THE EFFECT OF PORTACAVAL TRANSPOSITION ON HEPATIC CHOLESTEROL-7α-HYDROXYLASE ACTIVITY IN THE RAT

George S. BOYD and Margaret E. LAWSON
Department of Biochemistry, University of Edinburgh Medical School, Teviot Place, Edinburgh, EH8 9AG, Scotland

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1. Introduction

There is considerable interest in the role of the liver in the synthesis, secretion and catabolism of plasma lipoproteins. One important constituent of the plasma lipoproteins is cholesterol and recently attention has been drawn to the fact that the establishment of a portacaval anastomosis in humans reduces the plasma cholesterol concentration [1,2]. It seemed of interest to find out whether this operative procedure altered the hepatic catabolism of cholesterol.

Cholesterol-7α-hydroxylase is possibly the rate limiting enzyme in the catabolism of cholesterol to bile salts in rat liver [3,4]; it is a microsomal enzyme and requires NADPH and molecular oxygen [5]. The enzyme utilises cytochrome *P* 450 as the terminal oxidase [6] but there appears to be little correlation between the total liver microsomal cytochrome *P*450 content and the microsomal cholesterol-7α-hydroxylase activity [7]. The activity of this enzyme is enhanced following diversion of the enterohepatic circulation of bile salts [8] and the activity of this enzyme exhibits a marked diurnal rhythm [9–11]. The establishment of a portacaval anastomosis in the rat results in a decrease in the hepatic cytochrome *P*450 concentration [12–14]. Since this procedure alters the concentration of bile salts returning to the liver, such an effect might alter the activity of the liver microsomal cholesterol-7α-hydroxylase. It has been shown that portacaval anastomosis in the rat did not affect the bile salt pool size and the bile acid secretion rate [15,16]. In this paper we report the effects of portacaval anastomosis on the liver microsomal cholesterol-7α-hydroxylase activity and cytochrome *P*450 concentration in rat liver microsomes. This paper also contains an account of the effects of portacaval anastomosis on the plasma cholesterol concentration in the rat.

2. Materials and methods

Rats used were males of the Wistar strain weighing 150–200 g which were bred in the Edinburgh University Small Animal Breeding Establishment. The animals were fed a soft diet consisting of 70% wholemeal flour, 25% skimmed milk powder and 5% dried brewer's yeast. The animals were housed in a controlled environment of 12 h lighting and 12 h darkness at 20°C.

Portacaval anastomosis were performed on 54 animals using an end-to-side technique [17]. In a few animals a sham operation was performed to act as an operational control series. The animals had free access to food and water after the operative procedure The mean body weight of the operated animals decreased to 21% of the control animals 32 days after the portacaval shunt had been established.

Rats were killed at 9.00 a.m. at fixed periods after the establishment of the portacaval anastomosis. These periods varied from 10 days to 80 days after the operation. The animals were anaesthetised with ether and a blood sample taken from the heart. The liver was rapidly removed, weighed and homogenized in 0.154 molar KCl (20% w/v) and microsomes prepared in the usual way [5].

Cholesterol-7α-hydroxylase assays were conducted

as previously described [7]. Microsomal cholesterol was determined by extracting 1 ml of each microsomal suspension with ethanol–acetone and the total cholesterol content of this extract determined by the enzymic method using cholesterol oxidase [18]. Plasma cholesterol was also determined by this cholesterol oxidase method. Cytochrome *P*450 concentration was determined in the microsomal suspension according to the procedure of Omura and Sato [19]. The protein content of the microsomes was determined by the method of Lowry [20].

3. Results

The post-operative mortality following portacaval anastomosis in this series of animals was very low. In the entire experiment lasting over 80 days after the operation only 10% of the animals died. In this series of animals as in other studies reported [16] there was a marked decline in the body weight of the operated animals and after 20–30 days the decrease in body weight plateaued at a new fairly steady state. Again, as in other reported series [16], there was a marked change in the liver weight of the animals, see table 1. It was possible to confirm that as a result of portacaval anastomosis there was a marked decrease in the liver microsomal cytochrome *P*450 content [12–14] (fig.1).

