ELECTRON MICROSCOPY AND X-RAY SCANNING MICROANALYSIS OF NEEDLE BIOPSY MATERIAL FROM HUMAN LIVER

G. YASUZUMI, M.D.

From the Electron Microscope Research Laboratory, Department of Anatomy, Nara Medical College, Kashihara City, Nara, Japan

ABSTRACT

A study has been made of the fine structure of hepatic parenchymal cells of human biopsy material in a case of pancreatic tumor with obstructive jaundice. Dense particles about 60 A in diameter have been found in the cytoplasm, which are considered to be ferritin molecules by electron microscopy. They are encountered throughout the cytoplasmic matrix and are often aggregated in electron-transparent areas, most of which are enclosed by an apparently single-layered membrane. Identification of the elemental iron has been pursued by the application of the x-ray scanning microanalyser which reveals a quantitative value within 1.0 per cent of the pure iron sample. The use of x-ray scanning microanalysis enables one to obtain accurate data from extremely small and precisely defined volumes of biological specimens.

INTRODUCTION

In the course of an electron microscope study of the liver in infectious hepatitis in humans (1), the author examined a liver biopsy specimen obtained from a case of pancreatic tumor with obstructive jaundice in order to compare the fine structures of hepatic parenchymal cells in both diseases. It has been observed that isolated as well as aggregated dense particles about 60 A in diameter are present in the cytoplasm of the hepatic cells in the biopsied specimen. These particles have dimensions and profiles characteristic of the iron hydroxide micelles of ferritin molecules as previously reported (2–11).

Recently, it has been confirmed that the x-ray scanning microanalyzer can be usefully applied to analyze the elemental iron localized in the cytoplasm of testicular nutritive cells in pond snails, which has been revealed by histochemical technique (12). In that study, it was impossible to perform a quantitative analysis of the iron because the element was present in too small amounts. In the present study, a quantitative analysis of iron has been carried out on liver cells by recording the intensity of the x-ray emission on a pen recorder and comparing it with the intensity of the emission from a specimen of pure iron element irradiated at the same beam current.

MATERIALS AND METHODS

A biopsy specimen of the liver from one patient was available for electron microscopy. The patient, a 49-year-old housewife, was admitted to Nara Medical College Hospital because of jaundice which had developed in early July 1961. The liver specimen was taken on August 20, 1961. The laboratory tests revealed the following: Van den Bergh, direct 24.2 mg/dl, indirect 7.8 mg/dl; icterus index 90; Kunkel 7 units; thymol 5 units; CCF (+); BSP 50 per cent; total protein 7.8 per cent; alkaline phosphatase 12.0 mg per cent; syphilis test (+). A diagnosis of tumor of the head of the pancreas was made on ventrotomy

on December 13, 1961. The jaundice was clearly traced to a constriction of the common bile duct caused by the tumor. The patient had never received whole blood or taken iron-containing drugs.

The liver specimen was cut into tiny blocks (1 mm in greatest dimension) which were fixed for 30 minutes at 4°C in 1 per cent osmium tetroxide adjusted with veronal-acetate buffer to pH 7.4. After fixation, the specimen (without washing in distilled water) was directly dehydrated in a series of increasing concentrations of ethyl alcohol, and embedded in a mixture of methyl and n-butyl methacrylates, or in Epon 812. Sections were cut on a Porter-Blum microtome, with glass knives, and mounted on copper grids coated with formvar. The sections were then stained according to Watson's lead acetate procedure (13), with slight modification, and a thin coat of carbon evaporated onto them. They were examined in an Akashi-TRS-50E₁ electron microscope.

Procedures for x-ray scanning microanalysis were as follows: sections 1 μ or more thick were mounted on copper grids with a supporting membrane; they were stained (without removing the plastic) with 0.5 per cent basic fuchsin solution to identify individual cells, and covered with a carbon film; they were then examined with an Akashi x-ray scanning microanalyzer, model Tronalyzer-TRA type, with an accelerating voltage of 22 kv and a probe current of 0.35 μA (12).

RESULTS

This description will be limited essentially to observations on hepatic parenchymal cells of the biopsy material. Most of the cytoplasm of the cells contains large aggregates of electron-opaque particles, some of the aggregates measuring up to 0.6 μ in cross-section (Figs. 1 and 3). These particles are found in electron-transparent areas which are frequently surrounded by an apparently single-layered membrane (Fig. 1). It is evident

that the dense particles, which are about 60 A in diameter, are found throughout the cytoplasmic matrix (Figs. 1 to 4). The 60-A dense particles appear to be regularly spherical in shape at low magnification (Figs. 1 to 3); some of them reveal the characteristic ferritin pattern at higher magnification (Fig. 4). Although the tetrad form is clearly visible, it is infrequent. Other profiles, such as rings, triads, and pentads, are also demonstrated. These variations presumably are oblique or lateral projections of quadrimicellar configuration as already suggested by Richter (6). No dense particles of this size have ever been observed in the nuclei of the parenchymal cells (Figs. 3 and 5).

