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ELECTRON MICROSCOPE STUDY ON THE INFECTIOUS AND SERUM HEPATITIS

by

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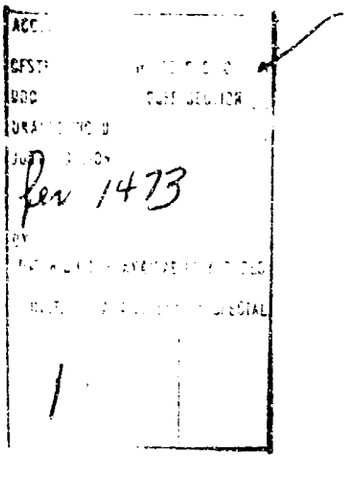

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Abstract

In 26 cases of serum hepatitis from the observations of 80 cases of serum hepatitis and infectious hepatitis, the dense virus-like particles approximately 200 Å in diameter, isolated or in clusters, were observed in the cytoplasm of the hepatic parenchymal cells and of the developing fibroblasts. Moreover, the intranuclear inclusion bodies formed by invagination of the nuclear envelope were found in several hepatic parenchymal cells in cases of serum hepatitis. Many of the inclusion bodies were revealed to contain dense particles which were similar or smaller as compared with th virus-like particles appearing in the cytoplasm. The dense particles within the inclusion seemed to appear associated with the ATPase reaction product.

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1. Purpose of the Investigation

The purpose of the study is to identify viral particles from biopsy material or corpse materials in cases of infectious or serum hepatitis with the aid of electron microscopy and x-ray scanning microanalysis or electron probe microanalysis. Moreover, the structural changes of the liver cells occurring in such diseases are revealed in relation with the liver function.

During the past one year we have tried to observe several enzyme activities of cell organelles in the parenchymal cells of human liver biopsy materials from patients with serum or infectious hepatitis. In the course of such study, several unusual bodies of different sizes and forms have been found in a series of the parenchymal cell nuclei which were prepared to observe their ATPase activities. The description of such structural pattern and a discussion of its functional significance form the topics of this report.

2. Materials and Methods

Biopsy specimens of the human liver were available for electron microscopy from patients those were admitted to Nara Medical University Hospital or to Osaka Prefectural Hospital, and diagnosed as serum or infectious hepatitis from the clinical findings and from the results of the liver function test. The liver biopsy materials were cut with a razor into smaller blocks and fixed either in 1.25 % glutaraldehyde or in 6.25 % glutaraldehyde followed by 1 % osmium teroxide (31), each being adjusted to pH 7.2 with 0.1 M cacodylate buffer.

Small slices or frozen sections of the single fixation material were treated according to a modifided (43) Wachstein-Meisel procedure (36). Some material was incubated in substrate-free medium as control, and subssequently immersed in lead nitrate. The specimens incubated were then washed for 5 minutes in 7.5 % sucrose and postfixed for 1 hour in 1 % osmium teroxide buffered to pH 7.2 with 0.1 M cacodylate buffer. After fixation all the specimens were dehydrated in a series of increasing concentrations of alcohol and embedded in epoxy Epon resin (23). Thin sections were cut on a Porter-Blum microtome or an LKB ultrotome with glass knives. They were mounted on Formvar-coated copper specimen grids and stained either singly with saturated aqueous uranyl acetate (37) or doubly with saturated aqueous uranyl acetate followed by lead nitrate (28). Thse sections were examined in an electron microscope of Hitachi Co., model HU-11C, or an electron microscope of Japan Electron Optics Co., model JEM-7, or an Akashi electron microscope, model TRS-80.

3. Results

In the present materials obtained from 26 cases of serum hepatitis, which were selected from the observations of 80 cases of serum and infectious hepatitis, the dense particles

approximately 200 Å in diameter have been observed in the cytoplasm of the hepatic parenchymal cell and the developing fibroblast. These particles are worth demonstrating here, for purpose of comparison with particulate elements appearing in the intranuclear inclusion bodies. Such dense particles appear isolated or in clusters in the cytoplasmic vesicles or in the cytoplasmic matrix which is often surrounded by a triple-layered unit membrane (Figs. 2 and 3). The particles are clearly differentiated from the glycogen granules which are characteristically organized into 400 to 600 Å rosettes (Final Report: J-203-2). These particles are similar in size and locus to the virus-like particles described recently by Babudieri and coworkers in cases of human infectious hepatitis (3).

