The Effect of Linoleic Acid, Peroxidation and Antioxidants on Induction of Cytochrome P-450 in Rat Liver

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Phenobarbitone administration causes a massive synthesis of cytochrome P-450 and the associated microsomal hydroxylating (drug-metabolizing) enzyme activity in the rat liver (Conney, 1967).

If rats are fed on a purified diet containing 20% casein, the response to phenobarbitone is only half as great as in rats fed on stock pellets (cytochrome P-450, 55 ± 15 nmol/g of liver in comparison with 113 ± 18 nmol/g; Marshall & McLean, 1969, 1971). Addition of 15% herring oil to the purified diet leads to full recovery of the ability to respond to phenobarbitone.

A 10% portion of linoleic acid [crude linoleic acid; Sigma (London) Chemical Co., London S.W.6, U.K.] was added to the purified diet in three ways. First, the diet was made up and allowed to peroxidize at room temperature for 3 days in air; secondly, the linoleic acid was mixed with the antioxidant butylated hydroxyanisole (1 mg/ml) before addition to the diet. Thirdly, the diet was mixed and kept cold until put into feed pots. The peroxidized diets allowed full induction of cytochrome P-450. The diets containing butylated-hydroxyanisole-stabilized linoleic acid had only slightly more effect than fat-free diet, and the diets stored in the cold before feeding had an intermediate effect (cytochrome P-450 values were 49 ± 11 nmol/g for fat-free diet, 133 ± 17 nmol/g for oxidized linoleic acid, 74 ± 9 nmol/g for linoleic acid + butylated hydroxyanisole and 95 ± 13 nmol/g for linoleic acid stored cold). The diets without the antioxidant peroxidized rapidly in the feed pots.

We conclude that maximal induction of cytochrome P-450 by phenobarbitone requires the presence of dietary peroxidized lipids, either peroxidized fatty acids or else peroxidized sterols (Marshall & McLean, 1971). It seems likely that this induction effect is related to detoxication of these reactive molecules, which are present in most natural diets.

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Isolation of Dense Bodies Containing Acid Hydrolases and Iron from Rat Spleen

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Bowers, Finkenstaedt & de Duve (1967) and Bowers & de Duve (1967*a,b*), investigating the distribution of lysosomal enzymes in normal rat spleen by using density-gradient isopycnic centrifugation in a linear sucrose gradient in 0.2M-potassium chloride, showed the presence of three distinct populations. These populations were designated L15, L19 and L30 to denote their model densities of 1.15, 1.19 and 1.30 respectively. The L30 group occurred associated with nuclei, and the possibility that this fraction resulted from agglutination of L19 lysosomes with nuclei was not eliminated.

The present work describes the isolation of a nuclei-free heavy-lysosome fraction, rich in iron, which probably corresponds to the L30 fraction described above.

Male and female Wistar rats from a pathogen-free inbred colony were used for the experiments. The animals were killed, and the spleens were removed and placed in ice-cold 0.25M-sucrose adjusted to pH 7.0 with sodium hydrogen carbonate. The pooled spleens from ten rats were blotted dry and passed through a tissue press (average pore size 1 mm diam.). A weighed portion of the brei was homogenized in 0.25M-sucrose with a Potter-Elvehjem Teflon-on-glass homogenizer and centrifuged at 700g for 10 min. The supernatant was removed, the nuclear pellet rehomogenized in 0.25M-sucrose and the mixture centrifuged at 300g for 10 min. The supernatant was again removed and added to the initial postnuclear supernatant. This extraction of the nuclear pellet was repeated three times. The combined postnuclear supernatants were then fractionated according to the method of Appelmans, Wattiaux & de Duve (1955). The heavy-mitochondrial fraction was further sub-fractionated by means of isopycnic density-gradient centrifugation by using a linear sucrose gradient of density 1.10–1.28 layered on a cushion of dense sucrose ($\rho = 1.30$). After centrifugation at 130 000g for 3.5 h two major bands were visible plus a rusty-brown pellet ($\rho > 1.30$). Acid phosphatase, cathepsin D, β -glucuronidase, protein and iron were determined in the pellet, the total homogenate and fractions obtained by differential centrifugation. The results indicated a lysosomal origin for the pellet. Electron microscopy revealed that it