Virchows Arch. A Path. Anat. and Histol. 374, 215–227 (1977)

Virchows Archiv A

Hepatocyte Giant Mitochondria: An almost Constant Lesion in Systemic Scleroderma

G. Feldmann[1,4], M. Maurice[1], J.M. Husson[2], J.N. Fiessinger[2], J.P. Camilleri[3], J.P. Benhamou[1], and E. Housset[2]

[1] Unité de Recherches de Physiopathologie Hépatique, Hôpital Beaujon, F-92118 Clichy;
[2] Chaire de Pathologie Vasculaire and
[3] Service Central d'Anatomo-pathologie, Hôpital Broussais, F-75014 Paris;
[4] Département d'Histologie-Embryologie, Faculté de Médecine Xavier-Bichar, France

Summary. Liver electron microscopic studies were performed in 14 patients with systemic scleroderma. In 13 of these patients, giant mitochondria were demonstrated in the hepatocytes. This ultrastructal abnormality was present whatever the type and duration of the disease and was also present even when the liver was histologically normal. The mechanism of formation of giant mitochondria in systemic scleroderma is unknown.

Key words: Ultrastructure – Liver – Hepatocyte – Mitochondria – Gigantism – Systemic scleroderma.

Introduction

Systemic scleroderma is characterized by alterations of the subcutaneous connective tissue and visceral lesions affecting mainly esophagus, lungs, kidneys and heart (Tuffanelli and Winkerlman, 1961; D'Angelo et al., 1969). The liver is histologically normal or is affected by slight nonspecific lesions (Bartholomew et al., 1964; Monckton-Copeman and Medd, 1967; Buffet and Husson, 1974), except in the small number of cases in which scleroderma is associated with primary biliary cirrhosis (Murray-Lyon et al., 1970; Reynolds et al., 1971; O'Brien et al., 1972; Geffroy et al., 1973; Levrat et al., 1973). In this study, we confirm that histological lesions in the liver are either absent or slight and nonspecific but we describe an almost constant ultrastructural lesion of the hepatocytes, represented by mitochondrial gigantism.

Material and Methods

1. Material

Our material consisted of liver specimens obtained by needle biopsy from 14 female patients suffering from systemic scleroderma. The age of the patients ranged from 39 to 77 years. Using the classifica-

Offprints requests to Unité de Recherches de Physiopathologie Hépatique, Hôpital Beaujon, F-92118 Clichy, France (Dr. G. Feldmann)

Table 1. Type of scleroderma, age and interval from the onset of the disease and the time of the liver biopsy

Patients	Type of systemic sclero-derma[a]	Age (years)	Interval from the clinical onset of the disease and the time of the liver biopsy (years)
1	1	42	5
2	1	42	3
3	1	77	5
4	1	45	10
5	1	39	9
6	2	65	41
7	2	72	40
8	2	44	15
9	2	49	10
10	2	51	10
11	2	44	7
12	2	49	30
13	2	64	19
14	3	47	1

[a] According to the classification of Barnett and Coventry (1959)

tion of Barnett and Coventry (1959), scleroderma was of type 1 in 5 patients (patients 1–5); type 1 is characterized by subcutaneous lesions only affecting the fingers and by rare visceral lesions. Scleroderma was of type 2 in 8 patients (patients 6–13); type 2 is characterized by subcutaneous lesions affecting the hands and multiple visceral lesions, but with slow progression. Scleroderma was of type 3 in one patient (patient 14); type 3 is characterized by generalized subcutaneous and multiple visceral lesions with rapid progression. The interval between the clinical onset of scleroderma and the time of the needle biopsy is given in Table 1.

There was no clinical manifestation indicating disorder of the liver in any of these patients. Conventional liver function tests were normal in all patients except for a moderate BSP retention in 3 (patients 4, 5, 12) and slight increase of the SGPT level in 2 (patients 3, 10) (Table 2). In 7 patients, systemic scleroderma was associated with the Sjögren syndrome (patients 3, 5–7, and 12–14).

HBs antigen was looked for by the complement-fixation method but could not be found in any of the patients. Tissue antibodies were investigated in all patients except patient 2, according to the methods described by Doniach et al. (1966); nuclear antibodies were demonstrated in 6 patients; smooth muscle antibodies were present in 2; antibodies to thyroid and antibodies to gastric parietal cells were present in patients 3 and 5 respectively; mitochondrial antibodies were absent in all patients.

Alcohol daily intake was not superior to 50 g in any of the patients; no patient took oral contraceptives. No known hepatoxic drug was given to the patients before liver biopsy.

2. Methods

Part of the liver fragment was fixed in Duboscq-Brasil's fluid and embedded in paraffin. Five-micron thick sections were stained with hematein-eosin-safran, Masson's trichrome and Gordon-Sweet's method for reticulin.

Another part of the fragment was cut in one-mm^3 blocks, which were immediately fixed in a 2% glutaraldehyde solution buffered with phosphate buffer 0.2 M pH 7.4 for 90 min at 4° C; the blocks were then washed in phosphate buffer for 24 to 48 h and post-fixed in a 1.5% osmium tetroxide solution buffered with veronal buffer pH 7.2 for 60 min at 4° C. After dehydrata-

Table 2. Liver function tests

Patients	BSP (%) (percent retent at 45 min)[a]	Serum bilirubine (mg/100 ml)[b]	Alcaline phosphatases (I.U.)[c]	SGPT (I.U.)[d]
1	4	0.7	4	5
2	4	0.3	16	5
3	5	0.6	41	52
4	25	0.6	8	10
5	14	0.6	26	10
6	4	0.3	26	6
7	5	0.6	18	12
8	4	0.8	16	10
9	5	0.6	14	10
10	5	0.3	43	86
11	4	0.3	7	18
12	21	0.7	42	10
13	5	0.4	31	10
14	4	0.3	30	17

[a] Normal: 0–5%
[b] Normal: 0.3–0.8 mg/100 ml
[c] Normal: 50–100
[d] Normal: 5–20

tion, the blocks were embedded in epoxy resin. One-micron thick sections, stained with toluidine blue, were made for orientation. Ultrathin sections, stained with uranyl acetate and lead citrate, were made on three randomly selected blocks and were examined with an electron microscope (Siemens Elmiskop I A).

