BILIARY OBSTRUCTION and WOUND HEALING, INFECTION, RENAL FUNCTION and BLOODCOAGULATION

An experimental study in rats

PROEFSCHRIFT

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To Joke, Jolien and Jan Jaap
To my parents.

PREFACE

Surgery in patients with obstructive jaundice is associated with significant morbidity and mortality, which has been attributed to the poor general condition of these patients. Apart from liver failure the main postoperative problems observed in patients with obstructive jaundice are: increased infection rate, renal failure and disturbances in wound healing and blood coagulation. The pathophysiology of these dysfunctions in relation to obstructive jaundice is still obscure.

The surgical approach in obstructive jaundice has been controversial for many years. Should primary operation be carried out in the jaundiced patient, or should biliary drainage be performed before the definitive operation? Proponents of biliary drainage claim improvement of the general condition of the patient and decreased postoperative morbidity and mortality. However, acceptable low operative mortality is possible without preoperative biliary drainage, as has been reported by others. The results of prospective clinical studies on the effects of preoperative biliary drainage using transhepatic drainage, do not show any beneficial effects of drainage on postoperative mortality or morbidity. Both surgical and non-surgical biliary drainage (percutaneous transhepatic biliary drainage or insertion of endoprosthesis at the time of ERCP) carry considerable risk of septic or other complications.

Investigations on liver histology and liver functions in patients and experimental animals with obstructive jaundice have been presented in the literature. Relief of the biliary obstruction is accompanied by a rapid, but not always complete, recovery of changes of liver histology and liver functions. Results of clinical and experimental studies on the effects of obstructive jaundice on wound healing, infection, renal function and blood coagulation are conflicting. The evaluation of these effects is complicated by the presence of other factors that accompany obstructive jaundice, such as anorexia with weight loss, and cancer.

Studies on the effects of biliary decompression on wound healing, infection and blood coagulation have not been reported in patients or experimental animals. Therefore, we do not know whether biliary drainage can restore these functions to a safe level before the definitive operation in the same time as is, for instance, required to lower the serum bilirubin to normal levels.

The results of a retrospective study in 67 patients after pancreatoduodenectomy or total pancreatectomy in our surgical department neither confirmed nor refuted the claims by other authors that preoperative biliary drainage contributes to a decrease in major postoperative complications.

Before biliary drainage can be accepted as a routine preoperative measure

to reduce operative risk, studies in experimental animals on the effects of biliary obstruction and subsequent decompression are necessary.

The aim of the present study is to investigate the effects of biliary obstruction on wound healing, infection, renal fuction and blood coagulation in a controlled study in rats. The effects of biliary tract decompression on the same parameters were also studied by relieving the surgical obstruction in order to come to certain propositions on the use of preoperative biliary drainage in patients with obstructive jaundice.

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PART A
REVIEW OF THE LITERATURE

CHAPTER 1
GENERAL INTRODUCTION

Later other surgeons, who normally performed pancreatoduodenectomy in one stage, suggested that it is possible that some of the patients who died after this operation would have had a better chance of survival if a preliminary decompression of the biliary tract had been accomplished (Warren et al 1962). The two stage operation is advised in jaundiced patients with cancer of the head or body of the pancreas by Ross (1959). This author stated that simple exterior drainage of the gallbladder will rehabilitate the patient without disarranging the anatomy. In another retrospective study, postoperative mortality was found to be higher in 196 patients who underwent an one stage pancreatoduodenal resection than in 30 patients after a two stage procedure. None of the patients had hepatic or renal insufficiency after the two stage operation (Monge et al 1964).
After analyzing the correlation between jaundice and operative mortality in both benign and malignant diseases of the biliary tract, a two stage operation was also recommended by Maki et al (1966) and Sato et al (1968). In malignant biliary diseases operative mortality was significantly higher in jaundiced patients than in non-jaundiced patients. The mortality increased proportionally with the increase of preoperative serum bilirubin. In patients with benign disease the overal mortality was much lower than in patients with a malignant tumor, but the mortality was higher in jaundiced patients. However, a higher mortality was also reported by the same authors for the two stage operation (9.3%) than for the one stage operation (4.3%). According to these authors this was possibly due to the fact that the highly jaundiced patients who underwent the two stage operation were in a significantly worse condition (Sato et al 1977).
Whipple used a drainage procedure involving a cholecystogastrostomy to the anterior surface of the stomach, well away from the pylorus. In order to avoid subsequent stenosis and cholangitis he made an anastomotic opening of at least 2 cm in diameter (Whipple et al 1935). Other surgical drainage procedures that have been used are cholecystocutaneostomy (Ross 1959, Maki et al 1966) and choledochostomy with the use of a T-tube (Wise et al 1976).

More recently percutaneous transhepatic biliary decompression and biliary decompression by endoscopic sphincterotomy and insertion of endoprostheses at the time of endoscopic retrograde cholangio-pancreatography have been used. These two procedures have the great advantage that laparotomy is unnecessary, and hence disturbance of the anatomy can be avoided.

Percutaneous drainage of the obstructed biliary tract was first used in 1962 in patients with longstanding obstructive jaundice. In these patients a neoplastic obstruction was diagnosed by the cholangiogram and most patients were operated on within a few hours of the procedure, but in one of the 30 patients a temporary drainage procedure was carried out for 5 days (Glenn et al 1962). The first series of patients successfully treated by non-surgical biliary decompression was not reported until 1974 (Molnar and Stockum 1974). Since then many reports on this procedure have been published.

In a large retrospective study (Nakayama et al 1978) operative death rate was compared in a group of patients who had undergone percutaneous transhepatic drainage, as the first step in the treatment of malignant biliary obstruction, with another group of patients without preoperative biliary drainage: 69 patients underwent operation 8 to 70 days after successful decompression, when the serum bilirubin level was reduced below 85 μmol/l, and 148 patients underwent operation without prior biliary tract decompression. The operative mortality was 28.3% without and 8.2% with biliary drainage ($p < 0.05$). In another retrospective study (Denning et al 1981) postoperative complications occurred in 7 out of 25 patients who underwent preoperative biliary decompression, 4 were fatal. Whereas in 32 patients without preoperative biliary decompression 18 patients had complications, including 8 fatalities. Sepsis, haemorrhage, renal failure and pulmonary complications were the major postoperative complications. The difference in postoperative complications between the two groups was statistically significant: 28% in the group with and 56% in the group without preoperative biliary drainage ($p < 0.05$). However, no difference in operative mortality was seen between these two groups (16% versus 25%). Norlander et al (1982) also studied the effect of percutaneous transhepatic drainage upon postoperative mortality following extensive biliary surgery. Fifty-eight patients were treated by percutaneous transhepatic drainage and 65 patients were operated on after diagnostic transhepatic cholangiography without drainage. The mortality in the drainage group was 14% and 21% in the non-drainage group. This difference is not statistically significant.

All these studies above were retrospective studies. The first prospective controlled clinical trial on the effect of preoperative biliary drainage was performed by Hatfield et al (1982). Twenty-nine patients had preoperative biliary drainage and 28 patients had no drainage before surgery. Postoperative complications were equal in number in both groups. Operative mortality was also not different in the two groups, 4 patients dying in both groups.

Minor complications without fatalities have been reported by Glenn et al (1962), Molnar and Stockum (1974), Okuda et al (1974), Nakayama et al (1978), and Dooley et al (1979). In recent studies, however, serious complications after percutaneous transhepatic drainage have been described. The complications in these studies are summarized in table 2.

Table 2 Serious complications of PTD.

Authors	No. of patients	Complications	n	mortality related to procedure N	%
Tylen et al. 1977	146	Haemorrhage	4	1	0.7
		Bile leakage	2		
		Infection	1		
Mori et al. 1977	13	Haemorrhage	2	1	7.7
Hansson et al. 1979	105	Haemorrhage	2	2	1.9
		Bile leakage	3		
		Infection	9		
Clark et al. 1981	42	Haemorrhage	1	1	2.4
		Infection	2		
		Pneumothorax	1		
Berquist et al. 1981	50	Haemorrhage	4	1	2
		Infection	4		
		Haematothorax	1		
Mueller et al. 1982	188	Haemorrhage	6	3	1.6
		Infection	7		
		Pneumothorax	1		
McPherson et al. 1982	37	Bile leakage	3	2	5.4
		Infection	12		
		Perforation bowel	1		
Gouma et al 1983	114	Infection	9	3	2.6
		Perforation bowel	1		
		Pleural effusion	1		

The most frequent complication was infection. Almost all patients with choledocholithiasis and benign biliary strictures have an infected bile, but only one third of patients with malignant bile duct obstruction have positive bile cultures (Keighley 1982). After PTD the incidence of infected bile is found to be 88% (Cox et al 1978). This suggests that in many patients with malignant biliary obstruction the bile will become infected after PTD. Bleeding after multiple punctures in the liver was the second most frequent complication after PTD which sometimes required an emergency laparotomy. Other serious complications were duodenal perforation, biliary pleural effusion and pneumothorax. Fatal complications in these series occurred in 0.7 to 7.7% of patients.
From these reports the conclusion can be drawn that PTD does carry significant hazards. This is confirmed by Zilly et al (1980) who stated that optimistic reports regarding percutaneous cholangiography, even when the skinny Chiba needle is used, should be regarded with a degree of reserve.
Futhermore, extensive biliary or pancreatic surgery can be hazardeous or even impossible following haemorrhage or septic complications caused by preoperative biliary drainage.

1.2.3 Decompression with endoscopically introduced endoprostheses

Another non-surgical method of biliary drainage, in which the biliary drainage is performed at the time of endoscopic retrograde cholangiopancreatography (ERCP), has been reported more recently. ERCP was first described by McCune et al (1968). The technical success rate is about 90%. Complications being described in 2 - 3% and mortality in 0.1 - 0.2% of cases. The main cause of this mortality is septicaemia (Zimmon et al 1975, Cotton 1977).
Preoperative endoscopic sphincterotomy can be performed in patients with ampullary tumors without jeopardizing the surgical resection (Alderson et al 1981). A new endoscopic method of introducing a transpapillary drain after papillotomy was described by Soehandra and Reynders-Frederix (1980). The drain being fixed in the bile duct at one end. The other end hanging freely in the duodenum.
In a study by Riemann et al (1981), 4 patients were treated with a so-called pigtail catheter in the biliary tract and 7 patients with a nasobiliary drain. These endoscopic procedures were carried out to accomplish permanent drainage in inoperable tumors or as a temporary preoperative measure. Complications after these procedures were rarely observed.

Duodenoscopic insertion of biliary prostheses for the relief of malignant obstructive jaundice was also attempted in a series of 23 patients. (Cotton 1982). The procedure was unsuccessful in 5 patients. Five patients developed septic cholangitis within 2 weeks of the procedure, all of whom died of septicaemia.
In another study a large calibre endoprosthesis was used in 45 patients. In only one patient was it used preoperatively. Complications of cholangitis and fever ware seen in 11 of these patients (Huibregtse and Tytgat 1982). Preoperative biliary drainage by endoscopically introduced endoprostheses after sphincterotomy seems very promising. However the complications, especially cholangitis with septicaemia, also carry significant risks.

1.3 Effects of biliary obstruction on the liver, wound healing, infection, renal function and blood coagulation in patients and experimental animals

1.3.1 Liver

1.3.1.1. Histopathology

In the tissue sections of livers from patients with acute biliary obstruction there is dilatation of the centrilobular bile canaliculus, and bile thrombi are found within the lumen. Bile pigment granules may also be seen in the adjacent hepatocytes and in the Kupffer cells. Deposition of collagen fibers in the periductular region is followed by fibrosis and scarring around the bile ducts and ductules. Destruction of the layer of hepatocytes around the portal tract is seen, with associated leucocyte inflammation (Benjamin 1982).
Obstruction of the common bile duct in experimental animals (rats, dogs) is followed by focal hepatocyte necrosis, progressive hyperplasia of the bile ducts, atrophy of liver cells and slight interlobular fibrosis (Trams and Symeonidis 1957, Cameron and Hasan 1958, Aronson 1961). The extent of the histopathological changes varies with the period of obstruction and with the species of animals concerned.

1.3.1.2 Bilirubin

Bilirubin is a waste product of haemoglobin. Bilirubin is conjugated in the liver and excreted in the bile. The normal daily excretion is approximately 300 mg. The majority of conjugated bilirubin is excreted in the faeces after conversion to stercobilin and other degradation products. Initially, bilirubin is mainly converted to urobilinogen in the intestine by bacterial action. Urobilinogen and bilirubin are partially absorbed from the gut and returned to the liver for re-excretion. This process is the so-called "enterohepatic circulation".
Urobilinogen entering the systemic circulation is excreted in the urine. (Kune and Sali 1980).
Jaundice is the classical physical sign in biliary tract obstruction. The conjugated bilirubin level may be elevated only marginally in incomplete and intermittent obstruction.
In experimental animals a progressive rise in total serum bilirubin levels occurs after biliary obstruction. Serum bilirubin reached maximum levels in 3 days in rats (Birns et al 1962) and in 1 or 2 weeks in dogs (Koyama et al 1981) and persists at a constant level thereafter if complete obstruction is maintained.

1.3.1.3 Alkaline phosphatase

Alkaline phosphatase is the most frequently used biochemical test of bile duct patency. The highest levels of serum alkaline phosphatase of hepatic origin are found in patients with complete biliary obstruction. Minor or intermittent degrees of obstruction, however, also produce significant elevation of alkaline phosphatase (Benjamin 1982).
In experimental animals following complete biliary obstruction, a rapid increase of activity is seen. Maximum values are reached after varying lengths of time depending on the animal species. These values then remain at a constant level for some time. In more prolonged biliary stasis, the alkaline phosphatase activity level gradually falls, but never to normal levels (Aronson 1961).

1.3.1.4 Other liver enzymes

Gross elevation of the amino-transferases indicates hepatocellular damage in patients, and as an isolated biochemical finding may cast doubt upon a diagnosis of extrahepatic obstruction. Minor elevation of amino-transferase activity is common, however, in extrahepatic obstruction, and indeed the amino-transferase activity rises earlier than that of alkaline phosphatase in cases of acute obstruction. Levels fall to normal, or nearly normal, within several days, despite deepening jaundice. Gamma glutamyl transpeptidase tends to parallel alkaline phosphatase (Benjamin 1982).

1.3.1.5 Other biochemical parameters

Serum protein abnormalities are often seen in patients with liver disease. Depression of serum albumin is a common feature in patients with malignant obstruction of the biliary tract, particularly when there has been prolonged nutritional impairment or infection. Elevations of the globulins, particularly the IgG fraction, may be seen in patients with severe parenchymal liver disease due to long-standing obstruction (Benjamin 1982).

1.3.2 Wound healing

It is generally accepted that jaundice has a negative effect on wound healing. Jaundice results in poor accumulation of collagen for reasons that are still obscure (Hunt 1979). However, in some retrospective studies of abdominal wound healing no convincing evidence was found to substantiate the concept that jaundice is involved in the pathogenesis of abdominal wound dehiscence (Reitamo and Möller 1972, Keill et al 1973). However others found that, wound dehiscence or incisional hernia occurred more frequently in jaundiced than in non-jaundiced patients (Ellis and Heddle 1977, Irvin et al 1978, Armstrong et al 1984). Furthermore, it has been shown that prolyl hydroxylase activity, which reflects the rate of collagen synthesis, is decreased in the skin of patients with obstructive jaundice (Than Than et al 1974, 1977).
The effect of biliary obstruction on wound healing has been studied in experimental rats after ligation and division of the common bile duct (Bayer and Ellis 1976, Arnaud et al 1981). Bursting strenght of abdominal incisions

was lowered in these animals as compared with sham operated controls. Standard gastric incisions showed histological evidence of delayed healing, but the bursting strength was not lowered. However, no differences in mechanical strength of abdominal wounds or skin wounds were found between jaundiced and control rats in other studies (Greaney et al 1979, Than Than et al 1979).
A delay of migration of reticulo-endothelial cells and fibroblasts was found in bile duct obstructed rats (Lee 1972). Delayed accumulation of collagen in wounds of jaundiced rats was found (Greaney et al 1979) and also a significant decrease of the prolyl hydroxylase activity (Than Than et al 1977).

1.3.3 Infection and immune competence

Bacteriologic studies of the bile at the time of operation on patients with obstructive jaundice have revealed the presence of a variety of micro-organisms (Nielsen and Justesen 1976, Farnell et al 1981). Postoperative complications resulting from infection occur frequently (Pitt et al 1981) and are significantly associated with mortality (Dixon et al 1983).
Whether patients with obstructive jaundice have impaired immunity or not is not clear. The function of the reticulo-endothelial system seems to be impaired (Drivas et al 1976). In vitro the proliferative response of T and B lymphocytes to mitogens was reduced when bile acids are added (Gianni et al 1980), but the concentrations used in this study were higher than those found in patients with obstructive jaundice. Unconjugated bilirubin appeared to have an inhibitory effect on cellular immune response in man (Rola et al 1975). However, the enzymes associated with antibacterial activity were increased in leucocytes from patients with obstructive jaundice (Wardle and Williams 1980).
The only defensive mechanism against micro-organisms that has been studied in relation to obstructive jaundice in experimental animals is the function of the reticulo-endothelial system, which plays an important role in removing micro-organisms from the blood. This function is impaired in obstructive jaundice. Circulating bacteria and endotoxin remain in the circulation longer (Wardle and Wright 1970, Holman and Rikkers 1982).
However, an increased activity of the reticulo--endothelial system in jaundiced rats has also been reported (Halpern et al 1957, Lázár 1972, Arii et al 1983).

1.3.4 Renal function

Acute renal failure occurred more frequently following operation on patients who were severely jaundiced in studies by Dawson (1965) and Dixon et al (1983). However, an other study reported no significant correlations between the level of serum bilirubin and renal impairment (Bailey 1976). The cause of a higher incidence of renal failure in patients with obstructive jaundice remains uncertain. Possible influences are: the direct toxic effects of bilirubin (Dawson 1964, Baum et al 1969) and bile acids (Aoyagi and Lowenstein 1968), the changes in haemodynamics (Williams et al 1960, Dawson 1968) and the effects of endotoxin (Wilkinson et al 1976).
The pathophysiology of renal function following biliary obstruction has been studied in many experimental models. Biliary tract obstruction caused a decrease in glomerular filtration rate (Yarger 1976) and renal plasma flow in rats (Yarger 1976, Better 1980). However, an increased renal plasma flow has also been reported in rats (Allison et al 1978).
Bilirubin (Baum et al 1969) and bile acids (Aoyagi and Lowenstein 1968) "sensitised" renal tubular cells to ischaemia in jaundiced rats. Reduction in renal blood flow, caused by bleeding was more marked in jaundiced rabbits than in controls (Hishida et al 1980). Impaired renal function due to the presence of endotoxin in jaundiced animals has been reported (Hinshaw and Bradley 1957, Hinshaw et al 1961, Gillenwater et al 1963).

1.3.5 Blood coagulation

Haemorrhage, either from gastrointestinal or from intra-abdominal wounds, is one of the postoperative hazards in patients with obstructive jaundice leading to a high mortality (Gilsdorf and Spanos 1973, Braasch and Gray 1977, Pitt et al 1981). After correction of prolonged prothrombin time with vitamin K other coagulation defects are usually not detected by the commonly used coagulation tests. Despite this, excessive haemorrhage does occur at the time of surgery (Dixon et al 1984).
Few data on the origin of the disturbance of blood coagulation in cholestatic jaundice are available. Abnormal thrombotests and thrombin and reptilase times were found, suggesting an inhibition of the clotting mechanism independent of vitamin K deficiency (Dioguardi et al 1973). Episodes of haemorrhage in jaundiced patients have been found which were associated with endotoxaemia (Hunt et al 1982).
In cats with experimentally induced obstructive jaundice, the clotting of

normal plasma with thrombin was inhibited. Bilirubin glucuronides in concentrations above 8 mg % in vitro showed antithrombic activity, but other bile constituents were not active (Kopeć et al 1961).

1.4 Effects of biliary tract decompression on the liver, wound healing, infection, renal function and blood coagulation in patients and experimental animals with biliary obstruction

1.4.1 Liver

1.4.1.1 Histopathology

The changes in liver biopsy specimens in patients with obstructive jaundice disappear after removal of the obstruction. Needle biopsies of the liver taken 6 weeks after operation show regeneration of the liver. Apart from minimal portal fibrosis, the hepatic stroma is found to be normal (Blumgart 1978).
The changes in histology of the liver after relief of the biliary obstruction have been studied in rats and dogs. All histopathological changes in the liver after cholestasis, with proliferation of the bile ducts and fibrosis, recovered in the reverse order in which they occurred, after recanalisation of the common bile duct or after relief of the biliary obstruction (Trams and Symeonidis 1957, Koyama et al 1981).

1.4.1.2 Bilirubin

The level of serum bilirubin after biliary drainage in patients with biliary obstruction has been studied in detail. The fall in serum bilirubin level does not only correlate with recovery of liver function, but also with improvement of the general condition of the patient.
The serum bilirubin level before the drainage procedure is very variable, ranging form 26 to 713 μmol/l, with an average of 349 μmol/l in a study of 104 patients by Nakayama et al (1978). In another study in 25 patients with extrahepatic bile duct obstruction without evidence of liver disease, the mean level of serum bilirubin was 291 μmol/l (Hansson et al 1979).

The time required for normalization of the serum bilirubin after the various drainage procedures ranges from about two weeks to one month (Wiechel 1964, Hansson et al 1979, Riemann et al 1981, Huibregtse and Tytgat 1982). The return to normal bilirubin level is slower during the later phase of drainage, probably because bilirubin is then excreted mainly in the bile, and to a much lesser degree via the urine (Wiechel 1964). Serum bilirubin does not reach normal values in all drained patients (Maki et al 1966, Mori et al 1977, Alderson et al 1981, Denning et al 1981, McPherson et al 1982).
No correlation was found between the time required for normalization of the serum bilirubin level and duration of the biliary obstruction (Wiechel 1964). Patients with high initial bilirubin levels reach approximately the same level after two weeks of drainage as do patients with low initial bilirubin levels. Thus, the degree of jaundice at the start of percutaneous transhepatic drainage is possibly of minor importance for the reduction rate of serum bilirubin (Hansson et al 1979).
In contrast, Nakayama et al (1978) reported that patients who had been jaundiced for more than two months before receiving percutaneous transhepatic drainage, responded rather poorly in terms of reduction of serum bilirubin following drainage. The speed of reduction of serum bilirubin was not different in patients treated with percutaneous transhepatic drainage or with cholecystostomy (Nakayama et al 1978). In patients who had bile refeeding after percutaneous transhepatic drainage, the serum concentration of bilirubin was much lower than in patients without bile refeeding, three weeks after biliary drainage (Norlander et al 1982). This more rapid reduction of the jaundice has not been reported in patients with internal drainage using endoscopically introduced endoprostheses.
The elevated serum bilirubin level in rats with bile duct obstruction returned to normal within 3 days after removal of the obstruction (Birns et al 1962). In dogs the serum bilirubin concentration returned to normal at a rate depending on the duration of the obstruction (Aronson 1961, Koyama et al 1981).

1.4.1.3 Alkaline phosphatase

Alkaline phosphatase activity in the blood is always increased during biliary obstruction. After removal of the obstruction the fall of alkaline phosphatase levels is not gradual. For about 5 days enzyme activity declines rapidly, this is generally followed by an increase in which new maximum levels are reached after circa two weeks, followed by a continuous slow return to normal levels within a few weeks (Wiechel 1964). Alkaline phosphatase

decreased significantly during the first two weeks of transhepatic biliary drainage, but not thereafter in a study by Norlander et al (1982). Following endoscopic sphincterotomy in 5 patients elevated alkaline phophatase levels did not return to normal in the 11 to 36 days of decompression (Alderson et al 1981). In Riemanns study 17 patients were successfully drained preoperatively, with a "dramatic" decrease in levels of alkaline phosphatase (Riemann et al 1981).
In rats alkaline phosphatase returned to normal after release of the biliary obstruction (Birns et al 1962). Also in dogs alkaline phosphatase decreased rapidly after release of the biliary obstruction, irrespective of the duration of the obstruction (Aronson 1961, Koyama et al 1981).

1.4.1.4 Other liver enzymes

Increased SGOT and SGPT levels returned to normal in about half of patients following cholecystostomy (Maki et al 1966). The highest values of the transaminases, SGOT and SGPT, are found in the acute phase of the illness regardless of the nature of the disorder, whereas lower values have been observed in the chronic stages of the disease. Transaminase activity in serum usually began to return to normal after removal of the obstruction (Wiechel 1964). In Norlanders study gamma glytamyl transferase (γ GT) decreased after PTD during the first week, but not thereafter and did not return to normal levels (Norlander et al 1982).
After release of biliary stasis in dogs SGOT and SGPT activity returned to normal. The length of time in which the activity returned to normal depended on the values of SGPT and SGOT at the time of release of the obstruction and on the duration of the obstruction (Koyama et al 1981). Also 5-nucleotidase and adenosine triphosphatase values returned to normal after release of the biliary obstruction in rats (Birns et al 1962). However, albumin-globulin ratio and serum gamma globulin (Trams and Symeonides 1957), hepatic mitochondrial respiratory function and ketogenesis (Koyama et al 1981) were still abnormal after release of the biliary obstruction. This probably indicates that liver recovery is not complete.

1.4.2 Wound healing, infection and immune competence, renal function and blood coagulation

No reports on the effects of biliary decompression on wound healing in pa-

tients with obstructive jaundice have been found in the available literature. Sepsis, haemorrhage, renal failure and pulmonary complications were the major postoperative complications in a retrospective study by Denning et al (1981).
These complications occurred in 7 out of 25 patients that underwent preoperative biliary decompression and in 18 out of 25 patients without preoperative biliary decompression ($p < 0.05$). In a prospective controlled clinical trial, postoperative complications were equal in number in patients with or without preoperative biliary drainage (Hatfield et al 1982).
Also no reports are available in the literature considering the effects of biliary decompression on disturbances in wound healing, immune competence or blood coagulation in experimental animals. After relief of obstructive jaundice, following choledochoduodenostomy, in rats plasma urea concentration, renal plasma flow and filtration fraction were not significantly different from control values, as was the case in jaundiced rats (Allison et al 1978).

1.5. Conclusions after review of the literature

1. The necessity for preoperative biliary drainage before major surgery in patients with obstructive jaundice is still debatable.

2. Complications of non-surgical biliary drainage, both percutaneous transhepatic drainage and endoscopic biliary drainage are in general underestimated. These procedures do carry significant hazards, including mortality.

3. Histopathological changes in the liver caused by biliary obstruction are largely reversible in patients and experimental animals following relief of the biliary obstruction. Restoration of liver functions is not always complete.

4. Elevated serum bilirubin levels decrease after biliary drainage in patients and experimental animals, regardless of the duration of obstruction, but not always to normal levels.

5. The effects of biliary obstruction and biliary tract decompression on wound healing, infection, renal function and blood coagulation need to be studied, as disturbances of these functions occur frequently in patients following biliary surgery.

PART B
EXPERIMENTAL STUDY IN RATS

CHAPTER 2
BILIARY OBSTRUCTION AND WOUND HEALING

that the healing of a standard gastric incision and a parietal peritoneal defect was delayed when compared with controls, using histological criteria. The bursting strength of the abdominal incision was also lowered, but not that of the stomach wound. However, angiography revealed delay in angiogenesis in the gastric wounds of jaundiced animals. This study was carried out using animals who were killed on the second, fourth, sixth and eighth day, following operation. These results were confirmed in a similar study by Arnaud et al (1981). In another study, jaundice was induced in rats 2 weeks before a second operation at which standard abdominal incisions were made. The wounds in jaundiced and control animals showed no significant differences in mechanical strength during a 21-day period of study, but there was a significant delay in accumulation of collagen in wounds of jaundiced animals. It was suggested that the biochemical changes in the wounds of jaundiced animals do not interfere with wound repair and these findings cast doubt on the hypothesis that jaundice has an adverse effect on wound healing (Greaney et al 1979).

Rupture strength of skin wounds in jaundiced rats and sham operated rats was significantly lower than the rupture strength of skin wounds in normal rats. But, no difference was seen between sham operated and bile duct ligated jaundiced rats (Than Than et al 1979).

The pathophysiology of wound healing in obstructive jaundice was investigated in a study of the migration of reticulo-endothelial cells and fibroblast into early experimental granulomata in rats (Lee 1972). Delayed migration of reticulo-endothelial cells and fibroblasts during the first week of the development of a cotton-wool granuloma was found in the presence of obstructive jaundice.

Prolyl hydroxylase activity, which reflects the rate of collagen synthesis, was decreased in the skin of both patients and rats with obstructive jaundice (Than Than et al 1974, 1977). A significant delay of accumulation of collagen in wounds of jaundiced animals was also found by Greaney et al (1979).

To test the hypothesis that jaundice per se impairs wound healing, the growth of rat fibroblasts in vitro was studied (Taube et al 1981). Addition of bilirubin to the culture media was found to cause morphological changes in the fibroblasts, and impaired the growth of cells even in concentrations that were slightly above normal. Addition of sera of jaundiced patients to the culture media also caused similar changes.

The present experimental study in rats was initiated because reports in the literature are conflicting, as is indicated in the introduction. The aim of the study was firstly, to investigate the effect of biliary obstruction on wound healing in a controlled study in rats and secondly, to see whether decompression of the biliary tract will normalize a possible delay in wound healing.

2.2 Material and methods

2.2.1 Animals, anaesthesia and operative techniques

Animals

Male random bred Wistar rats (TNO Animal breeding facilities, Zeist, The Netherlands), weighing between 240 and 340 grams were used throughout the study. They were housed in groups of 5 or singly, in cages, and were fed ad libitum on commercial chow (Hope Farms) and water (pH $\pm$ 3).

Anaesthesia

Rats were anaesthetized in a plastic box ventilated with ethyl ether in air. After induction of anaesthesia, they were placed in a supine position on an operating board. Anaesthesia was maintained throughout the operative procedure by placing a glass beaker containing cotton-wool soaked in ether over their nostrils.

Production of biliary obstruction and sham operations

All operations were performed with clean, but not sterile instruments. The operations were carried out as follows: the abdomen of the rat was shaved and the skin was desinfected with 0,5% w/v chlorhexidine in 70% v/v ethanol. The abdomen was opened using a standard midline incision of 4.5 cm. Two retractors were placed in the abdomen, holding the liver lobes in the hilar area apart. The duodenum was then delivered through the abdominal incision and the lesser omentum was stretched by gentle traction. The bile duct was then dissected from the omentum and a Haemoclip® (Weck 25's small) was placed on the bile duct.
The use of this clip made it possible to obstruct, or desobstruct whenever necessary. This clip was applied with standard pressure using a special forceps with a tension screw and just distal to the junction of the hepatic ducts.
In a pilot study the use of this clip was investigated. It was possible to maintain a total occlusion of the bile duct for 14 days (fig 1.). Following this period recanalisation of the bile duct occurred in 80% of the animals,

as has been described earlier (Trams and Symeonidis 1957). This recanalisation could be demonstrated by means of cholangiography (fig. 2).
In those rats in which secondary desobstruction was not included in the experiment, the bile duct was divided between two ligatures slightly distal to the junction of the hepatic ducts.
The duodenum was then replaced in the abdominal cavity and the abdominal wall was closed with a one-layer continuous 2-0 silk suture(Ethicon EH 784).
Control groups of sham operated rats underwent the same operative procedure without clipping or ligating the common bile duct.
Relief of the biliary obstruction was achieved by removing the clip in a second operation (fig. 3). After dissecting the common bile duct free the clip was opened and carefully removed with the specially designed "Haemoclip®" clamp.
Control rats underwent a second sham operation, in which the common bile duct was manipulated. Care was taken to standardize the duration of anaesthesia and the degree of operative trauma in both groups.