The liver microsomal cholesterol-7α-hydroxylase activity was significantly elevated in all animals subjected to portacaval anastomosis. As shown in fig.1, the increase in the activity of this mixed function oxidase is apparent a few days after operation and the marked increment in the activity of this enzyme was significant 80 days after operation. In this study the activity of cholesterol-7α-hydroxylase, is markedly elevated despite the fact that the microsomal cytochrome *P*450 is significantly decreased.

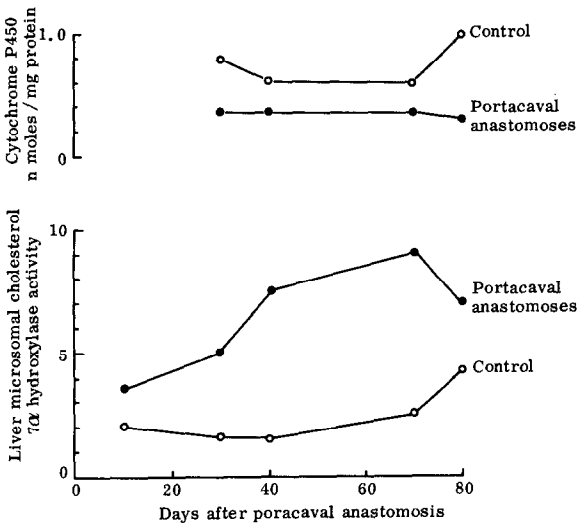

Fig.1. Effect of portacaval anastomoses on the liver microsomal cholesterol-7α-hydroxylase activity and the cytochrome *P*450 content. In this study there are 4 rats per group.

Since it is known that interruption to the entero-

Table 1
The effect of portacaval anastomosis (PCA) on the liver weight and on the liver microsomal cholesterol content in the rat

	No. of rats per group	Days after operation	Liver Weight	Liver weight / Body weight × 100	Liver microsomal cholesterol (μg/mg protein)
Control	4	30	13	3.7	22.5
Control	4	40	12.4	3.4	17.3
Control	4	70	12	3.9	15.7
Control	4	80	12	3.6	15.1
PCA	4	30	5.8	2.2	22.7
PCA	4	40	4.9	2.0	19.6
PCA	5	70	8.0	3.2	12.2
PCA	3	80			14.2

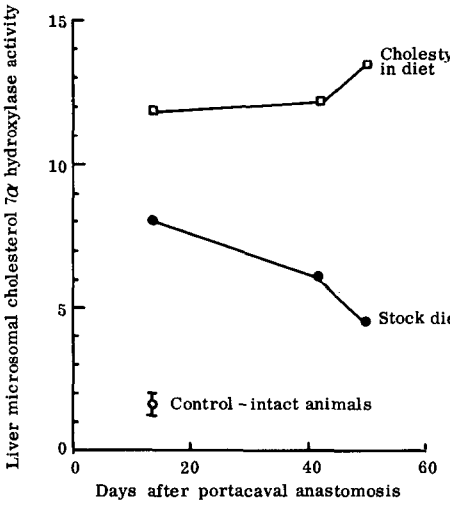

Fig.2. All the rats in this study were subjected to portacaval anastomosis and fed the stock diet thereafter. At fixed periods after the operation selected animals were fed the stock diet to which was added 4% cholestyramine resin to block the enterohepatic circulation of bile salts. These 'cholestyramine in diet' animals were killed 5 days later. The liver microsomal cholesterol-7α-hydroxylase activity was compared in the two groups of animals, the 'stock diet' and the 'cholestyramine in diet' groups. In this study there were 3 animals per group.