In several cases of serum hepatitis, intranuclear inclusion bodies have been found in the hepatic parenchymal cells, which are usually round or oval in shape, and seldom guiter-shaped. They range from 1.0 to 2.5 μ in size (Figs. 3, 4, 7 and 8), but the guiter-shaped is 6.5 μ in length, and 2.5 μ and 3.2 μ in each diameter (Fig. 9). All the inclusion bodies are bounded by a distinct double-layered membrane which is discontinuous in a similar way as in the nuclear envelope, and its outer layer appears associated occasionally with the condensed chromatin masses (Figs. 3 and 4). The inclusion bodies are characterized by containing degenerated mitochondria (Fig. 4) and vesicular elements of the agranular or granular endoplasmic reticulum in different shapes and sizes (Figs. 3, 4 and 8), sometimes showing a parallel arrangement of cisternal elements (Fig. 7). As the inclusion body increases in size, it becomes more complicated in structure, containing lipid droplet-like dense bodies, less dense microbody, and vesicular or vacuolar structures of varying size and form (Fig. 8). At the stage when the inclusion body becomes larger to show a guiter-shape, the included elements appear to be different from the morphologically well-defined cell organelles in the cytoplasm (Figs. 9 and 10). The most striking abnormal elements of the contents are dense particle-containing vesicles. The particles are variable in size between 140 Å and 200 Å in diameter (Figs. 4, 8 and 10).

The roughly round-shaped nucleolus 1.4 to 1.7 μ in diameter, appearing in the nucleus, which contains an inclusion body, consists mainly of tangled, dense nucleolonemata. It is noticeable that such nucleolus seems to have no particular relationship with the inclusion body (Figs. 3 and 7).

The karyoplasm containing an inclusion body is characterized by the relatively clear appearance, consisting of dispersed chromatin elements which are aggregated only in small amount, but not aggregated to form condensed chromatin masses (Figs. 3, 7 and 9).

Prior to the onservations on ATPase activities of the nucleus containing inclusion body, the activities have been observed in the nucleus which contains a large nucleolus consisting of tangled nucleolonemata, but has neither any inclusion bodies nor condensed chromatin masses. Such nucleus

appears to show clearly the deposits of lead phosphate in the nuclear pores (Fig. 5). In a tangential section through the nuclear envelope, the fine dense deposits of reaction product are observed on the nuclear envelope, and the most important feature is globular deposits measuring 460 to 560 Å in diameter. It is certain that these reaction sites correspond to the nuclear pores, on the basis of previous reports (41, 43). Although the deposits are too heavy to observe in detail the structure of the pores, many of the deposits seem to occur remarkably at the peripheral part of the pore, leaving a lighter inner space. But, one or two small deposits are found occasionally at a central point of the pore. Moreover, the deposits occur at the part surrounding the peripheral dense deposits, showing a less dense radial appearance (Fig. 6). Such appearance suggests that the ATPase reaction product has a close relationship with the structural elements constituting the pore, since Gall (14) has recently demonstrated by negative staining of isolated nuclear envelopes of several animal oocytes that the nuclear pores are octagonal rather than circular.

The ATPase activity is clearly detected also in the nuclear pores of the nucleus containing an inclusion body (Figs. 7, 9 and 10) in a similar way as in the nucleus containing no any inclusion bodies (Fig. 5). The limiting membrane and its pore-like spaces surrounding an inclusion body appear to be active sites of ATPase (Figs. 7, 8, 9 and 10), but deposits of the reaction product are smaller in amount as compared with those in the nuclear envelope (Figs. 3, 7 and 9). The precipitate occurs also in association with the membranes lining vesicular or vacuolar structures (Figs. 7, 9 and 10), and with the limiting membrane of lysosome-like bodies (Fig. 10). In an enalarged electron micrograph (Fig. 10) of a part of the guiter-shaped inclusion body (Fig. 9), it may be seen that unusual particles 140 to 200 Å in diameter appear associated with the deposits of ATPase reaction product: the particles are embedded in the dense deposits; they are attached closely to the depsits; and they are surrounded by the deposits.

4. Discussion

Intranuclear inclusion bodies have already been reported in the human and several animal hepatic parenchymal cells under a wide variety of conditions (1, 5-9, 13, 15, 17-21, 24-28, 30, 33-35, 38-40). On the other hand, a considerable number of electron microscope studies have been done in an effort to identify the unknown agents of human infectious or serum hepatitis in liver cells or body fluids of patients with viral hepatitis, and such studies have been summerized by Steiner et al. (32) and Babudieri et al. (3), but the intranuclear inclusion bodies have never been reported in the liver cells in such cases.

