Ultrastructural Localization of Thorotrast in the Reticuloendothelial System

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THE AFFINITY OF Thorotrast (colloidal thorium dioxide) for reticuloendothelial cells is well established. In immunologic experiments, it is commonly used to block the reticuloendothelial system (RES).[1,2] The mechanism of this effect, however, is still uncertain.[3]

By light microscopy, uptake of Thorotrast has been observed in the lymph nodes, spleen and liver.[4] When administered in large doses, the particles are believed to block the capillaries and small venules of the RES.[5] From electron microscopic studies on rat liver, it has been concluded that the Thorotrast particles are absorbed in vacuoles of the parenchymal cells and of the sinusoidal Kupffer cells.[6] Corresponding studies on spleen and lymph nodes have not been reported.

In a recent work on absorption in mesothelium,[7] we found Thorotrast particles to be located inside electron-dense bodies with the morphologic characteristics of lysosomes. The mechanisms whereby Thorotrast is further transported and retained are of considerable interest, and the present study was therefore undertaken (1) to study the cell types responsible for Thorotrast accumulation in the RES; (2) to examine the intracellular localization of Thorotrast in these cells; and (3) to obtain more information on the participation of cytoplasmic bodies in the cellular absorption of foreign material.

Materials and Methods

Thorotrast

Thorotrast (Fellows-Testagar, Detroit, Mich.) is a sterile, filtered, highly dispersed solution of 24–26% thorium dioxide (w/v) in dextrin and methylparaben. To avoid local cell damage at the injection sites, normal saline was substituted for the suspension medium.[7]

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Supported by grants from The Norwegian Council on Cardiovascular Diseases and from Anders Jahres Funds.

Accepted for publication Nov. 20, 1968.

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Experimental Procedure

Adult male and female rats, weighing from 250 to 350 gm., were used for the study. The animals were anesthetized with ether and alcohol (2:1) through an endotracheal catheter.[8] Thorotrast in a saline suspension was injected into one of the serous cavities, in the following volumes: pericardium, 0.3 cc.; pleura, 1 cc.; and peritoneum, 2 cc. The pericardial and pleural injections were performed through a left thoracotomy, while the peritoneal injections were made percutaneously, with a blunt needle. The thoracotomy and tracheostomy were closed immediately after injection. The animals were allowed a standard diet and free access to water.

Tissue Preparation

Fixation of tissues was initiated from 1 hr. to 16 days following injection of Thorotrast. The animals were perfused for 15 min. with 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2.[9] Simultaneously, fixative was applied directly onto the surfaces of: (1) the parietal surface of the serous membrane; (2) the upper mediastinal lymph nodes; (3) the spleen; and (4) the left lobe of the liver. Specimens were excised from the different tissues and kept in the glutaraldehyde solution for 2 hr. at 4° C. They were postfixed in osmium tetroxide and embedded in Epon 812. Sections were cut on LKB or Huxley microtomes, stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop I electron microscope.

Results

The experiments were well tolerated by all animals. Generalized toxic reactions or local cell damage were not observed.

The results from pericardial, pleural, and peritoneal administration of Thorotrast seemed identical, apart from some difference in the quantity of absorbed particles.

Serous Membranes

The pattern of absorption was essentially the same in the parietal mesothelium of the pericardium, pleura, and peritoneum. Most of the Thorotrast was absorbed through the cytoplasm by means of cytoplasmic vacuoles, but some particles were retained in electron-dense cytoplasmic bodies (Fig. 1). The latter displayed the morphologic features of lysosomes or phagolysosomes, with a single, triple-layered limiting membrane and an amorphous, electron-dense matrix (Fig. 1). Frequently, they seemed closely approximated to mitochondria.

Submesothelial Tissue

The thorium particles were not arrested by the mesothelial basement membrane. On the contrary, they were soon present between the collagen fibrils in the submesothelial area, either separately or in clusters (Fig. 1). In addition, Thorotrast was observed inside macrophages, both

in cytoplasmic vacuoles and in electron-dense bodies. With the lapse of time, increasing amounts of Thorotrast accumulated in the dense bodies, which showed great similarity with the dense bodies in the mesothelium.

Lymphatic Capillaries

Within the first 1–2 hr. after administration, Thorotrast particles had entered some of the lymphatics in the submesothelial tissue. Some particles occurred in the intercellular clefts between adjacent lymphatic endothelial cells. Others were present in cytoplasmic vacuoles, and a few were retained in dense bodies in the cytoplasm. The basement membrane showed frequent interruptions, and obviously did not prevent the thorium particles from entering the lumen.