Results

1. Histological Findings

The liver was normal in 10 patients (patients 1, 2, 4, 7–13). Steatosis was present in one patient (patient 14), portal sclerosis in 2 (patients 3 and 5) and cloudy swelling of some hepatocytes in 2 (patients 6 and 14).

2. Ultrastructural Findings

The main ultrastructural abnormality affected the mitochondria of the hepatocytes: this abnormality was present in all the patients except patient 9. Abnormalities in diameter, shape and structure were observed. The diameter of the mitochondria was abnormally increased: giant mitochondria with a diameter of 5 μ or more were seen. These giant organelles were usually roughly oval-shaped (Fig. 1); some round or irregular or elongated forms were also observed (Figs. 2 and 3). The structure of the mitochondria was markedly abnormal; the cristae were anarchically arranged: instead of being perpendicular to the inner mitochondrial membrane as they are in normal hepatocyte mitochondria, the cristae

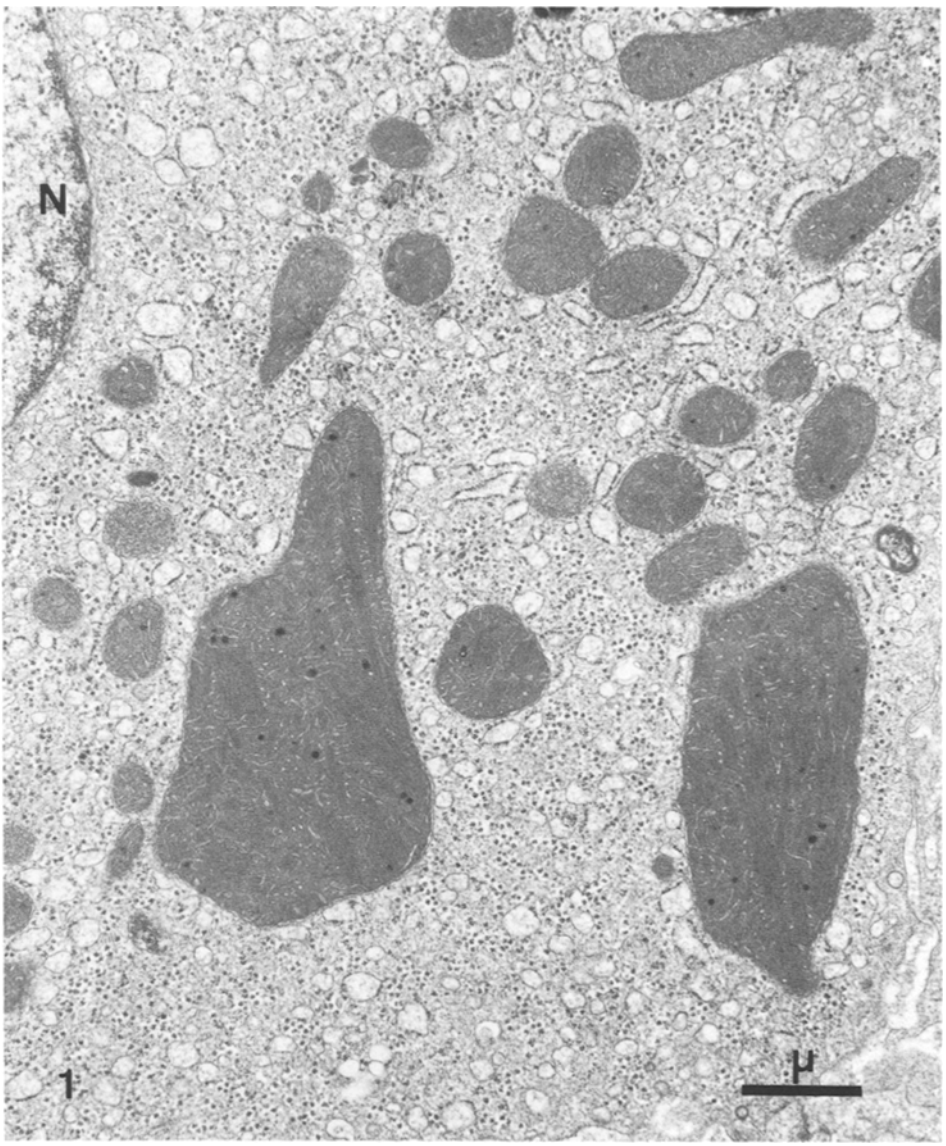


Fig. 1. Electron microscope appearance of a part of a hepatocyte in a patient with systemic scleroderma. Two giant mitochondria are visible; some cristae are anarchically distributed in the matrix. The other mitochondria are normal (*N*: Nucleus) (× 15,600)

were either parallel to the inner membrane or randomly distributed throughout the mitochondrial matrix (Figs. 1 and 2). Paracrystalline inclusions were observed in the matrix of some mitochondria in the neighbourhood of the cristae (Figs. 2 and 3); these inclusions could either run along the whole length of the mitochondrion or take the appearance of short linear segments randomly

