ENDOTOXAEMIA IN INTESTINAL DYSFUNCTION IN EXPERIMENTAL ANIMALS:

INTESTINAL ISCHAEMIA AND HYPERTHERMIA

by

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ABSTRACT

Endotoxins or lipopolysaccharides (LPS), highly toxic components of the outer membrane of gram-negative bacteria, are normally present in the mammalian gut lumen.

In this thesis, I investigated, in laboratory animals, whether these gut-derived endotoxins play a role in pathophysiology resulting from intestinal dysfunctions caused by intestinal ischaemia and heat-stress.

In primates, reperfusion of the splanchnic region after a temporary ischaemia was followed by a rapid increase in LPS concentration, first in the hepatic portal plasma and, ten minutes later, in the systemic arterial plasma.

Rises in plasma LPS concentrations during or following the temporary intestinal ischaemia was prevented by prophylactic administrations of corticosteroids, anti-LPS IgG antibodies and oral, non-absorbable, antibiotics - agents which appear to stabilize cellular membranes, aid the reticuloendothelial system in removal of LPS from the circulation and destroy the intestinal aerobic gram-negative bacteria respectively. In addition, administration of therapeutic anti-LPS antibodies also rapidly reduced the plasma LPS concentrations to baseline during an endotoxaemia.

In a control heat-stress model, elevations in plasma LPS concentration commenced at rectal temperatures greater than 41,5^{o}C. Like the intestinal ischaemia model, this occurred first in the hepatic portal plasma, and 10-15 minutes later, in the systemic arterial plasma. Peak plasma LPS levels of about 0,3 ng/ml, measured in heat-stressed primates, have proved in previous studies, to be toxic. A rapid decline in mean arterial pressure was

followed by increases in plasma LPS concentrations and heart rates. Reductions in splanchnic blood flow and consequent local ischaemia coupled with thermal injury to the intestinal wall and the liver, may have permitted rises in plasma LPS concentration. Furthermore, as in the ischaemia model, prophylactic administrations of corticosteroids, anti-LPS IgG antibodies, and oral, non-absorbable antibiotics prevented a rise in plasma LPS concentration. Of importance, prophylaxis with intravenous corticosteroids and anti-LPS IgG antibodies increased the survival rates significantly in heat stroke in primates. In addition, monkeys having high titres of "natural" anti-LPS IgG antibodies had lower plasma LPS concentrations and survived the induced-heat stroke.

It is suggested that other pathophysiologic conditions which compromise the integrity of the gut wall would also lead to the development of an endotoxaemia, and that gut-derived endotoxins contribute to the pathogenesis of heat stroke and treatments with corticosteroids and anti-LPS IgG antibodies may prove beneficial in other endotoxin-related disorders.

PREFACE

These studies represent original work by the author and have not been submitted in any form to another University. Where use was made of the work of others it has been duly acknowledged in the text.

The research described in this thesis was carried out in the Department of Physiology, University of Natal Medical School, Durban, under the supervision of Professor SL Gaffin.

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CHAPTER 1

INTRODUCTION

Gram-negative bacilli have become the predominant etiologic agent of post-traumatic and nosocomial infection over the past three decades and the prevalence of the infection does not seem to be decreasing[1,2]. In addition, the overall mortality from bacteraemic and septic shock has also remained at unacceptable high levels of 30-80% world wide[3-10] despite improvements in supportive care, more effective chemotherapy with antimicrobial and other pharmacological agents[11,12]. In the U.S. alone, there are an estimated 200 000 - 400 000 septic deaths annually[13]. Furthermore, sepsis is the main cause of maternal deaths at the King Edward VIII Hospital, Durban, causing 78 out of 258 deaths per year[14]. The development of antibiotic resistance and the severity of the underlying disease may account, in part, for the failure of conventional treatment. The main component of the high mortality rate, however, is believed to be endotoxins (lipopolysaccharide (LPS), pyrogens), the highly toxic components of the outer membrane of many gram-negative bacteria, such as *Salmonella* spp., *Shigella* spp., and *Escherichia coli*[15-19]. Moreover, the clinical features of gram-negative bacteraemia - fever, transient leucopenia and leucocytosis, hypotension, disseminated intravascular coagulation (DIC) and, in high doses, vascular collapse, shock and death[1,3-5,7,20-22] - are similar to those caused simply by the intravenous administration of endotoxin[2-5,7,8,11,15-22].

The term endotoxin was introduced by Richard Pfeiffer in 1904 in studies on the toxic principles of *Salmonella typhosa (Salmonella typhi)* infection (cited in **17**). He found that one exotoxin secreted by the bacteria was destroyed by boiling and a second toxin - bound to the bacteria - was heat stable. He called the latter endotoxin.

Thus endotoxin, besides being highly toxic (for example, persisting plasma levels of 1 ng/ml or 10^{-12} molar in humans results in renal failure and death[23]) is also highly resistant to denaturation by most treatments[4]. Therefore, antibiotics, while killing the bacteria, may leave the endotoxin intact[3,4,7,10-12,24,25]. Of importance, it has been shown that a rapid destruction of the parent bacteria, using antibiotics, can cause a 2000 fold increase in plasma LPS concentration[25].

Besides circulating gram-negative bacteria, another important, readily-available source of LPS is the gut lumen. The mammalian gut is colonized from birth onwards with gram-negative bacteria and their endotoxins[16]. Under normal circumstances, the permeability properties of the gut mucosa is able to confine the LPS to the lumen[26]. However, normally small quantities of LPS do appear to leak through the gut wall and enter the portal circulation. These are subsequently detoxified mainly by the macrophages or Kupffer cells of the liver reticuloendothelial system (RES)[27-30]. If, for any reason however, the permeability property of the intestinal mucosa is breached, for example, by ischaemia[31-35], vasoactive agents (bradykinin, serotonin and histamine)[36], severe burns[37], ionizing radiation[38,39], hypoxia[40], hyperthermia[41-44], haemorrhage or hypovolaemia[28,45-47], then large quantities of LPS would be able to migrate into the circulation. On entering the circulation, some of the LPS combines with the high density lipoproteins[48,49] and specific anti-LPS[4,7] antibodies whilst the remainder are detoxified mainly by the Kupffer cells of the liver and the macrophages of the spleen[27-30]. However, should the RES become overwhelmed or even damaged a small leakage may give rise to an elevated concentration of LPS in the circulating blood and the whole cascade of biological effects previously described[50]. In addition, LPS may activate host mediation systems, including complement and

coagulation, as well as a number of cell types such as blood monocytes, neutrophils, platelets and tissue macrophages to produce the above injurious effects[4,22,49].

In the opinion of Onda et al.,[11] gram-negative intestinal flora play an important role in the pathophysiology of severe sepsis in digestive disorders, such as intestinal obstruction, peritonitis and bilary tract infection. Furthermore, the clinical and laboratory abnormalities that occur during intestinal strangulation and obstruction, namely, colitis X, acute salmonellosis or neonatal septicaemia in horses, are believed to resemble the syndrome of shock and coagulapathy produced by endotoxin administration[51]. Moreover, elevated levels of plasma LPS have been detected in haemorrhagic enteric dogs[52], horses with gastroenteritis[53] and patients with liver disease[54].

Oxygen availability is critical in preventing deterioration and death of cells lining the gut wall. According to Fine[50], blood-flow to the splanchnic regions becomes, in all forms of shock, seriously reduced. He goes on to state that "...endotoxemia will develop when splanchnic vasoconstriction has continued long enough to injure the endotoxin-detoxifying power of the reticuloendothelial system of the liver and spleen. The undetoxified endotoxin aggravates the vasoconstriction caused by the hypovolemia and so accelerates the collapse of the systemic circulation". Fine's view has been supported by a number of experimental findings[55-58]. In Fine's[59] opinion: "...an endotoxaemia can become a self-sustaining process by mobilizing more endotoxin from the gastro-intestinal tract". He and his co-workers demonstrated that when a minimal lethal dose of LPS is injected, at death the total amount of LPS assayed in the plasma and tissues was greater than ten-times the injected dose. They, therefore, suggested that LPS, on entering the circulating blood, induces the liberation of various vasoactive substances which, in turn, increase the permeability of the splanchnic blood vessels

and other membranes, allowing increased leakage of LPS across the gut mucosa into the portal circulation.

From the foregoing, it can be seen that a considerable amount of work has been done on intestinal ischaemia and the role of gut-derived endotoxins in the pathophysiology of shock episodes. However, no detailed investigations on the time-course of changes in plasma LPS concentrations have been carried out or the effects of chemotherapy or immunotherapy on this time course have been determined. One of the reasons for this situation has been the lack of an accurate, reliable and reproducible test for the determination of plasma LPS concentration, but with the recently-developed chromogenic substrate modification of the Limulus amoebocyte lysate assay[60,61] it is now possible to carry out such investigations, as the present thesis demonstrates.

The purpose of the research leading to this thesis was:

To determine the time course of changes in plasma LPS concentration in experimental animals subjected to a temporary occlusion of the superior mesenteric artery (SMA). In addition, attempts were also made:

a) to show the effects of treatment with anti-LPS antibodies and corticosteroids on this time course,

b) to show that the source of the endotoxins is the gastro-intestinal tract by reducing gut bacterial content using oral, non-absorbable antibiotics prior to occlusion of the superior mesenteric artery and by determining simultaneously the changes in portal and systemic arterial plasma LPS concentrations.

As a control it was decided to test whether another agent which damages the permeability property of the intestinal wall, such as an elevated core temperature (heat

stress/stroke)[4] would also lead to an endotoxaemia. Heat stroke is also regarded as a shock state which can become lethal if not recognized and treated promptly[62-69]. It is caused by elevation of body temperature and is especially likely to occur in hot, humid climates and is a serious clinical problem encountered in sports[70-77], military[78-81], occupational[82,83] and civilian medicine[84-87]. Without prompt recognition and immediate treatment, it carries a mortality rate of as high as 80%[68,69,79,83,84] and, at times, even with optimum treatment, heat stroke may cause death or permanent damage[88].

A number of studies in a heat stroke model were designed to:

a) determine the time course of changes in plasma LPS concentration during hyperthermia and heat stroke and to relate these changes to certain cardiovascular parameters,

b) establish the effects of prophylactic treatment with a corticosteroid on plasma LPS levels and on cardiovascular parameters,

c) establish that the source of the endotoxin during hyperthermia and heat stroke is the intestinal lumen by treating animals with an oral, non-absorbable antibiotic before being heat-stressed,

d) investigate the route of entry of endotoxin into the systemic arterial blood by determining simultaneously the changes in hepatic portal and systemic arterial plasma LPS concentrations, and finally, to

e) investigate whether prophylactic administrations of corticosteroids and equine anti-LPS IgG plasma improve the survival rate of primates subjected to heat stroke.

It is anticipated that these studies will show:

a) that an endotoxaemia may develop when the blood flow to the wall of the intestine becomes sufficiently reduced, for example, during occlusion of blood flow to the gut or during a generalized heat-stress,

b) that endotoxins may contribute to the pathogenesis of heat stroke, and

c) that the elevated plasma endotoxin levels so produced can be suppressed by prophylactic treatment with non-absorbable oral antibiotics, corticosteroids or anti-LPS IgG antibodies.

It is hoped that these results will contribute significantly to knowledge in medical science, especially in so far as gastro-intestinal dysfunction and endotoxaemia are concerned.

CHAPTER 2

REVIEW OF LITERATURE

2.0 INTRODUCTION

Literature pertaining to the general characteristics of LPS (structure, target cells, biological activities) will be reviewed initially, followed by that concerning the role of gut-derived endotoxins in pathophysiology of shock and finally literature concerning hyperthermia and heat stroke.

2.1 GENERAL CHARACTERISTICS OF LPS

2.1.1 Structure of the bacterial cell envelope, including the structure of LPS

The bacterial cell envelope, shown in Figure 1 basically consists of an inner cytoplasmic membrane, and a trilayer outer cell wall structure. The latter in turn consists of a peptidoglycan layer, a phospholipid-protein layer, and an outer most lipopolysaccharide (LPS) layer[89,90].

The bacterial cell cytoplasmic membrane surrounds the cytoplasm and functions in a manner similarly to that of the mammalian cell membrane[89,90]. Directly external to the cytoplasmic membrane of both gram-negative and gram-positive bacteria is a layer of peptidoglycan, consisting of interwoven polysaccharide chains held together by cross-linking polypeptides forming a rigid and highly porous shell[89,90]. This layer is freely permeable to most metabolites. In gram-negative bacteria, unlike those of the gram-positive, the cell wall also produces a unique outer membrane which is attached to the peptidoglycan layer by lipoprotein bridges[89,90]. This outer membrane has a complex molecular structure containing phospholipids, the characteristic lipopolysaccharides and matrix proteins.

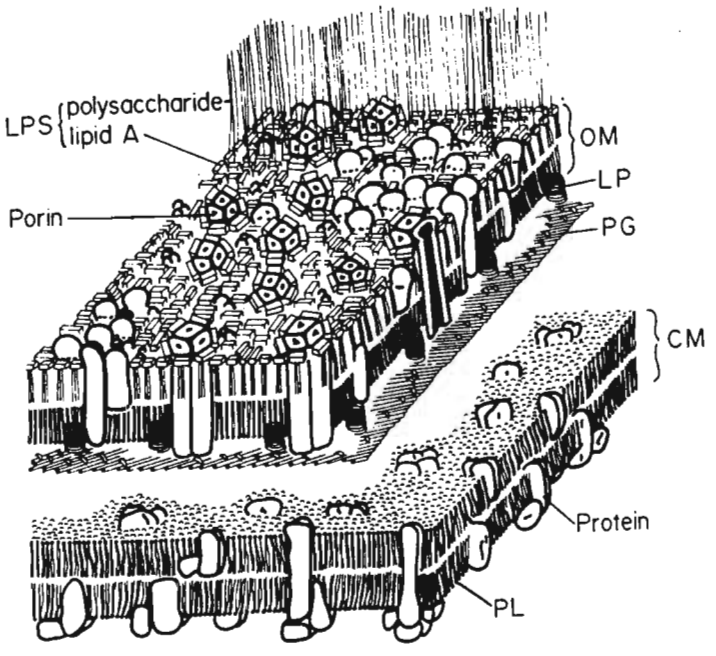

Figure 1. Schematic diagram of gram-negative bacterial cell envelope structure. LPS, lipopolysaccharide; OM, outer membrane; LP, lipoprotein; PG, peptidoglycan; CM, cytoplasmic membrane; PL, phospholipid (taken from Wheat[89]).

The outer membrane is asymmetrical, that is to say, all the LPS occur in the outer leaflet, in contrast to the phospholipid, which is found entirely in the inner leaflet of the outer membrane[89]. Some proteins occur in the outer leaflet; others form transmembrane channels known as porins.

General structure of lipopolysaccharide

The LPS from different groups of gram-negative bacteria conform to a common structural principle. They consist of a lipid component called lipid A and a covalently-bound polysaccharide portion. The polysaccharide component consists of two subcomponents called the "O"-specific polysaccharide (region I), which is the outermost layer of LPS, and "core" polysaccharide (region II) which lies between O-specific polysaccharide and lipid A (region III)[16,17].