The cytoplasm of these liver cells also contains a small number of roughly oval-shaped profiles which are filled with a homogeneously dense granular material and are surrounded by an apparently single membrane of greater density. These profiles correspond in size and structure to the so called lysosomes which possess high levels of acid phosphatase activity (14–19) in liver cells (Fig. 2). These structures have also been called large granules, microbodies (20), and cytosomes (21). The 60-A dense particles described above can also be seen within the lysosomes. A dense body with a matrix of non-homogeneous appearance, which is devoid of a partially surrounding membrane, appears infrequently in the cytoplasm (Fig. 2). This body is assumed to be a lysosomal derivative.

Many of the mitochondria seen in the cytoplasm of these cells are swollen; they are enveloped by a double-layered limiting membrane and contain a few cristae and a homogeneously fine granular matrix of intermediate density. No 60-A dense

FIGURE 1

An electron micrograph showing aggregates of dense particles in a parenchymal cell from the liver in a case of pancreatic tumor with obstructive jaundice. Parts of two adjacent parenchymal cells can be seen, being separated by their plasma membranes. Note the aggregates (AP) of dense particles in electron-transparent areas, some of which are surrounded by membranes (ME). Many dense particles (arrows) are found scattered in the cytoplasmic matrix. Large vesicular or vacuolar structures (V) are interpreted as distended elements of the endoplasmic reticulum. Many of the mitochondria (DM) seem to be degenerated, being almost devoid of their limiting membranes and cristae. The dense particles are not found in the endoplasmic reticulum. A few dense particles (P) can be seen in the degenerated mitochondria, but dense particle aggregates are not observed in them. A few Palade granules (PG) are found isolated in the cytoplasm or attached to the outer surfaces of the vesicular elements of the endoplasmic reticulum. × 53,000.
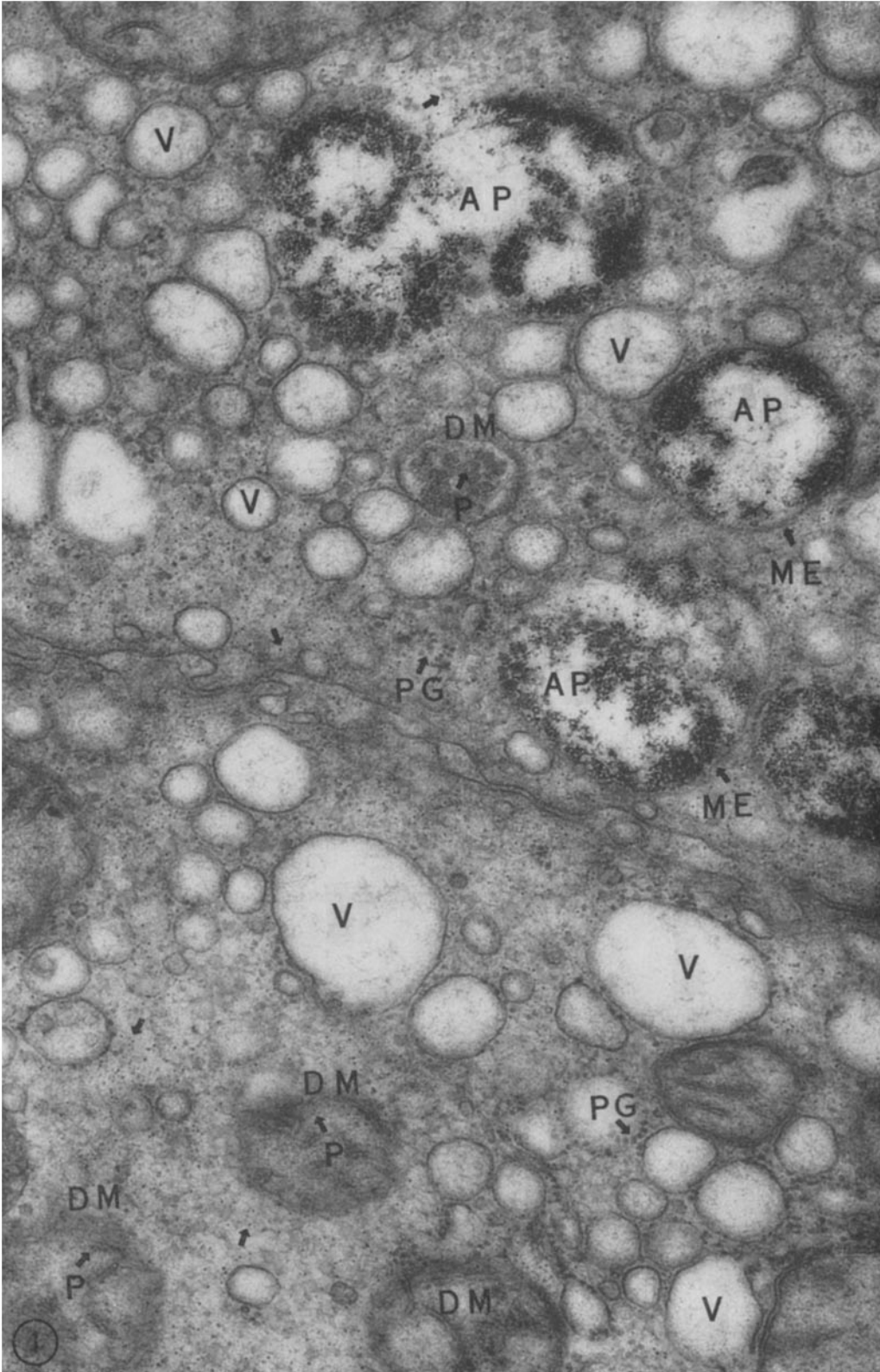
particles are found in the mitochondrial matrix (Figs. 2 and 3). Degenerating mitochondria are frequently encountered; they are small in size, almost devoid of limiting membranes, and have ill defined cristae. A few of the 60-A dense particles are found in the degenerating mitochondria (Figs. 1 and 2).