Regional Lymph Nodes

The first evidence of Thorotrast accumulation in regional lymph nodes was present 1–2 hr. following injection into either one of the serous cavities. Initially, particles were observed in the sinusoidal endothelium and in some of the macrophages in the periphery of the lymph nodes. They occurred mainly inside irregular vacuoles of larger size than is common in macrophages. Most of the vacuoles displayed a well-defined membrane, sometimes with an internal "coating."

With the lapse of time, Thorotrast particles accumulated in cytoplasmic bodies of numerous macrophages (Fig. 2–5), and in a few of the reticulum cells. These bodies exhibited a variable structure. Some were occupied by a dense, homogenous matrix, while others were large and contained inclusions or vacuoles (Fig. 2 and 3). Several bodies appeared to be packed with Thorotrast, while others presented scarce amounts of particles along the margin (Fig. 4). On some occasions, Thorotrast was localized inside small vacuoles surrounded by a common membrane, giving the appearance of a multivesicular body (Fig. 4). Frequently, cytoplasmic vacuoles and dense bodies were closely approximated, and fusion between the two types of organelles was often suggested (Fig. 3 and 4).

The above pattern of Thorotrast localization was observed at all time intervals from 4 hr. onwards. Even after 16 days, the macrophages and some of the reticulum cells contained Thorotrast inside cytoplasmic bodies of various appearance (Fig. 5). The other cell types present (i.e., lymphocytes, fibroblasts, monocytes, and plasma cells) did not contain thorium particles. Occasionally, some free particles were detected in the lumens of lymph capillaries.

Spleen

Thorotrast was present in the splenic pulp from 4 hr. on. The amount of particles seemed to increase during the first 24 hr. following administration. Their localization was essentially the same as in the lymph nodes—i.e., in the cytoplasm of macrophages and in a few of the reticulum cells and sinusoidal cells. The intracellular distribution also followed the previously described pattern. Initially, the particles seemed confined mainly to cytoplasmic vacuoles, although some dense bodies were labeled even at early stages. The Thorotrast-containing vacuoles frequently were large and irregular (Fig. 6). Later on, the tracer particles were accumulated inside different types of cytoplasmic bodies. At 1 week and at 16 days, the general picture was essentially the same as after 48 hr., indicating that the particles were retained in splenic macrophages and reticulum cells for an indefinite period of time.

Liver

Thorotrast accumulation was observed both in the parenchymal cells (hepatocytes) and in the sinusoidal endothelial cells (Kupffer cells).

Parenchymal Cells. At 6 and 12 hr., particles were present in vacuoles in the periphery of the cytoplasm, close to the space of Disse and to the intercellular space between the hepatocytes. These vacuoles usually were large and irregular, with a variable amount of internal coating. At 24 and 48 hr., thorium particles were observed inside dense bodies of different size and structures. Some were small and dense, with the typical appearance of liver lysosomes (Fig. 7); others were large, with varying degrees of vacuolation. Some presented a dense "eye" or other forms of inclusion, and corresponded to the concept of microbodies. Others possessed the features of multivesicular bodies. Interaction or fusion between lysosomes and Thorotrast-containing vacuoles seemed sometimes to occur (Fig. 7). At 4 days, the amount of Thorotrast inside the hepatocytes seemed to decrease, and the particles were observed mainly inside peribiliary dense bodies (Fig. 8). At 7 days, the cytoplasm usually was devoid of Thorotrast, but some few particles were detected in the lumens of biliary capillaries (Fig. 9).

Kupffer Cells. Thorotrast was observed inside most Kupffer cells at 6 and 12 hr. after injection, and at all later stages. During the first 24 hr., they were mainly localized inside vacuoles; later they seemed to accumulate in electron-dense bodies with the appearance of lysosomes (Fig. 10 and 11). Other cytoplasmic structures (e.g., the mitochondria, endoplasmic reticulum, and small vesicles) did not contain particles at any time interval. As observed in mesothelial cells and macrophages, the mito-

chondria were frequently lying in close approximation to the Thorotrast-containing bodies (Fig. 10 and 11).