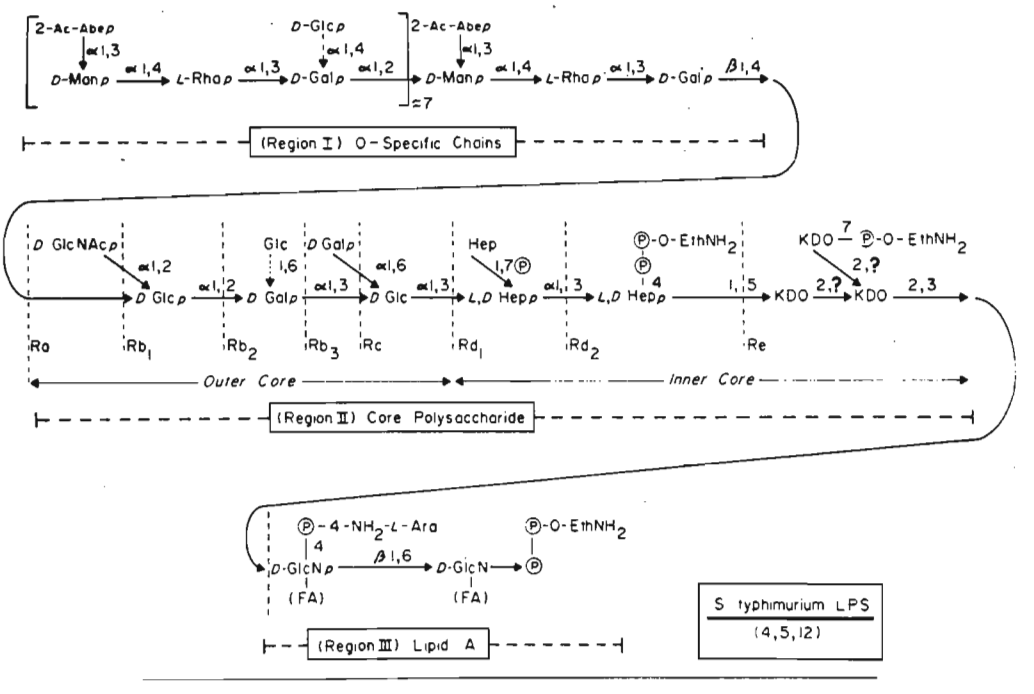

Figure 2: Structure of *Salmonella typhimurium* lipopolysaccharide (LPS). Vertical broken lines indicate limits of structure produced by Re to Ra mutants (rough form LPS). A question mark indicates uncertainty of bond (taken from Wheat[89]).

O-specific polysaccharide (region I)

The outermost layer of the LPS complex is composed of repeating oligosaccharide units of 3 to 4 monosaccharides [15-17,89,90]. This layer has antigenic properties which depend upon the sugar composition, sequence, linkage groups and additional substituents[15-17,89]. This layer is also referred to as the O-specific polysaccharide or the O-antigen[15-17,89]. The oligosaccharide determinants in the O-chain may function as receptors for bacteriophages[17,89].

Core polysaccharide (region II)

The middle "core" saccharide layer, is arbitrarily divided into "inner" and "outer" core regions. The inner core contains oligosaccharides of 2-keto-3-deoxyoctonic acid (KDO), heptose as well as phosphate and pyrophosphate-bound

ethanolamine[15-17,89]. The KDO is bound to the inner or lipid A region of the LPS complex. The outer core is composed of D-glucose, D-galactose and often N-acetyl-D-glucosamine. The core structures represent antigens and can induce the formation of core-specific antibodies. The core region shows much less diversity than the O-specific polysaccharide[17,89].

The discovery of rough (or R) mutants (because they form "rough" colonies as opposed to the "smooth" colonies of wild type bacteria) opened a new field for investigators in LPS research. LPS of rough gram-negative bacteria do not contain the O-specific polysaccharide region and some may lack parts of the core saccharide as well[15-17,89]. This is in contrast to the LPS from S (smooth) colonies which have complete LPS molecules. The mutants which are most defective in polysaccharide biosynthesis and which lack the greatest part of the LPS molecules are known as the Re-mutants[89].

Lipid A (region III)

The innermost layer of LPS complex, called lipid A (Figure 3) is bound by hydrophobic bonds to the cytoplasmic membrane and is covalently bound on its "outer" surface to the core. This in turn is bound to the polysaccharide component. All biologically active free lipid A's contain a 1-4'-diphosphorylated ß-1-6-linked-D-glucosamine disaccharide at C1 and C4' of the reducing and non-reducing glucosamine residues, respectively, and amide-bound D-3-hydroxyl fatty acids. The hydroxyl group at C6' serves as the attachment site for KDO and the core polysaccharide[89].

The lipid A's from a number of distinct groups of gram-negative bacteria show close structural relationships, but they are not identical[15-19,89,90] (see Figure 3). This variability in lipid A structure is due to the presence of various nonacylated polar head-groups bound to the phosphate residues at C4' and C1. These include phosphate, phosphorylethanolamine and D-glucosamine at C1 and 4-amino-4

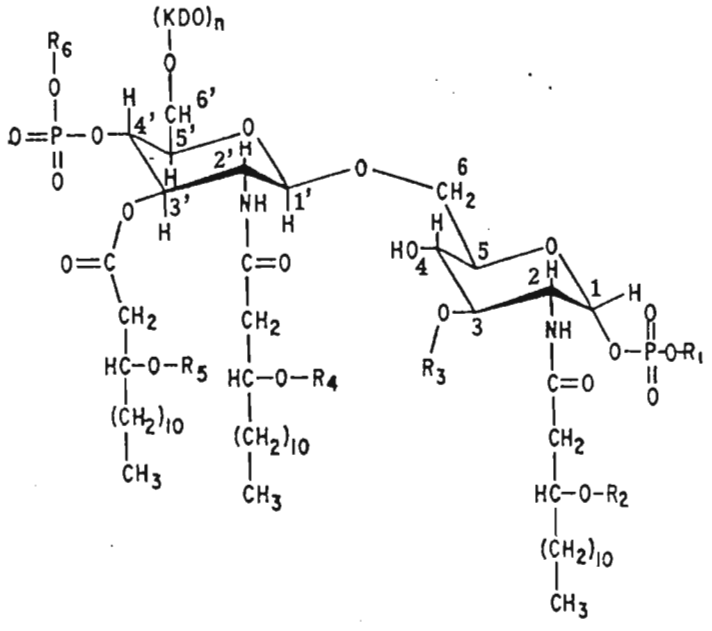

Figure 3. Chemical structure of naturally occurring lipid A with point of attachment of KDO group from the Re mutant of *S.typhimurium*. R_1 = H, phosphate or phosphorylethanolamine; R_2 = H or palmitic acid; R_3 = ß-hydroxymyristic acid; R_4 = lauric acid; R_5 = double ester, two ß-hydroxymyristic acids; R_6 = H or 4 amino-4-deoxy-L-arabinose in α-1-phosphate linkage, n = 2 or 3 (from Wheat[89]).

deoxy-L-arabinose at C4'. A limited variability is also due to the type of fatty acids present. For example, in *Salmonella* spp. the phosphate groups at C1 and C4' are partially substituted by phosphorylethanolamine and 4-amino-4 deoxy-L-arabinose respectively and in *E.coli* by phosphate and H respectively. The lipid A portion is responsible for the endotoxin properties of gram-negative bacteria[15-19,89].

In summary, a gram-negative bacterial cell envelope consists of an inner membrane, a peptidoglycan layer and an outer membrane. The LPS complex which occurs in the outer leaflet of the outer membrane is composed of an outer O-specific polysaccharide region, a middle core polysaccharide region and an inner lipid A region (shown schematically in Figure 4). All three regions of the LPS complex are antigenic but only the lipid A region is responsible for its toxic properties[15-18,89].

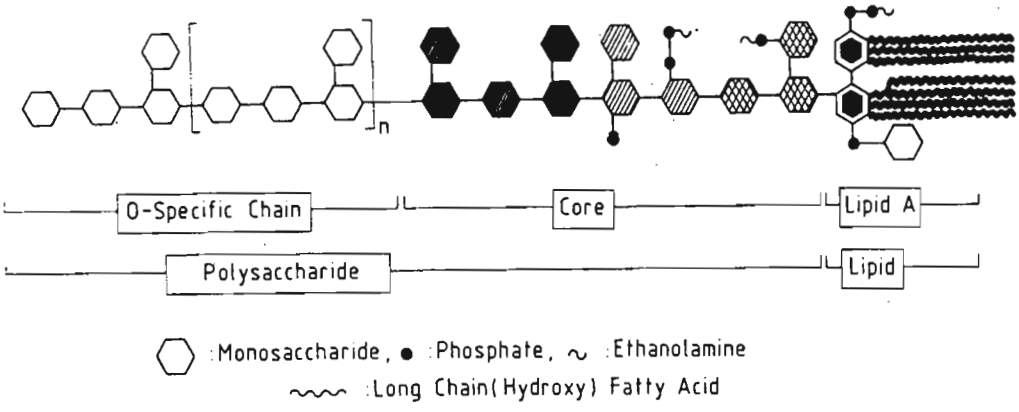

Figure 4. Schematic structure of a salmonella lipopolysaccharide (LPS) (from Rietschel et al.[16])

2.1.2 Clearance and detoxification and fate of LPS on entering the circulation

The fate of LPS on entering the circulatory system, either from a septic focus or from the gastrointestinal tract, is not completely understood[91]. According to Freudenberg et al.,[91] "...important questions such as whether resistance (to LPS), hereditary or acquired, has something to do with a better detoxification of the LPS molecule remain unanswered".

2.1.2.1 Distribution of LPS

Braude and his co-workers,[27] on injecting radio-labelled LPS intravenously, into experimental animals found that a large amount of the radioactivity initially appeared in the plasma. Within an hour of injection, the radioactivity in the plasma had fallen to about 60%, and within two hours to 50% of the original level. The period of rapid removal from the plasma was accompanied by fever, leucopenia and sometimes hypotension and diarrhoea. As the endotoxins left the plasma the largest percentage went to the buffy coat (platelets and leucocytes) and liver. In addition, the leucocyte count dropped precipitiously during the period LPS

accumulated in the buffy coat[27]. Braude and his co-workers[27] suggested that the heavy distribution of radio-labelled LPS in the liver, buffy coat and spleen could be related to the ability of phagocytic cells in these regions to remove the endotoxin from the circulation.

Mathison and Ulevitch[92] found that 0,5 to 2 minutes after injection of radio-labelled LPS, 35% of the radioactivity was contained in the buffy coat, 86% of which was associated with blood platelets and a smaller percentage with neutrophils and monocytes. The binding of LPS to platelets and leucocytes was followed by the disappearance of these cells from the circulation, indicating that a great deal of LPS is removed from the circulation by this route. Platelets to which LPS were bound were phagocytosed by liver Kupffer cells and splenic macrophages or remained as aggregates in the sinusoids of these organs[92]. The investigators, therefore, concluded that the binding of LPS to platelets may not play a major role in the development of DIC and shock. Radio-labelled LPS, when administered intravenously, accumulated mainly in the phagocytic vacuoles of macrophages in the liver and spleen. A similar distribution of radio-labelled LPS was observed by others[22,91,93,94].

To sum up, when a bolus of LPS is injected into animals it is initially distributed in the plasma. Thereafter, a large percentage goes to the buffy coat, the liver and spleen. A sharp drop in leucocyte count occurs during this time. The period of redistribution of LPS may be accompanied by fever, leucopenia, thrombocytopenia, hypotension and diarrhoea.

2.1.2.2 Clearance and detoxification of LPS

a) Role of the reticuloendothelial system (mononuclear phagocytic system) in clearance of LPS

The reticuloendothelial system (mononuclear phagocytic system[95]) is defined as a system of mononuclear cells that

are characterized by their intense phagocytic capabilities. The major portion of the RES consists of the macrophages located in the capillary sinusoidal beds of liver, spleen, lung and bone marrow. Hepatic macrophages (Kupffer cells), which line the sinusoids of the liver, contribute largely to the intravascular clearance of circulating particles, including endotoxins.

It is now generally accepted that the elimination of LPS from the circulation is effected mainly by the cells of the RES, especially the liver Kupffer cells[28,30,50,91-102]. Upon uptake of foreign substances, such as endotoxins, the cells of the RES enlarge and multiply, protecting the body and enabling it to withstand a second invasion more effecively[99].

Frank and his co-workers[55] found that perfusion of the portal vein or the superior mesenteric artery of an animal in haemorrhagic shock with a normal flow of oxygenated blood prevented death and the pathological changes which normally occur in "irreversible" haemorrhagic shock. Similar cross-perfusions when applied to other regions of the circulation in the above model, however, were not able to prevent death, indicating that the liver was essential for detoxification of LPS. In accordance with the above findings, Rutenberg et al.[96] showed that the lethality of endotoxin was reduced or eliminated when all or most of the endotoxin had to traverse the liver first before entering the systemic circulation. An 85% survival rate was obtained when endotoxin was injected into a branch of a mesenteric vein as opposed to 11% survival rate when it was injected into a systemic artery or vein[96].

It has been assumed that LPS, during its uptake by cells of the RES, is detoxified and degraded, but no conclusive evidence is as yet available that this is indeed the case[94]. However, no major degradation of LPS structure was apparent within 3 hours of being taken up by the Kupffer cells[49].

Furthermore, the presence of LPS was detected in the hepatocytes for up to 9 days after administration[94]. On the other hand, there is a possibility that the LPS in the hepatocytes is detoxified by bile acids[103].

b) Role of humoral agents in clearance and detoxification of LPS

In addition to LPS's binding with cellular elements in blood and tissues, they may also interact with certain humoral agents in plasma, leading to a possible detoxification of LPS[104,105]. However, the mechanism of LPS detoxification by humoral agents in the vascular compartment is a subject of controversy, and as yet, the plasma or serum components required have not been well characterized[106]. It has been established, nevertheless, that some of these agents are antibodies, the complement system and high density lipoproteins.

i) Role of antibodies (immunoglobulins)

Braude et al.[106] suggested that the susceptibility of rabbits to lethal challenge by LPS can be prevented by the presence of antibodies directed against the LPS molecule. Such "natural" antibodies were shown to be present in the serum of mice[107] and healthy human subjects and patients with immunological disorders[108]. Moreover, high serum anti-LPS titres were associated with increased survival from bacterial infections (80% survival with high titres vs 48% with low titres)[109,110]. It may be expected that "natural" antibodies would be eventually formed against the LPS of the bacteria present in the digestive tract because from the time of colonization the host is in contact with the LPS[111]. However, these "natural" antibodies become bound to or consumed by invading bacilli near the time of onset of septicaemia, and therefore greater turnover rates of the specific antibody are required during the course of the disorder to protect the body from bacterial invasion[112].

According to Braude[113], increased resistance to LPS toxicity can be generated by vaccination of animals or individuals with LPS or killed gram-negative bacteria. Furthermore, after certain infections with gram-negative bacteria enhanced resistance to LPS may also be found. Ziegler et al.[7,114] showed that the administration of antiserum against J5 mutants in rabbits at the onset of bacteraemia increased the survival rate from 3% to 33-69%.

Recently, Gaffin and his co-workers[115] showed that human anti-LPS antibodies increase the uptake of gram-negative bacteria by phagocytic cells. They also showed that in the presence of complement anti-LPS may also rapidly destroy a wide variety of gram-negative bacteria[116]. The administration of anti-LPS IgG antibodies directed specifically against LPS increased the survival rate of animal and human patients[7,14,35,52,53,117-121].