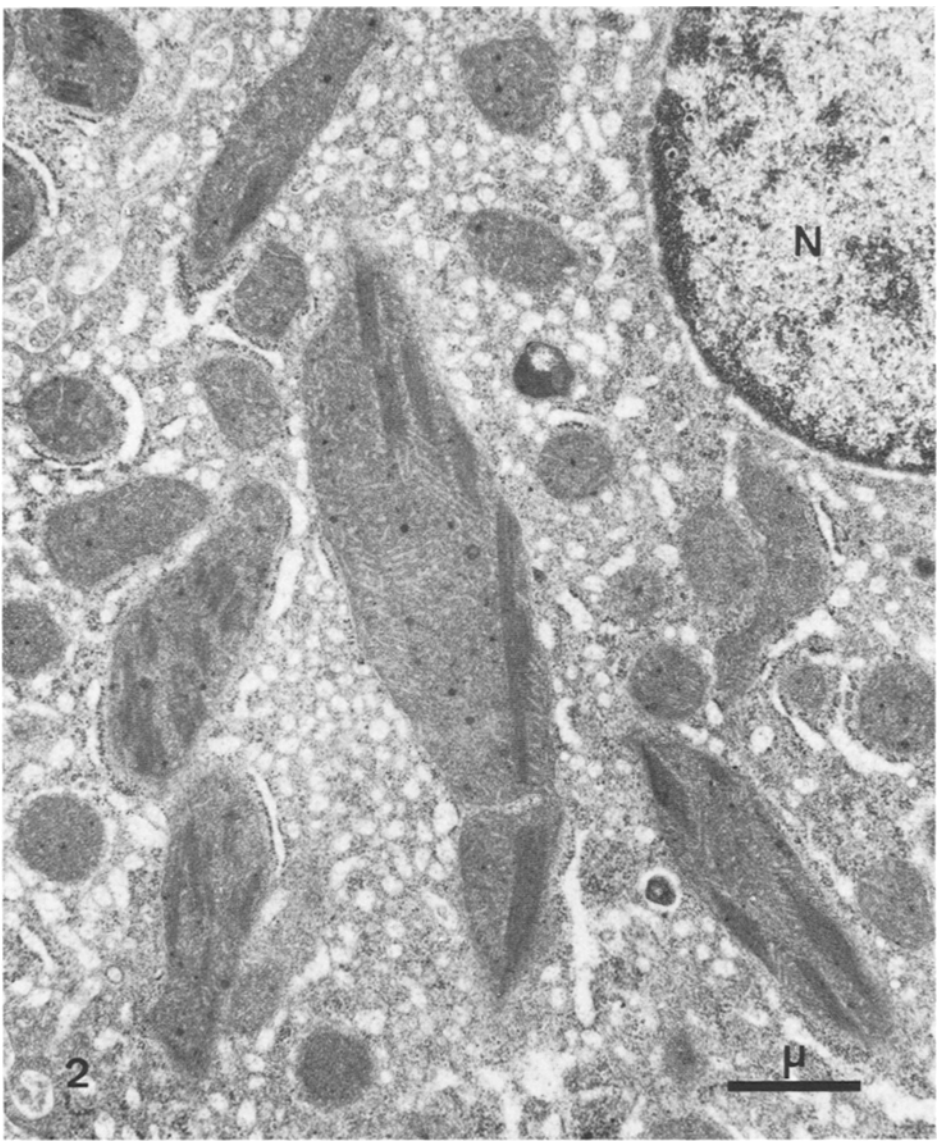
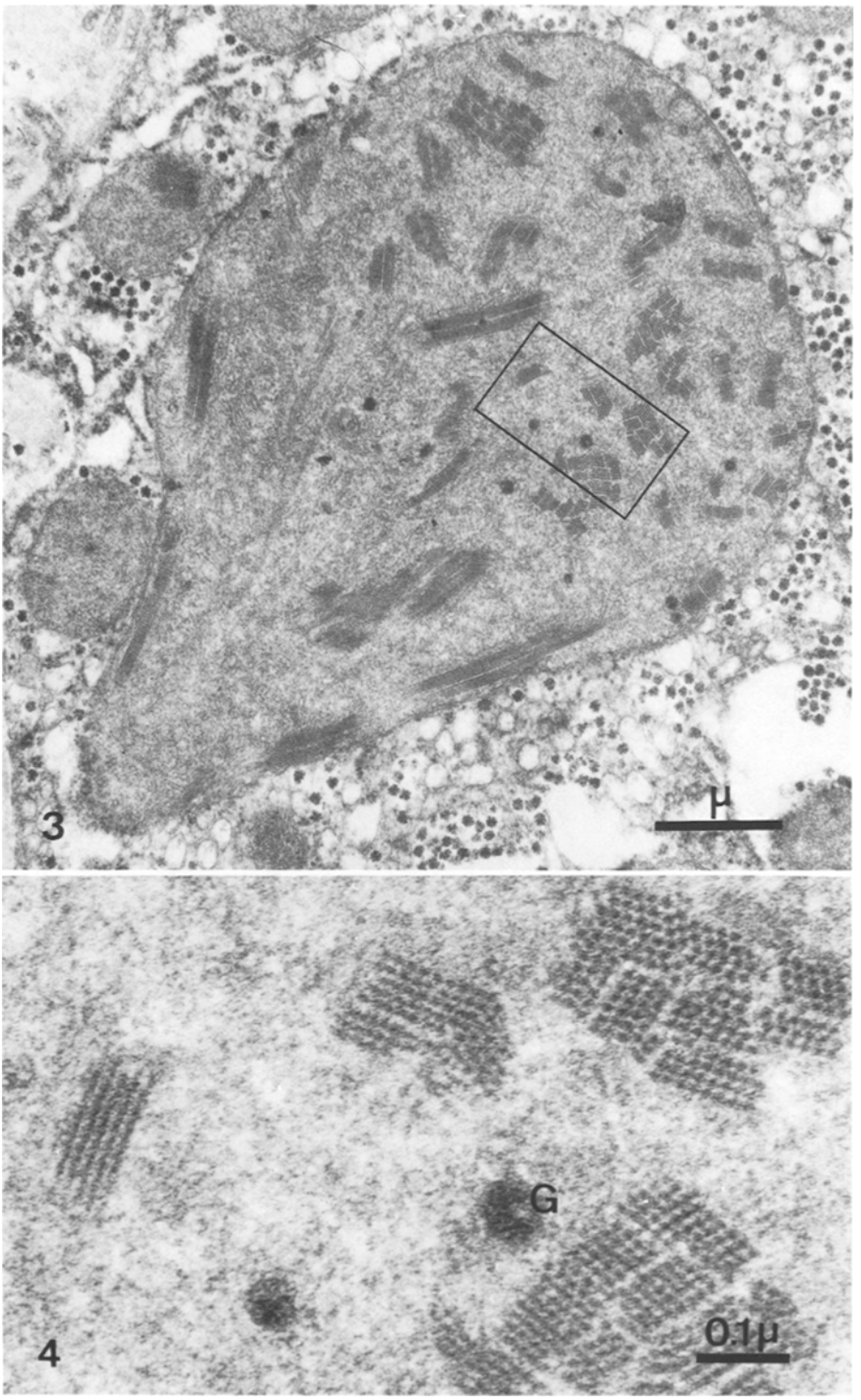