Discussion

Some of the thorium dioxide particles were retained inside electron-dense bodies in the mesothelium of the serous membranes. Most of the substance, however, traversed the mesothelium and its basement membrane, and entered the submesothelial tissue, the cytoplasm of macrophages and the lymph capillaries. These observations correspond well with previous studies on absorption of Thorotrast from serous cavities.[7,10-13] The mesothelial absorption was solely transcellular, while in lymphatic endothelium, some particles appeared also in the intercellular clefts.

The distribution of Thorotrast in the lymph nodes and in the spleen seemed identical. Particles occurred both in macrophages and in the reticulum cells, but seemed mainly to concentrate in macrophages. Initially, the thorium particles occurred inside cytoplasmic vacuoles. At later stages, they accumulated in various electron-dense bodies with the gross characteristics of lysosomes. Hence, the affinity of Thorotrast to lysosomal structures[7] seemed obvious. The possibility of interaction between the different cytoplasmic organelles is discussed below. The presence of lymphovenous communications could not be established from the present study. Serious doubt has been cast upon their existence in normal lymphoid tissue.

In the parenchymal hepatic cells, Thorotrast particles were first detected in the lumens of large vacuoles; thereafter they accumulated in electron-dense bodies of different morphologic pattern. The concentration of thorium particles inside these bodies may be of considerable interest. In previous studies on hepatic endocytosis, the uptake of thorium particles was confined to cytoplasmic vacuoles only.[6] Some of these vacuoles may represent heterophagosomes[16,17] or heterophagic vacuoles.[18] In the present study, some of the vacuoles were closely approximated to the small, dense, membrane-bound structures usually referred to as liver lysosomes,[19] and even seemed to merge with these. Other types of electron-dense bodies also participated in Thorotrast accumulation—e.g., the multivesicular and vacuolated bodies (Fig. 3, 4 and 7). Intermediate stages between these organelles were often suggested. It should be noted that some of the microbodies also contained thorium particles, particularly microbodies of the vacuolized type.[20]

This pattern of thorium dioxide localization in hepatic parenchymal cells strongly suggests interaction or even fusion between different cyto-

plasmic organelles. This pertains to the small, dense lysosomes, the cytoplasmic vacuoles, the multivesicular bodies, and the microbodies. Bruni and Porter [19] also suggest that interaction between these organelles takes place in normal rat liver. Studies on lipid absorption [21] indicate an incorporation into lysosomes, while protein is reported to accumulate in cytoplasmic vacuoles.[22]

The absorption of Thorotrast by the Kupffer cells of the liver sinusoids followed the same pattern as in macrophages and in endothelial and mesothelial cells. Similarities in the physiology of Kupffer cells and hepatocytes has been noted by previous authors.[23,24] This pertains even to studies on thorium dioxide absorption.[6]

In general, the uptake of thorium dioxide in different cells and tissues seemed to follow a common pattern. In the initial stages, the particles were absorbed into cytoplasmic vacuoles, which probably correspond to the concept of heterophagosomes [16,17] or heterophagic vacuoles.[18] Some of these vacuoles became closely related to electron-dense bodies with the characteristics of lysosomes.[17,30] At later stages, most particles were retained inside cytoplasmic bodies of different morphology—i.e., multivesicular bodies; large, dense bodies; microbodies; peribiliary bodies; and residual bodies. All of these may be regarded as secondary lysosomes [16,18] or phagolysosomes.[17] In this and most other studies, the membranes of these structures were well defined and did not appear damaged.

It is tempting to relate this lysosomal localization of thorium dioxide to the carcinogenic properties of Thorotrast. The development of thorotrastomas,[25] hepatomas, and endotheliomas [5] may possibly be related to Thorotrast accumulation in lysosomes of the cells in question. If so, the present study supports the suggestion of Duncan [26] that carcinogenesis and radiation effects are both caused by interference with the functions of lysosomes.

The effect of thorium dioxide upon endotoxin reactions,[1] on the bone marrow,[5] the adrenals,[27] and blood coagulation [28,29] may possibly be caused by similar mechanisms. Finally, it is suggested that the induction of reticuloendothelial blockade [1-3] may be brought about by the influence of Thorotrast upon lysosomal structures. Hence, this subcellular localization of Thorotrast may have a bearing upon its well-known effects in immunological experiments.

Summary

The accumulation of Thorotrast (colloidal thorium dioxide) in the reticuloendothelial system was studied by electron microscopy. Thoro-

trast was administered by injection into the serous cavities of adults rats.

At different time intervals, particles were observed in mesothelial cells, lymph capillaries, and in cells of the reticuloendothelial system. In lymph nodes and spleen, particles appeared in macrophages, reticulum cells, and sinusoidal cells. In the liver, both hepatocytes and Kupffer cells were seen to accumulate thorium particles.