To recapitulate in brief, "naturally" occurring anti-LPS antibodies may play an important role in aiding the clearance of LPS if high concentrations are present in the patient's serum[109,110]. In the absence of high concentrations of anti-LPS antibodies, the host's resistance may be increased by prophylactic administration of anti-LPS IgG antibodies or by vaccination. The use of anti-LPS IgG antibodies, as reported recently, increased the survival rate of septic and non-septic patients[7,14,35,52,53,117-121].

ii) Role of complement

The complement system is composed of a series of proteins and glycoproteins which are found in the plasma and circulate in the inactive form[105]. When activated they interact as an enzyme cascade and function as an immune effector of acute inflammatory response. Activation of the complement sequence of reactions may occur by two synergistic pathways; the "classic" pathway, set-off by antigen-antibody reactions or by the "alternative" or

properdin pathway which is independent of antigen-antibody reactions[105]. Endotoxins are capable of activating the complement system by both these pathways[105]. The classic pathway consists of three complement components, designated C1,C4 and C2. In the activation of the classic pathway the first component of complement (C1) binds to IgG or IgM molecules which are bound to antigens. The component C1 then acquires enzymatic activity which cleaves C4 and C2[105]. When C4 is cleaved, the larger part of the molecule (C4b) binds to proteins, e.g. those on cell surfaces. Component C2, in the presence of magnesium ions, then binds to C4b and is likewise cleaved by C1. The enzyme C2a (cleaved C2) reacts with C3 and C5. In its first reaction it binds C3 onto C4b (previously bound to the cell surface) and cleaves it into C3a and C3b. C3a, released into the fluid phase, has anaphylatoxin-like activity. C3b binds to polysaccharide structures on cell surfaces and serves as binding sites for C5. The latter when bound to C3b is cleaved by C2a to C5a and C5b. C5a is a potent chemotactic factor and anaphylatoxin[105]. C5b is the first step in the terminal or membrane attack complex and is capable of binding to membranes. The binding of C5b to the membrane sets up a series of reactions leading to the binding and assemblage of C5b,C6,C7,C8 and C9. This then leads to a lesion in the cell membrane and lysis of the cell[105].

In the alternative(properdin) pathway complement component C3 becomes activated by complex polysaccharides and lipopolysaccharides, such as zymosan, inulin and endotoxin. In the initial activation of the pathway C3 forms a complex with a plasma factor, factor B and this complex in turn becomes activated by another factor called factor D[105]. During this reaction C3 is cleaved. C3b then binds to the activator (e.g. endotoxin). Bound 3b complexes with factor B and the latter becomes activated by factor D. The complex C3bBb thus formed then activates properdin to form C3bPBb[105]. Both these (C3bBb and C3bPBb) cleave C5, eventually leading to the fixation of the membrane attack

complex (C5b,C6,C7,C8 and C9) to the activating agent[105]. When the complement system is activated several physiological effects including changes in vascular permeability, leucocyte "stickiness", DIC, thrombocytopenia, opsonization of microbial invaders and amplification of specific antibody activities may be produced[105]. It was recently shown that the complement system destroyed gram-negative bacteria if they became activated by the binding of LPS-specific antibodies to the LPS component of the bacteria[116]. However, the role of complement in the clearance and detoxification of LPS is not clear.

iii) Role of high density lipoproteins

As a consequence of their studies in experimental animals Skarnes and his co-workers,[122,123] suggested initially that the detoxification of LPS in the vascular compartment involved enzymatic degradation of LPS. They postulated that two proteins, both globulins, were implicated, one, a serum esterase, was thought to cause disaggregation of LPS, and the other was presumed to react enzymatically with the disaggregated LPS, rendering it non-toxic. However, other investigators were not able to detect any evidence of enzymatic degradation of LPS in the plasma[92,104,124].

Ulevitch and Johnston[104] observed that the interaction of LPS with serum resulted in the reduction of its buoyant density. They suggested that this was due to an interaction of LPS with lipids present in plasma or serum. This modification of LPS resulted in the loss of several endotoxin activities of native LPS, namely, loss in pyrogenicity, neutropenia and induction of anti-complementary activity[104]. Subsequently, it was shown that LPS binds to plasma high density lipoproteins (HDL) within 3 minutes of intravenous administration[48,49,92]. The binding of LPS with HDL in the plasma decreased the rate of clearance of LPS from the circulation, with the Re-form LPS being cleared faster than the S-form LPS[48,49,92]. A biphasic

clearance of LPS occurred after the intravenous injection of ^{125}I-LPS, into rabbits[49]. The initial rapid clearance was due to the speedy uptake of the radio-labelled LPS by the RES and, to a lesser extent, by granulocytes and adrenal glands, and an additional slower rate of the disappearance of LPS was due to the binding of LPS to high density lipoproteins in the plasma and subsequent uptake by the RES[49]. In contrast to earlier findings, however, Mathison and Ulevitch[92] showed that the LPS still retained its toxic activities even after complexing with HDL[49,92]. The injection of LPS-HDL complex into rabbits produced hypotension, DIC and lethality[49,92].

To sum up, recent findings, subject to further investigation, would suggest that LPS, even though bound to HDL, may still be injurious to the host and may induce target cells such as macrophages to release vasoactive and other toxic substances.

2.1.2.3 Fate of LPS after its uptake by the reticuloendothelial system (RES)

Whilst it is generally accepted that the cells of the RES are responsible for the removal of LPS from the circulation, very little information is available on the fate of LPS after its uptake by the RES[94].

Freudenberg et al.[94] determined the time course of distribution of LPS in the liver, lungs and kidneys of rats. They found that LPS first appeared in the liver where most of it also accumulated. They noticed that the accumulation of LPS in the liver was parallelled by alterations in liver histology. During the first 2-7 hours following intravenous injection, LPS was detected in the Kupffer cells and in the granulocytes in liver sinusoids and, subsequently, in hepatocytes as well[49,94]. Haemorrhagic necroses of the liver tissues with strong leucocytic demarcation were observed from the seventh hour onwards after LPS administration[94].

Freudenberg et al.[94] suggested that LPS may leave the liver cells via the bile duct to be excreted with the faeces, and this was confirmed by the discovery of radio-labelled molecules in the faeces of rats[94] and other animals[103].

In contrast to the liver, the lungs were free of LPS for the first 7 hours[94], but, 24 hours after administration, the lungs contained a large number of LPS positive cells. Radio-labelled LPS was present in a number of granulocytes, in interstitial cells (probably macrophages), in the capillaries of the alveolar walls and in some alveolar and bronchiolar macrophages[94,125]. The appearance of LPS in the lungs parallelled the pathological changes in the lungs (oedema of alveolar walls, perivascular and peribronchial tissue and infiltrations by neutrophils and mononuclear cells).

Insignificant amounts of LPS were detected in the kidneys[94]. Apart from enlargement of tubular lumen no other histological changes were evident in the kidneys[94]. Small amounts of radio-labelled LPS were found in the urine.

To sum up, LPS, after being injected intravascularly, is first taken up by the Kupffer cells and the granulocytes. After 2-3 hours LPS redistributes itself into the hepatocytes, to be excreted, still possibly in a toxic form, with bile into the gastrointestinal tract. The appearance of LPS in the liver parenchyma and in the lungs were followed by damage of these organs. In the lungs, LPS is detected later than in the liver. The presence of macrophages with phagocytosed LPS in the alveoli and bronchioles suggests that LPS may be excreted through the lungs as well. Small amounts of LPS may also be exreted via the urine.

2.1.2.4 Tolerance to LPS

The activities of the RES can be stimulated by the presence of certain non-immunoglobulin agents present in the serum or be reduced by the administration of certain pharmacological agents[27,97-101]. A single dose or repeated injections of sub-lethal amounts of LPS render experimental animals and humans less responsive to a second dose of LPS challenge (Schade et al.[22]). This phenomenon of increased resistance to LPS is called "tolerance". Zweifach and Thomas[97]. observed that animals made tolerant to endotoxin isolated from one bacterial species were also tolerant to endotoxins isolated from immunologically unrelated microorganisms. They suggested that the tolerance did not depend on the formation of specific antibodies and, postulating that it was due to a significant increase in stimulation of the phagocytic activities of the RES. Furthermore, they found that pre-treatment with zymosan, a lipopolysaccharide-protein complex derived from yeast cell walls, also increased tolerance to endotoxin. On the other hand, the administration of colloidal agents caused a blockade of RES function resulting in the complete loss of tolerance. Similar changes of the RES function were noticed by others[22,98-101]. However, according to Kim and Watson[126], "pyrogenic tolerance to endotoxin results from acquisition of classical immune mechanisms and not as a result of non-specific mechanisms". These researchers proposed that antibody assists the RES in the clearance and destruction of endotoxin and observed that the intravenous injection of endotoxin (100ug/kg) killed piglets which were colostrum-deprived and therefore antibody-poor. There were no deaths in those that received colostrum.

To sum up, the Kupffer cells of the liver RES appear to be the main sites of removal of LPS from the circulation. After being taken up by the Kupffer cells, LPS may pass into the hepatocytes to be excreted with bile into the faeces. Small

amounts of LPS may also be cleared by the macrophages in the spleen, lung and kidneys. Anti-LPS IgG antibodies, by binding with LPS, may enhance their removal by the cells of the RES. However, it is not certain whether LPS is detoxified during the process of its clearance from the circulation by the RES since no major degradation of phagocytosed LPS complex was detected within 3 hours of being administered to the animal[49]. Furthermore, LPS may form complexes with HDL in the plasma and the LPS-HDL complex is cleared at a slower rate from the circulation than "free" LPS[48,49,92]. Conflicting reports have appeared regarding the detoxification of LPS when it complexes with HDL[49,92,104,122,123]. It has been stated that LPS may also activate the complement system, causing physiological changes such as alterations in vascular permeability, leucocyte "stickiness", opsonization of microbial invaders and amplification of specific antibody activities[105]. The tolerance or increased resistance to LPS can be increased by injection of sub-lethal quantities of LPS into experimental animals and human. Two explanations have been offered to explain the phenomenon of tolerance. According to one, tolerance is due to the increased phagocytic activities of the cells of the RES[22,97-101] and according to the other, tolerance is accomplished by the formation of antibodies against the LPS[126].

2.1.3 Biological effects of LPS

2.1.3.1 Introduction

Endotoxins evoke a wide variety of biological responses in the host and are reported to affect almost all organ systems. However, in many cases it is the host's response to the gram-negative bacteria and their endotoxins, rather than the organism (or LPS) itself, which poses the ultimate threat to host tissues. Schade et al.[22] correctly stated that the reactions in the host to endotoxins are largely due to the action of endogenous mediators produced and released

by host cells in response to LPS activation rather than by LPS itself. Some of the mediators released and thought to play a role in the pathophysiology of endotoxin shock are: histamine[127-129], kinins[130], serotonin[131], epinephrine and norepinephrine[132,133], vasoactive intestinal polypeptide[134], phospholipase A_2[135], macrophage-insulin-like activity (MILA)[136], metabolites of arachidonic acid[137-143], opioids[144,145], interleukin-1[146,147], and cachectin[148]. In recent years, however, the last four have been given prominence in the literature and they will be discussed below.

i) Metabolites of arachidonic acid

The plasma concentrations of prostaglandins E and F increase early in lethal endotoxin shock. Inhibitors of their synthesis attenuate the haemodynamic events, though in most studies survival was not improved[137,138,149-151]. On the other hand, pretreatment with indomethacin, an inhibitor of prostaglandin synthesis, prevented the acute pulmonary hypertension and oedema, and increased the survival time in cats[152]. It was found that in the baboon indomethacin increased survival after LD_{73} endotoxin administration[153].

In addition to the role of prostaglandins on cardiovascular parameters, prostaglandin E_2 plays a significant role in induction of fever during endotoxaemia[139,146,147].

Attention has recently been directed towards thromboxane A_2 (TxA_2), prostacyclin (PGI) and leukotrienes. Their exact role in endotoxin shock, particularly in the mechanism of cardiovascular changes, is not known, although it is known that TxA_2 and PGI exert potent opposing effects, with TxA_2 being a potent vasoconstrictor and aggregator of platelets, and PGI a potent vasodilator and counteractor of platelet aggregation[22,140-142]. In some species both TxA_2 and PGI are released early in endotoxin shock and are associated with endotoxin - induced pulmonary changes[154]. In other species, TxA_2 is produced early in endotoxin shock, and is associated

with elevated blood pressure[155]. Yellin et al.[142] found that TxA_2 was invariably released early in endotoxin shock in all species, while PGI was elevated early, in some species in endotoxin shock whilst it was elevated later in other species, including man. It is therefore possible that TxA_2 is associated with haemodynamic changes which occur early in endotoxin shock and PGI with the late stage of endotoxin shock.

Rats deficient in essential fatty acids were totally refractory to endotoxin shock and exhibited 100% survival[140]. Moreover, prophylactic treatment with an inhibitor of thromboxane synthesis (7-IHA) improved the survival rate in endotoxin treated rats[141]. Some investigators, nevertheless, found that pre-treatment with a specific thromboxane synthetase inhibitor (OK 1581) failed to improve survival although it prevented pulmonary artery hypertension and a rise in plasma thromboxane level[156]. Still, none of the above inhibitors of prostaglandin synthesis have been used in septic and non-septic shock in man.

Though it has been found that leukotrienes are released during endotoxin shock[143] and are thought to play a role in endotoxin pathophysiology, their mechanism of action is obscure[22].

ii) Opiates (endorphins)

Holaday and Faden[144,145] found that endorphins cause, in part, the hypotension of shock since the opiate receptor antagonist, naloxone, increased the systemic arterial pressure in rodent endotoxin shock although survival was not improved. Others found that naloxone administration improved the arterial pressure, cardiac output, myocardial performance and survival in a canine endotoxin shock model[157]. The effects of naloxone appeared greatest in animals, which showed an early hypotensive response to endotoxin[145]. This suggests that endorphins are

predominantly released early in endotoxin shock and other factors become important subsequently in the pathophysiology of endotoxin shock[145]. In a recent study, it was shown that pretreatment with naloxone attenuated the endotoxin-induced decrease in superior mesenteric arterial blood flow, increases in portal venous and pulmonary arterial pressures, the fall in mean arterial pressure, cardiac index and the first derivative of left ventricular pressure ($LVdP/dt_{max}$) or maximal rate of rise of left ventricular pressure[158]. Furthermore, naloxone pre-treatment prevented the bloody diarrhoea and improved 24-hour survival in endotoxin administered dogs[158]. However, naloxone has not been used in human septic or non-septic shock.

Beta endorphins, one of the major endogenous opiates, are secreted from the pituitary gland and have been implicated as a cardiovascular depressant[13], i.e, they possibly act on the brain stem cardiovascular centres.

In summary, it appears that endorphins play a role in the pathogenesis of endotoxin shock especially on cardiovascular parameters. However, its exact mechanism of action in endotoxin shock and the use of naloxone have still to be determined[13].

iii) Interleukin-1 (IL-1)

It was initially proposed that fever was due to the ability of various substances, usually of microbial origin to stimulate phagocytic cells (especially the polymorphonuclear leucocytes) to synthesize and release a heat labile protein called endogenous pyrogen (EP)[159]. However, it recently became clear that endogenous pyrogens, in addition to inducing fever, is responsible for a number of other biological activities, including stimulation of T cells, increasing hepatic acute-phase protein synthesis, activating polymorphonuclear (PMN) leucocytes and affecting metabolic activities[160]. Because of the wide range of activities, endogenous pyrogens became known as interleukin-1 (IL-1)[146].