Recently, ATPase activities have been reported for the first time by the modified procedure (43) of Wachstein-Meisel method (36) within and adjacent to the nuclear pores of epithelial

Cells of the choroid plexus from an adult mouse brain. More recently, such activities have been detected in the nuclear pores of the Leydig cell in the young human testis, while it has been failed to reveal in the nuclear pores of the cell containing intranuclear inclusion bodies (41). Consequently, it seems pertinent to attampt a study devoted to ATPase activities within the pores of the nuclear envelope of nuclei which contain inclusion bodies in several shapes and sizes.

All of the intranuclear inclusion bodies demonstrated here were completely surrounded by a clearly discernible double-layered membrane. Although it was not demonstrated that this double-layered membrane was continuous with the nuclear envelope at one point, and that an opening was present connecting the interior of the inclusion with the cytoplasm, the outer membrane surrounding the inclusion body appeared to be associated with the chromatin elements, and ATPase activities were revealed at the points where seemed to correspond to the nuclear pores. Therefore, it seems that all the inclusion bodies examined represent invaginations of the nuclear envelope.

The cytoplasmic components engulfed by the invaginations were similar to the cell organelles but were sometimes not identical with those in the cytoplasm. Tentatively, based on the static electron micrographs, a line of development of inclusion bodies may be supposed as follows: the inclusion bodies seen in Figs. 3 and 4 represent early stages, Fig. 9 an advanced stage, and the other figures intermediate stages, since the bodies became more complex in structure concurrently with the increasing of their sizes. Engulfed cell organelles within the inclusion in advanced stages might have been degenerated as a result of such unfavorable environment as the decreased ATPase activity in the invaginated nuclear envelope. In the isolated rat livers perfused with glucagon, Ashford and Porter (2) found hepatocyte lysosomes containing cytoplasmic components in various stages of breakdown or hydrolysis. Some of the contents appearing in the Guiter-shaped inclusion body seem to be similar to lysosomes demonstrated by the authors mentioned above. But, it is well known that acid phosphatase is found exclusively in lysosomes since the work of De Duve (10). Therefore, whther or not the lysosome-like bodies are of acid phosphatase activities is a problem for further investigation.

Although the evidence presented did not provide an answer to the question of why the invagination of the nuclear envelope containing the cytoplasmic organelles or their derivatives occurred, at least one possibility will be considered briefly. The formation of invagination of the nuclear envelope is speculated to be dependent on an excellent mechanism by which the ratio between the surface of the nucleus and its volume is increased to perform nucleocytoplasmic exchanges of macromolecules in more active condition, as already suggested by Leduc and Wilson (21, 22). Such speculation may be supported by the presence of a large nucleololus consisting of dense nucleolonemata and by the clear appearance of karyoplasm,

containing only a few aggregated chromatin elements. Bearcroft (4), by histochemical techniques, showed that the nucleolar enlargement in yellow fever could be correlated with increased protein synthesis and probably represented the first reaction of injury. Yasuzumi et al. (42) suggested that the occurrence of tangled nucleolonemata might be associated with the protein and nucleic acid syntheses, on the basis of observations of nucleoli in several animal tissue cells under a variety of physiological conditions. By means of autoradiographic technique, it was suggested by Hay and Revel (16) that the dispersed DNP gel, diluted chromatin, is the metabolically active form of the DNP meshwork and that the condensed chromatin is relatively inert, at least with respect to nucleic acid and protein synthses. Thus, it may be concluded that the nucleus where the invagination of nuclear envelope occurred is in a metabolically active state, so far as the present material is concerned.

It was assumed by Yasuzumi et al. (41) that one of the causes leading to the formation of inclusion bodies in the aging human Leydig cell nuclei might be due to deficiency of ATPase activity within or adjacent to the nuclear pores. However, such case was clearly different from the present one, since abnormal protein or new and different protein was produced in the Leydig cell nucleus as a result of unfavorable environment.

Although it was assumed that the invagination of the nuclear envelope occurred to perform a more active metabolism, such formation is not compatible with current concepts of normal nuclear structure or function. Such structure may be the expression of nuclear modifications related with the viral particles. Many of the inclusion bodies were revealed to contain dense particles which are similar or smaller in size as compared with the virus-like particles appearing in the cytoplasm. It is interesting that the particles in the inclusion were associated with the ATPase reaction product, as reported by Epstein and Holt in herpes virus particles (12) and by De Thé in avian tumor virus particles (11).

In the intracytoplasmic inclusion bodies seen in neurons and microglial cells from the thalamus of mice infected with Japanese encephalitis virus, dense particles 210 to 250 Å in diameter were found, and they were considered to be premature virus particles, since mature ones were 570 to 580 Å in diameter (44). In a similar way, the particles found in the intranuclear inclusion bodies may be a precursor of the virus-like particles appearing in the hepatic parenchymal cell cytoplasm or in the developing fibroblast cytoplasm, though that must await further evidence.

