmic reticulum.

Degenerating changes in Ehrlich ascites tumor cells and liver tumor cells have been observed by Seeger (17, 18), Howatson and Ham (7), and Selby et al. (10). They have demonstrated appearance of many cytoplasmic vacuoles and lipid droplets. With increased vacuolization, a decrease of mitochondria was noted, and often small droplets, similar to free lipid in their density, were found in place of the mitochondrial cristae. In electron microscopy of human cancerous ascites cells, Yasuzumi and Sugihara (25) have succeeded in demonstrating remarkable degenerating changes of the mitochondria in their form and structure. Some mitochondria are swollen, and their center is often free of ridges and is occupied by a matrix of low density. The others decrease remarkably in size but increase in number; where there is an increase in the quantity of vesicles or vacuoles, there is a diminution in the mitochondria. This fact has been interpreted as evidence of a relation between the mitochondria and the accumulation of vesicles or vacuoles. In the degenerating Yoshida sarcoma cells, the mitochondria, transformed to dense masses or vacuoles, are accumulated in the part opposite the nucleus which has been displaced to the peripheral portion of the cell. This finding supports the previously reported results mentioned above. Cell necrobiosis of the Yoshida sarcoma, in which karyorrhexis and karyolysis occur, was characterized by the appearance of well-defined vacuoles within the cytoplasm. At all events, it seems clear that cell necrobiosis of the Yoshida sarcoma is due to a pathological specialization of such organelles as mitochondria, endoplasmic reticulum, and Golgi complex, in that their vacuolization renders impossible continuation of their normal function.

As to the role of the mitochondria in lipogenesis, a number of serious studies have been made in which mitochondria are found to be closely associated with lipid inclusions; but there is some disagreement concerning actual communication between the two (8–10, 13, 16). The present study deals with a pathological deposition of lipides, the so-called lipomatosis. In the degenerating Yoshida sarcoma cell, the association between mitochondria and lipid droplets has not been demonstrated; but it has been found that lipomatosis has occurred in the area where the mitochondrial bridges have been destroyed to more or less homogeneous masses, keeping their limiting membranes as a double-layered structure. At the beginning of lipomatosis, a homogeneous lipid droplet of moderate density is characterized by being surrounded by the osmiophilic myelin figures derived from the numerous intracytoplasmic lipoprotein systems. As the lipid droplets are accumulated, they become denser and denser, and their irregular shape gradually transforms to a round profile which demonstrates no dense myelin figures.

The most interesting feature of the nucleus has been presented in the present study in which the nuclear membrane has been completely separated from the nuclear surface. This seems to have occurred, following the shrinkage of the karyoplasm, the so-called "pyknosis." Such structure has never been seen with the electron microscope, the light or phase microscope as far as is known. The fine structure of the isolated nuclear membrane is similar to that of the endoplasmic reticulum. In the telophase of Yoshida sarcoma cells, Yasuzumi (22) has already found that the smooth-surfaced endoplasmic reticulum participates directly in the development of the nuclear membrane. The degenerating process of the naked nucleus proceeds to karyorrhexis and finally karyolysis.

According to a cytochemical analysis of pyknotic nuclear degeneration by Leuchtenberger (19), in the first stage of pyknosis there is no significant decrease of deoxyribonucleic acid content, al-

**Fig. 1.**—Section showing a portion of the nucleus and some part of the endoplasm in the normal Yoshida sarcoma cell. Note the complexity of cytoplasmic constituents close to the indented nucleus (N). The mitochondria (M) are swollen and electron lucent, being almost devoid of cristae. The mitochondrial membrane is destroyed at the points marked by the arrows, where the vesicles are closely applied to the mitochondrial surface. A great number of vesicles (V) of different sizes appear in the endoplasm, which contains a single granule or many smaller granules or substances of low electron density. The nucleus is enveloped in the double nuclear membrane and occupied with chromatic substance of low electron density. X44,000.
FIG. 2.—Portion of the normal Yoshida sarcoma cell, illustrating the cytoplasm near the nucleus (N). The mitochondria are seen sectioned longitudinally (LM) or transversely (TM) along their major axes. DM represents a destroyed mitochondrion. The circular or irregularly shaped vesicles represent the endoplasmic reticulum (ER). Fine fibrous elements (F) can be seen near the nucleus. A characteristic oval-shaped profile (OP), being enveloped in a discontinuous double membrane, appears in the juxtanuclear region. Its contents are composed of a heterogeneous substance of intermediate density. The nucleus, at the left upper corner, consists of a nearly homogeneous mass of fine granules, some of which appear to be arranged in rows and threads, particularly near the center. Some granules about 160 A in diameter are found congregated at its peripheral part. X37,000.

