determined from the amount of ^3H recovered and the specific radioactivity of the acetic anhydride.

This procedure was sensitive enough to measure the net synthesis of 7α -hydroxycholesterol in our incubations.

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The Cofactor Requirement for the Rat Liver Microsomal Cholesterol 7α -Hydroxylase Enzyme System

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The aerobic incubation of $[4\text{-}^{14}\text{C}]$ cholesterol and NADPH with 18000g (mitochondria-free) supernatant from rat liver homogenized in 154mM-potassium chloride gives rise to a single radioactive product, cholest-5-ene- 3β , 7α -diol or 7α -hydroxycholesterol. Incubation of the 105000g microsomal pellet under the same conditions gives rise to a spectrum of radioactive 'autoxidation' products of cholesterol, the formation of which can be inhibited by the 105000g supernatant.

This anti-oxidative activity is retained when the 105000g supernatant is heated at 95°C for 10 min, cooled and centrifuged to remove denatured protein. The cofactor present in this supernatant is diffusible on dialysis, labile to alkali and labile to prolonged heating at 95°C . This activity can be mimicked by certain thiols such as 2-mercaptoethylamine.

The conditions under which cholesterol is autoxidized (Mitton, Scholan & Boyd, 1971) are identical with those in which lipid peroxidation occurs (Ernster & Nordebrand, 1967; Wills, 1969) as measured by oxygen uptake and malonaldehyde

production. Heat-treated supernatant will inhibit lipid peroxidation measured by these criteria. The inhibition of cholesterol autoxidation and lipo-peroxidation by the supernatant factor(s) is removed by pretreating the supernatant with *p*-chloro-mercuribenzoate, thus indicating that thiol groups are necessary. Chromatography of freeze-dried heat-treated supernatant on Sephadex G-25 indicated the presence of one main thiol fraction. This fraction inhibits lipid peroxidation. Further chromatography of this thiol fraction on DEAE-Sephadex A-25 gives a single thiol peak with anti-lipo-peroxidation activity. This fraction has been examined by electrophoresis and shown to contain GSH, GSSG, aspartic acid, glutamic acid and u.v.-absorbing material. GSH alone or in combination with glutamic acid and aspartic acid does not have any anti-oxidative activity.

This suggests that GSH is one component of the cofactor involved in the efficient microsomal cholesterol 7α -hydroxylase system. Low concentrations of Mn^{2+} ions in the presence of GSH can imitate the activity of the heat-stable supernatant factor. However, the additional physiological components of heat-stable supernatant have still to be identified.

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Studies on the Site Specificity of a Hydroxy Steroid Dehydrogenase

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Crystalline preparations of 20β -hydroxy steroid-NAD oxidoreductase (EC 1.1.1.53) from *Streptomyces hydrogenans* (Hübener, Sahrholz, Schmidt-Thomé, Nesemann & Junk, 1959) show 3α -hydroxy steroid-NAD oxidoreductase activity towards various steroids (Pocklington & Jeffery, 1968), the reaction rate being greatly altered by a 17β -hydroxy or 16-oxo group in the substrate, and by the assay conditions (Gibb & Jeffery, 1970). To establish the relationship between the activities towards 3α - and 20β -hydroxy steroids, the rough estimates of kinetic constants provided by an earlier study (Pocklington & Jeffery, 1968) were not adequate. Values have now been obtained for three favourable substrates