hepatic circulation of bile salts in the rat results in an elevation of the liver microsomal cholesterol-7α-hydroxylase activity, it was of interest to establish whether these animals subjected to a portacaval anastomosis with elevated cholesterol-7α-hydroxylase activities would be affected by the introduction into their diet of a bile salt sequestering agent. Normal animals given such a sequestering agent (cholestyramine or cuemid) show a marked increase in liver microsomal cholesterol-7α-hydroxylase activity [21]. As shown in fig.2 animals subjected to a portacaval anastomosis and subsequently fed the stock diet containing 4% cholestyramine resin for a test period of 5 days had even higher liver microsomal cholesterol-7α-hydroxylase activities than animals subjected to portacaval anastomosis but fed the normal stock diet.

4. Discussion

The liver microsomal cholesterol-7α-hydroxylase enzyme system is a key factor in the rate of cholesterol degradation to bile acids [4,7,8,21]. It

Table 2
The effect of portacaval anastomosis in the rat in the presence and in the absence of dietary cholestyramine resin as a bile salt sequestering agent. Various parameters of cholesterol metabolism in plasma and in the liver have been studied

	Controls - intact animals on stock diet	Animals subjected to portacaval anastomosis kept on stock diet	Animals subjected to portacaval anastomosis fed stock diet containing 4% cholestyramine
Number of rats per group	16	9	9
Plasma cholesterol (mg/100 ml)	68	52	54
Liver cholesterol (mg/100 g wet weight)	190	204	216
Liver microsomal cholesterol (μg/mg protein)	17.7	18.5	19.2
Liver microsomal cholesterol (nmoles/mg protein)	46	48	50
Cholesterol-7α-hydroxylase activity (pmoles/min/mg protein)	16	38	70
Cytochrome *P*450 (nmoles/mg protein)	0.81	0.36	0.30

has been shown that diversion of bile salts from the liver by cannulation of the bile duct or by feeding bile salt sequestering agents increase the activity of this enzyme [21]. There is evidence that diversion of bile salts from the liver precedes the rise in activity of this oxygenase by many hours and the rise in the liver microsomal cholesterol-7α-hydroxylase may involve de novo protein synthesis [22].

In this study diversion of the portal blood to the vena cava presumably decreases, by dilution, the concentration of bile salts reaching the liver and as shown in this study there is an increase in the activity of this key oxygenase associated with cholesterol degradation. Furthermore, the rise in activity of this cholesterol-7α-hydroxylase was maintained throughout the 12 weeks of the experiment. If the rise in activity of this oxygenase was associated with a diminished concentration of bile salts reaching the liver, we wished to establish whether the liver could respond still further by lowering the plasma bile salt concentration. Animals subjected to portacaval anastomosis, fed for 5 days a diet containing 4% cholestyramine resin, exhibited a further increase in the liver microsomal cholesterol-7α-hydroxylase activity. This suggests that one factor involved in the control of the activity of cholesterol-7α-hydroxylase in liver may be the concentration of bile salts in the blood supply to that organ.

The liver microsomal cholesterol concentration tended to be higher and the plasma cholesterol concentration tended to be lower in the animals subjected to portacaval anastomosis. No attempt was made in this study to fractionate the plasma lipoproteins into separate groups and it is appreciated that there could be changes in the plasma lipoproteins which would not be shown up by plasma cholesterol measurements.

5. Conclusion

Portacaval anastomosis in the rat results in an increase in the activity of the liver microsomal cholesterol-7α-hydroxylase enzyme system. The increase in the activity of this oxygenase occurs despite a decrease in the total amount of cytochrome *P*450 in the liver microsomes after portacaval anastomosis. It is possible to increase further the activity of the cholesterol-7α-hydroxylase enzyme in these portacaval shunted animals by feeding them on a diet containing a bile salt sequestering agent. This suggests that one of the factors influencing the activity of the liver microsomal cholesterol-7α-hydroxylase enzyme may be the concentration of bile salts reaching the liver from the blood plasma. Portacaval anastomosis in the rat tended to achieve a small decrease in the plasma cholesterol concentration.

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