SEPTIC SHOCK:

Its influence on liver function and the significance of plasma fibronectin

by

PAUL M. NEWMAN

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Departments of Surgery and Biochemistry

University of Glasgow

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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AIMS

The experiments described in this thesis were undertaken to follow up the assertion (Fine, 1954) that endotoxin (widely accepted as a contributing factor in the clinical syndrome of septic shock) more often derives from the normal bacterial population of the intestine than from a focus of invading pathogens.

If this were the case, it would follow that the liver, constituting as it does the channel through which endotoxin necessarily passes, should be particularly exposed to damage. Assessment of the extent of such damage, therefore, seemed appropriate. The immediate difficulty was that the established tests of liver function, such as assays of plasma alkaline phosphatase, aspartate transaminase, alanine transaminase, and gamma-glutamyl transferase, or measurement of plasma bilirubin, while useful indicators of liver impairment for clinical purposes, do not provide any quantitative estimate of the loss of liver function. Such an estimate could, however, be obtained by measurement of clearance of antipyrine, which is exclusively metabolised by the cytochrome P450 system of the liver. An additional important factor was the effect of endotoxin on distribution of cardiac output, with particular regard to the splanchnic and hepatic circulation. Measurement of liver blood flow was therefore considered to be a valuable complementary measurement to that of antipyrine clearance. Sequential measurements of both indocyanine and antipyrine clearance in the presence of endotoxin should help to elucidate the role of the latter in disturbed hepatocellular function.

The second issue addressed by these experiments was the interrelationship between hepatocellular and reticuloendothelial function. Fibronectin assay was used to assess reticuloendothelial function in sepsis and, together with measurements of cardiovascular and respiratory status, to determine the effect of restoring to normal values depressed fibronectin levels by means of cryoprecipitate infusion.

SUMMARY

1. A commercially available immunoturbidimetric assay for plasma fibronectin has been modified to improve its sensitivity to the point where it can be used to measure the low levels frequently encountered in hospital practice.

2. A study of plasma fibronectin levels in healthy subjects revealed a Gaussian distribution. The mean for young males was higher than that for young females. In both sexes elderly subjects showed higher levels than young subjects, but the difference in males was less than that in females, so that the sex difference was reversed. Surgery caused a transient decrease. In addition, a more sustained decrease was seen in non-survivors.

3. An investigation of physiological and biochemical changes in patients suffering from septic shock was undertaken. It was found that in such patients indocyanine green clearance (which is normally regarded as an index of hepatic blood flow) was less than half the normal value. There was no significant difference in absolute values between patients who ultimately survived and those who succumbed, but, in general, the former showed an improvement in clearance during the first 24 hours in Intensive Care, while the latter showed a deterioration. Antipyrine half-life (which is generally taken as an index of the activity of the cytochrome P450 system) was prolonged to about twice the normal value. Plasma fibronectin levels were invariably low on admission to Intensive Care; consistently low or steadily falling levels generally presaged death.

4. The levels of plasma fibronectin in septic shock patients could be restored to normal by repeated infusions of cryoprecipitate. This treatment significantly reduced their plasma endotoxin levels and improved their urine output. It appeared also to improve mortality rate, at least in the first few days of the patients' stay in intensive care. Cardiac output, pulmonary artery wedge pressure, pulmonary vascular resistance and indocyanine green clearance were not significantly altered.

5. The observation that antipyrine half-life is extended in septic shock was followed up by an investigation of the effect of intravenous endotoxin administration on the cytochrome P450 system in the livers of rats. It was found that the activities of cytochrome b5, the cytochrome c reductases and cytochrome P450 itself were all reduced, the reduction being greatest with cytochrome P450. These effects were produced at endoxotin levels far below those required to lower the blood pressure.

6. The observation that indocyanine green clearance is greatly impaired in septic shock was followed up by infusing suspensions of Escherichia coli into pigs. This elicited the haemodynamic response similar to that seen in untreated septic shock patients: cardiac output was maintained initially and then fell; blood pressure fell gradually and progressively; tachycardia developed; and pulmonary vascular resistance rose sharply. Simultaneous infusion of colloid at a level sufficient to maintain left atrial pressure at normal levels (a form of treatment customary in septic shock) similarly produced effects comparable to those seen in patients: cardiac output increased; blood pressure was maintained initially but then dropped;

and tachycardia was less marked than in the absence of colloid administration. Whether colloid was infused or not, there was a rapid and progressive fall in plasma fibronectin. Indocyanine green clearance was decreased by 50%, perhaps because of impaired extraction by the liver rather than diminished hepatic blood flow. Blood lactate levels initially tended to fall but towards the end of the experiment rose dramatically, whether colloid was given or not.

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CHAPTER 1

INTRODUCTION

Though shock is a common clinical problem, it defies simple definition. Its cause is multifactorial, but inadequate tissue perfusion combined with a decrease in oxygen availability and utilisation are ubiquitous manifestations, regardless of the insult. Sepsis is now more frequently recognised as a cause, and a combination of sepsis and shock has an extremely poor prognosis. Many agents have been implicated in the pathogenesis of septic shock: Gram-positive bacteria, Gram-negative bacteria, fungi and viruses. The same clinical picture is observed regardless of the cause, and it is generally accepted that the haemodynamic disturbances are merely a reflection of the underlying metabolic derangement.

This chapter describes the history of shock, with emphasis on bacteraemia. Endotoxin is examined as a causative agent and its effects on metabolism are explored. The anatomy and physiology of the liver are described, and three facets of liver function are considered, microsomal cytochrome metabolism, hepatic blood flow and plasma fibronectin. Finally, fibronectin replacement is reviewed as a potential therapy in septic shock.

History of Shock

The concept of shock can be traced back to two unrelated reports published in the early eighteenth century. Hales (1733) observed that in exsanguinated animals there was venous constriction with a rise in venous pressure, while Le Dran (1743) described a condition whereby

the "capillary flow choked up" following haemorrhage caused by a gunshot wound. By the turn of the century the expression was in general use. Abernethy (1804) observed a patient who "sank in shock in consequence of the operation". Latta (1831) noticed many similarities in injured patients and those suffering from cholera, and suggested the involvement of toxic substances in his injured patients.

While wound infection had been recognised by Lister (1867) it was not until the end of the nineteenth century that the existence of blood-borne bacteria was demonstrated by Brill and Libman (1899), who described a condition of bacteraemia caused by Pseudomonas aeruginosa. At about the same time Ranberg (1899) showed the existence of "toxic shock" in animals injected with bacteria. Ten years later Jacob (1909) reported 39 cases of Escherichia coli sepsis in humans and implicated the biliary tract, urinary tract and gastrointestinal tract as portals of entry for bacteria.

Over the next decade much work was done to elucidate biochemical abnormalities during shock. McElleroy (1918) observed that acidosis was a symptom of shock, and Wright (1918) showed that a decreased blood flow leads to an accumulation of acid products in the tissues. By the end of the First World War it had been documented that bacterial infection following a gunshot wound produced "toxin". Debridement and the use of antigangrene serum was recommended (Vallee 1918). Shock after surgery had been described by Abernethy in 1824. A century later Felty and Keefer (1924) suggested that surgery was a predisposing factor in many cases of Escherichia coli sepsis. Furthermore, they were of the belief that the extent and location of the primary focus, as opposed to the mere fact of sepsis,

were the major prognostic factors. It was Fletcher (1947) who observed hypotension associated with sepsis. However, Waisbren (1951) recognised that hypotension was a prominent manifestation of severe sepsis and, in contrast to Felty and Keefer, Waisbren considered that sepsis itself was a major factor to be considered when assessing prognosis.

By this time Churchill (1951), amongst others, had realised that there were differences between septic shock and haemorrhagic shock. The latter tended to respond in the early stages, both in the clinical and experimental situation, to fluid administration (Burnett 1947), but once it was established fluid administration was ineffectual. This was termed the refractory stage. In contrast, septic shock was largely unresponsive to fluid therapy (Fine 1954).

Liver failure had been suspected as a major factor in the development of the refractory stage in haemorrhagic shock for a variety of reasons. Whipple (1920) showed that liver damage produced by chloroform caused severe shock. Blalock (1937) demonstrated that hepatic blood flow, as measured by bromosulphalein clearance, was reduced in haemorrhagic shock. Seligman (1948) showed that the refractory stage in haemorrhagic shock could be reversed in the dog by infusing its portal vein with arterial blood from a healthy donor. If the infusion was made into a systemic vein, there was no reversal of the refractory stage. Seligman suggested that the donor blood protected the shocked liver from hypoxia, preventing the release of a "vascular toxin" into the general circulation.

The existence of a vasodepressor substance in cold saline extracts from a shocked liver was shown by Shorr (1945). He believed that this vasodepressor substance was a normal body compound which was destroyed by the healthy liver and that prolonged ischaemia inhibited this process.

In haemorrhagic shock, Burnett (1947) observed that the greater the time lapse between blood loss and fluid administration, the less effective was the response. Fine (1951) thought that secondary invasion by endogenous bacteria might be the cause of this, and used a haemorrhagic shock dog model to extend Burnett's findings. Fine treated half his animals with antibiotics (aureomycin, neomycin or terramycin) prior to bleeding them, and found 88% survival in those that received antibiotics, and only 20% survival in those that did not (Fine 1951). Neomycin, which does not inhibit Clostridium welchii in the gut, was as effective as aureomycin and terramycin, which do. Equally, Clostridial toxoid administration, to eradicate any tissue Clostridia, failed to improve survival (Fine 1952). These experiments enabled Fine to rule out Clostridia as the causative organisms.

Fine thought that bacterial invasion by the normal flora of the gastrointestinal tract might cause the refractory state in haemorrhagic shock. To test this, he cultured blood from dogs during haemorrhagic shock, but found no evidence of invading organisms, even in the portal vein. He did, however, obtain positive cultures from blood taken from the portal vein, immediately after death or sacrifice, in both control and shocked animals, the latter giving many more positive cultures (Fine 1954). This suggested that bacteria from the gut obtain access to the portal vein and are normally removed by the liver as quickly as they invade.

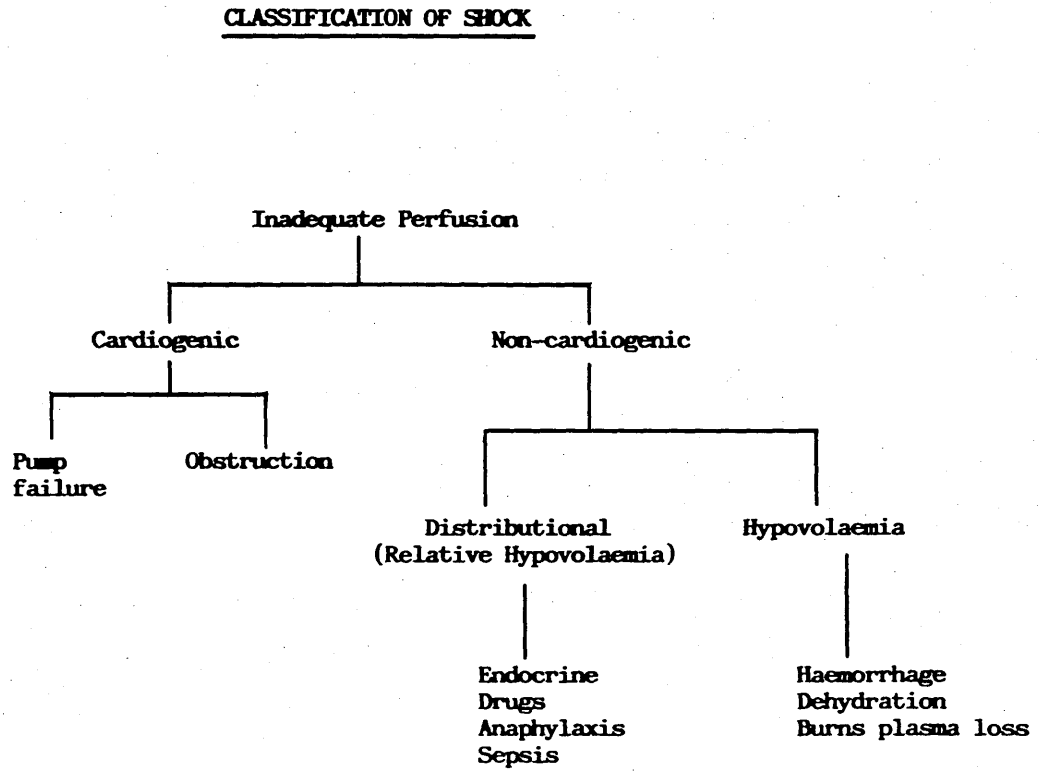
Septic Shock

Shock can be classified into broad categories as indicated in Figure 1; however, a number of underlying causes may be acting together. Its clinical features include the compensatory mechanisms: reflex tachycardia; vasoconstriction; tachypnoea; and redistribution of blood flow to vital organs at the expense of skin blood flow and gut blood flow.

Patients in cardiogenic or hypovolaemic shock have the same underlying problem, i.e., a reduced cardiac output, and they will exhibit many clinical features in common; tachycardia, hypotension, tachypnoea, cold clammy pale skin, oliguria, confusion and collapsed peripheral veins. In distributional shock the problem is a "relative hypovolaemia" brought about by an increase in the capacity of the vascular tree which is in turn due to a reduction in tone in the peripheral arterioles. Sepsis is the commonest cause of this condition.

The clinical picture in septic shock (at least in the early stages) differs from that seen in cardiogenic or hypovolaemic shock. The underlying pathophysiology is much more complex than was initially supposed, but in essence, patients with sepsis are initially hypercatabolic with a high cardiac output. One of the earliest changes is a reduction in peripheral vascular resistance accompanied by physiological arteriovenous shunting (both probably due to an action of endotoxin on small arterioles and precapillary sphincters). In the healthy state the sympathetic nervous system, acting through alpha receptors, maintains a degree of vascular tone. In septic shock

Figure 1


CLASSIFICATION OF SHOCK
Inadequate Perfusion
Cardiogenic
Non-cardiogenic
Pump failure
Obstruction
Distributional (Relative Hypovolaemia)
Hypovolaemia
Endocrine
Drugs
Anaphylaxis
Sepsis
Haemorrhage
Dehydration
Burns plasma loss

this is lost. Chernow (1985) has recently described a "down regulation" of the alpha receptors as an important factor in septic shock. However that may be, the reduction in peripheral vascular resistance leads to a reflex increase in cardiac output, with both increased chronotropy and inotropy. As a result, systemic blood pressure may initially be maintained, making the early diagnosis of septic shock difficult. At a later stage, fluid is lost from the capillary bed because of an increase in permeability, leading to hypovolaemia. In addition, myocardial depression may occur because of the release of myocardial depressant factors (Fisher et al, 1973). This, together with hypovolaemia, will obviously lead to a reduction in cardiac output. This mixed pathophysiology formerly led to the rather artificial distinction between early and late septic shock, with the terms warm or hyperdynamic being used to describe the early phase and cold or hypodynamic being used to describe the later phase. This distinction is artificial because many patients presenting with a "hypodynamic" picture - following aggressive resuscitation with fluid, oxygen and inotropes - will revert to a hyperdynamic state.

As stated above, the early diagnosis of septic shock can be difficult. Nevertheless it remains important to make the diagnosis early and to treat aggressively, since the mortality from septic shock is around 10% when treated early but rises to nearer 90% when treatment is delayed (Hanson 1978).

Endotoxin

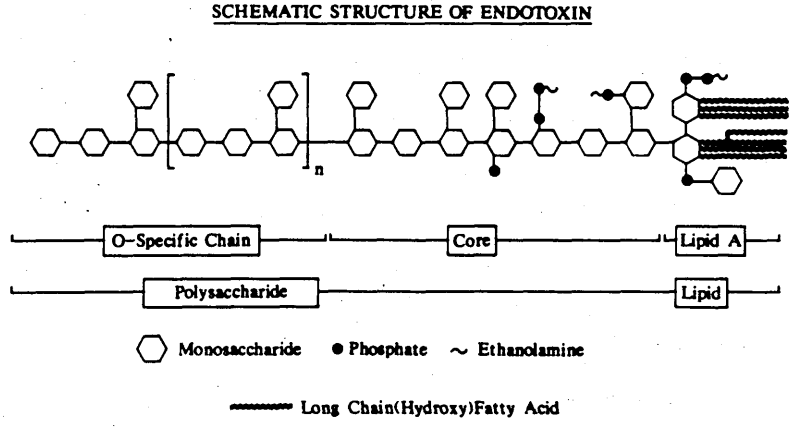
Fine has shown in experimental animals that whether shock is brought about by Gram-positive bacteria, Gram-negative bacteria or

haemorrhage, endotoxin is present in the circulation (Fine et al 1960, Fine et al 1970). This is true also of patients with Gram-negative sepsis and hepatic impairment (Fine 1972).

Structure

Endotoxins from a wide variety of Gram negative bacteria share a common structure. They consist of three distinct regions: a neutral polysaccharide part; an acidic polysaccharide core; and a lipid-rich part, the so-called "lipid A" (Westphal 1954) (Figure 2). The neutral polysaccharide consists of a heteropolysaccharide chain containing the repeating O-specific antigenic unit. The acidic heterooligosaccharide core links the O-antigen and lipid A. There is little structural variation in lipid A among endotoxins from taxonomically unrelated bacteria (Luderitz 1978). It appears to be the primary toxophore of endotoxin. This was demonstrated in experiments using rough and smooth mutants exposed to metabolic inhibitors at various stages during growth. These showed that neither the O - specific chain nor the core polysaccharide were required for the expression of endotoxicity (Kontrohr 1978). Moreover the absence of the nonacylated polar phosphate substituents of lipid A did not alter its toxicity (Rosner 1979). Rietschele et al (1982) have provided evidence that the toxic component is integrated in the acylated central disaccharide. However, little is known about the structural features which make it so toxic.

Figure 2

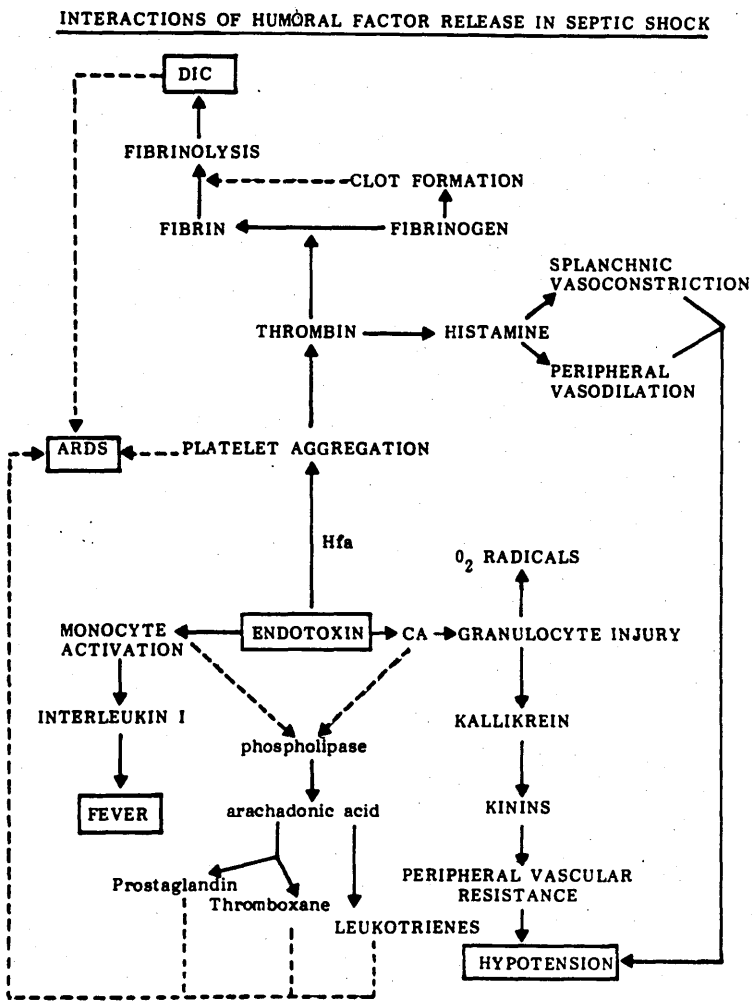

SCHEMATIC STRUCTURE OF ENDOTOXIN
n
O-Specific Chain
Core
Lipid A
Polysaccharide
Lipid
Monosaccharide
Phosphate
Ethanolamine
Long Chain(Hydroxy)Fatty Acid

Humoral factors in endotoxaemia

The microcirculation in the body is continuously being regulated to maintain a constant environment for the cells. Endotoxin is capable of disrupting this fine balance by stimulating the release of endogenous humoral factors. There is extensive, complex and poorly understood interaction between these factors (Figure 3), whose release can be brought about also by other antigens.

Endotoxin interacts primarily with components present in the blood to elicit, in animal experiments, the generalised Schwartzman reaction. This is characterised by intravascular clotting, especially in the glomerular capillaries, with consequent renal cortical necrosis (Husberg et al 1975). It is caused by the aggregation of platelets brought about by endotoxin, and initiation of the clotting cascade by production of the Hageman factor (factor XII) to produce thrombin, which converts fibrinogen to fibrin. This in turn is followed by the appearance of fibrin degradation products due to the action of thrombolytics, such as alpha-2 antiplasmin (Bennet et al 1984). It is these products, not the fibrin itself, that precipitate disseminated intravascular coagulation (String et al 1974). Thrombin also causes the release of histamine (Schumer et al 1970a), which leads to splanchnic vasoconstriction (Vick 1964) with stagnation of blood in the splanchnic bed and hepatic portal network (Weil et al 1956) and peripheral vasodilatation (Schumer 1970b). These effects, coupled with kinin production due to complement activation by endotoxin, increase capillary permeability which, together with peripheral vasodilatation, causes profound hypotension (Eskridge 1980).

Figure 3



Key

Hfa Hageman factor activation
CA complement activation
ARDS adult respiratory distress syndrome
DIC disseminated intravascular coagulation

The activation of complement by endotoxin produces up to 20 plasma proteins (Porter et al 1978) via a cascade system. These proteins are involved in host defence. There are two pathways of complement activation, the classical and alternative pathways. The former is activated by immunoglobulins, causing granulocyte injury and increased vascular permeability; the latter is activated by endotoxin and is involved in distinguishing "self" from "non self" as well as "injured self" permitting bacteriolysis (Fearon et al 1980). While both these pathways normally offer protection to the host, in overwhelming sepsis, or endotoxaemia, complement activation leads to a severe disruption of the body's biochemistry and physiology, with the consequent development of multiple organ failure (Jacob et al 1980).

Monocyte activation by endotoxin causes the release of interleukin 1 (Leucocytic pyrogen) which, in the presence of prostaglandin E, produces fever (Dinarello et al 1983), precipitates an inflammatory response and stimulates neutrophil oxidative metabolism and degranulation (Klemper et al 1978). Neutrophil activation due to interleukin 1 is believed to be independent of arachidonic products (Dinarello et al 1983), unlike fever production.

Membrane damage to monocytes, granulocytes and platelets by endotoxin stimulates phospholipase A activity, causing the release of arachidonic acid from tissue phospholipids. When metabolised, arachidonic acid produces cyclic endoperoxide derivatives and leukotrienes (Samuelsson et al 1979). In general these compounds are potent vasoconstrictors, particularly in the lung. The leukotrienes have been observed to cause pulmonary oedema (Drazen et al 1980)

following protein exudation from the microvascular structure. These products, together with those from fibrinolysis and the endotoxin-stimulated activation of leucocytes with consequent release of oxygen radicals, all lead to the development of the adult respiratory distress syndrome (Jacob et al 1980).

The Metabolic Response to Septic Shock

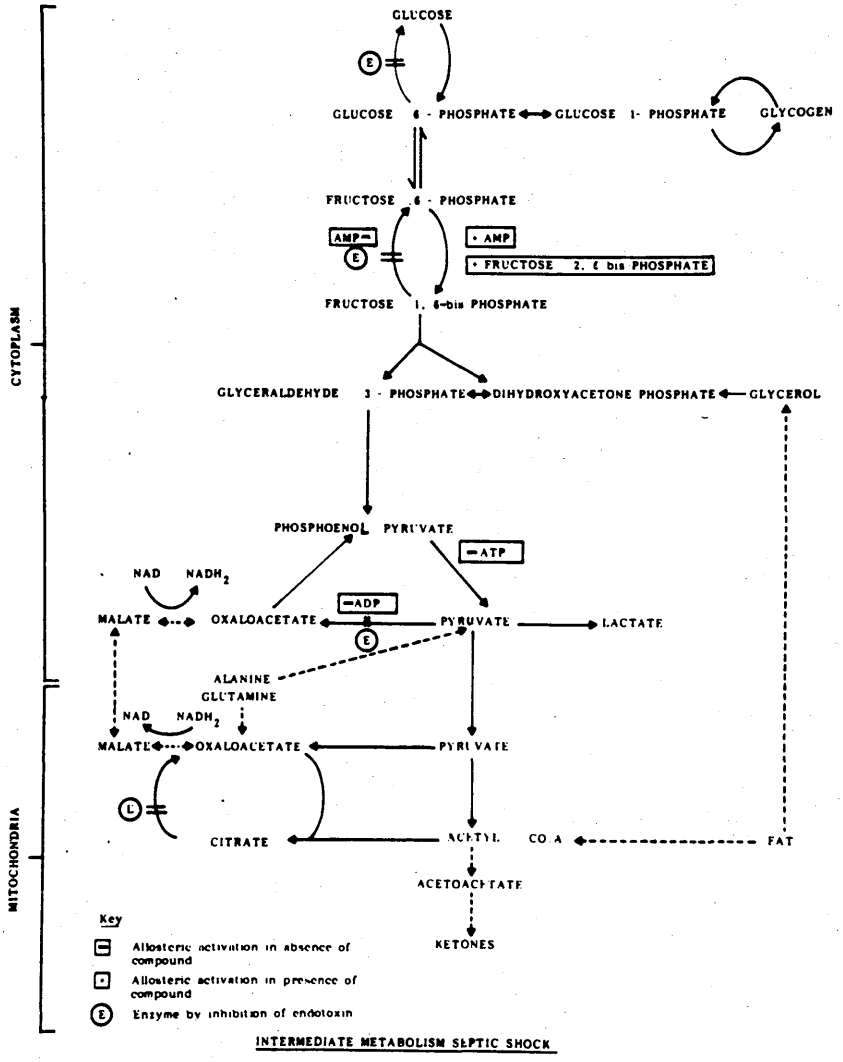
In septic shock the hyperdynamic phase may be regarded as an attempt to overcome microcirculatory disturbances caused by metabolic impairment. The transition to the hypodynamic phase is characterised by metabolic changes that are different from those of the hyperdynamic phase. Both phases are evident in the experimental and clinical situations.

Three aspects of intermediary metabolism are mainly affected - carbohydrate, protein and fat. While the responses in the latter two are largely mediated by hormones, carbohydrate metabolism appears to be directly affected by endotoxin. (Figure 4).

Carbohydrate metabolism

In the hyperdynamic phase there is an enhancement of glycogenolysis (Hamosh 1960) and lactate production (Smith et al 1975), with an inhibition of gluconeogenesis (Kutner et al 1974). These have the combined effect of producing hyperlactaemia followed by hypoglycaemia. This suggests that the hyperlactaemia is not anoxic in origin. It is thought that these changes may be due to adenylate cyclase activation by endotoxin. Gimbell et al (1984) demonstrated

Figure 4


CYTOPLASM
MITOCHONDRIA
GLUCOSE
GLUCOSE 6 - PHOSPHATE
GLUCOSE 1- PHOSPHATE
GLYCOGEN
FRUCTOSE 6 - PHOSPHATE
AMP
• AMP
• FRUCTOSE 2, 6 bis PHOSPHATE
FRUCTOSE 1, 6-bis PHOSPHATE
GLYCERALDEHYDE 3 - PHOSPHATE
DIHYDROXYACETONE PHOSPHATE
GLYCEROL
PHOSPHOENOL PYRUVATE
ATP
NAD
NADH2
ADP
MALATE
OXALOACETATE
PYRUVATE
LACTATE
ALANINE
GLUTAMINE
CITRATE
ACETYL CO A
FAT
ACETOACETATE
KETONES
Key
Allosteric activation in absence of compound
Allosteric activation in presence of compound
Enzyme by inhibition of endotoxin
INTERMEDIATE METABOLISM SEPTIC SHOCK

that the cell membrane has a binding site for endotoxin and that endotoxin will activate adenylate cyclase.

There is no direct evidence of impaired respiration in the hyperdynamic phase. Myrvold et al (1975) found no change in hepatic or skeletal muscle concentrations of adenosine triphosphate in canine septic shock. However, Mela et al (1975) showed a decrease in hepatic concentrations of adenosine triphosphate and a reduction in oxygen utilisation in rats following endotoxaemia. Their suggestion that this was due to defective blood supply must remain controversial: Lundsgaard et al (1972) found a large decrease in the levels of adenosine triphosphate in rat livers following endotoxaemia, yet the fall in systemic blood pressure was relatively small.

In the hypodynamic phase of septic shock and endotoxaemia, inadequate tissue perfusion and lack of oxygen cause production of lactate, predominantly in skeletal muscle and the gastrointestinal tract. The liver cannot convert this lactate back to glucose because the three irreversible reaction steps to gluconeogenesis are inhibited: glucose 6 - phosphatase; fructose 1, 6 bisphosphatase; and phosphoenolpyruvate carboxykinase (Ripple et al 1972, and Berry et al 1968, 1972). An inhibition of hepatic gluconeogenesis was demonstrated in endotoxic mice by McCallum et al (1972). By tracing the incorporation of ^{14}C isotope from ^{14}C-alanine into glucose, he found less than half of the label in blood glucose in those animals treated with endotoxin compared to the controls. The inhibition of gluconeogenesis in septic shock has been examined by other workers (Urbaschek et al 1977, Schumer 1975). However, Kutner et al (1983), using rats with septic shock due to peritonitis,

found that when assayed at optimal substrate concentrations and at pH 7.4, the activities of glucose 6 - phosphatase and fructose 1, 6 bisphosphatase were normal and that phosphofructokinase activity was increased.

Altered substrate cycling has also been implicated as a cause of reduced gluconeogenesis and increased glycolysis. In hypodynamic septic shock the concentrations of adenosine monophosphate and inorganic phosphate increase. This will result in a reduction in activity of fructose 1, 6 bisphosphatase and an increase in activity of phosphofructokinase due to allosteric interaction. This latter enzyme is also enhanced by fructose 2, 6 bisphosphate (Claus et al 1980). The fact that fructose 2, 6 bisphosphate is not a substrate for either aldolase or fructose 1, 6 bisphosphatase in rat liver (Pilkis et al 1981) and that it is decreased by the presence of glucagon in rat hepatocytes (Furuya et al 1980) suggest that this mediator may be involved in control of fructose 6 - phosphate and fructose 1, 6 bisphosphate substrate cycle. Indeed Pilkis et al (1981) have shown that rat liver phosphofructokinase is inactive at a physiological concentration of fructose 6 - phosphate unless other allosteric mediators, such as adenosine monophosphate and fructose 2, 6 bisphosphatase are available. The role of fructose 2, 6 bisphosphate in the control of glycolysis and gluconeogenesis in septic shock must remain highly speculative, as there is no evidence to show whether or not the level of this allosteric mediator is affected by the presence of endotoxin.

In endotoxaemia the conversion of hexose to pentose in the pentose pathway is stimulated (Filkin et al 1970). Endotoxin has been shown

directly to affect Krebs cycle. Schumer et al (1975) demonstrated, in liver homogenates, a block between pyruvate and acetyl CoA, resulting in a fall in citrate. Succinate dehydrogenase has been shown, in rabbits during endotoxaemia, to be inhibited (Kun et al 1948). These effects, plus the acute lack of oxygen, will reduce the output from the electron transport chain. This is compounded by the uncoupling action of endotoxin (Moss 1969). A reduction in the ATP : ADP ratio in primate liver has been demonstrated experimentally by Schumer et al (1975) during endotoxaemia.

A fall in the concentration of adenosine triphosphate will lead to the failure of the many ATPase transporter systems in the cell membrane (Mela et al 1970). Malfunction of the sodium ATPase pump leads to a loss of intracellular potassium, with an influx of both water and sodium into the cell and mitochondria (Lundsgaard et al 1972), destroying the integrity of the membranes and cristae (White 1973).

Fat Metabolism

Lipid metabolism in septic shock is difficult to interpret because lipolysis cannot be measured directly. Furthermore animals tend to show a different lipid response from man, making comparison between the experimental model and the clinical situation difficult.

In man lipid mobilisation during septic shock is a response to the need for energy (Clowes et al 1976). It is mediated, at least in part, by hormones, in particular the catecholamines (Benedict et al 1978) via the adenylate cyclase system. Endotoxin is also capable of activating adenylate cyclase (Gimbell 1984).