The intracellular distribution of Thorotrast seemed to follow a general pattern. Initially, the particles were localized in large cytoplasmic vacuoles, probably phagosomes (phagocytic vacuoles). With the lapse of time, they accumulated in secondary lysosomes (phagolysosomes) of different types, and seemed to be retained in these structures.

It is suggested that the lysosomal localization of Thorotrast in reticuloendothelial cells may be related to the carcinogenic and immunologic properties of this substance.

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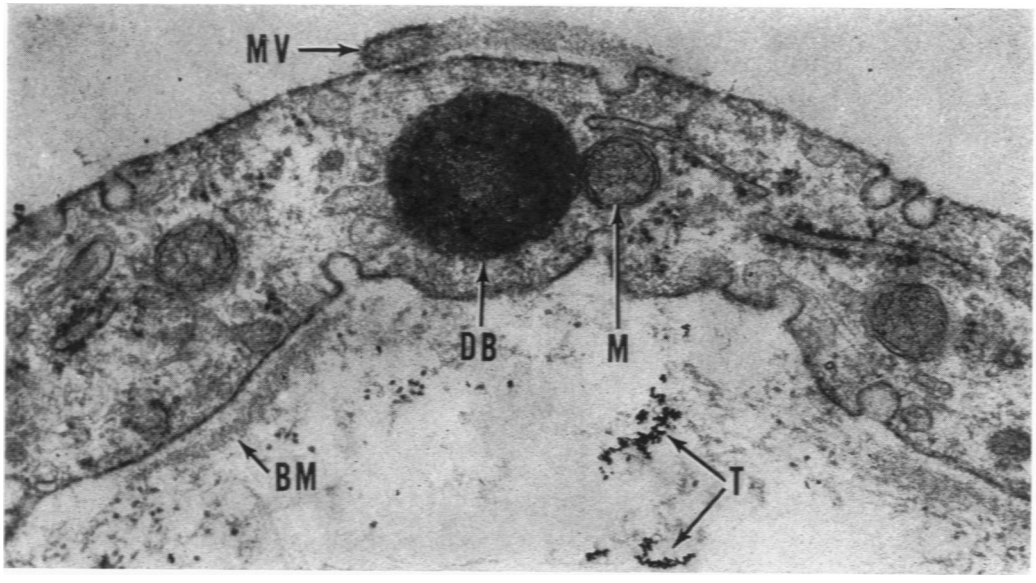
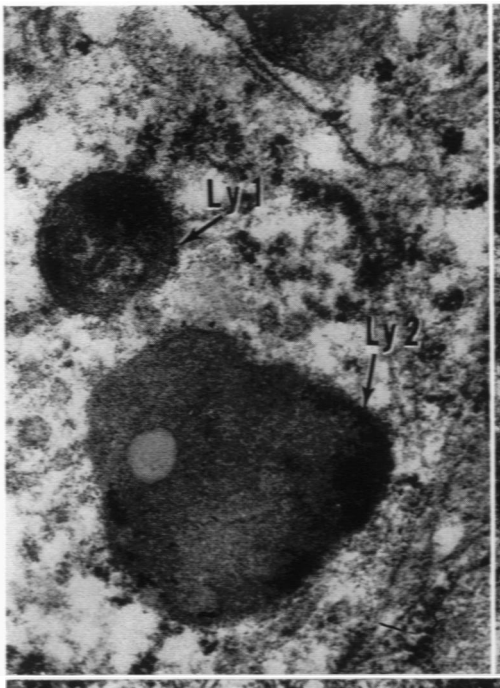


Fig. 1. Pericardial mesothelium, 30 min. after injection of thorium dioxide suspension into pericardial cavity. In the mesothelial cell, particles are present inside a lysosomal dense body (*DB*). A mitochondrium (*M*) is closely approximated to the dense body. Several free particles (*T*) are lying in the submesothelial space. The basement (*BM*) appears interrupted. Microvillus (*MV*). × 60,000.