The present study will be published in Experimental Cell Research in near future.

5. Summary

A series of intranuclear inclusion bodies appearing in the hepatic parenchymal cells in cases of serum hepatitis were

studied under consideration of ATPase activities on the electron microscopic level, in which virus-like particles 200 Å in diameter were found in the cytoplasm, and similar or smaller particles in size within some inclusion bodies. All the inclusion bodies were formed by invagination of the nuclear envelope, in which the pores of the nuclear envelope and its invaginated one were revealed to be active sites of ATPase. Possible function and relationship to viral infection of the intranuclear inclusion bodies were discussed.

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7. Explanation of Figures

Fig. 1. Dense particles approximately 200 Å in diameter isolated or in clusters are visible in the vesicles or in the cytoplasmic matrix surrounded by a triple-layered unit membrane (arrows). X 126,000.

Fig. 2. Dense particles 200 Å in diameter appear in the developing fibroblast. MF:Myelin-figure. X 90,000.

Fig. 3. Electron micrograph of a hepatic parenchymal cell nucleus, showing an intranuclear inclusion and a large nucleolus. The pores (arrows) are visible in the nuclear envelope as well as in the membrane surrounding the inclusion. The chromatin elements appear attached closely to the outer limiting membrane of the inclusion. X 34,000.

Fig. 4. An intranuclear inclusion contains several vesicles of different sizes and shapes, and mitochondrion (M) which seems to be in a stage of breakdown. A particles 200 Å in diameter is visible in a small vesicle (arrow). The pores (P) may be seen in the limiting membrane of the inclusion. The perichromatin granules (PG) can be seen in the chromatin mass attached to the outer limiting membrane of the inclusion. X 75,000.

Fig. 5. Part of the nucleus of the hepatic parenchymal cell incubated with ATP. The roughly round-shaped nucleolus consists of tangled nucleolonemata. The ATPase reaction deposits (arrows) occur in the pores of the nuclear envelope. X 51,000.

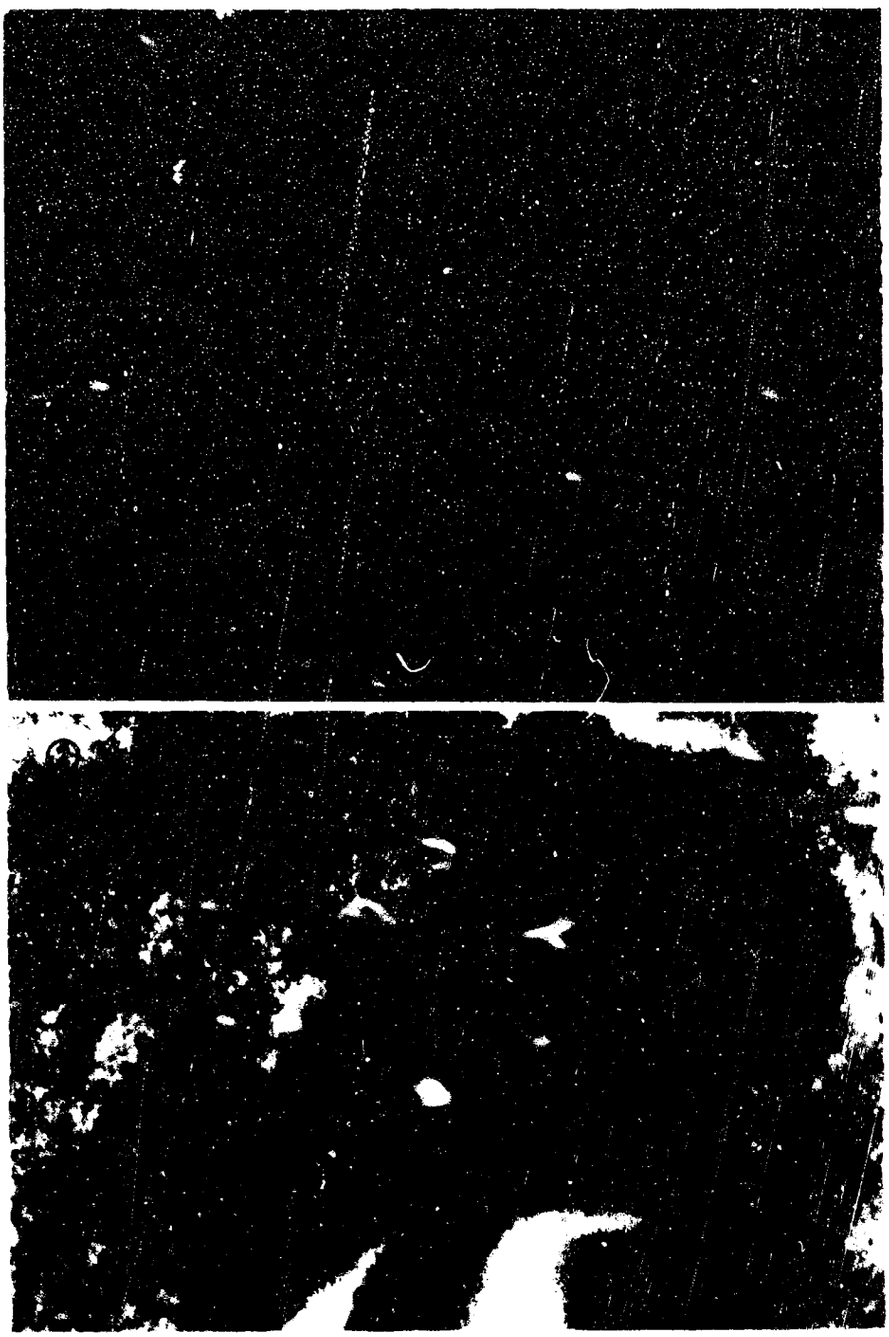
Fig. 6. Tangential section through the nuclear envelope of the hepatic parenchymal cell incubated with ATP. ATPase reaction product occurs as fine granular deposits on the nuclear envelope and as more dense globular deposits 460 to 560 Å in diameter in the pores of nuclear envelope. The less dense deposits (arrows) appear in a radial appearance around the dense precipitate. X 195,000.