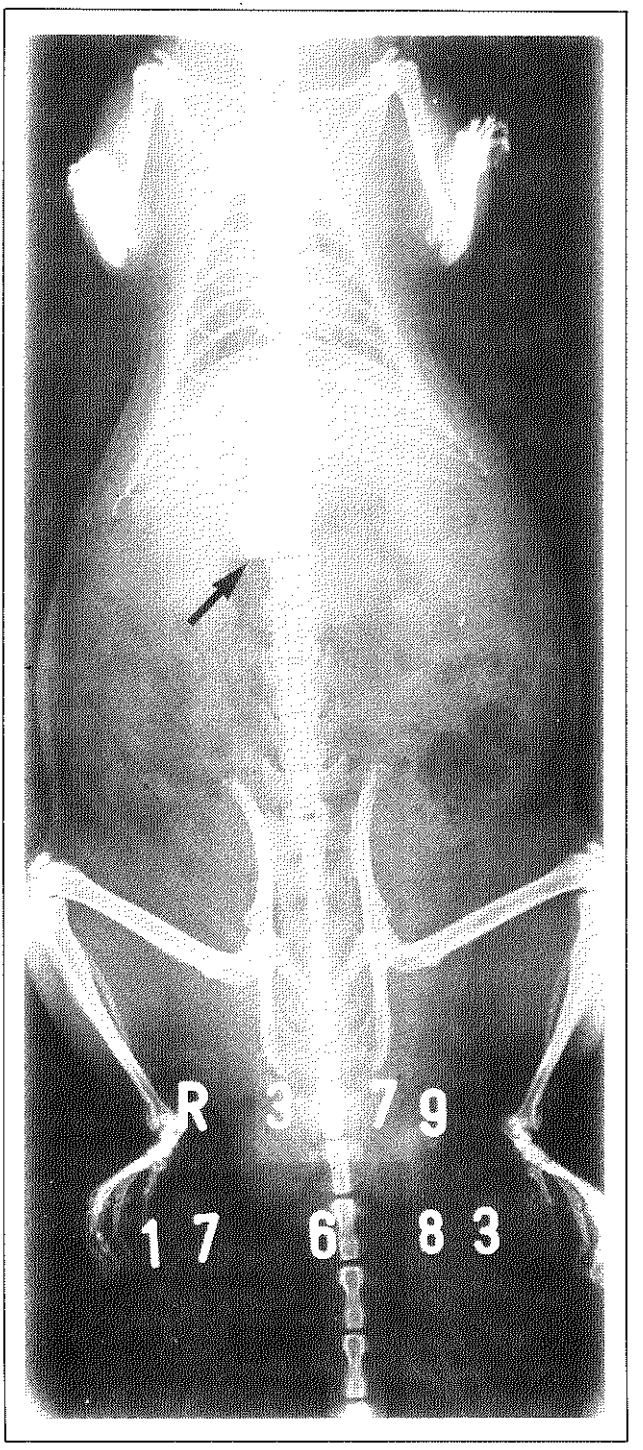


Fig. 1 Cholangiogram, after transhepatic injection of 2 cc barium sulphate, showing total occlusion of the common bile duct 14 days after biliary obstruction using a haemoclip (arrow).

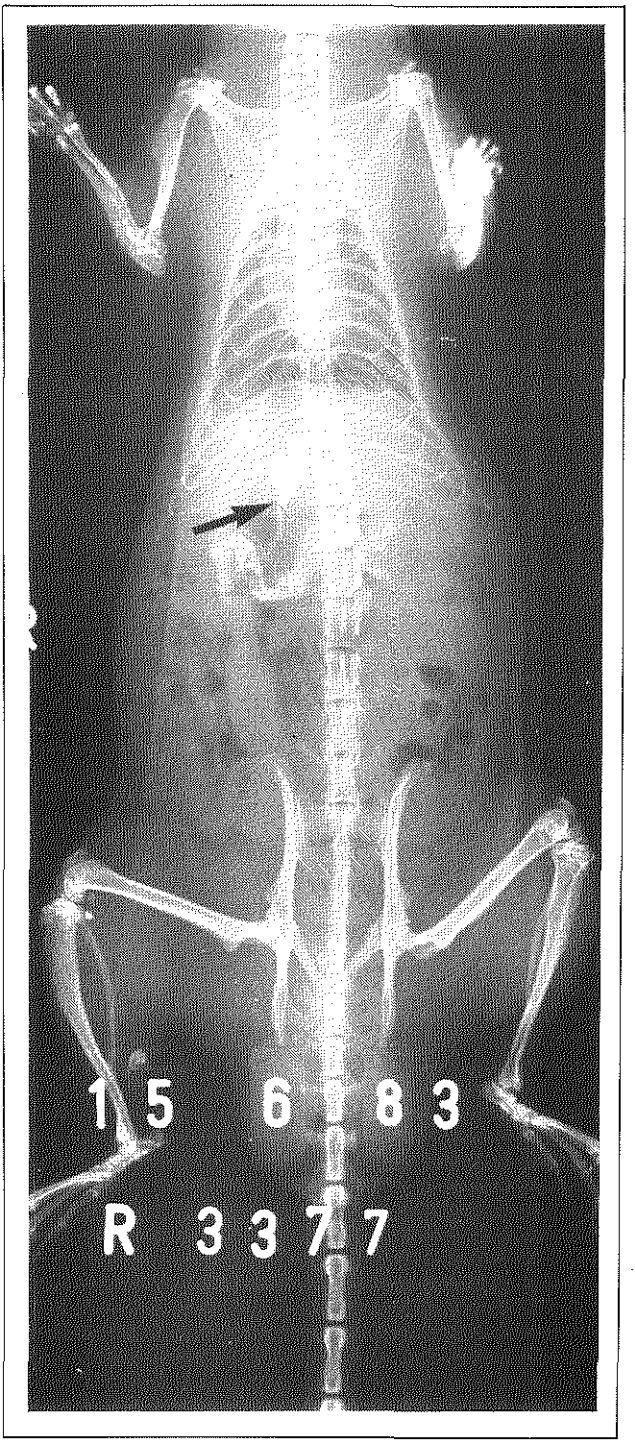


Fig. 2 Cholangiogram, showing recanalisation of the common bile duct 18 days after biliary obstruction without removal of the clip (arrow).

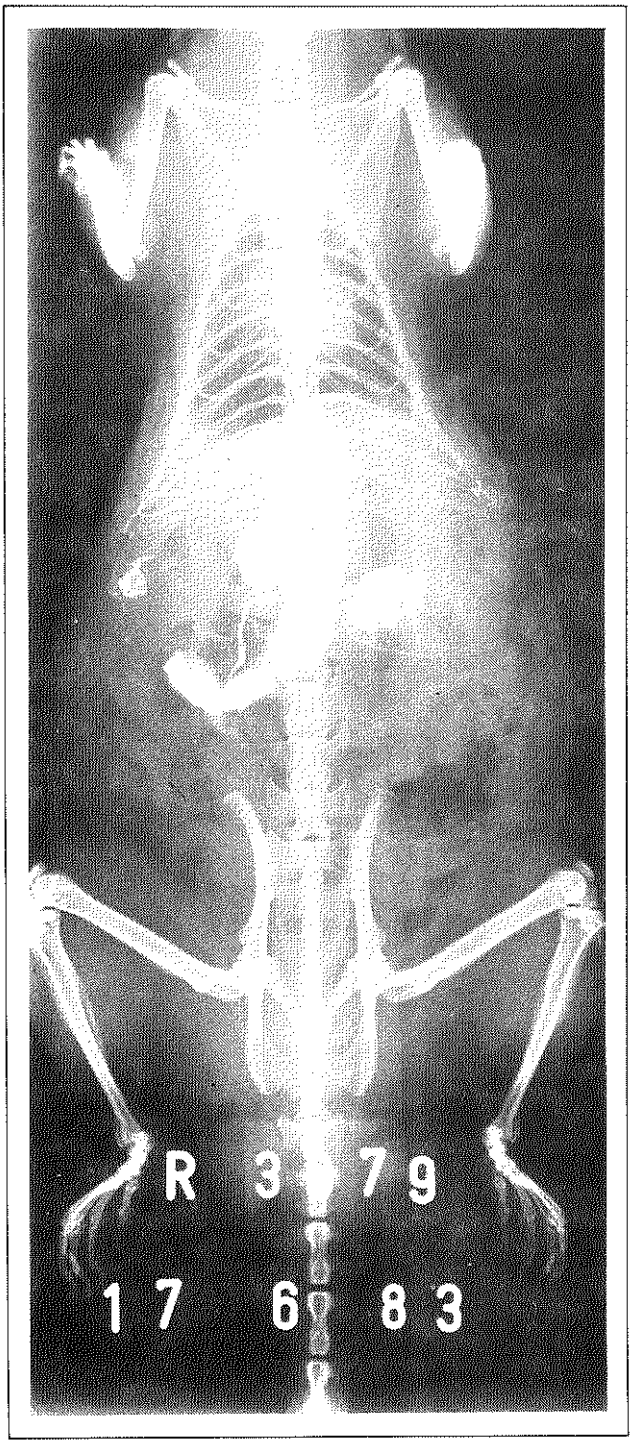


Fig. 3 Cholangiogram, showing patency of the common bile duct without leakage 15 minutes after removal of the clip. The common bile duct was obstructed during 14 days.

Two weeks after ligation or sham ligation of the bile duct or one week after relief or sham relief of two weeks obstruction or sham obstruction, a second or third laparotomy was performed. The duodenum was divided distally at 2 to 3 cm from the entry of the bile duct and reanastomosed in a single layer fashion using a 7-0 silk (Ethicon K803 H) continuous suture. The transverse colon was divided and reanastomosed in a single layer also using a 7-0 continuous suture.
The test wounds in the abdominal wall were made from the inside in the right and left abdomen. A standard incision of 1.5 cm in transverse direction through the muscles and fascia of the abdominal wall was made using a no. 20 scalpel blade. The subcutaneous layer and the skin above were left intact. The test wound was closed with two 4-0 silk sutures (Ethicon EH 782). The midline abdominal incision was closed again in a single layer using continuous 2-0 silk.

2.2.2 Autopsy

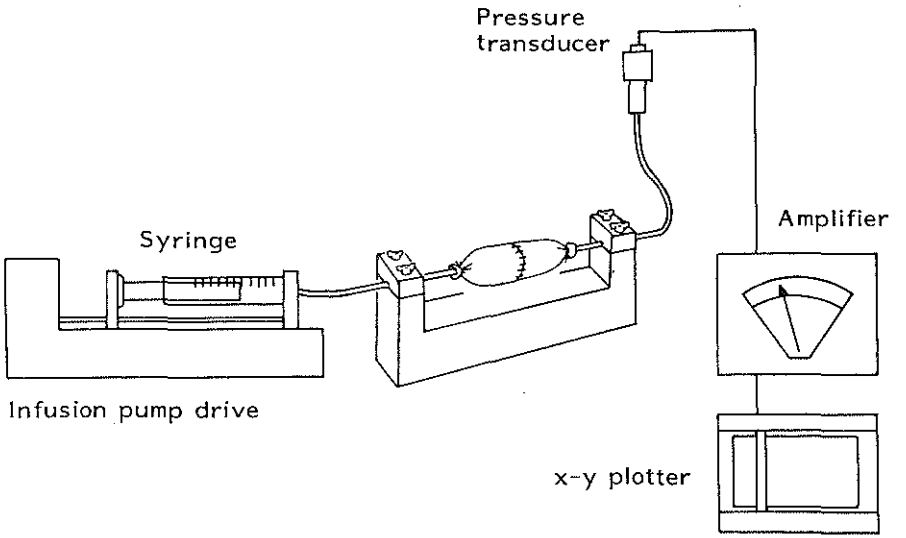
Experimental and sham operated rats were randomly sacrificed at either 3, 7 or 14 days after making the test wounds. The animal was anaesthesized with ether. After weighing and blood sampling the animal was sacrificed by bleeding. The abdomen was opened through the midline incision. The skin of the abdominal wall was carefully dissected from the muscular layer on both sides and the muscular layers with the test wound in the middle were removed from the animal. The specimens were stored for less than 1 hour in saline solution at room temperature before estimating the bursting pressure of abdominal wounds.
The colon was lifted and adhesions were carefully dissected, taking care not to damage the anastomosis. A piece of colon measuring approximately 5 cm long, with the anastomosis in the middle, was taken. The bursting pressure was measured immediately. The duodenal anastomosis was handled in a similar manner.

2.2.3 Measurement of bursting pressure

Duodenal and colonic anastomoses

A special apparatus was constructed in the "Central Research Workshop" of the Erasmus University, Rotterdam to measure the bursting pressure of the duodenal and colonic anastomoses (fig 4.).

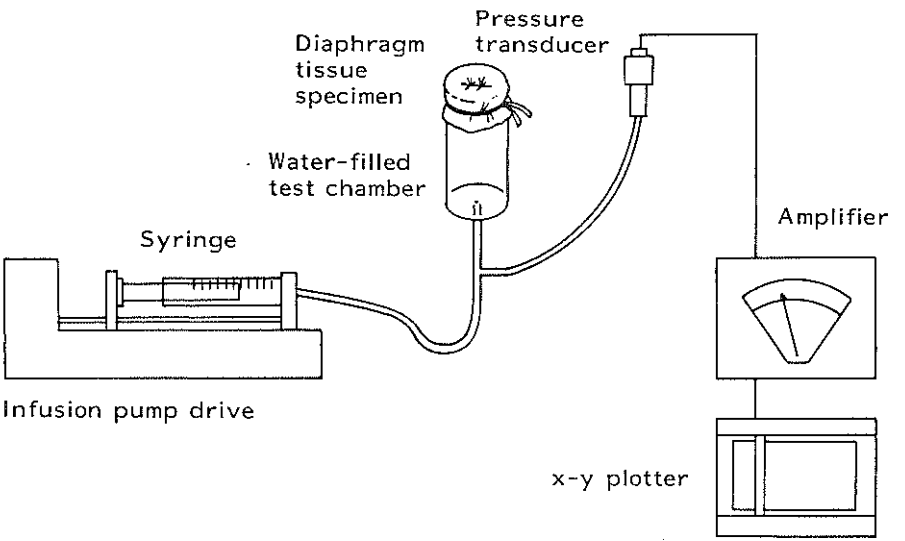
Fig. 4. Diagram of the apparatus for bursting pressure measurements of intestinal anastomoses.



The ± 5 cm long centrally anastomosed section from colon or duodenum was fastened tightly over two hollow tubes. One tube was connected to an infusion pump (B. Braun Melsungen AG) which infused water at a rate of 114 ml/hour. The other tube was connected to a pressure-transducer (Statham® P23 Db) and amplifier (VA 200 Peekel) which was in turn connected to an X-Y plotter (Tekman TE 200) for the purposes of graphic reproduction. Once set up, the whole system was flushed and calibrated before the pump was switch on. During the test the pressure in the system increased until leakage occurred. This provided an accurate pressure-volume curve which could be used in the analysis of the results.

The technique of testing the mechanical strength of abdominal wounds in vitro has been published by Van Noort et al (1978) and Greaney et al (1979). This technique is based on the measurements of mechanical stress occuring at the time of rupture of a diaphragm of abdominal musculature distended by water. In the present study bursting pressure of abdominal wounds was used instead of calculated "membrane rupture stress" (fig. 5).

Fig. 5 Diagram of the apparatus for bursting pressure measurements of abdominal wounds.



The muscular layer with central wound was placed over the test chamber as a diaphragm and fixed with a shoestring. The test chamber had a diameter of 20 cm. The test chamber was filled with water and pressurized with water at a constant rate by an infusion pump (570 ml/hour). This caused the diaphragm of tissue to distent, resulting ultimately in bursting of the tissue. The pressure developed in the test chamber was measured with a pressure-transducer and recorded on an X-Y plotter for permanent record.

2.2.4 *Pair-feeding*

In order to estimate whether the jaundice or the reduced nutritional intake was the reason for poor healing, one group of 20 rats were pair-fed. The control animals were operated on one day after the experimental rats. The animals were housed individually and a control sham operated rat was coupled with a jaundiced rat. The amount of food eaten by the jaundiced rat was weighed and the same amount of food was given to the control rat. Weight curves were obtained by measuring the weights of jaundiced and sham operated rats on each day of the study.

2.2.5 *Biochemistry*

Determinations of levels of serum bilirubin, albumin, alkaline phosphatase activity, glutamate-oxalo-acetate transaminase (SGOT), gamma glutamyl transpeptidase (γGT)were carried out on a computer directed analyser (Gilford System 3500) by standard methods.

2.2.6 *Experimental groups*

Group 1 (n=30, 15 experimental animals, 15 controls)
Two weeks after biliary obstruction or sham operation duodenal-, colonic anastomoses and abdominal wounds were made.
After 3 days the bursting pressures were measured.

Group 2 (n=20, 10 experimental animals, 10 controls)
Two weeks after biliary obstruction or sham operation duodenal-, colonic anastomoses and abdominal wounds were made. After 7 days the bursting pressures were measured.

Group 3 (n=20, 10 experimental animals, 10 controls)
Two weeks after biliary obstruction or sham operation duodenal-, colonic anastomoses and abdominal wounds were made. After 14 days the bursting pressures were measured.

Group 4 (n=30, 15 experimental animals, 15 controls)
Two weeks after biliary obstruction or sham operation, the obstruction was relieved or sham operation was performed. After one week of drainage duodenal anastomoses and abdominal wounds were made. After 3 days the bursting pressures were measured.

Group 5 (n=20, 10 experimental animals, 10 controls)
Two weeks after biliary obstruction or sham operation, the obstruction was relieved or sham operation was performed. After one week of drainage duodenal-, colonic anastomoses and abdominal wounds were made. After 7 days the bursting pressures were measured.

Group 6 (n=41, 21 experimental animals, 20 controls)
Two weeks after biliary obstruction or sham operation, the obstruction was relieved or sham operation was performed. After one week of drainage duodenal-, colonic anastomoses and abdominal wounds were made. After 14 days the bursting pressures were measured.

Group 7 (n=20, 10 experimental animals, 10 controls)
Two weeks after biliary obstruction or sham operation, the obstruction was relieved or sham operation was performed. At the same time duodenal-, colonic anastomoses and abdominal wounds were made. After 7 days the bursting pressures were measured.

Group 8 Pair-feeding group (n=20, 10 experimental animals, 10 controls)
The control animals were operated on one day after the experimental rats. The animals were housed individually and a control rat was coupled with a jaundiced rat. The amount of food eaten by the jaundiced rat was weighed and the same amount of food was given to the control sham operated rat. Two weeks after biliary obstruction or sham operation duodenal-, colonic anastomoses and abdominal wound were made. After 7 days the bursting pressures were measured.

Group 9 Starvation group (n=20, 10 experimental animals, 10 controls)
The control animals were kept on a starvation diet in order to produce more weight loss than the jaundiced animals. Two

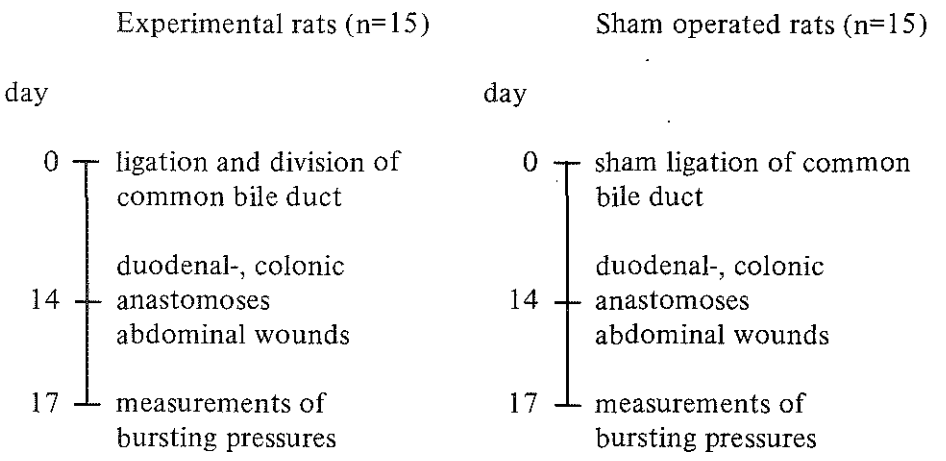
weeks after biliary obstruction or sham operation duodenal-, colonic anastomoses and abdominal wounds were made. After 7 days the bursting pressures were measured.

2.2.7 Statistical analysis

In the statistical analysis Student's t-test for unpaired data was used. When the term significant is used in the text this refers to statistical significance ($p < 0.05$ for the two sided test). Variance of the mean is expressed as the standard error of the mean (SEM).

2.3. Results

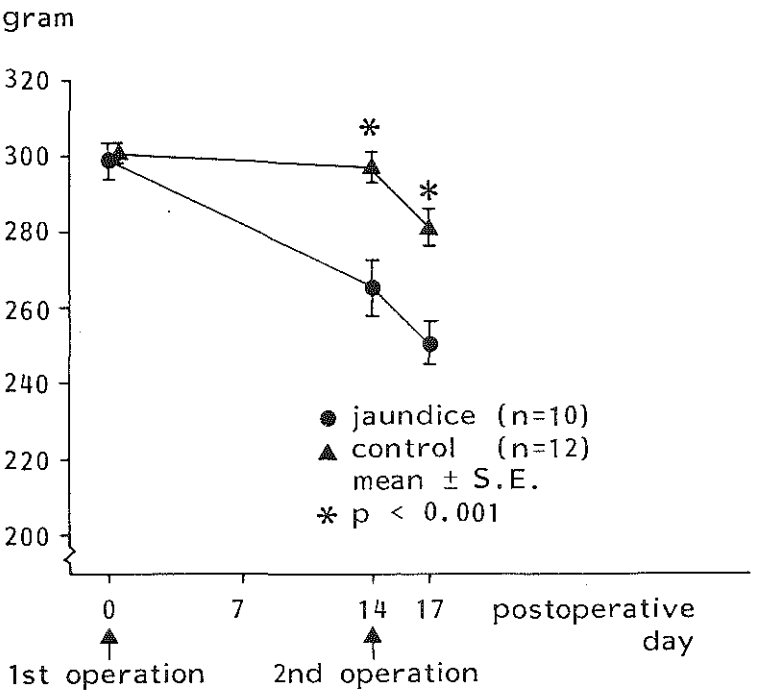
Group 1



Mortality in the jaundiced animals was 33% (5/15) and in the control animals 20% (3/15). These rats died within a few days of the operation, either from the effects of the anaesthesia or from biliary peritonitis in the jaundiced rats. These animals were excluded from further analysis. There was no mortality due to leakage of intestinal anastomoses.
Mean serum bilirubin was 161.1 ± 7.4 μmol/l in the experimental rats and 1.2 ± 1.4 μmol/l in the sham operated rats. Alkaline phosphatase was also

increased in the experimental rats when compared with the sham operated rats (962.5 ± 44.1 U/l and 281.7 ± 41.5 U/l respectively).
Weights of the animals are given in fig. 6. The experimental animals lost significantly more weight compared to the controls ($p < 0.001$). Serum albumin was significantly decreased in the jaundiced rats (25.6 ± 0.7 g/l) as compared with the controls (28.1 ± 0.8 g/l, $p < 0.05$).

Fig. 6 Mean body weights of jaundiced and control rats in group 1.

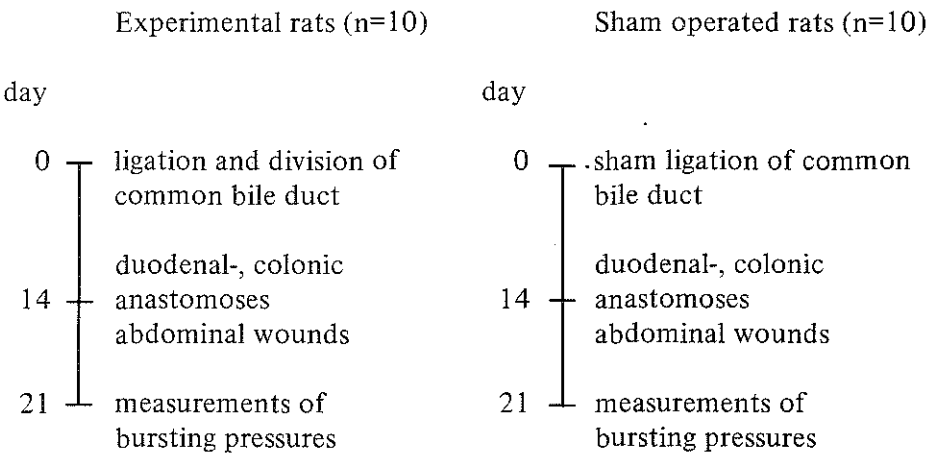


Bursting pressures tested 3 days after duodenal anastomoses and abdominal wounds were similar in both jaundiced and sham operated rats (table 3). It was not possible to test colonic anastomoses after 3 days, because these anastomoses were too weak and ruptured during dissection.

Table 3 Mean bursting pressures (± S.E.) in mm Hg in group 1 (3 days after making intestinal anastomoses and abdominal wounds).

Bursting pressure	Exp. animals	Controls	Stat. analysis
duodenum	44.4 ± 4.3	67.0 ± 10.0	n.s.
colon	–	–	–
abdomen right	221.5 ± 18.2	221.5 ± 20.8	n.s.
abdomen left	235.6 ± 30.9	238.2 ± 23.4	n.s.

Group 2



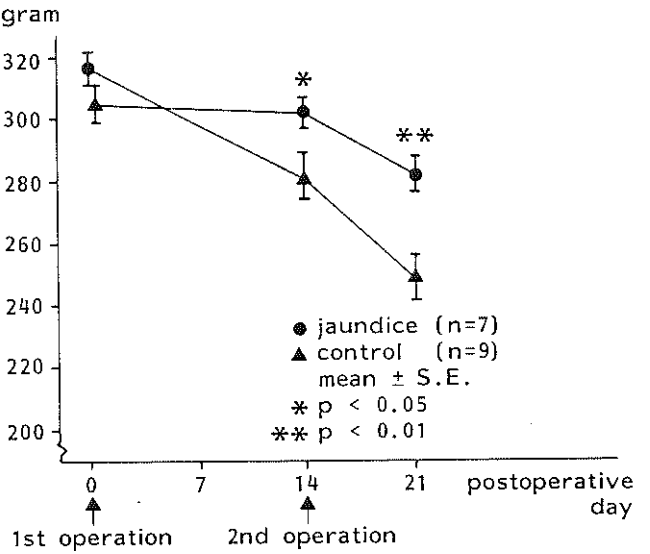
The mortality in the jaundiced animals was 33% (3/10) and in the control rats 10% (1/10). Causes of death were biliary peritonitis, bleeding and intestinal obstruction in the jaundiced rats and intestinal obstruction in the control rat.

Mean serum bilirubin was 143.3 ± 3.1 μmol/l in the experimental rats and 1.7 ± 1.3 umol/l in the controls. Alkaline phosphatase was 759.8 ± 27.3 U/l in the jaundiced rats and 248.3 ± 19.7 U/l in the sham operated rats.

Weights of the animals are given in fig. 7. The experimental animals lost

significantly more weight than the controls ($p < 0.001$). Albumin was significantly decreased in the jaundiced rats when compared with the controls (23.9 ± 0.6 g/l and 28.2 ± 0.6 g/l respectively, $p < 0.001$).

Fig. 7 Mean body weights of jaundiced and control rats in group 2.



Bursting pressures tested 7 days after duodenal anastomoses and abdominal wounds were significantly lower in the jaundiced animals. Bursting pressures of colonic anastomoses were still low in both jaundiced and control rats, showing no difference between the two groups (table 4).

Table 4 Mean bursting pressures (± S.E.) in mm Hg in group 2 (7 days after making intestinal anastomoses and abdominal wounds).

Bursting pressure	Exp. animals	Controls	Stat. analysis
duodenum	158.6 ± 32.3	275.0 ± 24.1	$p < 0.01$
colon	190.7 ± 17.0	238.8 ± 17.5	n.s.
abdomen right	382.1 ± 50.7	593.9 ± 47.7	$p < 0.01$
abdomen left	397.1 ± 58.5	582.8 ± 33.4	$p < 0.01$

Group 3

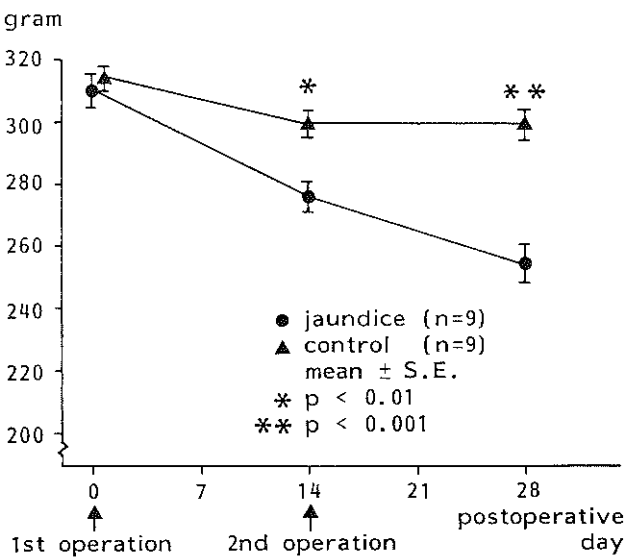
	Experimental rats (n=10)	Sham operated rats (n=10)
day		day
0	ligation and division of common bile duct	0 sham ligation of common bile duct
14	duodenal-, colonic anastomoses abdominal wounds	14 duodenal-, colonic anastomoses abdominal wounds
28	measurements of bursting pressures	28 measurements of bursting pressures

A mortality rate of 10%(1/10) was found in both the jaundiced and the sham operated rats. These rats died from biliary peritonitis and from the effects of anaesthesia.

Mean serum bilirubin was 170.9 ± 7.3 μmol/l in the experimental rats and 1.9 ± 1.2 μmol/l in the sham operated rats. Alkaline phosphatase was also increased in the experimental rats when compared with the sham operated rats (795.5 ± 26.8 U/l and 310.9 ± 19.2 U/l respectively).

The weights of the animals are given in fig. 8. The experimental animals lost significantly more weight than the controls ($p < 0.001$). Albumin was significantly decreased in the jaundiced rats (25.4 ± 0.8 g/l) when compared with the controls (32.9 ± 1.0 g/l, $p < 0.001$).

Fig. 8 Mean body weights of jaundiced and control rats in group 3.



Bursting pressures tested 14 days after duodenal-, colonic anastomoses and abdominal wounds were all significantly lower in the jaundiced rats than in the sham operated rats (table 5).

Table 5 Mean bursting pressures (± S.E.) in mm Hg in group 3 (14 days after making intestinal anastomoses and abdominal wounds).

Bursting pressure	Exp. animals	Controls	Stat. analysis
duodenum	211.9 ± 19.4	357.8 ± 18.5	$p < 0.001$
colon	208.8 ± 14.6	270.6 ± 10.2	$p < 0.01$
abdomen right	282.5 ± 22.4	624.4 ± 46.4	$p < 0.001$
abdomen left	316.9 ± 27.2	611.7 ± 39.4	$p < 0.001$

Summary of group 1, 2 and 3

The changes in serum bilirubin and alkaline phosphatase in jaundiced rats are show in fig. 9 and fig. 10.

Fig. 9 Changes in serum bilirubin in rats after biliary obstruction.

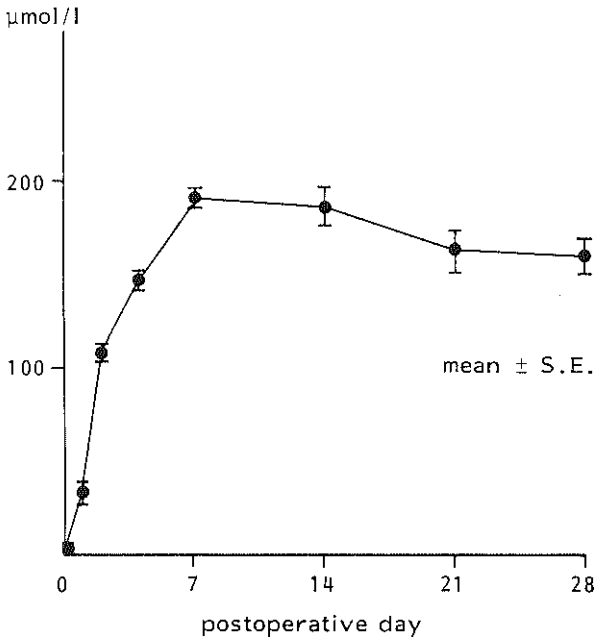
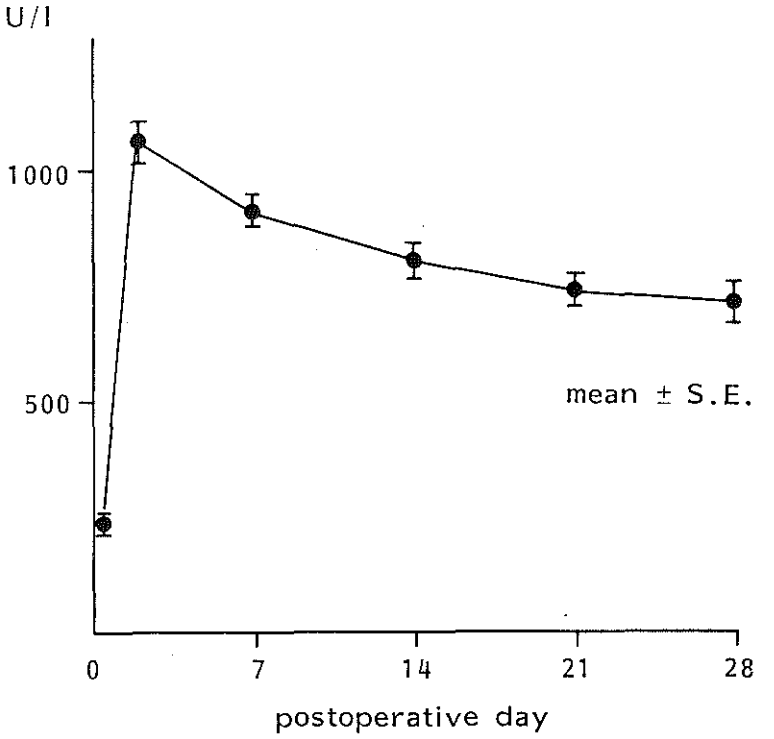


Fig. 10 Changes in alkaline phosphatase in rats after biliary obstruction.