Recent studies show that besides PMN leucocytes,[162] mononuclear phagocytes[146,147,160,161], endothelial[163] and other cell types also synthesize and release IL-1.

According to Dinarello and his co-workers[146,147,160], IL-1 is a primary mediator of host responses to infections and may exert its effects either directly or in concert with other host products. Endotoxin appears to be the most potent inducer of IL-1 production, in vitro, and its production may occur at levels of endotoxin below the sensitivity of the Limulus assay[146]. In addition to endotoxin, cachectin or tumour necrosis factor (discussed later) also induces the release of IL-1[163].

IL-1 initiates fever by inducing the release of prostaglandin E_2 in the thermoregulatory centre in the anterior hypothalamus[146,147,160]. Furthermore, IL-1 also increased the number of immature circulating neutrophils, and depressed the levels of serum iron and albumin concentrations[147,160]. Moreover, marked neutrophilia[164] and hypoalbuminaemia occur during endotoxaemia (see below - section 2.1.3.4).

IL-1 had a profound effect on vascular endothelial function. It activated endothelial cells in vitro to synthesize and release prostacyclin[165,166], and prostaglandin E_2[166]. It also stimulated the plasma membrane of endothelial cells so that neutrophils, monocytes and lymphocytes adhered to them[167]. In addition, IL-1 also induced an increase in tissue factor or procoagulent activity by endothelial cells[168], increasing coagulation and inducing the production of plasminogen activator inhibitor[169] which, further, exacerbated thrombosis. IL-1 increased the release of throboxane by PMN leucocytes and caused platelet aggregation[147]. These activities of IL-1 may be associated with disseminated intravascular coagulation, frequently

observed in endotoxaemia[170]. Thus the endothelial cells may be both a source and target for IL-1. However, the exact mechanism of action of IL-1 in the pathogenesis of endotoxin shock is not known.

From the foregoing we observe that IL-1 may be produced either directly in response to endotoxin or by the action of cachectin (tumour necrosis factor). In addition to producing fever, IL-1 shows a wide range of biological activities such as activation of lymphocytes and PMN leucocytes, increasing the number of PMN leucocytes, inducing blood coagulation and depressing serum iron and albumin concentrations.

iv) Cachectin or Tumour necrosis factor (TNF)

After challenge with endotoxin, high plasma levels of cachectin or tumour necrosis factor (TNF) were produced within minutes in experimental animals[171] and 60 to 90 minutes later in healthy human volunteers[172]. Cachectin is secreted by macrophages in response to endotoxin action, and travel via the circulation to distant sites in the body where it binds to specific receptors, exerting discrete metabolic effects[171,172]. The major tissue targets for cachectin include the liver, skin, kidneys, lungs, gastrointestinal tract and adipocytes[171]. Once induced by endotoxin, macrophages produce copious quantities of cachectin within minutes of intravenous administration in rabbits[171]. The hormone has a very short half-life of approximately 6 minutes[173]. Macrophages from endotoxin-resistant mice, in contrast to endotoxin-sensitive mice, failed to produce cachectin in response to endotoxin[174,175].

It is increasingly being accepted that cachectin plays a major role in the pathogenesis of endotoxin shock, and that it is responsible for most of the deleterious effects of endotoxaemia, such as, fever, metabolic acidosis, diarrhoea, hypotension and DIC, leading to death[148].

Administration of large doses of cachectin to animals directly mimics the clinical syndrome produced by endotoxin[174,175]. Rats to which low doses of cachectin were administered exhibited mild to moderate tachypnoea and hypotension, and then recoved[174]. When higher doses, were administered metabolic acidosis and haemoconcentration occurred, and the levels of lactate, glucose (transient) and potassium in the plasma were elevated, and death resulted from respiratory arrest. At necropsy, diffuse hyperaemia and punctate haemorrhages in the lungs, ischaemia of the bowel with haemorrhagic lesions or necrosis were observed. In addition, acute renal tubular necrosis was observed[174].

Passive immunization of experimental animals with anti-cachectin antibodies protected them from the lethal effects of endotoxin, but the animals still developed fever[174,176]. The fever was probably due to the action of IL-1 which, as mentioned above, is released in response to endotoxin. Cachectin is a potent pyrogen, causing fever both through a direct effect on the hypothalamic thermoregulatory centres, by inducing the release of prostaglandin E_2[177] and through the induction of IL-1 biosynthesis and release. Interaction of cachectin with endothelial cells (in vitro) leads to IL-1 production[178]. There is a biphasic fever in rabbits after the administration of intravenous cachectin[177]. The initial fever which peaked at 45-55 minutes after administration was produced by the cachectin itself and the second peak was produced by the IL-1 induced by cachectin[177].

Cachectin activates neutrophils, increasing their phagocytic activity and adherence to endothelial cells[179]. In addition, interaction of cachectin with vascular endothelial cells stimulated the production of tissue factor or procoagulent from these cells and inhibited the expression of thrombomodulin on endothelial surfaces thus prolonging coagulation and resulting in the formation of DIC associated

with endotoxaemia[180]. Stimulation of the release of IL-1 by cachectin may further augment the coagulation process in endotoxin shock.

After binding with tissue membranes (liver, skeletal muscle, adipocytes) cachectin suppressed the expression of several specific messenger RNA (mRNA) species and may have affected widespread changes in cellular metabolism[181]. Cachectin suppressed the biosynthesis of glycerophosphate dehydrogenase mRNA in rat adipocytes, with a parallel decreased production of glycerophosphate dehydrogenase[181]. In addition, it prevented the synthesis of lipoprotein lipase in adipose tissue, thereby preventing the uptake of exogenous triglyceride by fat cells[181,182] and causing the paradoxical hypertriglyceridaemia frequently observed in endotoxin shock (discussed later in the chapter - section 2.1.3.4).

Cachectin may also induce disturbances in carbohydrate metabolism during endotoxin shock. It was shown recently that a crude monokine preparation (from endotoxin-stimulated RAW 264.7 cells) and cachectin, but not IL-1, increased glycogenolysis and lactate production in muscle cells (in vitro)[183,184]. Moreover, the administration of cachectin caused elevated plasma levels of insulin, glucagon and catecholamines in rats[184]. The addition of a corticosteroid (dexamethasone) to a culture medium containing macrophages, prior to or at the time of treatment with endotoxin, completely inhibited an increase in cachectin level in the medium, whereas near normal quantities were present if it was added 2 hours after endotoxin[173]. This demonstrates that once cachectin synthesis has begun corticosteroid has no effect on its synthesis.

To sum up, research on the role of cachectin during endotoxaemia is still in its infancy and its mode of action is not very clear. However, it may well be playing an important role in the pathophysiology of endotoxin shock either directly or by inducing the release of other vasoactive agents, such as prostaglandins, IL-1 from target cells. In addition to being a pyrogen, it contributes to various perturbations in cardiovascular, haematological, endocrinological and metabolic parameters during endotoxaemia. Corticosteroid inhibits cachectin synthesis by macrophages, in vitro, if applied prior to or with endotoxin. This may explain why the early administration of corticosteroid is beneficial in endotoxin shock.

2.1.3.2 Effect of LPS on the cardiovascular system

The changes in the various cardiovascular parameters following a bolus administration of LPS or during septic shock are summarized in Table 1 and are discussed below:

a) Arterial blood pressure

Most investigators agree that hypotension follows the toxic insult of LPS[3-5,7,16,20-24,147,185,186]. However, the time course of its occurrence and the degree of response vary in different mammalian species[186]. There is usually an early profound fall in the arterial blood pressure in dogs[149,150,158,186-195], rats[144,196-198], cats[152,199,200], rabbits[186,201,202] and sheep[198,203-205] followed by transient slight recovery, and then a second progressive decline to shock levels.

According to some investigators[186], arterial pressure in monkeys does not show an acute, early fall as in other species, but shows instead a slight rise before falling to shock levels. According to other investigators, there is an initial marked decrease in arterial pressure which remains

TABLE 1: Effect of endotoxin on cardiovascular parameter of different mammalian species.

CARDIOVASCULAR PARAMETERS	DOG	RAT	CAT	RABBIT	SHEEP	MONKEY AND BABOON	MAN
Blood pressure: Arterial	Biphasic response - initial immediate drop (5-10min), followed by a transient, partial recovery and then second progressive decline to shock levels (149,150,158,186-195)	Similar to dogs, (144,196) but in some second decline absent (197,198)	Similar to dogs (152,199,200)	Early acute drop close to shock level followed by progressive return to control (186, 201,202) or remain stable and fall just before death (186) or a progressive fall throughout (202)	In some similar to dogs (203). In others no change or elevated and followed by a progressive decline (198,204,205)	Marked decrease throughout, (130, 206-209). In some an initial rise is followed by a fall to shock levels (186)	No change or a rise followed by a fall (186, 210)
Portal	Immediate increase either manitained at elevated level before returning to normal level (158,192,193) or initial increase is followed by progressive decline (187,188,191)	-	Decreases within 30 min and remained decreased (200) or slight rise, followed by fall to normal and late fall (186)	slight rise, followed by return to normal (186) or decreases and remained decreased (202).	-	Slight rise followed by return to normal (186)	-
Central venous	significantly reduced (193,194)	Slow decline throughout (197)	Initial increase followed by marked fall (200)	reduced throughout study (202)	-	-	-
Cardiac output	Immediate fall, followed by slight recovery and progressive fall (149,150, 158,186,187,191-193,195).	Sharp fall, followed by slight recovery (30 min) and progressive decrease (197)	Initial fall (2 hr) followed either by recovery and progressive decline (152,199) or progressive decline (200)	-	Immediate fall, followed by a return to near normal and then a progressive decline(203-205)	steady decline (207)	Fall, followed by a severe fall or initial increase followed by a fall (13,186,210)
LVdP/dt max (First derivative - left ventricle pressure)	Initial rapid fall, followed by slight recovery and then progressive fall (149, 150,158,192,193).	-	initial fall, followed by recovery towards control level (99,199)	-	-	-	-
Total periheral resistance	Rise followed by a rapid fall (30min) and progressive decline (186,191). In some initial fall, followed by a rise (150,191-193).	Initial rise followed by slight recovery (30min) then an increase and final decline (197)	-	-	Immediate decline followed by transient recovery and a second decline (203,204)	Variable: decrease in late stage(206,207)	Increase (210) and decreases in later phase of bacteraemic shock (207).
Heart rate	Variable: Immediate decline followed by recovery and then second decline (191)), or decline followed by a maintained low rate (158, (192-194)	Variable: after initial increase, remained elevated (197) or immediate fall followed by rise (198)	Variable: initial rise followed by progressive fall (152) or transient bradycardia followed by a rise (199)	-	Variable: raised (203) or initial tachycardia followed by steady decline (204)	Variable: constant or bradycardia (207) or increased (130)	-
Pulmonary artery pressure	Initial rise, followed by slow, progressive decline (158,186,188)	-	Marked rise. In survivors rise followed by decline (152)	Slight rise followed by return to normal (15min) (186)	Marked increase followed by decline (203,205)	-	-

low throughout the study period[206-209]. In man an intravenous administration of endotoxin or typhoid vaccine (whose toxic effects are believed to be caused by the LPS it invariably contains) may be followed by a latent or prodromal period of 30-90 minutes before any biological responses are noticed[186,210,211]. At the end of this period, depending on the dosage of endotoxin, core temperature begins to rise rapidly, with or without a typical chill. Following this period, there is usually a period of chilly sensation with shivering. During this latter phase the skin is cool and may be cyanotic because of vasoconstriction, and there may be a slight rise in arterial blood pressure[186,211]. The fever reaches its peak during the second or third hour and then begins to drop rapidly. Cutaneous vasodilatation, profuse sweating, pupillary constriction and a marked drop in arterial blood pressure occur during the phase of decreasing temperature[186,211]. Arterial pressure may remain low for several hours or may fall to shock levels[211].

It is possible that the decreased cardiac output due to a decreased venous return caused the initial, transient fall in blood pressure. It is believed that either splanchnic pooling of blood (dogs and cats[186-195,200]) or a generalized pooling (primates and other species[130,206,207]) was responsible for the decline in venous return.

The intravenous administration of endotoxin to dogs induced the release of histamine which caused constriction of the hepatic vein[186-189] with consequential splanchnic pooling. On the other hand, some researchers were not able to find any evidence of splanchnic pooling after the administration of endotoxin[212]. It was assumed that the generalized pooling in some species was due to the effect of histamine[127-129,213], kinins[130] (potent vasodilators[214]) and other vasoactive agents (discussed previously) released by the action of endotoxin.

The initial drop in blood pressure, possibly, activated the baroreceptor reflex and the sympathetic nervous system[185] or caused an increased release of angiotensin[215] (a potent vasoconstrictor[214]) and resultant transient recovery of blood pressure.

Furthermore, endotoxin, either directly[216,217] or by inducing the release of histamine[127-129,213] and oxygen free radicals from PMN leucocytes[218], damaged the permeability property of capillary endothelium and gave rise to extravasation of plasma[219,220] and the reduction in blood volume. The decreasing venous return and blood volume and vasodilatation of peripheral blood vessels (by the action of the released vasoactive agents, as discussed previously) led to a progressive decline in blood pressure to shock levels during the later stages of endotoxaemia.

According to Fine[50] and others[192,193], the refractory state of shock is the result of a reaction of the nervous system to bacterial endotoxins. Fine and his co-workers observed that denervation of the abdominal viscera (close to the coeliac plexus) prior to the administration of endotoxin preserved the integrity of the peripheral circulation and changed the mortality rate from 80% to 30% and under[50].