Spitzer et al (1973) showed that endotoxin induced lipolysis in adipocytes. However, Spitzer et al (1980) in a study of adipocytes isolated during septic shock demonstrated that lipolysis was due to hormonal influence.

The concentration of free fatty acids in plasma is believed to reflect their turnover (Romanosky et al 1980), but in the late stages of septic shock this relationship becomes distorted (Nordenstrom et al 1983). A rise in plasma free fatty acid concentrations in the early stages of both endotoxaemia and bacteraemia has been demonstrated in baboons (Horowitz 1972). However, in late septic shock free fatty acid concentrations are reduced and turnover decreased (Spitzer et al 1972). These effects are probably enhanced by insulin (Neufield 1980) and hyperlactataemia (Miller et al 1964), both of which are known to stimulate reesterification. The low levels of ketone bodies observed in septic shock (Wannemecher et al 1979) can be rationalised in view of the decreased levels of free fatty acids as these are the precursors of ketone bodies. Insulin has also been implicated in the inhibition of ketogenesis during septic shock (Neufield 1980).

The function of lipolysis is presumably to supply the body with fuel, in particular the heart. However Scott et al (1973) found that the uptake and metabolism by the myocardium of free fatty acids was reduced during endotoxaemia. Furthermore, lactate uptake was increased.

Protein Metabolism

In the early stages of shock there is altered hepatic protein synthesis (Wanacher et al 1972). The proteins involved are called the acute phase reactants. The response is complicated by the fact that synthesis of certain proteins is increased and others decreased. A net decrease in protein synthesis without any significant increase in protein catabolism is a normal stress response. However, in shock there is an increase in protein breakdown presumably due to the catabolic hormones. Such hormones have not been implicated in the acute phase reactants response (Bessey et al 1984).

The effect of these hormones is to make substrate available for energy production. However, as gluconeogenesis is blocked these substrates are of limited use. One such substrate is alanine and the plasma concentration of this amino acid has been shown to rise in patients with refractory shock (La Brosse 1967). Many other amino acids are found at increased levels in plasma during shock (Coran et al 1972).

The Liver in Septic Shock

Anatomy

The liver is the largest organ in the body, weighing approximately 1.5 kg. It is located in the right hypochondrium and extends across the epigastrium. It is a reddish-brown, wedge-shaped organ which is pliable but firm. The majority of the liver is protected by the thoracic cage. The convex upper surface fits under the lower

surfaces of the domes of the diaphragm. The postero-inferior surface moulds to the adjacent viscera, giving rise to an irregular appearance. The liver is divided into a large right lobe and a smaller left lobe by the falciform ligament. The right lobe is subdivided into the quadrate lobe and caudate lobe by the gall bladder and inferior vena cava. The porta hepatis is located on the posterior-inferior surface, and houses the right and left hepatic ducts, the right and left branches of the hepatic artery, the portal vein and several lymph nodes. (Figure 5).

Physiology

Blood is conveyed to the liver by the portal vein and hepatic artery, both of which enter at the porta hepatis. The hepatic artery transports oxygenated arterial blood arising from the aorta, while the portal vein is formed by the joining of the superior mesenteric vein, conveying venous blood from a large area of the gastrointestinal tract, and the splenic vein, bringing venous blood from the stomach, spleen, pancreas and gall bladder.

The portal vein divides into branches called the interlobular veins, which surround the lobules of the liver and supply blood to the sinusoids via vascular capillaries. The sinusoids, in turn, meet at the centre of the lobule and drain into the intralobular branch of the hepatic vein.

Similarly, the hepatic artery divides into branches which follow those of the portal vein between the lobules. Ultimately the hepatic artery blood enters the sinusoids and mixes with blood from the portal vein.

Figure 5

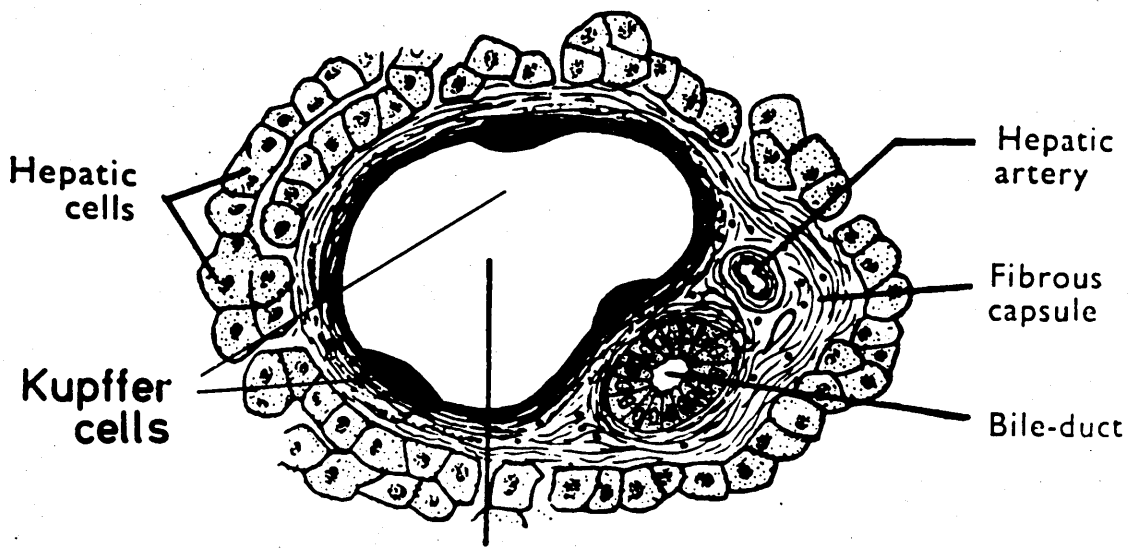


DIAGRAM OF THE HEPATIC STRUCTURE

These capillaries do not possess specific endothelial lining, which allows them to ramify between the hepatocytes. At intervals along the sinusoids are the stellate cells of Kupffer, which form part of the reticuloendothelial system.

In man, the liver receives 1500 ml of blood per minute, which is approximately a quarter of the cardiac output. One-fifth of the blood supply to the liver comes from the hepatic artery and the rest from the portal vein. Dye dilution studies show that the blood from the superior mesenteric vein enters mainly the right lobe of the liver, whereas splenic blood is distributed to the left lobe. The mean pressure in the hepatic artery is about 100 mm Hg while that in the portal vein is only 5 mm Hg. If there is increased capillary resistance to the portal flow, portal pressure will rise, causing retrograde congestion.

The presence of a dual blood supply to the liver can present problems in the measurement of hepatic blood flow. The existence of arteriovenous capillary connections between the portal vein and hepatic artery allows shunting between the two vessels, which also hinders hepatic blood flow measurements.

Liver Function in Septic Shock

The hepatocyte membrane has been shown to be damaged in endotoxaemia, as has the liver's capacity for detoxication (White et al 1973). The Kupffer cells are thought to be one of the sites of uptake of endotoxin (Jacob et al 1977). Hepatic phagocytosis is mainly responsible for the clearance of blood borne particles (Spence

et al 1972). This function is affected by the number and size of the particles, the presence of opsonins, the functional state of the Kupffer cells and the blood flow through the liver (Saba et al 1970).

Failure of the Kupffer cells would be expected to lead to endotoxin derived from the gut spilling over into the general circulation and causing severe damage (Nolan 1981). Histological examination of the Kupffer cells in patients who had died of septic shock revealed hyperplasia (Ledingham et al 1978), and Kupffer cell impairment has been reported in septic shock (Saba et al 1983). This impairment appears to be related to a fall in plasma fibronectin levels (Saba et al 1978). The latter is known to augment reticuloendothelial function by non-specific opsonisation (Saba et al 1978).

On the other hand liver function in patients with septic shock, as measured by standard tests, does not show any relationship with the onset of the condition, its severity or the patients prospects of survival (Ledingham et al 1978). There seemed therefore a need to measure liver function in septic shock in terms of its blood flow, its detoxifying capability and Kupffer cell function.

Liver Blood Flow Measurement

Direct electromagnetic measurement of liver blood flow (Kolin 1936) necessitates opening the abdomen and is therefore possible in patients only in the course of surgery. Indirect measurement is easy but does not discriminate between the contributions of the hepatic artery and portal vein. The clearance method is the simplest example of this technique, and is based on Fick's principle. A dye

which is taken up and cleared by the liver is injected into the blood stream and its half life in plasma used as an indicator of liver blood flow. Bromosulphalein was one of the first dyes to be used. However, it can be removed by tissues other than liver, particularly if the liver is diseased (Bradley 1950). Bromosulphalein also undergoes enterohepatic recirculation (Lorber et al 1953) and its plasma clearance does not always follow an exponential decay pattern (Caesar et al 1961). In contrast, the tricarbocyanine dye, indocyanine green, is eliminated exclusively by the liver, undergoes no enterohepatic circulation (Wheeler et al 1958) and follows an exponential rate of plasma clearance (Caesar et al 1961).

Despite these differences, the two dyes give similar measurements of liver blood flow in healthy human subjects (Caesar et al 1961). However, in subjects with liver disease, bromosulphalein tends to give a higher apparent flow than indocyanine green.

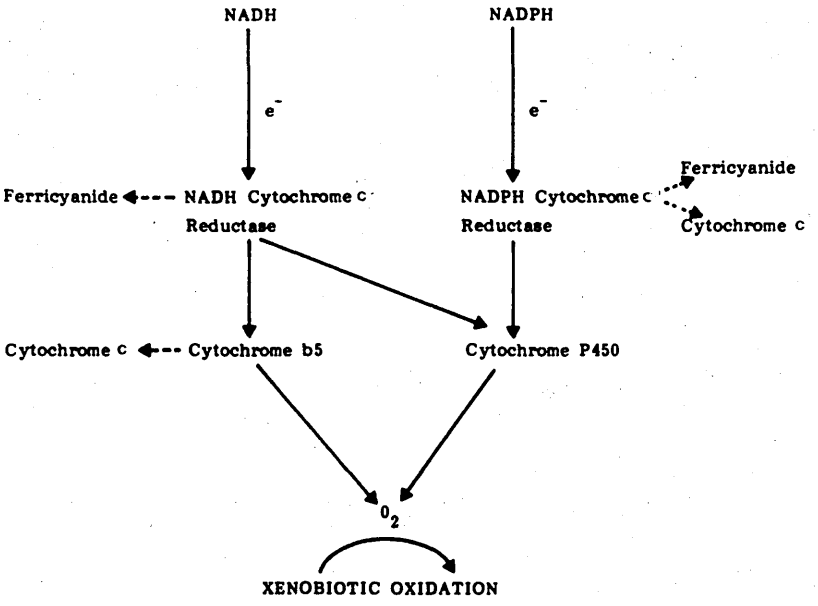
The use of indocyanine green for measuring liver blood flow has largely superceded bromosulphalein. When it is injected intravenously, 95% binds to albumin. Its disappearance from the plasma follows first order kinetics, therefore its half-life is constant. The greater part of the dye is removed from the blood during a single transit through the liver. Clearance is normal in acute hepatitis, but diminished in chronic hepatitis and cirrhosis, and diminished still further in alcoholic cirrhosis (Gilmore et al 1982).

Detoxication in the liver

Impairment of the liver's capacity for detoxication during endotoxaemia or septic shock might contribute to the high mortality observed in both these conditions.

Detoxication is brought about by the microsomal cytochromes of the hepatocyte. Commonly it takes the form of oxidation of a lipid-soluble compound to a more water soluble structure by the cytochrome P450-linked mono-oxygenase system. Almost all compounds which act as substrates for the cytochrome P450 system are lipophilic, probably because the enzyme system is an integral part of the lipid in the membrane. The cytochrome P 450 enzyme system is only found in certain cells, such as liver and lung. No cytochrome P 450 has been found in foetal liver, undifferentiated tissue (Dallner et al 1965) or certain types of hepatomas (Ikeda 1965). The electron transport system of the liver microsomes consists of cytochrome b5, cytochrome P 450 and their respective reductases (Figure 6). The main function of cytochrome b5 and its reductase is to supply reducing power to cytochrome P 450 to allow it to carry out the various oxidation reactions. Reducing power is abundant for such reactions of the microsomal system, as the endoplasmic reticulum of the hepatocyte is surrounded by the cytoplasm, where reducing equivalents in the form of NADPH are freely available. The components of this electron transfer system are amphipathic membrane proteins. (Ito et al 1968). Cytochrome P450 is largely hydrophobic (Alvares et al 1973) whereas cytochrome b5, NADPH cytochrome c reductase and NAPH cytochrome c reductase all contain a large hydrophilic component. (Spatz et al 1971, Welton et al 1973, Spatz et al 1973). The major hydrophilic parts, which are the catalytically active portions, protrude from the membrane into the cytoplasm (Takesue et al 1976). Although cytochrome P 450 is extremely hydrophobic, it must also extend into the cytoplasm to receive electrons from NADPH cytochrome c reductase.

Figure 6



Sequence of electron transfer in the microsomal electron transport system

(-----▶ Electron flow to non physiological electron acceptors)

During endotoxic shock, disturbances in drug metabolism and detoxication have been observed (Vaino 1973, Mori et al 1973 and Gordischeri et al 1966). The cytochrome P 450 content of human liver has been shown to be decreased in liver disease to a degree related to the severity of the condition (Brodie et al 1981). Many workers have used the half-life in plasma of antipyrine (Phenazone) as a measure of cytochrome P 450 activity in the hepatic microsomes. Antipyrine is metabolised by the microsomal cytochrome P450 system. (Poland et al 1970). McManus et al (1979) demonstrated good correlation in rabbits between hepatic microsomal metabolism of antipyrine in vitro and its half-life in vivo. Drugs known to inhibit microsomal cytochrome P450 function of the liver prolong the plasma half life of antipyrine (Vessel et al 1973, Welch et al 1975), whereas drugs that induce microsomal cytochrome P450 activity significantly shorten it (Stevenson et al 1972,).

The Kupffer Cell

The removal of circulating bacteria by the reticuloendothelial system was first reported by Wyssokowitsch in 1886. The failure of this system to remove bacteria and endotoxin from the blood has been implicated in the genesis of septic shock (Zweifach et al 1957).

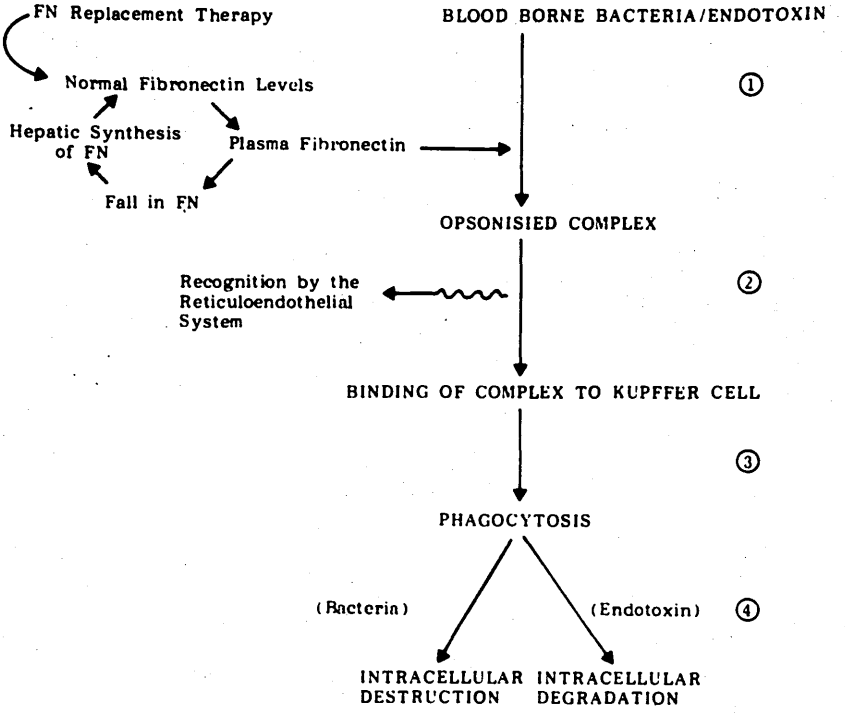
The reticuloendothelial system is composed of sessile macrophages located in the bone marrow, lung, spleen and liver. The liver is responsible for 85% of the body's total phagocytic activity (Saba et al 1970). It is the stellate cells of Kupffer that perform this function. They constitute 15% of the total hepatic population (Gates et al 1961). In animals, Kupffer cell function has been demonstrated using

carbon colloid (Biozzi et al 1965), lipid emulsion (Di Luzio 1964), bacteria (Stiffel et al 1970) and endotoxin (Di Luzio 1964). Human studies have also shown Kupffer cell clearance of bacteria and endotoxin in man. (Saba et al 1979). Clearance is dependent on factors such as liver blood flow, the concentration of foreign particles, the functional state of the Kupffer cells and the presence of opsonins (Saba et al 1970).

Phagocytosis depends on the ability of the reticuloendothelial system to differentiate between healthy indigenous tissue and diseased and foreign matter (Di Luzio et al 1971, Allen et al 1973). Opsonins have been implicated in this (Niehaus et al 1980, Saba 1979). Plasma fibronectin has been shown to act as an opsonin in the uptake of carbon by perfused liver (Filkins et al 1966). In vivo its concentration in plasma falls after intravenous injection of colloid. This is accompanied by depression of reticuloendothelial function, which can be reversed by the administration of exogenous fibronectin (Saba et al 1969, Cornell et al 1973). It seems reasonable to suppose that, when either live bacteria or endotoxin enter the blood stream, they bind plasma fibronectin to form an opsonised complex. This complex would be bound to the Kupffer cell membrane and ingested for intracellular destruction or degradation (Saba et al 1970, Saba et al 1975). (Figure 7).

The reticuloendothelial system appears to form a first line of defence against shock (Zweifach et al 1957, 1958, Zweifach 1960, 1964), and impairment occurs early in shock. Impairment or blockade of the system diminishes resistance to shock, whereas hyperfunction improves it. In experimental animals there is a close correlation

Figure 7



Rate Limiting Steps

1. Fibronectin
2. Hepatic blood flow
3. Kupffer cell integrity
4. Microsomal cytochrome P450 system

FN-Fibronectin

Sequence of physiological events involved in the clearance of bacteria and endotoxin from the blood by the stellate cells of Kupffer

between the degree of shock and the degree of reticuloendothelial impairment. In mild shock, impairment can be reversed to some extent. In contrast, in severe shock there is a rapid decline in reticuloendothelial function until death supervenes (Altura et al 1967, 1971, 1972). In rats administration of antiserum to fibronectin produces a decrease in phagocytosis in rough proportion to the dose given (Saba et al 1978).

Fibronectin

Firbronectin is a high molecular weight alpha-2 glycoprotein present in plasma (Morrison et al 1948), on cell surfaces (Yamada et al 1976) and in cell matrices (Hynes et al 1978). It exists in two forms - plasma and cellular fibronectin. Plasma fibronectin was first isolated by Morrison et al in 1948. Its peptide structure is different from that of cellular fibronectin (Hayashi et al 1981) but the amino and carboxy terminals are similar in both, suggesting that two separate genes may exist and that the differences are not due to postranslational modification. While both forms of fibronectin have structural differences which can be detected using monoclonal antibodies (Altherton et al 1981), they are immunologically indistinguishable using polyclonal antisera.

Plasma fibronectin is a dimer of two similar, but not identical, polypeptide chains, each weighing 220,000 daltons, which are linked by disulphide bridges (Mosesson et al 1975). In the aqueous environment the protein shows no evidence of alpha-helical content and only small amounts of beta-pleated sheet (Mosesson et al 1975). It appears to consist of large domains connected by randomly ordered

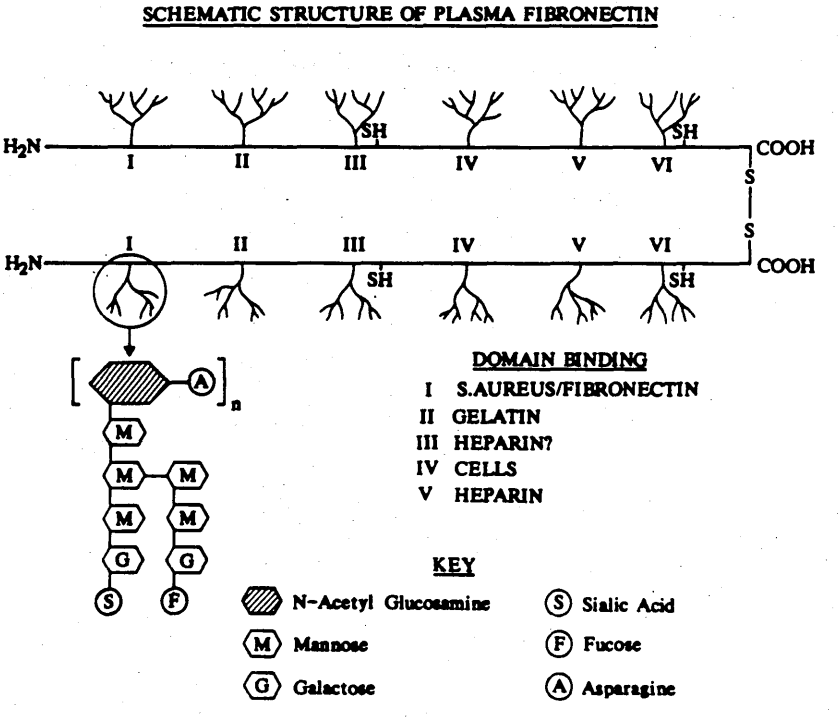
flexible polypeptide sequences, which would allow flexibility to the domains. These domains have been shown to have binding sites for Staphylococcus aureus (Mosher et al 1980) fibrin (Jilek et al 1978) collagen (Mosher et al 1980) heparin (Smith et al 1982) and fibronectin (Hormann et al 1980). (Figure 8). Although fibronectin is synthesised by many cells in the body, Tamkun et al (1983) demonstrated that the liver is the main source of synthesis for plasma fibronectin.

Fibronectin measurement involves the use of antiserum raised against human fibronectin in rabbits. The immunotrubidimetric technique has largely superceded electroimmunoassay because it offers immediate results, requires minimal amounts of plasma and can be performed at the bedside. Saba (1981) demonstrated that the immunoturbidimetric technique correlates well with the other types of assays available. Bioassays, on the other hand, offer the advantage of measuring functional activity. Such assays utilise liver slices (Saba et al 1969), peritoneal macrophages (Doran et al 1980), or Kupffer cells (Saba et al 1986) and measure the degree of opsonic activity. However, these assays are not suitable for bedside use and are time consuming.

Opsonic Function of Plasma Fibronectin

Fibronectin is believed to be an opsonin which will stimulate reticuloendothelial system function. Plasma fibronectin is capable of improving liver and spleen clearance of particulate matter (Saba 1970), as well as improving macrophage phagocytic activity (Saba et al 1975). A decrease in plasma fibronectin levels has been shown to

Figure 8


SCHEMATIC STRUCTURE OF PLASMA FIBRONECTIN
H2N
COOH
SH
I
II
III
IV
V
VI
S
DOMAIN BINDING
I S.AUREUS/FIBRONECTIN
II GELATIN
III HEPARIN?
IV CELLS
V HEPARIN
KEY
N-Acetyl Glucosamine
Mannose
Galactose
Sialic Acid
Fucose
Asparagine

be associated with reduced clearance of particulate matter (Kaplan et al 1976). However, Bevilacqua et al (1981) demonstrated that human macrophage binding of fibronectin-coated particles is reversible in the presence of trypsin, suggesting that opsonisation had not taken place. In contrast, Gudewicz et al (1980) using rat macrophages did demonstrate opsonisation. Bevilacqua et al (1981) also found that fibronectin stimulated monocyte binding of fibrinogen, but not opsonisation. However, Pommier et al (1983) found that sheep cells coated with immunoglobulin G were phagocytosed by monocytes in the presence of fibronectin, but if the sheep red cells were coated with immunoglobulin M opsonisation and phagocytosis did not take place.

When the reticuloendothelial system is blocked by an injection of gelatin colloid, further injections of colloid are not removed by the reticuloendothelial system (Saba 1970). This blockade results in reduced resistance to endotoxin and bacteria (Saba 1970). Saba observed that following the first injection of colloid plasma fibronectin levels fell (Saba 1970). When this work was repeated using bacteria, the same results were obtained, with the development of neutrophil-mediated pulmonary margination of bacteria (Saba et al 1975). The reduction in plasma fibronectin correlates well with reticuloendothelial impairment and this impairment can be reversed by restoring fibronectin levels to normal (Saba et al 1975). Animals with low fibronectin levels died, but death could be prevented if the fibronectin levels were restored to normal. In human studies involving sepsis, Lanser et al (1982) have shown that a fall in plasma fibronectin is not merely a reflection of the sepsis, but that the host defence mechanisms to sepsis are severely compromised. Reduced

levels of plasma fibronectin have been implicated in the development of disseminated intravascular coagulation. Bang et al (1973) observed that fibrin/fibronectin complexes develop in the blood as disseminated intravascular coagulation progresses. This suggests that the depleted fibronectin levels observed in sepsis may be secondary to disseminated intravascular coagulation in some cases.

Plasma Fibronectin in Disease

Plasma fibronectin levels have been reported to be altered in various diseases. They are increased in biliary cirrhosis, obstructive jaundice and the nephrotic syndrome (Stathakis et al 1981), and depressed in some cancer patients, particularly those with extensive liver metastases (Mosher et al 1978). In contrast, Stathakis et al (1981) did not observe this pattern in his cancer patients. Low levels of fibronectin are also observed following injury (Saba et al 1975), burns (Saba et al 1975), and major surgery (Blumenstock et al 1977). Low levels of fibronectin correlate well with the degree of disseminated intravascular coagulation (Mosher et al 1978), particularly in septicaemia. Richards et al (1983) reported that plasma fibronectin levels were reduced in intra-abdominal sepsis, and that three patients in his study who developed multiple organ failure had the lowest fibronectin levels. Ahlgren et al (1985) observed that reduced plasma fibronectin levels were a consistent finding in septic patients, and that a transient, as opposed to sustained, fall in plasma fibronectin signified a good prognosis. They also found that the degree of hypofibronectinaemia was more marked in Gram-positive sepsis than in Gram-negative sepsis.

In intensive care patients, Rubli et al (1983) found that those that were septic had a significantly lower fibronectin level than those that were not. In their septic patients mortality was lower in those patients with fibronectin levels above 200 mg ml^{-1}. Pott et al (1981) also found that plasma fibronectin levels were reduced in human septic shock.

The fall in plasma fibronectin levels observed in many conditions may be related to increased consumption, decreased synthesis or changes in turnover. While tissue damage will increase consumption and hypoxia will reduce synthesis, the reason for the fall in plasma fibronectin is not yet fully elucidated.

Fibronectin Replacement Therapy

Several authors have reported that hypofibronectinaemia is associated with a high mortality rate (Mosher et al 1978, Scovill et al 1978, Couland et al 1982) and it has been suggested that reversal of this deficiency may improve prognosis (Saba et al 1978). Depleted fibronectin levels observed in sepsis can be augmented by the administration of cryoprecipitate (Scovill et al 1978, Scovill et al 1979). These authors found an improvement in pulmonary arterio-venous shunt and an increased peripheral blood flow after cryoprecipitate administration. Saba et al (1980) observed that in septic patients cryoprecipitate infusions reduced pyrexia and increased oxygen delivery to the tissues.

The half-life of cryoprecipitate used by Scovill et al (1978) was approximately 36 hours in his patients. However, this finding is

difficult to interpret because no attempt was made to assess the severity of illness. This, and the absence of controls, and the small number of patients in such trials using cryoprecipitate as a source of fibronectin, must therefore make the value of cryoprecipitate in the treatment of sepsis and septic shock speculative.

While cryoprecipitate raises depleted fibronectin levels, it is also a source of many other proteins, such as fibrinogen and factor VIII - both of which may be reduced in sepsis due to disseminated intravascular coagulation. It is therefore difficult to attribute the improvements associated with cryoprecipitate administration solely to fibronectin. Saba et al (1986) attempted to circumvent this problem by using purified human plasma fibronectin. In a group of six patients with conditions including injury and sepsis, infusions of purified human fibronectin successfully raised plasma fibronectin levels. Opsonic activity, as measured by the peritoneal macrophage assay, was increased. Saba also administered this purified fibronectin to sheep in sepsis and observed improvements in systemic vascular resistance, pulmonary vascular resistance and blood pressure (Saba et al 1986).

Physiological Measurements

Electrocardiography was monitored via 3 standard electrodes and displayed on a Kone patient data monitor 565A.

Arterial blood pressure was measured via an indwelling arterial catheter connected to a disposable pressure transducer (Gould) with a continuous wave trace displayed on the Kone monitor.

Temperature: Core temperature was measured using a rectal thermistor probe. Peripheral temperature was measured using a thermistor probe attached to one of the toes and the respective temperatures displayed digitally on the Kone monitor.

Pulmonary artery pressure: This was measured via a pulmonary artery flotation catheter located in the pulmonary artery, connected to a disposable pressure transducer and the wave form displayed on the Kone monitor.

Pulmonary artery wedge pressure was measured by inflating the terminal balloon on the pulmonary artery flotation catheter, ensuring that a wedge trace was obtained on the Kone monitor. The wedge pressure was calculated at end-expiration.

Cardiac output was measured by injecting 10 ml of dextrose 5% at $0^{o}C$ through the proximal port of the pulmonary artery flotation catheter into the right atrium. The thermistor 4 cm behind the end of the pulmonary artery flotation catheter detected the resulting temperature change. Using a Kone cardiac output console (538) the cardiac output was calculated and displayed on the Kone monitor. Measurements were performed in triplicate and the mean value recorded.

Urine output was measured hourly via an indwelling urinary catheter.

Arterial and mixed venous blood gases: 2 ml samples of blood, from either the arterial line for arterial blood gas analysis or the distal port of the pulmonary artery flotation catheter for a mixed venous sample, were drawn into heparinised syringes containing 50 μl of heparin (1000 units ml^{-1}). The samples were analysed using a blood gas machine (radiometer ABL3).

Biochemical Measurements

Indocyanine Green Measurements

Clearance measurements

Indocyanine green was reconstituted in 10 ml of solvent immediately prior to use. 3 ml of blood was drawn from the indwelling arterial catheter or via a 21g butterfly needle and an intravenous bolus of indocyanine green (0.5 mg kg^{-1} body weight) was administered through the central venous catheter or by venepuncture. 1.5 ml blood samples were taken every 3 mins. for 21 mins. and placed in primed EDTA Eppendorf tubes. When an intravenous butterfly needle was used it was kept patent by flushing with 2 ml of heparin-saline (100 units ml^{-1}) after each blood sample.

Assay Procedure

This was identical to that described on Page **66**, with the exception that 1 μl of indocyanine green (5 mg ml^{-1}) was added to the plasma blank. The calculations are also identical.

Endotoxin Measurement

The method used for the measurement of endotoxin was the Limulus Amoebocyte Lysate assay utilising a chromogenic substrate (Harris et al 1983) using a one step incubation. In the presence of Limulus Amoebocyte Lysate activated by endotoxin, p-nitroaniline is released from the colourless synthetic chromogenic substrate, the rate being proportional to the amount of endotoxin present.

Blood Sampling

Full aseptic technique was observed including wearing a mask, hat, sterile gloves and a sterile gown. 20 ml of blood was collected using a 20 ml pyrogen-free syringe and a 21g needle, after the skin had been cleansed using 1% (w/v) iodine in alcohol. 10 ml of blood were placed in a pyrogen-free tube (Falcon 2054) containing 100 units of preservative-free heparin (Leo Labs). The sample was gently mixed with the heparin and placed immediately on ice. The remaining 10 ml of blood were sent for blood culturing. (Blood cultures were performed by the Western Infirmary's Bacteriology Laboratories).

The sample remained on ice for 30 mins. to allow partial sedimentation of the cells and was then centrifuged at 100 x g for 2 mins. (Medifuge). The platelet-rich plasma was removed using a 5 ml syringe with a quill needle and placed in a pyrogen-free tube (Falcon 2054) and frozen at -20^{o}C.

Assay Procedure

Disposable, pyrogen-free equipment was used throughout. All pipetting was performed using Gilson P20 and Gilson P200 automatic

pipettes. Sterile, individually-wrapped pipette tips (Sterilin) were utilised.

A commercially available kit (QCL 100) was used (M.A. Bioproducts) coupled with chromogenic substrate S2423 (Kabi Diagnostica). The kit contained 7 vials of lyophilised Limulus Amoebocyte Lysate (each vial was reconstituted with 1.4 ml of pyrogen-free water at the time of use), and 2 ng of lyophilised endotoxin (Escherichia coli 0111.B4) which was reconstituted with 1 ml of pyrogen-free water (this solution was stable at $2^{o}C$ for 31 days). Before use it was warmed to room temperature and vigorously mixed for 30 secs. Twenty five mg of lyophilised chromogenic substrate S2423 were reconstituted with 16.7 ml of pyrogen free water and divided into 33 aliquots of 500 μl placed in sterile microeppendorf tubes (Sterilin) and stored at $2^{o}C$. Prior to use the chromogen-substrate solution was mixed in 1:1 water with chromogen substrate buffer (0.05m Tris buffer pH 7.9).