The results of measurements of bursting pressures of enteral anastomoses and abdominal wounds in jaundiced and non-jaundiced rats at different times after operation are summarized in fig. 11, 12, 13 and 14.
Bursting pressures are lower in jaundiced rats than in non-jaundiced sham operated rats, indicating that wound healing is impaired in jaundiced rats. The difference is most striking in the bursting pressures of abdominal wounds (fig. 13 and 14), especially 14 days after incision.

Fig. 11 Mean bursting pressures of duodenum 3, 7 and 14 days after duodenal anastomoses (group 1, 2 and 3).

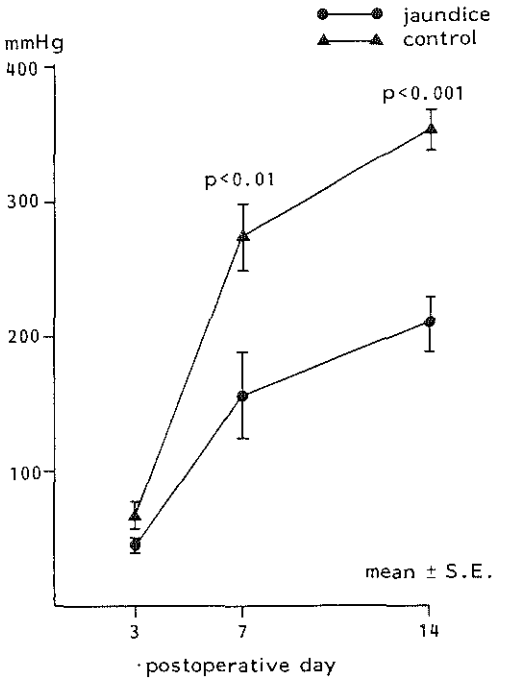


Fig. 12 Mean bursting pressures of colon 7 and 14 days after colonic anastomoses (group 2 and 3).

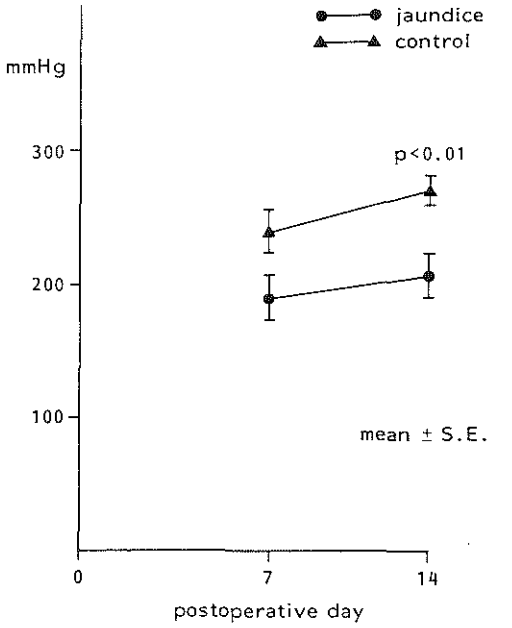


Fig. 13 Mean bursting pressures of right abdomen 3, 7 and 14 days after abdominal wounds (group 1, 2 and 3).

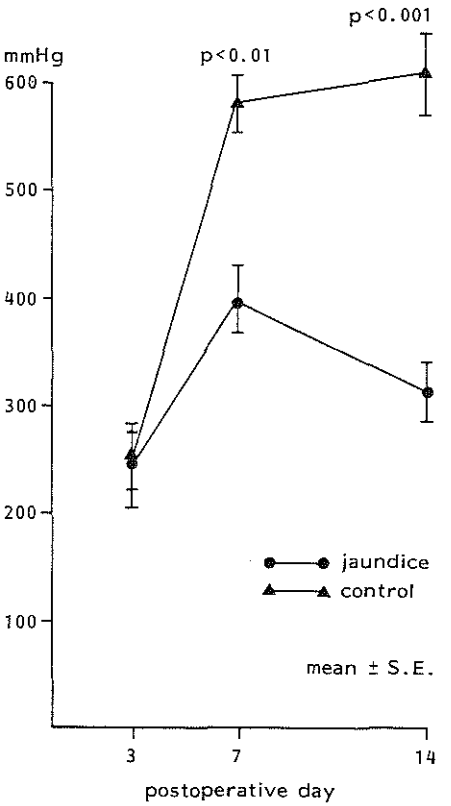
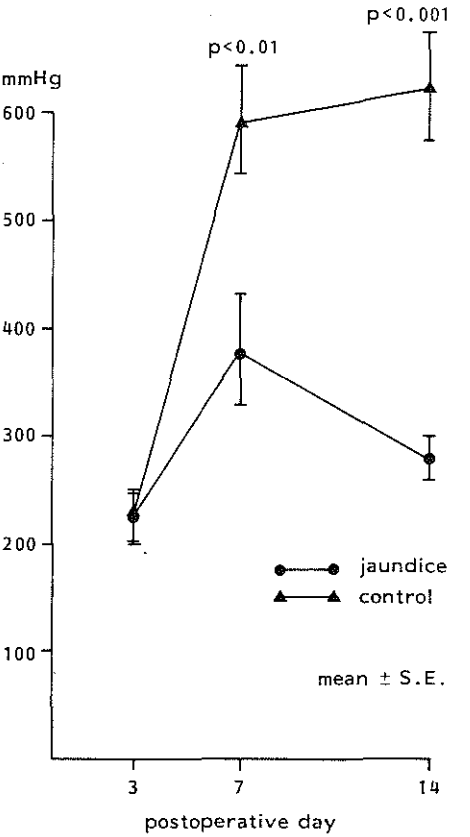
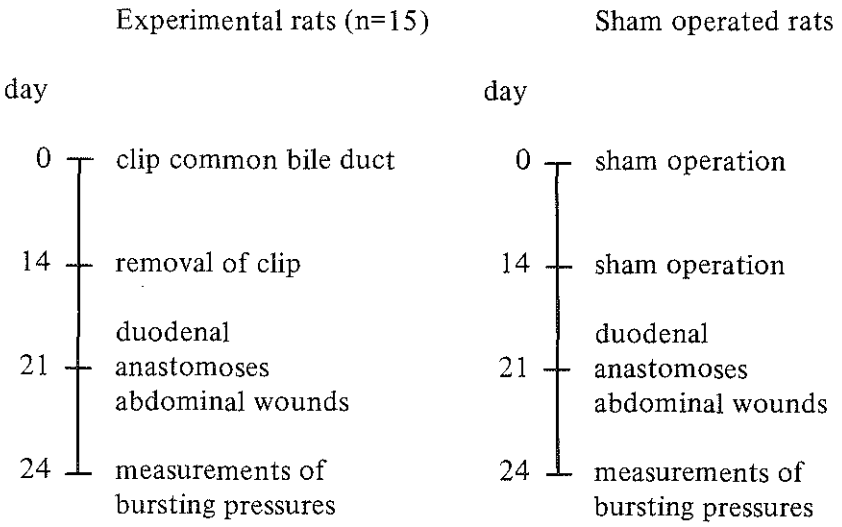


Fig. 14 Mean bursting pressures of left abdomen 3, 7 and 14 days after abdominal wounds (group 1, 2 and 3).



Group 4

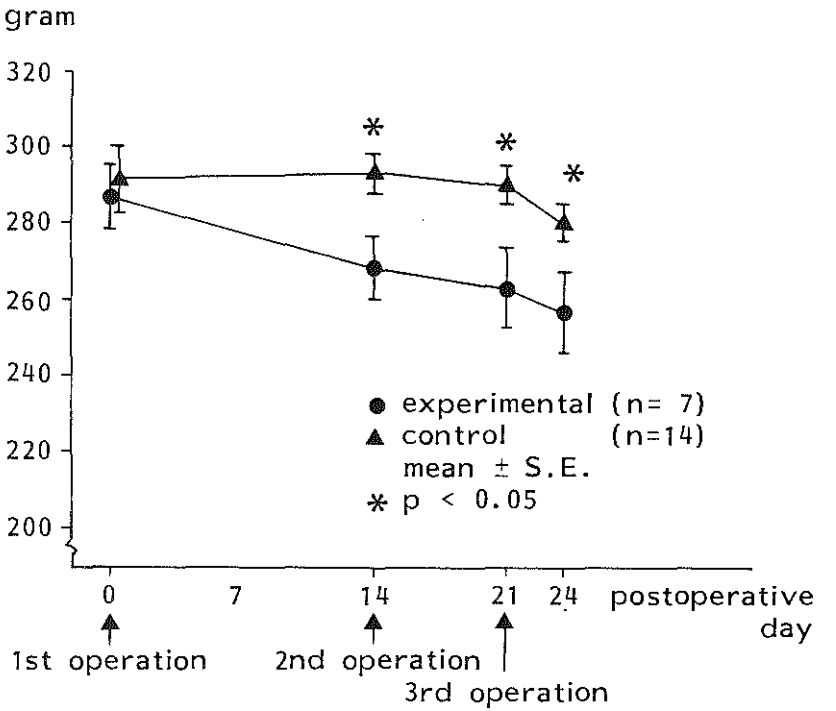


Mortality in the experimental animals was 53% (8/15) and in the control animals 7% (1/15). The high mortality in the experimental animals was caused by rupture of the enormously dilated common bile duct just above the clip, resulting in biliary peritonitis. One of the control animals died from leakage of the duodenal anastomosis.

Mean serum bilirubin 14 days after biliary obstruction was 183.1 ± 10.8 μmol/l in the experimental rats and 2.1 ± 1.9 μmol/l in the sham operated rats. Alkaline phosphatase was also increased in the experimental rats when compared with the sham operated rats (882.0 ± 68.7 U/l and 367.3 ± 11.5 U/l respectively).

The weights of the animals are given in fig. 15. During the 14 days of biliary obstruction the experimental animals lost significantly more weight than the controls ($p < 0.001$). Serum albumin was significantly decreased in the jaundiced rats (28.4 ± 1.1 g/l) when compared with the controls (32.0 ± 0.5 g/l, $p < 0.01$).

Fig. 15 Mean body weights of experimental and control rats in group 4.

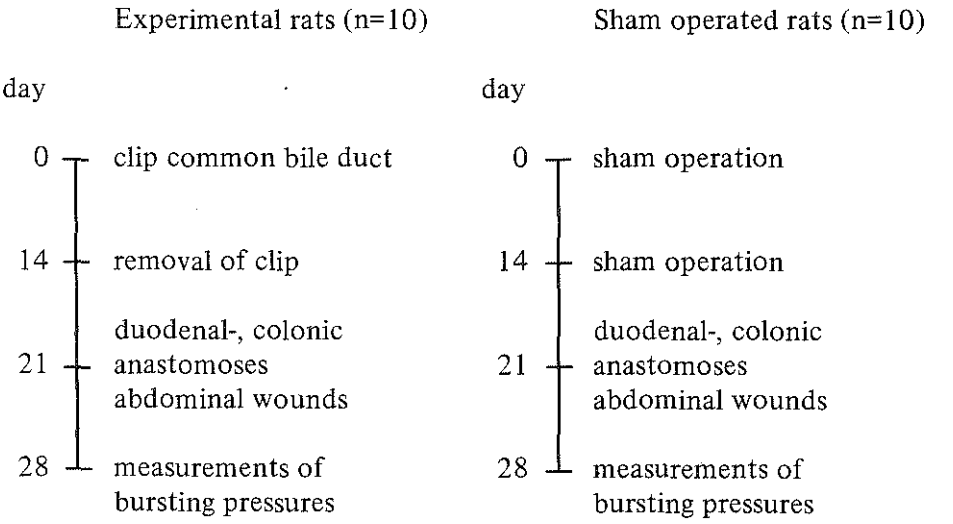


After relief of the biliary obstruction continuing until the end of the study (10 days) weight curves more or less paralled. Serum albumin was still significantly decreased in the experimental rats (27.6 ± 1.1 g/l) when compared with the sham operated animals (29.6 ± 0.4 g/l, $p < 0.01$).
Serum bilirubin returned to normal values after relief of the biliary obstruction in the experimental rats (2.4. ± 3.2 μmol/l), but serum alkaline phosphatase was still significantly increased in the experimental animals (335.0 ± 25.2 U/l) when compared with the sham operated animals (274.4 ± 12.2 U/l, $p < 0.05$).
Three days after the duodenal anastomoses and the making of the abdominal wounds, bursting pressures were only lowered in the duodenal anastomoses in rats which underwent relief of their biliary obstruction, when compared with control animals (table 6).

Table 6 Mean bursting pressures (± S.E.) in mm Hg in group 4 (3 days after making intestinal anastomoses and abdominal wounds following one week of biliary drainage).

Bursting pressure	Exp. animals	Controls	Stat. analysis
duodenum	59.2 ± 10.8	85.4 ± 6.8	$p < 0.05$
colon	–	–	–
abdomen right	207.1 ± 18.5	190.7 ± 19.8	n.s.
abdomen left	273.6 ± 35.0	253.9 ± 15.5	n.s.

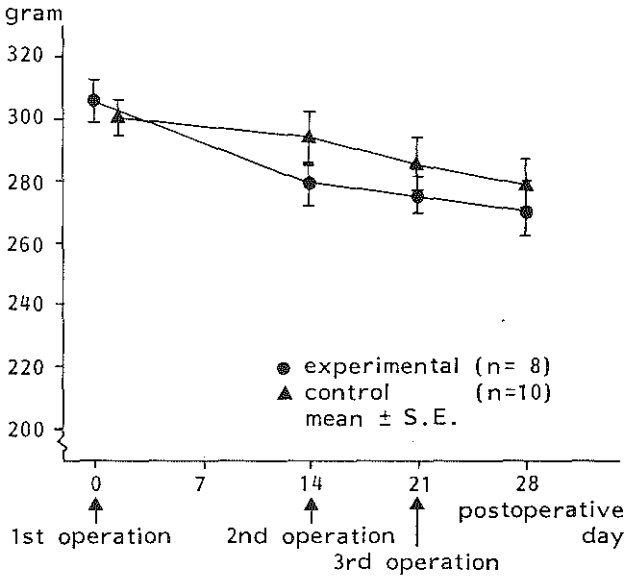
Group 5



Two of the 10 experimental animals died from biliary peritonitis after removal of the biliary obstruction. All control animals survived.
Mean serum bilirubin 14 days after biliary obstruction was 180.8 ± 8.4 μmol/l in the experimental animals and 2.2 ± 1.7 μmol/l in the sham operated rats. Serum alkaline phosphatase was increased in the experi-

mental rats when compared with the sham operated rats (871.8 ± 63.9 U/l and 410.8 ± 18.3 U/l respectively).
The weights of the animals are shown in fig. 16. During the 14 days of biliary obstruction the experimental animals lost significantly more weight than the controls ($p < 0.01$). Serum albumin values were similar in the two groups (31.8 ± 0.9 g/l in the experimental rats and 31.2 ± 0.5 g/l in the sham operated rats).

Fig. 16 Mean body weights of experimental and control rats in group 5.

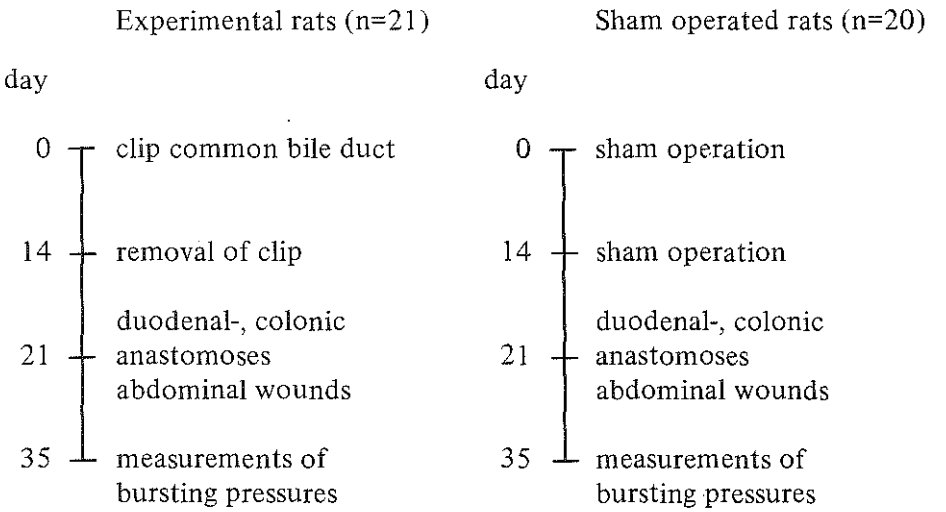


After relief of the biliary obstruction until the end of the study (14 days) the weight curves were parallel. Serum albumin, however, was significantly lower at the end of the study in the experimental rats (26.9 ± 0.4 g/l) when compared with the sham operated animals (29.1 ± 0.4 g/l, $p < 0.01$).
Serum bilirubin returned to normal values after relief of the biliary obstruction (2.5 ± 3.3 μmol/l), and the serum alkaline phosphatase was no longer significantly different in the experimental rats (399.8 ± 83.4 U/l) and controls (271.4 ± 12.1 U/l).
Bursting pressures 7 days after enteral anastomoses and abdominal wounds were the same in the experimental and control groups after relief of the biliary obstruction (table 7).

Table 7 Mean bursting pressures (± S.E.) in mm Hg in group 5 (7 days after making intestinal anastomoses and abdominal wounds following one week of biliary drainage).

Bursting pressure	Exp. animals	Controls	Stat. analysis
duodenum	266.9 ± 30.6	256.0 ± 33.9	n.s.
colon	240.0 ± 18.1	253.0 ± 17.0	n.s.
abdomen right	530.0 ± 33.9	562.0 ± 37.4	n.s.
abdomen left	613.7 ± 28.7	565.0 ± 41.8	n.s.

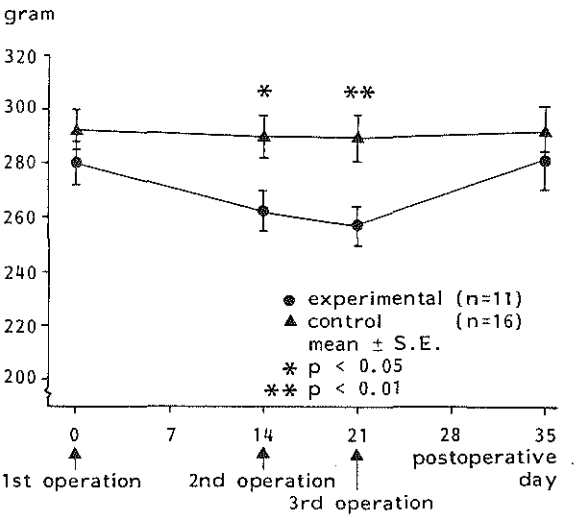
Group 6



A mortality rate of 48% (10/21) was found in the experimental animals and 20% (4/20) in the control animals. Mortality resulted from biliary peritonitis, effects of anaesthesia and bleeding.
Mean serum bilirubin 14 days after biliary obstruction was 195.9 ± 6.8 μmol/l in the experimental animals and 2.6 ± 2.1 μmol/l in the sham operated rats.

Serum alkaline phosphatase was increased in the jaundiced rats when compared with the sham operated rats (908.1 ± 46.3 U/l and 334.2 ± 16.4 U/l respectively).
The weights of the animals are given in fig. 17. During the 14 days of biliary obstruction, weight loss of the jaundiced rats was significantly higher than weight loss in the control animals ($p < 0.01$). Serum albumin was significantly decreased in jaundiced rats (28.8 ± 0.8 g/l) when compared with controls (33.0 ± 0.9 g/l, $p < 0.01$).

Fig. 17 Mean body weights in experimental and control rats in group 6.



After relief of the biliary obstruction continuing until the end of the study (21 days) there was an increase of weights in the experimental animals (17.3 ± 6.4 grams).
At the end of the study serum albumin was no longer decreased in the experimental rats (32.7 ± 2.0 g/l) as compared with the sham operated rats (33.4 ± 1.1 g/l).
Serum bilirubin returned to normal values after relief of the biliary obstruction (1.9 ± 0.8 μmol/l) and serum alkaline phosphatase was not significantly different in the experimental rats (386.3 ± 40.2 U/l) in comparison to the controls (370.2 ± 16.4 U/l).
After relief of the biliary obstruction bursting pressures of enteral anastomoses and right abdominal wounds 14 days after operation were not different in the experimental and the control animals. The bursting pressures of the left abdominal wounds were lower in the experimental rats when compared with the sham operated rats ($p < 0.05$, table 8).

Table 8 Mean bursting pressures (± S.E.) in mm Hg in group 6 (14 days after making intestinal anastomoses and abdominal wounds following one week of biliary drainage).

Bursting pressure	Exp. animals	Controls	Stat. analysis
duodenum	293.0 ± 13.9	298.3 ± 15.2	n.s.
colon	246.8 ± 16.0	275.3 ± 12.7	n.s.
abdomen right	459.1 ± 28.0	545.6 ± 33.8	n.s.
abdomen left	452.3 ± 36.0	558.0 ± 26.9	$p < 0.05$

Summary of group 4, 5 and 6.

The changes in serum bilirubin and alkaline phosphatase in the experimental rats are shown in fig. 18 and 19. After removal of the biliary obstruction serum bilirubin returned to normal levels within 3 to 4 days. Alkaline phosphatase also decreased to normal levels, but at a slower rate.

Fig. 18 Changes in serum bilirubin in rats after biliary obstruction followed by removal of the biliary obstruction.

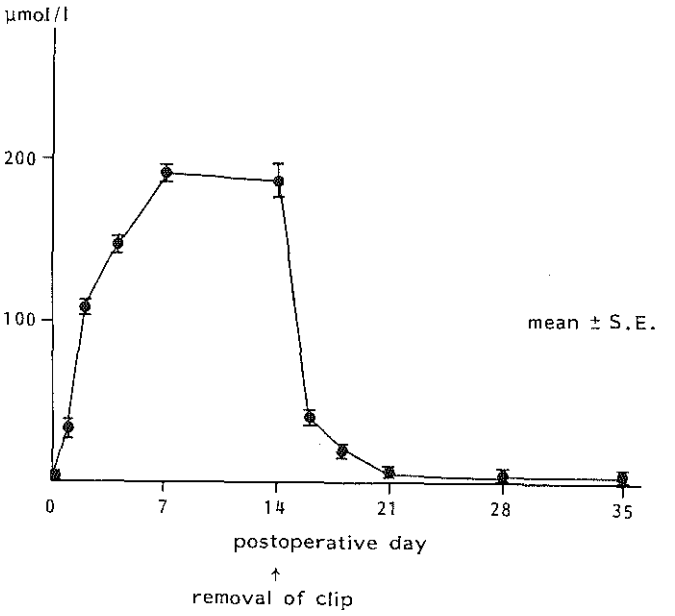
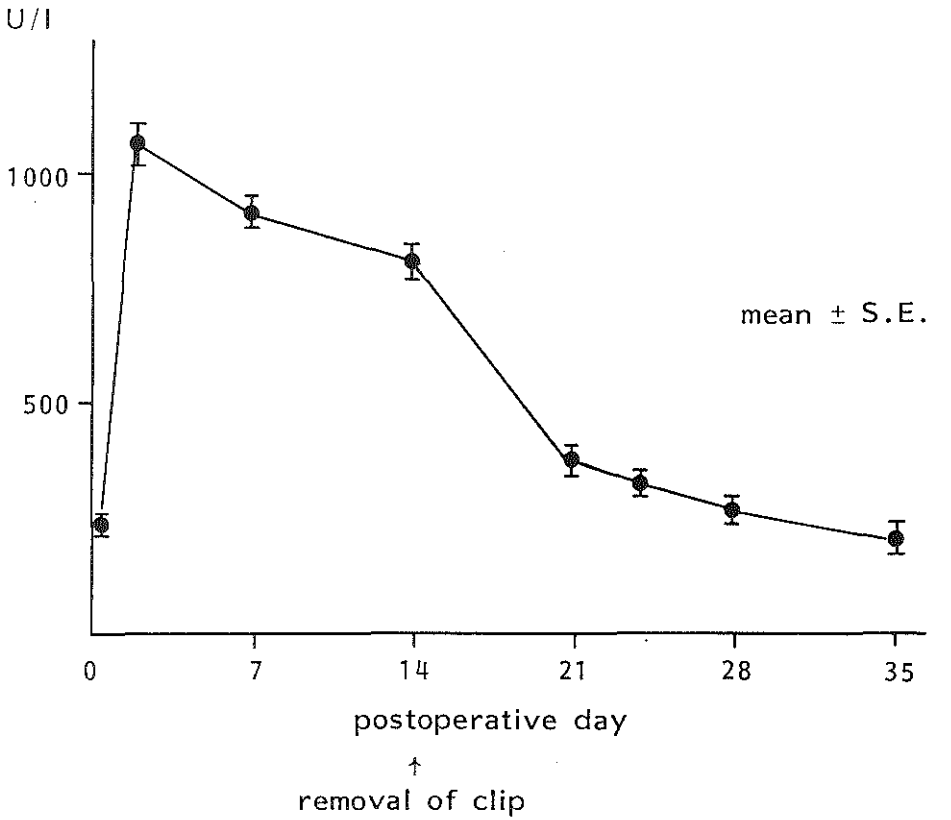


Fig. 19 Changes in alkaline phosphatase in rats after biliary obstruction followed by removal of the biliary obstruction.



The results of measurements of bursting pressures of duodenal-, colonic anastomoses and abdominal wounds in both the experimental and the sham operated rats at different times after operation are summarized in fig. 20, 21, 22 and 23. No significant differences, apart from the bursting pressures of the duodenal anastomoses 3 days after operation and of the left abdominal wounds 14 days after operation were found between the two groups, indicating that wound healing returns to normal after relief of the biliary obstruction.

Fig. 20 Mean bursting pressures of duodenum 3, 7 and 14 days after duodenal anastomoses following one week of biliary drainage (group 4, 5 and 6).

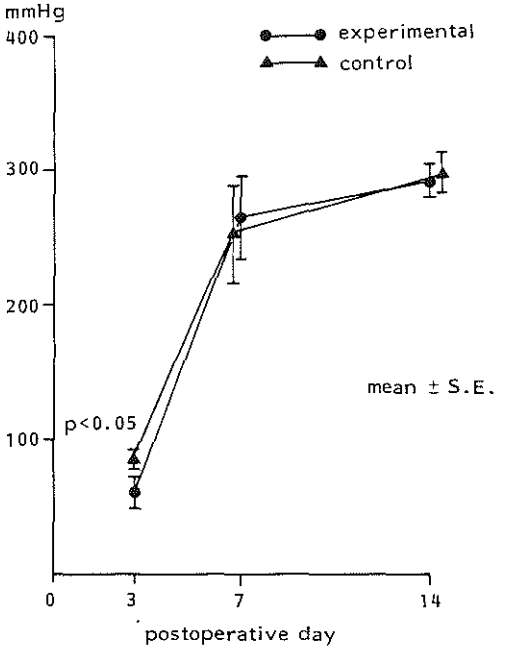


Fig. 21 Mean bursting pressures of colon 7 and 14 days after colonic anastomoses following one week of biliary drainage (group 5 and 6).

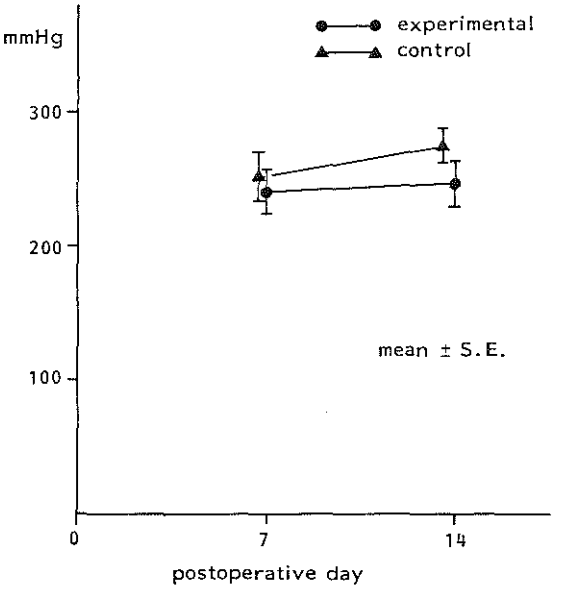


Fig. 22 Mean bursting pressures of right abdomen 3, 7 and 14 days after abdominal wounds following one week of biliary drainage (group 4, 5 and 6).

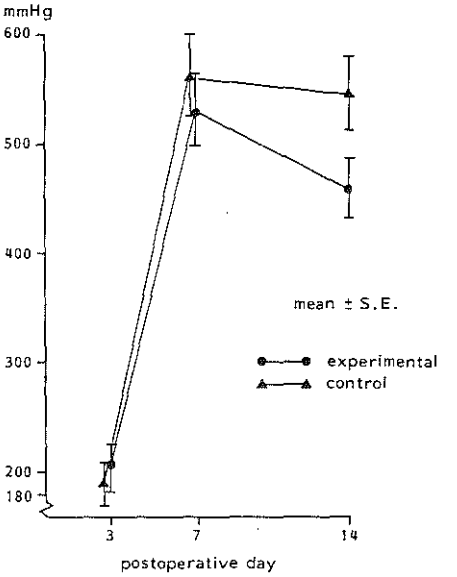
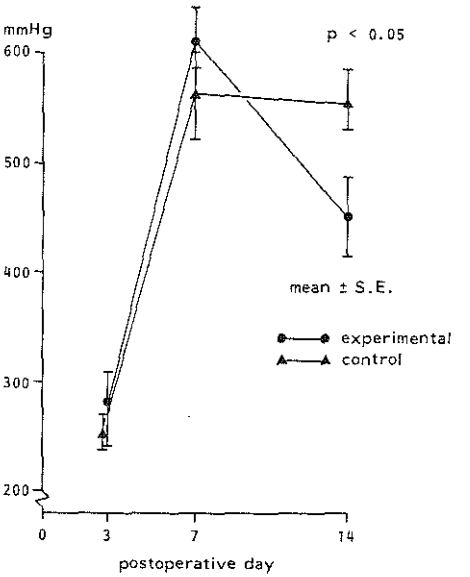
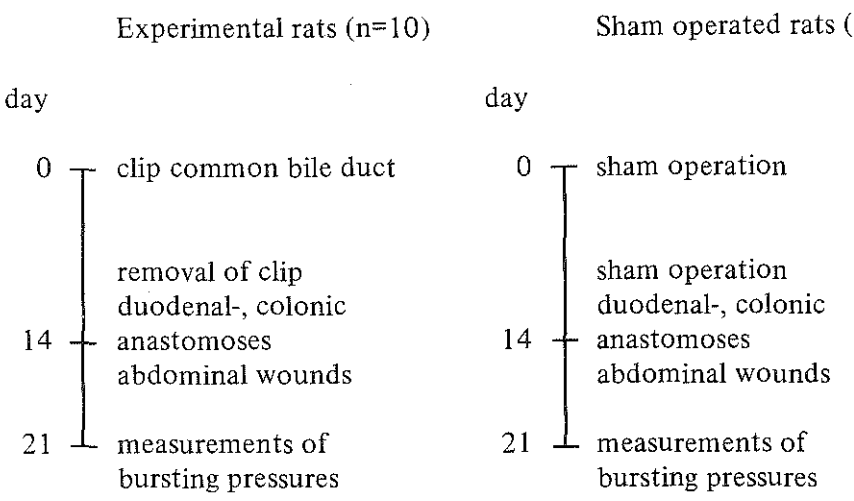


Fig. 23 Mean bursting pressures of left abdomen 3, 7 and 14 days after abdominal wounds following one week of biliary drainage (group 4, 5 and 6).



Group 7



In this group three of 10 experimental animals died and all control rats survived. The mortality was caused in all cases by biliary peritonitis after removal of the clip.

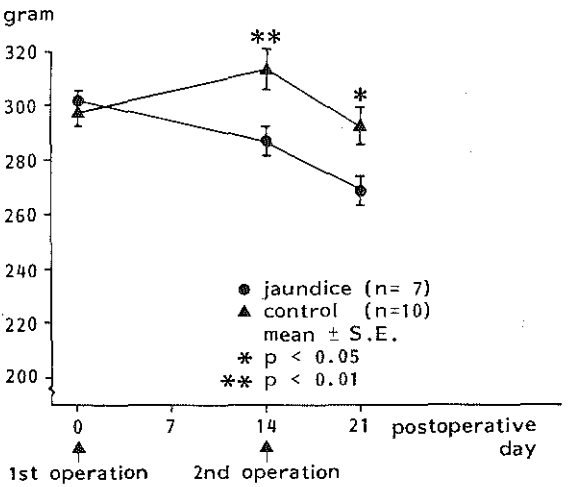
Mean serum bilirubin 14 days after biliary obstruction was 188.2 ± 8.3 μmol/l in the experimental rats and 2.4 ± 1.4 μmol/l in the sham operated rats. Alkaline phosphatase was also increased in the experimental rats when compared with the sham operated rats (890.4 ± 37.3 U/l and 461.6 ± 27.5 U/l respectively).