Fig. 2 and 3. Lymph node macrophage, 24 hr. Different types of lysosomes at higher magnification. Some small bodies *(Ly 1* and *Ly 3)* display a dense, homogeneous matrix. The large body *(Ly 5)* contains several small and one large vacuole, and is closely connected with *Ly 3* and *Ly 4.* A single vacuole is present in *Ly 2* and in *Ly 4,* indicating that these bodies may represent intermediate stages between small dense and large vacuolated bodies. The dense granules of *Ly 5 (arrows)* are possibly remnants of a primary lysosome that has fused with a phagosome in the formation of a phagolysosome (phagocytic vacuole, secondary lysosome). **Fig. 2,** × 45,000; **Fig. 3,** × 45,000.

Fig. 4. Lymph node macrophage, 48 hr. Thorotrast is present inside a large dense body with homogeneous matrix *(Ly 1),* in a vacuole-containing body *(Ly 2),* and in a multivesicular body *(MVB).* Several Thorotrast-containing vacuoles *(V)* are merging with, or separating from, the multivesicular body. Note the arrangement of thorium particles in *Ly 1* and *Ly 2,* close to the lysosomal membrane. × 15,000.

Fig. 5. Lymph node, 16 days. Thorotrast is still present in lysosomes of macrophage. Nucleus *(N).* × 30,000.



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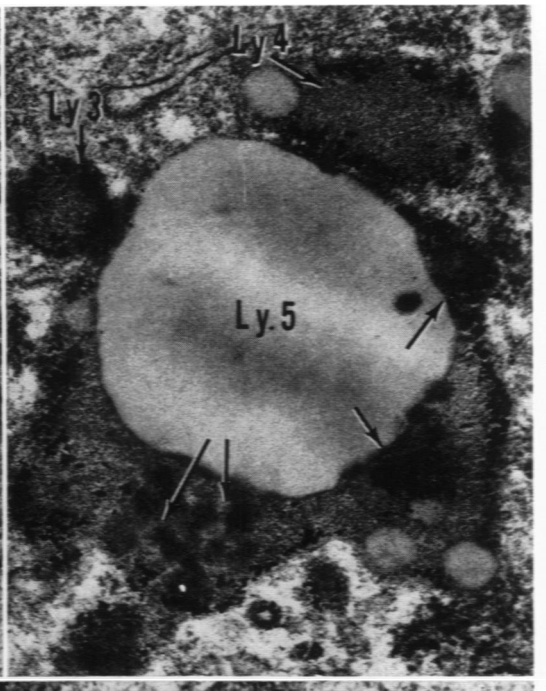

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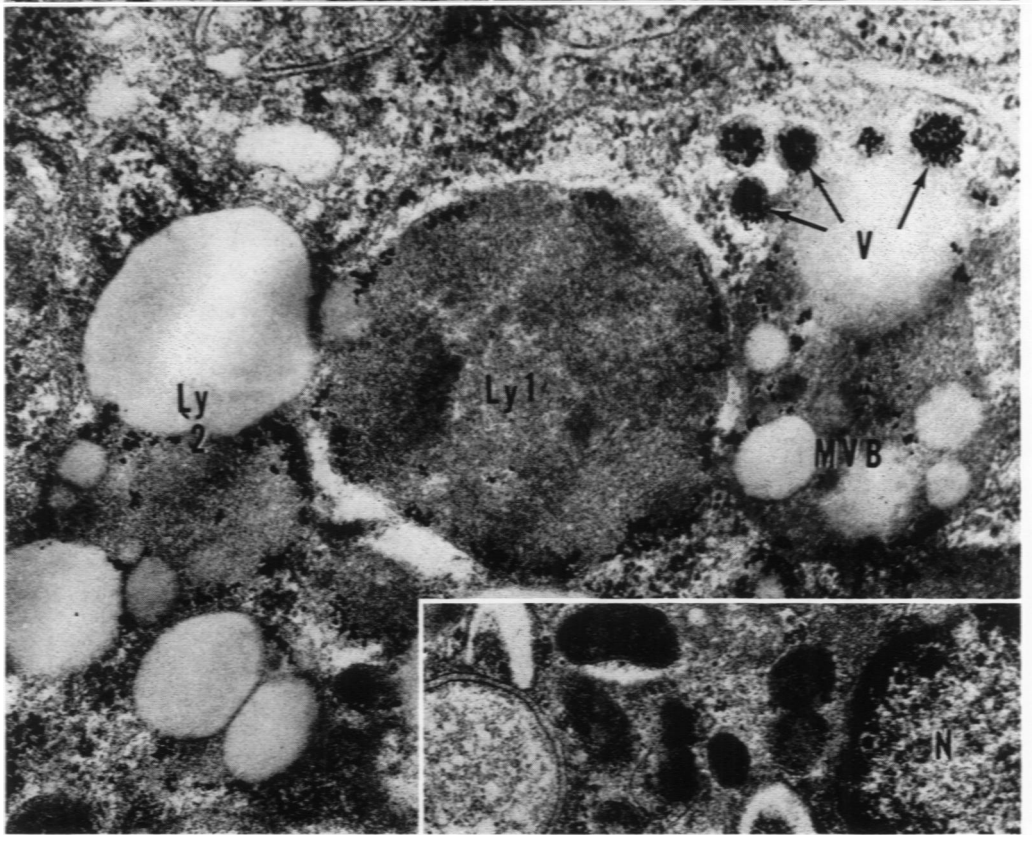