Plasma extracts were obtained by adding 100 μl of platelet-rich plasma to 300 μl of extraction buffer (50 mM Tris, 10 mM $MgCl_2$, 15 mM NaCl to pH 7.4) in a sterile microeppendorf tube (Sterilin) and heating to $100^{o}C$ for 1 min. The resulting precipitate was sedimented by centrifuging at 13,000 x g for 2 mins. using a microcentrifuge (Microcentaur).

Fifty microlitres of plasma extract were added to a sterile 1.5 ml microeppendorf tube containing 15 μl of Limulus Amoebocyte Lysate and 35 μl of chromogenic substrate mixture pre-warmed to $37^{o}C$. This was done in triplicate. In addition, one of the tubes (designated sample control) contained 50 μl of 50% (w/v) ethanoic acid. The

samples were incubated at 37°C for 40 mins. (20 mins. in the case of high endotoxin concentration), and the reaction terminated, in the other two tubes with 50 µl of 50% (w/v) ethanoic acid. 125 µl of each sample was transferred to a flat bottom microtitre plate and the absorbance read at 410 nm using a microtitre reader (Dynatech Labs). The standard curve for the assay was performed at the same time as the test samples. Endotoxin (Escherichia coli 0111.B4) was added to healthy control plasma to give five concentrations, with a range of 5 pg ml^{-1} to 100 pg ml^{-1}. The assay procedure was as described.

Measurement of Antipyrine in Plasma

Plasma antipyrine concentrations were assayed using the method of (Shargel 1979).

Procedure

Six hundred milligrams of antipyrine were administered intravenously through the central venous line. Ten millilitres of blood were drawn from the arterial line immediately prior to injection and at 3, 5, 8, 12, 24, 36 and 48 mins. after the injection. Samples were placed in lithium heparin tubes and centrifuged at 2000 x g for 5 minutes. Plasma was transferred into a plain tube and frozen at -20°C.

Preparation of standard and plasma samples

Standard samples The standard samples were prepared using a stock solution of 50 mg of antipyrine in 50 mls of methanol. The following concentrations were prepared from the stock solution: 0.4 mg ml^{-1}, 0.3 mg ml^{-1}, 0.2 mg ml^{-1}, 0.1 mg ml^{-1} and 0.05 mg ml^{-1}. Nine

hundred and fifty microlitres of deionised water, 50 μl of the appropriate standard and 100 μl of methanol containing 10 μg of 4-methyl antipyrine (to serve as the internal standard) were added to a 15 ml glass test tube.

Test samples The test samples were prepared by adding to each 15 ml test tube 1 ml of the appropriate plasma sample and 100 μl of methanol containing 10 μg of 4-methyl antipyrine.

Extraction Procedure

The standard and test samples prepared using the above methods were brought to pH11 with 0.25 ml of 0.5 M sodium hydroxide and 7.5 ml dichloromethane added. The test tubes were sealed with parafilm and mixed by vortexing for 60 secs. They were centrifuged at 1000 x g for 5 mins. and the aqueous layer was removed using a Pasteur pipette. The dichloromethane layer was decanted into a fresh test tube and evaporated using a vortex evaporator at 40^{o}C. The residue was reconstituted in 200μl of methanol.

HPLC Measurement of Antipyrine

The reconstituted samples were loaded into an injector carousel (Rheodyne 7125). Each run consisted of 5 standards and 24 test samples all in duplicate. The first two samples and the last two samples were identical and served as a quality control. The injector was programmed to inject 10 μl of samples every 6 minutes into the HPLC column (C 18 micro-bondopac Waters number 13) which was connected to a Waters single pump solvent metering system with a mobile phase consisting of 50% methanol and 50% water. The flow

rate was 1.5 ml min^{-1} with a pressure of 2500 p.s.i. Antipyrine was detected at 250 nm spectrophotometrically (Cecil CE 212 UV). The data was recorded on a chart recorder (Phillips 8251) set at 1 cm min^{-1}. A standard curve was constructed and the antipyrine concentrations derived from this.

Plasma Fibronectin Measurements

Plasma fibronectin was measured using the modified method detailed on Page 69. A 1.5 ml blood sample was drawn through the indwelling arterial line via a three way tap following the removal of a large flush sample. The sample was transferred to a primed Eppendorf tube containing 2 mg of EDTA and centrifuged at 13,000 x g for 2 mins. in a microcentrifuge (Microcentaur). To a reaction volume of 500 μl, containing 1:21 dilution of the fibronectin antiserum and buffer, 5 μl of plasma was added. The optical density at 365 nm was read at 1 minute and again at 11 minutes using a Pye unicam SP450 spectrophotometer. Two standard samples were also assayed at the same time and plasma fibronectin concentration was calculated.

Scoring Systems

Scoring systems have proved extremely useful in classifying patients according to the severity of disease, allowing comparison between patients or groups of patients. Two scoring systems were used in the present study - the APACHE II (Knaus et al 1985) and the Sepsis Score (Elebute & Stoner 1983).

APACHE II score

This scoring system is applicable to any disease process. It has been extensively studied in the Intensive Care situation. The score consists of three parts: acute physiology component, age points component, and a chronic health evaluation. These are added together. The score has been used to evaluate prognosis and when calculated at intervals it allows an assessment of response to treatment. In the present study, scores were calculated at the time of admission to allow comparisons between groups. (Table 1).

Sepsis Score

Sepsis Score is a well proven method of assessing the degree of sepsis. It is calculated on the basis of four clinical features of the septic state:

Local effects of tissue infection

Pyrexia

Secondary effects of sepsis

Laboratory data

Scores for these features were assessed according to Table 2. The sepsis score is their sum.

Table 1

APACHE II Score

A Acute Physiology Score

	+4	+3	+2	+1	0
Temperature (^{o}C)	≤29.9 ≥41	30-31.9 39-40.9	32-33.9	34-35.9 38.5-38.9	36-38.4
Mean Blood Pressure (mm Hg)	≤49 ≥160	130-159	50-69 110-129		70-109
Heart Rate (beats min^{-1})	≤39 ≥180	40-54 140-179	55-69 110-139		70-109
Respiratory Rate (breaths min^{-1})	≤5 ≥50	35-49	6-9	10-11 25-34	12-24
Oxygenation (PaO_2 mm Hg)	≤PO_2 55	PO_2 55-60		PO_2 61-70	PO_2 70
Arterial pH	≤7.15 ≥7.7	7.15-7.24 7.6-7.69	7.25-7.32	7.5-7.59	7.33-7.49
Serum Sodium (mmol l^{-1})	≤110 ≥180	111-119 160-179	120-129 155-159	150-154	130-149
Serum Potassium (mmol l^{-1})	≤2.5 ≥7	6-6.9	2.5-2.9	3-3.4 5.5-5.9	3.5-5.4
Serum Creatinine (mg 100 ml^{-1})	≥3.5	2-3.4	0.6 1.5-1.9		0.6-1.4
Hematocrit (%)	≤20 ≥60		20-29.9 50-59.9	46-49.9	30-45.9
White Blood Count (total cu.mmx1000)	≤1 ≥40		1-2.9 20.-39.9	15-19.9	3-14.9

B Age Points

Age (Yrs)	Points
44	0
45-54	2
55-64	3
65-74	5
75	6

C Chronic Health Points

If the patient has a positive Chronic Disease History (history of severe insufficiency or is immuno-compromised)

a for non-operative or emergency post-operative patients - 5 points

b for elective post-operative patients - 2 points

Table 2

Sepsis Score

A Scoring of local effects of tissue infection

Attribute	Score
Wound infection with purulent discharge/ enterocutaneous fistula	
Slight purulent discharge	2
Heavy purulent discharge	4
Peritonitis	
Localised peritonitis	2
Generalised peritonitis	6
Chest infection	
Clinicial or radiological signs without productive cough	2
Clinical or radiological signs with purulent sputum	4
Full clinical manifestations of pneumonia	6
Deep-seated infection	6

B Scoring of pyrexia

Attribute	Score
Maximum daily temperature (^{0}C)	
36-37.4	0
37.5-38.4	1
38.5-39	2
39	3
36	3
	Add
Minimum daily temperature 37.5	1
If 2 or more temperature peaks above 38.4 in 1 day	1
If any rigors occur in a day	1

C Scoring of secondary effects of sepsis

Attribute	Score
Obvious jaundice (in the absence of established hepatobiliary disease)	2
Metabolic acidosis	
Compensated	1
Uncompensated	2
Renal failure	3
Gross disturbance of mental orientation/ level of consciousness	3
Bleeding diathesis (from disseminated intravascular coagulation)	3

D Scoring of Laboratory Data

Attribute	Score
Blood culture	
Single positive culture	1
Two or more positive cultures separated by 24 hrs	3
Single positive culture and history of invasive procedure	3
Single positive culture and cardiac murmur and/or tender enlarged spleen	3
Leucocyte count (x 10^9 l^{-1})	
12-30	1
30	2
2.5	3
Haemoglobin level in the absence of obvious bleeding (g dl^{-1})	
7-10	1
7	2
Platelet count (x 10^9 l^{-1})	
100-150	1
100	2
Plasma albumin level (g l^{-1})	
31-35	1
25-30	2
25	3
Plasma total bilirubin level in the absence of clinically obvious jaundice	
25 umol l^{-1}	1

CHAPTER 3

ANIMAL EXPERIMENTS

Experiments on Rats

Male Sprague-Dawley rats weighing 150-200 grams were supplied by Bantin and Kingman. Each rat had a carotid intra-arterial line and a jugular intravenous line inserted following surgical exposure. Forty-eight hours later the carotid line was connected to a pressure transducer for monitoring of arterial blood pressure and endotoxin was infused over a period of four hours via the jugular line. For each dose of endotoxin rats were sacrificed at specified times and the livers removed for enzyme examination.

Surgical Procedure for the Insertion of the Carotid and Jugular Lines

The carotid line was constructed from two pieces of polythene tubing (Portex); the part that was to be inserted into the artery had an internal diameter of 0.35 mm and a length of 15 mm. This was sealed to the second piece which had an internal diameter of 0.58 mm and a length of 65 mm. The jugular line was constructed in a similar manner but using smaller bore tubing, the insertion part having an internal diameter of 0.26 mm and the second part having an internal diameter of 0.58 mm. Both lines were sterilized in activated glutaraldehyde for 10 minutes and rinsed in sterile 0.9% sodium chloride. One hour prior to surgery the rat was given 2μg of atropine sulphate. Anaesthesia was induced and maintained with diethyl ether using the open drop method. The thorax, head and neck were shaved and swabbed with 1% (w/v) iodine in alcohol. The rat was placed in the supine position with the limbs extended and taped to sterile

drapes. The instruments to be used in the procedure had been sterilized by autoclaving.

Using a pair of scissors a mid-line thoracic incision was made, the strap muscles were split and the left carotid artery was located by blunt dissection. The fascia was stripped away and the vagal nerve carefully separated. The artery was tied distally with a 4/0 silk ligature and a loose tie placed 15 mm proximally. An aneurysm clip was applied just above the proximal tie. (The vessel was kept tense by placing a pair of artery forceps over the distal tie). Using a pair of iris scissors a small incision was made in the superior side of the artery just above the clip. This was dilated with a pair of microforceps and the carotid line, stiffened with a stylet, was threaded into the lumen of the vessel. Using the micro-forceps the vessel and the line were gently clamped and the aneurysm clip removed. The line was slowly threaded up the vessel by delicately releasing the grip on the forceps. The proximal tie was tightened to secure the line and the stylet was removed, causing a flash back of blood into the catheter which indicated correct positioning. The line was flushed with heparin/saline (10 units ml^{-1}) and sealed using a sterile stainless steel pin. It was sutured to the strap muscle, run subcutaneously round the back of the head, brought out through the skin and secured with a 4/0 silk suture.

The same procedure was followed for the insertion of the line into the right external jugular vein. Once the lines had been inserted the incision was closed using 4/0 silk. The rat was given 50 μg of morphine sulphate intravenously and was returned to a warm cage. Solid food was withheld but free access to 10% glucose in water was permitted.

Blood pressure measurement and endotoxin infusion

Forty-eight hours after surgery the rat was placed in a metabolic cage. The carotid and jugular lines were each connected to tubing encased in a flexible tight-coiled spring, held in the vertical position and attached to a counter balance weight. This permitted the rat free run of the cage while preventing kinking of the tubing. The arterial line was connected to a pressure transducer (Gould), which was calibrated at the beginning of each experiment. Using a chart recorder (Device) set at 0.025 mm sec^{-1}, a continuous pulsatile arterial pressure trace was recorded. The line was flushed every five hours with 0.1 ml of heparin/saline (10 units ml^{-1}) to prevent clotting. The jugular line tubing was connected to a 1 ml syringe which contained either physiological saline or endotoxin. This was administered intravenously over 4 hours using a syringe driver (Graseby/165A). The rat was allowed to settle and had free oral access to 10% dextrose. For each infusion a separate animal was sacrificed at 5, 10, 15, 20, 30 or 50 hours by cervical dislocation. The liver was removed immediately, flushed with phosphate-buffered saline to remove blood, frozen in liquid nitrogen and stored at $-70^{o}C$.

Each experiment was carried out in duplicate and 2 rats were studied simultaneously. Arterial blood pressure changes were read off the recorded pulsatile pressure waveforms. The assays of cytochrome P450, NADPH cytochrome c reductase, NADP cytochrome c reductase and cytochrome b5 were performed at a later date. Rat corticosterone was measured by the M.R.C. Blood Pressure Unit at the Western Infirmary.

Microsomal Cytochrome Enzyme Assays

Microsomal preparation

Rat liver, frozen at -70^{o}C, was thawed in 20 ml of ice-cold 0.15 M potassium chloride, 0.025 M potassium phosphate buffer pH 7.5. The tissue was homogenised using 10 strokes of a Potter-Elvejhem homogeniser with a teflon pestle (clearance 0.1 to 0.15 mm). The homogenate was centrifuged at 1000 x g for 10 mins. at 4^{o}C to sediment nuclei, and for a further 15 mins. at 24,000 x g at 4^{o}C to sediment mitochondria.

The post-mitochondrial supernatant was centrifuged at 105,000 x g for 60 mins. at 4^{o}C in Beckman tubes (39411-127-0584) using an ultracentrifuge (MSE high speed 75). The lipid layer was removed and the supernatant decanted. The microsomal pellet was washed three times in the same buffer and assayed for the respective enzymes and protein content. Microsomal suspensions were prepared by dispersing the pellet in the same buffer as used for homogenization to give a final concentration equivalent to 10 μg protein ml^{-1}. For each assay the microsomal suspensions were further diluted five-fold with the buffer appropriate to the assay in question.

Determination of Protein

The method of Lowry et al (1951) was used. This method relies on the formation of a coloured complex between alkaline copper phenol reagent and tyrosine and tryptophan residues of the protein, which

can be measured by optical density. 50 parts 4% (w/v) Na_2HCO_3 in 0.1 M sodium hydroxide were added to 1 part of 1% (w/v) potassium disodium tartrate and 1 part 0.5% (w/v) copper sulphate. 1 ml of protein solution containing no more than 150 μg of protein was added to 2 ml of the above reagent, mixed and left to stand for 10 mins. This was followed by the addition of 1 M Folin Ciocaltean reagent, and the solution was mixed rapidly and left to stand for 30 mins. The optical density was read at 750 nm and a standard curve was constructed using 0.2 - 1 ml aliquots of crystalline bovine serum albumin (200 μg ml^{-1} made up to 1 ml).

Cytochrome P450 Assay

The method used was that described by Estabrook and Werringloer (1978) which relies on the principle that the carbon monoxide adduct of reduced cytochrome P450 is characterised by the presence of an absorbance band at around 450 nm. A 1 ml microsomal suspension in 50 mM Tris buffer pH 7.4 magnesium chloride 5 mM each of two cuvettes, and maintained at 30°C. Using a dual beam spectrophotometer (SP2-800 Pye Unicam), a base line recording was made. Both cuvettes were gassed with carbon monoxide for 2 minutes; 1.6 mg sodium dithionite was added to one and both were gassed again with carbon monoxide for 2 minutes. The difference spectrum between the cuvettes was recorded between 450 nm and 500 nm. The change in absorbance at 450 nm relative to 490 nm was used to calculate the concentration of cytochrome P450 using a millimolar extinction coefficient of 91 cm mM^{-1}.

Cytochrome b5 Assay

The method used was that described by Estabrook and Werringloer (1978). It is based on the change in absorption when cytochrome b5 is reduced by NADH. A 1 ml microsomal suspension in 50 mM Tris buffer pH7, 5mM magnesium chloride was pipetted into each of two cuvettes and maintained at $30^{o}C$; 5 μl of 0.1 mM NADH were added to one of the cuvettes and the changes in absorption at 409 and 426 nm were recorded. The algebraic sum of these changes was used to calculate the concentration of cytochrome b5 using a millimolar extinction coefficient of 185 cm mM^{-1}.

NADPH Cytochrome c Reductase

The method used was that that described by Strobel and Dignam (1978). The reductase-catalysed reduction of cytochrome c was followed spectrophotometrically, by measuring the increase in absorbance at 550 nm. The assay mixture consisted of 300 mM of potassium phosphate buffer PH 7.7, 40 nM of cytochrome c, and was maintained at $30^{o}C$. The reaction was initiated by addition of 5 μl of 20 mM NADPH. The rate of cytochrome c reduction was calculated using the millimolar extinction coefficient of 21 cm mM^{-1} at 550 nm.

NADH Cytochrome c Reductase

The method used was that described by Mihari and Sato (1978). It depends on the ability of the enzyme to catalyse the reduction of ferricyanide by NADH. The assay mixture consisted of 0.1 M potassium phosphate buffer pH 7.5, 0.1 mM NADH, 1 mM potassium

ferricyanide and 500 μl of microsomal preparation in a final volume of 2 ml. It was maintained at 30^{o}C. The reaction was initiated by the addition of 10 μl of NADH and the reduction of ferricyanide was measured by a decrease in absorbance at 420 nm. The rate of reduction was calculated using a millimolar extinction coefficient increment of 1.02 cm mM^{-1}. One unit of activity is defined as that causing the reduction of 1 μM of ferricyanide per minute under these conditions.

Experiments on Pigs

These were designed to establish the effects of Escherichia coli administered intravenously on the pig's haemodynamic state, hepatic microsomal cytochromes, plasma fibronectin and indocyanine green clearance and extraction.

Anaesthesia

Pigs weighing 20-30 kilogrammes were premedicated with azaperone 4 mg kg^{-1} one hour prior to surgery. During this time the animal was transferred from the holding location to the surgical laboratory. The ear was cleansed with alcohol and a 16 gauge polyethylene cannula was inserted into an ear vein. Anaesthesia was induced with morphine 1 mg kg^{-1} and midazolam 0.75 mg kg^{-1} given intravenously by bolus injection. The animal was placed supine on the operating table and given suxamethonium 100 mg intravenously to achieve muscle relaxation. Endotracheal intubation was performed using a straight bladed laryngoscope and a 6.0 mm cuffed endotracheal tube. Anaesthesia was maintained using 70% nitrous oxide in oxygen and 0.5% halothane, supplemented with an infusion of morphine 1 mg kg^{-1} hr^{-1} and vecuronium 1 mg kg^{-1} hr^{-1}, the latter maintaining good muscle relaxation without adverse effects on the cardiovascular system. A Palmer pump was used to administer intermittent positive pressure ventilation with a tidal volume of 200-300 mls x 12 breaths per minute. Depth of anaesthesia was judged by pupil size, heart rate and blood pressure. When adequate anaesthesia had been established, a cannula was inserted into the femoral artery to allow direct measurement of arterial blood pressure. A pulmonary artery

flotation catheter was inserted via the external jugular vein to allow measurement of pulmonary artery pressure, pulmonary artery wedge pressure, cardiac output and mixed venous blood gases, and a laparotomy was performed to permit liver biopsy for microsomal cytochrome determinations.

Surgical procedure

Full aseptic technique was used, including a full surgical skin preparation using 1% (w/v) iodine in alcohol, sterile instruments and disposable paper drapes. During the surgical procedure the animal received 20 ml kg^{-1} hr^{-1} of 0.9% sodium chloride via the ear vein cannula.

Cannulation of femoral artery

A skin crease groin incision was made using diathermy (RadioTom 612). The adductor magnus and the adductor longus muscles were separated to expose the femoral artery in its sheath. The sheath was stripped, using the open-close blunt scissor technique, and the artery ligated distally with a 4/0 silk tie. An incision was made into the lumen of the artery and a 6 French polyethylene catheter inserted and advanced. The catheter was secured in position with a 4/0 tie and connected to a sterile disposable pressure transducer (Gould), for monitoring of intra-arterial pressure. The wound was covered with sterile swabs.

Insertion of pulmonary artery flotation catheter

A short transverse cervical incision over the carotid and jugular vessels was made using diathermy. The incision was deepened

through the platysma and the internal jugular vein exposed by blunt dissection, anterior to the sternomastoid muscle. The vein was ligated proximally using a 4/0 silk tie and a loop tie was placed distally. A small incision was made into the lumen of the vein. The pulmonary artery flotation catheter (7 French Swan Ganz) was primed with heparin saline (4 units ml^{-1}) and connected to a pressure transducer linked to an oscilloscope for display of pressure wave forms. The integrity of the balloon was checked and the catheter was inserted into the vessel under continuous pressure monitoring, gently advanced into the right atrium, and the balloon inflated. It was further advanced into the right ventricle and then into the pulmonary artery, until a pulmonary artery wedge phase was obtained. The balloon was deflated and the catheter pulled back slightly and finally positioned. A 6 French polyethylene catheter was inserted into the right atrium for central venous monitoring. Both lines were secured with 4/0 silk ties and the wound covered with sterile swabs.

Liver Biopsy

A laparotomy was performed using an upper mid line incision. The liver was exposed and a biopsy (100 grams) taken from the distal end of the right lobe. Haemostasis was achieved by inserting vertical mattress sutures (cat gut on a 3 mm round bodied needle) behind the biopsy line and tightening after the biopsy was removed. The biopsy itself was immediately flushed with phosphate buffer saline and frozen in liquid nitrogen. The laparotomy wound was closed in a continuous single layer with 2/0 ethilon.

Insertion of posterior vena cava catheter

The femoral vein was exposed through the same incision as the femoral artery. It was ligated distally with a 4/0 silk tie, and a small incision made through its wall. A 6 French polyethylene catheter was threaded up the vein into the inferior vena cava and positioned at the junction of the hepatic veins and vena cava. The position was confirmed, and corrected if need be, at laparotomy, and the catheter secured with a 4/0 silk tie.

Vena cava choke

At laparotomy the vena cava was exposed just above the right kidney by retracting the small bowel to the left. The vena cava was dissected with great care and a long 7 French catheter passed round it just below its junction with the hepatic veins. Both ends of this catheter were threaded through a long piece of plastic tubing and brought out through the laparotomy incision. Occlusion of the vena cava was achieved by sliding the outer tubing down the catheter, holding the latter firmly.

Experimental procedure

Following the surgical procedure, which lasted 30-45 mins., a small warming blanket was placed over the abdomen and the body covered with paper drapes. The animal was left for an hour to stabilize and at the end of this period a full haemodynamic assessment was made, including the pulse, blood pressure, electrocardiogram, pulmonary artery pressure, pulmonary artery wedge pressure, cardiac output, arterial blood gases and mixed venous blood gases. This assessment

was repeated hourly throughout the experiment. Blood lactate and plasma fibronectin were also measured at the start of the experiment and at two hourly intervals thereafter. Indocyanine green clearance and indocyanine green extraction ratios were measured on selected animals at the beginning and end of the experiment. A second liver biopsy was also taken at the end of the experiment.

Selection of muscle relaxant

As this experiment was designed to observe the effects of Escherichia coli on the liver and cardiovascular system, it was important that the drugs administered should not adversely affect them. It was found that pancuronium, atracurium, suxamethonium and tubocurarine all led to cardiovascular instability (Page **133**). However, vecuronium did not cause cardiovascular instability. It was the drug accordingly chosen as the muscle relaxant.

Experimental design

Following the one-hour stabilising period, the animals were divided into three groups, all of which were maintained under anaesthesia for 10 hours or until death. Throughout they received a continuous infusion of 0.9% sodium chloride at a rate of 5 ml kg^{-1} hr^{-1}:-

A control group which received no Escherichia coli infusion ;

E. coli group which received an infusion of 2×10^8 cells kg^{-1} Escherichia coli over a two hour period immediately following stabilisation;

E. coli colloid group which received a similar Escherichia coli infusion to the above but were given colloid fluid (Haemaccel) to maintain a normal pulmonary artery wedge pressure.

In all three groups, the tidal volume and inspired oxygen concentration were altered as necessary to maintain normal PCO_2 and PO_2.

Bacteriological procedures

Long term storage of cultures. A master culture of Escherichia coli ATC number 33985 was stored at -20^{o} as a 50% (w/v) glycerol suspension. Agar cultures were grown once a month from this and stored at $4^{o}C$. The latter were then used to prepare fresh cultures as needed.

Bacterial growth. Esherichia coli were grown in media containing 16 grams bacto-trypone, 10 grams yeast extract and 5 grams sodium chloride at pH 7.4. The culture was prepared by inoculating 100 ml of sterile medium with a single colony from the stationary phase agar cultures described above, and then incubating the medium at $37^{o}C$ with vigorous aeration in an orbital incubator (Gallenkamp) at 175 r.p.m.

Cell harvesting. The cells were harvested in the logarithmic phase by centrifuging 20 ml of media in a sterile tube at 3000 x g for 10 mins (Beckman TJ6) at $4^{o}C$. The pellet was washed once with 0.9% sodium chloride.

Purification of Porcine Plasma Fibronectin

The method used to isolate the porcine plasma fibronectin was that of Morgenthaler et al (1984) which involves gentle elution conditions.

Method

Five grams of CNBr-activated sepharose 4B (Sigma) was washed with water, resuspended in 30 ml 1mM HCl for 10 minutes, and washed in a sintered funnel using 5 x 30 ml 1mM HCl followed by 30 ml of carbonate buffer (0.1 M Na HCO_3 sodium chloride 0.5 M to pH9). It was then added to 100 ml of carbonate buffer containing 100 mg gelatin (Sigma type 1). The resulting slurry was mixed at $4^{o}C$ for 4 hours. The unreacted groups were blocked with ethanolamine (0.1 M). The resulting gelatin sepharose 4B matrix was poured into a column 0.9 x 15 cm (Pharmacia), and the matrix washed with 50 ml of potassium phosphate buffer saline and sodium citrate (10 mM of potassium phosphate buffer 135 mM sodium chloride 0.01 M of sodium citrate brought to pH 7.2). Using a syringe driver (Graseby 165A) set at 1 ml min^{-1} the washings were collected in a LKB fraction collector in 5 ml aliquots. 50 ml of porcine plasma at $4^{o}C$ was added to the column using the syringe drivers set at 2 ml per min. and 5 ml fractions collected. Elution was carried out using the phosphate buffer saline and sodium citrate. However, the pH was changed from 7.2 to 5.5. Both the buffer and the column were maintained at $30^{o}C$ and 5 ml fractions were again collected. The optical density at 280 nm for each 5 ml fraction was determined spectrophotometrically (Pye unicam SP800 UV). The fractions containing fibronectin were

pooled, and the optical density of this pooled fraction measured at 280 nm to determine the protein concentration. Protein concentration was more accurately determined using the Lowry technique (Page **51**). The pooled protein fractions' homogeneity was assessed by electrophoresis using a 5% polyacrylamide slab gel containing 1% sodium dilauryl sulphate and 2-mercaptoethanol. The gel was stained with Coomassie blue for 2 hours and destained with acetic acid.

Development of Porcine Fibronectin Immunoturbidimetric Assay

A variety of different antiserum concentrations, plasma concentrations, and reaction times were examined in an effort to optimise assay conditions. Human fibronectin antiserum (Boehringer) at dilutions of buffer of 1:20, 1:15, 1:10, 1:7.5 and 1:5 were reacted with each of the following porcine fibronectin standards: 50 μg ml^{-1}, 100 μg ml^{-1}, 200 μg ml^{-1}, 300 μg ml^{-1}, 400 μg ml^{-1} and 500 μg ml^{-1}. Each standard was tested at a volume of 5 μl, 10 μl, 25 μl, 50 μl and 75 μl. For each assay the optical density was read at 1 min., 5 mins., 10 mins., 20 mins., 30 mins., 40 mins., 50 mins. and 60 mins. All assays were carried out in triplicate. The change in optical density between 1 minute and each other time was calculated and the results expressed as change in optical density at 365 nm.

Physiological Measurements

Arterial Blood Pressure Measurement

Arterial blood pressure was measured via an indwelling catheter in the femoral artery connected to a pressure transducer. This was kept patent by a continuous slow flush of heparin saline (4 units ml^{-1}), from a pressure bag. The pressure was displayed on an oscilloscope (Diascope 2) and also digitally on a Mingograph (82). The latter component was capable of producing a permanent copy of the pressure wave form. The monitoring system was balanced and calibrated every hour.

Pulmonary Artery Pressure Measurement

Pulmonary artery pressure was measured via the pulmonary artery flotation catheter located in the pulmonary artery and connected to a disposable sterile pressure transducer. Similar techniques to those of the arterial line were employed for patency of the catheter, measurement of pressure and recording of the information.

Pulmonary Artery Wedge Pressure Measurements

Pulmonary artery wedge pressure was measured by inflating the balloon at the end of the pulmonary artery flotation catheter until the pulmonary artery pressure wave form changed to a wedge trace. This was held over two respiratory cycles and a permanent copy of the trace made. Pulmonary artery wedge pressure was calculated at the end of the respiratory expiratory phase.

Cardiac Output Measurements

Cardiac output was measured using the thermal indicator dilution method. A chilled solution is injected into the blood and the resulting drop in temperature is recorded at a downstream site. The cardiac output is calculated using the Stewart-Hamilton equation modified to take into account a change in temperature, rather than a change in particle concentration.

The temperature of the dextrose 5% injected was $0^{o}C$. This temperature was selected as it reduces the instrument and physiological noise. The temperature of the blood was measured using the thermistor at the end of the pulmonary artery flotation catheter. Ten microlitres dextrose was injected into the right atrial line. An Edwards cardiac output computer (9520A), connected to the thermistor of the pulmonary artery flotation catheter, gave a digital reading of the calculated cardiac output. The procedure was repeated twice and the average cardiac output taken. The electrocardiogram was observed throughout the procedure for the presence of ectopic beats, which can significantly alter cardiac output measurement.

Electrocardiography

The electrocardiogram was measured continuously using 3 electrodes connected to an oscilloscope (Diascope 2).

Heart Rate Measurement

Heart rate was calculated from both the electrocardiogram and the arterial blood pressure traces and a permanent copy stored on the Mingograph.

Temperature Measurement

Core blood temperature was measured via the pulmonary artery flotation catheter thermistor and the temperature displayed on the Edwards cardiac output computer.

Arterial Blood Gas and Mixed Venous Blood Gas Measurements

Arterial blood gases and mixed venous blood gases were measured using a blood gas analyser (Radiometer IL). Blood was drawn into a 2 ml syringe, primed with 50 units of heparin in the dead space, from the femoral artery catheter via a three-way tap after a flush sample had been drawn. The same method was used to obtain a mixed venous blood sample from the distal port of the pulmonary artery flotation catheter. The blood gas analyser was calibrated at the start of every experiment with a high gas calibration (10% CO_2) and a low gas calibration (5% CO_2). The pH electrode was calibrated prior to every measurement.

Haemoglobin Measurement

Haemoglobin was measured using the co-oximeter (IL 182) using blood obtained for arterial blood gas measurement.

Biochemical Estimations

Indocyanine Green

Clearance measurements. Indocyanine green was reconstituted in 20 ml of solvent immediately before use. About 5 ml of blood were drawn through the arterial line via a three-way stop valve and 1.5 ml aliquots dispensed into each of three Eppendorf tubes primed with 2 mg EDTA. An intravenous bolus of indocyanine green clearance 0.5 mg kg^{-1} body weight was injected via the distal port of the pulmonary artery flotation catheter. Blood samples (1.5 ml) were drawn every three minutes for 21 minutes after the injection and transferred to Eppendorf tubes.