Fig. 2. In some hepatocytes, some giant elongated mitochondria with paracrystalline inclusions are present. These inclusions are often in the neighbourhood of the cristae (*N*: Nucleus) (× 17,400)

distributed throughout the organelle. Each paracrystalline inclusion was formed of 6 to 8-nm thick filaments disposed periodically with a periodicity of approximately 10 nm (Fig. 4). More complicated paracrystalline inclusions with a pentagonal array were also visible.

In some organelles, tubular linear structures with a diameter of 40 nm were


3
μ
G
4
0.1μ

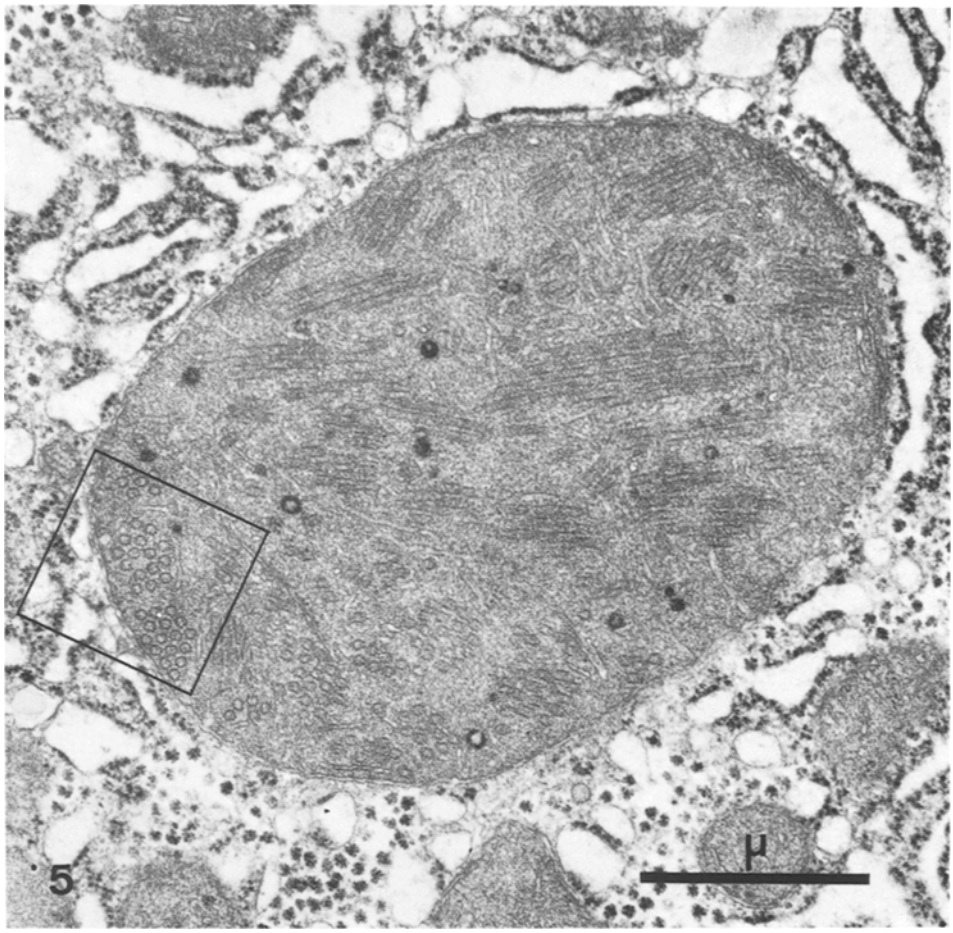


Fig. 5. Round giant mitochondrion with tubular linear structures. These structures are clustered in groups of 2 to 6 tubules with different orientations. Their appearance and their diameter are different from the cristae. Some large granules with a central hole are also present (×30,000)

