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Prolonged bile duct obstruction: a new experimental model for cirrhosis in the rat

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Summary. Hepatic morphological abnormalities were examined in rats whose bile ducts had been either cannulated and then obstructed or irreversibly ligated for 5, 10, 15 and 28 days or longer. Throughout the experiment most of the morphological changes observed in the cannulated group were comparable to those in the ligated group. Portal inflammation and marginal bile duct proliferation were noted with the same frequency in both groups. Biliary obstruction for 15 days or more led to cirrhosis. After 28 days obstruction, five out of six cannulated rats and four out of six ligated animals respectively developed cirrhosis. The development of cirrhosis was progressive and associated with ascites. It is concluded that in the rat the morphological sequelae of long term cholestasis induced by either cannulation and obstruction or ligation of bile ducts are similar and are accompanied by cirrhosis. The advantages of this experimental model for the study of human cirrhosis are discussed.

Keywords: cirrhosis, bile duct obstruction, cholestasis

The effects of conventional bile duct ligation on hepatic morphology have been examined extensively since the publication of the earliest histological studies approximately 50 years ago (Cameron & Oakley 1932; Cameron & Hasan 1958; Trams & Symeonidis 1957). However, the time of development of secondary biliary cirrhosis following ligation of the common bile duct appears to vary widely. Some authors found that cirrhosis developed after 3–5 weeks (Cameron & Hasan 1958) while others were unable to show evidence of cirrhosis after 14–40 days of bile duct ligation in the rat (Cartter 1966; Franco *et al.* 1979; Johnstone & Lee 1976; MacDonald & Pechet 1961; Moritz & Snodgrass 1972). Despite the high morbidity and mortality of cirrhosis (Galambos 1979) currently there is no reliable experimental model for the study of human cirrhosis. The most commonly used method of producing experimental cirrhosis involves multiple doses of the hepatotoxin carbon tetrachloride (CCl_4) (Miura *et al.* 1982; Proctor & Chatamra 1982, 1983). Many problems and questions arise, however, which throw doubt on the adequacy of this experimental model as a copy of the human disease (Tamayo 1983).

Unexpectedly, (J. Kountouras, P.J. Scheuer & B.H. Billing, unpublished data) cirrhosis developed when rats were subjected to complete bile duct obstruction for 2 weeks or more using the technique described by Accatino *et al.* (1979, 1981) in their studies on post-cholestatic choleresis. This technique, which enables bile flow to be re-established subsequently, is easy to perform and allows

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physiological studies on bile to be carried out. The animals survive for many weeks and the method appears to have advantages over the standard experimental procedure using CCl_4 (Tamayo 1983). The aim of the present study was, therefore, to assess the morphological liver changes and in particular cirrhosis induced by bile duct obstruction in rats, comparing cannulation and ligation, so as to determine the value of an obstructive model for the study of human cirrhosis.

Materials and methods

Animals. Male rats of the Sprague-Dawley strain, bred at the Royal Free Hospital School of Medicine, were maintained on standard laboratory diet (41B cubes) and initially weighed 250–300 g.

Experimental. The rat model described by Accatino *et al.* (1979, 1981) was used. Under ether anaesthesia a 2-cm incision was made just below the xiphoid process. A polyethylene cannula (Portex Ltd, Hythe, Kent, UK, ID 0.28 mm, OD 0.61 mm) was inserted into the proximal portion of the bile duct and held in position with three silk sutures. Bile flow was assessed for 10 min to ensure patency of the cannula. The distal portion of the cannula was then obstructed with 3 knots, brought out through the lower end of the midline incision and buried subcutaneously in the right lower quadrant. The abdomen was closed and the animal allowed to recover. Operative mortality was low. Four groups of animals were studied after 5 ($n=5$), 10 ($n=5$), 15 ($n=5$) and 28 ($n=6$) days of bile duct obstruction. Two further rats were studied after 42 and 50 days of obstruction respectively.

Another set of rats was subjected to double ligation of the common bile duct, with section between the two ligatures, under light ether anaesthesia. These animals were studied after 5 ($n=5$), 10 ($n=5$), 15 ($n=5$), 28 ($n=6$), 36 ($n=1$), 42 ($n=1$), 52 ($n=1$) and 53 ($n=1$) days of bile duct ligation.