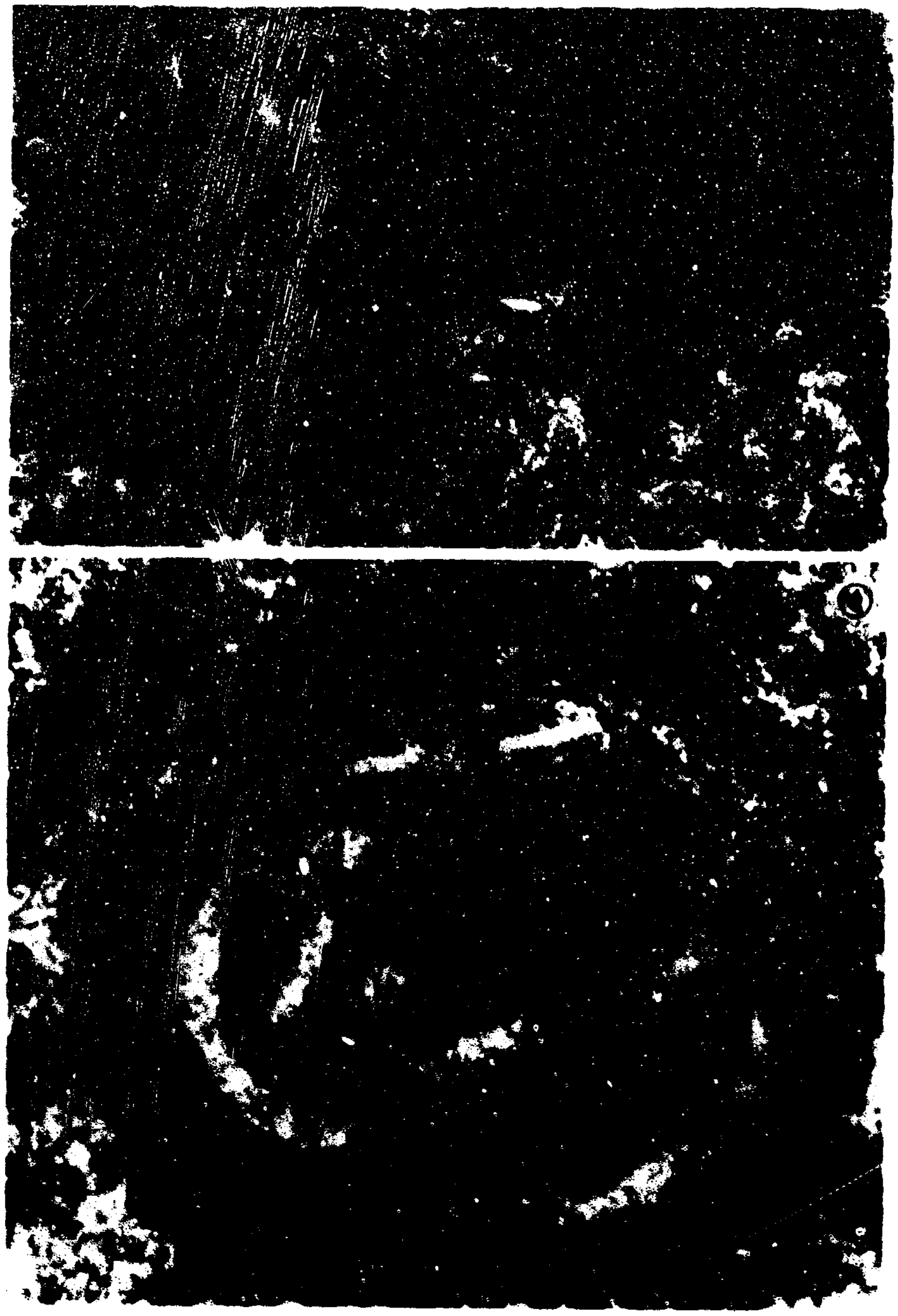
Fig. 7. Part of the nucleus of the hepatic parenchymal cell incubated with ATP, showing a large nucleolus and an inclusion in the nucleus. The ATPase reaction deposits (arrows) occur in the pores of the nuclear envelope as well as on the surface of a vacuole occupying the most part of the inclusion. The precipitate (P) occurs also in a small amount on the membrane surrounding the inclusion. The peripheral part of the inclusion consists of the elongated granular or agranular endoplasmic reticulum. X 45,000.

