Unsaturated lipid droplets in liver sinusoids lining cells in a case of Reye-Morgan-Baral syndrome

A. P. Águas[1]

Unidade de Microscopia Electrónica, Faculdade de Medicina de Lisboa, Hospital de Santa Maria, Lisboa (Portugal), 20 November 1978

Summary. The author reports a case of Reye-Morgan-Baral syndrome presenting osmiophilic-dense lipidic bodies in lining cells of hepatic sinusoids. The different lipidic saturation level between the content of these bodies and the one of lipid droplets of hepatocytes could represent a different response, between hepatocytes and lining cells of liver sinusoids, to excessive circulating lipids.

It has been pointed out that, in spite of the large number of published papers on Reye-Morgan-Baral syndrome, the liver ultrastructural studies are relatively scarce[2]. The present paper reports the presence of osmiophilic-dense bodies in the lining cells of the hepatic sinusoids in a case of grade II Reye-Morgan-Baral syndrome[3].

Material and methods. A 15-month-old boy was admitted to the hospital because of a history of vomiting and diarrhoea. 1 day after the admission he became somnolent and progressively lethargic, till deep lethargy. Hepatomegaly, with no jaundice, was present. SGOT was 206 U Wroblewski/ml (normal, 5–40 U Wroblewski/ml). 3 days after, the neurological symptoms disappeared and 1 month later the infant showed no clinical or laboratorial abnormalities.

The fragments for ultrastructural observation were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffered to pH 7.3, postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer, pH 7.3, dehydrated in graded ethanols and propylene oxide solutions, and embedded in Epon 812. Ultrathin sections were double-stained with uranyl acetate and lead citrate, and examined under a Philips EM 300 electron microscope.

Results. Increased fat content was observed in all the hepatocytes, as variable sized droplets measuring 0.1 up to 8.0 µm. They presented the typical low electrondensity of the triglycerides. Alterations of the mitochondria were observed with flocculation and expansion of matrix, reduction in the number of the cristae and decreased number of matricial dense granules. The endoplasmic reticulum presented scanty profiles of the rough variety and slight increased amount of the smooth variety, mainly consisting of vesicles. The amount of glycogen was decreased.

Proliferation and hypertrophy of the Kupffer cells was also present. Almost all of these cells contained round, non-membrane-bounded inclusions with a high electrondensity, clearly distinct from that of the lipid droplets of adjacent hepatocytes (figure 1). These osmiophilic-dense bodies usually occupied most of the cytoplasm of the cell and they measured 0.1–0.5 µm (figure 2). Osmiophilic-dense bodies were also observed in some endothelial cells as smaller sized droplets. No low electrodense lipid droplets, similar to those observed in hepatocytes, were seen in the lining cells of liver sinusoids.