Measurement of indocyanine green extracted from blood in a single passage through the liver. Blood flow in the inferior vena cava was obstructed just below the liver by applying the choke described. In this way the inferior vena cava above the choke received only hepatic venous blood. After an interval of 60 seconds, base line blood samples (6 ml) were drawn from both the arterial and the vena cava catheters. The choke was released immediately after the blood sample had been drawn. A single bolus of indocyanine green 0.5 mg kg^{-1} per body weight was administered, as in the clearance studies, and a continuous infusion of indocyanine green at a dose of 25 mg over 30 minutes was set up to produce a steady state. At 5 minute intervals the choke was reapplied and further arterial and caval blood samples withdrawn. Determination of indocyanine green in these samples permitted calculation of the percentage extracted in passage.

Indocyanine green estimation. The Eppendorf tubes were centrifuged in a micro-centrifuge at 13,000 x g for 2 minutes. Indocyanine green concentrations were estimated spectrophotometrically at 805 nm (Pye unicam SP6-450). All samples were read against the plasma blank, as were two standards prepared by adding respectively 2 μl and 1 μl of dye (2.5 mg ml^{-1}) to 1 ml of plasma blank. The calculated plasma indocyanine green concentrations were plotted against time on semi-logarithmic paper, a straight line fitted by eye, and extrapolated to zero. From this plot the initial concentration (C_o) and the half-life ($t_{\frac{1}{2}}$) could be measured.

Calculation of clearance. The initial concentration of the indocyanine green (C_o) and the injected dose can be used to calculate the volume (V) of plasma throughout which the indocyanine green was distributed.

$$V = \frac{D}{C_o}$$

The observed half-life ($t_{\frac{1}{2}}$) is related to the volume of plasma cleared (Cl) per minute thus:-

$$t_{\frac{1}{2}} = \frac{0.693 \times V}{Cl}$$

Hence

$$Cl = \frac{0.693 \times V}{t_{\frac{1}{2}}}$$

The volume of plasma cleared per minute can be converted to the corresponding volume of blood by using the haematocrit.

Extraction of indocyanine green

The mean arterial-hepatic venous extraction ratio (E) was calculated from the 3-4 steady state measurements of arterial (a) and hepatic venous (hv) dye concentrations according to the formula:

$$E = \frac{a - hv}{a}$$

Porcine Plasma Fibronectin Assay

Porcine plasma fibronectin was measured using the kinetic immunoturbidimetric technique based on the measurement of an antigen - antibody reaction. This assay was developed using commercially available sheep antiserum to human fibronectin. (Boehringer). (Page **62**). This was cross-reacted with standard porcine fibronectin purified by affinity chromatography (Page **61**).

Blood Collection

Blood (1.5 ml) was drawn from an arterial line via a three-way stop valve, following a large flush sample, and placed in an Eppendorf tube (1.5 ml) primed with 2 mg EDTA and 60 units of aprotinin. The samples were centrifuged at 13,000 x g for 2 minutes in a micro-centrifuge and the plasma removed, to be assayed or stored at -20^{0}C awaiting assay.

Assay Procedure

In the immunoturbidimetric assay the antiserum was diluted in a ratio of 1:10 in a solution containing 66 mM of phosphate buffer (Na_2HPO_4 and KH_2PO_4) pH 7.4 containing 3% polyethylene glycol 6000 and 1% Brij 35.

Five hundred microlitres of the antibody buffer mixture was pipetted into a disposable semi-micro cuvette maintained at 25°C, and 50 ml of plasma or standard added. With each batch of assays a set of fibronectin standards ranging from 50 mg l^{-1} to 500 mg l^{-1} were included as controls. Following the addition of the plasma or standard the optical density (O.D.) at 365 nm was read at 1 min. and at 30 mins. using a spectrophotometer (Pye unicam SP6-450). The observed difference (Delta O.D. = O.D. 30 min - O.D. 1 min) between the two optical densities was used to determine porcine plasma fibronectin level from a standard calibration curve. Each assay was carried out in duplicate.

Porcine Plasma Lactate Estimation

Blood Collection

One and a half microlitres of blood was drawn through the arterial catheter via a three-way stop valve, and placed immediately into an Eppendorf tube primed with 3 mg of fluoride oxalate. The sample was spun in a microcentrifuge at 13,000 x g for 2 mins. and the plasma transferred to a plain tube and assayed.

Assay Procedure

Two microlitres of NAD/carbonate buffer, containing 63 mM of L-glutamate, and 4.6 mM NAD to pH10, was mixed with 40 μl of plasma. This was split into two aliquots of 1.02 ml in semi-micro cuvettes. Twenty microlitres of lactate dehydrogenase (1632 units ml^{-1}) and alanine transaminase (102 units ml^{-1}) were added to one micro-cuvette (sample blank) and 20 ul of 3.2 M ammonium sulphate to the other. The optical density (O.D.) at 365 nm was measured, spectrophotometrically (Pye unicam SP6-450) immediately after mixing and again at 15 mins. The reaction was carried out at room temperature. The change in optical density (Delta O.D.) between the sample blank and the test was calculated, and the concentration of lactate was calculated using the relationship:-

Delta O.D. x 15.3 = lactate concentration in mM.

The test was carried out in duplicate.

CHAPTER 4

THE OBSERVATIONS ON PATIENTS IN SEPTIC SHOCK

Modification of the Kinetic Immunoturbidimetric Assay for Human Plasma Fibronectin

The primary objective of this work was to establish the value, if any, of the measurement of plasma fibronectin levels in the diagnosis of septic shock, and the usefulness of cryoprecipitate administration in elevating these and improving the patient's chance of survival. Before starting, it was necessary to find the best possible assay for plasma fibronectin. A commerical kit was available from Boehringer, but its range far exceeded the physiological reference range and it was insensitive and inaccurate at the low levels which are of particular clinical interest. There were no published data on its precision, accuracy and sensitivity and the manufacturers themselves could find no unpublished data in their files. Moreover, the cost per assay was approximately £2.00. It seemed important to try to improve the assay and if possible to make it less expensive. It proved possible to do this by reducing the reaction volume and increasing the antiserum dilution. (Technical details of the procedure will be found on Page43).

Reduction in reaction volume

Using the manufacturer's recommended antiserum dilution (i.e. 1 : 11) the reaction volume could be reduced first to 500 μl and then to 250 μl simply by inserting a small platform under the semi-micro

cuvette holder. Each standard was assayed five times, the optical density being read at 365 nm, spectrophotometrically (Pye unicam SP8-200), at 1 minute and 11 minutes. Table 3 shows the mean optical density, standard error and coefficient of variation obtained for the five assays carried out for each fibronectin standard using 1000, 500 and 250 μl reaction volumes. Although the accuracy and precision obtained using the two smaller volumes were both comparable with the 1000 μl volume of the original method, the 250 μl volume could not be used with the manual Pye SP450 spectrophotometer used in the Intensive Care Unit for bedside estimations. The 500 μl reaction volume was therefore used routinely.

Increase in Antiserum dilution

Using a reaction volume of 500 μl, the manufacturer's recommended antiserum dilution (1 : 11) was compared with two further dilutions (1 : 21 and 1 : 31). A cryoprecipitate sample and a plasma sample were assayed in triplicate by a nephelometric technique (carried out by the West of Scotland Blood Transfusion Service) and fibronectin values of 1050 mg l^{-1} and 410 mg l^{-1} respectively were obtained. Serial dilutions of these two standards were then prepared using buffer, and each dilution assayed five times with each of the antiserum dilutions. The values obtained, with their coefficients of variation, are shown in Tables 4 (cryoprecipitate) and 5 (plasma) and the coefficients of variation for each dilution are plotted in Figures 9 (cryoprecipitate) and 10 (plasma). If a coefficient of variation below 7% is deemed acceptable, the useful ranges for each antiserum dilution are as follows:-

1 : 11	275-850 mg l^{-1}
1 : 21	110-530 mg l^{-1}
1 : 31	90-150 mg l^{-1}

From the above it can be seen that the 1 : 11 dilution adequately covers the reported normal ranges for plasma fibronectin, but not the low levels of fibronectin, such as have been reported in patients suffering from sepsis. The 1 : 31 dilution performs best in the sub-physiological range of fibronectin levels, but is not sufficiently accurate over the range of normal fibronectin levels. Overall the 1 : 21 dilution seemed an acceptable compromise.

The reduction in reaction volume to 500 ul together with the increase in antiserum dilution to 1 : 21 increased the number of assays which could be performed from one kit four-fold. Figure 11 shows a comparison between the standard assay (1 : 11 dilution of antiserum and 1000 μl reaction volume) and the modified assay (1 : 21 dilution of antiserum and 500 μl reaction volume), when used to assay in triplicate 170 separate plasma samples for fibronectin level. A good correlation was observed across the range with a regression coefficient value of 0.993, although in the sub-physiological range the relative scatter was greater, in keeping with the coefficients of variation within this range shown above.

External factors and their influence on the fibronectin assay

The effect of lipid

As this assay is a turbidimetric technique it is important to take account of the possible errors due to plasma lipid. A healthy male

subject was fasted overnight and a blood sample obtained by venepuncture. He then consumed a meal high in fat and provided intravenous blood samples every 30 minutes for 8 hours. Figure 12 shows the results obtained when these samples were assayed for fibronectin. It would appear that the presence of lipid does result in an increase in the apparent fibronectin level.

A second experiment was carried out on patients receiving Intralipid (Kabivitrum) as part of a total parenteral nutrition regimen. Blood was withdrawn through arterial cannulae just before they received 500 mls of 10% Intralipid, administered over 8 hours or 16 hours. Blood samples were taken hourly for the next 24 hours and assayed for fibronectin. Figure 13 shows the results. In patients receiving Intralipid over 8 hours, the plasma was lipaemic to the naked eye and the assay was affected. In patients receiving Intralipid over 16 hours, the plasma was not obviously lipaemic and the assay was not affected.

Effect of heparin

It was reported by Saba et al (1978) that heparin interferes with the fibronectin assay, producing erroneously low results. As many of the blood samples from patients would be obtained through an indwelling arterial line which would be continuously flushed with heparinised saline (1 unit ml^{-1} at 3 ml hr^{-1}), it was important to find out if obtaining blood in this fashion introduced an error into the estimation of plasma fibronectin. Accordingly comparisons were made between simultaneous venous and arterial samples taken from patients, the venous sample by clean venepuncture and the arterial sample from an

arterial line following the removal of a 2 ml flush sample. Table 6 shows the results; it can be seen that with an adequate flush sample there was no difference between arterial and venous fibronectin levels.

Construction of Reference Ranges for Plasma Fibronectin

A reference range was established using samples from 144 young healthy control subjects all of whom were free from illness and taking no medication. There are few data in the literature on fibronectin levels in an elderly population and, since many patients admitted with septic shock are elderly, it was essential to establish also an acceptable reference range for them. Samples were obtained from 103 elderly healthy patients who had been hospitalised for social, as opposed to medical, reasons and had no evidence of illness or malignancy and had not received any recent blood transfusion. Figure 14 shows the results from both groups. The mean level in the elderly population was significantly greater than in the young population (368 vs 306 mgs l^{-1}). Interestingly there were also significant sex differences in fibronectin levels, which reversed with age. These trends are consistent with the much less extensive data published by Strathakis et al (1981), though these included too few patients over the age of 50 to allow statistical analysis. Figure 15 shows the distribution curves for fibronectin level in the young and elderly healthy populations in the present study.

Table 3

Accuracy and precision of the fibronectin assay at varying reaction volumes.

Fibronectin		1000 µl		500 µl		25 µl	
(mg l^{-1})	n	mean	C.V.	mean	C.V.	mean	C.V.
1000	5	0.286	9.3	0.274	8	0.291	10.75
750	5	0.254	4.9	0.261	5.5	0.254	4.5
500	5	0.193	5.3	0.184	5.10	0.189	6.3
250	5	0.091	11	0.092	10.5	0.087	13.6
125	5	0.042	20	0.045	22	0.0043	28
62.5	5	0.018	37	0.016	54	0.009	49

Table 4

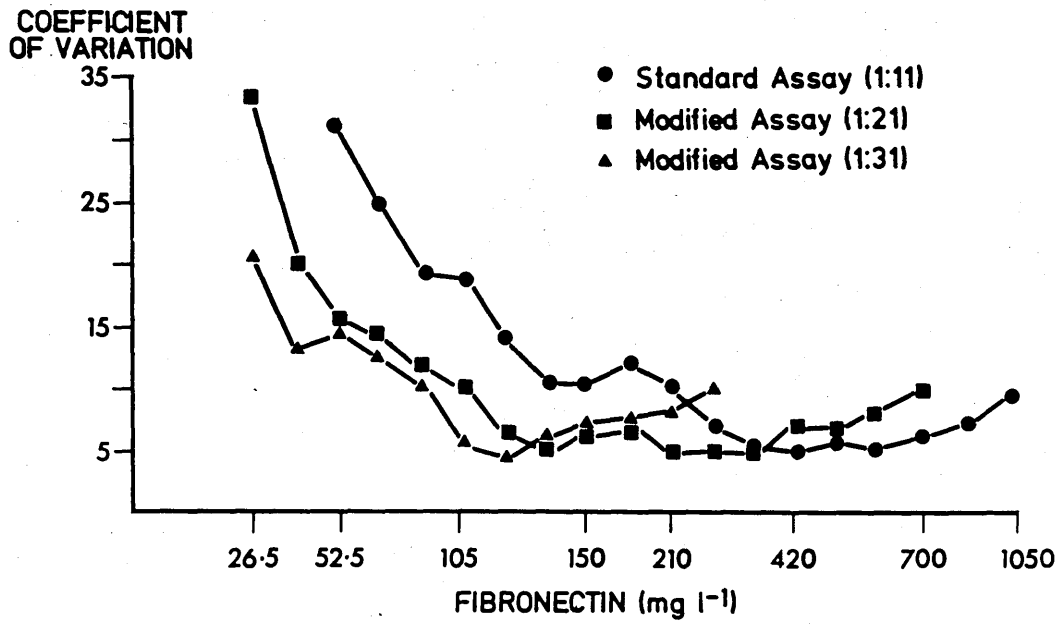
Accuracy and precision of the fibronectin assay
at varying antiserum dilutions using cryoprecipitate as a standard.

Fibronectin (mg l^{-1})

Calculated Fibronectin	n	Standard Assay Mean	C.V.	Modified Assay* Mean	C.V.	Modified Assay# Mean	C.V.
1050	5	901	9.08				
840	5	888	7.0				
700	5	750	5.94	780	9.9		
600	5	629	5.04	662	8.16		
525	5	516	5.9	556	6.9		
420	5	400	5.0	448	6.7		
350	5	348	4.72	339	4.9		
262	5	255	7.34	255	4.8	311	10.12
210	5	214	9.67	215	5.2	227	7.88
175	5	166	11.7	164	6.9	190	7.2
150	5	141	10.1	151	6.3	167	7.2
131	5	117	10.3	145	5.4	139	5.9
116	5	86	13.9	99	6.58	117	4.8
105	5	81	18.7	96	10.06	102	5.5
84	5	55	19.3	76	11.76	80	9.8
74	5	40	25	66	14.57	66	12.4
52.5	5	31	31	52.5	15.87	44	14.8
35	5			25	20.18	31	13.5
26.5	5			17	33.5	20	20.9

* 1 : 21 dilution of the antiserum
\# 1 : 31 dilution of the antiserum

Figure 9



Comparison of the coefficient of variation for the immunoturbidimetric fibronectin assay, utilising 3 different dilutions of fibronectin antiserum (1:11), 1:21) and (1:31) Serial dilutions was carried out on a known concentration of human Cryoprecipitate. Each sample was assayed five times for fibronectin.

Table 5

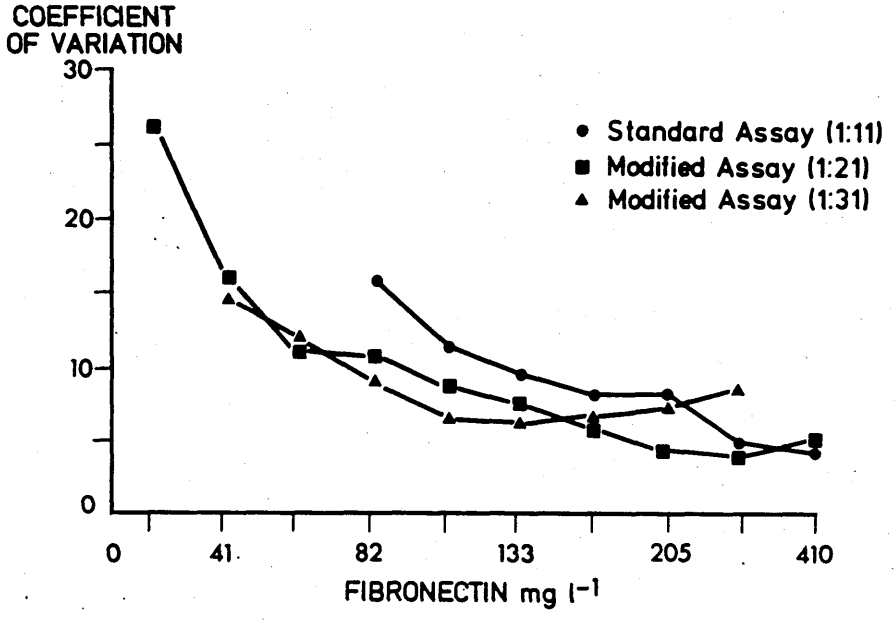
Accuracy and precision of the fibronectin assay
at varying antiserum dilutions using plasma as a standard.

Fibronectin (mg l^{-1})

Calculated Fibronectin		Standard Assay		Modified Assay*		Modified Assay#	
	n	mean	C.V.	mean	C.V.	mean	C.V.
410	5	394	4.15	433	5.08		
273	5	259	4.81	269	3.8	305	8.59
205	5	195	8.1	190	4.2	222	7.22
166	5	149	8.0	159	6.0	176	6.7
133	5	118	9.34	138	7.5	433	6.08
102	5	79	11.32	97	8.6	106	6.18
82	5	58	15.66	71	10.4	85	9.3
55	5	31	20.42	45	11	48	11.8
41	5			28	15.9	29	14.4
27	5			16	26.1	16	26.11

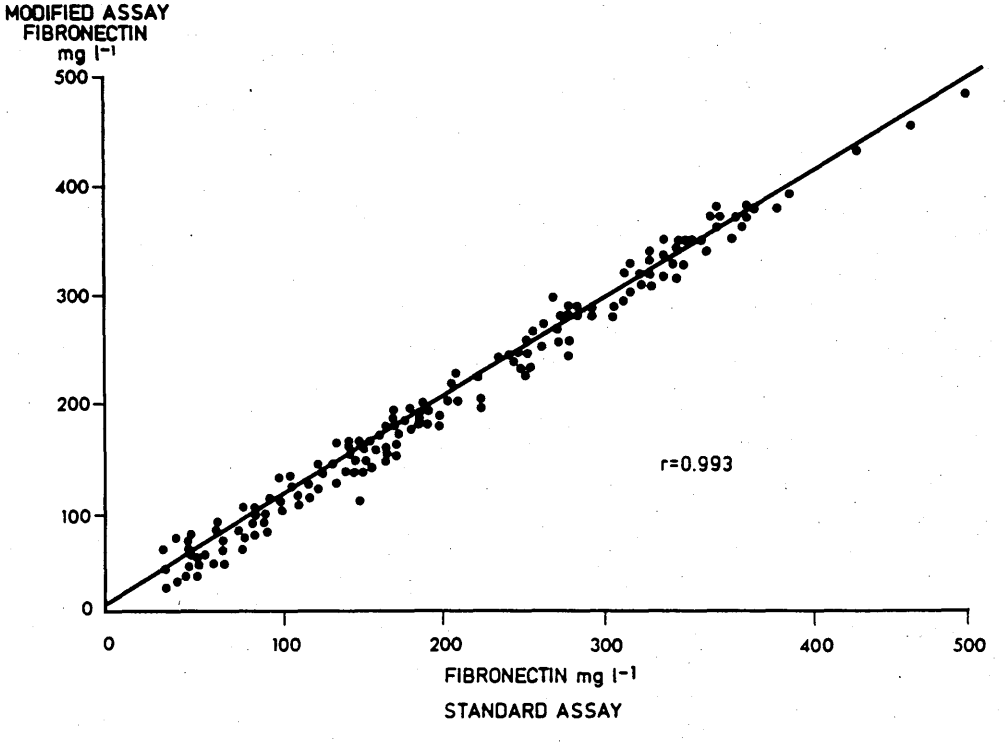
* 1 : 21 dilution of the antiserum
\# 1 : 31 dilution of the antiserum

Figure 10



Comparison of the coefficient of variation for the immunoturbidimetric fibronectin assay, utilising three different dilutions of fibronectin antiserum (1:11,1:21 and 1:31). Serial dilution was carried out on a known concentration of human plasma fibronectin. Each sample was assayed five times.

Figure 11



Comparison between the standard immunoturbidimetric assay(1.11 dilution of antiserum) and the modified immunoturbidimetric assay (1.21 dilution of antiserum)for human plasma fibronectin.

Table 6

The effect of heparin on plasma fibronectin estimations.

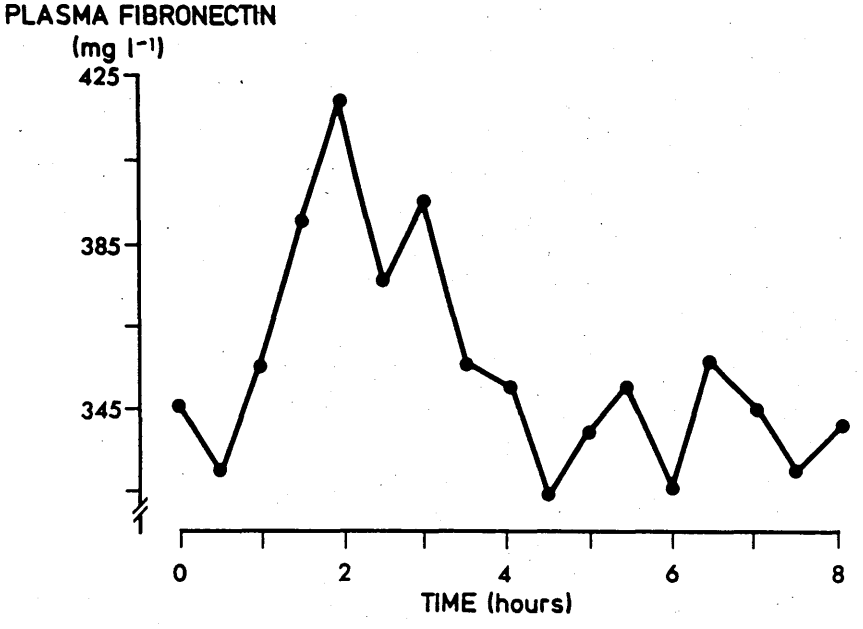
Plasma fibronectin (mg l^{-1})

Patient	Venous Sample	Arterial sample
1	245	258
2	184	156
3	225	210
4	286	278
5	300	275
mean	248	235.4
S.D.	46.9	52.6

Venous sample v. arterial sample - not significant*

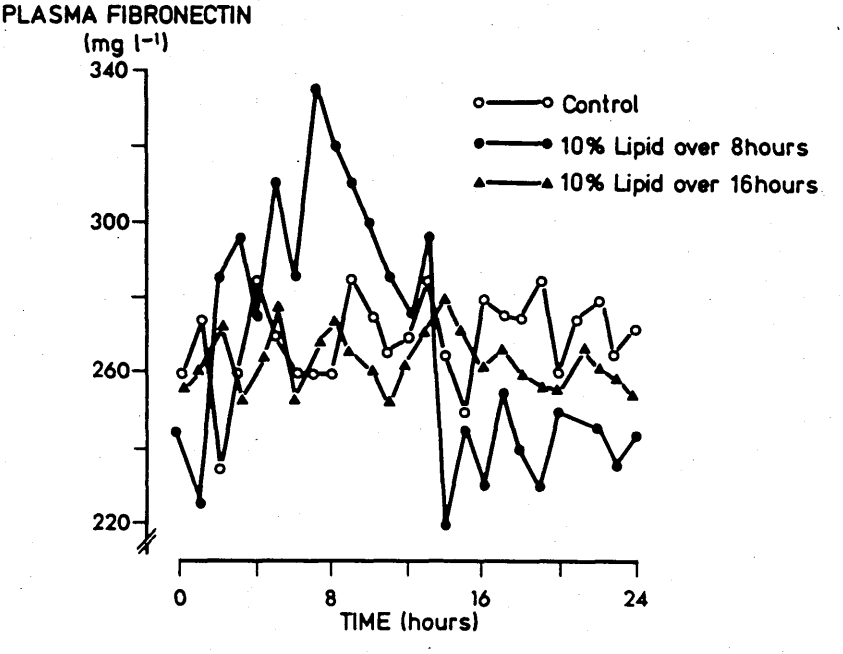
* Student's t test (paired)

Figure 12



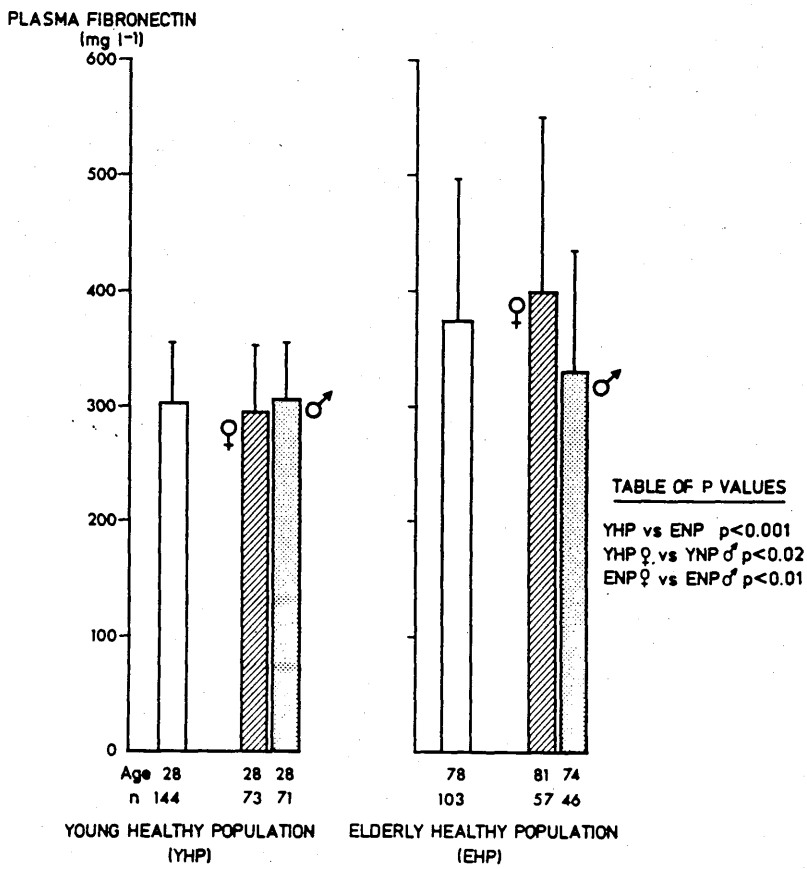
The effects of lipid on plasma fibronectin estimation. A healthy subject was fasted overnight and a food injested following base line plasma fibronectin estimation.

Figure 13



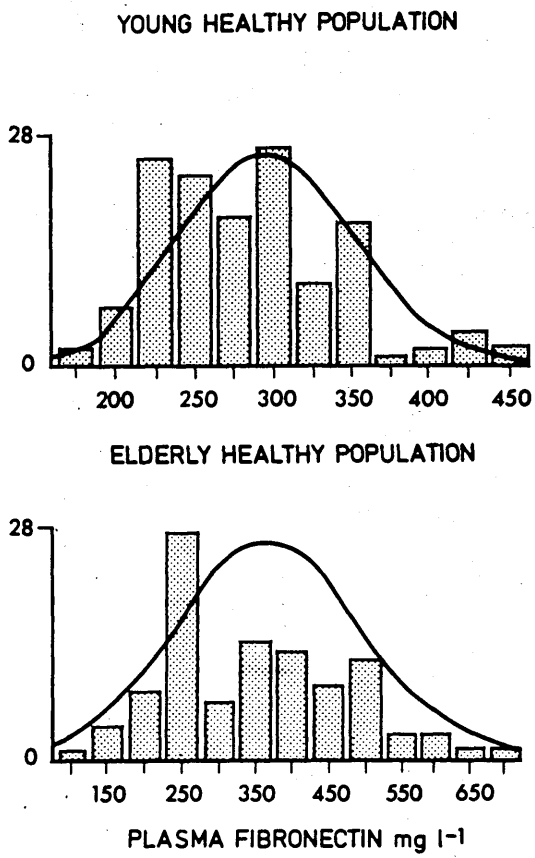
The effects of lipid on plasma fibronectin. Lipid 10%(500ml) was administered intravenously over 8hours, and on the next day over 16 hours; following a base line plasma fibronectin estimation.

Figure 14



THE EFFECTS OF AGE AND SEX ON PLASMA FIBRONECTIN IN A YOUNG HEALTHY POPULATION AND AN ELDERLY HEALTHY POPULATION.

Figure 15



NORMAL DISTRIBUTION STATISTIC OF PLASMA FIBRONECTIN FOR A YOUNG HEALTHY POPULATION AND A HEALTHY ELDERLY POPULATION.

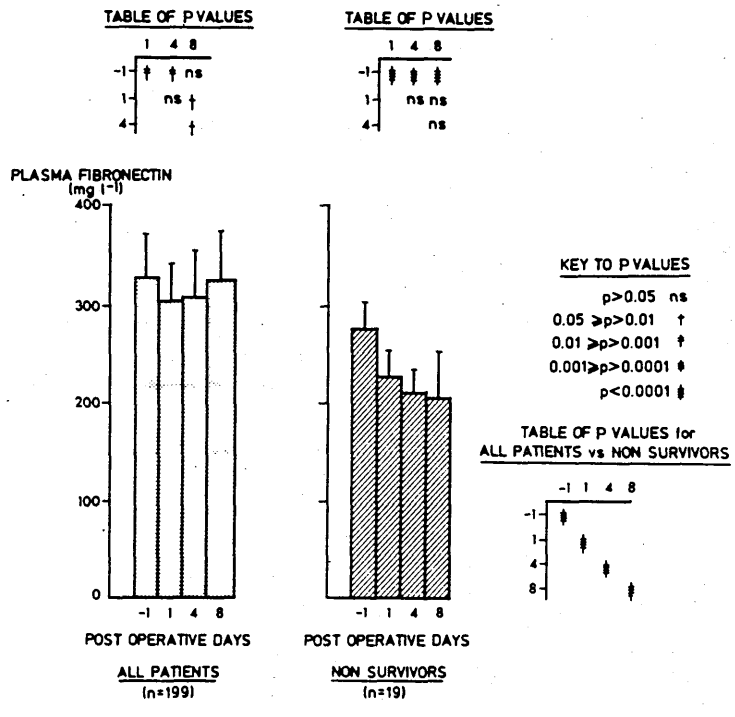
Effect of Surgery on Plasma Fibronectin

As most of the patients admitted to Intensive Care in septic shock have undergone surgery, it was important to determine the effect this might have had on their plasma fibronectin. Accordingly patients undergoing either routine or emergency laparotomy (except for appendicectomy) had blood samples taken on the pre-operative day, or during the immediate pre-operative period, and on the first, fourth and eighth post-operative days, for estimation of plasma fibronectin. In total, 199 patients were studied. The results are shown in Figure 16, which also shows separately the results from 19 patients who subsequently died. The mean pre-operative value for the group as a whole was 325 mg l^{-1}. The level had fallen significantly by the first post-operative day, it remained low on the fourth post-operative day but returned to normal by the eighth. In the patients who did not survive, the pre-operative level (275 mg l^{-1}) was significantly below the mean for the group as a whole. Again the level fell post-operatively but showed no reversal by the eighth. Figure 17 shows the distribution curves for fibronectin on each of the days studied.

Effect of Sepsis on Plasma Fibronectin

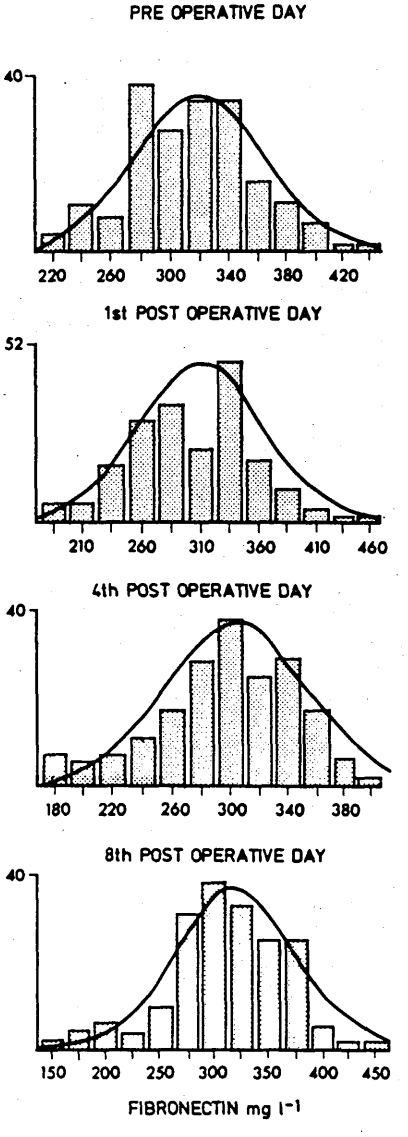
Patients admitted to Intensive Care with a diagnosis of sepsis but who were not in shock had plasma fibronectin levels measured twice a day throughout their stay in Intensive Care. Patients were divided into non-survivors (Figure 18) and survivors (Figure 19). Both groups showed a fall in plasma fibronectin, but the survivors showed a reversal of this trend as their condition improved.