Recently Kayama[192,193] hypothesized that the hypotensive effect of *E.coli* endotoxin was mediated through a central autonomic blood pressure-regulating circuit by stimulation of central α-adrenergic receptors. He noticed that intravenous administration of a new pharmacological agent (RA 642) with hypertensive properties, acted on centres in the central nervous system and beneficially reversed the hypotension and the decreased renal blood flow caused by endotoxin.

b) Portal venous pressure

In the dog there is an immediate, significant increase in portal venous pressure which is either maintained at elevated levels for about an hour before falling to normal levels[158,192,193] or the initial increase is followed by a progressive decline[187,188,191]. The increase in portal venous pressure is indicative of the pooling of blood in the splanchnic bed, (mainly in the liver)[186-188,191]. It was proposed by a number of investigators that the splanchnic pooling was caused by an intense vasoconstriction of the hepatic vein[185-189] (discussed above). The portal venous pressure, in the other species, either decreased initially and then continued to decrease slowly[200] or rose slightly before decreasing[186,202]. It is possible that if the pooling of blood occurs in species other than dogs, it is not localized to the splanchnic bed, but it is generalized[207].

c) Central venous pressure

In most species the central venous pressure decreased after the administration of LPS[193,194,197,202], except in the cat where an initial increase was reported[200]. The presence of a depressed central venous pressure suggests that failure of the heart is not primarily responsible for the hypotension during endotoxaemia[200].

d) Cardiac output

In all species the cardiac output shows either a progressive or a marked decline during endotoxaemia[186,207,210] or there is an immediate rapid fall, followed by a recovery towards normal levels and then a progressive decline[149,150,152,158,186,187,191-193]. As in the case of the mean arterial pressure, septic shock in man is often manifested, first by, a hyperdynamic or "high flow" state, then by a hypodynamic or "low flow" circulatory state[23,186]. The late stage of shock is always characterized by a fall in

cardiac output[186,210], which is probably due to a decrease in venous return[158,186,191,207,209] or on the depressant action of endorphins on the cardiovascular system (discussed previously). This decrease in venous return, as discussed above, has been attributed to peripheral pooling of blood, especially in the splanchnic bed[186-195,200], or to a generalized, continuous pooling in all tissues[130,206,207], or to splanchnic pooling coupled with extravasation of plasma and cellular elements[191,195]. However, according to Park et al.,[212] inadequate venous return is not the crucial factor in endotoxin shock because the measurement of the diameter of portal vein during endotoxin shock did not show an increase but a decrease in diameter, suggestive of a lack of splanchnic pooling. Park et al.[212] suggested that the decreased cardiac output was due to impairment of cardiac function.

e) First derivative of left ventricular pressure ($LVdP/dt_{max}$)

Following a bolus injection of LPS the first derivative of left ventricular pressure ($LVdP/dt_{max}$), an index of left ventricular contractility, showed a rapid fall initially, followed by a recovery towards control level[149,150,158,192,193,199] and a progressive fall thereafter[149,150,158,192,193]. These data suggest that decreased left ventricular contractility occurs during the early and late stages of endotoxaemia[221]. However, according to other investigators, a primary cardiac failure does not occur during the early stages of endotoxin shock but may occur in the late stages[186,197,207,222,223].

f) Total peripheral resistance

After LPS is administered parenterally, variable changes in total peripheral resistance (TPR) have been reported (Table 1). In some studies on dogs, the administration of

endotoxin, resulted in an initial increase in TPR followed by a rapid decline within 30 minutes, thereafter by a progressive fall[186]. Other studies show an initial fall in TPR followed by a rise[150,191-193]. In rats, intravenous administration of endotoxin caused an initial transient increase followed by a transient decrease towards normal[197]. Thereafter, the TPR increased slightly before declining again. In sheep, the TPR showed an immediate decline after the administration of endotoxin[203,204]. This was followed by a transient recovery and then a second decline. In man and other primates the TPR always fell in the late stages of endotoxin shock[206,207,210]. An increase in TPR is indicative of vasoconstriction in localized or generalized areas of the systemic circulation whilst a decrease is indicative of vasodilatation[207].

The hyperdynamic state of shock is associated with an early vasodilatation, and the hypodynamic state, first, with vasoconstriction and, prior to death, vasodilatation.

g) Heart rate

In most studies, responses of heart rate to endotoxin have been variable (Table 1). However, following an intravenous administration of endotoxin, the heart rate in dogs always showed an immediate fall[158,191-194]. Thereafter the heart rate was either maintained at low levels[192-194] or it recovered slightly before declining again[191]. In rats, cats and sheep, the heart rate either increased and remained at a high level[197,203] or it fell initially and then rose again[198,199,204]. Non-human primate either showed no change in heart rate or had bradycardia[207] or increased rates[130].

h) Pulmonary artery pressure

In most species the pulmonary artery pressure rose initially and then declined progressively[152,158,186,188,190,202,203-

[205]. According to Parrat and Sturgess[151] the acute pulmonary changes, viz., hypertension and decreased pulmonary compliance that occur in cats within a few minutes of endotoxin administration, ultimately contribute to the severity of the shock phase. This state is caused by the obstruction of blood flow due to endotoxin-induced platelet and granulocyte "plugs" formed in arterioles and capillaries[164,224-226] (discussed below) as well as by vasoconstriction[151].

i) Blood flow

Changes in regional blood flows, in response to endotoxin, have not been studied as intensively as other cardiovascular parameters.

1) Kidney

Intravenous injection of endotoxin showed a biphasic (an initial rapid fall, followed by a slight recovery and then a secondary progressive fall) response in renal blood flow in dogs[192]. The decrease in renal blood flow sometimes gave rise to acute renal failure with severe oliguria or anuria[54,213]. Furthermore, endotoxin caused a redistribution of blood flow within the kidneys, itself[227]. Endotoxin resulted in a five-fold increase in blood flow to the medulla at the expense of a reduced flow to the cortex[227]. This reduced cortical blood flow could further compromise glomerular function, leading to anuria and kidney failure.

2) Splanchnic circulation

Lillehei et al.[228] found in experimental animals that the blood flow to the superior mesenteric artery (SMA) fell almost to zero within an hour after profound shock had been induced by endotoxin, haemorrhage or epinephrine. Similar changes in splanchnic blood flow were observed by

others[57,58,158,181,229,230]. Infusion of sub-lethal quantities of endotoxin into a branch of the mesenteric artery in pigs caused intense margination of leucocytes (that is, adherence of leucocytes to the wall of blood vessels) and sluggish blood flow[216]. Similar experiments on rabbit and guinea pigs showed a degranulation of mast cells adjacent to blood vessels, suggesting that they had released histamine and serotonin[217]. Coincident with this, arterioles constricted, venules dilated and blood flow became sluggish[217].

This decreased splanchnic blood flow could cause ischaemic or hypoxic damage to the intestinal mucosa, resulting in leakage of LPS into the circulation.

3) Skin

After a bolus injection of endotoxin to guinea pigs blood flow to the skin was reduced[231], the cause being cutaneous vasoconstriction as a result of a drop in body temperature in order to prevent further heat loss[231]. Skin blood flow is usually decreased in human septic shock[232,233]. It has been suggested that the reduction in cutaneous blood flow in septic shock is due to the release of vasoactive substances[233]. However, as the following discussion indicates, the reduced skin blood flow is due more likely to a direct action of endotoxin or to the action of endogenous pyrogens on the hypothalamic thermoregulatory centres.

4) Brain

Administration of endotoxin was associated with an early (within 2 hours) reduction in blood flow (by 30-40%) in several regions of the dog's brain. The flow was decreased by 39-52% at the end of 4 hours[234-236]. Others have reported a decrease in common carotid artery blood flow[193]. A fall in cerebral perfusion pressure was associated with the early

fall in blood flow, but the subsequent fall was due to a paradoxical increase in cerebral vascular resistance in the face of a falling blood pressure and constant arterial pCO_2 and pH and decreased pO_2. However, the mechanism involved in the increased vascular resistance is not known; it could be due to the action of endotoxin-induced release of vasoactive agents. The reduced blood flow to the brain coupled with a concomitant hypoglycaemia (to be discussed later) may result in malfunction of nervous tissues especially in the brain stem where the major physiological control centres are located[233]. This may be a contributing factor to the irreversibility of endotoxin shock[234].

Prophylactic treatment with high doses of steroids (methylprednisolone sodium succinate, 30 mg/kg) either completely prevented or ameliorated the endotoxin-induced decreases in regional blood flows[235,236]. In addition, treatment with steroids elevated the arterial glucose concentration. The preservation in regional blood flows by steriods was due to decreased regional vascular resistances in the brain[235,236]. The beneficial effects of steroids on brain blood flow may also be due to inhibition of cachectin and IL-1 release.

To recapitulate in brief, within five minutes of being challenged with endotoxin, the cardiac output and arterial blood pressure decrease profoundly in most animal models (Table 1). At this time the total peripheral resistance is increased in dogs and rats, the implication being that in these models, there is either a pooling of blood, which is not indicated by the change in TPR, or the pumping action of the heart is compromised, or both activities are happening simultaneously. Endotoxin also induces a decline in the central venous pressure, implying a diminished venous return. Following the decline, the blood pressure and cardiac output recovered transiently before falling again to shock levels. The TPR in sheep and primate models showed a

decline throughout, indicating peripheral vasodilatation and possibly peripheral pooling of blood. A decreased blood flow to the splanchnic region might lead to a release of additional endotoxin from the gut lumen. Furthermore, the mechanisms involved in the haemodynamic dysfunctions during endotoxaemia appear to be very complex and the exact mechanism may be very difficult to identify.

2.1.3.3 Haematological disturbances

The haematological alterations observed following an intravenous bolus injection of endotoxin into experimental animals and during endotoxin or septic shock in man are summarized in Table 2 and discussed below.

2.1.3.3.1 Thrombocytes (platelets)

Bacterial endotoxins are known to have a profound effect on blood thrombocytes or platelets in a number of species. The mechanism of platelet-endotoxin interaction depends on the presence or absence of specific LPS receptors on the platelet membrane[241]. Blood platelets from rats, rabbits, dogs and guinea pigs appeared more responsive to endotoxins than those from human and sub-human primates because platelets from the latter lack immune adherence receptor sites in their membranes[241].

It can be seen from Table 2 that the response to endotoxin is an early thrombocytopenia followed by a slow recovery[22,92,170,225,226,237-241]. However, in a few cases biphasic responses were reported: an immediate decrease in platelet count, followed by a transient partial recovery, and a second decline[201,242]. In primates, on the other hand, either no change[130,208,241] or a progressive decline in platelet count[243] was reported.

Endotoxins caused platelet swelling, sphering and

Table 2: Effects of endotoxin on thrombocyte and leucocyte counts, coagulative changes, fibrinogen level, clotting time and haematocrit in experiment animals.

Blood Component	Effects of endotoxin
Thrombocyte (platelet count)	Early thrombocytopenia (within 5-15min) followed by slow recovery (22,92,170,225,226,237-241) or biphasic change: immediate decrease, followed by a partial recovery and a second decline (201,242) or in primates - no change(130,208, 241) or progressive decrease (243)
Leucocyte count	Extreme leucopenia (5 min) followed by depressed count (130,225,227,238) or extreme leucopenia followed by slow recovery or leucytosis (164,211,241,244).
Disseminated intravascular coagulation	Occurs early in endotoxin shock (22,170,211,224,226, 238,244-247)
Fibrinogen level	Initial decline which remains at low level (170,226) or is followed by a gradual elevation to above normal level (244)
Clotting time	Depressed (238,244) or prolonged (226)
Haematocrit	Increased (109,170,203,220,225) or no change (207,208,216)

degranulation[248], as well as aggregation or clumping and the release of platelet constituents including ADP, platelet factor 3, and vasoactive amines such as histamine[241], serotonin[249] and thromboxane A_2[140-142].

The early, rapid drop in circulating platelet count in experimental animals, in response to endotoxin, depended on serum complement[238,241,245,246]. Endotoxin activates both the alternate and classical complement pathways but the activation of the classical pathway is an absolute requirement for the development of thrombocytopenia[238,242]. In addition, the reduced platelet count could also be due to platelet aggregation. Endotoxin, in the presence of complement, activated PMN leucocytes to release oxygen free radicals[218]. Oxygen free radicals (in vitro) induced platelet aggregation over and above that caused by LPS alone and also released serotonin from platelets[249]. Platelet aggregation could also be caused by TxA_2 released from platelets[250] and macrophages[140-142] by the action of endotoxin. Platelet aggregation could cause "plugging " of capillaries resulting in reduced blood flow and the possible development of ischaemia and tissue damage[224,226].

2.1.3.3.2 Leucocytes

An early response by white blood cells to intravenous administration of endotoxin is leucopenia, especially neutropenia. Following an initial leucopenia the cell count either remained depressed for 3 to 4 hours[130,225,237,238] or showed a slow recovery to normal levels[164,211,241,244]. This recovery was often accompanied by a large number of immature leucocytes[164,241]. The mechanism for the observed leucopenia is not clear. Stetson[164], however, suggested that the early disappearance of neutrophils could be due to sequestration of these cells in capillaries, particularly in the lung. In the presence of complement components (C5a) endotoxin may activate PMN leucocytes to release TxA_2, which enhances the

adherence of these cells to endothelial surfaces[251]. These vasoactive agents could also enhance platelet aggregation[22] and cause cardiovascular derangements. In addition, PMN leucocyte-endotoxin interaction could also lead to the release of interleukin-1[162].

2.1.3.3.3 Disseminated intravascular coagulation (DIC)

One of the major effects of endotoxin in the circulation is triggering of the blood coagulation system[170,211,238,239,241,242,244-247].

The mechanism by which endotoxin accomplishes this is not fully understood[252], but it is felt that both the intrinsic and extrinsic coagulation pathways, are involved[241,253] (Figure 5). In the intrinsic pathway, endotoxin activates Hageman factor (factor XII) which then triggers a cascade of reactions resulting in the formation of a fibrin clot[241,252,253]. For the extrinsic pathway, tissue factor (procoagulant activator or thromboplastin) is required[241,252]. Endotoxin binds with and activates monocytes and granulocytes which then secrete tissue factor (procoagulant activator), which in turn combines with factor VII resulting in a chain of coagulative processes, eventually resulting in blood coagulation[241] (Figure 5).

Of late, there has been a growing awareness of the active role of vascular endothelium in the regulation of haemostasis. Exposure of cultured endothelial cells to endotoxin induced the release of tissue factor from these cells[252,254]. Endotoxin activated PMN leucocytes to release oxygen free radicals which caused endothelial damage[255-257]. According to Schorer et al.[256] damage to endothelial cells exposes tissue factor, or procoagulant activator, or thromboplastin which combines with factor VII, discussed above.

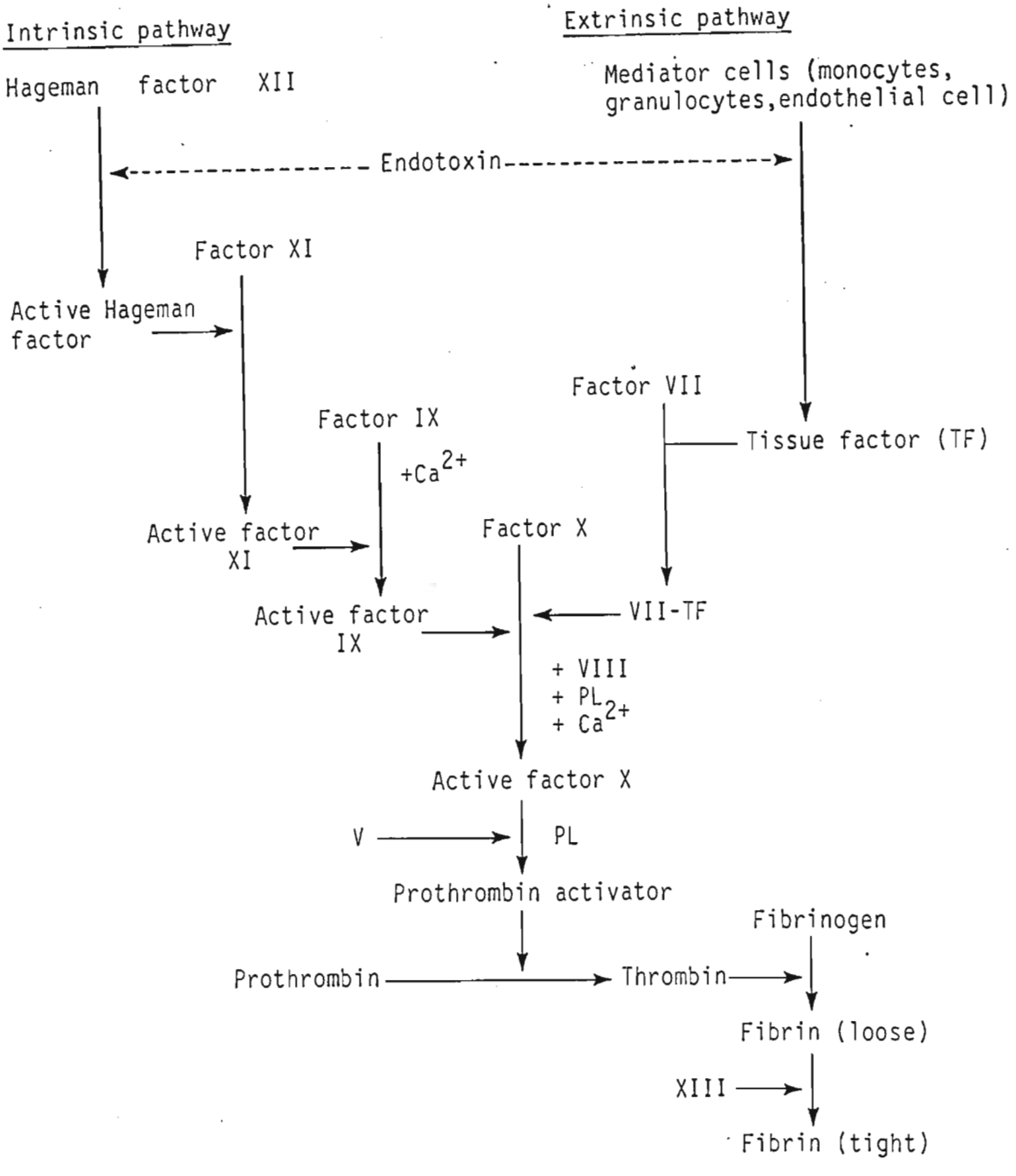

Figure 5: Schematic representation of the activation of the intrinsic and extrinsic coagulation pathways by endotoxin (haemostatic factors are indicated by Roman numerals, PL = platelet phospholipid).