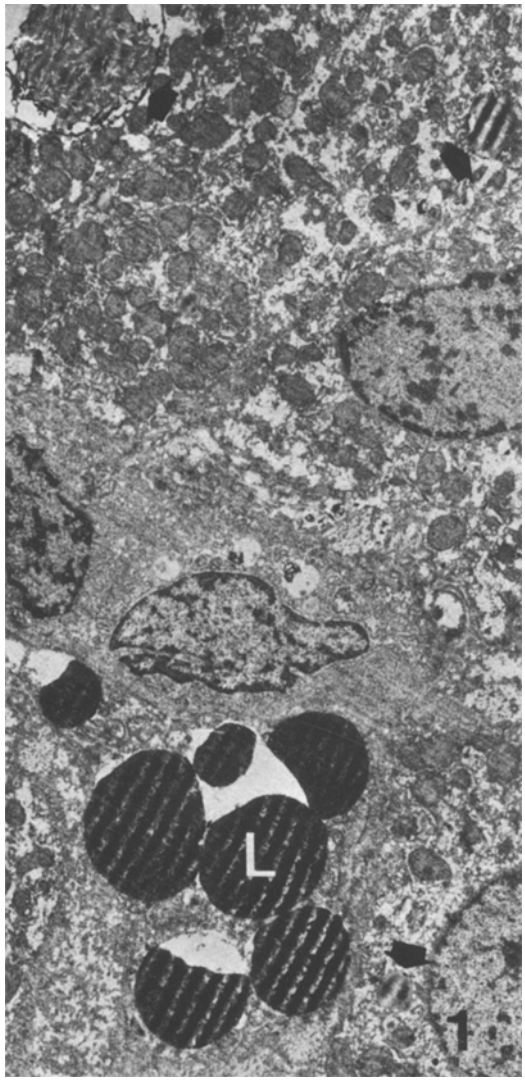

Fig. 1. Osmiophilic-dense bodies (L) in lining cells of a hepatic sinusoid, presenting a higher electrodensity than in those (arrows) found in adjacent hepatocytes. × 4800.

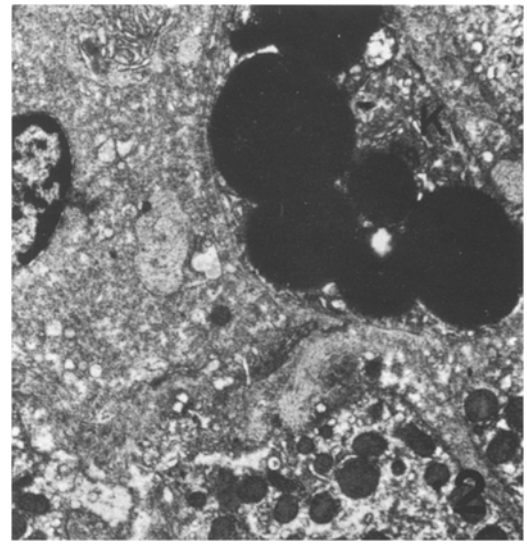

Fig. 2. A Kupffer cell (K) with most of its cytoplasm filled with osmiophilic-dense bodies. × 8100.

Discussion. Fatty degeneration of the viscera is a pathological abnormality of Reye-Morgan-Baral syndrome[4,5]. Lipid droplets have been reported in the liver, both in the hepatocytes and in the Kupffer cells, in this situation[3]. They have been described as having a low osmiophilic feature suggesting being formed by triglycerides[3]. In the case studied we found lipid droplets in the hepatocytes with the same morphological features, as has been described by others in Reye-Morgan-Baral syndrome. However, some lining cells of the liver sinusoids contained high osmiophilic lipid droplets. Osmiophilic-dense bodies in Reye-Morgan-Baral syndrome were previously observed only in the pericytes, near the cerebral vasculatures[6]. The high osmiophilic nature of these inclusions are generally believed to correspond to unsaturated lipids[7,8]. The different saturation level of the lipidic content of the droplets observed in the liver cells can be related with the increased mobilization of fatty acids in Reye-Morgan-Baral syndrome[8], and with a possible different response of the hepatocytes and of the lining cells of liver sinusoids to excessive circulating lipids. The removal of circulating lipids may be one of the mechanisms involved in the formation of osmiophilic-dense bodies in the lining cells of hepatic sinusoids, as it is assumed that these cells have a high endocytic activity[9,10].

1 Acknowledgments. This work was supported in part by A.N.A. Foundation of Lisbon. The author is indebted to Dr J.O. Soares and Dr J.F. Moura Nunes for helpful advice, to Mrs M.T. Correia and to Mrs M.E. Pereira for technical assistance, and to Mrs M.I. Condado for typing the manuscript.

2 T.C. Iancu, W.H. Mason and H.B. Neustein, Human Path. *8*, 421 (1977).
3 W.K. Schubert, J.C. Partin and J.S. Partin, in: Progress in Liver Diseases, vol. IV, p. 489, Ed. H. Popper and F. Schaffner. Gune and Stratton, 1977.
4 R.D.K. Reye, G. Morgan and J. Baral, Lancet *2*, 749 (1963).
5 D.M.O. Becroft, Br. med. J. *2*, 135 (1966).
6 L.W. Chang, E.F. Gilbert, W. Tanner and H.L. Moffat, Arch. Path. *96*, 127 (1973).
7 W. Stoecknius and S.C. Mahr, Lab. Invest. *14*, 1196 (1965).
8 J.R. Casley-Smith, J. R. Microsc. Soc. *87*, 463 (1967).
9 R.E. Brown and G.E. Madge, Arch. Path. *94*, 475 (1972).
10 E. Wisse, J. Ultrastruct. Res. *38*, 528 (1972).
11 I. Carr, Z. Zellforsch. Mikrosk. Anat. *89*, 328 (1968).