Figure 16



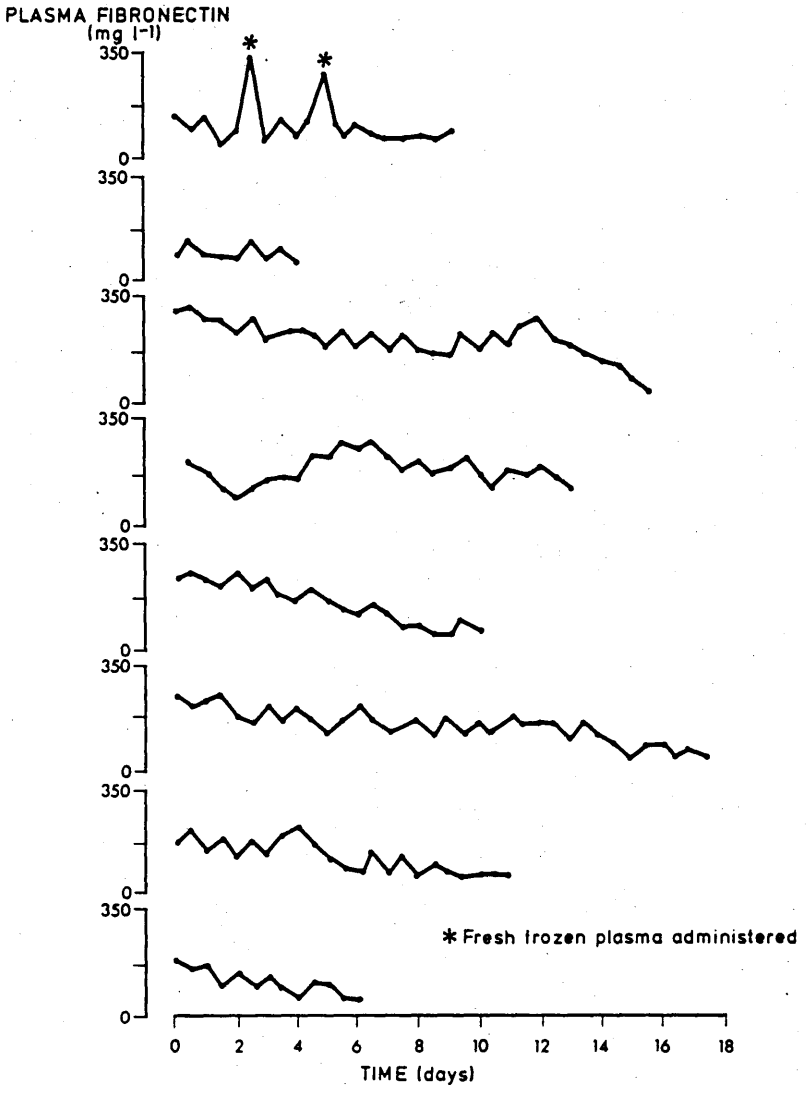
PLASMA FIBRONECTIN LEVELS (mean ± S.D.) ON 199 CONSECUTIVE PATIENTS UNDERGOING EITHER ROUTINE OR EMERGENCY LAPAROTOMY. PLASMA FIBRONECTIN WAS MEASURED PRE-OPERATIVELY AND THREE TIMES POST OPERATIVELY.

Figure 17



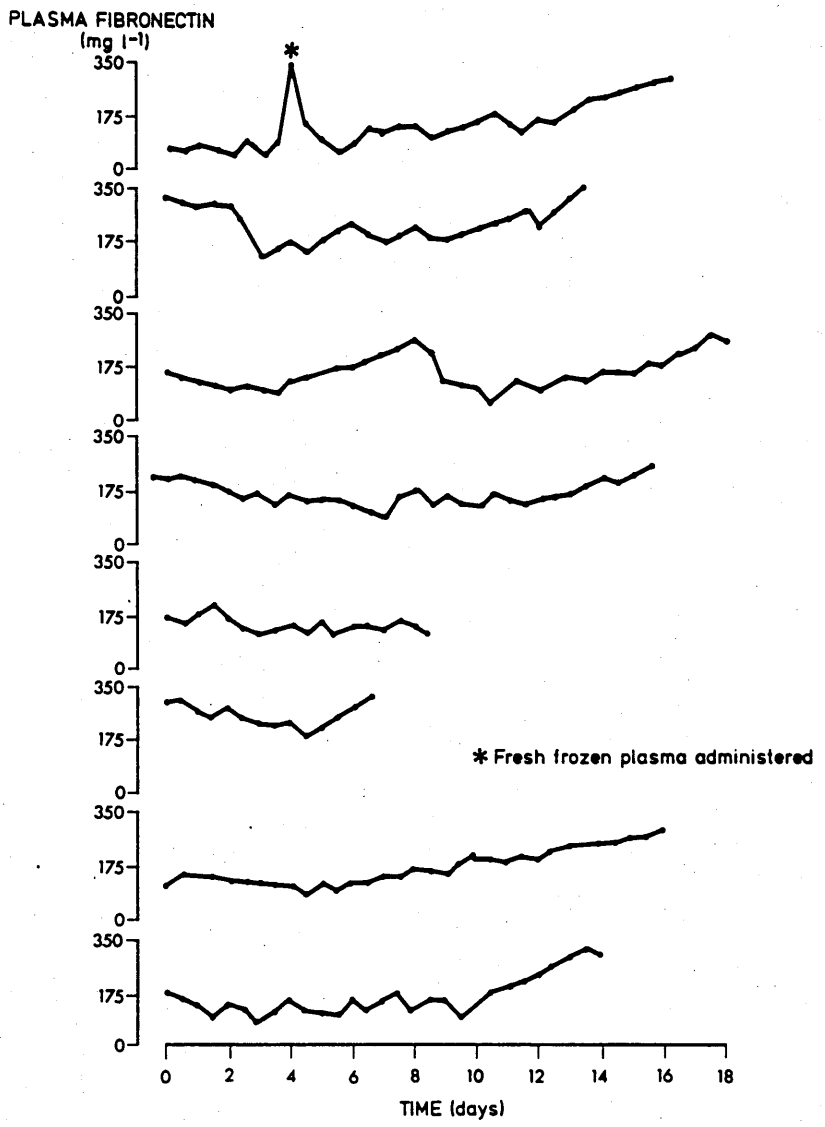
NORMAL DISTRIBUTION STATISTICS OF PLASMA FIBRONECTIN FOR LAPAROTOMY PATIENTS.

Figure 18



Serial plasma fibronectin levels in patients with severe sepsis who did not survive.

Figure 19



Serum plasma fibronectin levels in patients with severe sepsis who survived.

Cryoprecipitate Replacment of Fibronectin in Septic Shock

This study was designed to look, first, at the effects of septic shock on hepatic metabolism, plasma fibronectin and circulating endotoxin and, secondly, to evaluate the use of cryoprecipitate as a source of fibronectin in the treatment of septic shock.

Entry criteria

The patients in this study were all inpatients in the Intensive Care Unit of the Western Infirmary, Glasgow. The criteria for admission to the study were as follows:-

Proven septicaemia or a proven major source of sepsis

with

Systolic blood pressure less than 90 mm Hg.

and

Oliguria (less than 30 ml hr^{-1}).

and

Core/peripheral temperature gradient over $4^{o}C$.

and

Clinical features of shock

tachypnoea, tachycardia, confusion and pallor.

Admission procedure

Patients meeting these criteria were prospectively randomised by a closed envelope technique to either a control or treatment group. Both groups received routine Intensive Care therapy and, in addition, the treatment group (hereafter referred to as cryoprecipitate group) received an infusion of cryoprecipitate of a volume calculated to raise the plasma fibronectin level above 300 mg l^{-1}. Cryoprecipitate

was subsequently given whenever the fibronectin level fell to less than 200 mg l^{-1}, until the patients consumption of fibronectin decreased and the plasma fibronectin level remained stable in the normal range (or until the patients left the Intensive Care Unit). On the first day of treatment, patients in the control group received an infusion of plasma protein solution equivalent in volume to the infusion of cryoprecipitate that would have been given had the patient been in the cryoprecipitate group. Plasma protein solution can be regarded as cryoprecipitate-depleted plasma (it has a mean fibronectin level of 15 mg l^{-1}). On subsequent days, because the fibronectin level in the control group remained low, it was obviously inappropriate to calculate the volume of plasma protein solution to be given in the same way. However, since the volume of cryoprecipitate given to a patient in the treatment group on average was 400 mls, this volume of plasma protein solution was given on a daily basis to patients in the control group. These volumes of colloid given to the two groups were allowed for by the consultants in charge when calculating the patient's fluid requirements for the day.

Investigations carried out on admission

1. Plasma fibronectin level
2. Indocyanine green clearance
3. Antipyrine clearance
4. Plasma endotoxin level
5. Blood cultures
6. A full range of physiological measurements including pulse, blood pressure, core and peripheral temperature, cardiac output, arterial and mixed-venous blood gases, pulmonary

artery pressure, pulmonary artery wedge presure, central venous pressure and urine output

7. Sepsis score and APACHE II score
8. Routine laboratory tests i.e. biochemistry, haematology, bacteriology and blood cross matching.

Subsequent investigations

1. Plasma fibronectin levels were measured four-hourly throughout the study period in both the control and cyroprecipitate groups. The cryoprecipitate group also had fibronectin levels measured immediately before cryoprecipitate infusion and 30 to 60 minutes afterward. The control group had fibronectin levels measured before and 30 to 60 minutes after the infusion of plasma protein solution that was given in lieu of cryoprecipitate
2. Endotoxin measurements; these were initially performed four-hourly while the patients were unstable. They were also performed before and after the infusion of cryoprecipitate or plasma protein solution. (Obtaining samples for endotoxin measurement depended on reasonable peripheral venous access since these samples cannot be drawn from indwelling vascular lines)
3. Indocyanine green clearance was measured eight-hourly
4. The full range of physiological measurements, as listed above, were carried out four-hourly and, in addition, before and after colloid infusion
5. Blood cultures were performed at least daily

Results

Table 7 shows comparative data for the control and cryoprecipitate groups at the time of entry to the study. it can be seen that the groups were well matched for all the criteria examined. Both the APACHE II score and Sepsis Score indicate that the protocol had selected an extremely sick group of patients.

Selected Case Histories

R.McD. 45 years. Male. Gram-negative septicaemia following trauma. Control group. Non survivor.

This patient had been admitted to Intensive Care following multiple trauma; his injuries included chest trauma, multiple long bone fractures and traumatic amputation of one arm. He developed a proven Gram-negative septicaemia after seven days in Intensive Care, presumed to have come from a pulmonary source. Figure 20 shows a profile of measurements made for this patient. Plasma fibronectin, indocyanine green clearance, cardiac output, urine output, core temperature and plasma endotoxin are plotted against time. The initial fibronectin level of 140 mg l^{-1} rapidly fell to below 100 and remained at that low level until he succumbed. The values for indocyanine green clearance were low (normal being 1000-1500 mls min^{-1}) particularly when the high cardiac output is taken into account. He was anuric and remained pyrexial until his death. Significant levels of endotoxin were present in the plasma and climbed steadily.

T.B. 61 years. Male. Staphylococcus aureus septicaemia from prostatic abscess. Control group. Survivor.

This patient was admitted to Intensive Care direct from theatre having gone into septic shock during the operative drainage of a prostatic abscess. Staphylococcus aureus was subsequently isolated from both the abscess pus and the blood stream. Figure 21 shows the profile for this patient. The initial plasma fibronectin level of 230 mg l^{-1} fell to 100 mg l^{-1} but returned to the normal range before the patient's discharge from Intensive Care. The indocyanine green clearance values were close to normal, cardiac output was high and urine output rose to normal after a period of anuria immediately following admission. The patient's haemodynamic status stabilised following early resuscitation. The following day, however, there was a second cardiovascular collapse, at which time a high peak of endotoxaemia (100 pg ml^{-1}) was observed, as shown in Figure 21. The patient again stabilised rapidly and left Intensive Care the following day.

D.B. 51 years. Female. Necrotising fasciitis. Cryoprecipitate group. Non survivor.

This patient was admitted to Intensive Care in profound septic shock secondary to necrotising fasciitis. She was never sufficiently well to allow surgical debridement to be carried out. Her initial fibronectin level of 80 mg l^{-1} was raised following the infusion of 25 units of cryoprecipitate, but, as can be seen from Figure 22, this fibronectin was rapidly consumed, necessitating a further infusion of cryoprecipitate soon after. Indocyanine green clearance started at a low level and deteriorated. The patient was initially hyperdynamic but the cardiac output later fell. High circulating levels of endotoxin

were present throughout, the levels steadily rising until the time of death.

I.McD. 56 years. Female. Necrotising fasciitis. Cryoprecipitate group. Survivor.

This diabetic lady was admitted to Intensive Care in profound septic shock and following stabilisation was taken to theatre for surgical debridement of the involved fascia. Figure 23 shows her profile. The initial fibronectin level of 125 mg l^{-1} was raised to over 400 mg l^{-1} by the infusion of 20 units of cryoprecipitate. Indocyanine green clearance was initially low but improved slightly over the course of the patient's stay. She had a slightly elevated cardiac output and a low level of pyrexia, which settled after several days. High levels of endotoxin were present in the plasma throughout the first few days, but a decrease in this level of endotoxaemia was frequently observed following the infusion of cryoprecipitate, as can be seen from Figure 23. The patient's stay in Intensive Care was prolonged and she had a high requirement for cryoprecipitate infusion over a considerable length of time. The plot of fibronectin levels show that the rate of removal of fibronectin from the plasma gradually decreased as her condition stabilised. At the end of the time period covered by Figure 23 the patient was discharged to another hospital. Her fibronectin level at this stage had stabilised but was still low; her core temperature had also risen again following a period of apyrexia and this was interpreted as being due to low grade sepsis in some of the surgical wounds. Intermittent samples taken in the hospital to which she was transferred showed that the fibronectin level eventually rose spontaneously back to the normal level.

Q.E. 28 years. Male. Staphylococcus aureus septicaemia from septic arthritis. Cryoprecipitate group. Survivor.

This patient was admitted to Intensive Care with a staphylococcal septicaemia secondary to septic arthritis which developed following an arthroscopy and knee exploration. He was initially extremely ill and haemodynamically very unstable but improved after three to four days and subsequently survived. His initial fibronectin level of 120 mg l^{-1} was raised by the infusion of cryoprecipitate. Again the profile in Figure 24 shows that the rate of consumption of fibronectin gradually decreased as the patient's condition improved; the volumes of cryoprecipitate required gradually decreased and he was eventually weaned from cryoprecipitate infusion on the fifth day. Following this, the fibronectin level stabilised, then rose back to within the normal range. Indocyanine green clearance was initially extremely low and remained low for five days before gradually improving. Again, this patient had intermittent peaks of endotoxaemia, which appeared to fall following cryoprecipitate infusion. (The presence of these peaks of endotoxaemia in patients Q.E. and T.B., both of whom had Gram-positive septicaemia, will be commented on later in the discussion section).

The Effect of Cryoprecipitate and Plasma Protein Solution Infusions on a Variety of Physiological and Biochemical Variables

The cryoprecipitate infusions, and the plasma protein solution given to control patients in lieu of cryoprecipitate, were given as part of the patient's fluid requirements, and not in addition to those requirements. The rate of infusion was adjusted so as not to alter the pulmonary artery wedge pressure. Tables 8 and 9 show a range of

variables measured before and 30 to 60 minutes after infusion of either plasma protein solution (Table 8) or cryoprecipitate (Table 9). Following cryoprecipitate there was, as expected, a significant rise in plasma fibronectin levels. An improvement in urine output and a fall in the level of pyrexia were also observed, both of these changes being significant. Interestingly, there was also a significant fall in the level of endotoxaemia. There was a tendency for indocyanine green clearance to improve and for pulmonary vascular resistance to fall, although neither of these changes achieved statistical significance.

Following plasma protein solution infusion no significant change was observed in any of the variables measured.

Indocyanine Green Clearance from Plasma

For the method used to calculate indocyanine green clearance see Page **38** . Table 10 shows the mean initial indocyanine green clearance and mean 24-hour indocyanine green clearance for all the patients in the septic shock study; values are also given separately for the control and cryoprecipitate groups and for survivors and non-survivors. The mean initial indocyanine green clearance for all patients was 688 ml min^{-1}, which was significantly lower than the accepted normal value of greater than 1200 ml min^{-1}. The initial values were lower in the cryoprecipitate group than in the control group but this difference did not achieve statistical significance; nor was there any significant difference between the initial values for the survivors versus non-survivors. Of the seven patients who survived, all but two showed an improvement in clearance over the

first 24 hours; of the sixteen who died, all but one showed a deterioration. Such changes may therefore have a prognostic value.

Antipyrine Half-Life

Table 11 shows the results of antipyrine half-life analysis for all the patients in the septic shock study. The normal antipyrine half-life in plasma for healthy subjects is in the region of 10 hours (Boobis et al, 1980); the mean antipyrine half-life in the septic shock patients was significantly prolonged at 28.7 hours. There were no significant differences between the control and cryoprecipitate groups or between survivors and non-survivors.

Outcome in Septic Shock Study

For the purpose of this study, mortality was defined as total hospital mortality and not merely mortality within the Intensive Care Unit. Table 12 shows the details of randomisation and outcome in the control and cryoprecipitate groups. In total, 23 patients were included in the study: of the eleven in the control group, two survived; and of the twelve in the cryoprecipitate group, five survived. This mortality difference did not achieve statistical significance when tested by Chi-squared analysis. Figure 25 shows a plot of the cumulative mortality for each group against time. There were more early deaths in the control group than in the cryoprecipitate group and this difference in mortality was significant on day 2 and day 3. Using Fishers' exact analysis, the **P** values were 0.03 for day 2 and 0.01 for day 3.

Table 7

Comparative data for control and cryoprecipitate groups in the septic shock study.

	Control Group mean ± S.D.	Cryoprecipitate Group mean ± S.D.	P Value*
Age	56 ± 10.1	54 ± 13.6	N.S.
Sex M : F	6 ± 5	5 ± 7	N.S.
Mean Blood Pressure (mm Hg)	52 ± 7.5	55 ± 11.6	N.S.
Pulmonary Shunt	0.13 ± 0.06	0.14 ± 0.07	N.S.
A - a Gradient (mm Hg)	246.9 ± 69.7	266.6 ± 98.6	N.S.
Plasma Fibronectin ($mg\ l^{-1}$)	127.6 ± 69.4	97.0 ± 35.6	N.S.
Indocyanine Green Clearance ($ml\ min^{-1}$)	766 ± 497	589 ± 364	N.S.
Apache II Score	19.3 ± 3.1	21.5 ± 5.9	N.S.
Sepsis Score	21.5 ± 7.2	22 ± 4.8	N.S.

* Student's t test (unpaired)
N.S. not significant

Figure 20

R. McD 45yrs ♂ GRAM NEGATIVE SEPTICAEMIA FOLLOWING TRAUMA.CONTROL.NON SURVIVOR.

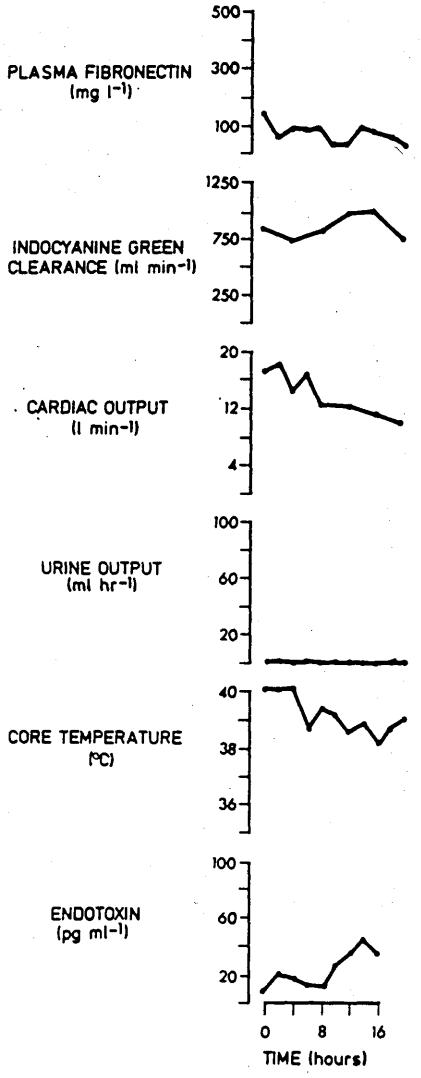


Figure 21

T.B. 60YRS ♂ GRAM POSITIVE SEPTICAEMIA FROM PROSTATIC ABSCESS.CONTROL SURVIVOR.

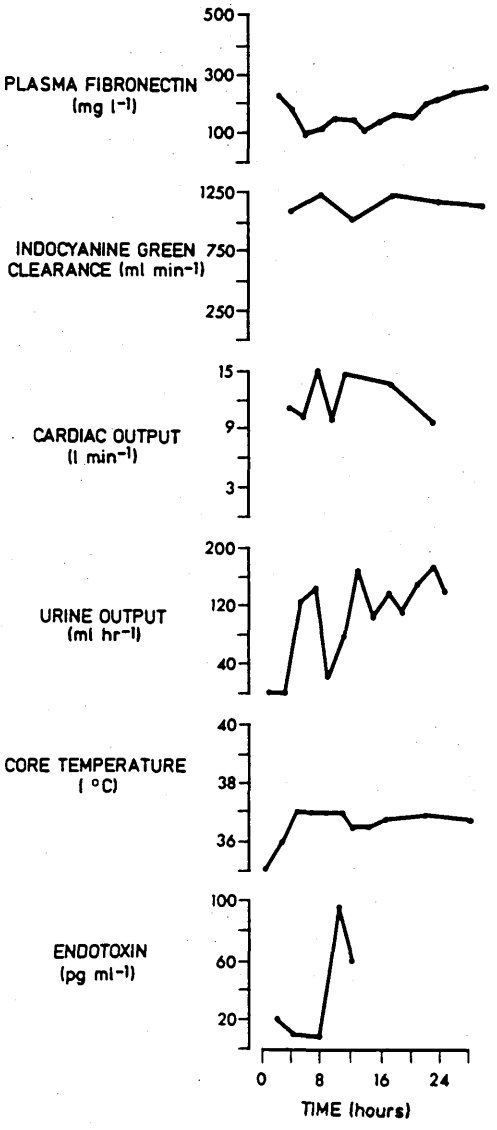


Figure 22

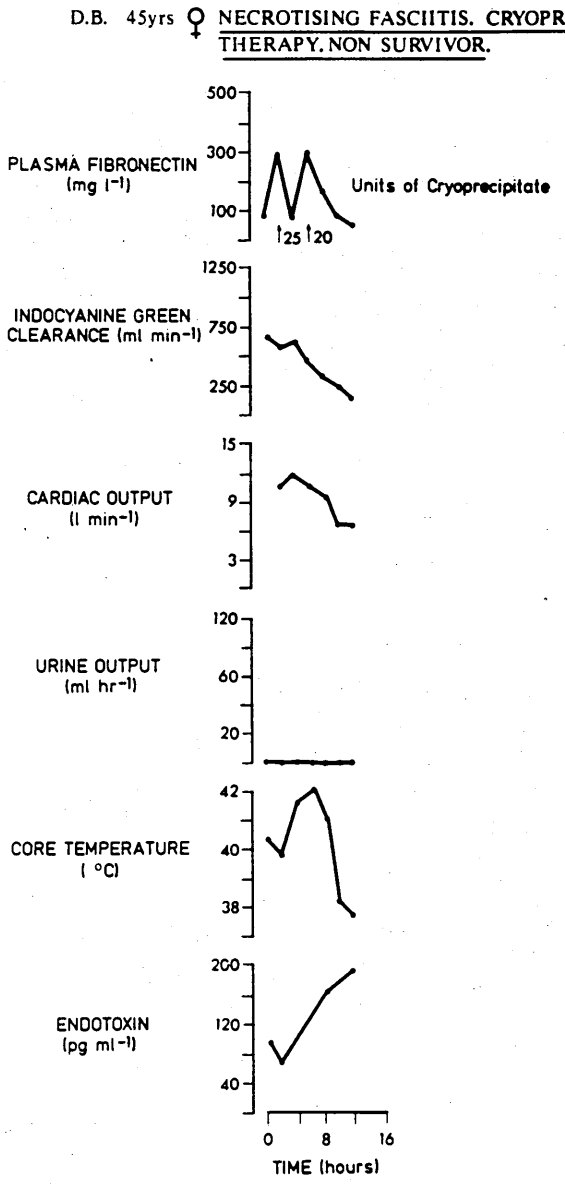

D.B. 45yrs ♀ NECROTISING FASCIITIS. CRYOPRECIPITATE THERAPY. NON SURVIVOR.
PLASMA FIBRONECTIN (mg l⁻¹)
500
300
100
↑25 ↑20
Units of Cryoprecipitate
INDOCYANINE GREEN CLEARANCE (ml min⁻¹)
1250
750
250
CARDIAC OUTPUT (l min⁻¹)
15
9
3
URINE OUTPUT (ml hr⁻¹)
120
60
20
CORE TEMPERATURE (°C)
42
40
38
ENDOTOXIN (pg ml⁻¹)
200
120
40
0
8
16
TIME (hours)

Figure 23

I. McD 56yrs ♀ NECROTISING FASCIITIS. CRYOPRECIPITATE THERAPY SURVIVOR.

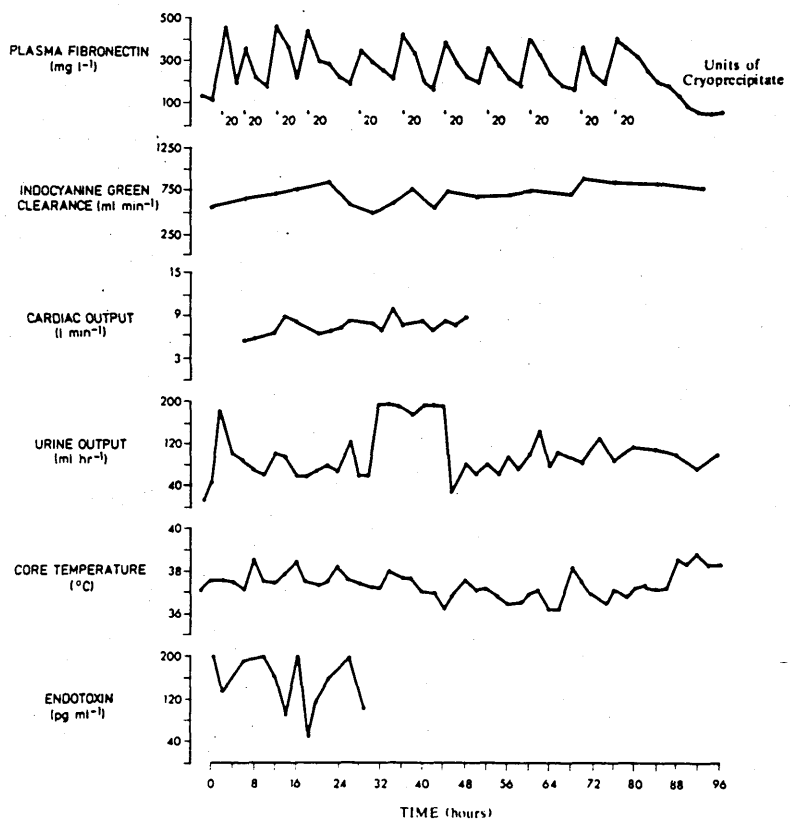


Figure 24

Q.E. 28YRS: ♂ S. AUREUS SEPTICAEMIA FROM SEPTIC ARTHRITIS. CROPRECIPITATE THERAPY. SURVIVOR.

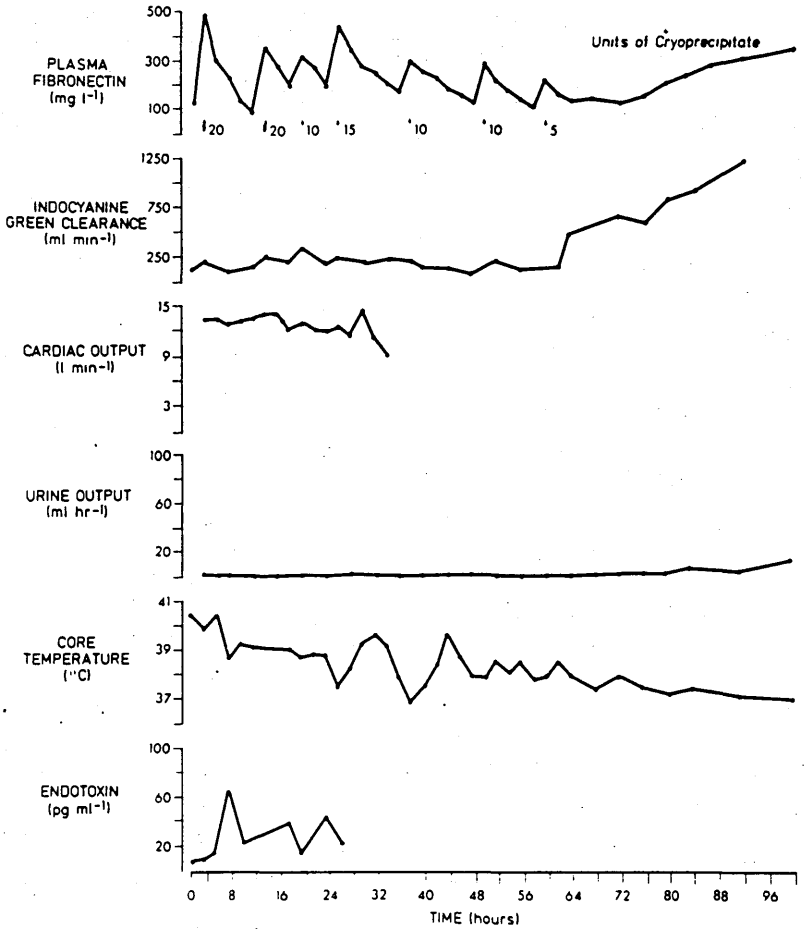


Table 8

The effect of plasma protein solution on physiological variables.

	Pre Infusion mean ± S.D.	Post Infusion mean ± S.D.	P Value*
Urine Output ($ml\ min^{-1}$)	53 ± 70.2	40 ± 47	N.S.
Core Temperature ($0^{o}C$)	38.23 ± 1.16	38.07 ± 1.24	N.S.
Indocyanine Green Clearance ($ml\ min^{-1}$)	564 ± 434	516 ± 426	N.S.
Cardiac Output ($l\ min^{-1}$)	9.39 ± 2.83	9.08 ± 1.88	N.S.
Pulmonary Artery Wedge Pressure (mm Hg)	11.21 ± 4.66	10.95 ± 3.35	N.S.
Pulmonary Vascular Resistance ($dyne.\ sec\ cm^{-5}$)	88.6 ± 55.2	93.6 ± 73.6	N.S.
Endotoxin ($pg\ ml^{-1}$)	15.8 ± 12.29	25.23 ± 23.44	N.S.
Plasma Fibronectin ($mg\ l^{-1}$)	117.1 ± 32.4	125 ± 72.1	N.S.
Mean Blood Pressure (mm Hg)	67.6 ± 13.3	69.8 ± 18.3	N.S.

* Student's t test (paired)
N.S. not significant

Table 9

The effect of cryoprecipitate on physiological variables.

Mean values for all infusions given to all patients in cryoprecipitate group.

	Pre Infusion mean ± S.D.	Post Infusion mean ± S.D.	P Value*
Urine Output ($ml\ min^{-1}$)	77.7 ± 49.6	123.7 ± 75.5	= 0.001
Core Temperature ($0^{0}C$)	38.3 ± 1.2	37.9 ± 0.9	< 0.05
Indocyanine Green Clearance ($ml\ min^{-1}$)	734 ± 419	818 ± 464	N.S.
Cardiac Output ($l\ min^{-1}$)	8.4 ± 2.44	8.19 ± 2.36	N.S.
Pulmonary Artery Wedge Pressure (mm Hg)	14.52 ± 4.04	13.91 ± 3.86	N.S.
Pulmonary Vascular Resistance (dyne. sec cm^{-5})	89.6 ± 43.2	63.8 ± 27.2	N.S.
Endoxotin ($pg\ ml^{-1}$)	86.25 ± 77.82	40.8 ± 49.5	< 0.05
Plasma Fibronectin ($mg\ l^{-1}$)	166.4 ± 59.45	432 ± 58.5	> 0.0001
Mean Blood Pressure (mm Hg)	74.72 ± 10.9	77.6 ± 11.84	N.S.

