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Case Reports

Storage of Proteins in the Rough Endoplasmic Reticulum of Human Hepatocytes in a Patient with Normal Blood Proteins, on Oral Contraceptives

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Summary. Aspects of protein storage in the rough endoplasmic reticulum of hepatocytes, comparable with those reported in α_1-antitrypsine (AAT) deficiency, have been observed in the course of jaundice in a woman presenting no evident abnormality in AAT or other blood proteins. In light microscopy, most hepatocytes contained characteristic globular inclusions but they were PAS negative and did not react with anti-AAT antibodies. This storage of protein ceased at the time the jaundice disappeared. Prolonged treatment with high doses of contraceptive steroids may have been involved in this peculiar reaction of the hepatocytes.

Key words: Hepatocyte – Rough endoplasmic reticulum – Protein storage – Oral contraceptives.

Introduction

An accumulation of proteinacious material can be observed in cisternae of the rough endoplasmic reticulum (rER) of hepatocytes in subjects with chronic deficiencies of circulating α_1-antitrypsin (AAT), and storage of AAT has been demonstrated cytoimmunologically in these cells (Sharp, 1971; Liebermann et al., 1972; Feldmann et al., 1974). Cells storing this glycoprotein contain characteristic, PAS positive globular inclusions of various sizes. This report concerns a transitory but general and very marked storage of proteinacious material in the rER of hepatocytes in a case of jaundice. This jaundice was probably iatrogenic and occured in a young woman presenting no alterations in levels of blood AAT. Most hepato-

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cytes appeared to contain PAS negative globular inclusions with the light microscope. They did not react with antibodies against AAT. Such a systematic transitory modification in hepatocytes has not, to our knowledge, been previously reported in either human pathology or experimental work.

Materials and Methods

1. Case Report. Mrs. H.I., 28 years old, who had been taking an oral contraceptive for 4 years (Ovariostat: mestranol, 0.075 mg and lynestrol, 2.5 mg), received, during a three week period, anti-inflammatory treatments of intramuscular ACTH (Synathène) and aspirin (Aspegic) combined with vitamin B-12 for lumbosacral pain and asthenia of unknown origin. She was then admitted to hospital on October, 1975, with symptoms of acute appendicitis accompanied by the onset of jaundice. The appendix was removed; its macroscopic appearance was normal, and the biliary ducts were not obstructed. A postoperative septicemia (staphylococus aureus and moraxella) responded rapidly to antibiotics, but the jaundice was exacerbated (bilirubin: 9.9 mg%, essentially in the conjuged form (9 mg), alkaline phosphatase: 112 i.u but with only mild increase in transaminases). Sedimentation rate was elevated. Examination of blood proteins reveled that the greatest change was an increase in gamma globulins (27.7%) concurrent with a relative decline in serum albumin (24.7%), without marked variation in the other fractions. Tests for antibodies against mitochondria, actin and liver were all negative, as were radioimmunologic and electroimmunodiffusion tests for HBS antigen (Australia). The jaundice decreased spontaneously, despite a retroperitoneal abscess, which occured after the interruption of antibiotic therapy and required further surgery. The patient was completely recovered in 4 months. Restablishment of the menstrual cycle occured 3 months after contraceptives were stopped (at the beginning of the illness).

Apart from variations in gamma globulins and serum albumin, analyses of blood proteins during the course of the disease revealed no evidence of abnormality of any defined fraction. AAT level were checked three times by the immunologic dosage technique of Mancini and were always in the normal range: 330 mg%, 230 mg% and 223 mg respectively (normal values: 200–400 mg%).

2. Techniques. Samples of liver were obtained by needle biopsy 6 weeks, 6 weeks and 4 months after the onset of jaundice. Part of each sample was fixed in formalin and embedded for routine histopathologic observations. Fragments for electron microscopic examination were fixed in 5% glutaraldehyde in 0.1 phosphate buffer at pH 7.4 and embedded in a mixture of Epon and Araldite. Semithin sections of this material were stained by several techniques for light microscopic control of electron microscopic observations. Ultrathin sections were double-stained with uranyl and lead citrate. Immunocytochemical tests, using the "sandwich method", were performed on formol-fixed material.

