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Ultrastructural changes induced by pregnenolone nitrile in the rat liver

In rats, intoxication with indomethacin, digitoxin (Selye, 1970a), cyclophosphamide (Selye, 1970b) and various other drugs can be prevented by pregnenolone nitrile (3β-hydroxy-20-oxo-5-pregnene-16α-carbonitrile). So far, this compound has been found to be the most active inhibitor of intoxication among over 300 steroids tested in our laboratory. Its ability to cause liver hypertrophy and increased pentobarbitone clearance in the blood is consistent with the view that this steroid nitrile acts through microsomal enzyme induction (Selye, 1970a,b). Therefore, it seemed of interest to determine whether this compound causes ultrastructural alterations in the hepatocytes.

Female ARS/Sprague-Dawley rats (Madison, Wisconsin, U.S.A.) of average weight 100 g and maintained on freely available Purina Laboratory Chow and tap water were used. Pregnenolone nitrile (10 mg in 1·0 ml of water) was administered orally, by soft rubber catheter, twice daily for 5 days. The animals were killed by destruction of the medulla oblongata on the 6th day, 16 h after the last gavage. A section of the liver was excised, minced, fixed in Millonig's osmium solution and processed for electron microscopic studies, as described elsewhere (Kovacs, Blascheck & Gardell, 1970; Gardell, Blascheck & Kovacs, 1970).

Pregnenolone nitrile-treated animals exhibited a marked proliferation of the smooth-surfaced, with a relative decrease of the rough-surfaced, endoplasmic reticulum (Fig. 1A and B). It could not be ascertained whether this increase was due to degranulation and transformation of the rough-surfaced into the smooth-surfaced endoplasmic reticulum, or whether it represented *de novo* synthesis. Long, smooth-surfaced lamellae were seen in some places; these were the originally ribosome-studded membranes which had undergone degranulation. The mitochondria were somewhat swollen and, in some cases, they assumed a homogeneous appearance with the disappearance of the cristae. Lipid content increased moderately, and the microvilli in the bile canaliculi seemed to be hypertrophied.

Proliferation of the smooth-surfaced endoplasmic reticulum is not a specific effect of pregnenolone nitrile; it is also induced by various other compounds, such as phenobarbitone (Fouts & Rogers, 1965), tolbutamide (Remmer & Merker, 1965), spironolactone (Kovacs & others, 1970), norbolethone (Gardell & others, 1970) and certain other steroids (Horvath, Kovacs & others, 1970). It has been assumed that this change indicates activation of various microsomal drug-metabolizing enzymes in the liver (Fouts & Rogers, 1965; Conney, 1967). However, further investigations are required to clarify the pathophysiological significance of smooth-surfaced reticulum hypertrophy and of the role played by pregnenolone nitrile in causing proliferation of smooth membranes.

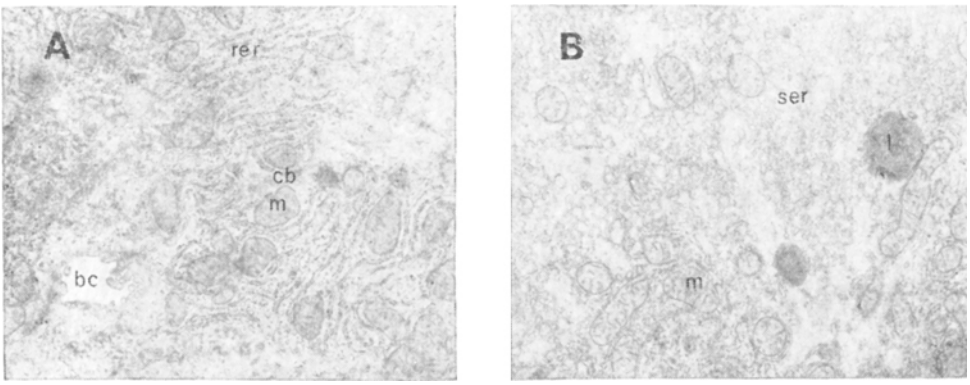

FIG. 1.A. Untreated rat. Portions of two hepatocytes showing characteristic features, r e r: rough-surfaced endoplasmic reticulum; m: mitochondrion; b c: biliary canaliculus; c b: cell border; × 13,300.

B. Pregnenolone-treated rat. Accumulation of smooth-surfaced endoplasmic reticulum can be seen in a portion of hepatocyte, s e r: smooth-surfaced endoplasmic reticulum; m: mitochondrion; l: lipid; × 12 000.

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