Cannulated and ligated animals were killed and their livers and spleens removed. Before this, a femoral arterial cannula was inserted and arterial blood samples (600–800 μl) were collected for bilirubin estimation.

Determination of bilirubin. Plasma bilirubin concentrations were determined by the diazo method of Michaelsson *et al.* (1965).

Morphological studies. Liver tissue for light microscopy was fixed in formal saline and stained with haematoxylin and eosin, for reticulin fibres, and by the van Gieson method for collagen.

Statistics. All results are expressed as means $\pm$ SEM. The unpaired Student's *t*-test was used to analyse the difference between groups. Values of $P<0.05$ were considered to indicate a significant difference.

Results

Changes in body, liver and spleen weight

During the immediate postoperative period, i.e. after 5 days, both the cannulated (group A) and ligated (group B) cholestatic rats showed a slight but insignificant decrease in body weight of 3% and 4.5% respectively (Table 1). In Group B, body weight remained substantially unchanged until the 10th day. Thereafter, there was an increase in body weight in both groups which became statistically significant after 15 and 28 days of obstruction. There was no significant difference in body weight between the two groups. After more than 28 days of obstruction the body weights increased further as ascites developed, values of 423 g (42 days) and 327 g (50 days) for the cannulated rats and a mean value of 392 ± 44.2 g for the four ligated animals being obtained.

There was a progressive increase in liver weight with time and this became significant in group A from 5 days, and in group B from 10 days (Table 2). There was no significant difference in liver weight between the two groups. A marked increase in spleen weights

Table 1. Changes in body weight (mean ± SEM) in rats with bile duct obstruction

Duration of obstruction (days)	Body weight (g)			
	Group A: cannulated rats		Group B: ligated rats	
	Initial	Final	Initial	Final
0	256.7±2.7			
5	269±5.9	261±9.0	262±5.5	250±8.0
10	259±6.2	267±10.8	278±11.0	272±9.9
15	278±2.5	291±6.7*	269±4.0	293±5.1**
28	267±5.5	330±9.4***	256±6.7	329±13.6**

Comparison with initial values: *$P<0.05$; ** $P<0.005$; *** $P<0.0005$.

was also observed in both groups (Table 2). After more than 4 weeks obstruction the liver weight decreased markedly to 4.4 (42 days) and 3.7 g/100 g of body weight (50 days) in group A and a mean value of 4.5±0.3 g/100 g body weight in group B. Animals with more than 1 month bile duct ligation had a mean spleen weight of 0.6±0.15 g/100 g body weight, whereas the cannulated rats had spleen weights of 0.3 and 0.4 g/100 g body weight at 42 and 50 days respectively.

Plasma bilirubin concentration

In both groups of obstructed animals there was a rise in plasma bilirubin concentration which tended to stabilize between 10 and 15 days and then decreased (Table 3). The percentage of the mean plasma bilirubin concentration in the conjugated form was relatively constant in both groups during the 4 weeks postoperative period (87.0±1.5%).

After more than 30 days bile duct ligation the mean total and conjugated bilirubin concentrations were 114±18.5 and 99±18.6 μmol/l respectively. After cannulation for 42 and 50 days, the plasma bilirubin concentrations were 4 and 131 μmol/l respectively.

Histological changes

In general, similar light microscopic changes were noted in both groups of cholestatic rats (Table 4).

After 5 days obstruction in both groups there was bile duct proliferation, mild oedema and an acute inflammatory reaction in portal areas. Fibrosis was slight and limited to the regions of new bile duct formation. Portal tracts were not linked and the basic lobular architectural pattern remained intact. There was a light infiltrate of neutrophil leucocytes around bile ducts; rarely these cells were present within a bile duct lumen. Hepatocytes contained numerous mitotic figures and occasionally a random focal area of cell necrosis was seen in the parenchyma. Sinusoidal cells were prominent.