Numerous agranular vacuoles or vesicles also appear in the cytoplasm; they may be expanded tubules of the endoplasmic reticulum (Figs. 1 to 3). A small number of Palade granules (ribonucleoprotein) are found attached to the outer surfaces of the vesicular elements or isolated in the cytoplasmic matrix (Figs. 1 to 3). The dense particles are not visible in the vesicular or vacuolar elements of the endoplasmic reticulum.

It is to be noted that glycogen granules have never been observed in the parenchymal cells of the present specimen (Figs. 1 to 3).

In the parenchymal cells stained with 0.5 per cent basic fuchsin solution, a pale nucleus with one or two nucleoli is seen surrounded by a remarkably reddish cytoplasm. The limitations between individual cells are clearly delineated (Fig. 6). Thus, it is possible to assay the chemical components of any part of the cell with the x-ray scanning microanalyzer. With the aid of an optical microscope, a fine electron-probe about 1 μ in diameter is directed first at the cytoplasm and then at the nucleus. The procedure of microanalysis has already been described in detail (12). The image formed by the Fe K_α emission from these liver cells demonstrates that iron elements are present in small clusters or scattered throughout the cytoplasm (Fig. 7). No such image formed

by the Fe K_α emission has been demonstrated for the nucleus. Fig. 8 shows clearly an Fe K_α spectrum obtained from the point marked by the arrow in Fig. 7. A quantitative analysis of the elemental iron in the cytoplasm has been carried out (Fig. 9) by recording the intensity of the emission on a pen recorder and comparing it with the intensity of the emission from a specimen of the pure iron element irradiated at the same beam current. In the cytoplasm examined the concentration of iron has been revealed to be within 1.0 per cent of the pure iron sample.

DISCUSSION

Some interesting features have been observed in the cytoplasm of the hepatic parenchymal cells in a case of human pancreatic tumor with obstructive jaundice. Among these are the following: glycogen granules are no longer seen; there seems to be a significant decrease in the amount of ribonucleoprotein; all the mitochondrial profiles tend to have the same roughly oval shape, which is taken to mean that they have a nearly ovoid form; bodies interpreted as degenerated mitochondria are frequently encountered, but their size is decreased and their limiting membranes and cristae are becoming disintegrated; the smooth surfaced endoplasmic reticulum is remarkably expanded into vesicles or vacuoles of different sizes and shapes. Changes in the fine structure of the organelles in these cells will be discussed in a future paper comparing these changes with those occurring in hepatic cells in various diseases of human liver.

