Portal and systemic bacteraemia and endotoxaemia in liver disease¹

D. R. TRIGER², T. D. BOYER³, AND J. LEVIN

From the University of Southern California School of Medicine, Los Angeles, California, and the Johns Hopkins University School of Medicine and Hospital, Baltimore, Maryland, USA

SUMMARY Using a percutaneous transhepatic technique, blood was obtained from the portal veins of 30 patients with various hepatic disorders and examined for the presence of bacteria and endotoxin. Simultaneous samples also were drawn from hepatic and peripheral veins. In three cases, portal vein cultures grew diphtheroids, which were of doubtful significance, while all hepatic and peripheral cultures were sterile. Endotoxin was detected in seven portal vein samples; in none of these patients were the hepatic or peripheral blood samples positive. In three cases, only peripheral blood samples were positive for endotoxin. It was concluded that portal bacteraemia occurs as infrequently in patients with liver disease as in those without. Portal endotoxaemia was detected in patients with all degrees of liver disease but, even in patients with moderately severe portal hypertension, the liver may remain an effective filter of endotoxin.

The idea that the liver may act as a filter of gastrointestinal tract bacteria that have entered the portal vein has long interested investigators. Although Schatten *et al.* (1953) reported a significant number of positive portal vein cultures, other workers (Coblentz *et al.*, 1954; Taylor, 1956; Orloff *et al.*, 1958) have reported very few positive results and the general consensus has been that most of the recovered organisms were contaminants. These studies have usually been performed on blood samples taken under general anaesthesia, either during laparotomy or umbilical vein catheterisation (Dencker *et al.*, 1974). However, Coblentz *et al.* (1954) investigated a series of patients with advanced malignant disease, using a blind technique of percutaneous transhepatic portal venography under local anaesthesia, and obtained similarly negative results.

The situation in liver disease may, however, be different, as cirrhotic patients have an increased

incidence of septicaemia and spontaneous bacterial peritonitis of presumed enteric origin (Conn, 1975). Although this may be secondary to the impaired ability of the cirrhotic liver to kill bacteria (Rutenburg *et al.*, 1959), an additional possibility is that increased numbers of bacteria may be present in the portal vein. The presence of such bacteria might be important in the pathogenesis of liver disease, as the induction of experimental nutritional cirrhosis in rats can be delayed considerably by pre-feeding them with neomycin (Rutenburg *et al.*, 1957).

More recently, clinical and laboratory studies have suggested that the liver may act as a barrier to the passage of endotoxin from the gastrointestinal tract to the systemic circulation and that, after liver injury, this barrier may be ineffective (Ravin *et al.*, 1960; Farrar and Corwin, 1966; Nolan 1975). Studies in man have shown that systemic endotoxaemia correlates with the renal failure associated with acute hepatic failure (Wilkinson *et al.*, 1974). However, controlled studies of the presence of endotoxin or bacteria in the portal vein of patients with liver disease are lacking, largely because of the difficulty of obtaining blood samples.

A technique which gives easy and reliable access to the portal venous system in patients under local anaesthesia (Boyer *et al.*, 1977) has hitherto been used only for direct portal pressure measurement. Portal vein blood also can be aspirated and, using this technique, we have undertaken a study of portal vein bacteria and endotoxin in a series of patients

¹This project was supported in part by Research Grant HL-01601 from the National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland USA.

²Address for correspondence: Dr D. R. Triger, Department of Medicine, Hallamshire Hospital, Sheffield S10 2JF, England.

Dr Triger was the recipient of a Medical Research Council Travelling Fellowship.

³Present address: 113 McLellan Avenue, San Mateo, California 94403, USA.

Received for publication 26 April 1978

with liver disease. This paper presents the results of the study.

Methods

PATIENTS

Blood samples were obtained from 30 patients during the evaluation of their wedged hepatic vein and portal vein pressures. Written informed consent was obtained from each patient and the project was approved by the Ethical Committee of the John Wesley County Hospital. Fifteen patients with alcoholic liver disease were studied, comprising five with alcoholic cirrhosis, one with steatosis, and nine with alcoholic hepatitis $\pm$ cirrhosis. The other 15 had non-alcoholic liver disease (six chronic aggressive hepatitis, four primary biliary cirrhosis, two chronic persistent hepatitis, one each with idiopathic portal fibrosis, methotrexate-induced cirrhosis, and pancreatic carcinoma with hepatic secondaries). All diagnoses were confirmed by liver biopsy. No patients were receiving antibiotics (including neomycin) at the time of the study and patients with evidence of systemic infection were specifically excluded. Urine culture was performed on all patients, to exclude asymptomatic urinary tract infection. Two patients with chronic active hepatitis were receiving corticosteroids and one of these also was taking azathioprine. Another patient had been taking azathioprine until three months before study.