After 10 days or more of bile duct obstruction, portal areas became expanded by fibrosis and bile duct proliferation, and linked to form a fibrous network. Marginal bile duct proliferation and concentric periductal fibrosis were noted. The ductular proliferation extended beyond the limits of the portal tracts to invade the liver parenchyma. A few Mallory bodies were seen in hepatocytes. Bile infarcts were occasionally noted, mainly in periportal areas. They were characterized by

Table 2. Changes in liver and spleen weights (mean ± SEM) in cannulated/obstructed (group A) and ligated (group B) cholestatic rats

Duration of obstruction (days)	Liver weight (g/100 g body weight)		Spleen weight (g/100 g body weight)	
	Group A	Group B	Group A	Group B
0	4.8 ± 0.1		0.2 ± 0.01	
5	5.2 ± 0.1*	5.2 ± 0.2	0.3 ± 0.01*	0.3 ± 0.01
10	5.8 ± 0.4*	6.1 ± 0.3**	0.4 ± 0.02***	0.5 ± 0.10**
15	6.3 ± 0.2***	6.2 ± 0.2**	0.5 ± 0.05**	0.6 ± 0.03***
28	6.2 ± 0.3**	6.5 ± 0.3**	0.6 ± 0.04***	0.7 ± 0.03***

Comparison with control values: * $P<0.05$; ** $P<0.005$; *** $P<0.0005$.

necrosis of hepatocytes and accumulation of fibrin. Small amounts of bile were occasionally seen within portal tracts.

After 15 days of biliary obstruction fibrous connective-tissue septa bridged portal areas and frequently extended into the lobules. As a result, the normal lobular pattern was disorganized, but well-defined nodules indicative of cirrhosis were seen in only one rat of group A. However, after 28 days obstruction five of the six cannulated rats (group A) and four of the six ligated animals (group B) respectively had cirrhosis (Figs 1 & 2). Narrow zones of oedema and ductular proliferation were seen at the junctions of parenchyma and septa. Concentric fibrosis was

Table 3. Changes in plasma total bilirubin concentration (mean ± SEM) in cannulated/obstructed (group A) and ligated (group B) cholestatic rats

Duration of obstruction (days)	Plasma total bilirubin (μmol/l)	
	Group A	Group B
0	5 ± 0.3	
5	219 ± 5.0	182 ± 10.1*
10	218 ± 9.1	224 ± 27.5
15	221 ± 8.1	234 ± 6.3
28	160 ± 5.8	161 ± 8.2

Comparison between two groups: * $P<0.01$.

Table 4. Summary of light microscopic changes in bile duct cannulated/obstructed (group A) and ligated (group B) cholestatic rats

Duration of obstruction (days)	Bile duct proliferation Groups A and B 0→++++	Fibrosis Groups A and B 0→++++	Cirrhosis			
			Group A		Group B	
			Number of rats	Cirrhosis	Number of rats	Cirrhosis
5	+	+	5	0	5	0
10	++	++	5	0	5	0
15	+++	+++	5	1	5	0
28	++++	++++	6	5	6	4
>30	++++	++++	2	2	4	4