In the cytoplasm of these hepatic parenchymal

FIGURE 2

An electron micrograph showing scattered dense particles in parenchymal cells of the liver in a case of pancreatic tumor with obstructive jaundice. The scattered dense particles (P) are clearly visible in the cytoplasmic matrix. Many of the mitochondria (M) are swollen, being surrounded by a sharply outlined, double-layered membrane. They are provided with a few cristae in a homogeneously fine granular matrix. An intramitochondrial dense granule can be seen in the degenerated mitochondrion (DM). Numerous electron-transparent, roughly circular profiles of different sizes seem to be distended elements of the endoplasmic reticulum (ER). The lysosome (LY) has a dense granular matrix and is sharply outlined by an apparently single limiting membrane. The dense body (DB), part of which is surrounded by a single membrane, may be a lysosomal derivative. A few particles (P) are visible in the degenerated mitochondrion, the lysosome and the lysosomal derivative (DB). A small amount of the rough surfaced endoplasmic reticulum is present, as well as a few Palade granules (PG) free in the cytoplasmic matrix. The area defined by a rectangle is presented in Fig. 4 at higher enlargement. X 53,000.
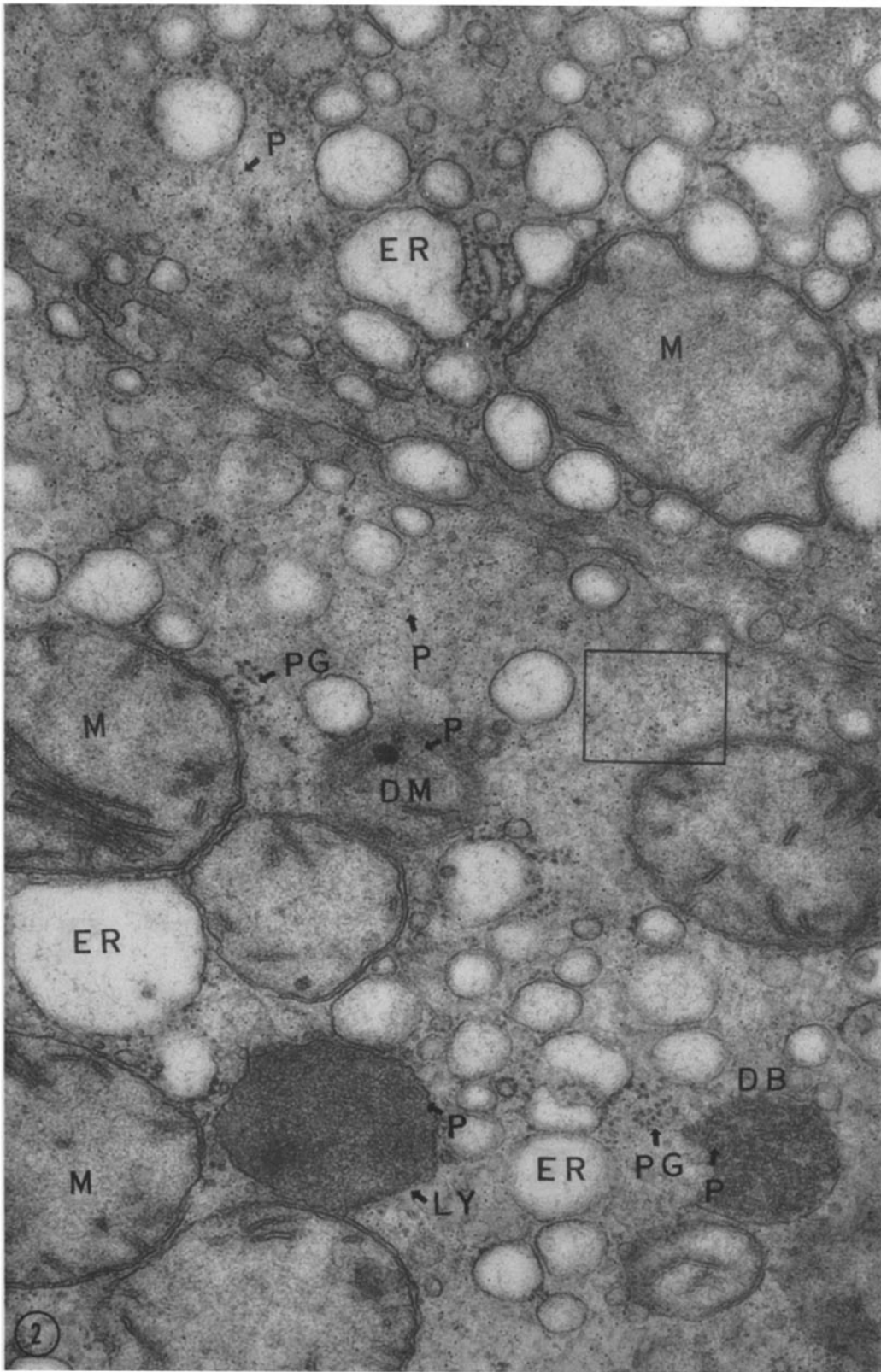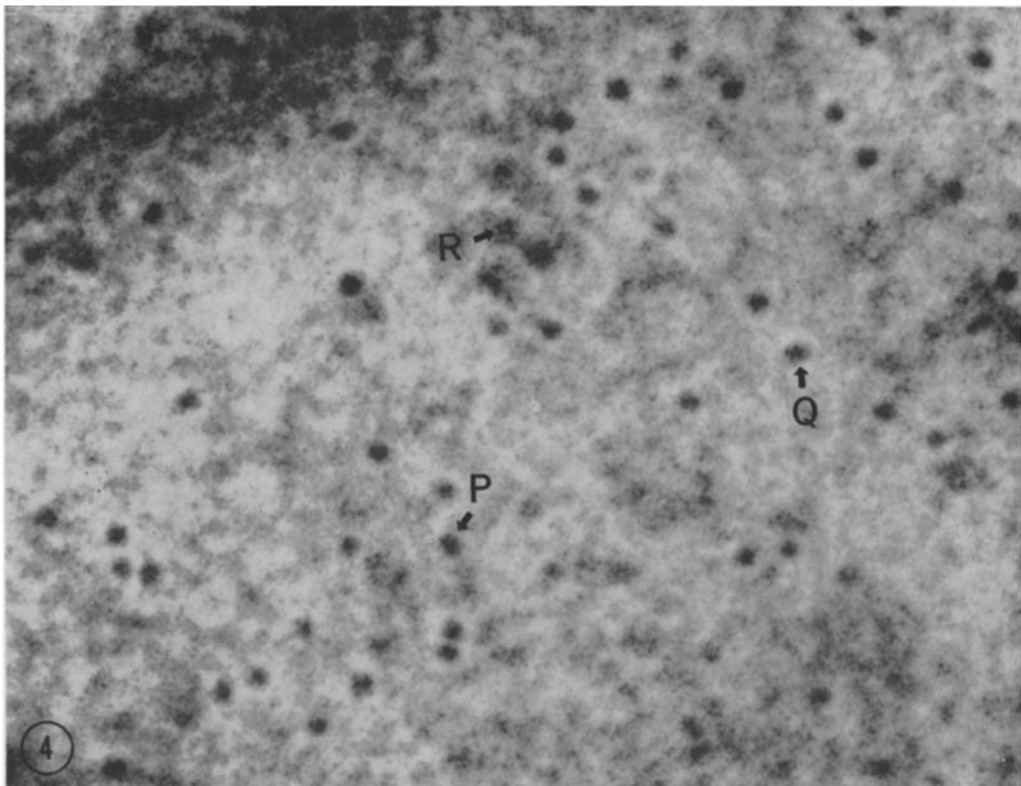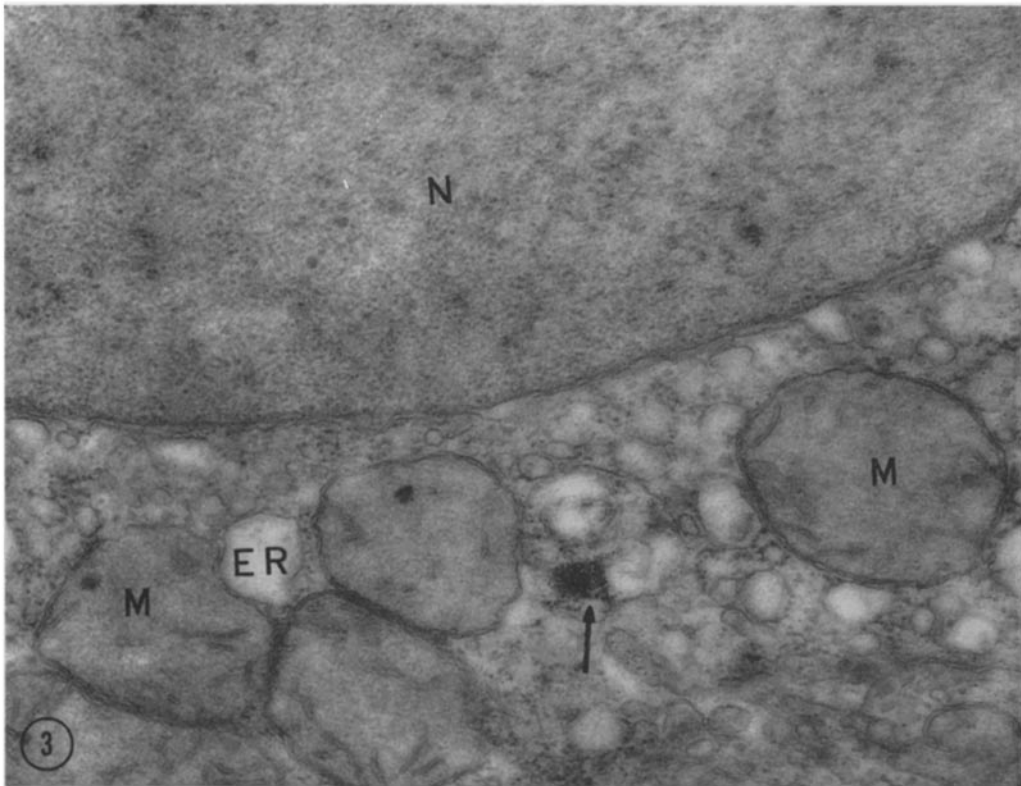
cells, isolated as well as aggregated dense particles about 60 A in diameter have been observed. These particles are interpreted to be ferritin molecules by their patterns in the electron microscope. Since the patient had never received whole blood or taken iron-containing drugs, it is assumed that the ferritin molecules have originated from the intracellular breakdown of hemoglobin. Kerr and Muir (10), Moore et al. (22), and Richter (11) have observed ferritin molecules in the nuclei, but in the present study there has been no evidence of ferritin in the nuclei.