Disseminated intravascular coagulation (DIC) has long been recognized as a serious complication of gram-negative septicaemia and endotoxaemia[170,225,240,241,258] and has often been labelled as a major cause of irreversibility and death in these disease states[226,246]. Fibrin and fibrin complexes resulting from DIC were observed to plug microvessels in critical organs, including the kidney, gastrointestinal mucosa, lungs, brain and the heart[3,224,226,227,246,256,258]. Renal dysfunction secondary to plugging of glomeruli is a common and often fatal event of endotoxin shock[226,227,246,258]. It has been proposed that DIC occurs during the normotensive phase of sepsis and endotoxaemia and this may be followed by ischaemia and hypoxia in an organ[226,245,246].

A peculiar effect of DIC is that the patient frequently haemorrhages in a number of places because many of the clotting factors are consumed and too few remain to allow for normal haemostasis[3,245]. This could explain the increased clotting time[226] and fibrinogen levels[170,226,244] noted in some studies.

To conclude, endotoxin causes early thrombocytopenia, leucopenia and disseminated intravascular coagulation. These changes could aggravate the cardiovascular changes caused by the previously described endotoxin-induced release of vasoactive agents. Adhesion of leucocytes to endothelial surfaces may narrow lumen of blood vessels and cause sluggish blood flow. In addition, the presence of aggregated platelets, and leucocytes and fibrin clots may plug arterioles and capillaries in organs such as kidneys, the gastrointestinal tract, lungs, heart, and brain leading to damage to these organs. It has been postulated that these haematological changes cause the irreversibility of shock and death in endotoxaemia.[226,246]

2.1.3.4 Metabolic disturbances

The prominent metabolic changes reported to occur in experimental animals following a bolus administration of endotoxin or in septic patients are listed in Table 3, which shows a progressive deterioration of energy production, accompanied by lacticacidaemia and metabolic acidosis, during endotoxin shock.

Glucose dishomeostasis is a common finding in experimental animals and septic patients[198,208,259-264]. The early responses to endotoxin are marked by hyperglycaemia[198,208,260-264], lacticacidaemia[198,208,211,259-264] and, to a lesser extent, leakage of potassium ions from interior of cells[279,280].

Following the early hyperglycaemic phase, glucose level in blood decreases and hypoglycaemia appears to be one of the metabolic hallmarks of endotoxaemia during the late or terminal stages[198,208,230,259-264].

It is believed that the early hyperglycaemia, which may initiate an hyperinsulinaemic response[230,260,270,271], is due to increased hepatic glycogenesis[263,266,268]. Transient hyperinsulinaemia occurs during the early phase of endotoxaemia[230,260,270,271]. Some investigators have suggested that the early hyperinsulinaemia exacerbates the hypoglycaemic episode. Still, hyperglycaemia does not always occur during endotoxaemia[265], and this has led to the conclusion that endotoxin induces macrophages to release a monokine with insulin-like activity (MILA) which increases the rate of glucose uptake by cells, and which may stimulate pancreatic ß cells to secrete insulin[260,264]. The increased glucose uptake by peripheral tissues coupled with a decreased hepatic gluconeogenesis caused the blood glucose level to drop, resulting in hypoglycaemia. Other

Table 3: Metabolic changes in septic patients and in experimental animals following a bolus injection of endotoxin.

CONSTITUENT	EARLY PHASE	LATE (> 3 hr) PHASE
Glucose:		
. Blood level	Normal (hyperdynamic state) or hyperglycaemia (198, 208,260-264) or progressive hypoglycaemia (265)	Hypoglycaemia (198,208 230,259-264)
Utilization	Elevated (263,266,267)	Elevated (263,266,267)
Hepatic Gluconeogenesis	Increased during fasted state (263,266).	Impaired during fasted state (261,263,264).
	Mobilization of liver glycogen - fed state (263).	Impaired - fed state(263, 268,269).
Glycogen level	Diminished (263,266,269)	Diminished (263,269).
Lactate level	Increased (198,208,211, 259-264)	Increased (198,208,211, 230,259-264)
Insulin concentration	Increased (230,260,270 271).	Normal or decreased (270,271).
Glucagon concentration	Elevated (1 hr post endotoxin (272)	Elevated (272).
Fat Metabolism:		
Serum Free fatty acid concentration	Elevated (low dose LPS) and decreased (with high dose LPS) (273)	Some as early phase (273)
Serum triglyceride concentration	minimally increased (with low dose) and increased with high dose (273,274)	same as early phase (273,274)
Oxygen consumption	Decreased (209,275,276)	Recovered (275) or remain decreased (276)
Blood pH	dropped (211,230,277)	dropped (211,230,277
Serum albumin level	-	hypoalbuminaemia (8)
Serum calcium level	-	hypocalcaemia (8)
Serum phosphate level	-	hypophosphataemia (278)

investigators believed that alterations in glucose metabolism are due to the effects of cachectin[183,184].

It has been suggested that the decreased oxygen consumption and elevated plasma lactate levels are due to inadequate tissue perfusion[275,276]. The altered glucose metabolism during endotoxaemia may be a crucial factor in the development of lethal endotoxaemia[270]. Moreover, the elevated glucagon concentration caused by elevated catecholamines released during the early stage and by hypoglycaemia during the late stage of endotoxaemia may aggravate the shock state[272].

The administration of low doses of endotoxin (0,3 and 0,9 mg/kg) to Rhesus monkeys produced increments in serum free fatty acids and minimal changes in triglyceride levels[273]. On the other hand, high doses of endotoxin (2,8-9,0 mg/kg) failed to produce an increase in serum free fatty acids but resulted in increased serum triglyceride levels[273]. Elevating total serum lipid levels (mainly triglycerides) occurred in patients with gram-negative infections [274]. Researchers have suggested that endotoxin exerts its effects on lipid metabolism by interfering with the activation of the lipid clearing enzyme, lipoprotein lipase[182]. This effect may be due to the release of cachectin which depresses lipoprotein lipase activity[181].

Other metabolic alterations reported during endotoxaemia are hypoalbuminaemia[8], hypocalcaemia[8] and hypophosphataemia[278] (Table 3). However, neither the mechanism involved in degradation of these substances nor their role in endotoxin shock is clear, but hypoalbuminaemia may cause extravasation of plasma by reducing intravascular osmolarity.

2.1.3.5 Gastrointestinal disturbances

The gastrointestinal complications reported during endotoxin shock include vomiting, diarrhoea and gastrointestinal bleeding[51,212,225,228]. In addition, mucosal lesions[51,212,225], haemorrhagic ulceration of the gastrointestinal mucosa[226] and sloughing of the superficial mucosal layers[226] were detected in endotoxin and septic shock. Possibly, the reduced splanchnic blood flow or elevated local histamine concentrations and other vasoactive agents caused these gastrointestinal dysfunctions[281]. Of 35 patients (who subsequently died with the clinical features of septicaemia but were <u>not</u> bacteraemic), 20 were reported to have had lethal amounts of LPS either in the blood or in the tissues[282]. Endotoxaemia was often associated with a high incidence of gastrointestinal bleeding[282]. Liver damage is also a common finding in septic and endotoxin shock[3,4,94,102,283-288]. The administration of endotoxin to experimental animals itself causes liver damage including necrosis and haemorrhage[94,283-288]. Freudenberg and his co-workers[94] noticed that both the smooth and rough form LPS caused necrosis of liver tissue with haemorrhages. According to Bertok[103], LPS is inactivated by bile acids and he stated that bile acids are significant factors in bacterial LPS detoxification in the liver as well as in the intestine. Therefore, any damage to the liver may decrease bile production and result in imperfect intestinal detoxification of LPS so that more endogenous LPS can enter the circulation[103].

It is possible that lesions of gastrointestinal mucosa may allow the migration of LPS from the gut lumen into the portal circulation. In the presence of a damaged liver, LPS would overflow into the systemic circulation, resulting in the elevated levels.

2.1.3.6 Kidney and Lung

Wilkinson et al.[54] found that most patients with cirrhosis of the liver had elevated plasma LPS concentrations and that these patients showed no evidence of gram-negative infection. The increased absorption of endotoxins (owing to decreased bile secretion[103]), together with impaired hepatic clearance could have accounted for the endotoxaemia[54]. Most of the cirrhotic patients also developed renal failure. The researchers have, therefore, suggested that the renal failure was caused by endotoxin. It was found that patients treated with polymyxin B, (an antibiotic with specific anti-endotoxin properties) recovered from the renal failure. Rats with experimentally-induced obstructive jaundice developed renal failure if they also received a sub-lethal dose of endotoxin[289]. In support of this, endotoxin was administered to rats with portacaval anastomosis, resulting in severe damage to the kidney with fibrin deposits in the glomeruli and tubular necrosis[227]. Normally the endotoxins would have been taken up by the RES of the liver, but in the presence of liver damage the endotoxin "spilled" over into the systemic circulation where it either directly or indirectly induced the release of vasoactive agents which, in turn, reduced renal blood flow. It was assumed that the latter, together with endotoxin-induced DIC and platelet aggregation, resulted in the development of tubular necrosis and renal failure[54].

Throboxane A2 and prostacylin-like materials were released from perfused, isolated rabbit kidneys, pretreated with endotoxin[290]. A generalized Shwartzman reaction developed in rabbits following a second intravenous injection of LPS. The generalized Shwartzman reaction is characterized by fibrin deposits in terminal vascular beds of visceral

organs[211,291]. As a result, blood supply to the affected tissue is interrupted, focal necrosis and interstitial haemorrhages ensue[291]. Although the fibrin deposits may occur in liver sinusoids and pulmonary capillaries, it is the widespread occlusion of the renal glomeruli resulting in bilateral cortical necrosis of the kidneys that is the most frequent pathognomonic lesion of the Shwartzman reaction. The Shwartzman reaction does not occur naturally in rabbits, nor comparable lesions are commonly encountered in man[291]. Light microscopic studies of the kidneys revealed local necrosis, leucocyte infiltration and vascular dilation during the Shwartzman reaction. Ozsan et al.[290] suggested that labile cyclo-oxygenase metabolites of arachidonic acid released from the LPS-pretreated kidneys could be responsible for the production of the generalized Shwartzman-like phenomenon. The reduced blood flow[54,192] or microthrombi formed in renal blood vessels[252] could give rise to oliguria, anuria and renal lesions. These changes could aggravate the shock state.

Adult respiratory distress syndrom (ARDS) is a common and serious complication of septic shock[215,225,292]. The term ARDS is used to describe a multitude of clinical entities (shock, trauma, sepsis) whose final common pathway is hypoxaemia and pulmonary oedema[293-295]. ARDS has been reproduced in experimental animals by the administration of endotoxin[292,296,297]. Bisio et al.[292] observed that acute endotoxaemia resulted in histopathological changes in the lung which were consistent with ARDS seen in septic shock. Cuevas et al.[32] found that lung lesions were present in all animals with persistent endotoxaemia. Furthermore, Freudenberg et al.[94] noticed that both smooth and rough forms of LPS caused histological changes in the lung (oedema of alveolar walls, perivascular and peribronchial tissue with infiltration of neutrophils and mononuclear cells) which became easily detectable 24 hours after endotoxin administration. The mechanism involved in lung lesions and hypoxaemia is

not clear. The platelet and granulocyte "plugs" (see section 2.1.3.3, above) formed in pulmonary capillaries[164,224-226,295] may result in ischaemia and increased capillary permeability[297]. The latter, together with pulmonary hypertension[151,215], may lead to pulmonary oedema and histopathological changes[154,295,297], and the loss of hypoxic pulmonary constrictor response is thought to cause the hypoxaemia[295,296].

To sum up, it can be concluded from the foregoing that almost all organ systems are in some way affected by endotoxin. In addition, endotoxin also induces host's cells to release endogenous pyrogens which in turn, raises the body temperature and causes fever[147,159,177,298-300] (this will be discussed in section 2.3.5). However, Kass et al.,[301] are of the opinion that "...factors other than endotoxin are more critical than endotoxin in determining the lethal outcome of sepsis".

2.2 GUT - DERIVED ENDOTOXINS IN SHOCK

2.2.1 Introduction

The mammalian gastrointestinal track from the duodenum to the colon is normally populated with bacterial organisms from birth[16,302,303]. The concentrations of bacteria increase from 10^1 to 10^3 organisms per gram net weight in the duodenum to 10^9 to 10^{11} per gram net weight in the colon[302]. Gram-negative bacteria are the normal flora in the small intestine and in the colon[302], hence they contribute to the intestinal endotoxins. Fine and his co-workers[34] found in rabbits that an aerobic gram-negative bacterial count of 10^9 to 10^{10} per gram faeces was equivalent to an average of 6 mg endotoxin per gram faeces (range 3-12 mg/g). Under normal circumstances, the permeability properties of the gut prevents the migration of endotoxins into the circulation, but endotoxins may

gain access to the circulating blood if the mucosal barrier is breached. Before reviewing the literature on the role of gut-derived endotoxin in endotoxin shock, two relevant aspects, namely, the normal gut microflora and the splanchnic circulation, will first be reviewed.