TECHNIQUE

Portal vein blood was sampled using the technique described for percutaneous transhepatic vein pressure measurement (Boyer *et al.*, 1977). Briefly, this was carried out under local anaesthesia in fully conscious patients by inserting a Chiba needle into the patient from a point in the mid-axillary line, just below the costophrenic angle toward the T11-12 interspace. The position of the needle was monitored by fluoroscopy. Fifty per cent diatrizoate meglumine (Hypaque) was injected through the needle while it was withdrawn slowly through the liver substance. By this technique, entry into a blood vessel or bile duct could be readily identified by the character and direction of flow of the contrast material. Once blood could be easily aspirated, the needle was cleared of all contrast material and blood was withdrawn into a heparinised, disposable syringe. Ideally, 10 ml of blood was aspirated. Two millilitres was injected directly into an anaerobic culture tube. The remaining 8 ml was centrifuged and the plasma divided into two equal parts before being placed in separate endotoxin-free tubes. These tubes were coded and stored at -20°C .

Bile ducts of normal calibre were entered twice

and a normal gall bladder once during the study. In each case the needle was removed and a clean needle inserted along a different track. In all three cases, the portal blood which was subsequently aspirated was sterile.

Hepatic vein blood samples were obtained through a hepatic vein catheter and were processed similarly. A new catheter was used for each procedure. Initially, peripheral vein samples were obtained through an antecubital vein, using fully aseptic techniques. Early studies showed that samples obtained *via* the hepatic vein catheter were consistently endotoxin-free and, therefore, subsequent samples of peripheral venous blood were obtained at the end of each study, as the catheter was being removed.

Collection tubes, glass pipettes, and transhepatic needles were rendered endotoxin-free by heating at 180°C for three hours. Preliminary testing confirmed that the contrast material was endotoxin-free, and a 1:10 dilution of Hypaque did not prevent the detection of known concentrations of endotoxin with the Limulus assay.

BACTERIAL CULTURE

Samples of portal vein, hepatic vein, and peripheral blood were collected in Anaport gassed-out tubes (Scott Laboratories, Fiskville, Rhode Island) and transported immediately to the microbiology laboratory. Conventional media for aerobic and anaerobic cultivation were inoculated from each specimen. The anaerobic plates for primary isolation (brain heart infusion blood agar and vitamin K-hemin solution blood agar) were incubated in a Gaspak jar (Baltimore Biological Laboratories, Cockeysville, Maryland) for 48 hours, before periodic examination over 14 days. Direct smears for gram stain also were obtained at the time of inoculation.

ENDOTOXIN

Endotoxin was detected with the Limulus amoebocyte lysate assay, as first described by Levin and Bang (1969). All samples of plasma were extracted initially with chloroform for four hours to remove inhibitors. (Levin *et al.*, 1970). 0.05 ml of the middle cloudy layer, which resulted after centrifugation of the emulsion of plasma and chloroform, was mixed with 0.05 ml of amoebocyte lysate, incubated at 37°C for four hours, and kept at room temperature for an additional 20 hours. The assay was read at hourly intervals during the initial four hours and the final reading was performed after 24 hours. Gelation, increased viscosity, or a definite increase in turbidity with flocculation were interpreted as positive reactions for endotoxin or endotoxin-like activity. Concurrent controls were performed with sterile, pyrogen-free 0.9% NaCl (Cutter Laboratories,

Berkeley, California) and were uniformly negative. The lysate used was capable of detecting 0.00005 ug/ml of *E. coli* endotoxin (026:B6, Difco Laboratories, Detroit, Michigan) in NaCl and 0.0001 ug/ml in plasma. All samples were coded and assays were reported without knowledge of clinical data or source of sample.

Initial determinations were carried out using only one of each pair of stored tubes of plasma but, in order to test the reproducibility of the assay and the reliability of the endotoxin-free tubes, 10 of the duplicate tubes (including both positive and negative samples) were recoded and tested blindly. Positive and negative reactions were entirely reproducible, although there was a slight inconsistency in the degree of positivity of one sample.

Results

BACTERIA

Satisfactory samples for culture were obtained from peripheral veins in all 30 cases, portal veins in 28, and hepatic veins in 13 (samples were not taken routinely from the hepatic vein in the early part of the study). All peripheral and hepatic vein samples were sterile, but three positive cultures were obtained from portal vein samples (Table 1). Two grew aerobic diphtheroids and an anaerobic diphtheroid was cultured from the third. Further classification of these bacteria was not attempted.