Effect of ionic environment on densities of membrane-associated particles in presynaptic membranes observed in freeze-fractured synaptosomes

Yukio Takano and Hiro-o Kamiya

Department of Pharmacology, School of Pharmaceutical Sciences, Fukuoka University, Fukuoka 814 (Japan),
30 October 1978

Summary. The effects of high-K and high-Ca in the incubation medium on membrane-associated particles of the presynaptic membranes were examined. There was a marked increase in the density of protoplasmic fracture face after incubation in the high-K or high-Ca medium.

Membrane-associated particles have been demonstrated on cell membranes by the freeze-fracture method[1-3]. These particles have been observed in presynaptic membranes of nerve endings of the central nervous systems in vertebrates[4-8]. These particles seem to consist of protein or glycoprotein[9,10], and especially in synapses, they may be channels for ionic movement across the membrane[7,11-14]. It has also been observed that alteration in pH, temperature[15] or calcium concentration[16,17] can induce translational movement of particles in various membranes. However, the function of membrane-associated particles is still unknown.

In the present study, the effects of potassium and calcium in the incubation medium on membrane-associated particles of the presynaptic membranes were examined. For this, pinched off nerve endings (synaptosomes) isolated from guinea-pig whole brain were incubated in potassium-rich (high-K) or calcium-rich (high-Ca) medium, and then they were fractured through the presynaptic membrane by freeze-fracture technique.

Materials and methods. Guinea-pigs, weighing 250-300 g, were decapitated, the whole brain was rapidly removed, and the synaptosomes were prepared by a slight modification of the procedure of Hajós[18]. Purified synaptosomes were incubated in control medium, or high-K, high-Ca, or Ca-free medium at 30 °C for 30 min. The control medium consisted of 140 mM NaCl, 5 mM KCl, 4 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 10 mM glucose adjusted to pH 7.6 with 20 mM tris-HCl buffer. In the high-K and high-Ca media, part of the NaCl was replaced by an equimolar amount of KCl or $CaCl_2$ to give final concentration of 55 mM high-K and 40 mM high-Ca, respectively. For the Ca-free medium, 1 mM EGTA was used. After incubation, the synaptosomes were recovered by centrifugation at 20,000 × g for 30 min and fixed in 5% glutaraldehyde with 0.1 M cacodylate buffer for 5 h. They were then washed with 0.1 M cacodylate buffer and immersed in 30% glycerol overnight.

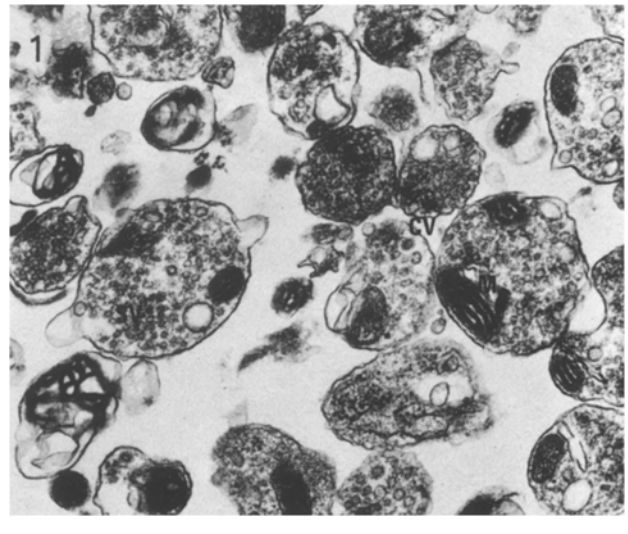

Fig. 1. Conventional thin-section of normal synaptosomes. Many uniformsized synaptic vesicles (SV), some coated vesicles (CV) and mitochondria (M) can be seen in the synaptosomes. × 24,300.