Fig. 3. In this round giant mitochondrion, numerous paracrystalline inclusions are visisble; their appearance is different depending on the section. Some inclusions are also present in a few normal-sized mitochondria (×17,600)

Fig. 4. This figure is an enlargment of the area enclosed in Figure 3. The paracrystalline inclusions are formed of filaments periodically disposed (*G*: Granule) (×132,000)

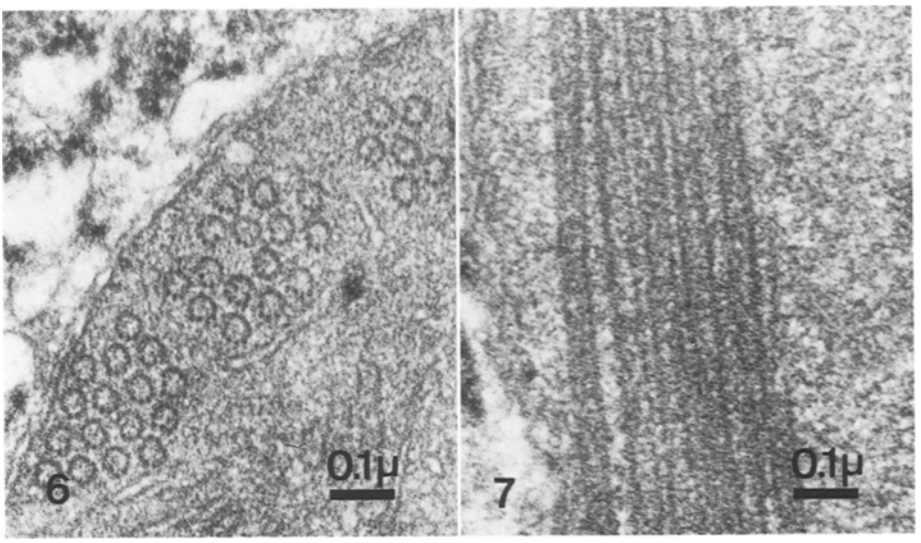


Fig. 6. This figure is an enlargment of the area enclosed in Figure 5. On a cross-section, some dots are visible in the wall of the tubules. The thickness of the wall is about 5 nm (×84,000)

Fig. 7. Appearance of the tubules in a longitudinal section. The diameter of the tubules is about 40 nm; a space of 10 to 20 nm separates 2 tubules (×84,000)

present (Figs. 5 and 8); their length did not exceed 1.5 μ, although longer tubules were sometimes seen. Most often, the tubules were clustered in small groups of 2 to 6 with a space of 10 to 20 nm between two tubules (Figs. 5 and 7). The thickness of the wall was approximately 5 nm; in some perpendicular sections, some dots were visible in the wall (Fig. 6); a central core was also sometimes present inside the tubule. No obvious relationship between the tubules and the cristae was observed, although some cristae were very near by the tubules. In general, the paracrystalline inclusions were absent in the mitochondria when the tubular structures were present; however, some giant mitochondria with both tubules and inclusions were seen (Fig. 8).

In some mitochondria numerous large electron-dense granules with a central hole were present in the matrix (Figs. 1, 3, 5 and 8). However, the membrane space and the two membranes of the organelles, were normal. Most often, giant mitochondria were in clusters of 2 or 3 in the same hepatocyte, the other mitochondria being generally normal (Figs. 1 and 2). In 11 of the 13 patients, giant mitochondria were estimated to be present in about 20% of the hepatocytes. They were less frequent in 2 patients (patients 2 and 8).

The other organelles of the hepatocytes were normal. Moderate steatosis was observed in patient 14. Increase in collagen fibers in Disse's space was observed in patient 3 and 5; the structure of these collagen fibers was normal. A dilatation of the endoplasmic reticulum was seen in patients 6 and 14.

No mitochondrial abnormalities were observed in the biliary cells, the endothelial cells, the Kupffer cells, the lipocytes or the fibroblasts.