Weights of the animals are shown in fig. 24. In the 14 days of biliary obstruction weight loss of the jaundiced rats was significantly higher than in the sham operated rats ($p < 0.001$). Serum albumin values were not significantly different between the two groups (27.7 ± 0.7 g/l in the jaundiced rats and 29.8 ± 0.8 g/l in the control rats).

After relief of the biliary obstruction weight curves were parallel until the end of the study (7 days).

Serum albumin was 28.6 ± 0.6 g/l in the experimental rats and 30.5 ± 0.7 g/l in the sham operated rats at the end of the study.

Fig. 24 Mean body weights of jaundiced and control rats in group 7.

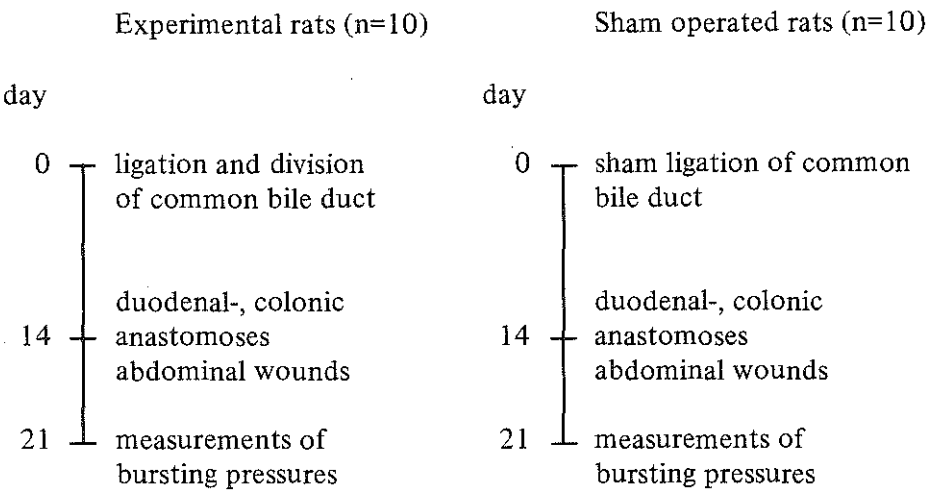


Serum bilirubin returned to normal values after relief of the biliary obstruction (2.7 ± 1.8 μmol/l) and serum alkaline phosphatase was also not significantly different in the experimental animals (470.6 ± 92.1 U/l) and the control animals (322.5 ± 22.6 U/l) at the end of the study.
Bursting pressures tested 7 days after duodenal anastomoses and abdominal wounds, made at the same operation in which the clip was removed, were significantly lowered in the experimental group when compared with the control group. There was no difference between the bursting pressures of the colonic anastomoses in the two groups (table 9).

Table 9 Mean bursting pressures (± S.E.) in mm Hg in group 7 (7 days after removal of the biliary obstruction and at the same operation made intestinal anastomoses and abdominal wounds).

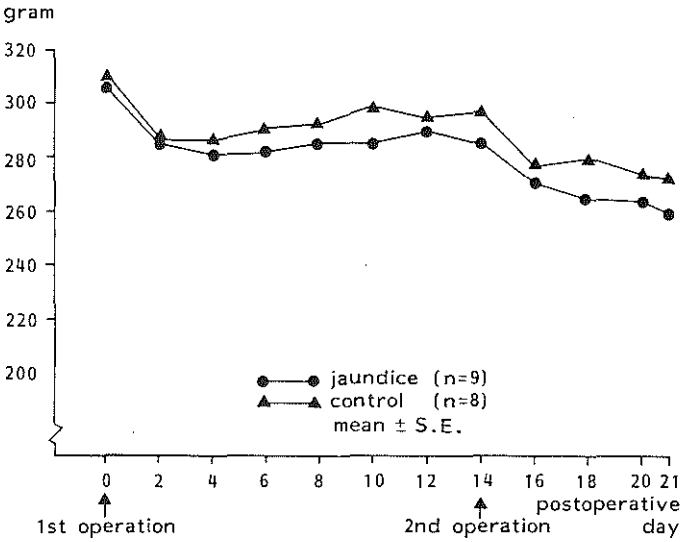
Bursting pressure	Exp. animals	Controls	Stat. analysis
duodenum	248.6 ± 6.1	286.3 ± 10.1	$p < 0.01$
colon	224.3 ± 20.4	242.3 ± 10.6	n.s.
abdomen right	495.7 ± 30.0	610.0 ± 37.1	$p < 0.05$
abdomen left	507.1 ± 33.2	598.0 ± 26.0	$p < 0.05$

Group 8 (Pair-feeding group)



One of 10 jaundiced animals and 2 of 10 control rats died. The reasons for these deaths were not clear.
Mean serum bilirubin was 162.1 ± 9.1 μmol/l in the jaundiced rats and 2.1 ± 0.2 μmol/l in the sham operated rats. Alkaline phosphatase was 913.2 ± 68.2 U/l in the jaundiced rats and 344.4 ± 49.6 U/l in the control rats. Both SGOT and γ GT were significantly increased in the jaundiced rats (263.7 ± 19.5 U/l and 50.9 ± 8.5 U/l respectively) when compared with the sham operated rats (97.7 ± 15.9 U/l and 4.9 ± 1.5 U/l respectively).
The weights of the animals are given in fig. 25. The mean weights of the jaundiced and the control rats were not statistically different throughout the entire study. Weight loss during the 21-day study was not statistically different in the jaundiced and the pair-fed control rats. However, serum albumin was significantly decreased ($p < 0.001$) in the jaundiced rats (23.1 ± 0.9 g/l) as compared with the control rats (30.1 ± 1.0 g/l).

Fig 25. Mean body weights of jaundiced and pair-fed control rats in group 8.

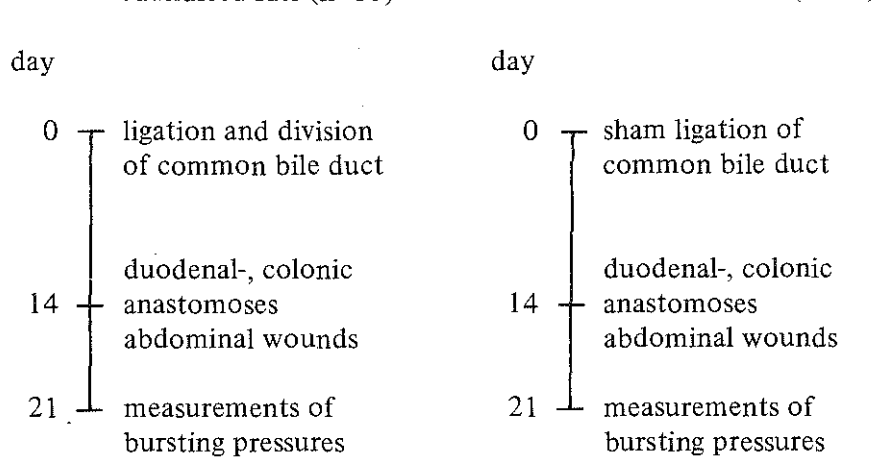


Bursting pressures tested 7 days after making the abdominal wounds were significantly lower in the jaundiced rats when compared with the sham operated rats. Enteral anastomoses, however, showed no significant difference in bursting pressure between either groups (table 10).

Table 10 Mean bursting pressures (± S.E.) in mm Hg in group 8 (7 days after making intestinal anastomoses and abdominal wounds in jaundiced and pair-fed sham operated rats).

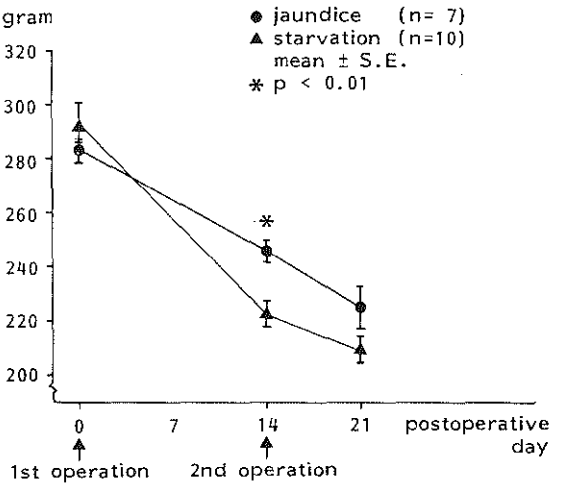
Bursting pressure	Exp. animals	Controls	Stat. analysis
duodenum	231.4 ± 24.4	287.5 ± 12.7	n.s.
colon	203.6 ± 10.1	220.7 ± 19.0	n.s.
abdomen right	385.6 ± 23.0	614.3 ± 28.3	$p < 0.001$
abdomen left	400.0 ± 30.4	648.6 ± 29.3	$p < 0.001$

Group 9 (starvation group)



Three of 10 jaundiced animals died, all the starved rats survived. Causes of death were leakage of the colon anastomosis, effects of anaesthesia and in one instance, unknown.
Mean serum bilirubin was 171.5 ± 10.3 μmol/l in the jaundiced rats and 2.1. ± 0.3 μmol/l in the sham operated rats. Alkaline phosphatase was 872.9 ± 68.0 U/l in jaundiced rats and 204.9 ± 10.9 U/l in the control rats. The mean body weights of the animals are shown in fig. 26. The sham operated animals, who were kept on a starvation diet, lost significantly more weight than the jaundiced animals ($p < 0.01$). However, serum albumin was significantly decreased in the jaundiced rats (23.2 ± 0.8 g/l) when compared with the sham operated animals (33.3 ± 0.9 g/l, $p < 0.001$).

Fig. 26 Mean body weights of jaundiced and starved sham operated rats in group 9.



Bursting pressures tested 7 days after abdominal wounds were significantly lowered in the jaundiced rats when compared with the animals which had been starved. Bursting pressures of enteral anastomoses were not different in the two groups (table 11).

Table 11 Mean bursting pressures (± S.E.) in mm Hg in group 9 (7 days after making intestinal anastomoses and abdominal wounds in jaundiced and starved sham operated rats).

Bursting pressure	Jaundiced rats	Starvation rats	Stat. analysis
duodenum	224.0 ± 17.4	273.8 ± 20.6	n.s.
colon	190.0 ± 13.7	209.4 ± 21.7	n.s.
abdomen right	318.3 ± 26.0	421.0 ± 21.7	$p < 0.01$
abdomen left	385.0 ± 21.6	479.0 ± 30.1	$p < 0.01$

2.4 Discussion

The evaluation of the effect of biliary obstruction on wound healing in man is complicated by the presence of factors such as malignancy or anorexia with malnutrition (Irvin et al 1978, Armstrong et al 1984). In order to separate the effects of different factors it is necessary to use animal models. In the present study we used the male random bred Wistar rat to investigate the effect of biliary obstruction on wound healing.

Trams and Symeonidis (1957) showed that after simple ligation of the common bile duct in rats recanalisation can occur after 14 to 28 days by invagination of the distal into the proximal stump of the common bile duct. This recanalisation, however, does not occur in all rats after bile duct ligation. In our opinion this model is not suitable for investigation of the effect of biliary drainage because the time of recanalisation can not be predicted. Another method to relieve a previously made biliary obstruction has been described in dogs by Aronson (1961) and in rats by Birns et al (1962). In these models a short plastic tube was used that was slit lengthwise. The tube was clamped around the common bile duct and fixed with a ligature. The biliary obstruction was relieved by cutting the ligature. This method has been used to cause biliary obstruction for maximally 3 days in rats, but its use after longer periods of obstruction has not been studied (Birns et al 1962).

Ryan et al (1977) performed choledochoduodenostomy in rats 2 weeks after ligation and division of the common bile duct, using a teflon splint. This procedure is technically difficult, time consuming and the risk of leakage of the anastomosis leading to biliary peritonitis is considerable.

For the present study we decided to use a Haemoclip®. This Haemoclip® is easy to put on the common bile duct after dissecting free the duct. A similar Haemoclip® has also been used in our laboratory to make a reversible obstruction of the ureters in rats (Provoost and Molenaar 1981). In a pilot study total obstruction of the rats common bile duct was achieved with this Haemoclip®. Approximately 14 days after clipping the common bile duct, recanalisation - the same process of invagination as described by Trams and Symeonidis (1957) - occurred in 80% of the rats. The obstruction and recanalisation were visualized by cholangiography (fig. 1, 2).

After removal of the clip with a special forceps the serum bilirubin level decreases to normal in 3 to 4 days. Alkaline phosphatase also decreases to normal levels, but more slowly. Histopathological changes of the liver will disappear in the reverse order to which they occur (Trams and Symeonidis 1957, Birns et al 1962, Ryan et al 1977). The use of this clip was associated with a high mortality (10-50%)

in some of the experimental groups, death being mainly due to biliary peritonitis. The mortality rate after ligation and division of the common bile duct, as described by Lee (1972) was somewhat lower (10-33%). The mortality rate of the sham operation was 10% and was mainly caused by the effects of anaesthesia. The mortality due to leakage of the intestinal anastomoses was negligible.

In pilot studies done by our group the effect of biliary obstruction on the healing of laparotomy wounds in rats was evident, since all rats with a permanent obstruction longer than 8 weeks developed an incisional hernia. The development of incisional hernias in jaundiced rats has also been found in other strains of rats (Brown Norway and Wag/Ry rats) used in our laboratory. To our knowledge, this observation has not previously been published, except for a remark on jaundiced rats with a high percentage of burst abdomen and incisional hernia by Ellis (1977). Such a high frequency of incisional hernia is not reported with other factors known to influence wound healing, for instance extreme malnutrition or corticosteroides.

The healing of duodenal anastomoses was investigated in the present study because small intestinal anastomoses are frequently used in biliary surgery. The healing of colonic anastomoses was studied to compare the effect of biliary obstruction on the healing of colonic anastomoses with the effects of other factors such as tumor growth (De Graaf 1981), malnutrition (Daly et al 1972, Irvin 1978, Ward et al 1982) and uremia (Shindo and Kosaki 1982), which have been studied in detail.

The intestinal anastomoses were performed with non-resorbable sutures and these sutures were not removed before measuring the bursting pressures to prevent damage to the anastomoses. The abdominal wounds were made from the inside without cutting the subcutis and skin. The process of wound healing could not, therefore, be influenced by external factors as infection due to licking or gnawing of the wounds by the rats.

The bursting pressures of both intestinal anastomoses and abdominal wounds were significantly lower in the jaundiced rats 14 days after the wounds were made, compared with the sham operated rats. Seven days after making the wounds the duodenal anastomoses and abdominal wounds had significantly lower bursting pressures, whereas after 3 days there were no differences between the two groups.

The delayed wound healing in obstructive jaundice found in this study is in accordance with the reports of Bayer and Ellis (1976) and Arnaud et al (1981), but in contrast to findings of Greaney et al (1979). The latter used the same technique to measure the bursting pressures of abdominal wounds as in our study. The length of the abdominal wounds in Greaney's study was 1 cm, while in our study the length was 1.5 cm. It is possible that a length of 1 cm is too small to reach significant differences in bursting

pressures in wounds of jaundiced and control rats. Longer laparotomy wounds were used for measuring the bursting pressures in the other studies (Bayer and Ellis 1976, Arnaud et al 1981).

In the present study we found that jaundiced rats lost significantly more weight when compared to sham operated rats. This loss of weight of jaundiced rats was not reported in studies of Bayer and Ellis (1976), Ryan et al (1977), Greaney et al (1979), Arnaud et al (1981). However, significant weight loss was reported in a study by Holman and Rikkers (1982) on obstructive jaundice and host defence failure and in a study by Yarger (1976) on obstructive jaundice and kidney function. Since weight loss itself can influence wound healing adversely (Daly et al 1972, Ward et al 1982) we studied wound healing in jaundiced and pair-fed sham operated rats, who had similar weight losses. From this study we found that weight loss in jaundiced rats is the result of anorexia rather than of malabsorption, since jaundiced rats eat much less food than control rats and the weight curves of pair-fed rats were parallel. Jaundiced rats eat practically nothing in the first days after obstruction of the common bile duct, during the period in which the serum bilirubin level rises. After this period jaundiced rats regain some appetite, but they still eat less than sham operated rats.

The anorexia disappears after relief of the biliary obstruction. The weight curves of the experimental rats were parallel to those of the control rats. In these two groups with similar weight loss due to pair feeding we found that the bursting pressures of the abdominal wounds were significantly lower in jaundiced rats, as compared with sham operated rats. The bursting pressures of the intestinal anastomoses were similar in the two groups. Hyperbilirubinaemia thus seems to be of greater importance to the healing of abdominal wounds than to the healing of intestinal anastomoses.

Reports on the effect of malnutrition on the process of wound healing are unanimous. A delayed healing of a colon anastomosis was found in rats with weight loss of 2% of body weight as compared with control rats that gained 8% of body weight in a study by Daly et al (1972). Lower bursting pressures of colon anastomoses were reported in rats with 12.5% loss of body weight (Ward et al 1982). In another study a significantly delayed healing of intestinal anastomoses was only found in rats with weight loss of more than one third of body weight (Irvin 1978).

The fact that hyperbilirubinaemia plays an important role in the healing of abdominal wounds can be concluded from our study (group 9) in which jaundiced rats had significantly less weight loss than sham operated rats, who were put on a starvation diet. Even then lower bursting pressures of the abdominal wounds were found in jaundiced rats in comparison to sham operated rats, who had a total weight loss of 28.5% of the initial weights. A different response to weight loss of parietal and visceral tissues has been

suggested by Harkness et al (1958) and Caback et al (1963). These authors found that visceral collagen was maintained in larger amounts after malnutrition than collagen in skin. Collagen is of great importance for the strength of a wound (Cronin et al 1968, Gottrup 1981 a and b) and collagen is produced by fibroblasts. It has been shown that bilirubin has a toxic effect on growing fibroblasts in vitro (Taube et al 1981). It is possible that a larger increase of fibroblasts in parietal tissue is the cause of the greater sensitivity for the toxic effect of bilirubin of abdominal wounds than intestinal anastomoses.

The poorer nutritional state of jaundiced rats was also evident from the significant decrease of the serum albumin levels. This decreased albumin level was probably not only caused by the lower food intake, but also by a reduced synthesis due to the disturbances in liver function. This can be concluded from the starvation and pair-feeding study in which jaundiced rats had a significant lower serum albumin than sham operated rats.

The effect of biliary tract drainage on wound healing has not been previously studied. In the present study delayed wound healing in jaundiced rats improved quickly after biliary drainage. Almost no differences in bursting pressures of intestinal anastomoses and abdominal wounds measured after 3, 7 and 14 days were found in the experimental and sham operated rats after biliary tract decompression. All rats had normal bilirubin levels after biliary drainage.

The improvement of wound healing after biliary drainage takes some time. When the intestinal anastomoses and abdominal wounds were made simultaneously with the relief of the biliary obstruction, a delayed healing of duodenal anastomoses and abdominal wounds is still found.

After biliary drainage the nutritional state of the rats improved, because the weight curves of the rats after drainage were parallel or even rising and the serum albumin level was increasing.

These findings clearly show the beneficial effect of biliary drainage on the process of wound healing in rats with biliary obstruction.

CHAPTER 3
BILIARY OBSTRUCTION AND INFECTION

3.1 Introduction

Cultures of bile at the time of operation on patients with obstructive jaundice frequently reveal a wide variety of micro-organisms. Passants of the small intestine, E.Coli, Streptococcus faecalis and Klebsiella, are the most common (Nielsen and Justesen 1976, Farnell et al 1981).
An overall incidence of positive bile cultures of 33% in 870 patients was reported in elective biliary surgery by Cox et al (1978). The reported incidence of bacteria in the bile is extremely variable in other studies ranging from 8 to 42% and depending on the type of patients being studied and the use of antibiotics preoperatively (Anderson and Priestly 1951, Flemma et al 1967, Engström et al 1971, Maddocks et al 1973). The incidence of positive cultures is much higher in patients with partial obstruction than in patients with total obstruction (Kune and Schutz 1974, Nielsen and Justesen 1976, Dye et al 1978, Cox et al 1978, Keighley 1982). After biliary tract decompression, however, 88% of the patients had positive cultures in the study described by Cox et al (1978).
Micro-organisms that cause postoperative cholangitis and septicaemia are usually the same as those are cultured from the bile at the time of the operation (Keighley 1977, Cox et al 1978).
The pathogenesis of bacteria in the bile is incompletely understood. Four different routes of invasion have been proposed (Scott and Khan 1967, Kune et al 1974):

1. The entero-hepatic route.
   Colonic organisms travel via the portal blood to the liver, are excreted in in the bile, and proliferate in the stagnant bile of a diseased biliary tree.
2. The ascending route.
   Organisms appear in the duodenum, traverse Vater's papilla, and ascend the biliary tree.
3. The haematogenous route.
   Blood-borne organisms are carried to the diseased biliary tree, where proliferation occurs.
4. The lymphatic route.
   Organisms from the gut are transported into the lymphatics of the liver and biliary tract.

None of these theories can completely explain all observations made regarding bile cultures in the various diseases affecting the biliary tree.
Wound infection after biliary surgery occurred by direct spread of the bacteria rather than by haematogenous dissemination in patients studied by Gallagher et al (1982).
Postoperative septic complications occur frequently in patients with ob-

structive jaundice (Pitt et al 1981) and are associated with a significant mortality (Dixon et al 1983). After pancreatoduodenectomy septic complications are a major problem. Anastomotic breakdown, especially from the pancreatojejunostomy, leading to septicaemia carries a high mortality (Gilsdorf and Spanos 1972, Warren et al 1975, Braasch and Gray 1977).

Although the high frequency of postoperative septic complications in patients with obstructive jaundice is explained by the presence of infected bile, especially after manipulation of the biliary tract, it is not clear whether patients with obstructive jaundice have impaired defensive mechanisms against infection.

It has been shown in vitro that the proliferative response of T and B lymphocytes to mitogens is reduced by addition of bile acids (in concentrations higher than found in patients with obstructive jaundice, Gianni et al 1981). Unconjugated bilirubin appears to have an inhibitory effect on cellular immune responses in man (Rola et al 1975). On the other hand enzymes associated with antibacterial activity are increased in leucocytes from patients with obstructive jaundice (Wardle 1980).

The phagocytic capacity of the reticulo-endothelial system which plays an important role in removing micro-organisms from the blood (Saba 1970, Jacob et al 1977) is impaired in patients with cholestasis (Drivas et al 1976). The phagocytic function of the reticulo-endothelial system has been studied in rats by means of colloidal carbon injection 3, 7, 14 and 21 days after ligation and division of the common bile duct. (Holman and Rikkers 1982). The corrected plasma disappearance rate (α-value) was significantly depressed 21 days after biliary obstruction as compared with control rats, but not after 3, 7 and 14 days. Mortality following i.v. injection of E.Coli endotoxin was increased in 21 days obstructed rats when compared to controls, suggesting a low phagocytic activity of the reticulo-endothelial system.

The reticulo-endothelial clearance of microaggregated albumin and ^{51}Cr labelled endotoxin was significantly reduced in rats with obstructive jaundice 3 days after ligation of the common bile duct just before its entry into the duodenum. Mortality after i.v. injection of E.Coli endotoxin was 18 out of 20 jaundiced animals and 2 out of 10 control animals (Wardle and Wright 1970).

On the other hand the phagocytic capacity (k-value) in rats was increased in 5 to 38 days jaundiced rats as compared with control rats. Phagocytic capacity was measured with carbon particles in this study (Halpern et al 1957). In a more recent study the phagocytic capacity was also found to be significantly increased in jaundiced rats after injection of ^{51}Cr endotoxin as compared with controls (Arii et al 1983).

The present experimental study in rats was initiated because the reports in the literature are conflictiong, especially with regard to phagocytic funtion of the reticulo-endothelial system in animals with biliary obstruction.
The aim of the study was:

1. to study the effect of biliary obstruction on mortality by induced bacteriaemia and endotoxaemia in a controlled study in rats;
2. to investigate the effect of relief of the biliary obstruction on this mortality induced by bacteriaemia;
3. to study the capacity of the reticulo-endothelial system, and some aspects of the cellular and humoral immunity in jaundiced rats.

3.2 Material and methods

3.2.1 Animals, anaesthesia and operative techniques

Animals

Male inbred Wag/Ry rats (W.R.) weighing between 220 and 340 grams and male inbred Brown Norway rats (B.N.) weighing between 220 and 360 grams were used throughout the study (TNO animal breeding facilities, Zeist, The Netherlands). They were housed in groups of 5, in cages, and were fed ad libitum on commercial chow (Hope Farms) and water (pH ± 3).

Anaesthesia

Rats were anaesthetized with ether as described in chapter 2. In the carbon clearance test and in the endotoxin clearance test anaesthetic Nembutal® (0.1 mg/100 gram body weight i.p.) was used after induction of anaesthesia with ether.

Production of biliary obstruction and sham operations

For permanent biliary obstruction division of the common bile duct between two ligatures was performed. In rats to be subjected to relief of the obstruction the common duct was clipped. Sham operated control rats were also obtained (see chapter 2).

Klebsiella pneumoniae serotype 4B, cultured for 20 hours at 37° in Todd Hewitt broth was used. Inocula of 10^5, 10^6, 10^7 and 10^8 Klebsiella pneumoniae were administered intraperitoneally in 4 groups of 3 non-operated rats. Mortality was examined after 24, 48 and 72 hours (table 12).

Table 12 Mortality after i.p. injection of 10^5, 10^6, 10^7 and 10^8 Klebsiella pneumoniae.

		Cumulative mortality		
Inoculum	No. of rats	< 24 h	<48 h	<72 h
10^5	3	0	0	0
10^6	3	0	0	0
10^7	3	0	2	3
10^8	3	3	0	0

Inocula of 10^7 Klebsiella pneumoniae were used for the further mortality studies. Blood cultures were taken after death. In one group bacteria were counted in the peripheral blood 30 hours after intraperitoneal injection of Klebsiella pneumoniae.
Escherichia Coli endotoxin 0.127 B8 (Difco) was also used in the mortality studies. The LD50 dose was determined in a pilot study (table 13) and this dose (3 mg/100 gram bodyweight) was injected intravenously via the penile vein in a singel bolus.

Table 13 Mortality after i.v. injection of 5 mg, 4 mg and 3 mg/100 gram body weight endotoxin.

		Cumulative mortality		
Dose	No. of rats	< 24 h	< 48 h	< 72 h
5 mg	6	6	0	0
4 mg	4	3	3	4
3 mg	4	0	2	2

3.2.3 Carbon clearance test

This test is based on the work of Biozzi, Halpern, Stiffel and Benacerraf (in Mononuclear phagocytes,ed. R. van Furth, 1970).
Colloidal carbon particles, which are stable in the bloodstream and uniform in size (Talens ink, Koninklijke Talens B.V. The Netherlands), were injected intravenously via the penile vein (Stuart et al 1973). The carbon particles are removed by the sessile intravasculair phagocytes in the liver and spleen. The Kupffer cells of the liver take up approximately 90% and the splenic macrophages 10% . Provided the dose is standardized, the rate of removal is a measure of reticulo-endothelial activity. Very small doses of colloid are a measure of hepatic blood flow rather than phagocytic function. If the blood concentration of carbon is plotted against time, the removal follows an exponential function for doses of carbon above the so-called critical dose. Above this critical concentration, the rate of phagocytosis depends on the initial concentration, and is proportional to the concentration in the blood, and inversely proportional to the amount of carbon already phagocytosed. The logarithm of blood concentration against time gives a straight line, the slope of which, the constant k, measures the rate of phagocytosis.
Within a given species, the same dose of colloid gives similar values of k. For each species, variations in k have been shown to be related to differences in weight of the liver and spleen. A corrected phagocytic index is a constant obtained from the following formula:

$$\alpha = \sqrt[3]{K}\,\frac{W}{WLS}$$

(W = bodyweight)

(WLS = weight of liver and spleen)

Carbon doses of 20 mg/100 gram bodyweight in W.R. rats and 40 mg/100 gram bodyweight in B.N. rats in a volume of 1cc saline solution (NaCl 0.9%) were administered via the penile vein. Blood samples (10 μl) were obtained before and 2, 5, 10, 15 and 20 minutes. after injection from a tail cut and mixed with 20 ml of a 0.1% solution of acetic acid. The optical density of each sample was determined in a spectrophotometer (Gilford 240) at 635 nm and compared to standards (blood density before injection of colloid) to determine blood concentration of colloidal carbon.
After the clearance test the animals were sacrificed and liver and spleen were removed. Liver and spleen were weighed individually and the phagocytic index (k-value) and the corrected phagocytic index (α -value) were calculated.

3.2.4 Endotoxin clearance test

Escherichia Coli endotoxin 0127.B8 (Difco) was labelled with ^{51}Cr. The method of preparation was based on the study of Braude et al (1955). 100 μ^{51}Ci $CrCl^3$ (spec. act: 350 mCi/mg Cr) in 0.9% w/v NaCl was added to 5 mg endotoxin and suspended in 5 ml buffer containing 5 mmol/l Na-Phosphate and 0.9% w/v NaCl (pH 7.0). After incubation for 24-36 hours at room temperature the endotoxin was precipitated by adding ethanol to a concentration of 80% by volume and separated by centrifugation at 2000 x for 15 minutes. After removal of the supernatant, the precipitate was dissolved again in 2 ml phosphate buffer (10 mM, pH 7.0). The sample was precipitated and dissolved at least 4 times with ethanol and buffer respectively until radioactivity in the supernatant was less than 0.1% of the radioactivity in the endotoxin precipitate. Labelled endotoxin was finally dissolved in 0.9% w/v NaCl containing 5 mM phosphate (pH 7.0). Specific activity was 15 μ Ci/mg endotoxin.
Approximately 0.3 mg of endotoxin in 1 ml was used in each experiment and was administered via the penile vein. Blood samples (50 μl) were obtained from a tail cut before and 2, 4, 6, 10 and 15 minutes after injection of the preparation. Radioactivity (counts/10 minutes) was measured by a gamma counter (Searle, model 1195R) in each blood sample. From these data the phagocytic index (k-value) was calculated in the same manner as in the carbon clearance test.

3.2.5 Serum biochemistry and haematology

Determinations of serum bilirubin were carried out in all experimental animals on a computer directed analyser by standard methods. Leucocytes and lymphocytes were counted with a Toa Electric cell counting system.

3.2.6 Cellular and humoral immunity tests

3.2.6.1 Leucocyte counts in peripheral blood

The number of lymphocytes and granulocytes were calcultated from the total and differential counts.

3.2.6.2 Number of lymphoid cells in various lymphoid organs

Rats were sacrificed and the spleens, thymi, mesenteric lymph nodes and left femurs were removed and placed in HBSS (Hank's balanced salt solution). The spleens, thymi and mesenteric lymph nodes were cut into small pieces with a pair of scissors and the fragments were pressed through a sieve consisting of nylon gauze.
Bone marrow cells were obtained by flushing the femurs with HBBS. A bent needle was inserted into the distal end of the shaft and moved slightly up and down to break the small bone spicules. Bone fragments were removed by filtering the cells throught a nylon sieve.
The number of lymphoid cells was counted.

3.2.6.3 Haemagglutination assay (HA)

After one week of biliary obstruction rats were immunized with 10^8 sheep red blood cells (SRBC) intraperitoneally. The anti-SRBC titers were determined one week later using the haemagglutination assay according to Mitchell et al (1969).

3.2.6.4 PHA stimulation

PHA-P (Pharmacia) in concentration of 0.1, 0.2, 0.4, 0.8 and 1.6 μgr/ml were used. 0.02 μl of each concentration was added to cultures. RPMI 1640 supplemented with L-glutamine, antibiotics, 10^{-5} M 2-mercaptoethanol and 10% foetal calf serum was used. $1\frac{1}{2}.10^5$ spleen cells in a total volume of 200 μl medium and PHA-P were incubated at 37° and 5% CO_2 in a humidified atmosphere.
Cells were harvested on day 4. 0.8 μCi of methyl-3H-Thymidine was added to each culture 14-16 hours before harvesting. Harvesting was performed on an automatic cell harvester (Dynatech) and 3H-uptake was measured on a B-counter.
The results were expressed as counts per minute.

3.2.7 Experimental groups

Group 10 (n=29, 15 experimental animals, 14 controls)
Two weeks after biliary obstruction or sham operation in W.R. rats inocula of 10^7 Klebsiella pneumoniae were injected intraperitoneally. Mortality was examined after 24, 48 and 72 hours.

Group 11 (n=25,15 experimental animals, 10 controls)
Two weeks after biliary obstruction or sham operation in W.R. rats the obstruction was relieved or sham operation was performed. One week later inocula of 10^7 Klebsiella pneumoniae were injected intraperitoneally. Mortality was examined after 24, 48 and 72 hours.

Group 12 (n=8, 5 experimental animals, 3 non-operated controls)
The number of bacteria in the peripheral blood was counted 30 hours after intraperitoneally injection of 10^7 Klebsiella pneumoniae in jaundiced W.R. rats 14 and 28 days after biliary obstruction and in control W.R. rats.