Results

1. Light Microscopy

The first and second biopsies appeared identical. There was no morphologic evidence of cholestasis in the bile canaliculi, which might be explained by the considerable diminution of jaundice by the time biopsies were collected. No inflammatory or fibrotic reactions were seen in the portal spaces or along vascular sinuses. Most hepatocytes, except in small centrolobular zones where cells appeared normal, contained a large spheroidal inclusion (Fig. 1) which often displaced the nucleus toward the periphery of the cell. In parafin sections these inclusions were stained pale rose with hematoxylineosin, blue with Masson's trichrome and were PAS-negative. Some were rather poorly delimited. In semithin sections of material embedded in Araldite-Epon, the spheroidal inclusions were always well delimited;

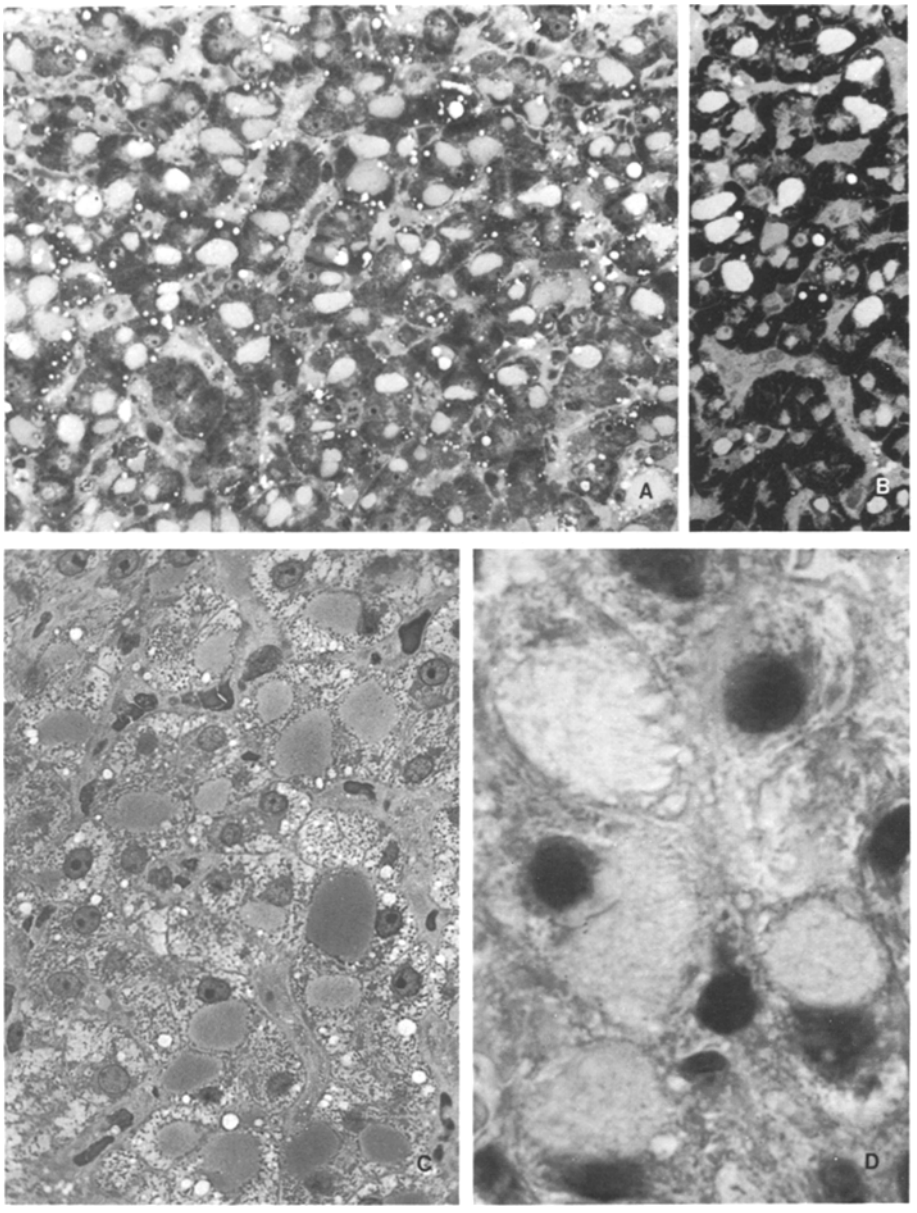

Fig. 1 A–C. Semithin sections of the samples prepared for electron microscopy stained with toluidine blue (**A**, × 330), PAS (**B**, × 330), and hematoxylineosin (**C**, × 575). D: paraffin section stained with Masson's trichrome (× 1500). The globular inclusions in the hepatocytes are very numerous and often larger than the nuclei. They are PAS negative in contrast with the intensely staining glycogen (**B**)