By the application of the x-ray scanning microanalyzer, Yasuzumi et al. (23) succeeded in showing an Fe K_α spectrum in human erythrocytes. Boyde et al. (24) demonstrated that the microanalyzer could be used to obtain quantitative and qualitative element analysis of differences in the surface zone of tooth enamel and suggested its possible use for studying changes in this zone that occur with advancing age. Furthermore, the microanalyzer has been applied to elemental iron localized in the PAS-positive granule, in testicular

nutritive cells of a pond snail, which has been revealed by cytochemical reaction (12). The present study shows that x-ray scanning microanalysis of thick sections, together with the electron microscope patterns, can be employed in the determination of elemental iron in hepatic parenchymal cells. Richter (4–7) has traced the fate of iron compounds injected into animals and has demonstrated the transformation patterns of the iron compounds, within individual cells, to ferritin and hemosiderin at the molecular level. Although it is impossible to differentiate organic and inorganic iron compounds by the x-ray scanning microanalyzer, it is possible, in this case, to measure the amount of elemental iron and to show that it occurs in small regions of the cytoplasm in concentrations up to 1 per cent of the pure iron.

[Bibliography will appear on p. 431.]

FIGURE 3

A part of the nucleus and the cytoplasm of the parenchymal cell of the liver in a case of pancreatic tumor with obstructive jaundice. Dense particles and a dense particle aggregate (arrow) can be seen in the cytoplasm. The dense particles are not found in the nucleus (N), perinuclear space, mitochondria (M), or endoplasmic reticulum (ER). X 43,000.