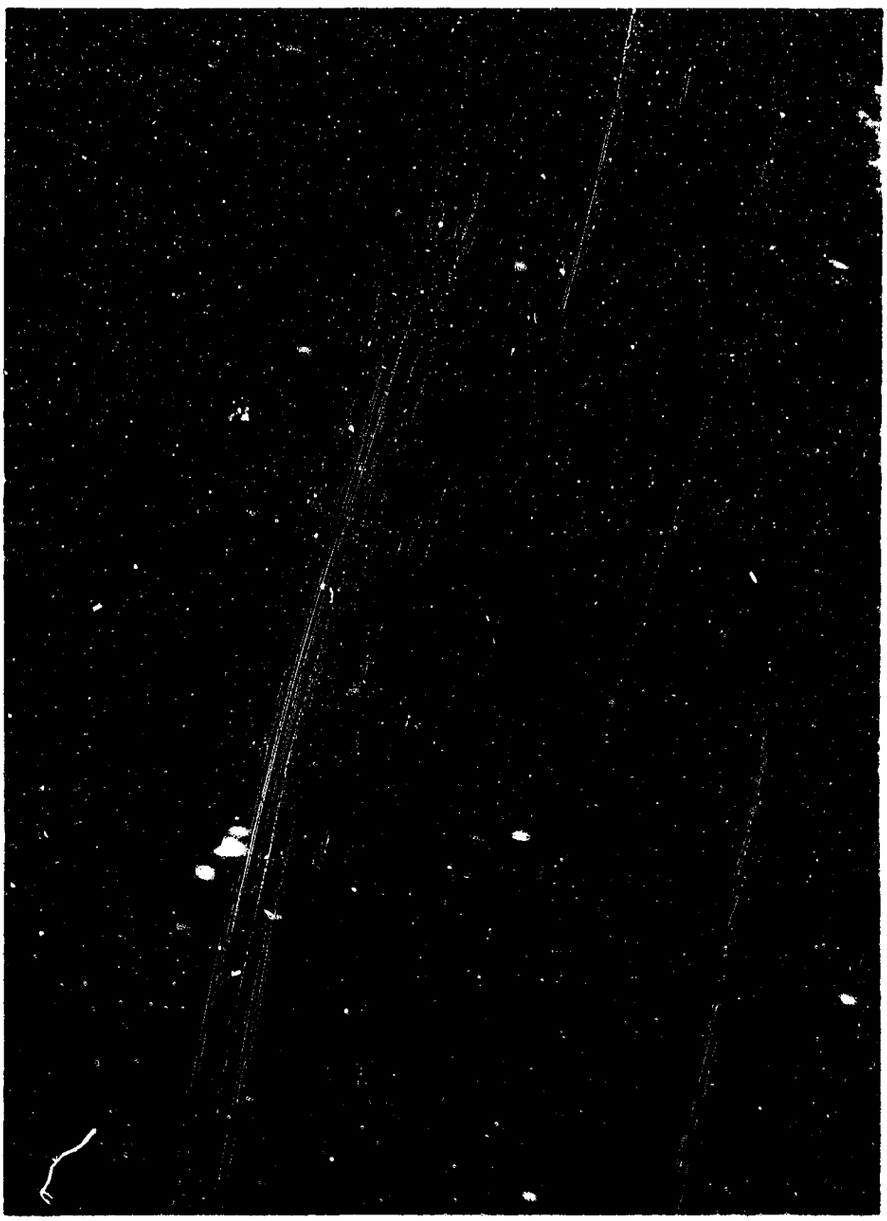
Fig. 8. An inclusion body in the hepatic parenchymal cell nucleus incubated with ATP. The ATPase reaction deposits occur on the membrane surrounding the inclusion, and remarkably at a point marked by P. Most of the contents in the inclusion are in a stage of breadown, though a degenerating microbody (MB) and the endoplasmic reticulum are found. Dense particles 140 to 200 Å in diameter (arrows) can be seen in small vesicles. A homogeneously dense body (L) may be lipid body. X 56,000.