* Student's t test (paired)
N.S. not significant

Table 10

Indocyanine green clearance (ICG) from plasma in septic shock study patients.

Initial values were at time of randomisation to study.

	Initial ICG clearance mean ± S.D.		24 hour ICG clearance mean ± S.D.		P value*
Control group (n = 11)	733	533	612	478	< 0.05
Cryoprecipitate group (n = 12)	589	362	577	331	N.S.
Overall	688	436	622	387	< 0.05
Survivors (n = 7)	548	297	648	331	N.S.
Non-survivors (n = 16)	721	512	562	440	< 0.01

* Student's t test (unpaired)

Initial ICG clearance	24 hour ICG clearance
control v. cryoprecipitate N.S.	control v. cryoprecipitate N.S.
survivors v. non-survivors N.S.	survivors v. non-survivors N.S.

N.S. not significant

Table 11

Antipyrine half-life in septic shock patients.

	Antipyrine half-life (hrs) mean ± S.D.	P Value*
Control group (n = 11)	32.1 ± 12.6	N.S.
Cryoprecipitate group (n = 12)	24.9 ± 7.0	N.S.
All patients (n = 23)	28.7 ± 10.7	
Survivors (n = 7)	25.3 ± 9.4	N.S.
Non-survivors (n = 16)	30.4 ± 11.21	

* Student's t test (unpaired)
N.S. not significant

Table 12

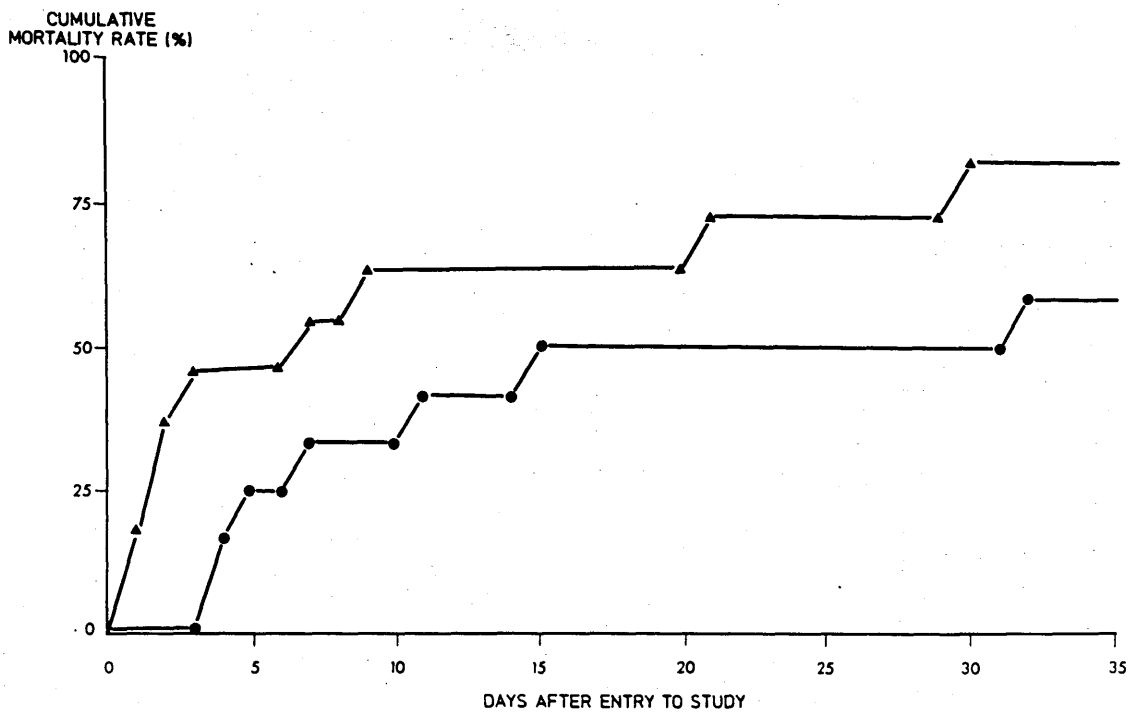
Mortality data for septic shock study.

	Control group	Cryoprecipitate group
n	11	12
survivors	2	5
non-survivors	9	7

Control group v. Cryoprecipitate group - not significant*

* Chi square test

Figure 25



CUMULATIVE MORTALITY CURVES FOR SEPTIC SHOCK STUDY PATIENTS;
▲——▲Control Group (n=11),●——●CYROPRECIPITATE GROUP (n=12)
Ultimate Mortality; Control Group=82%,CYROPRECIPITATE GROUP=58%.

Summary of Clinical Studies

The results described above could be summarised by saying that the administration of cryoprecipitate to patients in septic shock:

1. does, as one would anticipate, raise the plasma fibronectin level;
2. increases output of urine approximately twofold;
3. probably, but less certainly, reduces plasma endotoxin levels and brings core temperature a little closer to normal;
4. probably produces a modest improvement in liver function as reflected by indocyanine green clearance;
5. does not, however, improve the impaired capacity to metabolise antipyrine; and,
6. may improve survival.

CHAPTER 5

THE OBSERVATIONS ON ANIMAL EXPERIMENTS

The Effect of Endotoxin on Rats

One of the most striking observations in the clinical studies was that the septic shock patients had a greatly impaired ability to clear antipyrine. This seemed to merit further investigation in animals. Antipyrine is normally taken up in the liver and oxidised by the microsomes. It seemed of interest to establish whether the impairment of this process in septic shock was due to the endotoxin in some way diminishing microsomal function. This was investigated in rats.

Preliminary studies were carried out to assess the effect of storage and the effect of morphine administration on the microsomal cytochrome activity in the liver. At the same time, corticosteroid response to the surgical stress of cannulation was measured in order to determine the length of time required for stabilisation.

Effect of Storage at -70^{0}C on hepatic microsomal cytochrome activity

Five healthy animals were sacrificed by cervical dislocation without prior instrumentation or anaesthesia. The liver was removed and divided into four parts. Three of the four were frozen in liquid nitrogen and subsequently stored at -70^{0}C. The remaining part was placed on ice and the microsomal preparation and cytochrome assays carried out immediately. The stored samples were removed for

microsomal preparation and cytochrome determination after 6, 12 and 24 weeks. Table 13 shows the results; storage had no effect on the activity of any of the cytochrome enzymes measured.

Effect of morphine on hepatic microsomal cytochrome activity

In the main experiment, morphine sulphate was to be given as an analgesic after the implantation of cannulae. In order to assess the effect, if any, on cytochrome activity, two groups, each of five rats, were used. One group received 100 μg of morphine sulphate intravenously while the other group received saline. Forty-eight hours later the animals were sacrificed, the livers removed and hepatic microsomal cytochrome activity assayed. The results are shown in Table 14 It can be seen that morphine had no discernible effect.

Effect of surgical stress on rat plasma corticosterone levels

Five rats were subjected to the surgical procedure described on Page **48** and blood samples were withdrawn through the jugular line immediately after surgery, and 4, 24, 48 and 72 hours later, for measurement of plasma corticosterone levels. Figure 26 shows the results obtained. Corticosterone levels were very high at time zero and at 4 hours, but fell rapidly thereafter. In the main experiment, a stabilisation period of 48 hours was chosen as a compromise between, on the one hand, allowing stress (as reflected in corticosterone levels) to subside and, on the other, avoiding the risk of blood clotting in the cannulae.

Table 13

The effect of storage at -70°C on rat hepatic cytochrome activity.

		Time from sacrifice			
		0	6 wks*	12 wks*	24 wks*
	n	5	5	5	5
Cytochrome P450	mean	0.91	0.97	0.94	0.90
(n mol mg protein^{-1})	S.D.	0.054	0.046	0.063	0.027
Cytochrome b5	mean	0.39	0.37	0.41	0.38
(n mol mg protein^{-1})	S.D.	0.041	0.031	0.028	0.026
NADPH cytochrome c reductase	mean	0.126	0.119	0.123	0.128
(μmol min^{-1} mg protein^{-1})	S.D.	0.018	0.026	0.017	0.009
NADH cytochrome c reductase	mean	0.096	0.099	0.093	0.101
(μmol min^{-1} mg protein^{-1})	S.D.	0.02	0.016	0.022	0.014

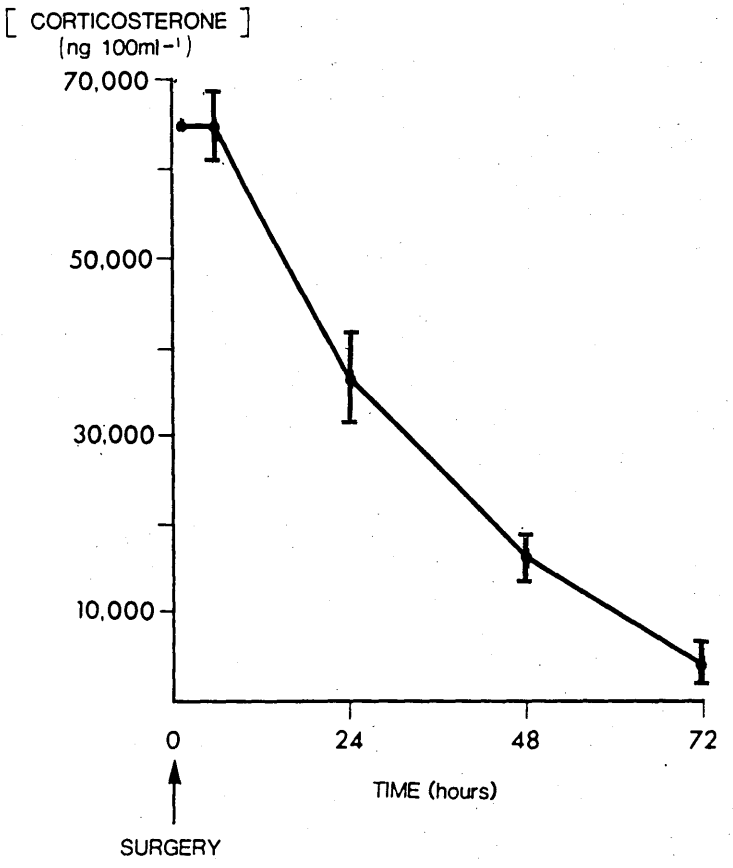
* Samples stored at -70°C

Table 14

The effect of morphine on rat hepatic cytochrome activity.

		Saline	Morphine
	n	5	5
Cytochrome P450	mean	0.94	0.99
(n mol mg protein^{-1})	S.D.	0.046	0.053
Cytochrome b5	mean	0.44	0.41
(n mol mg protein^{-1})	S.D.	0.029	0.022
NADPH cytochrome c reductase	mean	0.116	0.124
(μmol min^{-1} mg protein^{-1})	S.D.	0.022	0.017
NADH cytochrome c reductase	mean	0.104	0.095
(μmol min^{-1} mg protein^{-1})	S.D.	0.016	0.023

Figure 26



THE EFFECTS OF SURGICAL STRESS ON THE RATS ADRENAL- CORTICOTROPHIC RESPONSE. (mean ± S.D.)

Effect of endotoxin and hypovolaemic shock on hepatic microsomal cytochrome activity

This experiment was designed to test the effect of varying doses of endotoxin on hepatic microsomal cytochrome activity, with a view to distinguishing the haemodynamic and metabolic effects of endotoxin. Twenty rats were used as controls. Following line insertion and a 48-hour stabilisation period, they each received a 0.5 ml infusion of 0.9% saline intravenously over 4 hours. Four animals were sacrificed at each of the following time intervals: 5, 10, 20, 30 and 50 hours. The livers were removed and stored for microsomal cytochrome assay. Table 15 shows the results of the cytochrome assays and the mean arterial pressure readings. There are no significant changes in either.

The rats who received endotoxin also had a forty-eight hour stabilisation period following line insertion. They were grouped according to the does of endotoxin administered with 12 rats in each group. The doses of endotoxin were:-

5×10^{1} mg kg^{-1}
5 mg kg^{-1}
5×10^{-1} mg kg^{-1}
5×10^{-2} mg kg^{-1}
5×10^{-3} mg kg^{-1}
5×10^{-4} mg kg^{-1}
5×10^{-5} mg kg^{-1}

Two animals were sacrificed at each of the following times: 5, 10, 20 and 50 hours from the start of the endotoxin infusion. None of the animals receiving 5×10^{1} mg kg^{-1} survived to 10 hours, so the results for the animals sacrificed at 5 hours are shown in Table 16. For each of the other endotoxin doses, the results of the cytochrome activity

assay and blood pressure measurement are shown graphically in Figures 27 to 32.

The larger doses of endotoxin (down to 50 mg kg^{-1}) produced a consistent fall in mean arterial pressure and a roughly parallel fall in the cytochromes. These effects were diminished as the dose was reduced (Table 16, Figures 27, 28 and 29). Lower doses of endotoxin had no consistent effect (Figures 30, 31 and 32). The remarkable parallelism between blood pressure and cytochrome activities immediately raises the question whether the former is determining the latter. To test this, a further group of twelve rats were subjected to hypvolaemic shock by withdrawal of 2 ml of blood at 0,5 and 10 hours, i.e. 6 ml in total. The results are shown in Figure 33. The loss of blood resulted in a precipitatous fall in mean arterial pressure, but this was not accompanied by comparable falls in cytochrome activities.

Figure 34 summarises the effects of the various doses of endotoxin and hypovolaemic shock on blood pressure and hepatic microsomal cytochrome activity. From the results, it appears that cytochrome P450 is more sensitive than the other cytochromes measured to the effect of endotoxin. It was not only reduced by a greater percentage at each dose but was also affected by smaller doses of endotoxin than the other cytochromes. A fall in mean arterial blood pressure was observed down to and including 5×10^{-2} mg kg^{-1} of endotoxin. In doses smaller than this, no change in blood pressure was observed; but cytochrome b5 activity was still reduced at 5×10^{-3} mg kg^{-1} and cytochrome P450 activity was reduced at 5×10^{-3} and 5×10^{-4} mg kg^{-1}. The induction of hypovolaemia produced a marked fall in blood pressure, but only minimal changes in cytochrome activity. Thus the

haemodynamic and metabolic effect of endotoxin can be separated, and the reductions observed in cytochrome activity appear to be, at least in part, independent of the associated hypotension.

These observations are consistent with the limited information in the literature: the control value obtained for the cytochrome P450 assay agrees with the results of Takeda et al (1984) and Falzon et al (1984). The cytochrome b5 results agree with those of Takeda et al (1984). Finally, Takeda et al (1984) induced sepsis in rats by caecal ligation then measured cytochrome activity following sacrifice at 72 hours. They found reductions in cytochromes P450 and b5 and also NADPH cytochrome c reductase of a similar magnitude to those seen in the present study in the group of rats receiving 5 mg kg^{-1} of endotoxin when sacrificed at 50 hours. (Figure 27).

Conclusion

It is apparent that endotoxin does impair the activity of liver microsomal cytochromes, especially of cytochrome P450, and that this is not secondary to the concomitant fall in mean arterial pressure.

Table 15

Rat cytochrome activity and blood pressure following saline administration.

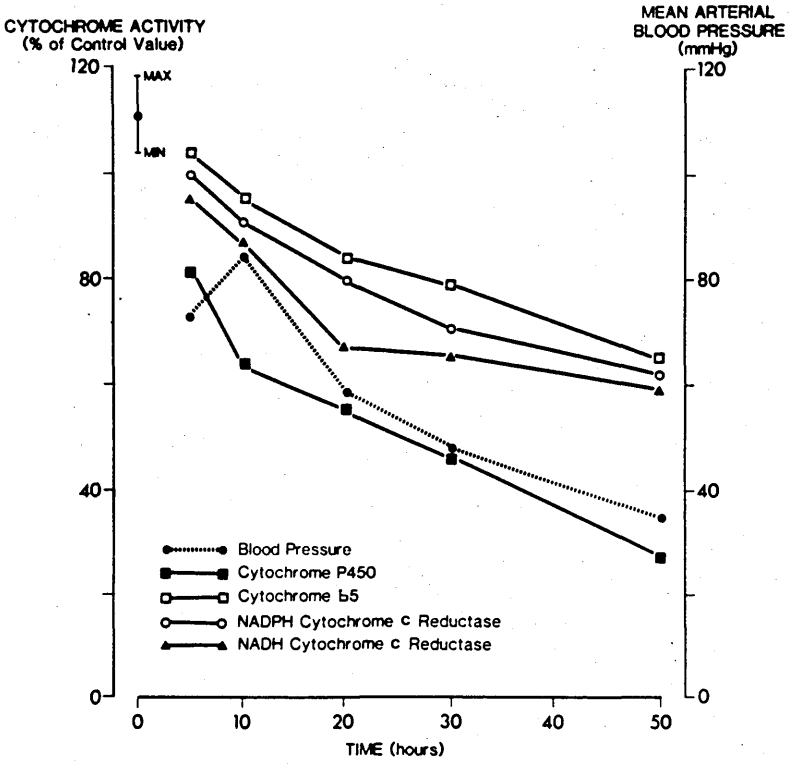
		Sacrifice time (hours) from start				
		5	10	20	30	50
	n	4	4	4	4	4
Cytochrome P450	mean	0.95	0.98	0.92	0.95	0.96
(n mol mg protein^{-1})	S.D.	0.061	0.064	0.054	0.026	0.066
Cytochrome b5	mean	0.42	0.39	0.42	0.44	0.46
(n mol mg protein^{-1})	S.D.	0.026	0.033	0.033	0.041	0.046
NADPH cytochrome c reductase	mean	0.137	0.125	0.125	0.13	0.115
(μmol min^{-1} mg protein^{-1})	S.D.	0.018	0.021	0.015	0.26	0.02
NADH cytochrome c reductase	mean	0.098	0.115	0.105	0.108	0.10
(μmol min^{-1} mg protein^{-1})	S.D.	0.021	0.014	0.019	0.011	0.015
Mean arterial blood pressure	mean	110	108	115	106	110
(mm Hg)	S.D.	8.46	3.68	6.73	7.76	9.12

Table 16

Rat cytochrome activity and blood pressure following endotoxin (5×10^{1} mg kg^{-1}) administration.

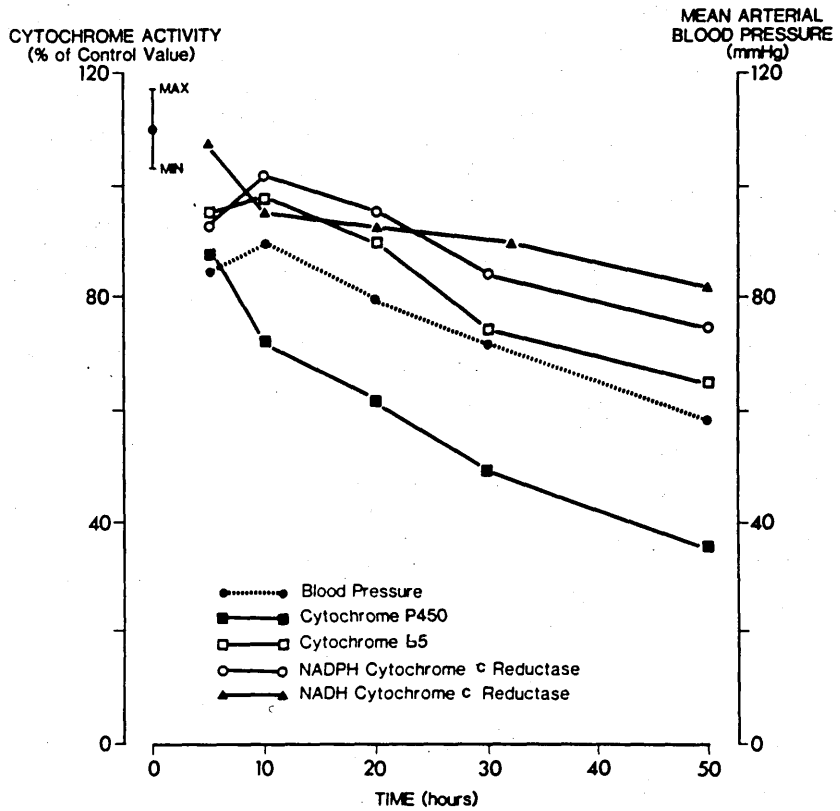
Cytochrome P450 % of control	56
Cytochrome b5 % of control	81
NADPH cytochrome c reductase % of control	85
NADH cytochrome c reductase % of control	31
Blood pressure % of control	62

Figure 27



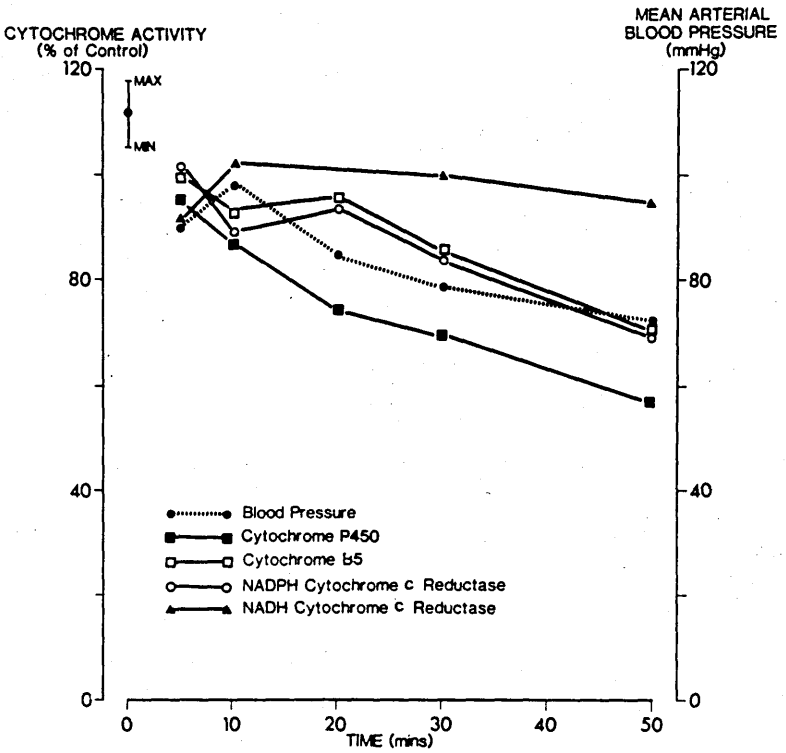
THE EFFECT OF ENDOTOXIN, $5 mgkg^{-1}$ ADMINISTERED INTRAVENOUSLY ON BLOOD PRESSURE AND HEPATIC CYTOCHROME ACTIVITY AT SELECTED TIME INTERVALS. EACH TIME REPRESENTS A SEPARATE PAIR OF ANIMALS.

Figure 28



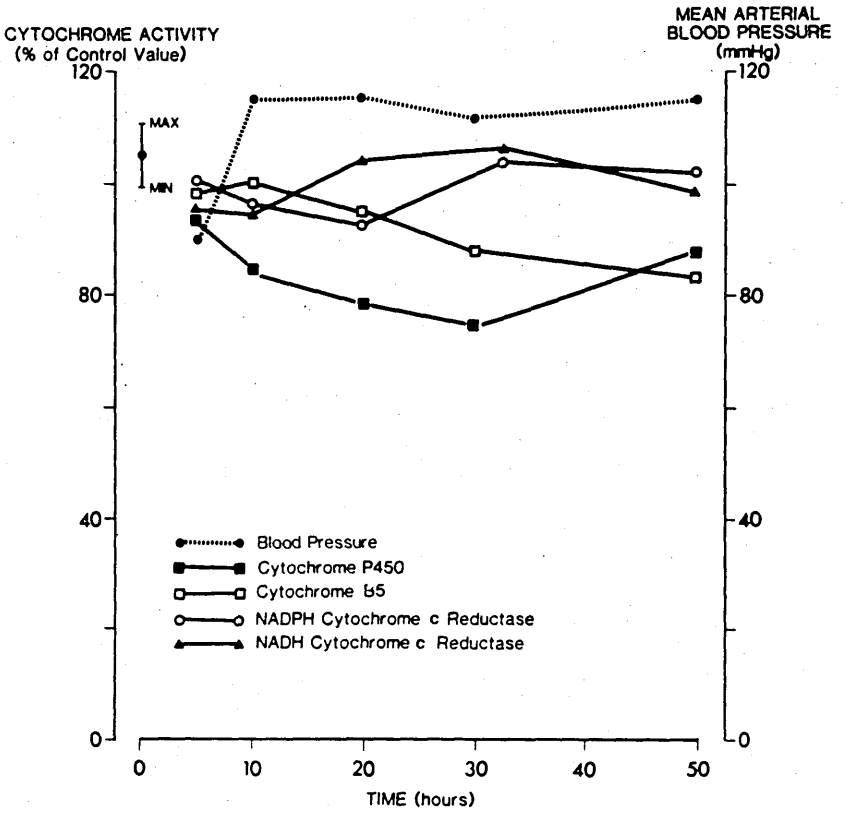
THE EFFECTS OF ENDOTOXIN 5×10^{-1} mg kg^{-1} ADMINISTERED INTRAVENOUSLY ON BLOOD PRESSURE AND HEPATIC CYTOCHROME ACTIVITY, AT SELECTED TIME INTERVALS. EACH TIME REPRESENTS A SEPARATE PAIR OF ANIMALS.

Figure 29



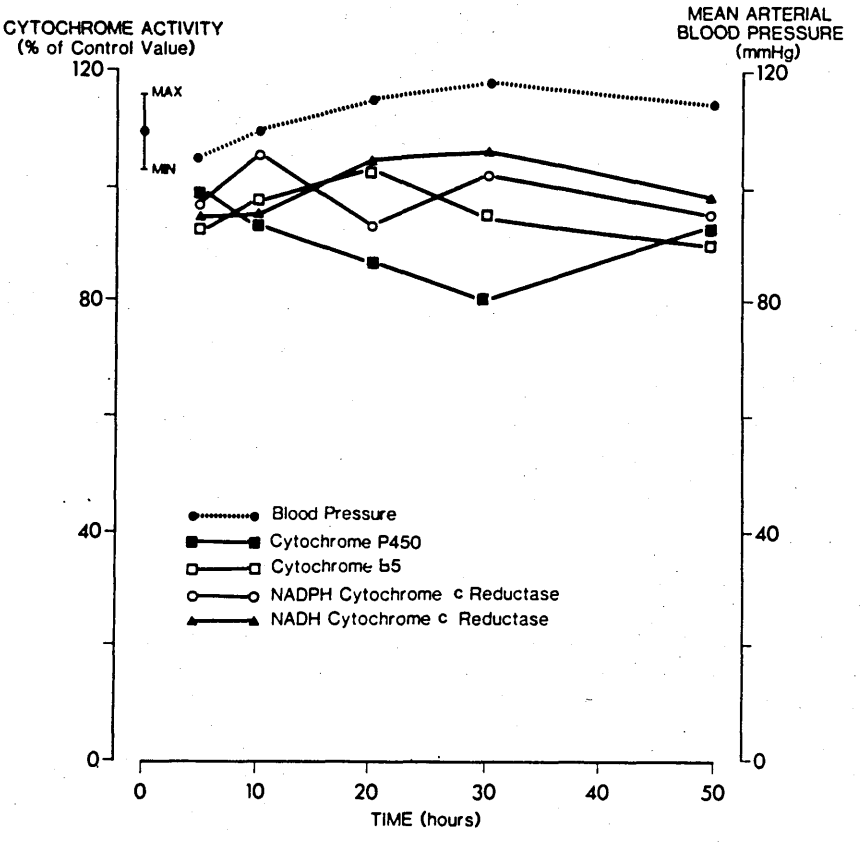
THE EFFECTS OF ENDOTOXIN, 5×10^{-2} mg kg^{-1} ADMINISTERED INTRAVENOUSLY, ON BLOOD PRESSURE AND HEPATIC CYTOCHROME ACTIVITY, AT SELECTED TIME INTERVALS. EACH TIME REPRESENTS A SEPARATE PAIR OF ANIMALS.

Figure 30



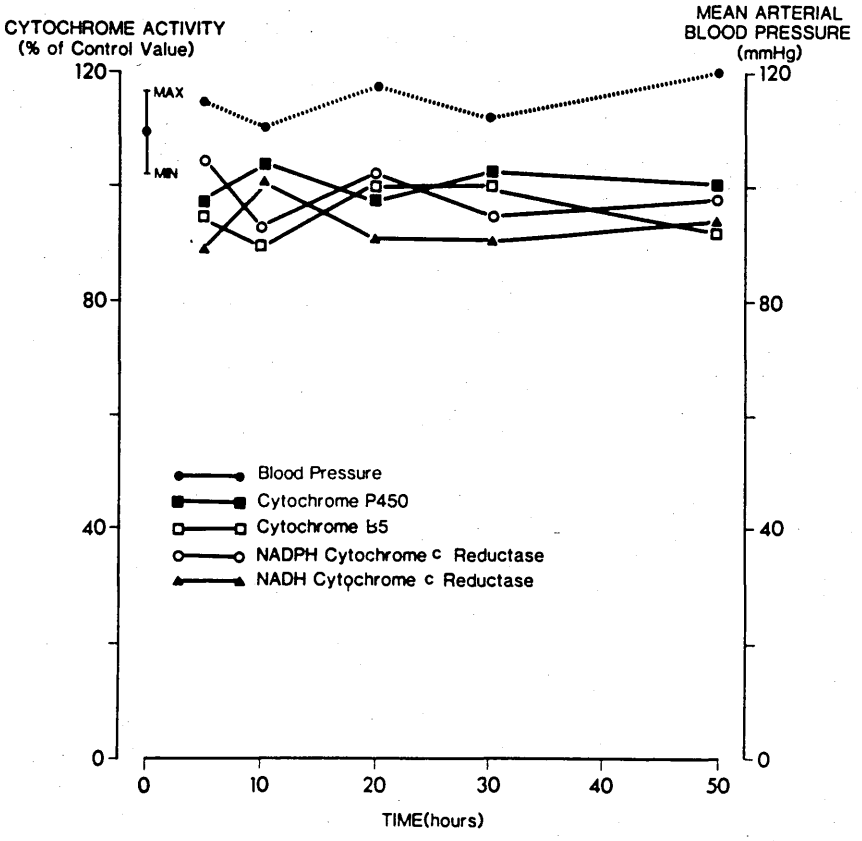
THE EFFECTS OF ENDOTOXIN, 5×10^{-3} mg kg^{-1} ADMINISTERED INTRAVENOUSLY, ON BLOOD PRESSURE AND HEPATIC CYTOCHROME ACTIVITY AT SELECTED TIME INTERVALS. EACH TIME REPRESENTS A SEPARATE PAIR OF ANIMALS.

Figure 31



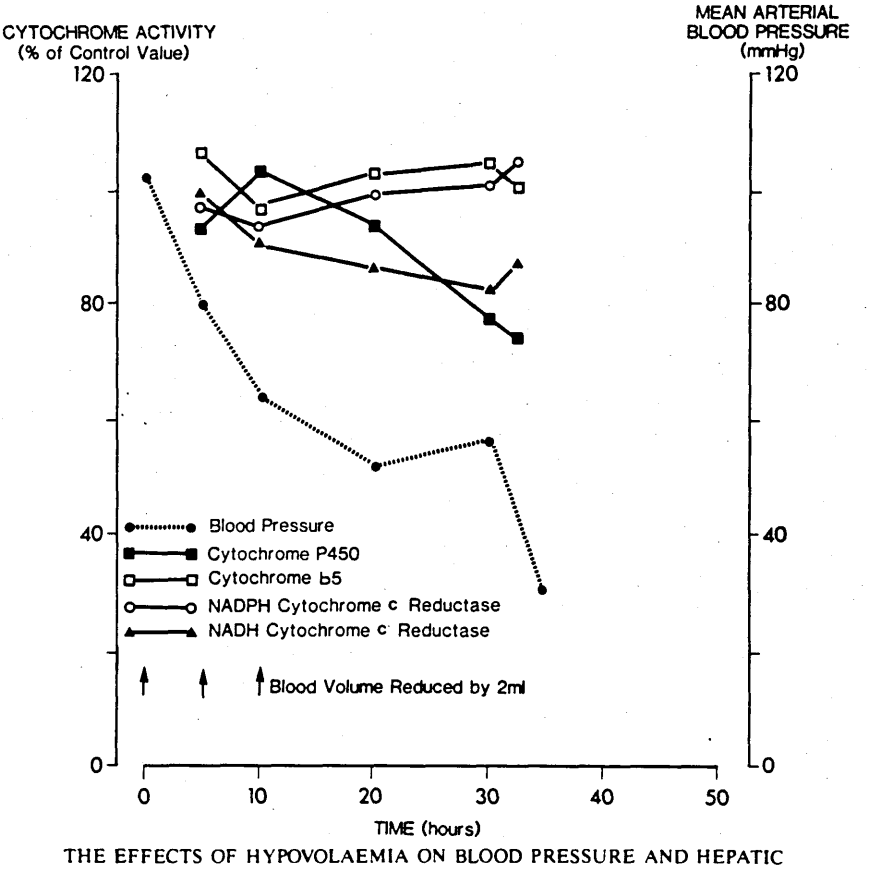
THE EFFECTS OF ENDOTOXIN, 5 x 10^{-4} mg kg^{-1} ADMINISTERED INTRAVENOUSLY, ON BLOOD PRESSURE AND HEPATIC CYTOCHROME ACTIVITY AT SELECTED TIME INTERVALS. EACH TIME REPRESENTS A SEPARATE PAIR OF ANIMALS.