2.2.2 The gut microflora

The mammalian gut lumen is normaly populated by both aerobic and anaerobic bacteria[16,302]. The anaerobic gram-negative bacilli are the predominant micro-organisms of the gut in most species and constitute about 99% of the total faecal count[302]. Many of these anaerobic organisms, previously considered to be harmless, are now accepted as opportunistic pathogens that may give rise to clinical disorders when the host's resistance is reduced[302,303]. The lipopolysaccharide component of most gram-negative anaerobic bacteria, however, unlike the aerobic organisms, lack the heptose and 2 keto-3-deoxyoctonate and, as such, their biological activity is weak compared to the aerobic facultative gram-negative bacteria[302,304]. Nevertheless, the presence of anaerobic gram-negative organisms contributes to the normal physiological state by maintaining good body health, i.e., they prevent the growth of other potentially pathogenic bacteria - a phenomenon known as bacterial interference[302,303,305]. In the chicken, *Lactobacillus* spp. present in the crop plays a role in the regulation of its intestinal flora population, e.g., if the *Lactobacillus* spp. population is destroyed the coliform count increases[304]. A similar symbiotic relationship exists in the rat, where the lactobacilli display specificity in their adhesion to the intestinal epithelial cells[305]. Furthermore, colonization by *Lactobacillus* spp. at the mucosal site may significantly limit the penetration and crossing of the mucous membrane by potentially-pathogenic organisms[303]. Suppression of the anaerobic flora by indiscriminate use

of antimicrobial agents or by irradiation may result in the rise of one or more of the potentially pathogenic species in the gastrointestinal tract, hence contributing to a large pool of LPS[303]. Animals which lacked the conventional aerobic gram-negative flora or those in which the gram-negative flora were suppressed, survived major gastrointestinal injuries as opposed to those having normal gram-negative intestinal bacteria[34].

Smith et al.[306] made differential counts of the faeces of calves, lambs, piglets, rabbits and human babies at frequent intervals, from birth onwards. They found that the bacterial flora at first developed in a remarkably similar manner in all species, but as the animals grew older considerable differences became apparent. For example, lactobacilli and bacteroids usually colonized the intestines later, but they often persisted in large numbers over a longer time. *Staphylococcus aureus* was never isolated from the faeces of calves, lambs, piglets and rabbits but was found in the faeces of many human babies. The variations in bacterial flora in different species were due to differences in composition of the diet[306]. The faecal flora of rats fed a chow diet contained significantly larger numbers of anaerobic and aerobic *Lactobacillus* spp. than those fed meat alone[307]. In contrast, diets high in meat reduced the number of *Lactobacillus* spp. but increased the coliform organisms[307]. The latter appear to be more frequently involved in bacteraemic death[307]. Diet therapy, for example, by decreasing the amount of meat intake and substituting a vegetable diet, may be beneficial in conditions likely to cause endotoxaemia (intestinal surgery, gram-negative bacteraemia, haemorrhagic and burn shocks, X-irradiation). Dietary manipulation to increase the coliform count prior to X-irradiation produced 100% deaths compared to 60% deaths in rats fed a normal diet[307].

Intestinal microflora also pose a problem during intestinal surgery[308,309]. The number of bacteria in the intestine can be reduced by a mechanical preparation; e.g., whole bowel irrigation or the making use of elemental diets[309] or by the administration of oral non-absorbable antibiotics such as neomycin[309] or kanamycin[308,309]. Interestingly, neither of these antibiotics influences the anaerobic flora of the colon[309]. This factor may be of importance because the increase in anaerobic flora may discourage invasion by the pathogenic organisms (interference phenomenon)[303,304].

By way of recapitulation, it may be stated that the mammalian intestinal lumen is populated with both aerobic and anaerobic gram-negative bacteria. However, the biologic activity of LPS from the anaerobes is weaker than that of the aerobes. The normal permeability properties of the intestinal mucosa prevent the entry of LPS into the circulating blood, but these may gain access to the circulation if the intestinal wall becomes damaged, for example, by surgery. The aerobic/facultative anaerobic gram-negative bacterial population can be suppressed by using oral, non-absorbable antibiotics such as kanamycin.

2.2.3 Splanchnic circulation

2.2.3.1 Physiological anatomy

The splanchnic circulation supplies a complex series/parallel arrangement of organs that includes the gastrointestinal tract (stomach, small and large intestines), spleen, pancreas and liver[214,310]. The coeliac, superior and inferior mesenteric arteries (collectively known as the splanchnic artery) and their interconnecting branches supply the splanchnic organs except for the liver, which is supplied by the hepatic artery. The venous blood from the gastrointestinal and

splenic regions drain into the portal vein. The liver and other splanchnic organs receive approximately 25% of the resting cardiac output and they have the largest regional circulation at rest[214,310]. The splanchnic region is a major region from which blood flow can be redistributed to other regions[214,310,311] as occurs during heat stress.

2.2.3.2 Control of splanchnic blood flow

Challenge to the cardiovascular system by a variety of stresses such as hyperthermia, exercise, and low cardiac output states, causes vasoconstriction of the blood vessels in the splanchnic region in order to maintain blood pressure and to redistribute cardiac output[310,311]. All the splanchnic organs are richly innervated by sympathetic vasoconstrictor fibres[310,311]. Like other tissues and organs, local regulatory mechanisms are thought to control blood flow in the splanchnic region[214,310]. In the intestines, blood flow through the mucosa and submucosa is controlled separately from blood flow to the musculature[310]. Various peptide hormones (vasoactive intestinal peptide, gastrin, secretin and cholecystokinin) cause mucosal vasodilatation[214,311]. Furthermore, some of the gastrointestinal glands, according to Guyton[214] release kallidin and bradykinin at the same time as they release their hormones. The kinins are powerful vasodilators.

2.2.3.3 Autoregulatory escape

Electrical stimulation of the splanchnic nerves (sympathetic) cause vasoconstriction, particularly in the intestines[214,310-314]. In cats, if stimulation of the splanchnic nerves is prolonged, blood flow returns towards control levels and this mechanism is called "autoregulatory escape"[214,310-314]. In rats and cats,

electrical stimulation of left splanchnic nerve for 3-4 minutes caused an initial constriction of submucosal arterioles and this was followed by partial escape[314]. Superficial mucosal flow was also reduced eventually to a complete cessation and the mucosa appeared blanched[314]. This was followed by a partial escape when the flow increased slightly[314]. The mechanism of autoregulatory escape is not clear, but it is thought to be due to accumulation of certain metabolites, the exact nature of which is not known[312-314]. In the intestine, autoregulatory escape is attended by redistribution of blood flow from the outer mucosal region, which remains constricted, to the submucosa[310]. However, in dogs, autoregulatory escape is not evident and stimulations of the splanchnic nerves cause a persistent fall in splanchnic blood flow[313]. Similar changes in splanchnic blood flow were produced by infusion of the mesenteric vessels with catecholamines and vasopressin[315,316]. In man, according to Rowell[310], intense splanchnic vasoconstriction can persist for a long time during severe stress and no net escape may be evident.

Guth[314] quotes from a paper by Beaumont 1833: "In ...predisposition, from whatever cause ... fear, anger or whatever depresses or disturbs the nervous system - the villous coat (of the stomach) becomes sometimes red and dry at other times pale and moist, and loses its smooth and healthy appearance". The administration of endotoxin or epinephrine or induction of shock by haemorrhage caused blood flow in the superior mesenteric artery to fall almost to zero within an hour[228]. It is possible that the reduced blood flow is responsible for the gastrointestinal disturbances and damage which occur during endotoxaemia.

To sum up, the splanchnic circulation acts as a reservoir for the storage of blood and it vasoconstricts whenever the cardiovascular system is challenged by a variety of

stresses[310,311]. This response is important in blood pressure regulation and for the redistribution of cardiac output, for example, during heat-stress - to the skin, and during exercise - to the skin and skeletal muscles[310,311]. In rats and cats, during prolonged stimulation of the sympathetic splanchnic nerve, the blood flow to the intestine is initially reduced and later increases to almost normal levels, and this phenomenon is known as autoregulatory escape. In man, according to Rowell[310] splanchnic vasoconstriction can be maintained for a long time and no escape may be evident. Maintenance of a vasoconstriction state for prolonged periods may damage the permeability properties of the gut mucosa, allowing gram-negative bacteria and their endotoxins to enter the circulating blood.

The literature on control of blood flow during heat-stress will be reviewed later in this chapter.

2.2.4 Intestinal ischaemia - patho-physiological changes and shock

2.2.4.1 Introduction

Over the past 2 to 3 decades, much interest has been directed to the role of ischaemia of the gastrointestinal tract during shock. It is known that the shock that develops in experimental animals upon or soon after occlusion of the superior mesenteric artery (SMA) is released is indistinguishable from the irreversible shock caused by hypovolaemia or bacterial endotoxins[50,317].

2.2.4.2 Physiological and pathological changes during intestinal dysfunction

(a) Cardiovascular changes

Flynn et al.[318] described the splanchnic artery occlusion (SAO) shock as a lethal form of circulatory shock which is directly attributable to prolonged ischaemia of the splanchnic region. The cardiovascular changes which occur during splanchnic artery occlusion or superior mesenteric artery occlusion (SMAO) shock are shown in Table 4.

No changes in cardiovascular parameters were observed whilst the splanchnic or the superior mesenteric arteries were occluded[319-323]. However, in most cases, the mean arterial pressure declined soon after the occlusion was removed and death occurred several hours later[31,34,50,317,319-323]. In addition, a drop in cardiac output[317,324-327], depressed left ventricular function[320,323,326], depressed heart rate[325], decreased blood volume[34] and pulmonary vasoconstriction[324] were also observed. However, in a few instances no change in mean arterial pressure[327], heart rate[326] or left ventricular function[328] was noticed.

The actual trigger mechanism which results in the cardiovascular derangements, shock and death during superior mesenteric artery occlusion or splanchnic artery occlusion shock is a matter of controversy. Whilst, most investigators agree that toxic substances or vasoactive agents are released from the ischaemic and hypoxic tissues, no consensus has been reached on the type of toxic substance or substances involved[318-335].

Table 4: Cardiovascular changes during splanchnic artery or superior mesenteric artery occlusion shock.

Cardiovascular parameter	Effect of SAO or SMAO shock
Mean arterial pressure	reduced soon after removal of occlusion (31,34,50, 317, 319-323) or no change (327)
Cardiac output reduced	(317,324-327)
Left ventricular contractility	depressed (320,323,326) or no change (328)
Heart rate	reduced (325) or no change (326)
Pulmonary vessels	vasoconstriction (324)
Blood volume	reduced (34)

Fine and his co-workers[50] postulated that the reduced blood flow to the intestine caused the mucosal barrier to be breached, thus allowing the leakage of LPS into the circulation. In the presence of an impaired RES function, LPS spilled over into the systemic circulation resulting in cardiovascular derangements[50]. This presumably further accentuated the reduced blood flow. Moreover, using the qualitative gelation test, increased plasma endotoxin levels were detected after superior mesenteric artery occlusion[31], immersion burns[34,37], prolonged haemorrhage[28] and administration of vasoactive substances[36]. Rabbits subjected to superior mesenteric artery occlusion shock, immersion burns, or intravenous endotoxin injection showed a progressive hypotension, hypovolaemia and anuria until death[34]. The plasma volume deficit at the end of 1 hour superior mesenteric artery occlusion was 20%, and after releasing the occlusion the plasma volume decreased by 35% to 40%[34]. None of these changes were observed in rabbits

lacking intestinal gram-negative bacteria or in which the counts were very low following similar insults[34]. These animals virtually all survived[34].

Rabbits pretreated with oral kanamycin or rabbits lacking gram-negative bacteria had decreased mortality rate following superior mesenteric artery occlusion[34], immersion burns[34,37] and haemorrhagic shock[28]. In addition, the administration of prophylactic antilipopolysacchride (anti-LPS) antibody protected rabbits against superior mesenteric artery occlusion shock and cats against haemorrhagic shock[117,119]. These data suggest that endotoxins which enter the circulation from the gut lumen possibly caused the observed cardiovascular changes, shock and death.

It was recently found that temporary ischaemia of the dog's colon allowed the migration of radio-labelled endotoxins, but not whole bacteria, from the intestinal lumen into the circulation[35]. Ischaemia of six hours was required to damage the gut wall enough to permit whole bacteria to translocate, whereas the LPS could translocate within a few minutes of intestinal ischaemia.

The route of entry of endotoxin during intestinal ischaemia from the intestinal lumen into the circulation is not certain. Some investigators showed that part of the endotoxin entered the systemic circulation by way of the lymphatics[102]. Others showed that endotoxin entered the systemic circulation via the peritoneal cavity and collateral blood vessels[329]. Other investigators, however, believed that bacterial endotoxins were not a lethal factor during superior mesenteric artery occlusion shock[318-323]. Janoff et al.[330] were the first to suggest that toxins released by the ischaemic or hypoxic intestinal tissue into the blood gave rise to hypotension, shock and death, but whilst they were not able to identify

the toxin precisely, they thought it might be serotonin[330]. Other researchers identified the toxin as a myocardial depressant factor (MDF) and postulated that it was released from the pancreas[320-322]. Lefer and his co-workers[320-322] showed, in *in vitro* and in *in vivo* experiments, that MDF had a negative inotropic effect on the heart. In addition, they found that MDF had a vasoconstrictor action on blood vessels in splanchnic region and also depressed RES activity[322]. The depressive effects of MDF on cardiac contractility was also verified by others[324,331]. Administration of synthetic glucocorticoids prevented the formation of MDF and also enhanced survival rate in superior mesenteric artery occlusion shock[322], and methylprednisolone prevented cardiovascular derangements[323]. These effects of corticosteroid may also be explained by its inhibitory action on the endotoxin-induced release of cachectin[173] and IL-1[160], both of which are released from mononuclear phagocytes and granulocytes.

Hinshaw and his co-workers[328], on the other hand, were not able to detect evidence of impairment of myocardial function during splanchnic artery occlusion shock. They perfused an isolated heart with blood from the splanchnic region of another dog subjected to 2 hours of splanchnic artery occlusion shock. No demonstrable depression of myocardial pumping action was detected in the recepient heart after the removal of the occlusion, but a profound drop in mean arterial pressure in the donor dog occurred soon after the removal. They suggested that other toxic substances were released from the ischaemic intestine, but not MDF. Bellamy et al.[332] were of the opinion that MDF was not responsible for the cardiovascular alterations, shock and death, as the time between onset of the ischaemic insult and death was too short for MDF to be released from the pancreas. Other researchers postulated that vasoactive substances, such as histamine[333], prostaglandins[318,325,334] or catecholamines (epinephrine,

norepinephrine and serotonin)[333,335] released during intestinal ischaemia, were responsible for the cardiovascular derangement. The overwhelming evidence seems to support the postulate of Fine[50] that, gut-derived endotoxins appear, during all forms of shock, to be responsible for the cardiovascular defects and other pathological changes.

(b) Intestinal damage

Mucosal lesions of the small intestine are characteristic features of ischaemic states and various other disorders such as haemorrhages and burns[34,37,50,317,323,325,336-341]. These lesions were described as extensive ulceration and necrosis with petechial haemorrhages in the mucosa. Some investigators have concluded that the lesions, start during complete or partial occlusion of the superior mesenteric artery, at the tip of the villi, and in severe forms the entire villus were more or less destroyed[336-340].