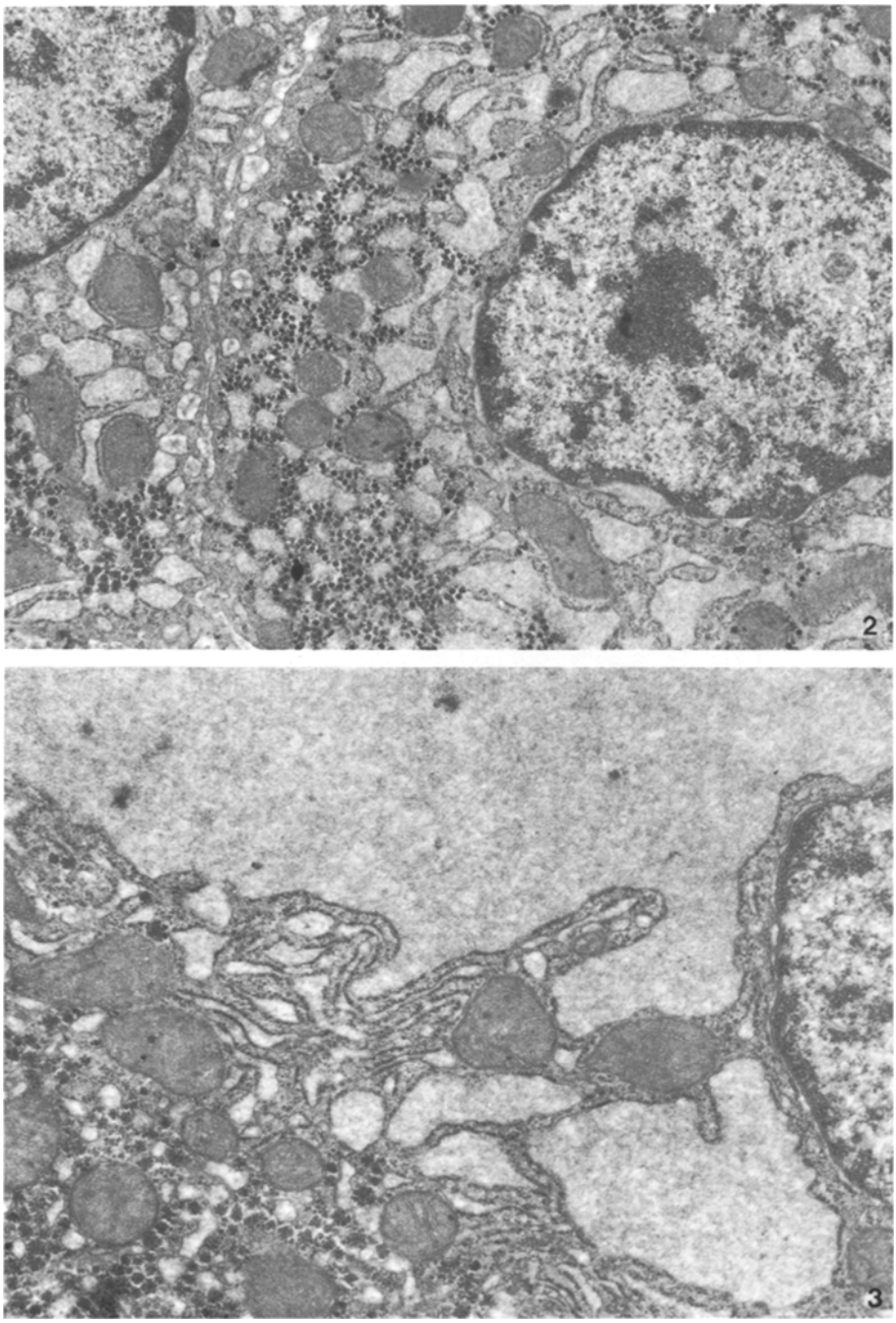

Fig. 2. Slight diffuse dilatation of the rER cisternae due to initiation of accumulation of a slightly electron-dense substance. × 11,500

Fig. 3. Large storage cavity of the rER, the wall of which is still partly studded with ribosomes and continuous with less dilated cisternae. × 19,000