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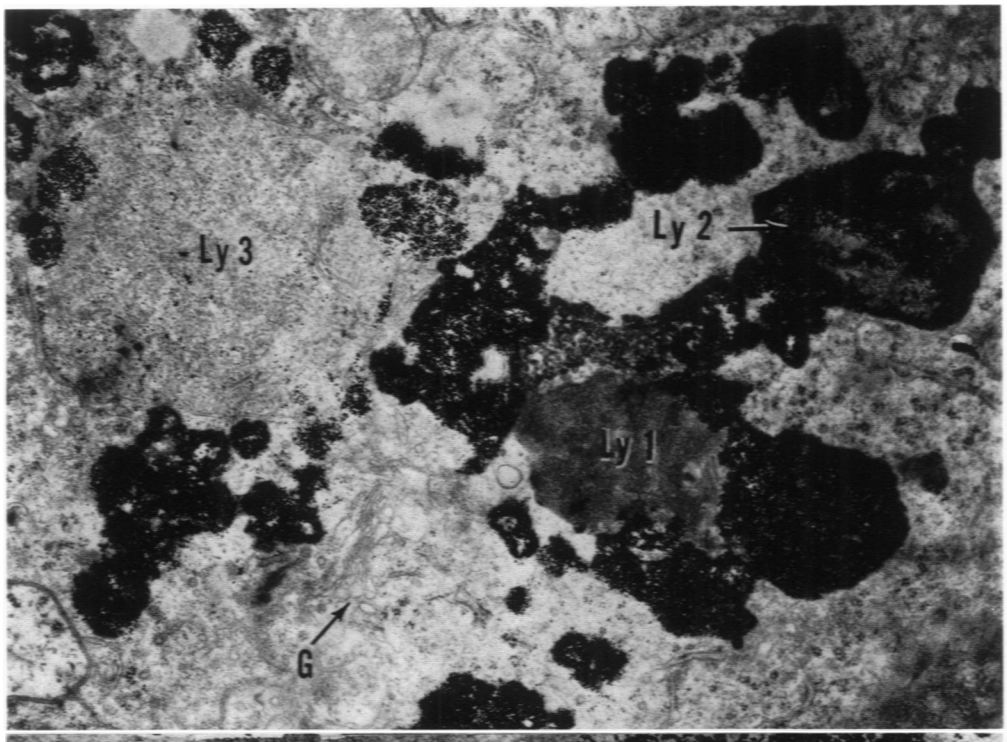
Fig. 6. Splenic macrophage, 48 hr. Thorotrast-containing vacuoles appear to fuse with lysosomes of different structure *(Ly 1, Ly 2,* and *Ly 3).* The amorphous, electron-dense matrix of the lysosome is clearly visible in *Ly 1,* while in *Ly 2* it is obscured by the thorium particles. *Ly 3* contains degraded cellular material and may represent a combined autophagic and heterophagic vacuole. Interaction between vacuoles and lysosomes takes place close to the Golgi apparatus *(G).* × 18,000.

Fig. 7. Hepatocyte, 24 hr. Many particles are still present within vacuoles *(V),* which probably represent heterophagosomes (heterophagic vacuoles). A nearby lysosome *(Ly 1)* displays a high electron density and a clearly defined membrane. This structure may correspond to the concept of a primary lysosome (see text). The other body *(Ly 2)* contains some electron-dense substance in addition to Thorotrast particles, and probably represents a phagolysosome (secondary lysosome). Mitochondria *(M).* × 48,000.