Figure 32



THE EFFECTS OF ENDOTOXIN 5×10^{-5}mg kg^{-1}ADMINISTERED INTRAVENOUSLY ON BLOOD PRESSURE AND HEPATIC CYTOCHROME ACTIVITY AT SELECTED INTERVALS EACH TIME REPRESENTS A SEPARATE PAIR OF ANIMALS.

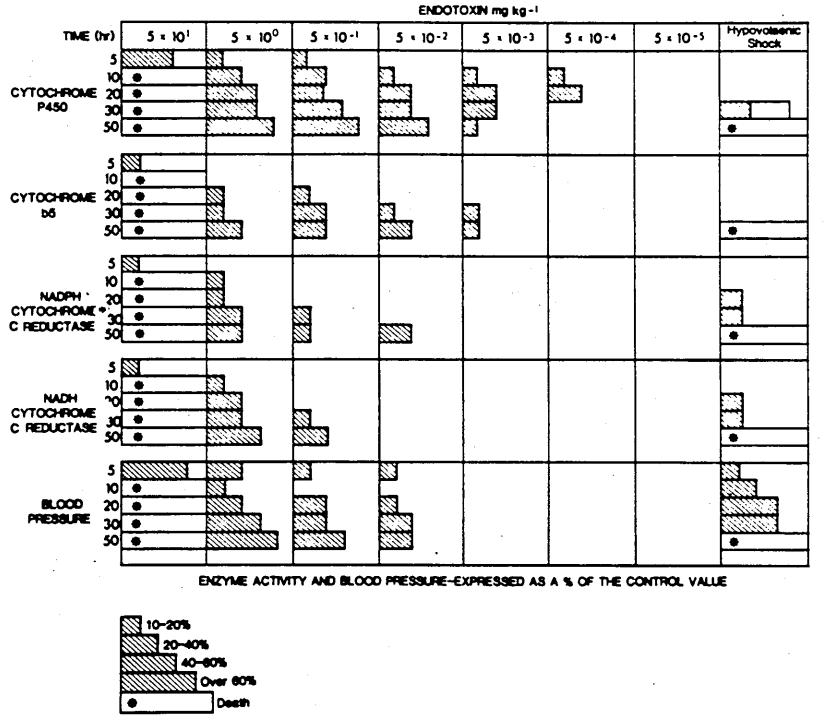
Figure 33



THE EFFECTS OF HYPOVOLAEMIA ON BLOOD PRESSURE AND HEPATIC CYTOCHROME ACTIVITY, AT SELECTED TIME INTERVALS. EACH TIME REPRESENTS A SEPARATE ANIMAL.

Figure 34

SUMMARY OF THE EFFECTS OF A VARIETY OF DOSES OF ENDOTOXIN ON BLOOD PRESSURE AND HEPATIC MICROSOMAL CYTOCHROME FUNCTION IN RAT.



Experimentally Induced Septic Shock in Pigs

Quite apart from the questions of antipyrine clearance and cytochrome P450 activity described in the preceding section, the clinical studies raised a number of issues which seemed to merit investigation in experimental animals. One of the most puzzling of these was that in some patients indocyanine green clearance was found to be very low, yet at autopsy or at laparotomy there was no evidence of ischaemia of the gut such as would be expected in the presence of a greatly reduced hepatic blood flow. This might point to a failure on the part of the liver to clear indocyanine green at its normal high rate of extraction. (In normal subjects 90% of the indocyanine green present in the blood is removed in a single passage through the liver). Such a hypothesis would be consistent with the evidence given in the preceding section for the effect of endotoxin in inhibiting some aspects of liver metabolism. A second question arose in relation to the low levels of fibronectin found in septic shock; were these evidence of impaired synthesis, on the one hand, or more rapid removal, on the other? To answer these and related questions, a study was undertaken of experimentally induced septic shock in the pig. The pig was chosen because a large animal model was required to allow a full range of instrumentation, and because, unlike the dog, it has a similar splanchnic and portal circulation to man. Before such a project could be undertaken, two preliminary problems had to be tackled: the fibronectin assay had to be modified for use with porcine plasma; and a muscle relaxant had to be selected which would not give rise to artefacts.

Development of the immunoturbidimetric assay for porcine fibronectin

Various dilutions of human fibronectin antiserum in buffer were reacted with various volumes of standard concentrations of purified porcine fibronectin. For each reaction the optical density was read at intervals to 60 minutes (for full details of the dilutions, standard concentrations, volumes and reaction times see Page 62). A selection of the results obtained are shown in Figures 35, 36 and 37 to illustrate the trends observed. Figure 35, in which the change in optical density against time is plotted at three different antiserum dilutions, shows that the change in optical density is similar with both the 1 : 5 and 1 : 10 dilutions. A 1 : 20 dilution however, produces a much smaller change in optical density. A 1 : 10 dilution was therefore chosen for use in the assay. Likewise, in Figure 36, in which the change in optical density with time is plotted for varying plasma volumes, the smallest volume of plasma which could be used without a marked reduction in the change in optical density observed was 50 μl. This was therefore used in subsequent assays. Figure 37, shows that the reaction is virtually complete by 30 minutes and no benefit is obtained by further prolongation of the reaction time.

The selection of a muscle relaxant

In attempting to obtain a stable haemodynamic profile in the control group, a number of different muscle relaxants were tested. This proved necessary since pancuronium, the one originally chosen, produced a marked increase in both heart rate and blood pressure. These effects of pancuronium and the other relaxants tested are

summarised in Table 17. Vecuronium was the only relaxant which had no effect on any haemodynamic measurements, making it the muscle relaxant of choice.

Porcine septic shock model

The procedure followed is described in detail in Page **55**. After insertion of the necessary cannulae, the animals were left for one hour to stabilise, and then observed for a further period of ten hours (or until death), anaesthesia being maintained throughout. They were divided into three groups:-

> the **control group** received only an infusion of 0.9% saline;
>
> the **E. coli group** received an infusion of Escherichia coli; and
>
> the **E. coli colloid group** received an infusion of Escherichia coli together with sufficient colloid to maintain pulmonary artery wedge pressure.

The survival of the three groups is shown in Tables 18 and 19. The **control group** were all alive at the end of the experiments. Those in the **E. coli** and **E. coli colloid groups** had all succumbed, but the latter had survived significantly longer than the former. Figure 38 shows the arterial PO_2. So far as possible, this was maintained at or above physiological levels by adjusting the concentration of the inspired oxygen, but this proved impossible in the case of the longest-surviving animals in the **E. coli group.** Figure 39 shows the core temperature for the three groups: there were no significant differences between them. Figure 40 shows the heart rate in the three groups. In the **control group** it was, as one might hope and

expect, reasonably constant, whereas in the **E. coli group** it increased steadily until death supervened. The **E. coli colloid group** also showed a rise, but after four hours this flattened out and in the longest-surviving animals there was actually a tendency for the rate to fall back towards normal. Figure 41 shows the mean arterial pressure in the three groups. Again, in the **control group** it was almost constant throughout the experiment whereas in the **E. coli group** there was an immediate and sustained fall. In the **E. coli colloid group** values fluctuated, but only at the end of the experiment did they fall significantly below the **control group.** Figure 42 shows the cardiac output in the three groups. Yet again the control values were quite constant. The **E. coli group** did not differ significantly from the **control group** for the first three hours, but thereafter output fell precipitately. In the **E. coli colloid group** there was an initial rise above the control levels, which was sustained for about five hours. Thereafter the values observed were close to the **control group.** Figure 43 shows the mean pulmonary artery pressure. Again in the **control group** this was reasonably constant, although there was perhaps evidence of a slight increase with time. The **E. coli group,** in contrast, showed a jump within an hour to a value more than twice the normal, and this high level was sustained until death. The **E. coli colloid group** behave almost identically. In contrast, pulmonary artery wedge pressure (i.e. left artrial pressure), shown in Figure 44, fell sharply in **E. coli group** animals while in the **control group** it remained steady and in the **E. coli colloid group** there was some evidence of an increase towards the end of the experiment.

These haemodynamic observations lead to several conclusions:-

1. The remarkably constant **control group** levels in each of the figures give grounds for confidence that the **control group** are being successfully maintained in a stable condition and therefore afford a secure basis to which the other groups can be related.
2. The **E. coli group** do indeed manifest the classical features of shock: tachycardia; falling arterial pressure; and falling cardiac output. All these changes are to some degree prevented or reversed by the administration of colloid. Presumably therefore they are all, in the main, consequences of hypovolaemia.
3. One of the most striking changes in the shocked animals was the sudden and sustained increase in pulmonary artery pressure. This was not affected by colloid administration, and presumably indicates an increase in the resistance to blood flow offered by the lungs.

Biochemical Estimations

Figure 45 shows the changes in fibronectin level. The **control group** values fell very slightly, whereas both the **E. coli** and **E. coli colloid groups** showed a precipitous fall. Here, as in the case of pulmonary artery pressure, colloid infusion did not diminish or reverse the effect of shock. The blood lactate levels are shown in Figure 46; the **control group** started at a slightly high level (2 mM) but fell steadily throughout the experiment. The level in the **E. coli group** remained steady for the first four hours and then rose rapidly. The **E. coli colloid group** also showed an increase from four hours onward, but to a lesser degree.

Indocyanine green clearance was measured in all groups at the beginning of the experiment and either at ten hours (in the **control group**) or before death (in the case of the **E. coli** and **E. coli colloid groups**). The results are shown in Table 20. In the **control group** there was no change in clearance; however, in both the **E. coli** and **E. coli colloid groups** clearance fell by about 40%. If clearance can be taken as an index of hepatic blood flow, this is a remarkable result since, in the course of the experiment, cardiac output fell by 50% in the **E. coli group** but was sustained in the **E. coli colloid group.**

It was important to know whether this was primarily due to a reduction in liver blood flow or a reduction in hepatic extraction. Table 21 shows the mean values for indocyanine green extraction, again at the initial and terminal phases, for the **control** and **E. coli colloid groups.** The initial extraction ratios obtained were much lower than those reported for the healthy situation in human subjects. There was no significant change in indocyanine green extraction in the **control group** between the initial and terminal phases; however, there was a significant fall in the **E. coli colloid group.** Taking the indocyanine green clearance and extraction results together, it would appear that the fall in indocyanine green clearance was primarily due to a reduction in extraction rather than a change in hepatic blood flow. From these results, therefore, one can be reasonably certain that the extraction ratio changes as a result of sepsis. Whether a change in hepatic blood flow is a contributory factor remains obscure.

Table 17

The effect of muscle relaxants on the cardiovascular system of the pig.

	Heart Rate	Blood Pressure	Cardiac Output
Pancuronium	↑↑↑	↑↑	↑
Suxamethonium	↑↑	↑	↑
Atracurium	-	↓	↓
Vecuronium	-	-	-
Curare	-	↓	-

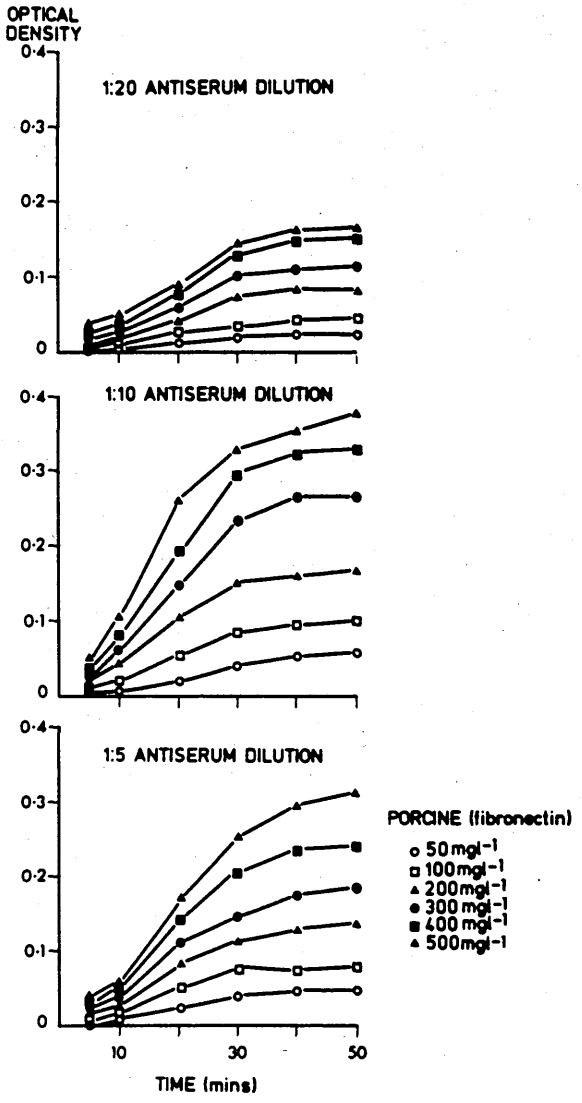
- = 20% change from baseline values

↑ ↓ = 20 - 50% change from baseline values

↑↑ ↓↓ = 50 - 75% change from baseline values

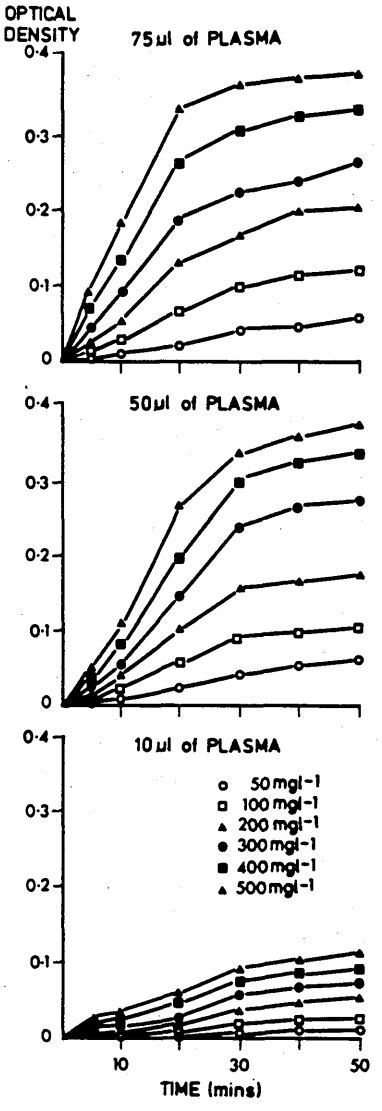
↑↑↑ ↓↓↓ = 75% change from baseline values

Figure 35



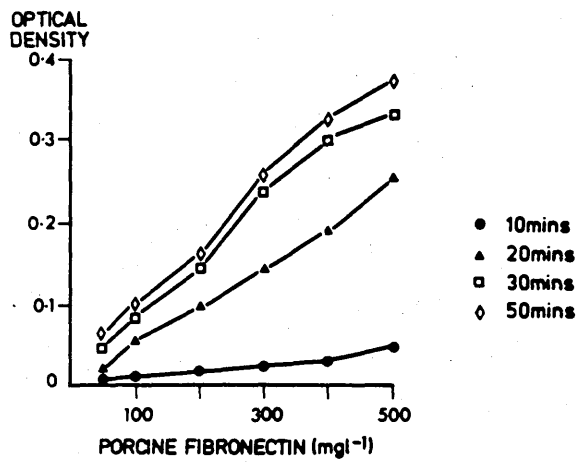
The effects of different human antiserum dilutions on the observed change in optical density at 365nm in the immunoturbidimetric assay for fibronectin. The reaction was carried out using 50µl of Porcine standard in a total volume of 1ml at 25°C

Figure 36



The effects of different volumes of Porcine standard on the observed change in optical density at 365nm in the immunoturbidimetric assay for fibronectin. The reaction was carried out using 1:10 dilution of human fibronectin antiserum in a total volume of 1ml at 25°C

Figure 37



The effects of different reaction times on the change in optical density at 365nm in the immunoturbidimetric assay for fibronectin. The reaction was carried out using a 1:10 dilution of human antiserum, 50µl of Porcine standard in a total volume of 1ml at 25°C

Table 18

Mean Survival Time for Pig Experiments.

	Time (hours)		
	n	mean	S.D.
Control group	10	10	-
E. coli group	6	4.48	1.2
E. coli colloid group	10	7.42	1.25

Control group v. E. coli group	$P > 0.0001^*$
Control group v. E. coli colloid group	$P > 0.0001^*$
E. coli group v. E. coli colloid group	$P > 0.0001^*$

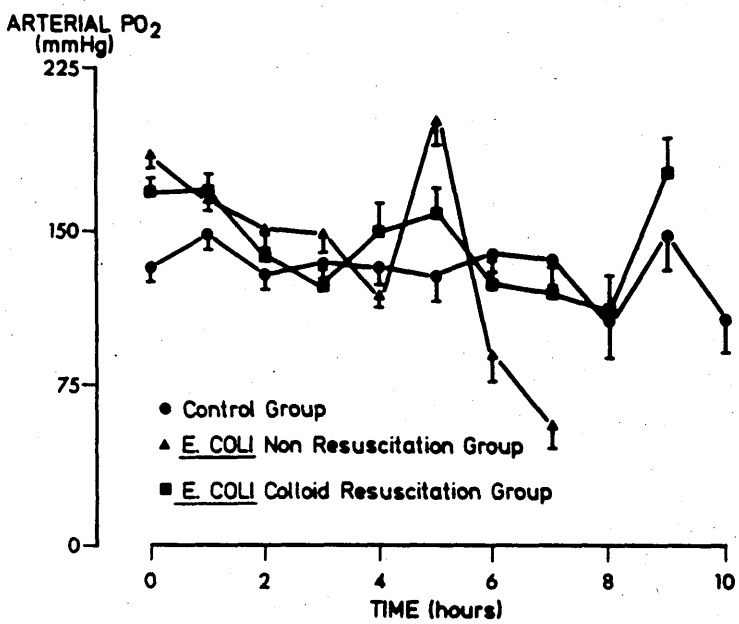
* Student's t test (unpaired)

Table 19

Individual Survival Times for Pig Experiments.

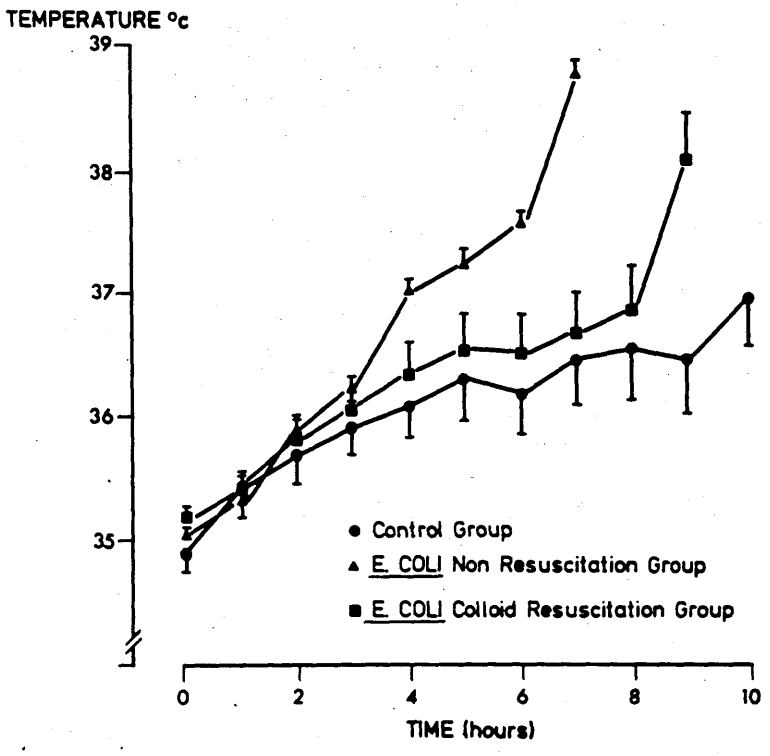
Time (hours)	Control Group	*E. coli* Group	*E. coli* colloid Group
1	10	6	10
2	10	6	10
3	10	6	10
4	10	5	9
5	10	4	9
6	10	3	7
7	10	1	6
8	10	0	4
9	10	0	1
10	10	0	0

Figure 38



Graph of Porcine arterial Po_2. The inspired oxygen concentration was regulated to maintain an optimal Pa o_2.

Figure 39



The effect of E. COLI infusion (2×10^8 organisms kg^{-1}) with and without colloid fluid resuscitation on Porcine core temperature (mean ± S.E.)

Figure 40

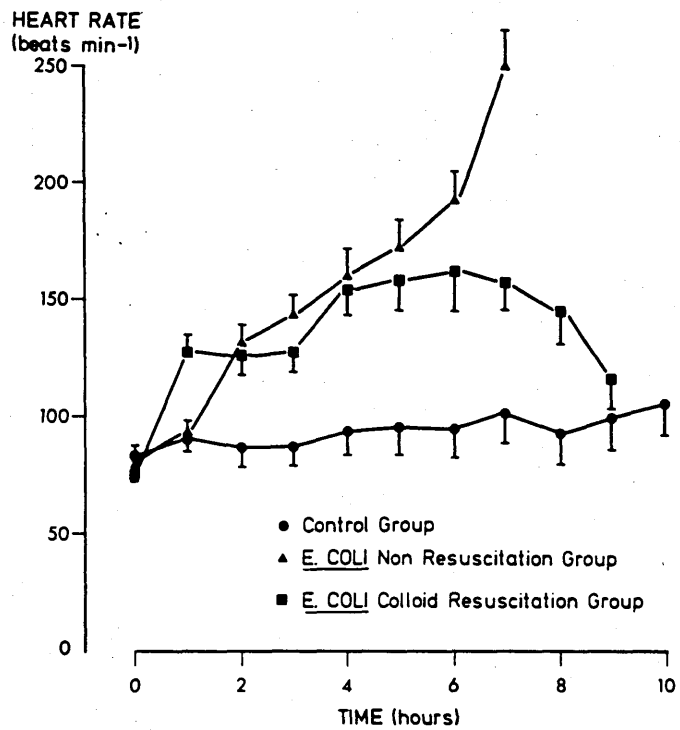
TABLE OF P VALUES

TIME (hours)

	0	1	2	3	4	5	6	7	8
● / ▲	NS	NS	NS	†	†	‡	†		
● / ■	NS	†	†	†	‡	†	†	†	†
▲ / ■	NS	NS	NS	NS	NS	NS	NS		

KEY TO P VALUES

P > 0.05 NS
0.05 ≥ P > 0.01 †
0.01 ≥ P > 0.001 ‡
0.001 ≥ P > 0.0001 ‡
P < 0.0001 ‡



The effects of E. COLI infusion (2×10^8 organisms kg^{-1}) with and without colloid fluid resuscitation on Porcine heart rate (mean ± S.E.)

Figure 41

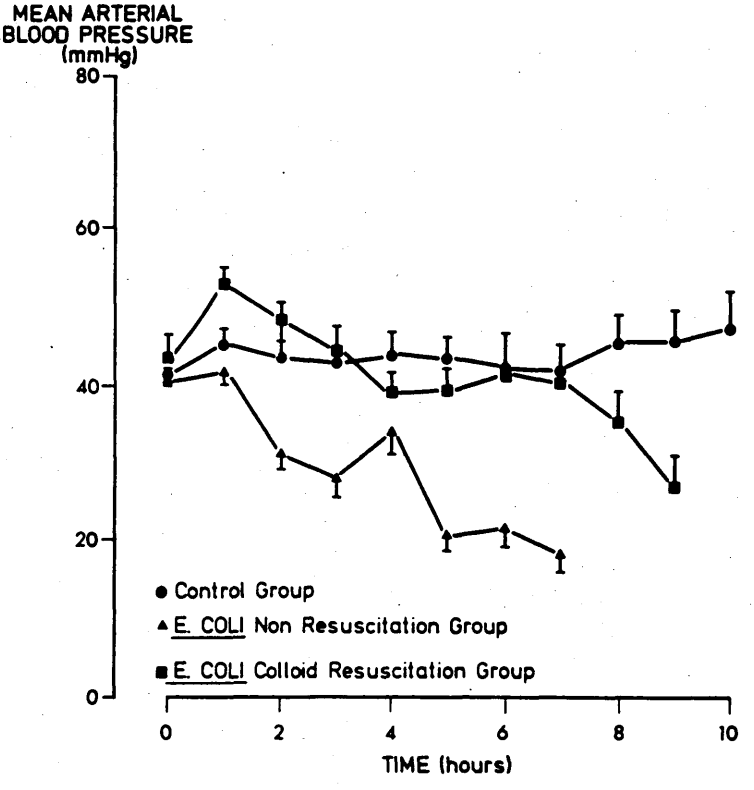
TABLE OF P VALUES

TIME (hours)

	0	1	2	3	4	5	6	7	8
● / ▲	NS	NS	†	‡	‡	‡	‡		
● / ■	NS	NS	NS	NS	NS	NS	NS	NS	†
▲ / ■	NS	NS	‡	‡	NS	NS	†	†	

KEY TO P VALUES

P>0.05 NS
0.05≥P>0.01 †
0.01≥P>0.001 ‡
0.001≥P>0.0001 ‡
P<0.0001 ‡



The effects of E.COLI infusion (2×10^8 organisms kg^{-1}) with and without colloid fluid resuscitation on Porcine mean arterial blood pressure (mean ± S.E.)

Figure 42

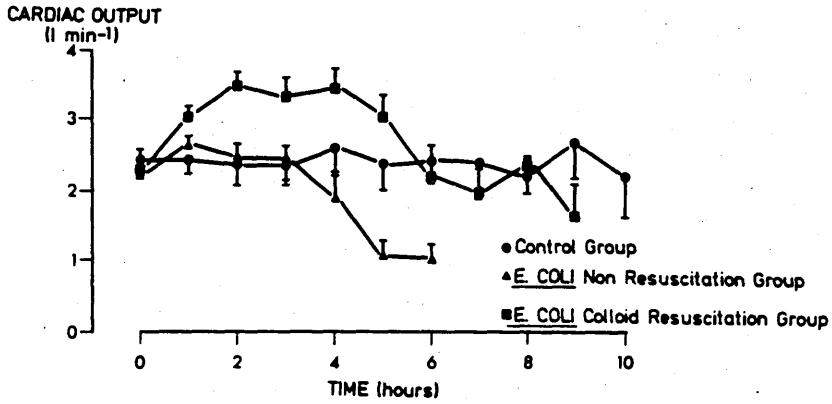
TABLE OF P VALUES

TIME (hours)

	0	1	2	3	4	5	6	7	8
●/▲	NS	NS	NS	NS	NS	NS	NS		
●/■	NS	†	†	†	NS	NS	NS	NS	NS
▲/■	NS	NS	†	†	†	†	†		

KEY TO P VALUES

P>0.05 NS
0.05≥P>0.01 †
0.01≥P>0.001 ‡
0.001≥P>0.0001 §
P<0.0001 §



The effects of E. COLI infusion ($2x10^8$ organisms kg^{-1}) with and without colloid fluid resuscitation on Porcine cardiac output (mean ± S.E.)

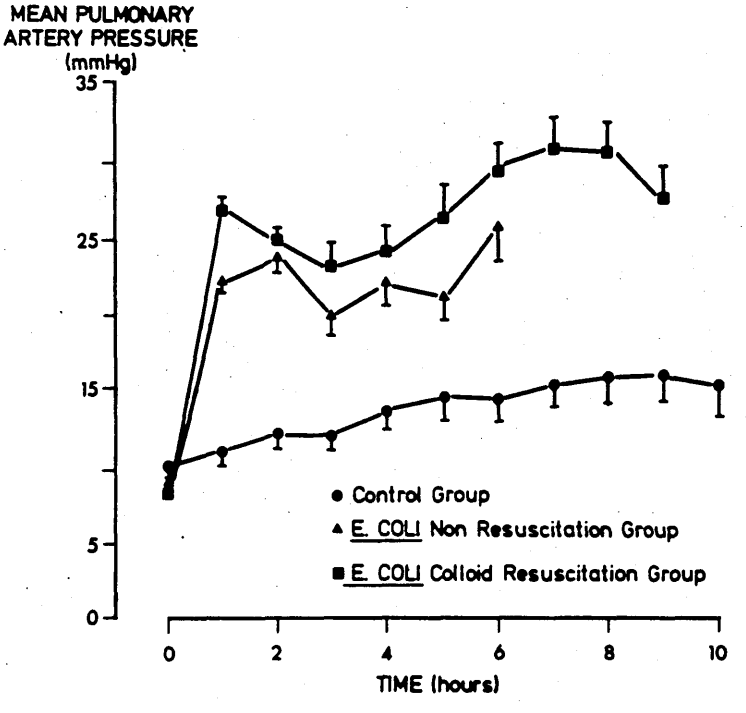
Figure 43

TABLE OF P VALUES
TIME (hours)

	0	1	2	3	4	5	6	7	8
●/▲	NS	‡	‡	‡	‡	‡			
●/■	NS	‡	‡	‡	‡	‡	‡	‡	‡
▲/■	NS	NS	NS	NS					

KEY TO P VALUES

P>0.05 NS
0.05≥P>0.01 †
0.01≥P>0.001 ‡
0.001≥ P>0.0001 ‡
P<0.0001 ‡



The effects of E. COLI infusion ($2x10^8$ organisms kg^{-1}) with and without colloid fluid resuscitation on Porcine mean pulmonary artery pressure (mean ± S.E.)

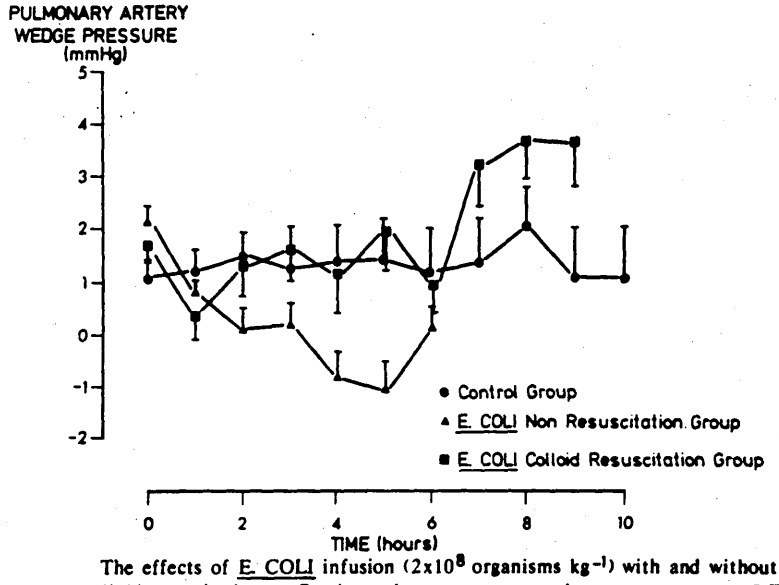
Figure 44

TABLE OF P VALUES
TIME (hours)

	0	1	2	3	4	5	6	7	8
●/▲	NS	NS	NS	NS	NS	†			
●/■	NS	NS	NS	NS	NS	NS	NS	NS	NS
▲/■	NS	NS	NS	NS	NS	†			

KEY TO P VALUES

P>0.05 NS
0.05≥P>0.01 †
0.01≥P>0.001 ‡
0.001≥P>0.0001 §
P<0.0001 §



The effects of E. COLI infusion (2×10^8 organisms kg^{-1}) with and without colloid fluid resuscitation, on Porcine pulmonary artery wedge pressure (mean ± S.E.)

