Rats were undernourished by restricting their mothers to an approx.-50% *ad-libitum* diet from the seventh day of gestation until weaning. All animals were fed *ad libitum* from 25 days, when undernourished young were 34% of the weight of normal rats. At killing (15–17 weeks) previously undernourished males were 69% and females 81% of the weights of normal animals. Finally there were eight groups of animals, separated according to (a) sex, (b) nutritional history (normal or previously undernourished) and (c) treatment (not stressed or stressed). Stressed females were killed 17 min after receiving an electric shock, and stressed males 15 min after being placed in a situation in which they had been shocked the day before. Plasma 11-hydroxy corticosteroids were determined (Mattingly, 1962) with corticosterone as standard.

Basal concentrations (means $\pm$ s.d.) (in rats that had not been stressed) did not differ significantly between normal and previously undernourished groups for either males (normal, $20.0 \pm 7.4 \mu\text{g}/100\text{ml}$, $n=19$; undernourished, $16.8 \pm 5.7 \mu\text{g}/100\text{ml}$, $n=14$) or females (normal, $33.2 \pm 18.9 \mu\text{g}/100\text{ml}$, $n=11$; undernourished, $30.5 \pm 15.7 \mu\text{g}/100\text{ml}$, $n=11$). Stressed normal males had significantly higher 11-hydroxy corticosteroid concentrations than did their previously undernourished counterparts (normal, $40.1 \pm 13.3 \mu\text{g}/100\text{ml}$, $n=21$; undernourished, $30.7 \pm 9.4 \mu\text{g}/100\text{ml}$, $n=17$; $P < 0.02$). Although early undernutrition did not significantly affect concentrations in stressed females (normal, $92.8 \pm 17.6 \mu\text{g}/100\text{ml}$, $n=15$; undernourished, $81.7 \pm 16.4 \mu\text{g}/100\text{ml}$, $n=12$; $0.1 > P > 0.05$) there was among these animals a significant correlation ($r = +0.625$, $n=26$, $P < 0.001$) between plasma 11-hydroxy corticosteroid concentration and body weight.

It is suggested that early undernutrition, in male rats at least, decreases their responsiveness to stressful stimulation as measured by plasma 11-hydroxy corticosteroid concentrations.

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An Assay Method for Cholesterol 7 α -Hydroxylase in Rat Liver Microsomal Fraction

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The first step in the conversion of cholesterol into cholic acid is thought to be the introduction of a 7 α -hydroxyl group into the ring system of cholesterol (Bergström, 1959). 7 α -Hydroxylase, the enzyme catalysing this reaction, is confined to the liver microsomal fraction and requires NADPH and molecular oxygen (Danielsson & Einarsson, 1966). Cytochrome *P*-450 is the terminal oxidase for this hydroxylation (Boyd, Scholan & Mitton, 1969). There is evidence to suggest that the 7 α -hydroxylation of cholesterol is rate-limiting in cholic acid formation (Danielsson, Einarsson & Johansson, 1967). Hence, by studying the effect of various physiological factors on the activity of 7 α -hydroxylase, it may be possible to obtain information about the regulation of bile acid formation in the liver.

The cholesterol molecule is susceptible to attack by molecular oxygen, with the formation of 7-oxo-cholesterol, 7 β -hydroxycholesterol, 7 α -hydroxycholesterol and other compounds. This oxidative process is enhanced in the presence of microsomal fraction fortified with NADPH. However, incubation of cholesterol with microsomal fraction from rat liver homogenized in the presence of EDTA (Shefer, Hauser & Mosbach, 1968) gives 7 α -hydroxycholesterol free from autooxidation products.

Current methods for assaying 7 α -hydroxylase activity are based on measurement of the incorporation into 7 α -hydroxycholesterol of radioactive cholesterol added to a suspension of microsomal fraction in the presence of a suitable medium. Such methods may give erroneous results because liver microsomal fraction contains appreciable amounts of cholesterol. It is possible that only a portion of this endogenous cholesterol acts as substrate for the enzyme, and that the proportion of the total endogenous cholesterol that is metabolically active varies under different conditions. It is also possible that added radioactive cholesterol mixes with the metabolically active pool to different extents depending on the way in which it is added. These considerations have led us to develop a more direct method for measuring 7 α -hydroxylase activity in microsomal suspensions.

[4-¹⁴C]Cholesterol was incubated with liver microsomal fraction and the 7 α -hydroxycholesterol synthesized was extracted and separated from other compounds by t.l.c. The steroid was located by radioautography, eluted and acetylated with [³H]acetic anhydride (Peterson & Eilers, 1965). The amount of 7 α -hydroxycholesterol was then

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 $[4-^{14}\text{C}]$ 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- $3\beta,7\alpha$ -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