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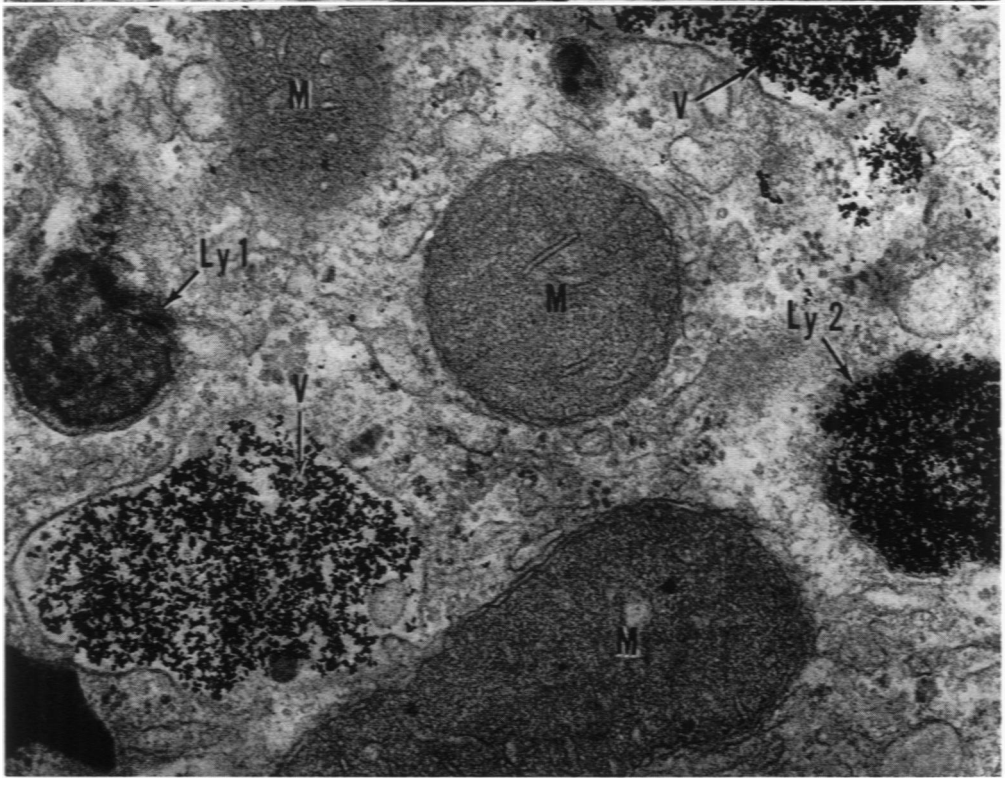

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Fig. 8. Liver parenchyma, 4 days. Three hepatocytes *(H)* surround a bile capillary *(BC)*. Small amounts of Thorotrast are present inside large lysosomes *(LY)*, close to the bile capillary. The rest of the cytoplasm is devoid of thorium particles. Intercellular spaces *(IS)*. × 24,000.

Fig. 9. Liver parenchyma, 7 days. Hepatocytes *(H)* contain no Thorotrast, but a few particles are present in the lumens of bile capillaries *(BC)*. Intercellular space *(IS)*. × 18,000.

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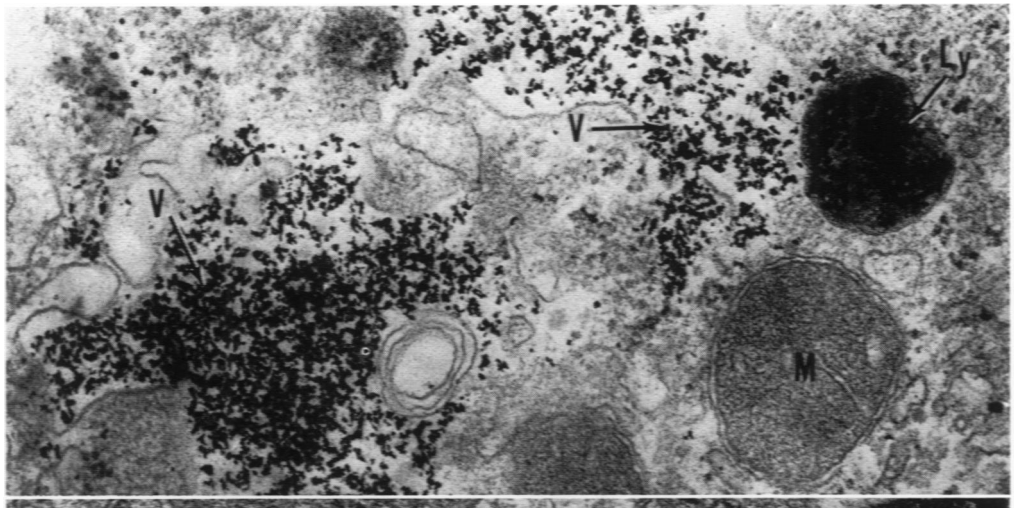
Fig. 10. Kupffer cell of hepatic sinusoid, 24 hr. Thorotrast within irregular vacuoles *(V)* and inside a lysosomal dense body *(Ly)*. Mitochondrium *(M)*. × 16,000.