ENDOTOXIN

Samples for endotoxin estimation were obtained from both portal and peripheral veins in all 30 patients and from the hepatic vein in 29. Hepatic vein catheterisation was unsuccessful in one case. Eleven samples were positive for endotoxin (Table 1). Seven of these were portal vein samples and in each

case the hepatic vein and peripheral vein samples were negative for endotoxin. In one patient in whom anaerobic diphtheroids were cultured from the portal vein blood, the same blood sample also was strongly positive for endotoxin. In three patients, only the peripheral blood sample was endotoxin positive. In each case, the blood was taken through the hepatic vein catheter, the hepatic vein sample obtained by the same route being endotoxin-negative. In one patient, the hepatic vein sample was positive for endotoxin, while peripheral and portal vein samples were negative. Table 2 shows the frequency of endotoxin-positive samples according to major disease diagnoses. Endotoxin was detected in the portal venous blood of patients with both alcoholic and non-alcoholic liver disease with comparable frequency.

Discussion

This is the first systematic study of portal vein blood in fully conscious patients with liver disease. In common with earlier bacteriological studies of patients without liver disease, we encountered occasional cultures positive for diphtheroids, which we were inclined to dismiss as contaminants, although such organisms may be pathogenic and cause septicaemia (Jobanputra and Swain, 1975; Weiner and Werthamer, 1975; Gerry and Greenough, 1976). The anaerobic diphtheroid cultured in case number 28 is of particular interest. The same blood sample was also strongly positive for endotoxin. It is noteworthy that injection of contrast material into the portal vein showed evidence of hepatofugal flow, a situation rarely encountered in hepatic cirrhosis. We cannot comment upon whether the association of this phenomenon with portal bacteraemia and endotoxaemia is more than coincidental.

Table 1 Summary of patients with endotoxaemia or bacteraemia

Case no.	Diagnosis	PVP (mm Hg > IVC)	Endotoxin			Culture
			PV	HV	Periph V.	
4	Chronic active hepatitis	ND	-	+	-	-
13	Primary biliary cirrhosis	20	+	-	-	-
15	Chronic persistent hepatitis	4	+	-	-	-
16	Primary biliary cirrhosis	9½	-	-	-	Aer. diphth.
19	Chronic active hepatitis	16½	-	-	-	Aer. diphth.
20	Chronic active hepatitis	3½	-	-	+	-
22	Alcoholic hepatitis + cirrhosis	12½	+	-	-	-
24	Alcoholic hepatitis + cirrhosis	27½	-	-	+	-
25	Alcoholic fatty liver	7½	+	-	-	-
27	Chronic active hepatitis	9	+	-	-	-
28	Alcoholic hepatitis + cirrhosis	16	+	-	-	Anaer. diphth.
29	Alcoholic hepatitis	15½	-	-	+	-
30	Primary biliary cirrhosis	12	+	-	-	-

PVP: portal venous pressure.

Normal portal vein pressure < 5 mm Hg above IVC (inferior vena cava).

ND: not determined.

Table 2 Relationship between detection of endotoxin and type of liver disease

	Source of sample			
	Portal	Hepatic	Peripheral	Total
Alcoholic liver disease	3/15*	0/15	2/15	5/45
Chronic active hepatitis	1/5	1/5	1/5	3/15
Primary biliary cirrhosis	2/4	0/4	0/4	2/12
Miscellaneous	1/6	0/5†	0/6	1/17
Total	7/30	1/29	3/30	

*Ratio indicates number of samples positive for endotoxin/total samples tested.

†Hepatic vein sample not obtainable in one case.

Although portal bacteraemia is unlikely to be a common event in liver disease, we cannot exclude the possibility that it occurs intermittently. Also, it may occur more frequently in patients with disease more advanced than those studied here.

In contrast, portal endotoxaemia appears to be relatively common (seven out of 30) in patients with liver disease. It occurred as frequently in non-alcoholic liver disease as in alcoholic liver disease; we have no data to suggest that it does not occur with similar frequency in patients without liver disorders. It would be unethical to contemplate portal vein sampling by this technique in healthy subjects. Three studies of portal endotoxaemia in patients undergoing abdominal surgery have shown a frequency of none out of 16 (Bailey, 1976), seven out of 15 (Prytz *et al.*, 1976), and 33 out of 34 (Jacob *et al.*, 1977). These apparent discrepancies may reflect differences in assay sensitivity, choice of subjects, or technique in obtaining samples. Prytz and colleagues (1976), noting a similar frequency of Limulus positive samples in arterial blood of ambulant patients with liver disease to that in portal vein blood of peptic ulcer patients at surgery, inferred that cirrhotic livers lose the capacity to filter endotoxin. Our observation that none of seven patients with portal endotoxaemia had hepatic or peripheral vein endotoxin does not support this hypothesis.