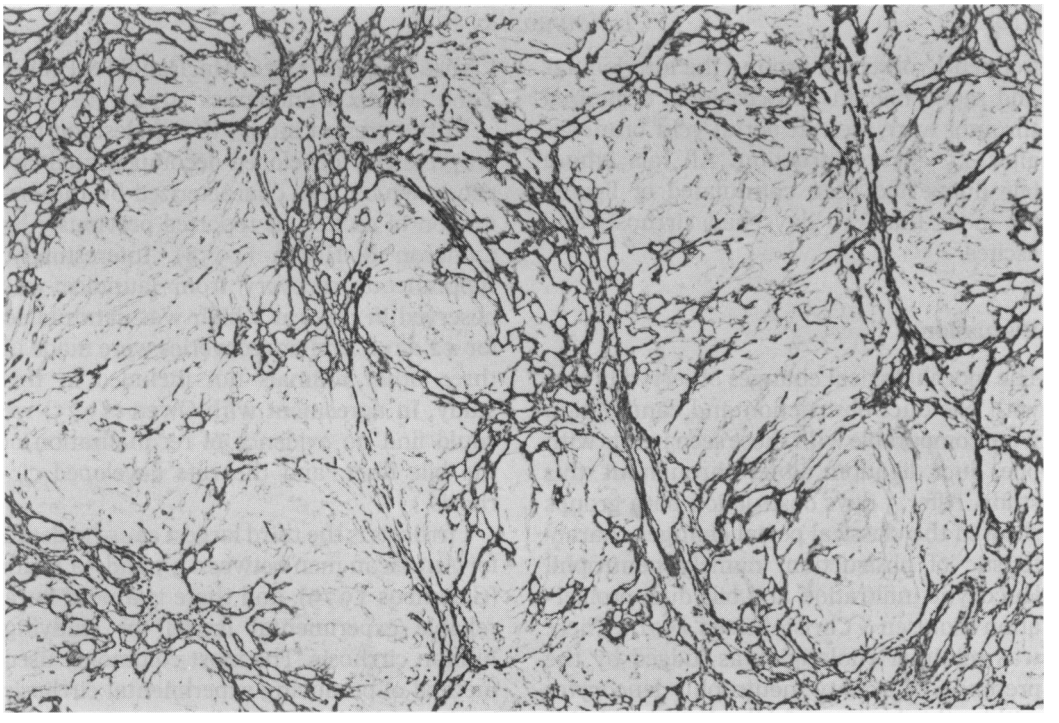

Fig. 1. Lobular architecture has been destroyed and nodules have formed, 28 days after bile duct cannulation and obstruction. Gordon & Sweets' reticulin × 75.

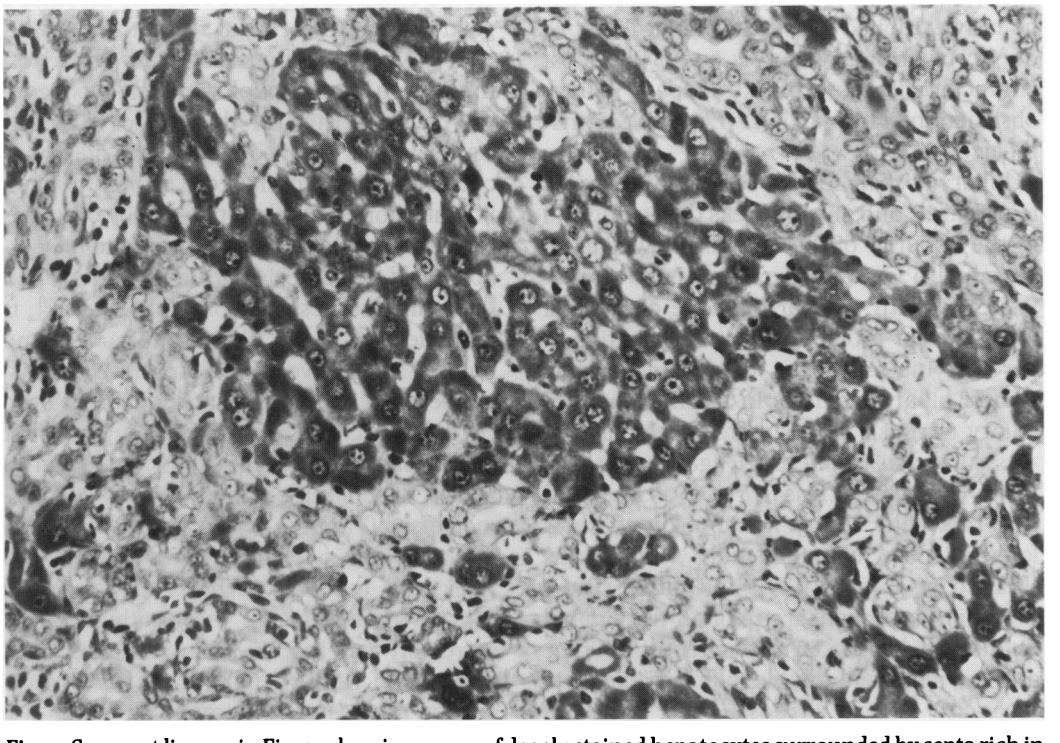

Fig. 2. Same rat liver as in Fig. 1, showing group of deeply stained hepatocytes surrounded by septa rich in proliferated bile ducts. Haematoxylin & eosin × 290.

frequently observed around the interlobular and septal bile ducts. Occasional organized thrombi were seen in portal vein branches after 15 days obstruction. All rats whose bile ducts had been cannulated or ligated for more than 30 days had cirrhosis and ascites.