Group 13 (n=17, 9 experimental animals, 8 non-operated controls)
Carbon clearance was measured in jaundiced W.R. rats 5, 11 and 18 days after biliary obstruction and in control W.R. rats.

Group 14 (n=30, 17 experimental animals, 13 controls)
Carbon clearance was measured in jaundiced and control B.N. rats 2, 7, 14, 21 and 28 days after biliary obstruction or sham operation. After sacrificing the rats, livers and spleens were weighed and the corrected phagocytic index (α -value) was also calculated.

Group 15 (n=19, 9 experimental animals, 10 controls)
Two weeks after biliary obstruction or sham operation in W.R. rats endotoxin (3 mg/100 gram body weight) was injected intravenously. Mortality was examined after 24 hours.

Group 16 (n=17, 9 experimental animals, 8 controls)
Endotoxin clearance was measured in jaundiced and control W.R. rats 7 and 14 days after biliary obstruction or sham operation. After sacrificing the uptake of the radioactivity was measured 1 hour after the injection of ^{51}Cr endotoxin in the liver, spleen, kidneys, hart, thymus, lungs and left femur.

Group 17 (n=10, 5 experimental animals, 5 controls)
Immunological studies were performed in W.R. rats following 2 weeks of biliary obstruction or 2 weeks after sham operation. One week after the biliary obstruction or sham operation the rats were inoculated with 10^8 SRBC.

3.2.8 Statistical analysis

Variances of the mean is expressed as the standard error of the mean (SEM). Differences in mean were analyzed with Student's t-test, and mortality data were tested by the Fisher test. The Fisher test was applied to compare two independent proportions, where the asymmetry of the null distribution was taken into account in the computation of the two-sided tail-probability p_2.

3.3 Results

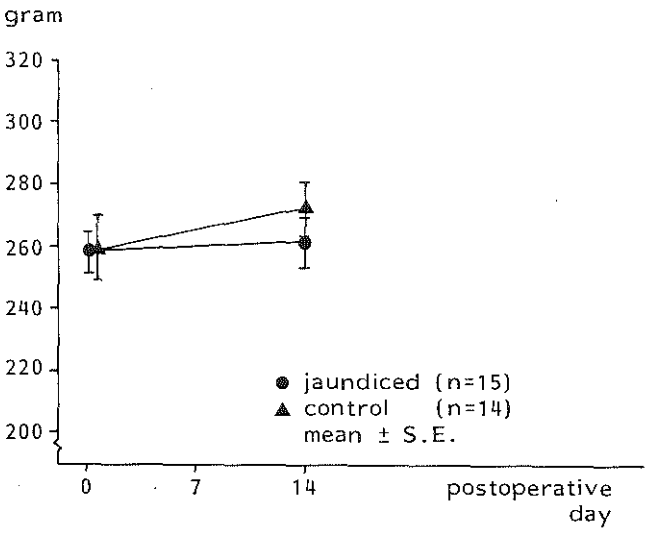
Group 10 Mortality study after injection of Klebsiella pneumoniae

Experimental rats (n=15)		Sham operated rats (n=14)	
day		day	
0	ligation and division of common bile duct	0	sham operation
14	i.p. injection of inoculum	14	i.p. injection of inoculum

Inoculum: 10^7 Klebsiella pneumoniae

There was no operative mortality in the experimental or sham operated group.
The body weights of the animals are given in fig. 27. During the study (14 days) the sham operated animals gained significantly more weight than the jaundiced animals ($p < 0.01$).

Fig. 27 Mean body weights of jaundiced and control rats in the mortality study (group 10).



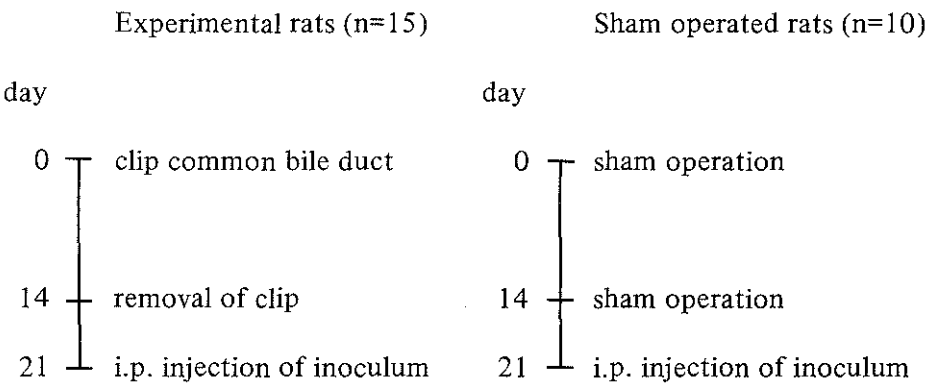
Mean serum bilirubin in the jaundiced rats 14 days after biliary obstruction was 153.1 ± 11.0 μmol/l.
A significant higher mortality was found in the jaundiced rats 24 and 48 hours after i.p. injection of 10^7 Klebsiella pneumoniae when compared with the sham operated rats (table 14). All dead animals had positive blood cultures for Klebsiella pneumoniae.

Table 14 Mortality after i.p. injection of 10^7 Klebsiella pneumoniae two weeks after biliary obstruction or sham operation.

		Cumulative mortality		
	N	< 24 h	< 48 h	< 72 h
Jaundiced rats	15	7*	12*	12
Sham operated rats	14	0	3	8

* $p < 0.01$

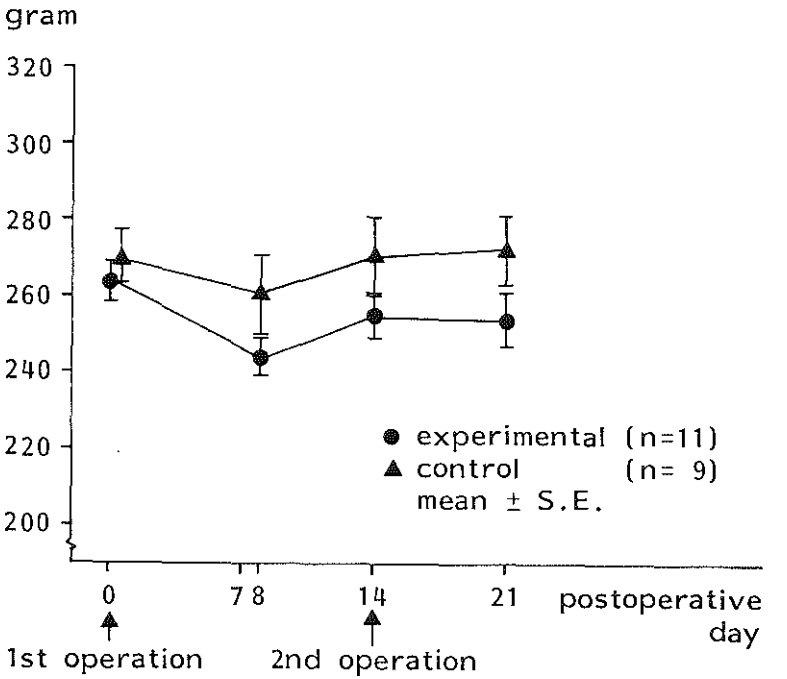
Group 11 Mortality study after injection of Klebsiella pneumoniae following biliary drainage.



Inoculum: 10^7 Klebsiella pneumoniae

Mortality in the experimental animals was 27% (4/15) and in the sham operated animals 10% (1/10).
The weights of the animals are shown in fig. 28. In the 14 days of biliary obstruction the experimental animals lost significantly more weight than the controls ($p < 0.001$). After relief of the biliary obstruction until the end of the study (7 days) weight curves more or less paralleled.

Fig. 28 Mean body weights of experimental and control rats in the mortality study (group 11).



Four-teen days after biliary obstruction mean serum bilirubin was 162.2 ± 21.9 μmol/l. After relief of the obstruction serum bilirubin level returned to normal values within 7 days.
Mortality after injection of 10^7 Klebsiella pneumoniae was not significantly different in the experimental and the control group after relief of the obstruction (table 15). All dead animals had positive blood cultures for Klebsiella pneumoniae.

Table 15 Mortality after i.p. injection of 10^7 Klebsiella pneumoniae one week after relief of the biliary obstruction.

		Cumulative mortality		
	N	< 24 h	< 48 h	< 72 h
Experimental rats	11	1	2	3
Sham operated rats	9	0	1	2

Group 12 Bacteria counts in peripheral blood

In this study the number of bacteria in 1 ml peripheral blood 30 hours after injection of 10^7 Klebsiella pneumoniae was counted 2 and 4 weeks after biliary obstruction.
Bacteria counts in the jaundiced rats were not found to be significantly higher than in the control rats (table 16).

Table 16 Number of bacteria in 1 ml peripheral blood 30 hours after injection of 10^7 Klebsiella pneumoniae.

	N	rat 1	rat 2	rat 3
Jaundice of 2 weeks duration	3	0	10	2.6×10^3
Jaundice of 4 weeks duration	2	0	8.1×10^5	–
Controls	3	0	75	1.2×10^5

Group 13 Carbon clearance test in W.R. rats

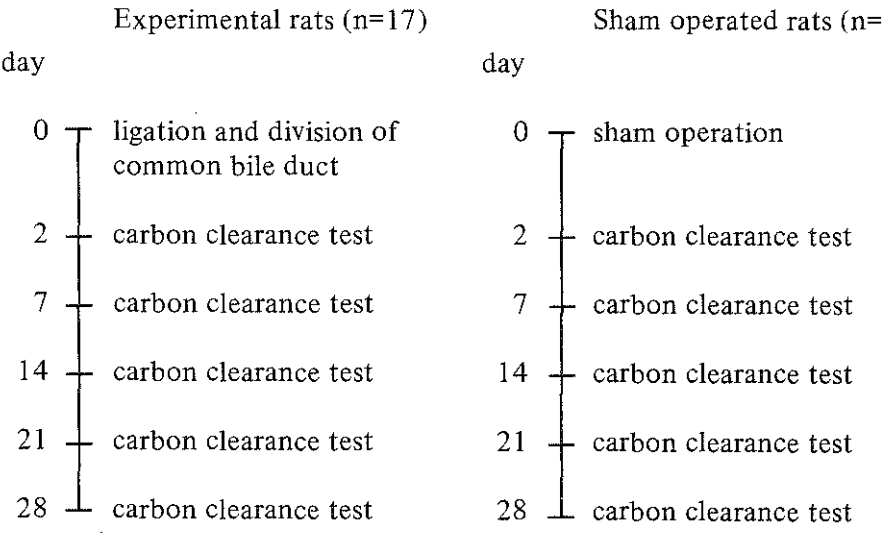
Clearance of carbon particles by the reticulo-endothelial system was measured at day 5, 11, 18 after biliary obstruction and compared with the

clearance in non-operated controls. It was found that the phagocytic index (k-value) was significantly increased in the jaundiced rats (table 17).

Table 17 Carbon clearance test in W.R. rats.

Duration of obstruction	N	Phagocytic index (k-value)	Statistical analysis
0 days (controls)	8	0.02 ± 0.004	
5 days	3	0.07 ± 0.006	$p < 0.001$
11 days	3	0.08 ± 0.012	$p < 0.05$
18 days	3	0.06 ± 0.0	$p < 0.05$

Group 14 Carbon clearance test in B.N. rats



Mortality in the jaundiced animals was 24% (4/17) and in the sham operated animals 0% . Carbon clearance was tested at day 2, 7, 14, 21 and 28 after ligation and division of the common bile duct and after sham ligation.

Phagocytic index (k-value) in jaundiced rats was significantly increased after 7, 14, 21 and 28 days of biliary obstruction (fig. 29). Spleen weights (fig. 30) and liver weights (fig. 31) were significantly increased in 14, 21 and 28 days jaundiced rats as compared with controls. The corrected phagocytic index (α -value) of the jaundiced rats was significantly decreased after 14 and 21 days of biliary obstruction as compared with the sham operated rats (fig. 32).

Fig. 29 Phagocytic index (k-value) in rats measured 2, 7, 14, 21 and 28 days after biliary obstruction or sham operation (group 14).

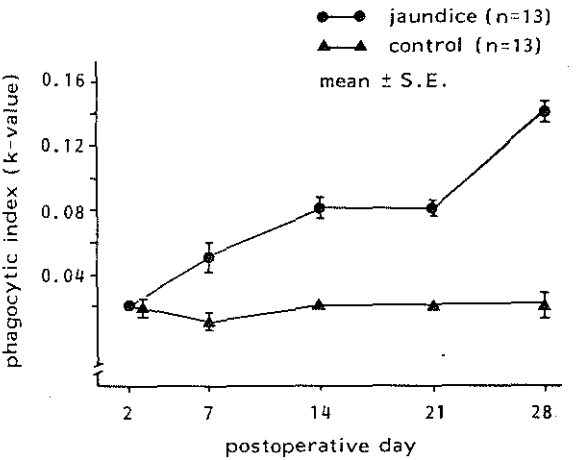


Fig. 30 Mean spleen weights of jaundiced and control rats (group 14).

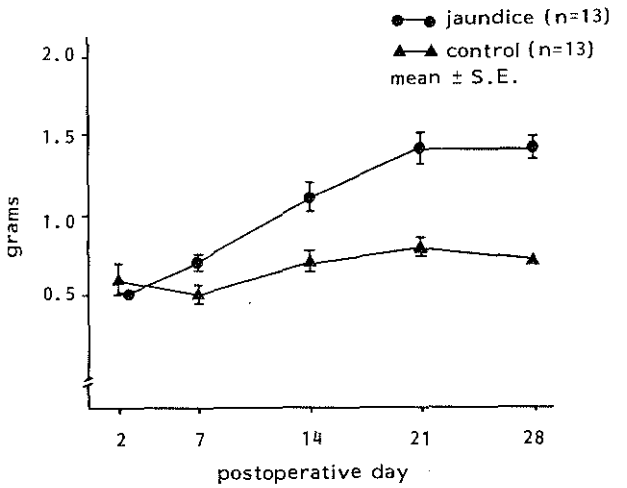


Fig. 31 Mean liver weights of jaundiced and control rats (group 14).

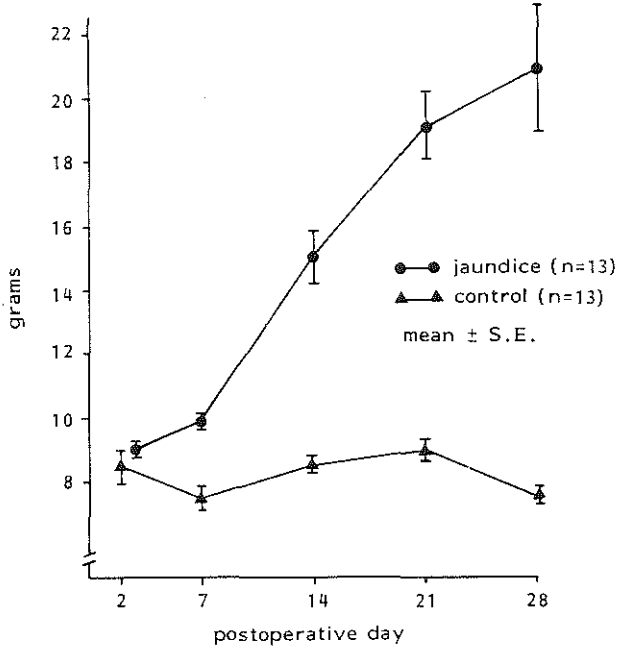
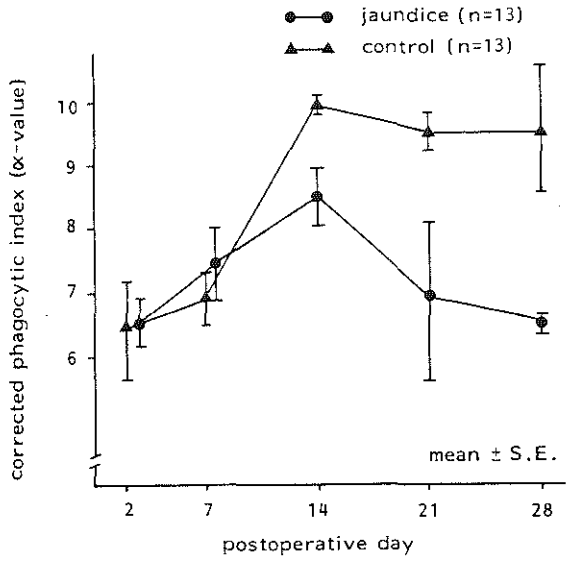


Fig. 32 Corrected phagocytic index (α -value) in rats measured 2, 7, 14, 21 and 28 days after biliary obstruction or sham operation (group 14).



A summary of the results of this study is given in table 18.

Table 18 Carbon clearance test in B.N. rats.

Experimental animals

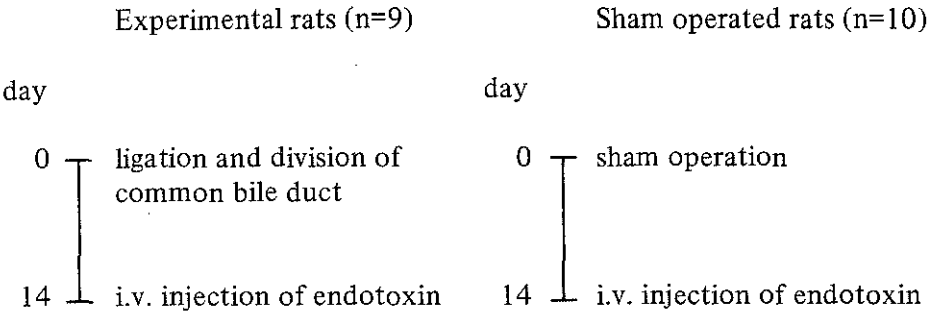
duration of obstruction	no. of jaundiced rats	weight loss (grams)	liver weight (grams)	spleen weight (grams)	phagocytic index (k-value)	corrected phagocytic index (α -value)
2 days	3	15.3±3.2	9.0±0.2	0.5±0.0	0.02±0.0 *	6.5±0.3
7 days	3	9.5±0.5	9.8±0.3	0.7±0.03	0.05±0.01 **	7.4±0.6 *
14 days	3	44.3±6.2	15.0±0.7	1.1±0.1	0.08±0.003 *	8.5±0.4 *
21 days	2	27.0±3.0	19.2±1.1	1.4±0.1	0.08±0.02 *	6.9±1.2
28 days	2	4.5±3.5	20.8±1.8	1.5±0.05	0.14±0.03	6.5±0.06

Sham operated animals

days after sham operation	no. of sham operated rats	weight loss	liver weight	spleen weight	phagocytic index (k-value)	corrected phagocytic index (α -value)
2 days	2	3.5± 1.5	8.5±0.6	0.6±0.1	0.02±0.005	6.4±0.8
7 days	3	+2.3± 5.2	7.5±0.4	0.5±0.03	0.01±0.003	6.9±0.4
14 days	3	15.3± 7.9	8.7±0.1	0.7±0.03	0.02±0.0	9.9±0.04
21 days	2	34.0±10.1	9.1±0.2	0.8±0.02	0.02±0.0	9.6±0.2
28 days	3	+2.3± 3.8	7.5±0.1	0.7±0.0	0.02±0.009	9.6±1.0

* $p < 0.05$ ** $p < 0.01$ mean ± S.E.

Group 15 Mortality study after injection of endotoxin



E.Coli endotoxin (3 mg/100 gram body weight)

There was no operative mortality in either group. Mean serum bilirubin 14 days after biliary obstruction was 152.4 ± 16.8 μmol/l.
A significantly higher mortality was found in the jaundiced rats 24 hours after injection of E.Coli endotoxin (3 mg/100 gram body weight) than in the sham operated rats (table 19).

Table 19 Mortality 24 hours after i.v. injection of E.Coli endotoxin two weeks after biliary obstruction or sham operation.

	N	Mortality
Jaundiced rats	9	9*
Sham operated rats	10	1

* $p < 0.001$

Group 16 Endotoxin clearance test in W.R. rats

Experimental rats (n=9)		Sham operated rats (n=8)	
day		day	
0	ligation and division of common bile duct	0	sham operation
7	endotoxin clearance test	7	endotoxin clearance test
14	endotoxin clearance test	14	endotoxin clearance test

Clearance of E.Coli endotoxin by the reticulo-endothelial system was measured at day 7 and 14 after biliary obstruction and compared with the clearance of the sham operated rats.
Phagocytic index (k-value) was significantly increased in the jaundiced rats 7 and 14 days after biliary obstruction as compared with the sham operated controls (table 20).
The uptake of radioactivity after injection of ^{51}Cr endotoxin was decreased in the spleens and the femurs of the jaundiced rats (table 21).

Table 20 Endotoxin clearance test in W.R. rats.

Experimental animals		
duration of obstruction	no. of jaundiced rats	phagocytic index (k-value)
7 days	5	0.08 ± 0.007*
14 days	4	0.15 ± 0.009**

Sham operated animals

days after sham operation	no. of sham rats	
7 days	4	0.06 ± 0.006
14 days	4	0.09 ± 0.005

* $p < 0.05$ ** $p < 0.001$ mean ± S.E.

Table 21 Uptake of radioactivity after injection of ^{51}Cr endotoxin in various organs

	Jaundiced rats (n=4)	Sham operated rats (n=4)
Liver	81.7 ± 3.1 %	82.8 ± 0.3 %
Spleen	6.3 ± 0.7 % **	11.7 ± 0.4 %
Lungs	11.2 ± 2.9 %	4.9 ± 0.2 %
Kidneys	0.3 ± 0.03 %	0.2 ± 0.02%
Hart	0.3 ± 0.3 %	0.07 ± 0.03%
Thymus	0.08 ± 0.04 %	0.03 ± 0.01%
Femur left	0.07 ± 0.02 % *	0.2 ± 0.03%

* $p < 0.01$ ** $p < 0.001$ mean ± S.E.

Group 17 Immunological studies

	Experimental rats (n=5)		Sham operated rats (n=5)
day		day	
0	ligation and division of common bile duct	0	sham operation
7	inoculation with 10^8 SRBC	7	inoculation with 10^8 SRBC
14	immunological studies	14	immunological studies

Jaundiced rats, after an obstruction period of 14 days, had significant increases in peripheral blood leucocytes, number of lymphocytes in the spleen, lymphocytes in the mesenteric lymph nodes (table 22) and an increase in lymphocytes PHA-stimulation of the total spleen (table 23).

Table 22 Immunological studies in jaundiced rats and control rats 14 days after biliary obstruction or sham operation.

Leucocyte counts	jaundiced rats	control rats	stat. anal.
total leucocytes	30.6 ± 2.2.10^6/ml	9.2 ± 0.2.10^6/ml	$p < 0.001$
lymphocytes	20.9 ± 2.0 "	8.4 ± 0.2 "	$p < 0.001$
granulocytes	10.4 ± 1.9 "	0.7 ± 0.1 "	$p < 0.01$
Lymphoid cells in various organs			
spleen	59.4 ± 9.6.10^6/ml	28.5 ± 2.0.10^6/ml	$p < 0.05$
thymus	537.5 ±45.6 "	506.3 ±39.5 "	n.s.
mesenteric lymph nodes	9.4 ± 0.8 "	5.8 ± 0.5 "	$p < 0.05$
left femur	9.4 ± 3.6 "	10.4 ± 1.3 "	n.s.
Haemagglutination assay			
mean titers	17 ± 5.7	16 ± 11.3	n.s.

mean ± S.E.

Table 23 PHA-stimulation in jaundiced and control rats 14 days after biliary obstruction or sham operation.

Stimulation per 150.000 cells		
concentrations μgr/ml	jaundiced rats	sham operated rats
0.1	2.942 ± 355 cpm	19.337 ± 3.579 cpm
0.2	8.924 ± 1.284 "	51.418 ± 8.145 "
0.4	104.206 ± 11.305 "	93.948 ± 11.096 "
0.8	83.289 ± 14.447 "	112.712 ± 8.596 "
1.6	25.929 ± 2.866 "	130.234 ± 18.853 "

Total spleen

concentration μgr/ml	jaundiced rats	sham operated rats
0.1	$1.2 \pm 0.3.10^7$ cpm	$3.6 \pm 0.6.10^7$ cpm
0.2	3.6 ± 0.8 " "	9.7 ± 1.6 " "
0.4	39.5 ± 4.0 " "	17.8 ± 2.2 " "
0.8	34.5 ± 9.0 " "	21.6 ± 2.8 " "
1.6	10.8 ± 2.7 " "	24.7 ± 3.9 " " *

* $p < 0.001$ mean $\pm$ S.E.

3.4 Discussion

Infection after biliary surgery is still an important cause of morbidity and mortality, despite the progress in prevention and treatment of microbial diseases. In obstructive jaundice infection may occur from operating in an infected area. In addition the performance of anastomoses carries the risk of intra-abdominal leakage leading to peritonitis. It is not clear from the literature whether patients with obstructive jaundice have an impaired resistance against infections.

In the present study an animal model is used to study the resistance against infection in obstructive jaundice. In this model jaundiced rats were intraperitoneally injected with Klebsiella pneumoniae, as these bacteria are frequently cultured in the infected biliary tract in man. Inocula of 10^7 Klebsiella pneumoniae were used, because in a pilot study this dose was found to be the most useful one.

The mortality after i.p. injection of 10^7 Klebsiella pneumoniae was significantly higher in jaundiced rats 2 weeks after biliary obstruction than in sham operated rats. The mortality was thought to be caused by septicaemia with Klebsiella pneumoniae, because Klebsiella pneumoniae was cultured from the blood of all rats that died. After a biliary obstruction of 2 weeks, followed by a biliary drainage during 1 week the mortality after i.p. injection of 10^7 Klebsiella pneumoniae was not different in the experimental and sham operated rats.

Endotoxin may play an important role in the pathophysiology of infections. In experimental studies in jaundiced rats an increased mortality was found after injection of a single dose of E.Coli endotoxin (Wardle and Wright 1970, Holman and Rikkers 1982). The increased mortality after injection of E.Coli endotoxin was confirmed in this study since 100% of the jaundiced rats died within 24 hours, whereas only 1 of 10 sham operated rats (10%) died.

Endotoxin is a component of the cell wall of gram negative bacteria and is liberated during autolysis of these bacteria. However, endotoxin may also be liberated by multiplying bacteria (Jorgensen and Smith 1974, Munford 1978).

Bacteria and endotoxin are for a great deal removed from the blood by the reticulo-endothelial system (Freedman 1960, Saba 1970). Kupffer cells constitute about 80 to 90% of fixed macrophages of the reticulo-endothelial system. Kupffer cells have the capacity to endocytose by pinocytosis and phagocytosis. Endotoxin is cleared in the liver by pinocytosis, whereas bacteria are cleared by phagocytosis (Jones and Summerfield 1982).

A decreased phagocytosis in patients with obstructive jaundice has been

suggested in a study of Drivas et al (1976). Nine of the 20 patients in this study had a decreased phagocytosis. However, 5 of these patients had a malignancy or liver metastases which may themselves cause a decreased phagocytosis (Old et al 1960). Patients with malignancy may also have loss of weight, which can cause a decreased phagocytosis (Juhlin 1958).
In this experimental study, involving 2 strains of rats, a significant increase of the clearance of carbon particles was found. These results are in accordance with the results of Halpern et al (1957) and Lázár (1972).
However, a decreased clearance of carbon particles was reported by Holman and Rikkers (1982). These authors used the corrected phagocytic index. This index is used to compare the phagocytosis in different species of animals, because of the differences in liver and spleen weight in proportion to the total body weight (Stiffel et al 1970).
It was shown in the present study that the liver and spleen weights increased in jaundiced rats, whereas the total body weights decreased. Thus the corrected phagocytic index (α-value) may drop, whereas the total capacity (k-value) can be increased.This drop in α -value was also found in our study.
The amount of bacteria in the peripheral blood 30 hours after intraperitoneal injection of 10^7 Klebsiella pneumoniae was not increased in jaundiced rats 2 and 4 weeks after biliary obstruction as compared with control rats. This indicates a normal phagocytosis of bacteria from the blood.
Endotoxin is also mainly cleared by the Kupffer cells. The spleen does not seem to play an important role in the detoxification of endotoxin since after splenectomy no increased mortality was found in rats after injection of endotoxin as compared with sham operated rats (Farrar and Corwin 1966, Holman and Rikkers 1982).
It has been shown that due to the absence of bile acids there is an increased absorption of endotoxin from the intestine (Kocsár et al 1969, Bailey 1976). The increased mortality in jaundiced rats may be explained by both an increased absorption of endotoxin from the intestine and a decreased clearance of endotoxin by the liver (Wardle and Wright 1970). However, in this study an increased clearance of ^{51}Cr labelled endotoxin was found in jaundiced rats 7 and 14 days after biliary obstruction as compared with sham operated rats. So the increased mortality does not seem to be caused by a decreased pinocytosis by the Kupffer cells.
It is more likely that the detoxification of endotoxin after pinocytosis is impaired or that liberation of toxic intermediary products, that arise after contact of endotoxin with the Kupffer cells (Nolan 1981), is the cause of the increased mortality. Examples of these intermediary products are prostaglandins and thromboxane. A rise of the level of prostaglandins in endotoxin shock has been reported in sheep (Cefalo et al 1980) and in rats

(Cook et al 1980).
Anti-prostaglandins, such as aspirin, indomethacin and 13-azaprostanoic acid, can decrease the mortality after injection of endotoxin in experimental animals (Fletcher et al 1977, Cook et al 1980). In jaundiced rats endotoxaemia was associated with significant elevations of thromboxane and prostaglandins in a study by Fletcher et al (1982). Pretreatment with indomethacin or prostacyclin produced significant improvement in the survival of jaundiced rats following endotoxin administration.
An other possibility is a reduction of intracellular killing of the phagocytosed bacteria caused by unknown factors. Katz et al (1984) found a significant decrease in the trapping of bacteria (E.Coli) in the liver and a significant increase in bacterial trapping in the lungs of jaundiced rats as compared with sham operated rats. These authors suggest that biliary obstruction decreases hepatic phagocytosis and increases pulmonary localization of viable E.Coli. However, a decreased uptake of ^{51}Cr labelled E.Coli endotoxin in the liver of jaundiced rats could not be demonstrated in the present study.
In order to get more insight into the immunological state of jaundiced rats some immunological studies were performed. No indications were found for a decreased resistance to infection, in fact the immunological system of jaundiced rats seemed to be stimulated: granulocytes and lymphocytes counts were increased in the peripheral blood. Lymphocytes in mesenteric lymph nodes and spleen were also increased in number. The PHA stimulation of the total spleen was raised in jaundiced rats.
In patients with obstructive jaundice increased function of polymorph leucocytes has been found (Wardle and Williams 1980) which also suggests a stimulation of the immunological system.

CHAPTER 4
BILIARY OBSTRUCTION AND RENAL FUNCTION

4.1 Introduction

Acute renal failure is believed to occur more frequently after operations on deeply jaundiced patients than after operations of comparable magnitude on patients who are not jaundiced (Williams et al 1960, Dawson 1965, Bailey 1976, Allison et al 1979). This postoperative complication after biliary surgery is significantly associated with mortality (Blamey et al 1983, Dixon et al 1983).

The exact mechanism of postoperative renal failure in patients or experimental animals with obstructive jaundice remains to be identified, but three factors may play a role:

1. The toxic effects of bilirubin or bile acids.
2. Changes in haemodynamics, both systemic and renal.
3. The effects of endotoxin.

1. The toxic effects of bilirubin or bile acids.

In Wistar rats 60 minutes of renal ischaemia, following a seven-day occlusion of the common bile duct, was found to produce a very severe renal lesion, which was fatal in 60% of the animals (Dawson 1964). This could not be reproduced by others (Baum et al 1969) in similar experiments using Gunn rats, that have a congenital absence of the enzyme glucuronyl transferase (Carbone and Grodsky 1957) and thus have an unconjugated hyperbilirubinaemia which remains unaffected by bile duct ligation.

It therefore seems likely that bilirubin diglucuronide, and not bile salts, unconjugated bilirubin or retained bile products are the cause of an increased sensitivity of the renal tubules to ischaemia.

Histological changes in dog kidneys have been reported after choledochocaval shunts in a study by Masumoto and Masuoka (1980). A marked deposition of bile pigment and exfoliation of epithelial cells was found in addition to swelling of epithelial cells in the proximal tubules. The protoplasm of proximal tubular epithelial cells protruded into the tubular lumen, causing a narrowing of the lumen. The authors conclude that these changes make it likely that bile pigment or accompanying elements were responsible for the disruption of renal parenchyma.

In another study in rats renal insufficiency that lasted for 4 days developed after infusion of sodium cholate and sodium taurocholate, followed by a 30 minute period of renal ischaemia. Rats that received bile acid infusions without renal ischaemia, and rats that received renal ischaemia without bile acid infusions did not develop renal insufficiency. Furthermore, rats that

received infusions of bilirubin before the period of renal ischaemia did not develop renal insufficiency (Aoyagi and Lowenstein 1968).
Bomzom et al (1979) studied the effects of bile acids on renal blood flow in baboons by infusing sodium taurocholate into the renal artery. The dose was equal to the blood concentration of bile acids occurring in obstructive jaundice. The intrarenal infusion of sodium taurocholate did not cause any significant changes in mean renal blood flow, cortical blood flow, or the percentage distribution of radioactivity to the cortex when compared with control values.