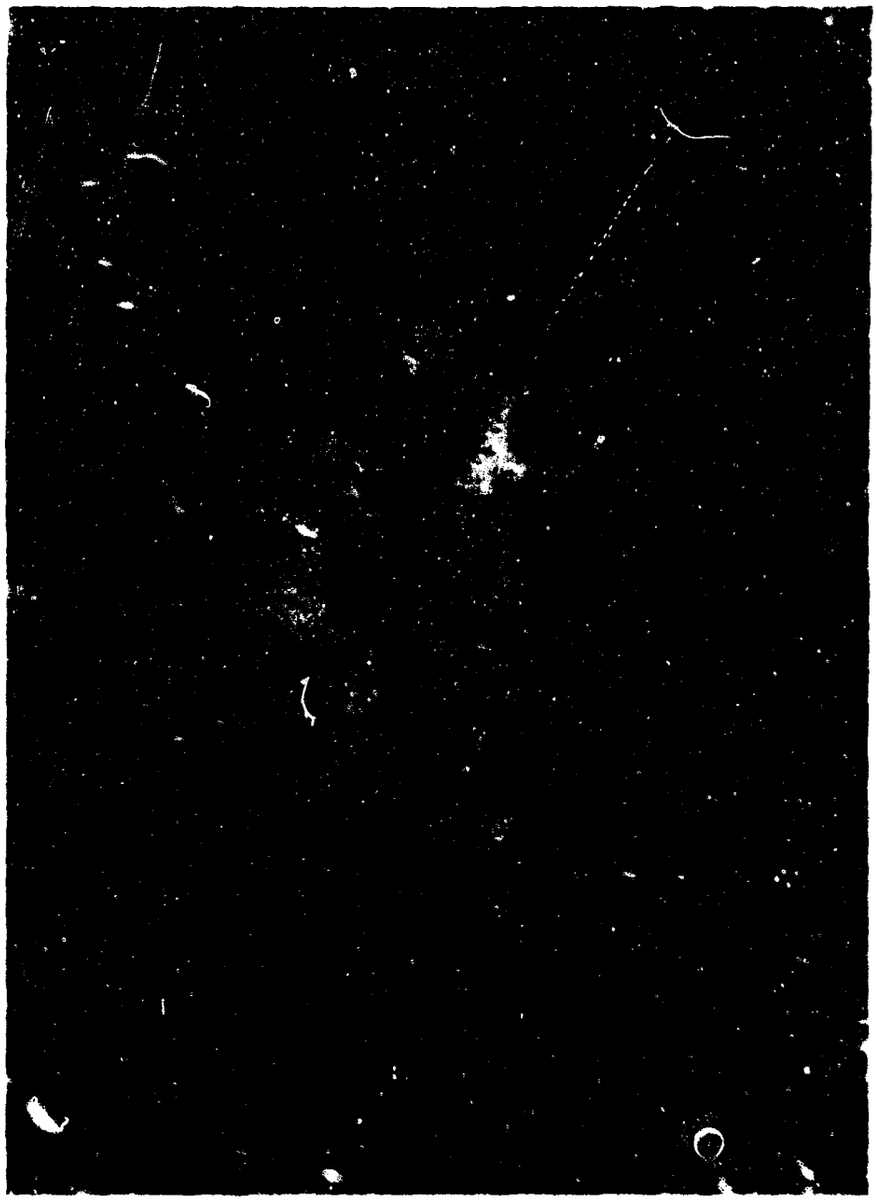
Fig. 9. Part of the nucleus containing a guiter-shaped inclusion body in a hepatic parenchymal cell incubated with ATP. The nuclear pores show clearly the ATPase reaction deposits, but the membrane surrounding the inclusion depicts a small quantity of the deposits in its pore-like spaces (arrows). Numerous degenerating lysosome-like bodies or vesicular structures can be seen within the inclusion. X 23,000.

