days is the period of maximum food intake in pregnant and lactating rats fed ad libitum.

Catalase specific activities in the liver, kidney and jejunal mucosa of virgin animals were 4.7 ± 0.74, 1.41 ± 0.36 and 0.11 ± 0.02 units/mg of homogenate protein respectively (means ± S.D.) and did not change significantly in each tissue during pregnancy and lactation. Palmitoyl-CoA oxidase specific activity in the kidney remained constant (Fig. 1), but in the liver there was a statistically significant increase (P < 0.02) in lactation. The pattern of change in the jejunum was different in that there was a decrease of approx. 50% in pregnancy (Fig. 1).

The ratio of palmitoyl-CoA oxidase/catalase specific activities in virgin rats is 20 times higher in the jejunal mucosa than it is in the liver, which suggests that long-chain fatty acid oxidation could be a more significant proportion of peroxisomal function in the jejunum than it is in the liver. Despite an increase in jejunal mucosal weight in pregnancy of 25%, the total palmitoyl-CoA oxidase activity in the mucosa decreased. If a major function of intestinal peroxisomes is to oxidize long-chain fatty acid then a reduced rate of oxidation could be a factor in the hyperlipidaemia of pregnancy. In lactating rats, the oxidase specific activity returned almost to the virgin control values in the jejunum but was significantly raised in the liver (Fig. 1). As jejunal mucosa and liver increased in weight by 140% and 80% respectively compared with controls, the total palmitoyl-CoA oxidase in the two tissues was markedly increased in lactation.

Peroxisomal enzyme changes in lactation could be a response to hyperlipidaemia. It is possible that enhanced peroxisomal β-oxidation is used to provide short-chain fatty acid for milk production, a possibility that is currently under investigation.

KAT THEILEN M. BOTHAM and GEORGE S. BOYD* Department of Biochemistry, University of Edinburgh Medical School, High Robson Building, George Square, Edinburgh EH3 9XD, U.K.

Previous studies on the effect of dietary cholesterol on the activity of the rate-limiting enzyme in bile acid synthesis, cholesterol 7α-hydroxylase, have given conflicting results, with some workers finding increased activity on feeding cholestero1 to rats (Boyd et al., 1969; Mitropoulos et al., 1973; Raicht et al., 1975) while others found no significant change (Shelcer et al., 1973; Björkhem & Danielson, 1975). In contrast, faecal excretion of bile acids, thought to represent bile acid synthesis in the steady state, has shown a consistent 2–3-fold increase on supplementation of the diet with cholesterol (Wilson, 1964; Beher et al., 1970; Raicht et al., 1975; Mathé & Chevallier, 1979). We have tried to resolve this conflicting evidence by measuring bile acid synthesis directly in vitro using isolated hepatocytes and in vivo in biliary-drained rats. In order to try to establish the fate of the excess cholesterol which accumulates in rat liver during cholesterol feeding (Gould, 1977) biliary cholesterol secretion was also determined.

Rats were given a diet containing 10% (w/w) olive oil (diet 1) or 10% (w/w) olive oil + 1% (w/w) cholesterol (diet 2) for at least 10 days, then either biliary drained by cannulation of the common bile duct (bile samples were collected every 2 h for 44 h) or used for the preparation of isolated hepatocytes as described previously (Botham et al., 1980). In cholesteryamine-feeding experiments diets 1 and 2 were supplemented with 4% (w/w) cholesteryamine for 4 days. The amounts of conjugated cholic, chenodeoxycholic and β-muricholic acid in the cell and bile samples were determined by radioimmunoassay (Beckett et al., 1978, 1979; Botham et al., 1983). Total bile acid synthesis in the cell experiments was calculated from the sum of the three individual bile acid determinations. Liver cholesterol content was estimated by using cholesterol oxidase (Richmond, 1973).

There was no significant difference in the total amount of bile acid synthesized in hepatocytes from cholesterol-fed as compared with control rats either when the rate of bile acid synthesis was basal (i.e. in rats given diets 1 or 2) or when it had been stimulated by feeding the bile acid sequestrant, cholesteryamine (Table 1), despite the fact that liver cholesterol levels were greatly elevated in the cholesterol-fed groups. In addition, biliary levels of conjugated cholic and chenodeoxycholic acid were not increased by prior feeding of cholesterol at any time point during 44 h of biliary drainage (four rats were used in each group). β-Muricholic acid is not quantitatively important in the bile of biliary-drained rats (Botham & Boyd, 1983). As biliary bile acid secretion is thought to represent bile acid synthesis after about 10 h of biliary drainage (Erickson, 1957), these results, together with those from the experiments with isolated hepatocytes, show that increased dietary cholesterol does not lead to increased bile acid synthesis.

*Deceased.

Vol. 13
The formation of cholic acid relative to chenodeoxycholic acid and its metabolite, $\beta$-muricholic acid, was decreased by cholesterol-feeding in hepatocytes from rats with a basal rate of bile acid synthesis (Table 1) and biliary cholic acid levels were significantly lower after 38-44 h of biliary drainage in the cholesterol-fed as compared with the control rats. These results support the finding of Gustafsson et al. (1975) that the activity of 7a-hydroxysterol-4-en-3-one-12a-hydroxylase, which controls the relative amounts of cholic and chenodeoxycholic acid formed, is depressed by dietary cholesterol. The secretion of cholesterol into bile in biliary-drained rats was also not significantly changed by cholesterol-feeding (six rats per diet). Our experiments, then, show that neither bile acid synthesis nor biliary cholesterol secretion are increased by cholesterol-feeding. Mathé et al. (1984) have found that hepatocytes isolated from cholesterol-fed rats excrete cholesterol-rich lipoproteins. It seems likely, therefore, that the excess cholesterol which accumulates in rat liver during cholesterol-feeding is largely removed by secretion into plasma as lipoproteins.


Chim. Acta 93, 145–150


Wilson, J. D. (1964) J. Lipid Res. 5, 409–417

Reversible phosphorylation of cholesteryl ester hydrolase

ROGER J. COLBRAN and STEPHEN J. YEAMAN

Department of Biochemistry, The University, Newcastle-upon-Tyne, NE1 7RU, U.K.

Cyclic AMP mediates the stimulatory action of corticotropin and lutropin on steroidogenesis in the adrenal cortex and corpus luteum respectively. One of the sites of action of cyclic AMP is postulated to be an increased phosphorylation of cholesteryl ester hydrolase by cyclic AMP-dependent protein kinase, thereby activating the enzyme and increasing the supply of non-esterified cholesterol to the mitochondrial side-chain cleavage system for conversion to pregnenolone (review in Boyd & Gorban, 1980). However, much of the work supporting this hypothesis has used only crude preparations of cholesteryl ester hydrolase, and as the enzyme had not been identified, direct phosphorylation was not demonstrated.

Cholesteryl ester hydrolase has recently been partially purified from both tissues and identified as having a subunit of Mr 84 000 (Cook et al., 1981, 1983). Interestingly the properties of the enzyme indicate that it is very similar or identical to the hormone-sensitive lipase of adipose tissue (Cook et al., 1982), an enzyme also regulated by a cyclic AMP-dependent protein kinase-catalysed phosphorylation.

Abbreviation used: SDS, sodium dodecyl sulphate.