FIGURE 4

Enlarged electron micrograph of the area defined by a rectangle in Fig. 2. Each dense particle is composed of smaller dense particles. The characteristic quadrimicellar configuration (Q), and other patterns such as ring (R), triad (T), and pentad (P) can be seen. X 360,000.
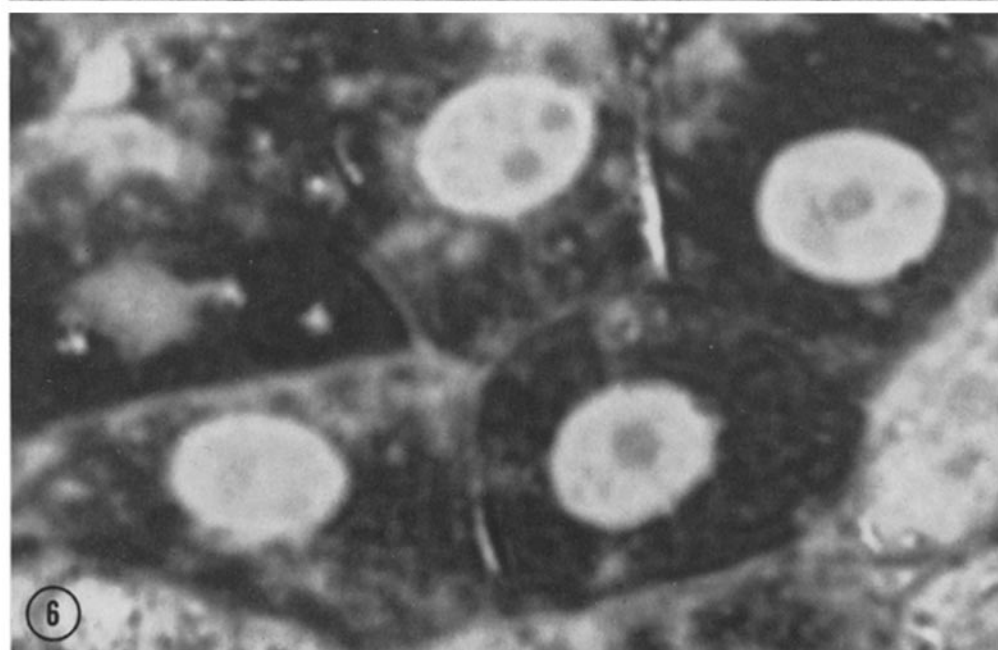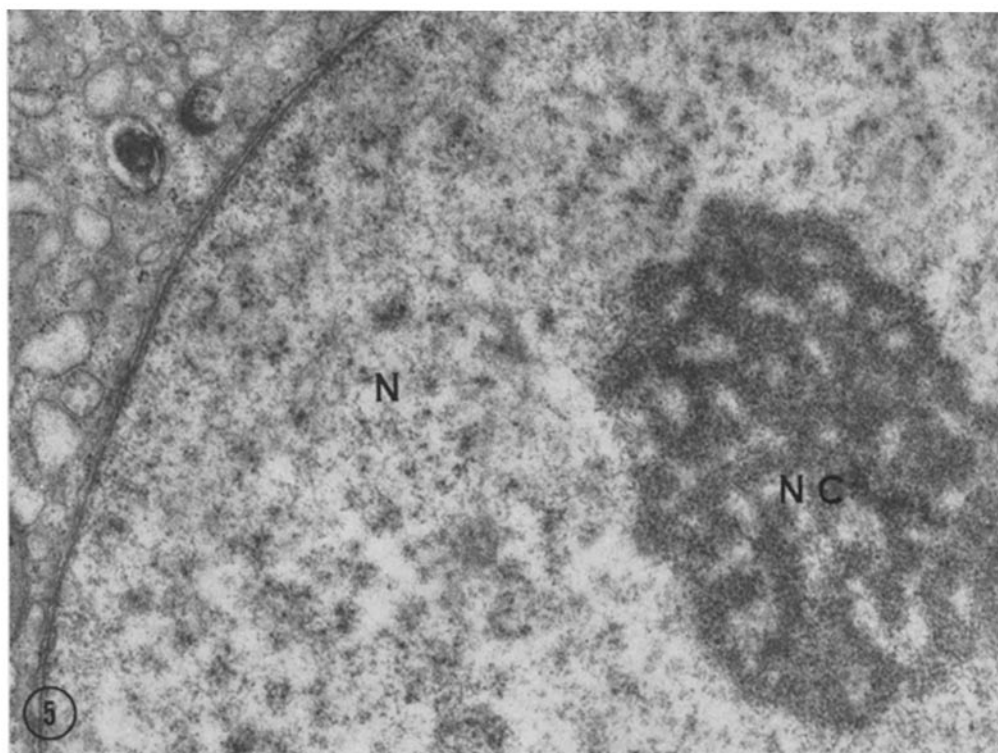
FIGURE 7
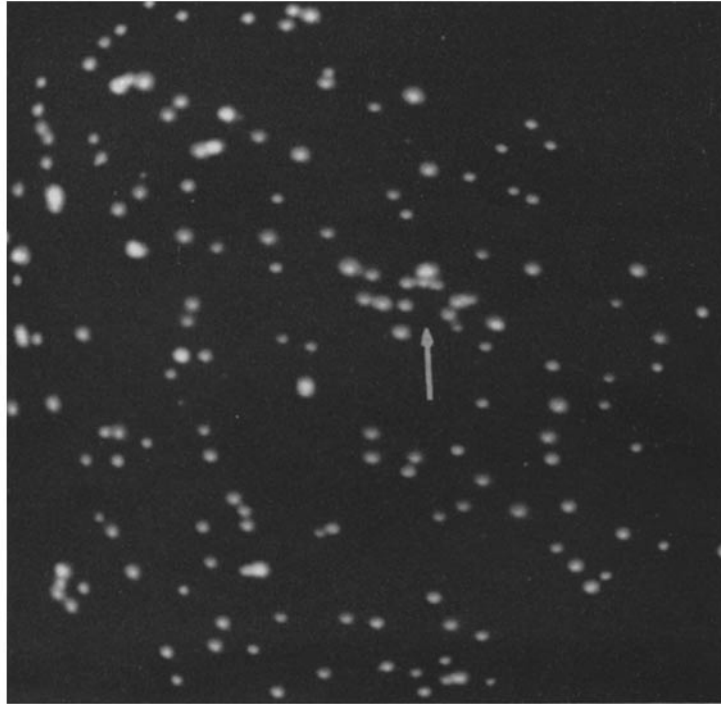
Scanning image produced by Fe K_α emission of the cytoplasm of the parenchymal cell of the liver demonstrated in Fig. 6. × 3,000.