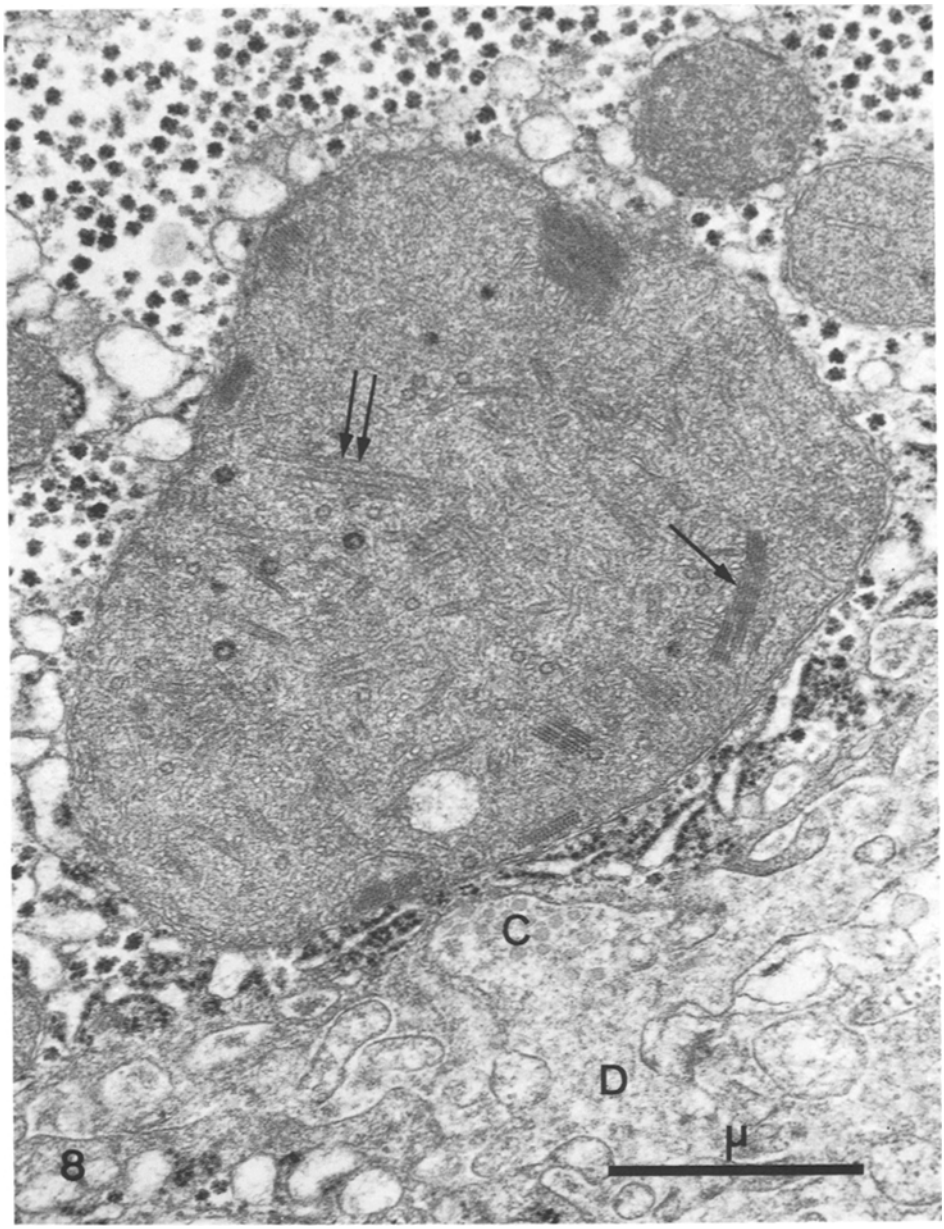


Fig. 8. In this giant mitochondrion, paracrystalline inclusions (arrow) and tubular structures (double arrow) are present (*D*: Disse's space; *C*: Collagen fibers) (× 33,500)

Discussion

Giant mitochondria were present in the hepatocytes of 13 out of the 14 patients we studied. The abnormality was not demonstrated in the other types of liver cells, and was associated with all types of scleroderma. The presence of such mitochondria was not related to the duration of the disease.

Hepatocyte giant mitochondria are a nonspecific liver lesion and have been demonstrated in numerous liver diseases, such as alcoholic hepatitis (Svoboda and Manning, 1964; Feldmann et al., 1970), viral hepatitis (Wills, 1968; Spycher and Rüttner, 1968), Gilbert's disease (Minio and Gautier, 1967; Feldmann et al., 1968), Dubin Johnson's syndrome (Minio and Gautier, 1967; Feldmann et al., 1970) Sanfilippo's disease (Haust, 1968), Wilson's disease (Sternlieb, 1968), recurrent jaundice in pregnancy (Adlercreutz et al., 1967; Van Haelst and Bergstein, 1970), hepatic porphyria (Jean et al., 1968), leptospirosis (Sandborn et al., 1966), amyloidosis (Thiéry and Caroli, 1961) and in the liver cells surrounding a hepatocellular carcinoma (Ghadially and Parry, 1966). Oral contraceptives are also known to induce mitochondrial gigantism (Perez et al., 1969; Martinez-Manantou et al., 1970). Giant mitochondria have also been described in histologically normal liver (David, 1964; Wills, 1965) and could not therefore, be regarded as an ultrastructural lesion (Wills, 1965). Nevertheless the notion that giant mitochondria do not constantly indicate a cellular dysfunction is questionable for the following two reasons: (1) this notion is based on a small number of reports with neither detailed clinical nor biochemical data (David, 1964; Wills, 1965); (2) giant mitochondria have not been demonstrated in an ultrastructural morphometric study on normal hepatocytes from adults whose age range was similar to that of the patients in the present investigation (Slabodsky-Brousse et al., 1974). Systemic scleroderma can thus be added to the list of the causes of giant mitochondria. It is noteworthy that giant mitochondria are present in almost all the patients with systemic scleroderma; in contrast they are demonstrated in only some patients suffering from the diseases mentioned above (with the exception of Wilson's disease, in which giant mitochondria are remarkably frequent (Sternlieb, 1968)). All the diseases accompanied by mitochondrial gigantism are diseases of the liver with histologically identifiable lesions of hepatic tissue and clinical manifestations and biochemical abnormalities related to the disease. In systemic scleroderma, the liver was histologically normal in 10 of 14 patients and liver functions test were normal in most of them.

In general the mitochondrial gigantism is accompagnied by other structural changes: paracrystalline inclusions were frequently seen within those altered mitochondria. The nature of these inclusions is not well established; optical diffraction studies performed by Sternlieb and Berger (1969) in Wilson's disease suggest that they are composed either of phospholipid micelles or protein molecules. In contrast to the paracrystalline inclusions, mitochondrial tubular structures have only occasionally been reported in liver diseases (Van Haelst and Bergstein, 1970). The fact that in some giant mitochondria both paracrystalline inclusions and tubular structures are present together suggest that they are related.