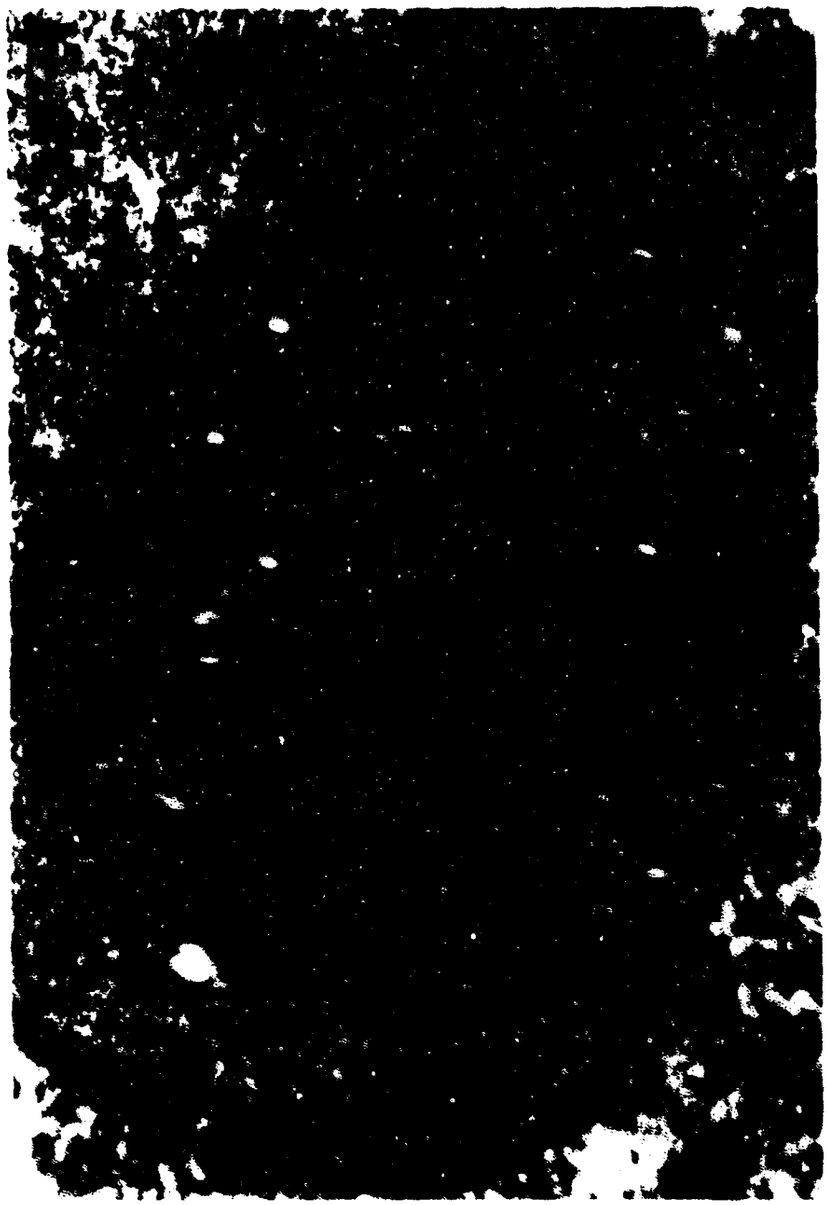
Fig. 10. An enlarged electron micrograph of a part of Fig. 10. The ATPase reaction deposits occur in a small amount in the pore-like spaces (large arrows) of the limiting membrane of the inclusion. Note that the deposits are found at the periphery of the vesicular structures or the lysosome-like bodies (small arrows). Dense particles 140-200 Å in diameter (P) appear in association with the ATPase reaction product. X 90,000.











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13. ABSTRACT

In 26 cases of serum hepatitis from the observations of 80 cases of serum hepatitis and infectious hepatitis, the dense virus-like particles approximately 200 Å in diameter, isolated or in clusters, were observed in the cytoplasm of the hepatic parenchymal cells and of the developing fibroblasts. Moreover, the intranuclear inclusion bodies formed by invagination of the nuclear envelope were found in several hepatic parenchymal cells in cases of serum hepatitis. Many of the inclusion bodies were revealed to contain dense particles which were similar or smaller as compared with the virus-like particles appearing in the cytoplasm. The dense particles within the inclusion seemed to appear associated with the ATPase reaction product. (Author)

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