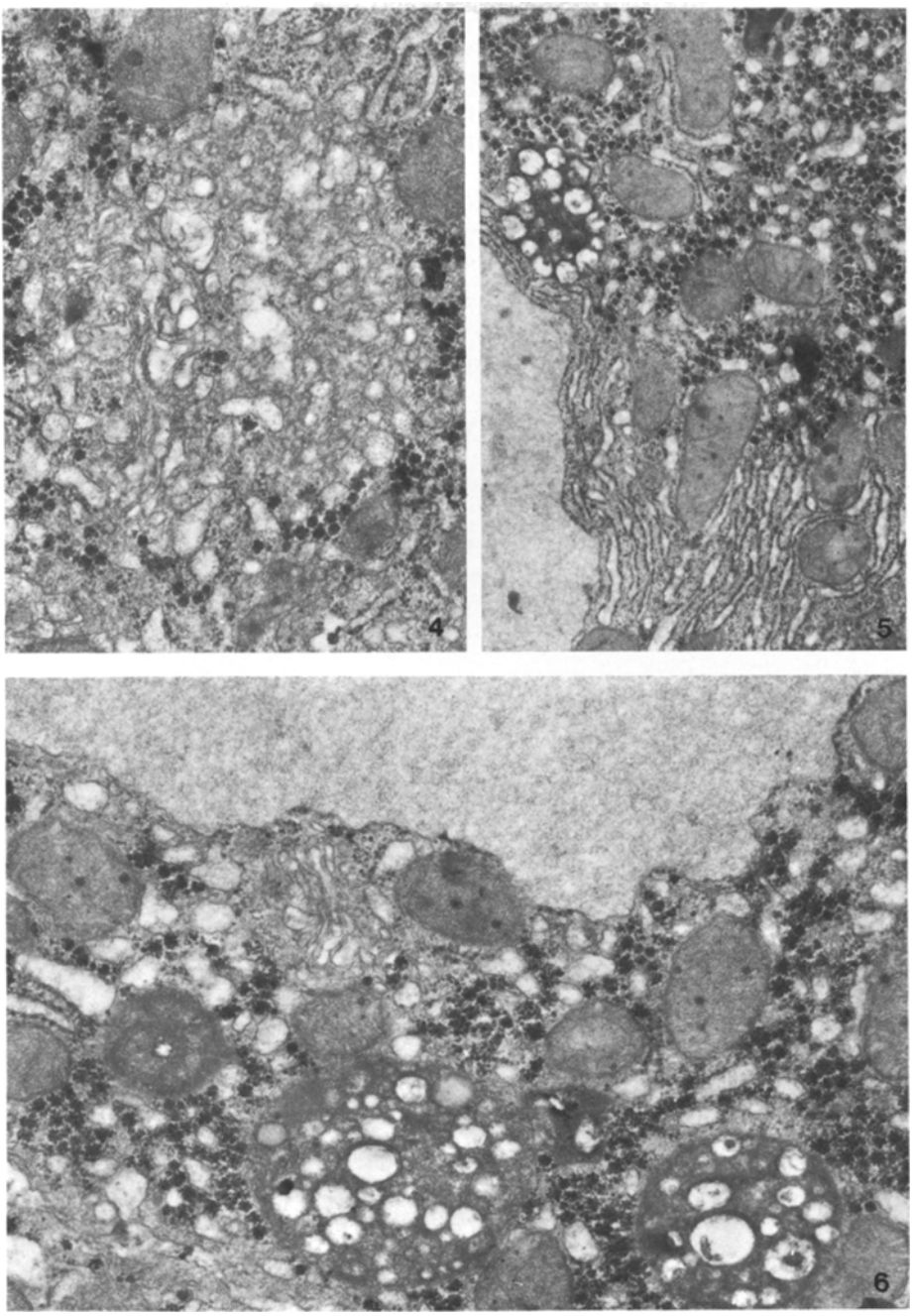

Fig. 4. Golgi apparatus: accumulation of vesicular profiles, some of which arise from fragmentation of cisternae of the rER (transitional elements). × 25,000

Fig. 5. Storage cavity (left), with normal-appearing neighbouring rER cisternae; numerous glycogen particles associated with the smooth ER. Peculiar dense body in the upper left. × 14,500

Fig. 6. Storage cavity (top); two peculiar dense vesicular bodies (bottom). × 25,000

they were eosinphilic and PAS negative. With the latter technique, they stood out as negative images in a cytoplasm intensely colored due to the abundance of glycogen, which is particularly well preserved in these preparations (Fig. 1 B).

In the third biopsy sample, taken after complete disappearance of jaundice, spheroidal inclusions were absent from the hepatocytes. Histologically the liver appeared completely normal.

All immunocytochemical tests with anti-AAT antibodies were negative. After exposure to antibodies to serum albumin, the abnormal hepatocytes, in contrast to normal liver which shows islets of fluorescent cells, reacted very irregularly and, with a few exceptions, very weakly. Only occasional randomly dispersed cells contained distinctly fluorescent inclusions. Similar weak and inconstant reactions were seen with anti-B_1 lipoprotein antibodies.