It is tempting to draw a parallel between the giant mitochondria observed

in almost all our patients and the known association between scleroderma and primary biliary cirrhosis (Murray-Lyon et al., 1970; Reynolds et al., 1971; O'Brien et al., 1972; Geffroy et al., 1973; Levrat et al., 1973), a liver disease in which mitochondrial antibodies are frequently demonstrated (Doniach et al., 1966); giant mitochondria might be the minor and primary biliary cirrhosis the major manifestation of the liver lesion induced by scleroderma. In fact, however this hypothetic link is not consistent with the following facts; 1) giant mitochondria are present in almost all patients with scleroderma, whereas the association of scleroderma and primary biliary cirrhosis is relatively uncommon (Murray-Lyon et al., 1970; Reynolds et al., 1971; O'Brien et al., 1972; Geffroy et al., 1973; Levrat et al., 1973); 2) only type 1 scleroderma has been described in association with primary biliary cirrhosis (Murray-Lyon et al., 1970; Reynolds et al., 1971; O'Brien et al., 1972; Geffroy et al., 1973; Levrat et al., 1973), whereas all types of scleroderma are associated with giant mitochondria; 3) the early lesion in primary biliary cirrhosis affects the ductules (Rubin et al., 1963; Foulk et al., 1964; Klion and Schaffner, 1966; Popper and Schaffner, 1970; Chedid et al., 1974), whereas giant mitochondria are observed only in the hepatocytes and not in the biliary cells; 4) to our knowledge, giant mitochondria have not been described in primary biliary cirrhosis in the early stages of the disease (Rubin et al., 1963; Foulk et al., 1964; Klion and Schaffner, 1966; Popper and Schaffner, 1970; Chedid et al., 1974); 5) in our patients with giant mitochondria, mitochondrial antibodies were not demonstrated.

We have no hypothesis to offer for the mechanism of mitochondrial gigantism in systemic scleroderma. It has been shown that serum from patients with systemic scleroderma contains a factor which induces chromosomal breakage in cultured lymphocytes (Emerit et al., 1971); this factor would exert its effect through activation of lysosomal enzymes (Emerit et al., 1973). Conceivably, similar factors, inducing alteration of another organelle, the mitochondrion, might also be present in scleroderma. This speculative mechanism would not account for the presence of giant mitochondria in the hepatocytes and not in the other types of liver cells. It is possible that giant mitochondria are present in other organs. However, to our knowledge, the skin is the only tissue studied so far by electron microscopy and no mitochondrial abnormalities have been observed in this tissue (Fleischmajer et al., 1972).

References

Adlercreutz, H., Svanborg, A., Anberg, A.: Recurrent jaundice in pregnancy. I. A clinical and ultrastructural study. Amer. J. Med. **42**, 335–347 (1967)

Barnett, A.J., Coventry, D.A.: Scleroderma: 1. Clinical features, course of illness and response to treatment in 61 cases. Med. J. Aust. **1**, 992–1001 (1959)

Bartholomew, L.G., Cain, J.C., Winkerlmann, R.K., Baggenstoss, A.: Chronic disease of the liver associated with systemic scleroderma. Amer. J. dig. Dis. **9**, 43–55 (1964)

Buffet, C., Husson, J.M.: Foie et sclerodermie généralisée. Nouv. Presse méd. **3**, 2701–2702 (1974)

Chedid, A., Spellberg, M.A., Debeer, R.A.: Ultrastructural aspects of primary biliary cirrhosis and other types of cholestatic liver disease. Gastroenterology **67**, 858–869 (1974)

D'Angelo, W.A., Fries, J.F., Masi, A.T., Shulman, L.E.: Pathologic observations in systemic sclerosis (scleroderma). A study of fifty-eight autopsy cases and fifty-eight matched controls. Amer. J. Med. **46**, 428–440 (1969)

David, H.: Submikroskopische Ortho- und Pathomorphologie der Leber, p. 56. Frankfurt: Pergamon Press 1964

Doniach, D., Roitt, I.M., Walker, J.G., Sherlock, S.: Tissue antibodies in primary cirrhosis, active chronic (lupoid) hepatitis, cryptogenic cirrhosis and other liver diseases and their clinical implications. Clin. exp. Immunol. **1**, 237–262 (1966)

Emerit, I., Housset, E., Grouchy, J. de, Camus, J.P.: Chromosomal breakage in diffuse scleroderma. A study of 27 patients. Rev. Europ. Et. clin. biol. **14**, 684–694 (1971)

Emerit, I., Levy, A., Housset, E.: Sclerodermie généralisée et cassures chromosomiques. Mise en évidence d'un "facteur cassant" dans le sérum des malades. Ann. Génét. **16**, 135–138 (1973)

Feldmann, G., Oudéa, P., Domart-Oudéa, M.C., Molas, G., Fauvert, R.: L'ultrastructure hépatique au cours de la maladie de Gilbert. Path. et Biol. **16**, 943–953 (1968)

Feldmann, C., Oudéa, P., Molas, G., Domart-Oudéa, M.C., Fauvert, R.: L'ultrastructure des hépatocytes au cours des cirrhoses alcooliques. Presse méd. **78**, 409–414 (1970)

Feldmann, G., Molas, G., Groussard, O., Domart-Oudéa, M.C.: Die Ultrastruktur der Leber bei chronischem idiopathischem Ikterus. Münch. med. Wschr. **39**, 2–7 (1970)

Fleischmajer, R., Damiano, V., Nedwich, A.: Alteration of subcutaneous tissue in systemic scleroderma. Arch. Derm. **105**, 59–66 (1972)

Foulk, W.T., Baggenstoss, A.H., Butt, H.R.: Primary biliary cirrhosis: reevaluation by clinical and histologic study of 49 cases. Gastroenterology **47**, 354–374 (1964)