Fig. 11. Kupffer cell, 7 days. Thorium dioxide appears within 2 lysosomal structures with quite different morphology. *Ly 1* may represent a phagolysosome, while *Ly 2* contains large vacuoles, and is probably a late stage in the development of a residual body. Space of Disse *(SD)*. Red blood cell *(RBC)* in sinusoid. Nucleus of Kupffer cell *(N)*. × 15,000.



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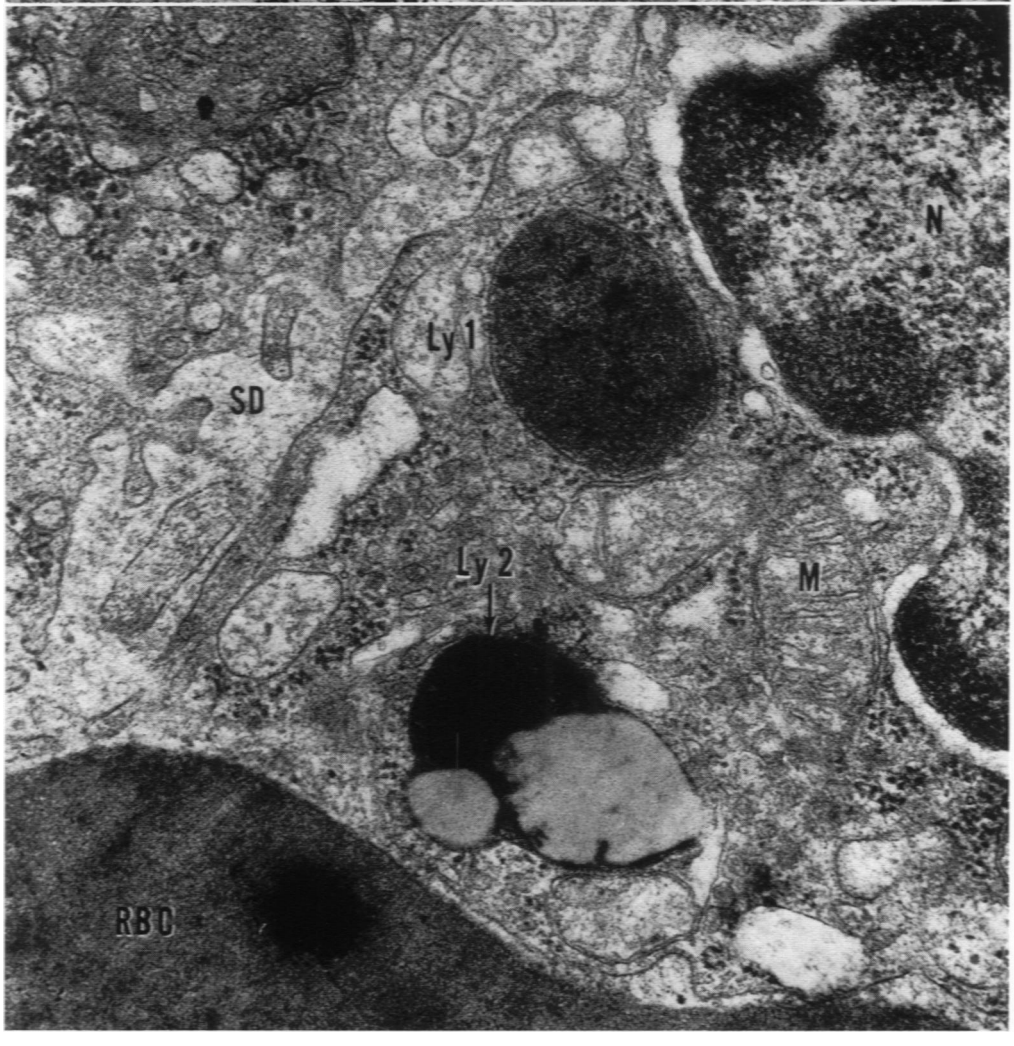

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