The finding of peripheral vein endotoxaemia in three patients was unexpected. All three samples were obtained through the hepatic vein catheter while it was being withdrawn and, as all three hepatic vein samples were endotoxin-free, the possibility of contamination at the time of sampling is remote. Two patients had alcoholic hepatitis and fever and it is tempting to speculate that endotoxin was associated with the cause of the fever. However, seven other patients with alcoholic hepatitis and pyrexia were endotoxin-negative. The third patient had chronic active hepatitis and, although in remission and not receiving therapy at the time of the study, she relapsed three months later. It has been assumed that

endotoxaemia associated with liver disease originates in the gastrointestinal tract (Caridis *et al.*, 1972) but its occasional production elsewhere could account for isolated positive peripheral vein samples.

Contamination at the time of sampling could account for the positive samples obtained in this study. The extreme sensitivity of the Limulus test makes this a possibility, although every effort to avoid contamination was made. Skin contamination at the site of entry was minimised by thorough cleansing, and the same procedures were adopted in both the antecubital fossa and over the liver. Ability to reproduce the results with samples which were retested, in addition to validating the laboratory assay, is strong evidence against the positive results being due to contamination of the tubes. Furthermore, previous studies from our laboratory did not demonstrate endotoxaemia in normal persons (Levin *et al.*, 1970).

Our technique depends upon the use of contrast material to identify the portal venous system, raising the possibility that the presence of Hypaque might affect the detection of endotoxin and bacteria. Although Hypaque is bactericidal to *E. coli in vitro*, *in vivo* studies in urine have not shown any effect in concentrations up to 25% (Narins and Chase, 1971).

The relatively low frequency of portal endotoxaemia does not exclude it as an important clinical entity, as it may occur only intermittently. In more severe liver disease, portal endotoxaemia may be more common, and may occur to such a degree that the liver is no longer able to filter endotoxin. Certain complications of liver disease are associated with systemic endotoxaemia, notably renal failure (Wilkinson *et al.*, 1974), ascites (Tarao *et al.*, 1977) and consumption coagulopathy (Liehr *et al.*, 1975) and are usually found with very advanced hepatic damage. The latter two complications contraindicate portal vein sampling by our technique but there were no clinical, biochemical or histological features to distinguish the patients with positive endotoxin tests (either peripheral or portal) from the others.

Our findings suggest that even the damaged liver can clear endotoxin from the circulation. Although this appears to contradict observations in experimental animals (Ravin *et al.*, 1960; Greene *et al.*, 1961; Farrar and Corwin, 1966), such comparisons may not be valid because of the widely differing conditions for inducing endotoxaemia and liver damage.

We are grateful to Dr Telfer Reynolds and Dr Allan Redeker for permission to study their patients and to Dr H. Canawati for assistance with the blood

cultures. Francine Cortesy Levin provided excellent technical assistance.