FIGURE 5

Part of the nucleus (N) of a parenchymal cell of the liver shows clearly the double-layered nuclear envelope, amorphous karyoplasm, and nucleolus (NC) consisting of tangled nucleolonemata. No dense particle aggregates are visible in the nucleus (N). × 31,000.

FIGURE 6

A thick section of the liver stained with 0.5 per cent basic fuchsin solution. The nuclei of parenchymal cells appear unstained, some of which contain one or two nucleoli. The cytoplasm is stained in reddish tone. × 1,500.
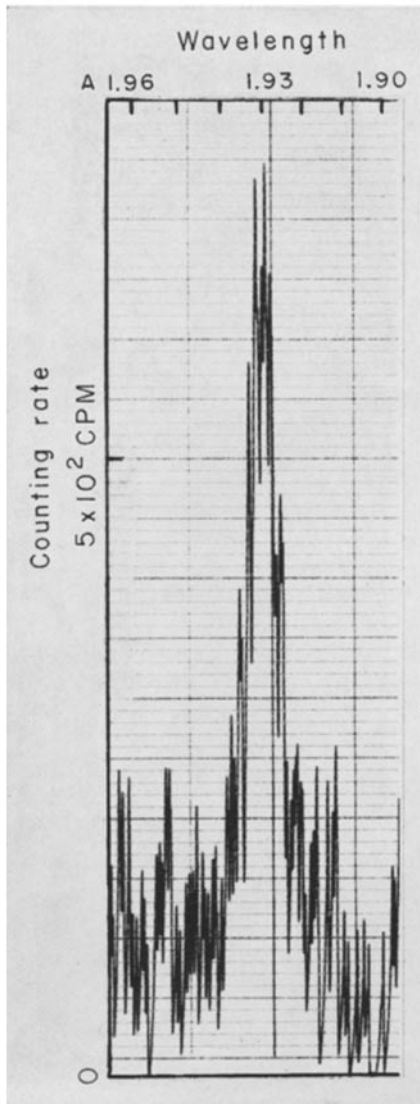
FIGURE 8

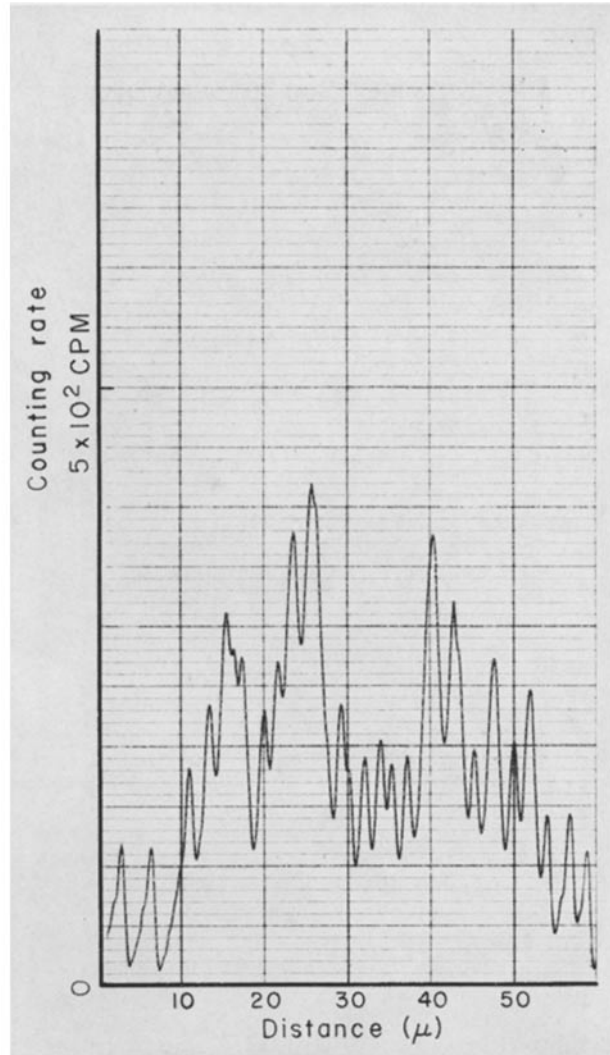Fe K$_\alpha$ spectrum at the point marked by the arrow in Fig. 7.

FIGURE 9

Fe K$_\alpha$ spectrum showing iron distribution in the cytoplasm of the hepatic parenchymal cell.

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