Geffroy, Y., Colin, R., Hemet, J., Galmiche, J.P., Guillard, J., Hecketsweiler, P.: Cirrhose biliaire primitive et sclérodermie. Med. Chir. Dig. **2**, 282–286 (1973)

Ghadially, F.N., Parry, E.W.: Ultrastructure of a human hepatocellular carcinoma and surrounding non-neoplastic liver. Cancer (Philad.) **19**, 1989–2004 (1966)

Haust, D.M.: Crystalloid structure of hepatic mitochondria in children with heparitin sulfate mucopolysaccharidosis (Sanfilippo type). Exp. molec. Path. **8**, 123–134 (1968)

Jean, G., Lambertenghi, G., Pranzi, T.: Ultrastructural study of the liver in hepatic porphyria. J. clin. Path. **21**, 501–507 (1968)

Klion, F.M., Schaffner, F.: Electron microscopic observations in primary biliary cirrhosis. Arch. Path. **81**, 152–161 (1966)

Levrat, M., Descos, L., Trepo, C., Vandenput, F., Lesbros, F., Moulin, G., Barthélémy, C.: Association sclérodermie, syndrome de Sjögren et cirrhose biliaire primitive. Arch. Mal. Appar. dig. **62**, 495–500 (1973)

Martinez-Manantou, J., Aznar-Ramon, R., Bautista-O'Farril, J., Gonzalez-Angulo, A.: The ultrastructure of liver cells in women under steroid therapy. II. Contraceptive therapy. Acta endocr. (Kbh.) **65**, 207–221 (1970)

Minio, F., Gautier, A.: L'ultrastructure du foie humain lors d'ictères idiopathiques chroniques. IV. Mitochondries de morphologie inhabituelle et "inclusions cytoplasmiques paracrystallines" hepatocytaires. Z. Zellforsch. **78**, 267–279 (1967)

Mölbert, E., Marx, R.: Electronenmikroskopische Untersuchungen am Lebergewebe beim Rotor-Syndrom. Acta hepato-splenol. (Stuttg.) **13**, 160–175 (1966)

Monckton-Copeman, P.W., Medd, W.E.: Diffuse systemic sclerosis with abnormal liver and gall-blader. Brit. med. J. **1967III**, 353–354

Murray-Lyon, I.M., Thompson, R.P.H., Ansell, I.D., Williams, R.: Scleroderma and primary biliary cirrhosis. Brit. med. J. **1970II**, 258–259

O'Brien, S.T., Eddy, W.M., Krawitt, E.L.: Primary biliary cirrhosis associated with scleroderma. Gastroenterology **62**, 118–121 (1972)

Perez, V., Gorosdisch, S., DeMartire, J., Nicholson, R., DiPaola, G.: Oral contraceptives: long-term use produces fine structural changes in liver mitochondria. Science **165**, 805–807 (1969)

Popper, H., Schaffner, F.: Non suppurative destructive chronic cholangitis and chronic hepatitis, In: Popper, H., Schaffner, F. (eds), Progress in liver diseases, pp. 336–354 New York: Grune & Stratton 1970

Reynolds, T.B., Denison, E.K., Frankl, H.D., Lieberman, F.L., Peters, A.L.: Primary biliary cirrhosis with scleroderma, Raynaud's phenomenon and telangiectasia. New syndrome. Amer. J. Med. **50**, 302–312 (1971)

Rubin, E., Schaffner, F., Popper, H.: Localization of the basic injury in primary biliary cirrhosis. J. Amer. med. Ass. **183**, 331–334 (1963)

Sandborn, E.B., Côté, M.G., Viallet, A.: Electron microscopy of a human liver in Weil's disease (leptospirosis ictero-haemorrhagica). J. Path. Bact. **92**, 369–374 (1966)

Slabodsky-Brousse, N., Feldmann, G., Brousse, J., Dreyfus, P.: Etude stéréologique de la fréquence des mitochondries géantes hépatiques dans la maladie de Gilbert. Comparaison avec le sujet normal. Biol. Gastroenterol. **7**, 179–186 (1974)

Spycher, M.A., Rüttner, J.R.: Kristalloide Einschlüsse in menschlichen Lebermitochondrien. Virchows Arch. B Zellpath. **1**, 211–221 (1968)

Sternlieb, I.: Mitochondrial and fatty changes in hepatocytes of patients with Wilson's disease. Gastroenterology **55**, 354–367 (1968)

Sternlieb, I., Berger, J.E.: Optical diffraction studies of crystalline structures in electron micrographs. II. Crystalline inclusions in mitochondria of human hepatocytes. J. Cell Biol. **43**, 448–455 (1969)

Svoboda, D.J., Manning, R.T.: Chronic alcoholism with fatty metamorphosis of the liver. Mitochondrial alterations in hepatic cells. Amer. J. Path. **44**, 645–662 (1964)

Thiéry, J.P., Caroli, J.: Etude au microscope électronique de l'amylose hépatique primaire de-l'homme. Sem. Hôp. Paris **37**, 29–40 (1961)

Tuffanelli, D.L., Winkerlmann, R.K.: Systemic scleroderma: a clinical study of 727 cases. Arch. Derm. **84**, 359–371 (1961)

Van Haelst, U., Bergstein, N.: Electron microscopic study of the liver in so-called idiopathic jaundice of late pregnancy. Path. europ. **5**, 198–215 (1970)

Wills, E.J.: Crystalline structure in the mitochondria of normal human liver parenchyma cells. J. Cell Biol. **24**, 511–514 (1965)

Wills, E.J.: Acute infective hepatitis. Fine structural and cytochemical alterations in human liver. Arch. Path. **86**, 184–207 (1968)

Received December 11, 1976