References

- Bailey, M. E. (1976). Endotoxin, bile salts and renal function in obstructive jaundice. *British Journal of Surgery*, **63**, 774-778.
- Boyer, T. D., Triger, D. R., Horisawa, M., Redeker, A. G., and Reynolds, T. B. (1977). Direct transhepatic measurement of portal vein pressure using a thin needle. Comparison with wedged hepatic vein pressure. *Gastroenterology*, **72**, 584-589.
- Caridis, D. T., Reinhold, R. B., Woodruff, P. W. H., Fine, J. (1972). Endotoxaemia in man. *Lancet*, **1**, 1381-1386.
- Coblentz, A., Kelly, K. H., Fitzpatrick, L., and Bierman, H. R. (1954). Microbiologic studies of the portal and hepatic venous blood in man. *American Journal of Medical Sciences*, **228**, 298-300.
- Conn, H. O. (1975). Cirrhosis. In *Diseases of the Liver*, pp. 833-939. Edited by L. Schiff. Lippincott: Philadelphia.
- Dencker, H., Kamme, C., Norrby, C., March, P. A., Tylén, U. (1974). Examination for aerobic and anaerobic bacteria in human portal blood collected by transumbilical catheterization. *Scandinavian Journal of Gastroenterology*, **9**, 367-369.
- Farrar, W. E., and Corwin, L. M. (1966). The essential role of the liver in detoxification of endotoxin. *Annals of the New York Academy of Sciences*, **133**, 668-684.
- Gerry, J. L., and Greenough, W. B., III (1976). Diphtheroid endocarditis. Report of nine cases and review of the literature. *Johns Hopkins Medical Journal*, **139**, 61-68.
- Greene, R., Wiznitzer, T., Rutenburg, S., Frank, E., and Fine, J. (1961). Hepatic clearance of endotoxin absorbed from the intestine. *Proceedings of the Society for Experimental Biology and Medicine*, **108**, 261-263.
- Jacob, A. I., Goldberg, P. K., Bloom, N., Degenshein, G. A., and Kozinn, P. J. (1977). Endotoxin and bacteria in portal blood. *Gastroenterology*, **72**, 1268-1270.
- Jobanputra, R. S., and Swain, C. P. (1975). Septicaemia due to corynebacterium hemolyticum. *Journal of Clinical Pathology*, **28**, 798-800.
- Levin, J., and Bang, F. B. (1969). Clottable protein in Limulus: its localization and kinetics of its coagulation by endotoxin. *Thrombosis et Diathesis Haemorrhagica*, **19**, 186-197.
- Levin, J., Tomasulo, P. A., and Oser, R. S. (1970). Detection of endotoxin in human blood and demonstration of an inhibitor. *Journal of Laboratory and Clinical Medicine*, **75**, 903-911.
- Levin, J., Poore, T. E., Zauber, N. P., and Oser, R. S. (1970). Detection of endotoxin in the blood of patients with sepsis due to gram-negative bacteria. *New England Journal of Medicine*, **283**, 1313-1316.
- Liehr, H., Grün, M., Brunswig, D., and Sautter, Th. (1975). Endotoxaemia in liver cirrhosis: treatment with polymyxin B (Letter). *Lancet*, **1**, 810-811.
- Narins, D. J., and Chase, R. M., Jr. (1971). The effect of Hypaque upon urine cultures. *Journal of Urology*, **105**, 433-435.
- Nolan, J. P. (1975). The role of endotoxin in liver injury. *Gastroenterology*, **69**, 1346-1356.
- Orloff, M. J., Peskin, G. W., and Ellis, H. L. (1958). A bacteriologic study of human portal blood: implications regarding hepatic ischemia in man. *Annals of Surgery*, **148**, 738-746.
- Prytz, H., Holst-Christensen, J., Korner, B., and Liehr, H. (1976). Portal venous and systemic endotoxaemia in patients without liver disease and systemic endotoxaemia in patients with cirrhosis. *Scandinavian Journal of Gastroenterology*, **11**, 857-863.
- Ravin, H. A., Rowley, D., Jenkins, C., and Fine, J. (1960). On the absorption of bacterial endotoxin from the gastrointestinal tract of the normal and shocked animal. *Journal of Experimental Medicine*, **112**, 783-792.
- Rutenburg, A. M., Sonnenblick, E., Koven, I., Aprahamian, H. A., Reiner, L., and Fine, J. (1957). The role of intestinal bacteria in the development of dietary cirrhosis in rats. *Journal of Experimental Medicine*, **106**, 1-14.
- Rutenburg, A. M., Sonnenblick, E., Koven, I., Schweinburg, F., Fine, J., (1959). Comparative response of normal and cirrhotic rats to intravenously injected bacteria. *Proceedings of the Society for Experimental Biology and Medicine*, **101**, 279-281.
- Schatten, W. E., Desprez, J. D., and Holden, W. D. (1953). A bacteriologic study of portal-vein blood in man. *Archives of Surgery*, **71**, 404-409.
- Tarao, K., So, K., Moroi, T., Ikeuchi, T., Suyma, T., Endo, O., and Fukushima, K. (1977). Detection of endotoxin in plasma and ascitic fluid of patients with cirrhosis: its clinical significance. *Gastroenterology*, **73**, 539-542.
- Taylor, F. W. (1956). Blood-culture studies of the portal vein. *Archives of Surgery*, **72**, 889-892.
- Weiner, M., and Werthamer, S. (1975). Corynebacterium aquaticum septicemia. *American Journal of Clinical Pathology*, **64**, 378-381.
- Wilkinson, S. P., Arroyo, V., Gazzard, B. G., Moodie, H., and Williams, R. (1974). Relation of renal impairment and haemorrhagic diathesis to endotoxaemia in fulminant hepatic failure. *Lancet*, **1**, 521-524.