2. Electron Microscopy

The inclusions observed with the light microscope correspond to accumulations of amorphous, moderately electron-dense material in dilated cisternae of the rER. These dilated profiles generally had few ribosomes associated with their surface, and they were still in continuity with cisternae which appeared to be in earlier stages of accumulating the same material (Fig. 3). Normal-appearing parallel cisternae were present in other areas of the cytoplasm of most cells (Fig. 5); however, in some there was diffuse dilatation of all the rER (Fig. 2). In the midst of the amorphous material there were sometimes fine osmiophilic condensations which could be discerned with the light microscope in semithin sections also.

The Golgi complexes were strikingly modified: sparse saccular profiles were surronded by a large accumulation of vesicles with slightly electron-dense contents; transitional elements from rER were intermingled with vesicles of Golgi origin (Fig. 4). Peculiar bodies formed by voluminous aggregates of vesicles embedded in a osmiophilic matrix were regularly observed (Figs. 5 and 6). Glycogen particles were abundant and frequently associated with tubules of smooth ER (Figs. 5 and 6), a morphologic relationship commonly observed in hepatocytes and other cells actively synthetizing glycogen. The mitochondria appeared normal.

The ultrastructural appearance of the liver biopsy taken four months after disappearance of jaundice confirmed the impression gained by light mocroscopy. The rER and the Golgi complex appeared normal, glycogen was abundant and no dense vesicular agglomerates were found.

Discussion

Storage of protein in the rER of hepatocytes was more marked and more generalized (involving almost all the hepatocytes) in this subject than in subjects with genetic deficiency in circulating AAT (Fizz mutation). Moreover, these inclusions are PAS negative and have no special affinity for antibodies to AAT. Finally, the

three tests of AAT levels during the course of the disease all showed normal blood values. The transitory character of the protein storage, with complete disappearance after cessation of jaundice, does not suggest a genetic disturbance in the secretion of a defined type of protein. Our ultrastructural and cytochemical observations suggest rather that the inclusions correspond to accumulations of diverses proteins synthetized by liver cells, their composition being variable between cells.

Accumulation of material in rER is frequently observed in hyperstimulated glandular cells (e.g. "castration" and "thyroidectomy" cells in the hypophysis). As in these exemples, this phenomenon probably reveals in the hepatocytes a desequilibrium between release and synthesis of protein. The modifications of the Golgi apparatus (i.e. accumulation of vesicles originating from Golgi saccules together with transitional elements from rER), suggest concurrent abnormalities in release of secretory material and dynamic relations between the Golgi complex and rER. The peculiar dense vesicular bodies can be interpreted as morphologic evidence of catabolic regulation of unreleased secretory material. These ultrastructural modifications in sum suggest that in the case reported here, stimulation of protein synthesis was associated with an abnormaliy, probably primary, of mechanisms for release of both proteins and bile from the hepatocytes.

In certain species, e.g. the fish, in which the liver has a vitellogenic function, estrogens can cause storage of proteins in the rER of hepatocytes (Porte et al., 1960). Such modifications have not been described in livers of mammals after steroid treatment (see Altmann and Klinge, 1972). However, on the base of the clinical history, it seems that steroids may play a role in our case. It seems reasonable to ascribe the origine of the condition not to a single specific factor, infectious or medicamentous, but rather to the combination of anti-inflammatory and adrenal-stimulating therapy, together with infection and prolonged high doses of oral contraceptives. Adrenal and contraceptive steroids, associated with various humoral disturbances, may have had additive effects on the liver. It is known that 17 alkyl steroids (for example the anabolic steroids, methyltestosterone, norethandrolone and ethylestrenol) can provoke a benign choleostatic jaundice with disturbance of BSP test. Such jaundice has also been reported after treatment with contraceptive steroids; its development seem to bee facilited by other factors such as genetic or nutritional conditions (for review see Altmann and Klinge, 1976). It should be noted that the steroids mestranol and lynestrol, involved in this case and also present in other contraceptive products, are stabilized with an ethinyl group at the 17α position which inhibits their detoxication by the liver. Progressive accumulation of contraceptive steroids in the liver possibly could, under conditions which affect hepatic metabolism, initiate a generalized malfunction of the hepatocytes.

Relationships between clinical problems and oral contraceptives are not always evident, but it appears more and more probable that these steroids at least establish conditions which facilitate a variety of disease states. Our observations of a very peculiar reaction of the hepatocytes shows, in any case, the importance of this concept in the pathology of the liver, and at the same time it provides a contribution to the study of hepatic cytophysiology.

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