Discussion

The morphological changes observed in rats with bile duct obstruction and cannulation were comparable with those seen in rats with bile duct ligation alone throughout this study. After 5 days obstruction both groups showed the classical portal changes characteristic of obstruction, namely neutrophil leucocytic infiltration and bile duct proliferation (Poulsen & Christoffersen 1970). There was minimal cholangitis as judged by the presence of scanty neutrophil leucocytes within the lumens of bile ducts, and this probably reflects a low incidence of infection (Johnstone & Lee 1976). From 10 days after obstruction both cholestatic groups frequently exhibited the so-called marginal bile duct proliferation which is one of the most characteristic signs of extrahepatic biliary obstruction in human liver (Poulsen & Christoffersen 1970).

Biliary obstruction for 15 days or more led to cirrhosis, and after 4 weeks this was found in the majority of the animals. It was associated with features of extra-hepatic cholestasis and with the narrow zone of oedema and ductular proliferation at the junction of parenchyma and septa which is characteristic of biliary cirrhosis in man. Secondary biliary cirrhosis was reported previously in rats subjected to bile duct ligation for 3–5 weeks (Cameron & Hasan 1958) as well as in two rats with biliary ligation for 28 days followed by bilioduodenal anastomosis (Franco *et al.* 1979). In the latter study, however, a separate group of 28-day ligated animals did not develop cirrhosis. Other authors also were unable to find evidence of secondary biliary cirrhosis after 14 to 40 days in bile duct ligated rats (Cartter 1966; Johnstone & Lee 1976; MacDonald & Pechet 1961; Moritz & Snodgrass 1972).

In the present study, the cholestatic rats progressively developed decompensated cirrhosis which was characterized by the formation of ascites, as reported previously by Cameron & Hasan (1958). Interestingly, spontaneous recovery from jaundice was observed in one rat which was cannulated for 42 days; similar recoveries were made in three other animals not included in this study. In agreement with Owen (1975) we could find no evidence of recanalization of the bile duct, and the rats developed cirrhosis.

Cirrhosis is the third largest cause of death for American men between 35 and 54 years (Galambos 1979), and there is a need for a reliable experimental model for studying human cirrhosis. The most commonly used method of producing experimental cirrhosis is with multiple doses of the hepatotoxin CCl_4 given by subcutaneous, intramuscular or intraperitoneal injections, by nebulizer in a closed chamber, or more recently through a gastric tube (Proctor & Chatamra 1982, 1983). Many questions arise, however, relating to both the morphological and biochemical characteristics of this model (Tamayo 1983) which throw doubt on the adequacy of the model to simulate the human disease. Furthermore, there are two major obstacles to the production of a consistent and predictable yield of cirrhosis with this model: First, the individual response of animals to CCl_4 is variable, i.e. some show minimal or no fibrosis, some have a few thin connective-tissue septa, while others have fully developed cirrhosis; second, mortality during the first weeks of treatment is 30–60% and varies widely depending on the source of the animals used (Tamayo 1983). In addition, this method takes a relatively long time, 10–12 weeks, to produce cirrhosis (Miura *et al.* 1982). Moreover CCl_4 intoxication is now extremely rare in man (Zimmerman & Maddrey 1982; Tamayo 1983), and this toxin is seldom implicated in human cirrhosis.

Our results show that both cannula-

tion/obstruction and ligation induce a high yield of cirrhosis in rats obstructed for 1 month or longer. The morphological changes are comparable to those in human biliary cirrhosis. The techniques are easy to perform, and most animals survived in good health for many weeks. The initial increase in plasma bilirubin was followed by a decrease, as found by other investigators (Cartter 1966; Haber & Rees 1963). The duct obstruction was associated with portal hypertension indicated by splenomegaly, as reported previously by Proctor & Chatamra (1982), and with decompensated cirrhosis indicated by the development of ascites. This obstructive model therefore appears to have many advantages over the CCl_4 model and may prove to be a useful tool for studying human cirrhosis. An additional advantage of the cannulation method is that it permits bile secretion to be studied at various stages during the development of cirrhosis.

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