2. Changes in haemodynamics in obstructive jaundice.

The renal damage seen in postoperative renal failure in obstructive jaundice is acute tubular necrosis similar to that which complicates circulatory failure (Dawson 1968). General anaesthesia and surgical procedures are known to alter renal perfusion. Ligation of the common bile duct in laboratory animals may cause hypotension or an increased propensity to hypotension after minor bleeding (Cattel and Birnstingl 1967, Williams et al 1960, Aarseth et al 1979).
Shashe et al (1976) found a significant rise in mean cardiac index and a significant fall in mean total peripheral resistance 5 weeks after bile duct ligation in dogs. A significant decrease in cardiac output was also reported in rabbits after bile duct ligation. Renal blood flow and glomerular filtration rate were also reduced. Jaundiced rabbits had more marked reduction in mean blood pressure and mean renal blood flow after bleeding than sham operated rabbits (Hishida et al 1980).

3. Effects of endotoxin

Impaired renal function due to endotoxin has been reported in man (Wilkinson 1974, Bailey 1976) and experimental animals (Hinshaw et al 1961, Gillenwater et al 1963). This impairment is attributed to renal vasoconstriction that leads to a decreased renal perfusion.
The vasoconstriction is possibly due to both a direct local action of endotoxin on the renal vascular bed and to a secondary systemic release of vasoconstrictor agents during the systemic hypotension.
In an attempt to separate the haemodynamic from a possible nephrotoxic action of endotoxin the local effect of Salmonella thyphosa endotoxin was studied in a dog kidney perfused at a constant blood flow. In the kidney with constant blood flow no alterations in renal function after systemic or

local endotoxin injection were found, indicating that the main effect of endotoxin on the kidney is secondary to haemodynamic changes (Gillenwater et al 1963).
In patients with obstructive jaundice renal failure was associated with a positive limulus lysate assay, indicating endotoxaemia (Wilkinson et al 1976, Wardle 1975, Bailey 1976).
Endotoxin is probably cleared by the reticulo-endothelial cells after absorption from the gastro-intestinal tract (Greene et al 1961, Mori et al 1973). Bile salts that can prevent endotoxin absorption from the gut are absent in obstructive jaundice (Kocsár et al 1969, Bailey 1976). Increased absorption of endotoxin, together with impaired hepatic clearance (Wardle and Wright 1970, Drivas et al 1976) may therefore account for the endotoxaemia in obstructive jaundice.
The effect of preoperative bile salt administration on postoperative renal function in patients with obstructive jaundice was studied by Evans et al (1982) and Cahill (1983). A statistically significant decrease in renal failure was found in patients treated with bile salts before the operation. The incidence of endotoxaemia in untreated jaundiced patients was significantly higher than in non-jaundiced patients.
The difference disappeared after prophylactic oral administration of bile salts.

In the present study the effects of biliary obstruction on renal function were studied in rats by using a method to measure kidney functions repeatedly in the same animal.

4.2 Material and methods

4.2.1 Animals, anaesthesia, operative techniques

Animals

Male random bred Wistar rats (TNO Animal breeding facilities, Zeist, The Netherlands) weighing 360 to 460 grams were used throughout the study. They were housed in groups of 5, in cages, and were fed ad libitum on commercial chow (Hope Farms) and water (pH ± 3).

Anaesthesia

Rats were anaesthesized with ether as described in chapter 2. In the determination of the renal clearance anaesthetic Nembutal® (0.1 mg/100 gram body weight i.p.) was used after induction of anaesthesia with ether.

Production of biliary obstruction and sham operations

For permanent biliary obstruction the common bile duct was divided between two ligatures. Sham operated rats served as controls
(see chapter 2).

4.2.2 Kidney function test

The variables of kidney function examined included serum creatinine, blood urea nitrogen and renal clearance. Both the glomerular filtration rate (GFR) and the effective renal plasma flow (ERPF) were determined simultaneously (Layzell and Miller 1975, Provoost et al 1983). The GFR was measured using an intravenous injection of ^{51}Cr EDTA and a single timed blood sample taken 60 minutes after the injection.
The ERPF was determined by an identical technique, carried out simultaneously with the GFR measurement, using a intravenous injection of ^{125}I-iodohippurate (^{125}I-IOH). The clearances were then calculated from the formula:

$$C = \frac{V}{t} \cdot \frac{\ln P_o}{P_t}$$

where C is the clearance of ^{51}Cr EDTA (GFR) or ^{125}I-IOH (ERPF) (ml/min). V is the distribution volume of each substance (ml). P_t is the amount of radioactivity (cpm/ml) in the plasma sample taken at t = 60 min, and P_o is I/V, in which I is the injected amount of radioactivity (cpm).

4.2.3 Serum biochemistry

Determinations of levels of serum bilirubin, albumin, alkaline phosphatase activity, glutamate-oxalo-acetate transaminase (SGOT), gamma glutamyl

transpeptidase (γGT), creatinine, blood urea nitrogen were carried out on a computer directed analyzer (Gilford System 3500) by standard methods. The electrolytes, sodium and potassium, were determined by flame photometry (Klina Flame, Beckman).

4.2.4 Experimental group

Group 18 (n=30, 23 experimental animals, 7 controls)

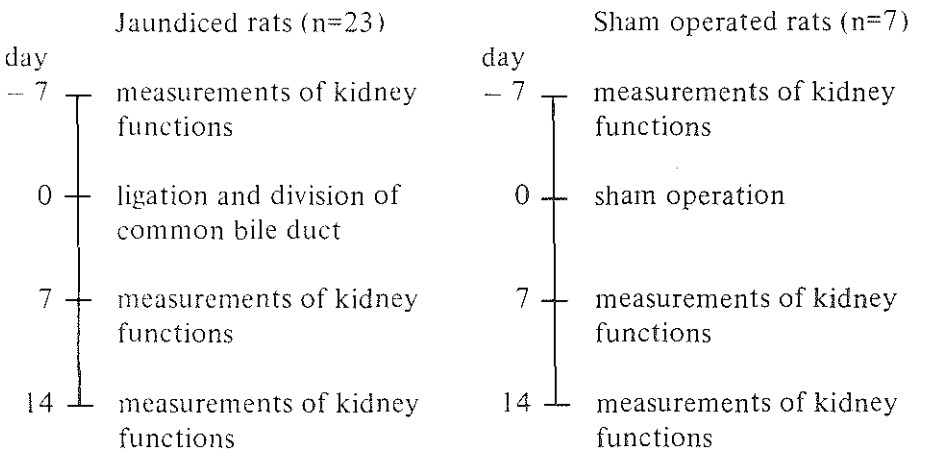
Serum creatinine, blood urea nitrogen, sodium, potassium, GFR and ERPF were estimated preoperatively and one and two weeks after bile duct division or sham operation. Bilirubin, alkaline phosphatase, SGOT, γ GT and albumin were determined one and two weeks after the operation.

4.2.5 Statistical analysis

Variance of the mean is expressed as the standard error of the mean (SEM). Differences in means were analyzed with Student's t-test for unpaired data.

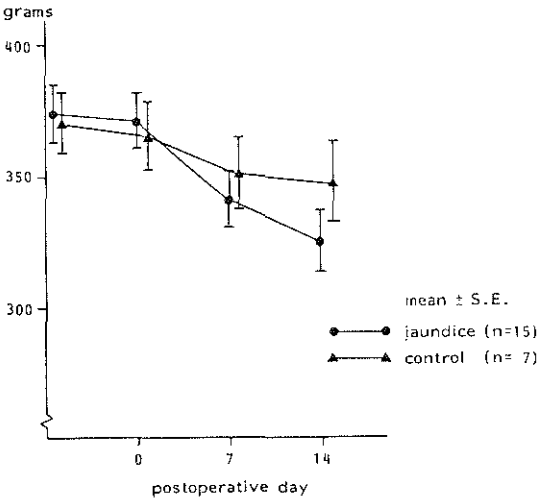
4.3. Results

Group 18



Mortality in the jaundiced animals was 35% (8/23) and 0% in the sham operated group. These eight rats died within a few days either from the effects of anaesthesia or from biliary peritonitis and have been excluded from further analysis.
The mean body weights of the animals are given in fig. 33. During the study (21 days) the experimental animals lost significantly more weight than the sham operated animals ($p < 0.05$).

Fig. 33 Mean body weights of jaundiced and control rats in group 18.



The results of the kidney function tests and the electrolytes are given in table 24 and the liverfunctions in table 25.
Preoperatively and one week after biliary obstruction or after sham operation the kidney functions and electrolytes did not differ in the two groups. Serum bilirubin, alkaline phosphatase, SGOT and γ GT were significantly increased one week after biliary obstruction. Serum albumin did not differ in the two groups.
Two weeks after biliary obstruction or sham operation the GFR was significantly decreased by 34% in the jaundiced animals as compared with the control animals ($p < 0.001$, fig. 34). The GFR per 100 gram body weight was decreased by 29% in the jaundiced animals ($p < 0.01$). ERPF was decreased by 20% in the jaundiced animals ($p < 0.05$, fig 35) and the filtration fraction (FF) was also decreased by 20% in the jaundiced animals as compared with the sham operated animals ($p < 0.01$).
However, serum creatinine, blood urea nitrogen and electrolytes were not different. Serum bilirubin, alkaline phosphatase and γ GT were increased in the jaundiced animals, but serum albumin was not different in both groups.

Table 24 Kidney functions and electrolytes (group 18).

	Preoperative		1 week postoperative		2 weeks postoperative	
	exp. (n=15)	sham (n=7)	exp. (n=15)	sham (n=7)	exp. (n=15)	sham (n=7)
GFR ml/min	2.69 ± 0.06	2.63 ± 0.07	2.20 ± 0.08	2.34 ± 0.10	1.76 ± 0.15 ***	2.67 ± 0.13
GFR/100g/ ml/min	0.73 ± 0.02	0.72 ± 0.03	0.65 ± 0.02	0.67 ± 0.02	0.55 ± 0.05 **	0.77 ± 0.02
ERPF ml/min	5.09 ± 0.07	4.92 ± 0.12	5.09 ± 0.10	5.05 ± 0.15	4.30 ± 0.27 *	5.39 ± 0.23
ERPF/100g/ ml/min	1.37 ± 0.03	1.33 ± 0.04	1.51 ± 0.05	1.45 ± 0.06	1.35 ± 0.10	1.56 ± 0.05
FF	0.53 ± 0.01	0.53 ± 0.01	0.43 ± 0.01	0.46 ± 0.01	0.40 ± 0.02 **	0.50 ± 0.01
Creat (μmol/l)	46.0 ± 4.4		56.8 ± 6.9	45.6 ± 6.5	61.9 ± 3.3	60.3 ± 7.8
Ureum(μmol/l)	6.0 ± 0.6		8.1 ± 0.8	7.1 ± 0.6	8.5 ± 1.1	8.0 ± 1.5
Na (μmol/l)	146.0 ± 3.0		146.7 ± 0.9	147.3 ± 0.6	146.9 ± 1.4	148.9 ± 1.2
K (μmol/l)	4.8 ± 0.2		4.8 ± 0.1	4.8 ± 0.1	4.8 ± 0.1	4.5 ± 0.2

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ mean ± S.E.

Table 25 Liverfunctions in group 18.

	1 week postoperative		2 weeks postoperative	
	exp. (n=15)	sham (n=7)	exp. (n=15)	sham (n=7)
Bilirubin	108.8 ± 19.2	2.6 ± 1.3	75.5 ± 16.8	16.0 ± 6.2
Alk. phospha-tase	602.6 ± 33.0	220.8 ± 12.4	599.7 ± 45.8	317.1 ± 30.4
SGOT (U/l)	227.3 ± 28.7	75.1 ± 18.6	209.6 ± 22.4	89.2 ± 12.6
γGT (U/l)	13.4 ± 2.1	3.6 ± 0.7	19.3 ± 2.7	8.0 ± 1.9
Albumin (g/l)	28.9 ± 0.5	26.6 ± 0.8	26.4 ± 1.3	25.5 ± 0.8

mean ± S.E.

Fig. 34 Mean glomerular filtration rate in jaundiced and control rats.

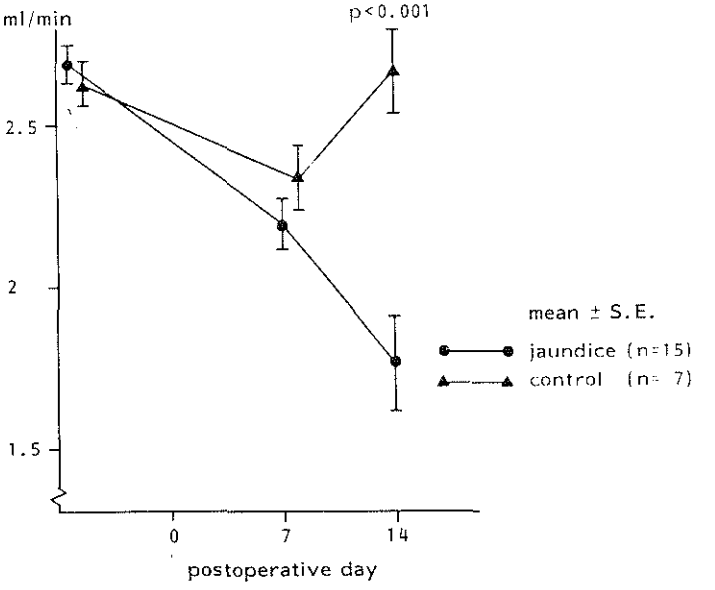
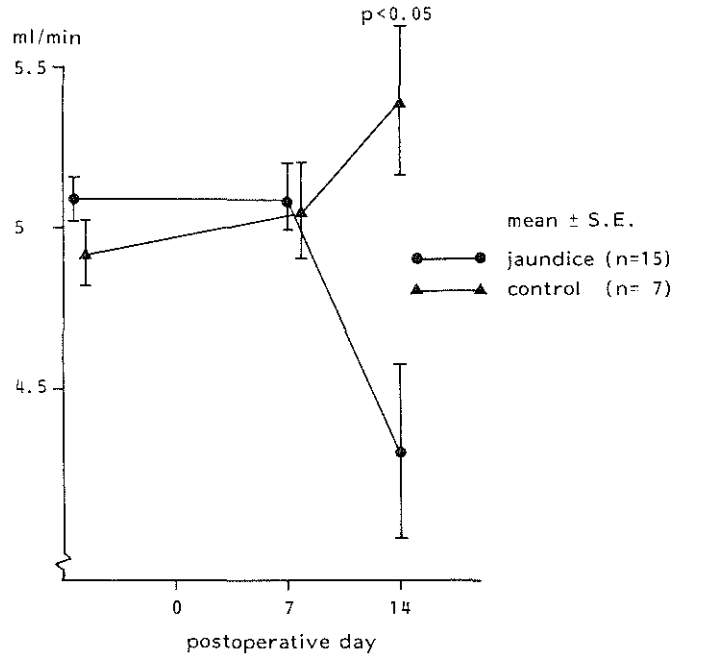


Fig. 35 Mean effective renal plasma flow in jaundiced and control rats.



4.4 Discussion

In earlier studies significant renal failure in experimental animals with biliary obstruction was found after induction of "extra factors". Examples of these "extra factors" are renal ischaemia (Dawson 1964), systemic hypotension (Williams et al 1960, Cattell and Birnstingl 1967) or infusion of endotoxin (Gillenwater et al 1963).
In experimental studies in rats, in which only an obstruction of the biliary tract is introduced, the results of kidney function tests are not unanimous. The results of the changes in glomerular filtration rate (GFR), (effective) renal plasma flow ((E)RPF) and filtration fraction (FF) in rats with obstructive jaundice without "extra factors" compared with sham operated rats, from 5 different studies, are summarized in table 26.

Table 26 Results of kidney function tests in 5 studies.

Author	duration of jaundice	method	GFR	(E)RPF	FF
Bank and Aynedjian (1975)	10-14 days	inuline/PAH urine clearance	=	=	=
Yarger (1976)	4-14 days	" "	⇓	⇓	=
Allison et al (1978)	21-28 days	" "	=	⇑	⇓
Better et al (1980)	8-10 days	" "	=	⇓	⇑
Present study	14 days	^{51}Cr EDTA/ ^{125}I-IOH	⇓	⇓	⇓

The advantage of the present study is that the GFR and ERPF can be determined several times in the same animal. So the changes of kidney functions at different times after biliary obstruction can be studied and a comparison can be made with the preoperative values. A disadvantage of

this study is that for the calculation of the GFR and ERPF a plasma clearance is used and not the standard inulin/PAH urine clearance. For the determination of the latter radical measures in the experimental animals are necessary, such as the introduction of an ureter catheter and intra-arterial catheters. These measures can influence the results of the kidney function tests because of haemodynamic changes.
In the present study decreased GFR and ERPF were found in rats with biliary obstruction of 2 weeks duration. However, this decrease of kidney functions was too small to influence the serum creatinine values. The decrease in GFR and ERPF was partly caused by the greater weight loss of jaundiced rats, since GRF and ERPF per 100 gram body weight were less decreased in jaundiced rats as compared with sham operated rats.
From this study the conclusion can be drawn that no serious renal failure developes in rats with only a biliary obstruction, without introduction of "extra factors". However, an increased sensitivity of the kidneys to damage in obstructive jaundice, as suggested by others (Dawson 1964, Aoyagi and Lowenstein 1968, Baum et al 1969, Yarger 1976, Bailey 1976, Allison et al 1979, Aarseth et al 1979, Better 1983) is also confirmed by our study. Haemodynamic changes associated with decreased renal perfusion may be the cause of a further worsening of kidney functions, leading to manifest renal failure. These haemodynamic changes do not seem to be caused by the toxic effects of endotoxin, since the clearance of endotoxin by the reticulo-endothelial system is increased in obstructive jaundice as has been shown in chapter 3. In a study by Fletcher et al (1982) it has been suggested that endotoxin induced thromboxane A2 production can cause renal fibrin deposition in obstructive jaundice and thus contribute to the pathogenesis of renal impairment.
From previous studies it is not clear whether an increased level of bile acids in the blood make the renal parenchyma more sensitive (Bomzom et al 1979).
The high incidence of renal failure in the past (Williams et al 1960, Dawson 1965), leading to mortality, may have been caused by a less intensive haemodynamic monitoring of jaundiced patients both during the operation and in the postoperative period.
Bilirubin itself is a powerful vasodilator and can lead to an obligatory loss of salt and water in the preoperative period.
Blood volume deficits of 1500 ml have been recorded in patients with biliary obstruction (Wardle 1975). More infusions of mannitol are needed in jaundiced patients in order to maintain urine flow rate, as compared with non-jaundiced patients after operations of similar magnitude (Allison et al 1979). Renal failure due to dehydration in patients with obstructive jaundice is also suggested by McPherson et al (1984).

In recent studies a decreased frequency of postoperative renal failure is reported (Blamey et al 1983). Also in the study in our own hospital of 67 patients, undergoing pancreatoduodenectomy or total pancreatectomy, only one patient was treated by temporary haemodialysis for renal failure (Snellen et al 1984). This probably indicates a more successful haemodynamic control of operated patients in general.

CHAPTER 5
BILIARY OBSTRUCTION AND BLOOD COAGULATION

5.1 Introduction

Haemorrhage, either from the gastrointestinal tract or from intra-abdominal wounds, is one of the hazards in postoperative patients with obstructive jaundice leading to a high mortality. Postoperative bleeding was reported in 18% of 88 patients undergoing pancreatoduodenectomy leading to mortality in 56% of the bleeding patients (Gilsdorf and Spanos 1972). Bleeding from the gastrointestinal tract and wound haemorrhage was found in 13.6% of 279 patients after pancreatoduodenectomy, with a mortality of 58% in a study by Braasch and Gray (1977). Pitt et al (1981) reported upper gastrointestinal haemorrhage in 5.8% of 155 patients after biliary surgery. The mortality in their series was 33.3%. In a statistical analysis of preoperative risk factors affecting mortality and morbidity in biliary tract surgery in 373 patients, by Dixon et al (1983), gastrointestinal bleeding was a postoperative complication significantly associated with mortality.

In our own study we found postoperative haemorrhage in 15% of 67 patients undergoing pancreatoduodenectomy or total pancreatectomy, with no mortality (Snellen et al 1984). Postoperative haemorrhage was seen in 21% of the jaundiced and 4% of the non-jaundiced patients (serum bilirubin above 50 μmol/l).

This finding is in accordance with the reports that the incidence of haemorrhage is increased in patients with serum bilirubin levels above 342 μmol/l (Braasch and Gray 1977) or above 100 μmol/l (Blamey et al 1983).

After correction of the prolonged prothrombin time in patients with obstructive jaundice, by administration of vitamin K, other coagulation defects can usually not be detected. Still, in the presence of normal coagulation as tested with standard coagulation tests, excessive haemorrhage may occur during surgery and in the postoperative period.

Little is known about the origin of coagulation disorders in cholestatic jaundice. Jedrychowski et al (1973) studied the fibrinolytic system in patients with primary biliary cirrhosis and large bile duct obstruction. They found that the plasma fibrinolytic activity (plasminogen activator activity) was decreased and that fibrinogen was increased more markedly in patients with large bile duct obstruction. It therefore was concluded that in patients undergoing surgery for large bile duct obstruction there may be an increased risk of thrombosis.

These findings of decreased fibrinolytic activity and high levels of clotting factors (fibrinogen, factor VIII) were confirmed by Dioguardi et al (1973). However, these authors also found an abnormal thrombotest, and pro-

longed thrombin and reptilase times, suggesting the presence of some degree of inhibition of the clotting mechanisms independent of vitamin K deficiency. In accordance with our own experience, bleeding is a greater problem than thrombo-embolism in the postoperative period in these patients. The incidence of deep vein thrombosis after biliary surgery is not higher than after surgical operations of comparable severity (Williams 1971, Kakkar et al 1972, Nicolaides et al 1972).
Others have found increased fibrinogen catabolism and a reduced fibrinolysis in patients with obstructive jaundice. It is suggested that endotoxaemia plays an important role in the intravascular coagulation of liver disease (Wardle 1974). In a study by Hunt et al (1982) the relation between endotoxaemia and disturbances of coagulation was investigated in patients with obstructive jaundice and in control patients. A positive endotoxin assay was as common in control patients as in jaundiced patients, but in the latter positive endotoxin assay was significantly associated with increased fibrin degradation product levels and soluble fibrin. It was suggested that it is the combination of jaundice and endotoxin which is associated with a higher incidence of gastrointestinal bleeding and not the endotoxin alone.
In experimental animals a decrease in the coagulability of blood was associated with fibrin deposition in the liver and the lung after two or three spaced injections of endotoxin (Kunz et al 1977). The influence of different bile constituents on the clotting of plasma or fibrinogen with thrombin was examined in patients with pronounced jaundice as well as in cats with experimental obstructive jaundice. Bilirubin glucuronides in concentration above 8 mg % showed antithrombic activity but other substances, such as free bilirubin, glucuronic acid and bile acid salts were inactive (Kopeć et al 1961).

The present experimental study in rats was initiated to examine the effect of biliary obstruction on coagulation in a controlled model, in order to clarify some of the questions on the presence of clotting disorders in jaundiced patients.

5.2 Material and methods

5.2.1 Animals, anaesthesia and operative techniques

Animals

Male random bred Wistar rats (TNO Animal breeding facilities, Zeist, The

Netherlands) weighing between 300 and 400 grams were used throughout the study. They were housed in groups of 5, in cages, or separately (rats used for thromboelastrography) and were fed ad libitum on commercial chow (Hope Farms) and water (pH ± 3).

Anaesthesia

Rats were anaesthetized with ether as described in chapter 2. In the tail bleeding test Nembutal® (0.1 mg/100 gram body weight i.p.) was used after induction of anaesthesia with ether.

Production of biliary obstruction and sham operations

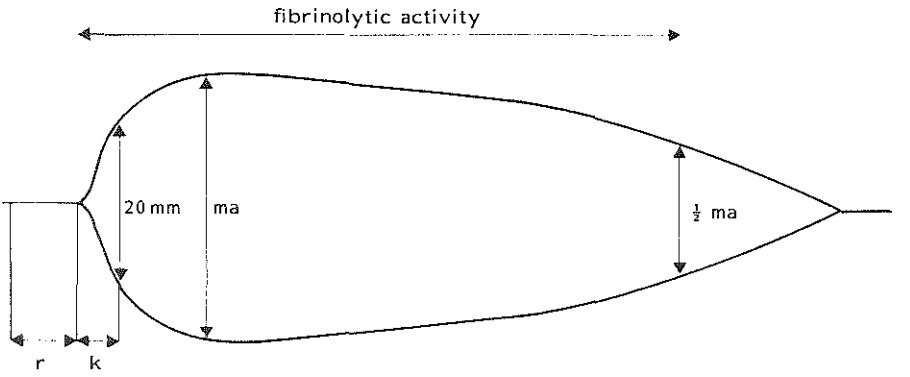
For permanent biliary obstruction ligation and division of the common bile duct was performed in experimental animals. Sham operated control rats were also obtained (see chapter 2).

5.2.2 *Coagulation analysis*

The following analyses were carried out:
Level of haemoglobin, number of platelets (Toa Electric cell counting system), Fibrinogen (Claus 1957), Thrombotest (Owren 1959), Normotest (Owren 1969), and Activated Partial Thromboplastin Time (APTT) was estimated with activated cephaloplastin reagent (Williams et al 1972).
Bleeding time was measured with the tail bleeding test as described by Dejana et al (1979) and Van Lanschot et al (1984). The rats were placed into a cage of a constant temperature (26 ± 1°C) with the tail in a vertical hanging position. After 10 minutes the tail was transsected at a diameter of 2 mm. The bleeding tail-tip was placed in 20 cc of water of same temperature for 10 minutes. Haemoglobin concentrations were measured by grating spectrophotometry (Beckman DB-6) and the amount of haemoglobin lost per tail was calculated.
To study the whole coagulation process under conditions that are constant and reproducable, thromboelastography was used (Vreeken 1957, De Nicola 1957). Blood obtained form a tail-cut was directly dripped into a cylindrical cuvet. Within 2 minutes the cuvet was placed in the thromboelastograph (Heilige, Germany). The piston was immediately lowered into the cuvet and 3 drops of paraffin oil were floated over the top, protecting the surface of the liquid from evaporation and air. The thromboelastogram was measured during 3 hours. Reaction time (r) in minutes, k-value (point were the curves of the thromboelastogram are 20 mm apart) in minutes,

maximal amplitude (ma) in mm and fibrinolytic activity (point were the curves reaches the half of the maximal amplitude) in minutes were measured (fig. 36).
Serum bilirubin was measured by standard methods on a computer directed analyzer (Gilford System 2500).

Fig. 36 Scheme of thromboelastogram, reaction time (r), k-value (k), maximal amplitude (ma).



5.2.3 Experimental groups

Group 19 (n=19, 10 experimental animals, 9 controls)
Two weeks after biliary obstruction or after sham operation the tail bleeding test was performed. After this test the animals were sacrificed by exsanguination. The blood was used for determinations of haemoglobin, platelets, thrombotest, normotest, fibrinogen, APTT and serum bilirubin.

Group 20 (n=27, 19 experimental animals, 8 controls)
After 7 to 21 days of biliary obstruction or sham operation blood was collected for thromboelastography and serum bilirubin determination.
In four "7 day jaundiced rats" epsilon amino capron acid (EACA, 0.8 mg/gram body weight) was injected intraperitoneally 2 hours after the first thromboelastogram. One hour later blood was taken for a second thromboelastogram.
Fibrinolytic activity was considered to be abnormal if this activity was shorter than 120 minutes.

5.2.4 Statistical analysis

Variance of the mean is expressed as the standard error of the mean (SEM). Differences in mean were analyzed with Student's t-test and fibrinolytic activity data were tested by the Fisher test. The results of the tail bleeding test were analysed with the Wilcoxon test.

5.3. Results

Group 19 Coagulation analyses

Jaundiced rats (n=10)		Sham operated rats (n=9)	
day		day	
0	ligation and division of common bile duct	0	sham operation
14	coagulation analyses	14	coagulation analyses

There was no operative mortality in either group. The jaundiced rats lost significantly more weight than the sham operated rats ($p < 0.05$). The jaundiced rats were found to have significantly increased thrombocytes and significantly decreased haemoglobin and fibrinogen levels. Tail bleeding was significantly increased in the jaundiced rats (Wilcoxon test was used). Thrombotest, normotest, and APTT were not significantly different in both groups (table 27).

Table 27 Coagulation analyses and serum bilirubin.

	jaundiced rats (n=10)	sham rats (n=9)	stat. anal.
Tail bleeding test	5.7 ± 2.0 mgHb/l	2.7 ± 0.7	$p < 0.05$
Haemoglobin	8.5 ± 0.2 mmol/l	9.3 ± 0.2	$p < 0.05$
Thrombocytes	1181.5 ± 49.5.10^9/l	946.3 ± 42.1	$p < 0.05$
Fibrinogen	2.1 ± 0.2 g/l	3.2 ± 0.3	$p < 0.01$
Thrombotest	23.6 ± 0.9 sec	23.0 ± 0.8	n.s.
Normotest	45.6 ± 1.3 sec	42.3 ± 2.4	n.s.
APTT	21.0 ± 0.9 sec	21.1 ± 0.7	n.s.
Bilirubin	159.6 ± 15.8 µmol/l	1.2 ± 0.4	$p < 0.001$

Group 20 Thromboelastography

In the jaundiced animals the fibrinolytic activity, measured as the length of time within which the curves reached 50% of the maximal amplitude, was significantly increased. The other parameters, reaction time, k-value and maximal amplitude, did not differ in the two groups (table 28).

Table 28 Results of thromboelastography.

	jaundiced rats (n=19)	sham rats (n=8)	statistical analysis
reaction time (r)	3.6 ± 0.2 min	4.4 ± 0.5 min	n.s.
k-value	1.7 ± 0.2 min	1.7 ± 0.2 min	n.s.
maximal amplitude (ma)	67 ± 2.6 mm	72 ± 1.9 mm	n.s.
fibrinolytic activity ⇓	13/19	1/8	$p < 0.05$

In 3 of the 4 jaundiced rats given EACA i.p. the fibrinolytic activity was no longer abnormal.

5.4 Discussion

According to the literature, patients with obstructive jaundice probably have an increased bleeding tendency (Gilsdorf and Spanos 1972, Braasch and Gray 1979, Pitt et al 1981, Dixon et al 1984). However, in the usual coagulation tests no abnormalities are found indicating an increased bleeding tendency. Abnormalities are reported indicating an increased risk of thrombosis in patients with obstructive jaundice (Jedrychowsky et al 1973, Wardle 1974).

In order to examine the mechanism of haemostasis in obstructive jaundice, different factors were studied in jaundiced and sham operated rats. The bleeding time was determined with the tail bleeding test as described by Dejana et al (1979) and Van Lanschot et al (1984). Other possible methods to determine bleeding time in rats are the muscle bleeding test (Meuleman et al 1982), the determination of the bleeding time after transsection of the mesenteric vessels under the microscope and the determination of the bleeding time after a standard liver resection (Zoucas et al 1982). A technical problem in all these methods is that a standardized performance is almost impossible. In the present study the tail bleeding test was used because the performance of this test is the least difficult to standardize.

In our study, bleeding time also showed large differences per group. However, if the Wilcoxon test was used on these results, a statistically prolongend bleeding time was found in jaundiced rats as compared to sham operated rats.

The advantage of using this tail bleeding test is that all mechanisms that play a role in haemostasis are studied, namely, the vascular reaction, the function of thrombocytes, blood coagulation and fibrinolysis. In studies of haemostasis using only the usual coagulation tests, certain parts of the coagulation mechanism are examined and not the entire process of haemostasis.