Figure 45

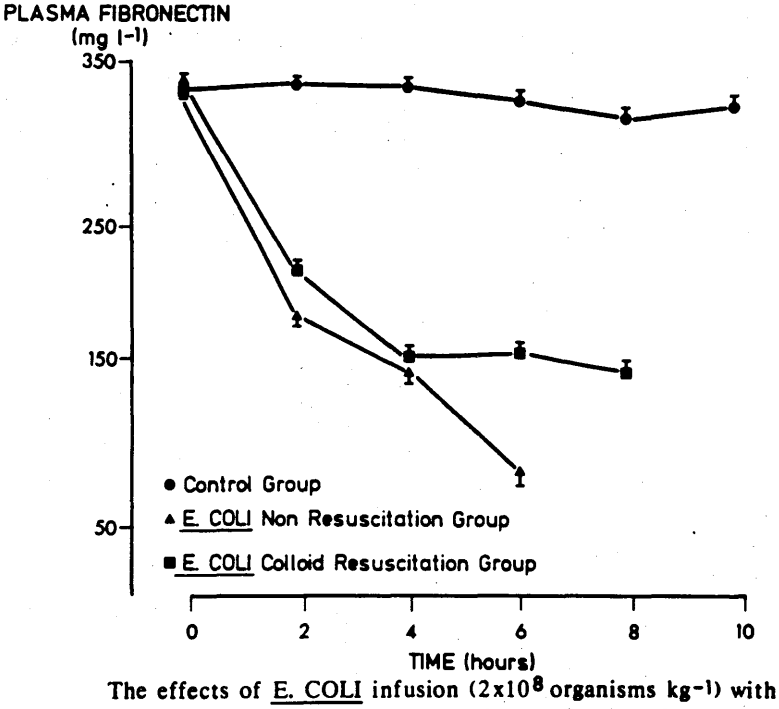
TABLE OF P VALUES

TIME (hours)

	0	2	4	6	8
● / ▲	NS	‡	‡		
● / ■	NS	‡	‡	‡	‡
▲ / ■	NS	NS	NS		

KEY TO P VALUES

P>0.05 NS
0.05≥P>0.01 †
0.01≥P>0.001 ‡
0.001≥P>0.0001 ‡
P<0.0001 ‡



The effects of E. COLI infusion (2×10^8 organisms kg^{-1}) with and without colloid fluid resuscitation on Porcine plasma fibronectin (mean ± S.E.).

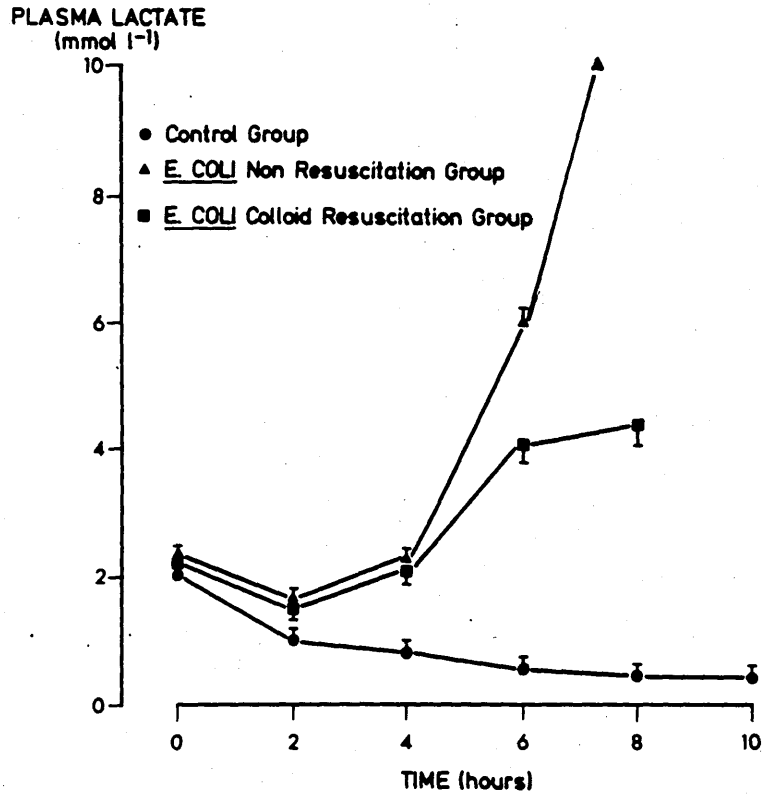
Figure 46

TABLE OF P VALUES

	TIME (hours) 0	2	4	6	8
●/▲	NS	+	+	‡	
●/■	NS	+	‡	‡	‡
▲/■	NS	NS	NS	NS	

KEY TO P VALUES

P>0.05 NS
0.05≥P>0.01 †
0.01≥P>0.001 ‡
0.001≥P>0.0001 ‡
P<0.0001 ‡



The effects of E. COLI infusion ($2x10^8$ organisms kg^{-1}) with and without colloid fluid resuscitation on Porcine plasma lactate (mean ± S.E.)

Table 20

Indocyanine green clearance following E.coli administration.

The effect of E. coli infusion (2×10^8 organisms kg^{-1}) with or without colloid resuscitation on porcine hepatic blood flow as measured by indocyanine green clearance (ICGC)

	Initial ICGC (ml min^{-1})		Terminal ICGC* (ml min^{-1})	
	n	mean ± S.D.	n	mean ± S.D.
Control Group ●	5	379 ± 40.5	5	380 ± 24.5
E. coli Group ▲	5	329 ± 31.3	4	222 ± 20.5
E. coli colloid group ■	5	346 ± 34	5	200 ± 25

Table of P Values#

● v. ●*	Not significant
▲ v. ▲*	= 0.001
■ v. ■*	= 0.001
● v. ▲	Not significant
● v. ■	Not significant
■ v. ▲	Not significant
●* v. ▲*	= 0.001
●* v. ■*	= 0.001
▲* v. ■*	Not significant

* Terminal measurement corresponds to 10 hours in the control group and final reading on both other groups.

\# Student's t test (unpaired)

Table 21

Indocyanine green extraction following *E. coli* administration.

The effects of *E. coli* infusion (2×10^8 organisms kg^{-1}) with colloid resuscitation on indocyanine green extraction (ICGE) expressed as a ratio in the pig.

	Initial ICGE		Terminal ICGE*	
	n	mean ± S.D.	n	mean ± S.D.
Control group ●	5	0.38 ± 0.027	5	0.38 ± 0.29
E. coli colloid group ■	5	0.38 ± 0.031	5	0.148 ± 0.024

Table of P Values#

● v. ●*	not significant
● v. ■	not significant
■ v. ■*	>0.0001
●* v. ●*	>0.0001

* Terminal measurement corresponds to 10 hours in the control group and final reading in the other group.

\# Student's t test (unpaired)

DISCUSSION

The Assay of Plasma Fibronectin

There are several assay techniques available for fibronectin: radial immunodiffusion, radioimmunoassay, electroimmunoassay and laser nephelometry, as well as immunoturbidimetry. Of these, the most popular have been electroimmunoassay as described by Laurell (1966) and nephelometry as described by Gressner and Wallraff (1980). Both are sensitive and reproducible; their major limitation is the typical 24 to 48 hour time delay before results are obtained. The immunoturbidimetric technique, on the other hand, can be performed in 10 to 15 minutes. It was chosen in the present study because of the need for a rapid bedside assay in septic shock, where the physician needs information about the patient's condition immediately. It has been used by other authors and been found to compare favourably with both the Laurell technique (Saba 1981) and nephelometry (Gressner and Wallraff 1981). The main problem in its use, in spite of its popularity, has been a lack of information on its accuracy and precision.

The present study has shown that the commercial immunoturbidimetric kit (Boehringer) performs well enough to be considered a useful clinical test. However, the standard assay, as recommended by the manufacturers, is not suitable for accurate measurement of concentrations below the normal range. The modifications made to the assay at the start of the present study have remedied this defect. Consequently it is now possible to assay

fibronectin accurately and precisely even when, as in septic shock, its concentration is substantially below the levels found in healthy subjects.

Human fibronectin studies

The data from the studies of the effect of age and sex on plasma fibronectin levels have been discussed on Page**77/8.** In the study of patients undergoing laparotomy (Figure 16) the finding of a transient fall in fibronectin levels following surgery is in agreement with published data from other authors (Chadwick et al 1984 and Gauperaa et al 1985). In Gauperaa's study the pre-operative fibronectin levels were lower than those quoted by other authors and the fall after surgery more marked, perhaps because he used serum rather than plasma samples. This is likely to lead to lower fibronectin levels, because of fibronectin's high affinity for fibrin. Binding to fibrin, and involvement in coagulation following surgery, may partly explain the fall in fibronectin observed after laparotomy, but it may also be attributable in part to sequestration of fibronectin at the site of surgical injury, brought about by its high affinity for collagen (Reese et al 1982).

In the present study, the group of patients who died following laparotomy had significantly lower pre-operative fibronectin levels, and post-operatively these declined steadily (Figure 16). O'Connel et al (1984) found that, in medical Intensive Care Unit, patients initial fibronectin levels were significantly lower in patients who subsequently died than in patients who survived to leave the unit; and Richards et al (1983) reported that patients who developed significant

intra-abdominal infection and multiple organ failure post-operatively had significantly lower fibronectin levels pre-operatively than patients who did not develop complications. In our laparotomy study, however, the difference in fibronectin levels between survivors and non survivors, whilst statistically significant, was insufficient to allow any discrimination on the basis of fibronectin levels alone.

In the present study of patients with sepsis but not in shock it was found that fibronectin levels fell, and remained low, in non-survivors (Figure 18), but returned towards normal in survivors (Figure 19). Most, but not all, other authors agree with this finding; this will be discussed in more detail on Page **170.** Klim et al (1986) have recently examined various coagulation factors in surgical Intensive Care Unit patients, in an attempt to distinguish between septic and non-septic patients. They found that by measuring fibrinogen, partial thromboplastin time, plasminogen and fibronectin, then using rank discriminant analysis it was possible to discriminate between septic and non-septic patients. Fibronectin level was the most important single variable for classification, and fibronectin level alone correctly predicted the outcome in 90% of patients in their study. They concluded that measurement of fibronectin should become routine for patients in a surgical Intensive Care Unit.

Septic Shock Study

The APACHE II and Sepsis Scoring systems were applied to all patients at the time of admission to the septic shock study. The APACHE II score is a powerful method in assessing prognosis and has been widely adopted for use in intensive care (Knaus et al 1981, Bion

et al 1985). The Sepsis Score is more specifically for grading the severity of sepsis and in this study, when combined with the APACHE II score it provided a useful means of assessing the comparability of patient groups. For the control and cryoprecipitate groups the mean APACHE II scores were respectively 19.3 and 21.5 and the mean sepsis scores 21.5 and 22. (Table 7). These scores show that the two groups were well matched and underline the severity of illness in these patients. All other variables examined (age, blood pressure, arterio-aveolar oxygen gradient, plasma fibronectin and indocyanine green clearance) also show that the two groups were well matched.

All patients had low fibronectin levels at the time of admission to the study, and in the control patients consistently low or steadily falling levels were associated with a fatal outcome. In patients receiving cryoprecipitate, plasma fibronectin was elevated by an average of 135 mg l^{-1} per pool of 10 units of cryoprecipitate infused. During their first 24 hours in Intensive Care, patients often had to be given cryoprecipitate two or three times but, as they stabilised, it usually became possible to predict from consecutive plasma fibronectin estimations what their requirement would be. This usually allowed cryoprecipitate to be given only once a day. Cryoprecipitate infusion had a significant effect on a variety of physiological variables: urine output; core temperature; endotoxin and plasma fibronectin (Table 9). However, it was not followed by an increase in cardiac output or pulmonary artery wedge pressure, suggesting that the beneficial effects were non-attributable to volume expansion. An alternative, more attractive, hypothesis would be that the fibronectin, (or some other protein in the cryoprecipitate), is stimulating clearance of endotoxin by the reticuloendothelial system; and that the other

beneficial effects are a consequence of lower plasma endotoxin levels.

The overall mortality rate was lower in cryoprecipitate-treated patients than in control patients (7/12 vs 9/11). Early mortality was lower in the cryoprecipitate-treated group with a statistically significant difference in mortality rates on day 2 and day 3 following admission to the study (Figure 25). There have been relatively few controlled studies of cryoprecipitate therapy in septic shock patients, and, in general, other authors' experiences in this field have been disappointing (Hesselvik et al 1986, Thijs, personal communication). There is, however, a major difference between the procedure followed in our study and that adopted by Hesselvik et al, in that they gave cryoprecipitate to their patients only on day 1 and day 2. As can be seen from the patient profile shown in Figures 20 to 24, plasma fibronectin levels rapidly fall back to a sub-normal range after the infusion of cryoprecipitate, especially when the degree of sepsis is severe and the patients are unstable. Saba et al (1984) observed in their study of cryoprecipitate administration to patients who had suffered injury that the reversal of fibronectin deficiency after infusion of cryoprecipitate was more sustained in non-septic patients than in septic patients. This is in keeping with our own observations, and suggests that fibronectin is more rapidly removed from the blood stream in severe sepsis. From the patient profiles (Figures 20 to 24) it would appear that the rate of this removal decreases as the patient's condition improves. The reasons for this "consumption" will be discussed later on Page **172**.

In summary, the clinical observations in this study have shown:

(1) the immunoturbidimetric technique is an appropriate assay for use in a study of fibronectin replacement;

(2) Plasma fibronectin levels are:-

higher in the elderly than in the population at large

transiently decreased following surgery

decreased by sepsis

(3) Plasma fibronectin levels are very low in septic shock;

(4) In patients in septic shock, cryoprecipitate infusion elevates plasma fibronectin and can be used to maintain a normal plasma fibronectin level; it has beneficial effects on endotoxin levels, urine output and core temperature;

(5) Mortality from septic shock may be reduced by cryoprecipitate therapy.

The Rat Experiments

Patients in septic shock receive an overwhelming variety of drugs, most of which are metabolised in the liver by the cytochromeP450 system (Estabrook et al 1978). It seemed of interest, therefore, to include in the clinical study measurements of the activity of this system. This was done by determining antipyrine clearance. In all the patients this proved to be abnormally slow (average half-life of 28.7 hours whereas the accepted healthy value is 10 hours). It was important to establish if this reflected damage to the cytochrome P450 system of the patient's liver. There are plausible alternative explanations. Conceivably this system was so busy metabolising the other drugs given to the patient that it had no time to deal with the antipyrine. It could be that sepsis impaired antipyrine uptake rather than antipyrine metabolism. The rat experiments showed that, provided sufficient doses of endotoxin were given, the cytochrome P 450 system was indeed damaged; that this damage was greatest in the case of cytochrome P450 itself; and that no comparable effect was produced by haemorrhagic shock. Moreover the extent of the impairment (about 70%) would be sufficient to explain the magnitude of the increase in antipyrine half-life seen in septic shock patients (Page**100**).

As pointed out by Werringloer (1982), even small changes in cytochrome b5 or in the cytochrome c reductases could have a major effect, since they may be the rate limiting enzymes for mono-oxygenase reactions. Endotoxin is know to bind strongly to any cell membrane through its lipid A. Once within the cell, its sites of

attachment and attack are less well known. Schumer et al (1970) and Silver (1981) have shown that the membrane of endoplasmic reticulum was disrupted in rat liver after exposure to endotoxin. This may explain the finding in the present study that cytochrome P450 activity was more affected than the activity of the other enzymes, since it is highly hydrophobic and is an integral protein within the membrane of endoplasmic reticulum (Ito et al 1968). Cytochrome b5, NADPH cytochrome c reductase and NADH cytochrome c reductase, on the other hand, have large hydrophilic components, are less membrane-bound, and might therefore be expected to be less affected by endotoxin.

Other authors have looked at the effect of endotoxin on microsomal cytochrome activity, but the effects of a wide range of doses of endotoxin have not been studied nor have the haemodynamic and metabolic effects of endotoxin been separated. Falzon et al (1984) reported that, following intraperitoneal endotoxin, cytochrome P450 activity fell to 60% of the control level, cytochrome b5 activity fell to 80% of the control level, NADH cytochrome c reductase also fell to 80%, while NADPH c reductase fell to 60%. Interestingly, when the dose of endotoxin administered was increaed from 3.5 mg kg^{-1} to 6 mg kg^{-1}, the inhibition of cytochrome activity was less marked, in contrast to the results in this study where a clear dose response was discernible over a wide range of doses of endotoxin. Bissell and Hammaker (1976) reported that, after the intraperitoneal injection of 1.5 mg kg^{-1} of salmonella endotoxin, cytochrome P450 activity was decreased. There was little effect at five hours, but by 22 hours cytochrome P450 activity was reduced by half. The marked inhibition of the cytochrome P450 system after endotoxin

administration obviously has important implications in the clinical field, since many of the drugs administered to patients in septic shock are metabolised by it. The fact that inhibition is produced by a dose of endotoxin 100 times less than that needed to bring about a fall in blood pressure is also important. It implies, if it can be extrapolated to the clinical situation, that by the time septic shock is signalised by hypotension, the patient will already have suffered serious impairments of at least one function of the liver.

The Pig Experiments

Many animal models of septic shock have been described in the literature, and a variety of species have been used. They have been subjected to infusion of endotoxin or live Escherichia coli or to such procedures as caecal ligation. They have followed a variey of time courses, and they have, not surprisingly, led to a variety of haemodynamic results.

In this study the aim was to examine the effect of septic shock on liver function. It was convenient to use an animal that was not too different from man and to adopt an experimental design which would extend over a sufficient time to allow any change in liver function to develop, yet be short enough to allow the animal to be kept under anaesthesia throughout. It is well recognised that dogs respond to endotoxaemia with sudden systemic arterial hypotension, probably as a result of venous pooling of blood in the splanchnic circulation. This is now ascribed to a functional hepatic venous outflow sphincter (Hinshaw et al 1966). This appeared to rule out the use of dogs; the pig was the obvious alternative.

As techniques for inducing sepsis, caecal or appendiceal ligation were excluded for two reasons: first, they are so slow to take effect that the animal has to be allowed to recover consciousness before sepsis develops, and is therefore unprotected from the distress it occasions; and secondly, because the haemodynamic effects are very variable (Nxumulo et al 1978 and Gappos et al 1982). Endotoxin has obvious advantages in terms of reproducibility of dose and therefore of effects but in the case of 20 to 30 kg animals is prohibitively expensive. Hence the decision to infuse live Escherichia coli. To facilitate comparison with the clinical situation, three groups of pigs were studied:-

(1) **Control group**

cannulated and maintained under anaesthesia but otherwise intact;

(2) **E. coli group**

cannulated, maintained under anaesthesia and infused with live bacteria. This group was intended to represent the patient in septic shock and untreated;

(3) **E. coli colloid group**

cannulated, maintained under anaesthesia and infused with live bacteria, and also given sufficient colloid to maintain its pulmonary artery wedge pressure. This group was intended to represent the patient in septic shock given, as would normally be the case, sufficient colloid to maintain blood volume.

The **control group's** importance lies in the fact that it demonstrates that throughout the 10 hours of the experiment pulse, blood pressure,

cardiac output and pulmonary artery wedge pressure remain essentially constant. The **E. coli group** shows what might be expected in a patient in septic shock who received no treatment. The pulse rate rises sharply, the blood pressure falls less precipitiously but over a longer period and the cardiac output falls gradually. The pulmonary artery wedge pressure (more succinctly, the left atrial pressure) is initially maintained but, after some hours, declines also. These are the typical features of hypodynamic shock as seen by the clinician. In the **E. coli colloid group** there was again an increased pulse rate, but the increase was less than that seen when colloid was not given. The blood pressure was maintained for longer than in the absence of colloid. The cardiac output rose substantially, was sustained for some hours at this higher level, but ultimately declined to about the initial level. These manifestations are reminiscent of the hypodynamic phase of septic shock as seen by the clinician. The plasma lactate results indicate the degree of microcirculatory hypoperfusion that was present in the group of pigs receiving **E. coli colloid,** despite the hyperdynamic picture with a high cardiac output and normal blood pressure; the lactate values remained low and within the normal range in the **control group** while both **E. coli** and **E. coli colloid groups** showed a significant and marked rise in lactate levels.

Liver Function in Septic Shock

Multiple organ failure is common in critically ill patients suffering from septic shock and is a frequent cause of death in such patients. There is often no obvious sign of hepatic impairment, and attention and therapeutic effort are usually focused on other organs, particularly the lungs, kidneys and heart, since failure of these produces more obvious clinical signs. Fine (1954) suggested that the liver might be the primary target organ in patients suffering from shock, but this suggestion has not been widely followed up, perhaps because the standard liver function tests have been insufficiently sensitive to evaluate the degree of hepatic impairment (Ledingham 1985). In a retrospective study, Banks et al (1982) examined liver function tests in a group of 57 patients with septic shock, 27 of whom survived to leave Intensive Care. Liver tests within 48 hours of the diagnosis of septic shock showed marked but variable liver damage. The results obtained from patients who survived were not significantly different from those obtained for patients who died. Liver function tests, with the exception of bilirubin, failed to distinguish between survivors and non-survivors. Banks' study underlined the fact that, while there is considerable hepatic impairment in patients suffering from septic shock, the available tests of liver function are inadequate to allow any prediction of the outcome to be made during the early course of treatment. There was therefore a need to assess alternative techniques of measuring the degree of hepatic impairment. It was for this reason that in the study of clinical septic shock, both indocyanine green clearance from plasma and antipyrine half-life were calculated in all patients

(Figures 10 and 11). Indocyanine green clearance has customarily been used as an index of hepatic blood flow (Feely et al 1981) and antipyrine half life as an index of the activity of the cytochrome P450 system of the liver (Boobis et al 1981).

In the clinical septic shock study, initial values of indocyanine green clearance were very low in all patients (Table 10). There was no significant difference between patients who subsequently survived and those who did not. The trend in indocyanine green clearance over the first 24 hours does however appear to be of prognostic value. Over this period there is a significant change in indocyanine green clearance indocyanine green clearance can change dramatically over an even shorter time course (i.e. before or after cryoprecipitate infusion). "Static" liver function tests, such as bilirubin level, tend to change much more slowly; this is presumably because indocyanine green clearance is partly, perhaps predominantly, dependent on hepatic blood flow, which can change rapidly. The levels of indocyanine green clearance seen in septic shock patients were often so low that it seems improbable that they could be approximations of hepatic blood flow (since such low blood flows would result in massive necrosis of both the gut and the liver). It seems more likely that the extraction of indocyanine green was decreased in these patients, but to measure this it would have been necessary to insert a catheter into the hepatic vein. It was for this reason that further studies into the handling of indocyanine green by the liver were carried out in the pig. These showed that infusion of Escherichia coli brought about a 40% fall in indocyanine green clearance, regardless of whether colloid was administered or not. The fact that colloid administration had no effect on the rate of clearance is suprising in view of the fact that it

greatly increased cardiac output. This is, at the least, an indication that the impairment of clearance is not due to hypovolaemia. There is a parallel here with the impairment of cytochrome P450 function seen in rats infused with endotoxin (Figure 34).

The hypothesis that septic shock reduces the liver's ability to extract indocyanine green from the blood passing through it would also explain, in part at least, the fact that, although colloid administration greatly improved cardiac output in pigs infused with Escherichia coli, it produced no improvement whatever in indocyanine green clearance. It was obviously desirable to determine directly the proportion of indocyanine green extracted from the blood as it passed through the liver. This is however easier said than done, since, in the pig, the only way to sample blood leaving the liver is to occlude temporarily the posterior vena cava below the level of the liver and to withdraw the blood sample above the occlusion. This procedure is feasible, though not easy, in a healthy animal; in an animal in shock it is liable to provoke cardiac arrest. Such results as could be obtained, however, suggested that, even in the healthy animal, the proportion of indocyanine green extracted in passage through the liver was only 38% and that in septic shock it was reduced to 15%.

Just as indocyanine green clearance was retarded by a factor of 2 in septic shock, so also was antipyrine clearance. The compounds are both disposed of by the liver, but by quite different processes: indocyanine green is conjugated and secreted in the bile; antipyrine is oxidised by the cytochrome P450 system. It is possible that in septic shock a single mechanism is responsible for the impairment of both

processes. Reduction in hepatic blood flow is a possibility; but it has been shown above that this is probably not the main factor in the case of indocyanine green. Again, it is conceivable that the defect lies in the uptake of both compounds into the cell rather than their metabolism once inside, but it seems unlikely that two compounds with such very different structures and with such different half-lives (in normal subjects, 3 minutes for indocyanine green, 10 hours for antipyrine) should share a common means of entry into the hepatocytes. It seems much more reasonable to suppose that the depression of the cytochome system produced by endotoxin infusion in rats is paralleled by a similar depression in patients in septic shock and that this is sufficient to account for their inability to clear antipyrine.

Fibronectin

The literature contains conflicting reports on the effect of sepsis on plasma fibronectin levels. Some authors have reported a rise in the early stages (up to 24 hours) of sepsis induced in animals by intraperitoneal injection of bacteria (Lanser et al 1982 and 1983, Aukburg et al 1981). In contrast, all the clinical studies, with one exception (Stathakis 1981) have shown that plasma fibronectin is lower in patients with sepsis than in those without (Rubli et al 1983, Klim et al 1986, Boughton et al 1982). This fall appears to be most marked in critically ill patients with severe sepsis, especially if they have disseminated intravascular coagulation (Chadwick et al 1984, O'Connell et al 1984). These reports are in agreement with the work described in the present study.

The published animal results, however, with reports of rising fibronectin levels in the first 24 hours after intraperitoneal injection of bacteria, are in contradiction with the pig experiments in the present study (Page **151**) in which fibronectin levels fell dramatically within two hours of the infusion of Escherichia coli being started. This discrepancy can plausibly be explained by two considerations:

(1) It seems unlikely that severe sepsis would develop within 24 hours of intraperitoneal innoculation with bacteria, which was the technique used by Lanser et al (1982) and Aukburg et al (1981); whereas intravenous innoculation, as used in the present study, might be expected to elicit an immediate response. It is well known that severe sepsis is associated with

disseminated intravascular coagulation, and this in itself can reduce fibronectin levels.

(2) The rise in fibronectin following intraperitoneal innoculation of bacteria might well be due to the stress which would follow such a procedure. Owens and Cimino (1982) have shown that stress hormones such as cortisol increase hepatic synthesis of fibronectin.

It seems therefore safe to conclude that sepsis lowers plasma fibronectin levels in experimental animals as well as patients. The low levels of fibronectin observed in sepsis could be due to one or more of the following: increased intravascular consumption; decreased synthesis; or increased extravascular distribution. It has been reported that fibronectin is taken up by tissues at the site of injury or surgical trauma (Reese et al 1982) as a result of binding to exposed collagen. This may explain the fall in fibronectin following surgery, but not the much greater reductions in fibronectin level observed in sepsis. Severe sepsis, with its associated increase in capillary permeability, is likely to increase leakage of fibronectin into the extravascular space. It has been shown to increase leakage of albumin to the extravascular space up to three-fold (Fleck et al 1985), but since fibronectin is a much larger molecular weight than albumin (molecular weight 440,000 vs 69,000), it might be less affected by this permeability change. In patients with severe sepsis of long standing (i.e. more than 48 hours) with impairment of organ function, decreased synthesis of fibronectin is likely to be a contributory factor (Pussell et al 1985). These mechanisms by themselves seem inadequate to account for the precipitous fall observed in fibronectin level in the pigs in septic shock in the present

study. Increased consumption by some mechanism seems a more likely explanation. This consumption may be due to fibronectin being involved in opsonisation of circulating endotoxin and other bacterial cell wall debris, with subsequent clearance by the reticuloendothelial system. Fibronectin levels in septic patients are even lower when there is associated disseminated intravascular coagulation. In the pig, such coagulation does occur in sepsis induced by the technique used in the present work (Ramsay 1986, a private communication). It has been suggested that fibrin micro-aggregates are opsonised by plasma fibronectin and cleared by the reticuloendothelial system. Powell et al (1986) have shown that in patients who had undergone aortic surgery there was a post-operative fall in fibronectin level which was due to consumption of fibronectin in the formation of complexes with circulating collagen debris. This was found to an even more marked degree in pigs subjected to prolonged aortic cross clamping.

It would seem therefore that, in septic shock, fibronectin, as well as leaking out through the capillary wall, may be taken up:-

(1) In the opsonisation of endotoxin and bacterial debris

(2) In the opsonisation of fibrin in intravascular coagulation

(3) In formation of complexes with the breakdown products of collagen.

Endotoxaemia in Septic Shock

It has long been recognised that in experimental animals an injected dose of endotoxin is rapidly cleared by the reticuloendothelial system, in which the Kupffer cells of the liver are numerically preponderant. The processes of clearing the endotoxin itself and the fibrin degradation products from the disseminated intravascular coagulation it provokes, effectively saturate the reticuloendothelial system. The consequence is that a second dose, injected before the first is completely disposed of, is cleared very slowly, so slowly that it may persist long enough to cause serious organ damage and ultimately death. This so-called "Schwartzman phenomenon" demonstrates both the efficacy of the reticuloendothelial systems in dealing with a limited amount of endotoxin and the dire consequences when its capacities are exceeded.

There is some reason to believe that endotoxin may be present in the portal blood of normal healthy individuals. It is known that obstructive jaundice impairs the function of the reticuloendothelial system. Further Cahill (1983) has shown that obstructive jaundice increases the likelihood of substantial portal endotoxaemia, sometimes accompanied by systemic endotoxaemia. Taken together, these observations suggest that in normal healthy individuals there is some passage of endotoxin from Gram-negative organisms in the gut into the intestinal capillaries and thence to the portal circulation. This is normally intercepted by the Kupffer cells. If, however, Kupffer cell activity is depressed (e.g. by obstructive jaundice) some endotoxin may escape interception, pass into the systemic circulation

and, once there, cause organ failure (e.g. the renal failure sometimes seen in patients with obstructive jaundice). It has been shown, both in experimental animals and in patients, that cholestatic jaundice can indeed lead to the development of septic shock. (Pain et al 1985).

Furthermore, all patients in shock, whatever its cause, will at least in the early stages, have a substantially diminished blood flow through the gut and the liver, and an increased mucosal permeability in the former. These factors might simultaneously favour leakage of endotoxin from the gut to the portal blood, and hinder its removal by the Kupffer cells. In this connection it is notable that patients who have been diagnosed as in septic shock on the basis of clinical criteria are almost invariably found to have systemic endotoxaemia, although in many cases they have negative blood cultures. In the present study, endotoxaemia was detected at some stage in all patients. In particular, high endotoxin levels were detected in two patients with Gram-positive septicaemia (the case histories of these patients T.B. and Q.E. are given on Page**104**). Gram-positive organisms do not have endotoxin in their cell walls and neither of these patients at any stage gave positive cultures for Gram-negative organisms. It seems inescapable that their endotoxin must have been coming from bacteria in the gut.

If it is true that, in general, patients with septic shock have endotoxaemia, and that the endotoxin is derived from the patients own intestinal flora, it might be sensible to treat the condition by measures aimed at reducing levels of circulating endotoxin. In the present study, cryoprecipitate was shown to bring about this result: the other beneficial changes which it produces may be secondary to this reduction in endotoxin level.

CONCLUSIONS

The nature of shock is a complex subject in which generalisation is hazardous, and the following views are put forward with due diffidence.

1 It is generally agreed that in all forms of shock splanchnic blood flow is depressed and permeability of the intestinal mucosa increased. It seems reasonable to suppose that such circumstances would favour passage of endotoxin from the gut to the portal blood and impair the ability of the Kupffer cells to intercept it.

2 The clinical part of the present study has shown that endotoxaemia is an invariable feature of septic shock, even when the cause of the condition is infection with organisms which do not produce endotoxin; and that endotoxaemia in such cases is often not accompanied by detectable septicaemia. It seems reasonable, therefore, to suppose that septic shock is, in general, due to escape of endotoxin from the gut in quantities too great to be intercepted by the Kupffer cells of the liver.

3 In the course of the clinical part of this study it was noted that hepatocyte function, as reflected in clearance of indocyanine green and antipyrine, was greatly impaired. The evidence of the rat and pig experiments indicates that in both cases this was a direct effect of high circulating levels of endotoxin (and thus not related to the state of the Kupffer

cells). It may simply exemplify the damage endotoxin inflicts on a wide variety of organs and tissues.

4 If the views outlined in paragraphs 1 and 2 above are correct, it may be important in septic shock to try to reduce the endotoxaemia. In the present study it was found that this could be achieved by cryoprecipitate administration. The reduction in endotoxin levels was accompanied by improvements in urine output, and core temperature and there was also some evidence that survival was prolonged.

5 It would seem, therefore, that regular endotoxin assays, preferably at intervals as brief as every four hours, may be a useful means of monitoring the condition of septic shock patients, and that cryoprecipitate therapy may have a part to play in their treatment.

6 The mortality rate in patients suffering from shock depends to a large extent on the underlying cause; patients with uncomplicated hypovolaemic shock, if treated early, have a very good prognosis, while septic shock, especially if the diagnosis and treatment are delayed, has an extremely poor one. The mortality from septic shock has remained unchanged for many years, despite major advances in surgical techniques and technological medicine. Therefore it could be argued that any therapeutic manoeuvre which has any influence on mortality in septic shock, or even buys time for other treatments (such as surgery and antibiotics) to be successful, could be considered a significant advance in the management of patients with septic shock.

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