FIG. 3.—Electron micrograph showing a lipide droplet (L) in the cytoplasm of the degenerating Yoshida sarcoma cell. The osmiophile myelin figures (MF) are found around a lipide droplet (L) of moderate density. Degenerating mitochondria (Z) are found in the cytoplasm. The cytoplasmic matrix consists of a considerable number of small vesicles (V) and granular elements. X36,000.
FIG. 4.—Electron micrograph showing another part of the same section as Figure 3. Four lipide droplets \( (L) \) in irregular shape are found in the cytoplasm. Note single or more layered myelin figures \( (MF) \) appearing around the lipide droplets. Degenerated mitochondria \( (Z) \) are visible scattered in the cytoplasmic matrix, which consists of fine vesicles \( (V) \) or granular elements. Fine granules are visible in a group at the point marked by \( GG \). The fibrous elements \( (F) \) appear at the right upper corner. \( X33,000 \).
FIG. 5.—In an early stage of degeneration of the Yoshida sarcoma the nucleus (N) shows an oval-shaped profile in section, which is displaced to the peripheral zone of the cell. The nuclear membrane demarcates the nucleus from its surrounding homogeneously granular cytoplasm. The chromatic substance is moderately osmiophilic and granular, the granules being packed more closely in a peripheral zone. At the point marked by the arrow the nuclear membrane is disintegrated; thus, the karyoplasin comes in contact with the cytoplasmic matrix. The elongated endoplasmic reticulum (ER) is visible in a small amount. Four lipid droplets (L) appear at the periphery of the cytoplasm. Mitochondria (M) of different sizes and different density and vacuoles (V,A) of varying size are found in the cytoplasm opposite to the nucleus. S represents the degenerated mitochondrial substance. ×46,000.
FIG. 6.—The nuclear texture (N) is dark and consists of a closely packed chromatic substance. Part of the nuclear membrane (NM) appears isolated from the nuclear surface, which demonstrates the same structure as the endoplasmic reticulum (ER) appearing in the juxtanuclear region. The main cytoplasmic elements are round, oval, or elongated mitochondria (M) with matrix of intermediate density, numerous vacuoles (VA) bounded by a single membrane, and elongated endoplasmic reticulum (ER). Two substances (S) of moderate density appearing in the area surrounded by the elongated reticulum (ER) may be the degenerated mitochondrial masses. Mitochondria are frequently encountered as pairs at the points marked by the arrows. X44,000.
FIG. 7.—The nuclear membrane (NM) is found to be completely isolated from the oval-shaped profile of the nucleus (N). The nucleus is directly surrounded by a narrow space of the cytoplasm which shows a finely granular appearance. The elongated endoplasmic reticulum (ER) is found running parallel with the nuclear membrane or connected with the nuclear membrane and the degenerating mitochondria (M). ×65,000.
FIG. 8.—The nucleus breaks up to at least three fragments (PA, PB, and PC). The nuclear fragment profile (PA) is half enveloped in the nuclear membrane (NM). The isolated nuclear membrane is found parallel to the plasma membrane (PM). A great number of vacuoles (VA) of different sizes, being enveloped by single or double membranes, appear in a group. ×31,000.
though over half of it is depolymerized according to the test with methyl green stain. The protein content diminishes to nearly half of the normal level. Later on, there is a progressive loss in deoxyribonucleic acid.

The submicroscopic structure of the nucleus in the pyknotic stage becomes more compact, showing a fine reticular structure, than that of the normal. The submicroscopic structure of the nuclei becomes looser and less osmiophilic than that in earlier stages.

This seems to support the cytochemical analysis of Leuchtenberger (12).

REFERENCES

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