Another method that is used in order to study the entire coagulation mechanism is thromboelastography. This method has proved to be very useful and the variables of the thromboelastogram correlate very well with the usual laboratory tests. Besides it gives additional information about the process of haemostasis (Zuckerman et al 1981). Disadvantages of thromboelastography for clinical use are the long duration of the determination (minimal 3 hours) and the necessity to place the blood sample

into the apparatus within 2 minutes.
In the present study no indications were found for a decreased clot formation time and for disturbances in the proportions and structure of the clots in jaundiced rats. This can be deduced from the r, k and ma values, which do not differ in the experimental and control groups.
Also a normal APTT was found in jaundiced rats. The thrombotest and the normotest were not prolonged in jaundiced rats, so that a deficiency of vitamin K can be ruled out.
Jaundiced rats had a decreased fibrinogen, but there were no indications of an increased consumption of fibrinogen due to diffuse intravascular coagulation. In this disorder a decreased number of thrombocytes should be found. The appearance of diffuse intravascular coagulation with decrease of fibrinogen and thrombocytopenia was reported in rats after injection of endotoxin (Kunz et al 1977).
Diminished synthesis of fibrinogen secondary to liver function disorders is also unlikely, since vitamin K dependent coagulation factors with a shorter half-life elimination value were synthesized normally (normal thrombotest and normotest were found).
A decreased haemoglobin has been reported previously (Powell et al 1968), possibly as a result of increased haemolysis in obstructive jaundice. However, this cannot explain the prolonged bleeding time.
In our study an increased fibrinolytic activity was found using thromboelastography in jaundiced rats as compared with sham operated rats. Fibrinolytic activity was considered to be abnormal if a 50% decrease of the maximal amplitude was reached within the period of 120 minutes. The accelerated narrowing of the amplitude of the tromboelastogram can also result from an increased number of thrombocytes (Vreeken 1957). We indeed found an increased number of thrombocytes in jaundiced rats, but this increase in platelet can be ruled out as the cause, because the fibrinolytic activity was normalized after administration of epsilon amino capron acid. EACA is a synthetic opponent of fibrinolytic activity and does not influence thrombocytes (Boks, personal communication). The cause of this increased fibrinolytic activity is still obscure and needs further investigation.
From this study we can conclude that biliary obstruction in rats gives rise to a prolonged bleeding time and an increased destruction of the formed clot. However, the formation of the clot is not delayed. The increased destruction of the formed clot may possibly be the cause of the increased bleeding tendency in jaundiced patients.

CHAPTER 6
GENERAL DISCUSSION AND CONCLUSIONS

Malignant tumors of the extrahepatic biliary tract and the pancreas are often diagnosed at a late stage of the disease. Jaundice caused by the biliary obstruction in one of the first symptoms (Walsh et al 1982, Warren et al 1983, Alexander et al 1984). The tumor is found to be irresectable in about 85% of these patients (Brooks 1983) and consequently the prognosis is poor (Nakase et al 1977, Obertop et al 1983, Warren et al 1983).

Considering this poor prognosis and the technically difficult resection followed by a high operative mortality some authors have advocated a conservative approach (Crile 1970, Shapiro 1975). However, in the treatment of malignant tumors of the biliary tract and the pancreas the only chance of cure is resection of the tumor.

Reduction of the operative mortality is one way of improving the prognosis after these operation. Retrospective analyses of potential risk factors in patients undergoing major biliary and pancreatic surgery have shown that increased serum bilirubin levels have a significant association with postoperative morbidity and mortality (Gilsdorf and Spanos 1973, Pitt et al 1981, Dixon et al 1983, Blamey et al 1983).

A staged surgical approach with a preliminary bilio-digestive bypass in order to lower the serum bilirubin level and so to decrease the operative risk was first proposed by Whipple et al (1935) and later advocated by others (Ross 1959, Maki et al 1966, Sato et al 1968).

More recently non-surgical biliary decompression has been recommended during the preparation of jaundiced patients for surgery. The use of preoperative percutaneous biliary drainage before the definitive resection in jaundiced patients has been reported to lower the operative mortality (Nakayama et al 1978, Andrén-Sandberg and Ihse 1983) and postoperative morbidity (Denning et al 1981, Gundry et al 1984).

Preoperative biliary drainage by endoscopically introduced endoprostheses after sphincterotomy has also been shown to be succesful in lowering serum bilirubin levels (Riemann et al 1981, Cotton 1982, Huibregtse and Tytgat 1982). The series of patients treated by the latter technique are still small and no data are available on the effect on postoperative morbidity and mortality.

Prospective studies on the effect of preoperative biliary drainage on postoperative mortality following extensive biliary and pancreatic surgery have not, to date, shown any beneficial effect (Norlander er al 1981, Hatfield et al 1982). McPherson et al (1984) in a controlled prospective trial even found that operative mortality after biliary and pancreatic surgery was increased in patient who underwent preoperative transhepatic biliary drainage.

Studies on the correlation of biliary obstruction with postoperative complications after biliary surgery are complicated because many factors might be involved in these patients. The general condition of jaundiced patients is often poor and the postoperative problems that arise in malignant obstructive jaundice may be caused by the presence of a tumor, liver failure, increased bilirubin level or weight loss.

This weight loss can be caused by anorexia or by malabsorption due to the absence of bile acids in the small intestine. Anorexia may be caused by hyperbilirubinaemia, as has been shown in rats in the present study, but the presence of a malignant tumor may also play an important role in the origin of anorexia (Krause 1980, Wesdorp et al 1983).

To examine these factors separately, studies in experimental animals are necessary. However, other factors still remain that cannot be examined separately, for instance, hyperbilirubinaemia and failure of other liver functions.

Experiments in vitro can further elucidate the effects of different factors, such as the suppressive effect of bilirubin on growing fibroblasts (Taube et al 1981) and the stimulating effect of bilirubin on anti-bacterial activity in leucocytes (Wardle 1980). In these in vitro studies, however, non-physiological concentrations of bilirubin are often used.

In studies on the effects of hyperbilirubinaemia in vivo, both in man and in experimental animals, we have to take into account a combination of different factors and be careful with the interpretation of the results of these studies.

The present study in rats with biliary obstruction has been focused on these processes outside the liver that are involved most frequently in patients with obstructive jaundice: wound healing, infection, renal function and blood coagulation.

Impaired wound healing has been reported in jaundiced patients (Than Than et al 1974, Ellis and Heddle 1977, Irvin et al 1978, Armstrong et al 1984) and experimental animals with biliary obstruction (Bayer and Ellis 1976, Arnaud et al 1981). In the present study it was also clearly shown that rats with biliary obstruction have impaired wound healing.

It has been suggested that in patients with obstructive jaundice the impaired wound healing is due to the associated malnutrition and the presence of a malignancy and not to the raised serum bilirubin per se (Irvin et al 1978, Armstrong et al 1984). However, in the present experimental study impaired wound healing of abdominal wounds was not seen in pair-fed rats with equal weight loss as jaundiced rats, indicating that hyperbilirubinaemia impairs wound healing. The cause seems to be a toxic effect of bilirubin on growing fibroblasts (Taube et al 1981).

The effect of biliary obstruction was more pronounced on the abdominal

wounds than the intestinal wounds of the jaundiced rats. In all rats with prolonged jaundice an incisional hernia of the laparotomy was found.
The effect of biliary decompresion on wound healing has not been reported in patients or experimental animals before. In our study it was shown that after removal of the biliary obstruction bilirubin levels returned to normal within 4 days and further weight loss stopped. The healing of abdominal and intestinal wounds became normal after biliary decompression.
These results indicate that in patients with obstructive jaundice and extreme weight loss a biliary drainage before the definitive operation will probably have a beneficial effect on wound healing. In jaundiced patients in good general condition and without extreme weight loss it is probably justifiable not to perform a biliary drainage. Furthermore, at the time of the definitive operation the obstruction will be removed or bypassed and from that moment on the serum bilirubin level will fall.
Infection is a major cause of morbidity and mortality in patients with malignant biliary obstruction (Nakase et al 1977, Braasch and Gray 1977, Pitt et al 1981, Blamey et al 1983, Armstrong et al 1984). This high infection rate is partly the result of operating in an area with infected bile (Nielsen and Justesen 1976, Keighley 1977, Cox et al 1978, Farnell et al 1981). It has also been suggested that the increased frequency of postoperative septicaemia might be due to an impairment of the defence mechanisms of patients with obstructive jaundice (Rola et al 1975, Drivas et al 1976).
In the present study jaundiced rats were found to have an increased mortality after induction of infection or following endotoxin challenge. The cause of the decreased resistance against infection is still obscure. The explanations of other investigators that in obstructive jaundice endotoxin which is absorbed in greater amounts from the small intestine (Kocsár et al 1969, Bailey 1976), is phagocytosed insufficiently by the Kupffer cells (Wardle and Wright 1970, Holman and Rikkers 1982), does not seem to be justified following our finding that there is an increased phagocytosis of both carbon particles and ^{51}Cr labelled endotoxin by the liver.
In rats with biliary obstruction an increased pulmonary localisation of injected viable E.Coli has been reported and it has been suggested that this may be followed by a re-emergence of bacteria into the bloodstream and thus may be a potential mechanism predisposing to septicaemia (Katz et al 1984). However, in the present study we did not find an increased localisation of ^{51}Cr labelled endotoxin in the lung and the amount of bacteria in the peripheral blood was not found to be increased.
There are indications that intermediary products are released during phagocytosis of endotoxin, such as prostaglandins and thromboxane (Nolan 1981). Elevated levels of these intermediary products have been reported in

rats with obstructive jaundice and pretreatment with prostaglandin synthetase inhibitors or prostacyclin produced significant improvement in survival in jaundiced rats after endotoxin challenge (Fletcher et al 1982). These intermediary products may produce the toxic effects now ascribed to endotoxin.

After removal of the biliary obstruction in jaundiced rats and after 1 week of biliary drainage, we found that the mortality after induction of infection was no longer increased.

In patients with obstructive jaundice a biliary drainage procedure before the definitive operation seems indicated to increase resistance against infection.

Renal failure in patients with obstructive jaundice is another frequently reported complication associated with an increased mortality after biliary surgery (Dawson 1975, Wardle 1975, Blumgart 1978, Blamey et al 1983, Dixon et al 1983).

Preoperative percutaneous transhepatic biliary drainage of the obstructed biliary tree has been advocated, in order to improve the impaired renal function before surgery (Dooley et al 1979, Denning et al 1981).

The possible causes of renal failure in patients and experimental animals with biliary obstruction has been discussed in chapter 4. An increased "sensitivity" of the kidneys to damage in obstructive jaundice, as suggested in many studies (Dawson 1964, Aoyagi and Lowenstein 1968, Baum et al 1969, Yarger 1976, Bailey 1976, Allison et al 1979, Aarseth et al 1979, Better et al 1980, Bomzom and Kew 1983) was confirmed in our study, since we found a decrease in glomerular filtration rate and effective renal plasma flow in rats 2 weeks after biliary obstruction.

It has also been recommended to treat patients with obstructive jaundice with bile salts before surgery, in order to improve renal function (Evans et al 1982, Cahill 1983).

In patients with obstructive jaundice it seems indicated to perform a biliary drainage procedure before the definitive operation to protect renal function. On the other hand, it has been suggested that the improvements in renal function observed during preoperative transhepatic biliary drainage are largerly due to rehydration of the patient and not to the beneficial effect of the drainage (McPherson et al 1984).

Postoperative gastro-intestinal haemorrhage is a significant cause of morbidity and mortality after surgery to relieve bile duct obstruction (Gilsdorf and Spanos 1974, Braasch and Gray 1977, Pitt et al 1981, Dixon et al 1984). The cause of this increased bleeding tendency in patients with obstructive jaundice remains obscure; only abnormalities in blood coagulation indicating an increased risk of thrombosis in patients with obstructive jaundice have been reported (Jedrychowsky et al 1973, Wardle 1974).

In the present study we found an increased bleeding time in 2 weeks jaundiced rats. Furthermore, we had indications of an increased lysis of clots in rats with biliary obstruction using thromboelastography, whereas the formation of the clots was not found to be delayed. So obstructive jaundice may increase the risk of postoperative haemorrhage.

After a review of the literature we come to the conclusion that patients with obstructive jaundice have an increased morbidity and possibly an increased mortality after extensive biliary and pancreatic surgery. However, we cannot conclude that postoperative mortality and morbidity will decrease with the use of biliary drainage.

In patients with obstructive jaundice histological abnormalities of the liver are restored quickly after biliary drainage (Blumgart 1978). Liver functions also return to normal, or nearly normal, after an average of 2 to 3 weeks (Wiechel 1964, Nakayama et al 1978, Norlander et al 1982). This capacity of the liver to recover is also found in experimental animals with obstructive jaundice, following relief of the biliary obstruction (Aronson 1961, Birns et al 1962, Koyama et al 1981).

After biliary drainage the general condition of the patient can improve due to the restoration of liver functions, returning appetite and disappearance of malabsorption, if bile is refed to the patient in external drainage or reaches the bowel via internal drainage. Itching will disappear after decompression of the biliary tract, and wound healing and resistance to infection will return to normal. Renal function will not decrease further and the chance of postoperative haemorrhage may be reduced.

The disadvantages of biliary drainage must also be considered: the definitive operation must be postponed, but this delay of 2 to 3 weeks can be used for other diagnostic procedures in order to estimate resectability. The drainage procedure itself has complications that have been described in detail in the general introduction. It must be emphasized that these complications can be very serious, and even be fatal. Complications are more often seen after percutaneous transhepatic drainage than after endoscopic drainage. If technically possible, endoscopic biliary drainage is the procedure of choice. The choice also depends on the local experience with either method.

A possible complication is that the definitive operation cannot be carried out due to cholangitis after contamination of the biliary tract by the drainage procedure. In patients with total obstruction of the biliary tract the bile is infected in about 33% of the patients (Keighly 1982), whereas after biliary drainage infected bile is found in almost 90% of the patients (Cox et al 1978). Septic complications of percutaneous transhepatic biliary drainage can be reduced by the use of closed drainage systems (Blenkharn et al 1984).

After considering the advantages and disadvantages of preoperative biliary

drainage the conclusion can be drawn that preoperative drainage is indicated in patients with a poor general condition. These patients are often older than 70 years and already have an increased mortality-risk because of their age (Obertop et al 1982, Andrén-Sandberg and Ihse 1983). And so it is essential that the condition of these patients is improved before a definitive operation can be performed. For this improvement preoperative biliary drainage seems to be necessary.

In younger patients in good general condition, regardless of the depth of jaundice, preoperative biliary drainage is not necessary and the definitive operation should be performed as soon as possible. However, when a diagnostic PTC or ERCP is performed, the bile ducts should be drained if this is possible without additional hazards, to prevent further biliary stasis and lower the bilirubin level.

SUMMARY

The purpose of the study reported in this thesis, was to investigate the effects of biliary obstruction on wound healing, resistance to infection, renal function and blood coagulation. Disturbances in these processes are often reported in patients with obstructive jaundice, and may lead to post-operative complications and mortality. However, in a retrospective study of 67 patients undergoing pancreatoduodenectomy or total pancreatectomy in our own surgical department morbidity and mortality was not higher in patients with increased serum bilirubin levels (serum bilirubin > 50 μmol/l) than in non-jaundiced patients.
Preoperative biliary drainage has been advocated in an attempt to reduce these postoperative complications and to lower the mortality rate. These drainage procedures, however, may cause considerable complications and even death. To which extent biliary drainage can restore the disturbances in wound healing, resistance to infection, renal function and blood coagulation is not clear form the literature (chapter 1).

We studied the effect of biliary obstruction on wound healing in rats. This study is decribed in chapter 2. After a common bile duct obstruction for 14 days, duodenal- and colonic anastomoses and wounds in the right and left side of the abdomen were made. Bursting pressures of intestinal anastomoses and abdominal wounds were tested at day 3, 7 and 14. These were compared with bursting pressures of intestinal anastomoses and abdominal wounds of non-jaundiced rats after a sham operation. In jaundiced rats lower bursting pressures were found 7 days ($p < 0.01$) and 14 days ($p < 0.001$) after duodenal anastomoses, 14 days ($p < 0.05$) after colonic anastomoses and 7 days ($p < 0.01$) and 14 days ($p < 0.001$) after abdominal wounds.
To examine the effect of drainage of the biliary tract on wound healing, the common bile duct was obstructed with a clip. The clip was removed after 2 weeks of obstruction, following which the serum bilirubin decreased to normal level within 3 to 4 days. Intestinal anastomoses and abdominal wounds, made 7 days after removal of the clip, were tested at day 3, 7 and 14. Lower bursting pressures of the duodenum were only found after 3 days ($p \pm 0.05$). In all other groups the difference in bursting pressures between jaundiced and sham operated rats disappeared.
Because all the jaundiced rats lost significantly more weight than the sham operated rats, two groups were subsequently studied with identical weight loss due to pair-feeding. Bursting pressures of intestinal and abdominal wounds were tested at day 7. Abdominal wounds still showed delayed healing in jaundiced rats ($p < 0.001$), whereas the intestinal wound healing was identical in both groups.

From this study we conclude that rats with a biliary obstruction have delayed healing of wounds in the abdomen and the intestine. This effect disappears after drainage of the biliary tract. The delayed healing of intestinal wounds may be the result of the weight loss which accompanies obstructive jaundice, whereas the healing of abdominal wounds can be impaired by the jaundice itself.

In chapter 3 the effect of biliary obstruction on resistance to infection is described. In a study in rats mortality was examined after injection of a gram negative bacteria, Klebsiella pneumoniae, following a common bile duct obstruction for 14 days. The mortality was compared with the mortality of rats that were injected with Klebsiella pneumoniae 14 days after a sham operation. Mortality in jaundiced rats was significantly higher than in sham operated rats ($p < 0.001$). Mortality of jaundiced rats with a biliary obstruction for 14 days was also studied after injection of E.Coli endotoxin and compared with the mortality of sham operated rats. The mortality was significantly higher in jaundiced rats ($p < 0.001$).
To study the effect of drainage of the biliary tract on the resistance to infection in rats, the common bile duct was obstructed with a clip that was removed 14 days later. After removal of the clip the bile could flow freely to the duodenum. After 7 days of biliary drainage Klebsiella pneumoniae was injected. The mortality was not significantly different from that of sham operated rats.
It is known that Kupffer cells in the liver play an important role in the resistance against micro-organisms. In this study the clearance of carbon particles from the blood by the Kupffer cells was investigated. In a series of rats the common bile duct was obstructed during 2, 7, 14, 21 and 28 days, whereas another series of rats underwent sham operations. The clearance was significantly increased in jaundiced rats after 7, 14, 21 and 28 days obstruction compared with the clearance in sham operated rats. The clearance of ^{51}Cr labelled endotoxin of E.Coli was also studied after an obstruction of the common bile duct for 7 and 14 days. The clearance of endotoxin was significantly increased in jaundiced rats ($p < 0.05$) when compared with the clearance in sham operated rats.
After an obstruction of the common bile duct for 14 days, jaundiced rats had a significantly increased number of leucocytes in the peripheral blood ($p < 0.001$), an increased number of lymphocytes in the spleen ($p < 0.05$) and in the mesenteric lymph nodes ($p < 0.05$) and an increased PHA-stimulation of the total spleen ($p < 0.05$).
From this study we conclude that rats with a biliary obstruction have a higher mortality after induction of infection. After biliary drainage the

mortality is no longer increased. Obstructive jaundice is accompanied by an increased clearance of both carbon particles and endotoxin by Kupffer cells, and with an increase in blood leucocytes and lymphocytes. The increased sensitivity to infection is therefore not explained by failure of these defensive systems.

In chapter 4 the effect of biliary obstruction on renal functions in the rat is described. Renal functions were determined before, and 7 and 14 days after obstruction of the common bile duct. After one week no differences were found in renal functions between jaundiced and sham operated rats. Two weeks after biliary obstruction or sham operation a lower glomerular filtration rate was found in jaundiced rats when compared with sham operated rats ($p < 0.001$). At the same time a decreased effective renal plasma flow was found in jaundiced rats ($p < 0.05$). However, when these data were corrected for body weight only a decreased glomerular filtration rate was found. No significant differences were found in serum creatinine values of jaundiced and sham operated rats.
From this study we conclude that biliary obstruction alone, without introduction of other detrimental factors, causes only a minor decrease of certain renal functions in rats.

In chapter 5 the effect of biliary obstruction on blood coagulation in rats was described. Coagulation analyses were performed in rats with a common bile duct obstruction during 14 days and compared with coagulation analyses of sham operated rats. Jaundiced rats had a prolonged tail bleeding time ($p < 0.05$), an increased number of thrombocytes ($p < 0.05$) and a decreased fibrinogen ($p < 0.01$) when compared with sham operated rats.
Thromboelastography was performed in jaundiced rats after a biliary tract obstruction during 7 to 21 days and in rats after a sham operation. There were indications that the fibrinolytic activity was increased, whereas the time needed for the formation of the clot was not different in jaundiced or sham operated rats.
We conclude that these disorders in the mechanism of haemostasis might be the cause of the increased bleeding tendency in patients with obstructive jaundice.

In chapter 6 the conclusions drawn from this study are discussed in relation to the literature. Finally we recommend that a biliary drainage procedure must only be performed in jaundiced patients with a poor general condition. Because endoscopic biliary drainage causes less complications than percutaneous transhepatic biliary drainage this procedure is the treatment of choice.

SAMENVATTING

Het doel van het in dit proefschrift beschreven onderzoek was het bestuderen van de effecten van een galwegobstructie op wondgenezing, weerstand tegen infectie, nierfunctie en bloedstolling. Stoornissen in deze processen worden dikwijls gevonden bij patienten met een obstructie icterus, en kunnen leiden tot postoperatieve complicaties en mortaliteit. In een retrospectief onderzoek bij 67 patienten die een Whipple operatie of een totale pancreatectomie ondergingen in onze eigen chirurgische kliniek werd echter géén verhoogde morbiditeit en mortaliteit gevonden bij de icterische patienten in vergelijking met de niet-icterische patienten.
Preoperatieve galwegdrainage wordt door sommige auteurs aangeraden en toegepast met als doel deze postoperatieve complicaties te verminderen en de mortaliteit te verlagen. Galwegdrainage procedures kunnen echter zelf ook tot aanzienlijk complicaties en mortaliteit leiden. In hoeverre galwegdecompressie inderdaad leidt tot herstel van stoornissen in bovengenoemde processen is nog niet onderzocht (hoofdstuk 1).

In een experimenteel onderzoek bij ratten werd het effect van een galwegobstructie op wondgenezing bestudeerd. Dit onderzoek is beschreven in hoofdstuk 2. Na een ductus choledochus obstructie gedurende 14 dagen werden een duodenum- en colonanastomose en wonden in de rechter en linker kant van de buik gemaakt. De treksterkten van de darmnaden en buikwonden werden na 3, 7 en 14 dagen gemeten. Deze werden vergeleken met de treksterkten van darmnaden en buikwonden van niet-icterische ratten na een schijn operatie. Bij de icterische ratten was de duodenumnaad zwakker na 7 dagen ($p < 0.01$) en na 14 dagen ($p < 0.001$). De colonnaad was zwakker na 14 dagen ($p < 0.05$) en de buikwonden na 7 dagen ($p < 0.01$) en na 14 dagen ($p < 0.001$).
Om het effect van een galwegdrainage op wondgenezing te bestuderen, werd in een andere groep ratten de ductus choledochus geobstrueerd met een clip. Na 14 dagen werd deze clip verwijderd, waarna het serum bilirubine gehalte in 3 à 4 dagen daalde naar normale waarden. Zeven dagen na het verwijderen van de clip werden de duodenum- en colonnaden en buikwonden gemaakt en na 3, 7 en 14 dagen werden de treksterkten gemeten. Na 3 dagen werd alleen een verminderde treksterkte van het duodenum gemeten bij experimentel ratten ($p < 0.05$). In alle andere gevallen waren de verschillen in treksterkten tussen de groepen experimentele en schijn geopereerde ratten verdwenen.
Omdat icterische ratten een significant groter gewichtsverlies hadden dan ratten na de schijn operatie, werden twee groepen bestudeerd die een gelijk gewichtsverlies hadden door middel van gepaarde voeding. Zeven dagen na het maken van de darmnaden en buikwonden in beide groepen werden de

treksterkten gemeten. Alleen de buikwonden van de icterische ratten waren zwakker ($p < 0.001$).
Uit dit onderzoek werd geconcludeerd dat de wondgenezing bij ratten met een galwegobstructie verminderd is bij zowel buikwonden als bij darmnaden. Na een galwegdrainage wordt de wondgenezing weer normaal. De gestoorde genezing van darmnaden is mogelijk het resultaat van gewichtsverlies dat gepaard gaat met obstructie icterus, terwijl de genezing van buikwonden gestoord kan zijn door de icterus zelf.

In hoofdstuk 3 wordt het effect van een galwegobstructie op de weerstand tegen infectie beschreven. In een onderzoek werd de mortaliteit bestudeerd bij ratten, waarvan de ductus choledochus gedurende 14 dagen was geobstrueerd en waarna gram negatieve bacteriën, Klebsiella pneumoniae, werden ingespoten. De mortaliteit van icterische ratten was significant hoger dan die van schijn geopereerde ratten ($p < 0.001$). Ook werd de mortaliteit van ratten met een voorafgaande galwegobstructie gedurende 14 dagen bestudeerd na het inspuiten van endotoxinen van E.Coli en vergeleken met de mortaliteit van schijn geopereerde ratten. De mortaliteit was eveneen significant hoger bij icterische ratten ($p < 0.001$).
Om het effect van een galwegdrainage op de weerstand tegen infectie te bestuderen werd bij een groep ratten de ductus choledochus afgesloten met een clip die 14 dagen later werd verwijderd. Hierna kon de gal vrij afvloeien naar het duodenum. Na 7 dagen van galdrainage werd Klebsiella pneumoniae ingespoten. De mortaliteit werd bestudeerd en vergeleken met de mortaliteit van ratten die schijn operaties hadden ondergaan. De mortaliteit was niet significant verschillend in de twee groepen.
Het is bekend dat de Kupffer cellen in de lever een belangrijke rol spelen in het afweerproces tegen micro-organismen. In dit onderzoek werd de klaringssnelheid van carbon partikels uit het bloed door de Kupffer cellen bestudeerd bij ratten. Bij een serie ratten werd de ductus choledochus afgesloten gedurende 2, 7, 14, 21 en 28 dagen, terwijl een andere serie ratten een schijn operatie ondergingen. De klaringssnelheid was significant verhoogd bij icterische ratten na 7, 14, 21 en 28 dagen obstructie, vergeleken met de klaringssnelheid van schijn geopereerde ratten. Tevens werd op dezelfde wijze de klaringssnelheid van ^{51}Cr-gelabelde endotoxinen van E.Coli bestudeerd na een obstructie van de ductus choledochus gedurende 7 en 14 dagen. De klaringssnelheid van endotoxinen was significant verhoogd bij icterische ratten ($p < 0.05$) vergeleken met de klaringssnelheid van schijn geopereerde ratten.
Voorts hadden icterische ratten na een obstructie van de ductus choledochus gedurende 14 dagen een significant verhoogd aantal leucocyten in

het perifere bloed ($p < 0.001$), een verhoogd aantal lymfocyten in de milt ($p < 0.05$) en in de mesenteriale lymfeklieren $p < 0.05$) en een verhoogde PHA-stimulatie van de totale milt ($p < 0.05$).
Uit dit onderzoek werd geconcludeerd dat bij ratten met een obstructie icterus de mortaliteit na infectie verhoogd is. Na een galwegdrainage is de mortaliteit niet langer verhoogd. Obstructie icterus gaat gepaard met een verhoogde klaringssnelheid van carbon partikels en endotoxinen door de Kupffer cellen en met een toename van leucocyten en lymfocyten. De verhoogde gevoeligheid voor infectie kan derhalve niet verklaard worden door falen van deze afweermechanismen.

In hoofdstuk 4 wordt het effect van een galwegobstructie op de nierfuncties van de rat beschreven. De nierfuncties werden bepaald vóór, en 7 en 14 dagen na obstructie van de ductus choledochus. Na één week werden géén verschillen gevonden in de nierfuncties van icterische en schijn geopereerde ratten. Twee weken na de galwegobstructie of schijn operatie werd een lagere glomerulaire filtratiesnelheid gevonden bij icterische ratten ten opzichte van schijn geopereerde ratten ($p < 0.001$). Tevens werd na twee weken een verminderde effectieve renale plasma flow gevonden bij icterische ratten ($p < 0.05$). Wanneer echter deze nierfuncties werden gecorrigeerd naar het gewichtsverlies dan werd nog slechts een verminderde glomerulaire filtratiesnelheid gevonden. Er werden géén significante verschillen gevonden in kreatinine waarden van icterische en schijn geopereerde ratten.
Uit dit onderzoek werd geconcludeerd dat galwegobstructie alleen, zonder introductie van andere schadelijke factoren, slechts een geringe vermindering van bepaalde nierfuncties veroorzaakt bij ratten.

In hoofdstuk 5 wordt het effect van een galwegobstructie op de bloedstolling van de rat beschreven. Stollingsanalyses werden uitgevoerd bij ratten waarvan de ductus choledochus gedurende 14 dagen werd geobstrueerd en vergeleken met de stollingsanalyses van schijn geopereerde ratten. Icterische ratten hadden een verlengde staartbloedingstijd ($p < 0.05$), een verhoogd aantal thrombocyten ($p < 0.05$) en een verlaagd fibrinogeen gehalte ($p < 0.01$) vergeleken met schijn geopereerde ratten. Thromboelastographie werd verricht bij icterische ratten na een galwegobstructie gedurende 7 tot 21 dagen en bij ratten na een schijn operatie. Er werden aanwijzingen gevonden voor een verhoogde fibrinolytische activiteit, terwijl de voor de vorming van het bloedstolsel benodigde tijd niet verschillend was bij de icterische en schijn geopereerde ratten.

Er werd geconcludeerd dat deze afwijkingen in het stollingsmechanisme mogelijk de oorzaak zijn van de verhoogde bloedingsneiging bij patienten met een obstructie icterus.

In hoofdstuk 6 worden de conclusies die uit dit onderzoek zijn getrokken besproken en gerelateerd aan de bestaande literatuur.
Tot slot wordt een aanbeveling gedaan om alleen bij icterische patienten die in een slechte algemene conditie verkeren een preoperatieve galwegdrainage procedure te verrichten.
Omdat bij de endoscopische galwegdrainage minder complicaties optreden dan bij de percutane transhepatische galwegdrainage verdient deze hierbij de voorkeur.

References

AARSETH S, BERGAN A and AARSETH P. Circulatory homeostasis in rats after bile duct ligation. Scand J Clin Lab Invest 1979; 39:93-97.

ALDERSON D, LAVELLE MI and VENABLES CW. Endoscopic sphincterotomy before pancreaticoduodenectomy for ampullary carcinoma. Br Med J 1981; 282: 1109-1111.

ALEXANDER F, ROSSI RL, O'BRYAN M, KHETTRY U, BRAASCH JW and WATKINS E. Biliary carcinoma. Am J Surg 1984; 147: 503-509.

ALLISON MEM, MOSS NG, FRASER MM, et al. Renal function in chronic obstructive jaundice: a micropuncture study in rats. Clin Sci Mol Med 1978; 54: 649-659.

ALLISON MEM, PRENTICE CRM, KENNEDY AC and BLUMGART LH. Renal function and other factors in obstructive jaudice. Br J Surg 1979; 66: 392-397.

ANDERSON RE and PRIESTLEY JT. Observations on the bacteriology of choledochal bile. Ann Surg 1951; 133: 486-489.

ANDRÉN-SANDBERG A and ISHE I. Factors influencing survival after total pancreatectomy in patients with pancreatic cancer. Ann Surg 1983; 198: 605-610.

AOYAGI T and LOWENSTEIN LM. The effect of bile acids and renal ischemia on renal function. J Lab Clin Med 1968; 71: 686-692.

ARII S, SHIBAGAKI M, TAKAHASHI E, et al. Different response of reticuloendothelial system to endotoxin in obstructive jaundiced and cirrhotic rats. Eur Surg Res (Suppl) 1983; 15: 107.

ARMSTRONG CP, DIXON JM, DUFFY SW, ELTON RA and DAVIES GC. Wound healing in obstructive jaundice. Br J Surg 1984; 71: 267-270.

ARMSTRONG CP, DIXON JM, TAYLOR TV and DAVIES GC. Surgical experience of deeply jaundiced patients with bile duct obstruction. Br J Surg 1984; 71: 234-238.

ARNAUD JP, HUMBERT W, ELOY MR and ADLOFF M. Effect of obstructive jaundice on wound healing: an experimental study in rats. Am J Surg 1981; 141: 593-596.

ARONSON KF. Liver function studies during and after complete extrahepatic biliary obstruction in the dog. Acta Chir Scand (Suppl) 1961; 275: 1-114.

ASTON SJ and LONGMIRE WP JR. Management of the pancreas after pancreaticoduodenectomy. Ann Surg 1974; 179: 322-327.

BAILEY ME. Endotoxin, bile salts and renal function in obstructive jaundice. Br J Surg 1976; 63: 774-778.

BANK N and AYNEDJIAN HS. A micropuncture study of renal salt and water retention in chronic bile duct obstruction. J Clin Invest 1975; 55: 994-1002.

BAUM M, STIRLING GA and DAWSON JL. Further study into obstructive jaundice and ischaemic renal damage. Br Med J 1969; 2: 229-231.

BAYER I and ELLIS H. Jaundice and wound healing: an experimental study. Br J Surg 1976; 63: 392-396.

BENJAMIN IS. The obstructed biliary tract. In: Blumgart LH (ed.): The biliary tract. Churchill Livingstone Edinburgh 1982; Chapter 10: 157-182.

BERQUIST TH, MAY GR, JOHNSON CM, ADSON MA and THISTLE JL. Percutaneous biliary decompression: internal and external drainage in 50 patients. AJR 1981; 136: 901-906.

BETTER OS. Bile duct ligation: an experimental model of renal dysfunction secondary to liver disease. In: Epstein M (ed.): The kidney in liver disease. Elsevier New York 1983: 295-311.

BETTER OS, AISENBREY GA, BERL T, et al. Role of antidiuretic hormone in impaired urinary dilution associated with chronic bile-duct ligation. Clin Sci 1980; 58: 493-500.

BIRNS M, MASEK B and AUERBACH O. The effects of experimental acute biliary obstruction and release on the rat: a histochemical study. Amer J Path 1962; 40: 95-111.

BLAMEY SL, FEARON KCH, GILMOUR WH, OSBORNE DH and CARTER DC. Prediction of risk in biliary surgery. Br J. Surg 1983; 70: 535-538.

BLENKHARN JI, McPHERSON GAD and BLUMGART LH. Septic complications of percutaneous transhepatic biliary drainage. Am J Surg 1984; 147: 318-321.

BLUMGART LH. Biliary tract obstruction: new approaches to old problems. Am J Surg 1978; 135: 19-31.

BOMZON I and KEW MC. Renal blood flow in experimental obstructive jaundice. In: Epstein M (ed.): The kidney in liver disease. Elsevier New York 1983: 313-326.

BOMZON L, WILTON PB, MENDELSOHN D and KEW MC. Bile salts, obstructive jaundice and renal blood flow. Israel J Med Sci 1979; 15: 169-171.

BRAASCH JW and GRAY BN. Considerations that lower pancreatoduodenectomy mortality. Am J Surg 1977; 133: 480-484.

BRAUDE AI, CAREY FJ, SUTHERLAND D and ZALESKY M. Studies with radioactive endotoxin: I. the use of CR^{51} to label endotoxin of Escherichia Coli. II. correlation of physiologic effects with distribution of radioactivity in rabbits injected with lethal doses of E. Coli endotoxin labelled with radioactive sodium chromate. J Clin Invest 1955; 34: 850-866.

BROOKS JR. The case for total pancreatectomy. In Delaney JP and Varco RS (eds.): Controversies in Surgery II. WB Saunders Philadelphia 1983: 327-335.

CABAK V, DICKERSON JWT and WIDDOWSON EM. Response of young rats to deprivation of protein or of calories. Br J Nutr 1963; 17: 601-616.

CAHILL CJ. Prevention of postoperative renal failure in patients with obstructive jaundice: the role of bile salts. Br J Surg 1983; 70: 590-595.

CAMERON GR and MUZAFFAR HASAN S. Disturbances of structure and function in the liver as the result of biliary obstruction. J Path Bact 1958; 75: 333-349.

CARBONE JV and GRODSKY GM. Constitutional nonhemolytic hyperbilirubinemia in the rat: defect of bilirubin conjugation. Proc Soc Exp Biol Med 1957; 94: 461-463.

CATTELL WR and BIRNSTINGL MA. Blood volume and hypotension in obstructive jaundice. Br J Surg 1967; 54: 272-278.

CEFALO RC, LEWIS PE, O'BRIEN WF, FLETCHER JR and RAMWELL PW. The role of prostaglandins in endotoxemia: comparisons in response in the nonpregnant, maternal, and fetal models. Am J Obstet Gynecol 1980; 137: 53-57.

CHILD III CG, HINERMAN DL and KAUFFMAN GL JR. Pancreaticoduodenectomy. Surg Gynecol Obstet 1978: 147: 529-533.

CLARK RA, MITCHELL SE, COLLEY DP and ALEXANDER E. Percutaneous catheter biliary decompression. AJR 1981; 137: 503-509.

CLAUSS A. Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogenes. Acta Haematol 1957; 17: 237-246.

COOK JA, WISE WC and HALUSHKA PV. Elevated thromboxane levels in the rat during endotoxic shock. J Clin Invest 1980; 65: 227-230.

COTTON PB. Progress report ERCP. Gut 1977; 18: 316-341.

COTTON PB. Duodenoscopic placement of biliary prostheses to relieve malignant obstructive jaundice. Br J Surg 1982; 69: 501-503.

COX JL, HELFRICH LR, PASS HI, OSTERHAUT S and SHINGLETON WW. The relationship between biliary tract infections and postoperative complications. Surg Gynecol Obstet 1978; 146: 233-236.

CRILE G JR. The advantages of bypass operations over radical pancreatoduodenectomy in the treatment of pancreatic carcinoma. Surg Gynecol Obstet 1970; 130: 1049-1053.

CRONIN K, JACKSON DS and DUNPHY JE. Changing bursting strength and collagen content of the healing colon. Surg Gynecol Obstet 1968; 126: 747-753.

DALY JM, VARS HM and DUDRICK SJ. Effects of protein depletion on strength of colonic anastomoses. Surg Gynecol Obstet 1972; 134: 15-21.

DAWSON JL. Jaundice and anoxic renal damage: protective effect of mannitol. Br med J 1964; 1: 810-811.

DAWSON JL. The incidence of postoperative renal failure in obstructive jaundice. Br J Surg 1965; 52: 663-665.

DAWSON JL. Acute post-operative renal failure in obstructive jaundice. Ann R Coll Surg Engl 1968; 42: 163-181.

DAWSON JL. Renal failure in obstructive jaundice: clinical aspects. Postgrad Med J 1975; 51: 510-511.

DE GRAAF PW. The influence of tumorgrowth on wound healing. Thesis 1981, University of Leiden.

DEJANA E, CALLIONI A, QUINTANA A and DE GAETANO G. Bleeding time in laboratory animals: II. a comparison of different assay conditions in rats. Throm Res 1979; 15: 191-197.

DE NICOLA. Thrombelastography. Charles C Thomas Springfield Ill 1957.

DENNING DA, ELLISON EC and CAREY LC. Preoperative percutaneous transhepatic biliary decompression lowers operative morbidity in patients with obstructive jaundice. Am J Surg 1981; 141: 61-65.

DIOGUARDI N, MARI D, DEL NINNO E and MANNUCCI PM. Fibrinolysis in cholestatic jaundice. Br Med J 1973; 2: 778-779.

DIXON JM, ARMSTRONG CP, DUFFY SW and DAVIES GC. Factors affecting morbidity and mortality after surgery for obstructive jaundice: a review of 373 patiens. Gut 1983; 24: 845-852.

DIXON JM, ARMSTRONG CP, DUFFY SW, ELTON RA and DAVIES GC. Upper gastrointestinal bleeding: a significant complication after surgery for relief of obstructive jaundice. Ann Surg 1984; 199: 271-275.

DOOLEY JS, OLNEY J, DICK R and SHERLOCK S. Non-surgical treatment of biliary obstruction. Lancet 1979; ii: 1040-1043.

DRIVAS G, JAMES O and WARDLE N. Study of reticuloendothelial phagocytic capacity in patiens with cholestasis. Br Med J 1976; 1: 1568-1569.

DYE M, MACDONALD A and SMITH G. The bacterial flora of the biliary tract and liver in man. Br J Surg 1978; 65: 285-287.

ELLIS H. Wound healing. Ann R Coll Surg Engl 1977; 59: 382-387.

ELLIS H and HEDDLE R. Does the peritoneum need to be closed at laparotomy? Br J Surg 1977; 64: 733-736.

ENGSTRÖM J, HELLSTRÖM K, HÖGMAN L and LÖNNQVIST B. Micro-organisms of the liver, biliary tract and duodenal aspirates in biliary diseases. Scand J Gastroenterol 1971; 6: 177-182.

EVANS HJR, TORREALBA V, HUDD C and KNIGHT M. The effect of preoperative bile salt administration on postoperative renal function in patients with obstructive jaundice. Br. J. Surg 1982; 69: 706-708.

FARNELL MB, VAN HEERDEN JA and BEART RW JR. Elective cholecystectomy: the role of biliary bacteriology and administration of antibiotics. Arch Surg 1981; 116: 537-540.

FARRAR WE JR and CORWIN LM. The essential role of the liver in detoxification of endotoxin. Ann NY Acad Sci 1966; 133: 668-684.

FLEMMA JF, FLINT LM, OSTERHOUT S and SHINGLETON WW. Bacteriologic studies of biliary tract infection. Ann Surg 1967; 166: 563-572.

FLETCHER JR and RAMWELL PW. Modification, by aspirin and indomethacin, of the haemodynamic and prostaglandin releasing effects of E. Coli endotoxin in the dog. Br J Pharmacol 1977; 61: 175-181.

FLETCHER MS, WESTWICK J and KAKKAR VV. Endotoxin, prostaglandins and renal fibrin deposition in obstructive jaundice. Br J Surg 1982; 69: 625-629.

FORREST JF and LONGMIRE JP JR. Carcinoma of the pancreas and the periampullary region. Ann Surg 1979; 189: 129-137.

FREEDMAN HH. Reticuloendothelial system and passive transfer of endotoxin tolerance. Ann NY Acad Sci 1960; 88: 99-106.

GALLAGHER P, OSTICK G, JONES D, SCHOFIELD PF and TWEEDLE DEF. Intraoperative microscopy of bile: is it useful? Br J Sug 1982; 69: 473-474.

GIANNI L, DI PADOVA F, ZUIN M and PODDA M. Bile acid-induced inhibition of the lymphoproliferative response to phytohemagglutinin and pokeweed mitogen: an in vitro study. Gastroenterology 1980; 78: 231-235.

GILLENWATER JY, DOOLEY ES and FROHLICH ED. Effects of endotoxin on renal function and hemodynamics. Am J Physiol 1963; 205: 293-297.

GILSDORF RB and SPANOS P. Factors influencing morbidity and mortality in pancreaticoduodenectomy. Ann Surg 1973; 177: 332-337.

GLENN F, EVANS JA, MUJAHED Z and THORBJARNARSON B. Percutaneous transhepatic cholangiography. Ann Surg 1962; 156: 451-462.

GOTTRUP F. Healing of incisional wounds in stomach and duodenum: collagen distribution and relation to mechanical strength. Am J Surg 1981a; 141: 222-227.

GOTTROP F. Healing of incisional wounds in stomach and duodenum: functional interaction between normal and wounded tissue. Am J Surg 1981b; 141: 706-711.

GOUMA DJ, WESDORP RIC, OOSTENBROEK RJ, SOETERS PB and GREEP JM. Percutaneous transhepatic drainage and insertion of an endoprosthesis for obstructive jaundice. Am J Surg 1983; 145: 763-767.

GREANEY MG, VAN NOORT R, SMYTHE A and IRVIN TT. Does obstructive jaundice adversely affect wound healing? Br J Surg 1979; 66: 478-481.

GREENE R, WIZNITZER T, RUTENBERG S, FRANK E and FINE J. Hepatic clearance of endotoxin absorbed from the intestine. Proc Soc Exp Biol Med 1961; 108: 261-263.

GUNDRY SR, STRODEL WE, KNOL JA, ECKHAUSER FE and THOMPSON NW. Efficacy of preoperative biliary tract decompression in patients with obstructive jaundice. Arch Surg 1984; 119: 703-708.

HALPERN BN, BIOZZI G, NICOL T and BILBEY DLJ. Effect of experimental biliary obstruction on the phagocytic activity of the reticuloendothelial system. Nature 1957; 180: 503-504.

HANSSON JA, HOEVELS J, SIMERT G, TYLEN U and VANG J. Clinical aspects of nonsurgical transhepatic bile drainage in obstructive lesions of the extrahepatic bile ducts. Ann Surg 1979; 189: 58-61.

HARKNESS MLR, HARKNESS RD and JAMES DW. The effect of a protein-free diet on the collagen of mice. J Physiol 1958; 144: 307-313.

HATFIELD ARW, TERBLANCHE J, FATAAR S, et al. Preoperative external biliary drainage in obstructive jaundice. Lancet 1982; ii: 896-899.

HINSHAW LB and BRADLEY GM. Alterations in kidney weight produced by Escherichia coli endotoxin. Am J Physiol 1957; 189: 329-330.

HINSHAW LB, SPINK WW, VICK JA, MALLET E and FINSTAD J. Effect of endotoxin on kidney function and renal hemodynamics in the dog. Am J Physiol 1961; 201: 144-148.

HISHIDA A, HONDA N, SUDO M and NAGASE M. Mechanisms of altered renal perfusion in the early stage of obstructive jaundice. Kidney Int 1980; 1: 223-230.

HOFFMANN RE and DONEGAN WL. Experience with pancreatoduodenectomy in a cancer hospital. Am J Surg 1975; 129: 292-297.

HOLMAN JM JR and RIKKERS LF. Biliary obstruction and host defense failure. J Surg Res 1982; 32: 208-213.

HOWARD JM. Pancreatico-duodenectomy: 41 consecutive Whipple resections without an operative mortality. Ann Surg 1968; 168: 629-640.

HUIBREGTSE K and TYTGAT GN. Palliative treatment of obstructive jaundice by transpapillary introduction of large bore bile duct endoprosthesis: experience in 45 patiens. Gut 1982; 23: 371-375.

HUNT TK. Disorders of repair and their management. In: Hunt TK and Dunphy JE (eds.): Fundamentals of wound management in surgery. Appleton-Century-Crofts New York 1979: 111.

HUNT DR, ALLISON MEM, PRENTICE CRM and BLUMGART LH. Endotoxemia, disturbance of coagulation, and obstructive jaundice. Am J Surg 1982; 144: 325-329.

IRVIN TT. Effects of malnutrition and hyperalimentation on wound healing. Surg Gynecol Obstet 1978; 116: 33-37.

IRVIN TT, VASSILAKIS JS, CHATTOPADHYAY DK and GREANEY MG. Abdominal wound healing in jaundiced patients. Br J Surg 1978; 65: 521-522.

JACOB AI, GOLDBERG PK, BLOOM N, DEGENSHEIN GA and KOZZIN PJ. Endotoxin and bacteria in portal blood. Gastroenterology 1977; 72: 1268-1270.

JEDRYCHOWSKI A, HILLENBRAND P, AJDUKIEWICZ AB, PARBHOO SP and SHERLOCK S. Fibrinolysis in cholestatic jaundice. Br Med J 1973; I: 640-642.

JONES EA and SUMMERFIELD JA. Kupffer cells. In: Arias I, Popper H, Schachter D and Shafritz DA (eds.): The liver, biology and pathobiology. Raven Press New York 1982; Chapter 30: 507-523.

JORGENSEN JH and SMITH RF. Measurements of bound and free endotoxin by the Limulus assay. Proc Soc Exp Biol Med 1974; 146: 1024-1031.

JUHLIN L. The effect of starvation on the phagocytic activity of the reticuloendothelial system. Acta Physiol Scand 1958; 43: 262-274.

KAKKAR VV, SPINDLER J, FLUTE PT, CORRIGAN T, FOSSARD DP and CRELLIN RQ. Efficacy of low doses of heparin in prevention of deep-vein thrombosis after major surgery. Lancet 1972; ii: 101-106.

KATZ S, GROSFELD JL, GROSS K, et al. Impaired bacterial clearance and trapping in obstructive jaundice. Ann Surg 1984; 199: 14-20.

KEIGHLEY MRB. Micro-organisms in the bile: a preventable cause of sepsis after biliary surgery. Ann R Coll Surg Engl 1977; 59: 329-334.

KEIGHLEY MRB and BURDON DW. Identification of bacteria in the bile by duodenal aspiration. World J Surg 1978; 2: 255-259.

KEIGHLEY MRB, FLINN R and ALEXANDER-WILLIAMS J. Multivariate analysis of clinical and operative findings associated with biliary sepsis. Br J Surg 1976; 63: 528-531.

KEIGHLEY MRB. Infection and the biliary tree. In: Blumgart LH (ed.): The biliary tract. Churchill Livingstone Edinburgh 1982; Chapter 14: 219-235.

KEILL RH, KEITZER WF, NICHOLS WK, HENZEL J and DEWEESE MS. Abdominal wound dehiscence. Arch Surg 1973; 106: 573-577.

KNIGHT CD and GRIFFEN FD. Abdominal wound closure with a continuous monofilament polypropylene suture: experience with 1000 consecutive cases. Arch Surg 1983; 118: 1305-1308.

KOCSÁR LT, BERTÓK L and VÁRTERÉSZ. Effect of bile acids on the intestinal absorption of endotoxin in rats. J Bact 1969; 100: 220-223.

KOPEĆ M, DAROCHA T, NIEWIAROWSKI S and STACHURSKA J The antithrombin activity of glucuronic esters of bilirubin. J Clin Path 1961; 14: 478-481.

KOYAMA K, TAKAGI Y, ITO K and SATO T. Experimental and clinical studies on the effect of biliary drainage in obstructive jaundice. Am J Surg 1981; 142: 293-299.

KRAUSE R. Anorexia in cancer. Thesis 1980. University of Maastricht.

KUNE GA, HIBBERD J and MORAHAN R. The development of biliary infection: an experimental study. Med J Aust 1974; 1: 301-303.

KUNE GA and SALI A. Applied physiology in biliary surgery. In: The practice of biliary surgery. Blackwell Oxford 1980; Chapter 2: 32-35.

KUNE GA and SALI A. Biliary infections. In: The practice of biliary surgery. Blackwell Oxford 1980; Chapter 9: 288-316.

KUNE GA and SCHUTZ E. Bacteria in the biliary tract: a study of their frequency and type. Med J Aust 1974; 1: 255-258.

KUNZ F, CONSTANTINI R, SEMENITZ E, MIKUZ G, SCHMALZL F and HOLZKNECHT F. The production of disseminated intravascular coagulation (DIC) by spaced injections of endotoxin in nonpregnant, normolipaemic rats. Thromb Res 1977; 12: 119-130.

LAYZELL D and MILLER T. Determination of glomerular filtration in the rat using ^{51}Cr-EDTA and a single blood sample. Invest Urol 1975; 13: 200-204.

LÁZÁR G. Influence of splenectomy, partial hepatectomy and bile duct ligation on the granulopectic activity of the reticuloendothelial system. Acta Physiol Acad Sci Hung 1972; 42: 287-291.

LEE ECG. The effect of obstructive jaundice on the migration of reticuloendothelial cells and fibroblasts into early experimental granulomata. Br J Surg 1972; 59: 875-877.

LERUT JP, GIANELLO PR, OTTE JB and KESTENS PJ. Pancreaticoduodenal resection. Ann Surg 1984; 199: 432-437.

MADDOCKS AC, HILSON GRF and TAYLOR R. The bacteriology of the obstructed biliary tree. Ann R Coll Surg Engl 1973; 52: 316-319.

MAKI T, SATO T and KAKIZAKI G. Pancreatoduodenectomy for periampullary carcinomas: appraisal of two-stage procedure. Arch Surg 1966; 92: 825-833.

MASUMOTO T and MASUOKA S. Kidney function in the severely jaundiced dog. Am J Surg 1980; 140: 426-430.

McCUNE WS, SHORB PE and MOSCOVITZ H. Endoscopic cannulation of the ampulla of Vater: a preliminary report. Ann Surg 1968; 167: 752-756.

McPHERSON GAD, BENJAMIN IS, HABIB NA, BOWLEY NB and BLUMGART LH. Percutaneous transhepatic drainage in obstructive jaundice: advantages and problems. Br J Surg 1982; 69: 261-264.

McPHERSON GAD, BENJAMIN IS, HODGSON HJF, BOWLEY NB, ALLISON DJ and BLUMGART LH. Pre-operative percutaneous transhepatic biliary drainage: the results of a controlled trial. Br J Surg 1984; 71: 371-375.

McPHERSON GAD, BENJAMIN IS and BLUMGART LH. Improving renal function in obstructive jaundice without preoperative drainage. Lancet 1984; i: 511-512.

MEULEMAN DG, HOBBELEN PMJ, VAN DEDEM G and MOELKER HCT. A novel antithrombic heparinoid (Org 10172) devoid of bleeding inducing capacity: a survey of its pharmacological properties in experimental animal models. Thromb Res 1982; 27: 353-363.

MITCHELL MS, BOVE JR and CALABRESI P. Simplified estimation of mouse isohemagglutinins by microassay. Transplantation 1969; 7: 294-296.

MOLNAR W and STOCKUM AE. Relief of obstructive jaundice through percutaneous transhepatic catheter- a new therapeutic method. Am J Roentgenol Radium Ther Nucl Med 1974; 122: 356-367.

MONGÉ JJ, JUDD ES and GAGE RP. Radical pancreatoduodenectomy: a 22-year experience with the complications, mortality rate, and survival rate. Ann Surg 1964; 160: 711-722.

MORI K, MATSUMOTO K and GANS H. On the in vivo clearance and detoxification of endotoxin by lung and liver. Ann Surg 1973; 177: 159-163.

MORI K, MISUMI A, SUGIYAMA M, et al: Percutaneous transhepatic bile drainage. Ann Surg 1977; 185: 111-115.

MUELLER PR, VAN SONNENBERG E and FERRUCI JT JR. Percutaneous biliary drainage: technical and catheter related problems in 200 procedures. AJR 1982; 138: 17-23.

MUNFORD RS. Endotoxin(s) and the liver. Gastroenterology 1978; 75: 532-535.

NAKASE A, MATSUMOTO Y, UCHIDA K and HONDO I. Surgical treatment of cancer of the pancreas and the periampullary region: cumulative results in 57 institutions in Japan. Ann Surg 1977; 185: 52-57.

NAKAYAMA T, IKEDA A and OKUDA K. Percutaneous transhepatic drainage of the biliary tract. Gastroenterology 1978; 74: 554-559.

NICOLAIDES AN, DESAI S, DOUGLAS JN, et al: Small doses of subcutaneous sodium heparin in preventing deep venous thrombosis after major surgery. Lancet 1972; ii: 890-893.

NIELSEN ML and JUSTESEN T. Anaerobic and aerobic bacteriological studies in biliary tract disease. Scand J Gastroenterol 1976; 11: 437-446.

NOLAN JP. Endotoxin, reticuloendothelial function, and liver injury. Hepatology 1981; 1: 458-465.

NORLANDER A, KALIN B and SUNDBLAD R. Effect of percutaneous transhepatic drainage upon liver function and postoperative mortality. Surg Gynecol Obstet 1982; 155: 161-166.

OBERTOP H, BRUINING HA, EEFTINCK SCHATTENKERK M, EGGINK WF, JEEKEL J and VAN HOUTEN H. Operative approach to cancer of the head of the pancreas and the periampullary region. Br J Surg 1982; 69: 573-576.

OKUDA K, TANIKAWA K, EMURA T, et al: Nonsurgical, percutaneous transhepatic cholangiography - diagnostic significance in medical problems of the liver. Am J Dig Dis 1974; 19: 21-36.

OLD LJ, CLARKE DA, BENACERRAF B and GOLDSMITH M. The reticuloendothelial system and the neoplastic process. Ann NY Acad Sci 1960; 88: 264-280.

OWREN PA. Thrombotest: a new method for controlling anticoagulant therapy. Lancet 1959; ii: 754.

OWREN PA. The interrelationship between normotest and thrombotest. Farmakoterapi 1969; 25: 1-13.

PITT HA, CAMERON JL, POSTIER RG and GADACZ TR. Factors affecting mortality in biliary tract surgery. Am J Surg 1981; 141: 66-72.

POWELL LW, DUNNICLIFF MA and BILLING BH. Red cell survival in experimental cholestatic jaundice. Br J Haemat 1968; 15: 429-435.

PROVOOST AP, DE KEIJZER MH, WOLFF ED and MOLENAAR JC. Development of renal function in the rat: the measurement of GFR and ERPF and correlation to body and kidney weight. Renal Physiol 1983; 6: 1-9.

PROVOOST AP and MOLENAAR JC. Renal function during and after a temporary complete unilateral ureter obstruction in rats. Invest Urol 1981; 18: 242-246.

REITAMO J and MÖLLER C. Abdominal wound dehiscence. Acta Chir Scand 1972; 138: 170-175.

RIEMANN JF, LUX G, RÖSCH W and BEICKERT-STERBA A. Non-surgical biliary drainage - technique, indications and results. Endoscopy 1981; 13: 157-161.

ROLA-PLESZCZYNSKI M, HENSEN SA, VINCENT MM and BELLANTI JA. Inhibitory effects of bilirubin on cellular immune responses in man. J Pediatrics 1975; 86: 690-696.

ROSS DE. Cancer of the pancreas. Am J Gastroenterol 1959; 31: 517-526.

RYAN CJ, THAN THAN and BLUMGART LH. Choledochoduodenostomy in the rat with obstructive jaundice. J Surg Res 1977; 23: 321-331.

SABA TM. Physiology and physiopathology of the reticuloendothelial system. Arch Intern Med 1970; 126: 1031-1052.

SATO T, SAITOH Y, KOYAMA K and WATANABE K. Preoperative determination of operability in carcinomas of the pancreas and the periampullary region. Ann Surg 1968; 168: 876-886.

SATO T, SAITOH Y, NOTO N and MATSUNO S. Follow-up studies of radical resection for pancreaticoduodenal cancer. Ann Surg 1977; 186: 581-588.

SCOTT AJ and KHAN GA. Origin of bacteria in bile duct bile. Lancet 1967; ii: 790-792.

SHAPIRO TM. Adenocarcinoma of the pancreas. Ann Surg 1975; 182: 715-721.

SHASHA SM, BETTER OS, CHAIMOVITZ C, DOMAN J and KISHON Y. Haemodynamic studies in dogs with chronic bile-duct ligation. Clin Sci Mol Med 1976; 50: 533-537.

SHINDO K and KOSAKI G. Effects of chronic renal failure on wound healing in rats: I. biochemical study. II. microscopic study and hydroxyproline assay. Jap J Surg 1982; 12: 41-51.

SMITH R. Carcinoma of the pancreas: a radical or conservative philosophy. In: Najarian JS and Delaney JP (eds.): Hepatic, biliary and pancreatic surgery. London Year Book 1980: 373-379.

SNELLEN JP, OBERTOP H, BRUINING HA, EEFTINCK SCHATTENKERK M, EGGINK WF, JEEKEL J and VAN HOUTEN H. The influence of preoperative jaundice, bile drainage and age on the results of pancreatoduodenectomy and total pancreatectomy. Submitted for publication.

SNELLEN JP, OBERTOP H and WESTBROEK DL. The effect of obstructive jaundice on wound healing in the rat. Eur Surg Res (Suppl) 1984; 16: 62.

SNELLEN JP, OBERTOP H and WESTBROEK DL. The effect of obstructive jaundice on wound healing and infection in the rat. Proceedings of the 25th dutch federation meeting 1984: 382.

SOEHENDRA N and REYNDERS-FREDERIX V. Palliative bile duct drainage - a new endoscopic method of introducing a transpapillary drain. Endoscopy 1980; 12: 8-11.

STIFFEL C, MOUTON D and BIOZZI G. Kinetics of the phagocytic function of reticuloendothelial macrophages in vivo. In: Van Furth R (ed.): Mononuclear phagocytes. Blackwell Oxford 1970; Chapter 23: 335-381.

STUART AE, HABESHAW JA and DAVIDSON AE. The carbon clearance test for in vivo phagocytosis. In: Weir MD (ed.): Handbook of experimental immunology. Blackwell Oxford 1973; Chapter 24: 18-21.

TAUBE M, ELLIOT P and ELLIS H. Jaundice and wound healing: a tissue culture study. Br J Exp Path 1981; 62: 227-231.

THAN THAN, EVANS JH, RYAN CJ, SMITH DA, HARPER AM and BLUMGART LH. Rupture strength of skin wounds in jaundiced rats. Br J Exp Path 1979; 60: 107-110.

THAN THAN, McGEE JO'D and BLUMGART LH. Prolyl hydroxylase in the skin of patients with obstructive jaundice. J Clin Path 1977; 30: 1044-1047.

THAN THAN, McGEE JO'D, SOKHI GS, PATRICK RS and BLUMGART LH. Skin prolyl hydroxylase in patients with obstructive jaundice. Lancet 1974; ii: 807-808.

TRAMS EG and SYMEONIDIS A. Morphological and functional changes in the livers of rats after ligation or excision of the common bile duct. Am J Path 1957; 33: 13-25.

TYLEN U, HOEVELS J and VANG J. Percutaneous transhepatic cholangiography with external drainage of obstructive biliary lesions. Surg Gynecol Obstet 1977; 144: 13-16.

VAN HEERDEN JA, REMINE WH, WEILAND LH, McILRATH DC and ILSTRUP DM. Total pancreatectomy for ductal adenocarcinoma of the pancreas. Am J Surg 1981; 142: 308-311.

VAN LANSCHOT JJB, TEN BERG RGM and BRUINING HA. Effect of propanolol on surgical bleeding and hemostasis: an in vivo study in the rat. J Surg Res 1984; 36: 89-91.

VAN NOORT R, BLACK MM, GREANEY MG and IRVIN TT. A new in vitro method for the measurement of mechanical strength of abdominal wounds in laboratory animals. Engineering in Medicine 1978; 7: 217-221.

VREEKEN J. Thrombelastografie. Thesis 1957, University of Amsterdam.

WALSH DB, ECKHAUSER FE, CRONENWETT JL, TURCOTTE JG and LINDENAUER SM. Adenocarcinoma of the ampulla of Vater. Ann Surg 1982; 195: 152-157.

WARD MWN, DANZI M, LEWIN MR, RENNIE MJ and CLARK CG. The effect of subclinical malnutrition and refeeding on the healing of experimental colonic anastomoses. Br J Surg 1982; 69: 308-310.

WARDLE EN. Fibrinogen in liver disease. Arch Surg 1974; 109: 741-746.

WARDLE EN. Renal failure in obstructive jaundice: pathogenic factors. Postgrad Med J 1975; 51: 512-514.

WARDLE EN and WILLIAMS R. Polymorph leucocyte function in uraemia and jaundice. Acta Haemat 1980; 64: 157-164.

WARDLE EN and WRIGHT NA. Endotoxin and acute renal failure associated with obstructive jaundice. Br Med J 1970; 4: 472-474.

WARREN KW, CATTEL RB, BLACKBURN JR and NORA PF. A long term appraisal of pancreaticoduodenal resection for peri-ampullary carcinoma. Ann Surg 1962; 155: 653-662.

WARREN KW, CHOE DS, PLAZA J and RELIHAN M. Results of radical resection for periampullary cancer. Ann Surg 1975; 181: 534-540.

WARREN KW, CHRISTOPHI C, ARMENDARIZ R and BASU S. Current trends in the diagnosis and treatment of carcinoma of the pancreas. Am J Surg 1983; 145: 813-818.

WESDORP RIC, KRAUSE R and VON MEYENFELDT MF. Cancer cachexia and its nutritional implications. Br J Surg 1983; 70: 352-355.

WHIPPLE AO, PARSONS WB and MULLINS CR. Treatment of carcinoma of the ampulla of Vater. Ann Surg 1935; 102: 763-779.

WIECHEL KL. Percutaneous transhepatic cholangiography: technique and application. With studies of the hepatic venous and biliary duct pressures, the chemical changes in blood and bile and clinical results in a series of jaundiced patients. Acta Chir Scand (Suppl); 1964: 380: 1-99.

WILKINSON SP, GAZZARD BG, ARROYO V, MOODIE H and WILLIAMS R. Relation of renal impairment and haemorrhagic diathesis to endotoxaemia in fulminant hepatic failure. Lancet 1974; i: 521-524.

WILKINSON SP, MOODIE H, STAMATAKIS JD, KAKKAR VV and WILLIAMS R. Endotoxaemia and renal failure in cirrhosis and obstructive jaundice. Br Med J 1976; 2: 1415-1418.

WILLIAMS HT. Prevention of postoperative deep-vein thrombosis with perioperative subcutaneous heparin. Lancet 1971; ii: 950-952.

WILLIAMS RD, ELLIOTT DW and ZOLLINGER RM. The effect of hypotension in obstructive jaundice. Arch Surg 1960; 81: 334-338.

WILLIAMS W, BEUTLER E, ERSLEV A and RUNDLES R (eds.) Hematology. McGraw-Hill Book Company New York 1972: 1401.

WISE L, PIZZIMBONO C and DEHNER LP. Periampullary cancer: a clinicopathologic study of 62 patients. Am J Surg 1976; 131: 141-148.

YARGER WE. Intrarenal mechanisms of salt retention after bile duct ligation in rats. J Clin Invest 1976; 57: 408-418.

ZILLY W, LIEHR H and HÜMMER N. Chiba-needle percutaneous cholangiography - a method without risk to the patient? Endoscopy 1980; 12: 12-15.

ZIMMON DS, FALKENSTEIN DB, RICCOBONO C and AARON B. Complications of endoscopic retrograde cholangiopancreatography: analysis of 300 consecutive cases. Gastroenterology 1975; 69: 303-309.

ZINNER MJ, ROBINSON BAKER R and CAMERON JL. Pancreatic cutaneous fistulas. Surg Gynecol Obstet 1974; 138: 710-712.

ZOUCAS E, BERGQVIST D, GÖRANSSON G and BENGMARK S. Effect of acute ethanol intoxication on primary haemostasis, coagulation factors and fibrinolytic activity. Eur Surg Res 1982; 14: 33-44.

ZUCKERMAN L, COHEN E, VAGHER JP, WOODWARD E and CAPRINI JA. Comparison of thrombelastography with common coagulation tests. Thromb Haemostas 1981; 46: 752-756.

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