During partial occlusion of the superior mesenteric artery significant damage occurred not only during the ischaemic period but also during reperfusion of the tissue with oxygenated blood[336-340]. It was postulated that the latter injury was due to the presence of oxygen free radicals, generated by the enzyme xanthine oxidase formed from xanthine dehydrogenase during ischaemia or hypoxia[336-339]. The administration of allopurinol, which blocks the formation of xanthine oxidase, or the administration of superoxide dismutase, the free radical scavenger, reduced the mucosal damage following partial occlusion of the superior mesenteric artery[336-340]. On the other hand, during complete occlusion of the splanchnic arteries, pretreatment with either allopurinol or superoxide dismutase did not prevent the damage[336,338]. These findings indicate that whilst oxygen free radicals may be

responsible for the mucosal lesions produced by regional hypotension, other factors may play a more important role in causing structural alterations during arterial occlusion[336,338].

(c) Other changes

(i) Lung lesions

Following superior mesenteric artery occlusion, in rabbits[34,282] lungs at death were heavier than normal, extravasated fluid and red blood cells were present in the interalveolar septa and the alveolar spaces. Similar changes in the lungs appeared after intravenous infusion of endotoxins (discussed in section 2.1.3.6).

(ii) Blood pH

Changes in blood pH were negligible during the period of occlusion, but following release of the occlusion a marked acidosis occurred in the majority of experimental dogs subjected to superior mesenteric artery occlusion shock[317]. Similar acidosis was detected in animals receiving a bolus injection of LPS.

It can thus be seen that the pathological changes occurring during splanchnic artery occlusion or superior mesenteric artery occlusion shock are similar to those occurring during endotoxaemia.

To conclude, in most experimental animals, the removal of occlusion, after a temporary occlusion of either the splanchnic or superior mesenteric arteries, was followed by a drop in the mean arterial pressure, cardiac output and blood volume, depressed left ventricular function and heart rate and pulmonary vasoconstriction and death. It has been variously postulated that the entry of endotoxins

into the systemic circulation from the intestinal lumen or the release of MDF from the pancreas or the release of vasoactive agents from the ischaemic intestinal tissue were the cause of the cardiovascular dysfunctions and lethality. Furthermore, lesions in the intestinal mucosa, characteristic of ischaemia, and in the lungs and acidosis were also detected. Similar changes have also been reported during endotoxin shock.

2.3 HEAT STROKE

2.3.1 Introduction

The emergence of homeotherms can be regarded as a major evolutionary event in that it has liberated the animal from its environment. This has been accomplished by the development of a thermoregulatory mechanism which maintains the body temperature within narrow limits despite wide fluctuations in environmental temperature.

Thermal homeostasis is maintained by a delicate balance between heat gain and heat loss by the body. The body gains heat mainly from metabolic activities and, in a warm environment, from the surroundings as well. Under cool conditions the body loses heat to the environment by means of radiation, conduction, convection and the evaporation of sweat[214]. However, if the environmental temperature is equal to or is higher than that of the body surface, heat can only be lost by the evaporation of sweat from the surface of the skin and water from the respiratory system. The body temperature rises when heat gain exceeds heat loss. Elevated body temperature or hyperthermia may be due to increased metabolic heat production (for example, during increased physical work) or may be due to diminished heat-dissipating capacity brought about by a vasoconstriction of skin blood vessels or a combination of environmental factors, comprising elevated ambient

temperature, high humidity or a combination of both[342]. If this situation is allowed to continue for a prolonged period of time it could give rise to any of the three major clinical heat-related disorders: heat cramps, heat exhaustion or heat stroke, of which heat stroke is the most serious heat-related disorder, with reported mortalities of up to 80%[64,68,342,343].

2.3.2 Historical aspect

Heat stroke, also known as sunstroke, is one of the oldest known diseases[344]. Historical description of heat stroke dates back over 2000 years: in 24 B.C. a Roman army was almost destroyed by the effect of heat-stress in Saudi Arabia[345]. The ancients associated heat stroke with Siriasis[344] because of the biblical reference that it occurred coincidentally with the appearance of the dog star Sirius[344]. It would appear that heat stroke is also mentioned in the Bible; in the fourth book of Kings, Chapter IV, verses 18 to 20 a child's death is attributed to heat exposure: "When the boy grew up, he went out one day to his father among the reapers. He called to his father, oh, my head! my head!... when he was brought to his mother he sat on her lap till noon; then he died." A study from 1861 to 1926 of heat-related casualties in the United States Army revealed 38 deaths and 43 rendered invalid from the armed services[344]. In Mesopotamia, more than 400 British soldiers died during a heat wave in the summer months of 1917[346]. During world war II, in preparing military personnel for rapid deployment in tropical and subtropical climates, American recruits were exposed to a vigorous training programme, which resulted in 125 fatal cases of heat stroke[78]. In Peking, in July, 1943, 11 000 persons were said to have perished in the streets from heat stroke during a heat wave[344]. Recently the death of a British soldier during training provoked a

great deal of correspondence in the 1988 issue of Lancet[347].

Heat-stress also poses a problem among pilots[348] and recruits training in either the mission operated protective posture (MOPP) gear[349] or nuclear, biological and chemical (NBC) protective clothing[350]. Recently a recruit training in NBC protective clothing suffered from heat stroke[350]. However, civilians have suffered more from heat stroke than military personnel, who are usually young, healthy and well-conditioned[351-359]. A high incidence of heat stroke occurs every summer in Agra, India and most of the cases involve children in the age group 0-10 years[352]. Haseeb et al.[353] reported the tragic case in Kosti, Sudan of 187 deaths and 11 seriously ill patients out of 281 prisoners who had been locked in a ward which normally accommodates 16 inmates. It is of some interest to note that the deaths occurred overnight when, presumably, the temperatures were relatively lower than in the day. When the prison ward was opened in the morning, pools of sweat in the depressed abdomens of the bodies were observed[353].

The pilgrimage to Mecca in Saudi Arabia claims many heat stroke victims when the pilgrimage occurs during the summer months[354,355]. During the summer of 1959, 1960 and 1961 there were 1025 deaths related as a result of the heat[356], and in 1982, 1119 cases of heat stroke were admitted at treatment centres[355]. Khogali and Al-Marzoogi[357] estimated that an additional 800 victims might have died before they could receive treatment. Of the 1119 cases, 75% of the patients had rectal temperatures above 42^{o}C. In Mina, 9,5%, and in Arafat 5%, of the patients died[355].

Heat stroke may be subdivided into two forms, "classical"

and exertional[360]. The "classical" type usually occurs in epidemics or during a heat wave, while the "exertional" type is hyperthermia in which heat is produced by muscular work at a rate that exceeds the body's capacity to dissipate it[360].

As opposed to the "classical" type of heat stroke which afflicts mainly the elderly and the infant, the "exertional" type, occurs chiefly among young, motivated adults[360]. Shibolet et al.[81] reported 36 heat stroke cases in men engaged in physical exercise. In South Africa, serious heat illness, including heat stroke, is most frequent in the mining industry, for example, between 1968 and 1973, 128 gold miners developed heat stroke because the temperatures and humidity were high in the deep and ultra-deep levels of the mines[343].

In addition to the military and the mining personnel, those engaged in athletics are affected by heat stroke[70-76]. Among American high school athletes heat stroke remains, second to head and spinal injuries, the leading cause of death[77]. Casualties are more common in novices, who exceed their training efforts when racing, and in well-trained competitors who strive for improved performances by suddenly increasing their lead midway through a long distance run[76]. Hart et al.[72] reported that a 41-year old man had collapsed after 9 km of a 10 km "fun run" in Hamilton, Canada. The victim did not have any premonitary symptoms. Shortly after the 9 km mark he collapsed and was rushed to hospital within 20 minutes. He was in a comatose state, unresponsive to painful stimuli and sweated profusely. His pulse rate was 180 beats/minute, his blood pressure 140/90 mm Hg and his rectal temperature was 40,3^{o}C. Over the ensuing 24 hours he vomited and had diarrhoea. A diagnosis of heat-induced acute renal failure, rhabdomyolysis, disseminated intravascular coagulation and hepatic necrosis was made[72].

Heat injury is not always associated with hot, himid environments; it could occur in a cool environment as well. Sutton and Bar-Or[74] reported heat stroke in runners with a rectal temperature as high as 43°C during the "City - to - Surf" fun run in Sydney where the ambient temperature was as low as 10°C. Runners at greatest risk of hyperthermia are those who run the fastest (Dr T Noakes, University of Cape Town, Personal communication, 1987).

Apart from man, domestic animals, including poultry[361-363], trained and poorly- conditioned horses[364], dogs[365] and pigs[366] can be adversely affected by heat-stress. Commercial poultry producers suffer a considerable loss each year as a consequence of irreversible heat prostration[363]. The problem is particularly acute during the transportation of broilers to the processing plant[363].

Before reviewing the literature on the pathophysiology of heat stroke, literature concerning normal temperature control, elevated body temperature and the physiological responses to hyperthermia will first be reviewed.

2.3.3 Regulation of body temperature

2.3.3.1 General

The temperature of the deep tissues of the body (core temperature) in homeotherms usually remains constant, that is, within 0,6°C of the mean, day in and day out, despite wide fluctuations in the environmental temperature[214,367,368]. In man, the core temperature ranges from 37,3 to 37,6°C when measured rectally[214], and in birds about 40°C[369]. This constancy in body temperature is made possible because a balance between heat gain and heat loss is maintained.

2.3.3.2 The thermoregulatory mechanism

It is generally accepted that specific central and peripheral structures (various temperature receptors in peripheral tissues and in the hypothalamus) not only detect the absolute temperature but also the magnitude, direction and rate of change of temperature. Sites in the central nervous system take appropriate measures, directed to the effector systems, to keep the temperature in balance[370]. The thermoregulatory effector functions of most homeotherms can be conveniently classified into two general categories:

i. Physiological responses - these include autonomic responses, such as cardiovascular, respiratory, sweating and shivering, which depend upon the integrity of central and peripheral pathways but not the cerebral cortex[370].

ii. Behavioural functions - these require the cerebral cortex.

Only literature concerning the autonomic responses will be reviewed.

In general, in man, the primary physiological responses to a fall in body temperature consist of peripheral vasoconstriction and increased metabolic heat production in the form of shivering and to a rise in body temperature of peripheral vasodilatation and sweat secretion[370]. Vasoconstriction of cutaneous blood vessels decreases blood flow to the skin and thus minimizes heat loss to the environment, whilst dilatation of cutaneous blood vessels increases blood flow to the skin and, together with sweating facilitates heat loss to the environment.

A uniformly held concept of thermoregulation recognizes the integrator controlling these thermoregulatory responses as the preoptic - anterior hypothalamic (POAH) area[214,367-376]. Benzinger[368] described the anterior hypothalamus as the "Temperature eye". Nakayama et al.[377] were the first to report that thermosensitive neurones were present in the anterior hypothalamus. These neurones increased their firing rate during local heating of the hypothalamus[377]. Subsequently, a number of studies showed that some neurones in the POAH area respond to heating and cooling but most are insensitive to temperature changes[377-381]. The warm-sensitive neurones (comprising about 31% of the neurones in the POAH area) increased their firing rates with local warming and decreased with cooling[300,377-381]. Conversely, the cool-sensitive neurones (about 10%) increased their firing rate with local cooling and decreased with warming. Neurones which were insensitive to temperature changes possibly provided a stable reference signal, called the "set-point" for thermoregulatory control[370,377-381]. The "set-point" of the hypothalamic thermoregulatory system undergoes rhythmic changes with the diurnal and menstrual cycle[367].

In addition to the POAH area, thermosensitive units were shown to be present in the spinal cord, medulla oblongata, mid-brain and other CNS sites[367,375,376,382-384]. Furthermore, electrical stimulation of the median Raphe of the midbrain increased the firing rate of the warm-sensitive neurones and decreased the firing rate of the cold-sensitive neurones of the preoptic area[385]. Moreover, the neurones in the median Raphe of rats also responded to changes in local temperature[383]. It has been assumed that the median Raphe either detects the midbrain temperature or receives signals from the skin thermoreceptors and convey them to the thermosensitive neurones of the preoptic area[385].

The neurones in the POAH area not only receive synaptic inputs from the spinal cord and brain stem but are also influenced by them and, in addition, by inputs from the peripheral thermoreceptors such as those in the lower brain, spinal cord and the skin[370,385-395]. Because thermosensitive neurones are present in the POAH area it has been assumed that the hypothalamic temperature is the regulated temperature. The thermosensitive neurones in the POAH area were found to be more sensitive to changes in local, hypothalamic temperatures than to changes in the ambient temperature[378]. In addition, animals with lesions in the POAH area were unable to maintain a constant body temperature in cold or warm environments[367].

Recordings of single unit activity in the hypothalamus of rabbits during changes in local temperature revealed bell-shaped curves, peaking generally either above or below the usual range of body temperature (37-39^{o}C)[395]. The units which increased their firing rate as the temperature was increased between 37-39^{o}C were designated as warm-sensitive and those which showed the opposite response as cold-sensitive. The position of the maxima of the bell-shaped curve, above or below the usual range of body temperature determined whether the neurone was warm or cold unit[395]. Also the warm units decreased their firing rate above 41-42^{o}C[395]. This reversal in response of the warm units to temperatures above 41^{o}C supposedly explained the instability of the thermoregulatory system at high body temperatures[395]. In addition, intravenous administration of pyrogens inhibited the warm-sensitive units - about half of these units showed a decrease in both their spontaneous firing and temperature sensitivity and the remaining stopped firing completely. The cold sensitive units, on the other hand, either increased their firing rate or did not respond to the injections of pyrogens[395]. Of importance, most of the temperature insensitive units did not show any change[395]. Because of

the latter, these workers were unable to explain the role of the temperature insensitive units as "set-point" determinants[395]. In a recent study, it was found that local heating of the hypothalamus and the spinal cord increased the skin blood flow and elicited panting, and resulted in a fall in rectal temperature in unanaesthetized sheep[384]. Whilst intravenous administration of LPS from *E.coli* produced a decreased skin blood flow and caused shivering. The reduced blood flow to the skin was not influenced by local heating of the spinal cord and the hypothalamus during the rise in fever[384]. These findings support those of the above workers[395] that pyrogens affect the thermosensitive units of the hypothalamus.

Basically, the type of thermoregulatory responses depends on the sign and magnitude of the difference between the regulated temperature and the "set" or "reference" or "set point" temperature. This reference or "set-point" signal was provided by the neurones in the POAH area which were insensitive to changes in temperature[370,377-381]. It was postulated that the POAH area transduced its own temperature into neural impulses and then, by a continous comparison between the transduced temperature and the "set point" temperature, the rate of heat loss was either increased or decreased[370-372]. Furthermore, it was suggested that different sites in the hypothalamus may separately control cold-induced skin vasoconstriction and shivering as well as heat-induced skin vasodilatation. Shivering was stimulated directly by cold inputs from the skin to the posterior hypothalamus[390] whilst cutaneous vasoconstriction depended on the integrity of the preoptic area and possibly the vasoconstrictor area of the brain stem[373,374]. The anterior hypothalamus, where inputs from the warm-sensitive thermoreceptors synapse, and possibly also the medullary vasodilator area, are responsible for cutaneous vasodilatation and splanchnic vasoconstriction[374,375]. Cutaneous vasodilation increases

blood flow to the skin which, in turn, facilitates heat-dissipation to the environment by radiation, convection and conduction[214,368,371-375,384].

However, others believe that the hypothalamus is not the sole integrator of body temperature because stimulation of sites in the midbrain and spinal cord also elicited thermoregulatory responses[384,391-393]. Satinoff[391] proposed that thermoregulation is achieved through hierarchical set of integrators located at various levels in the CNS, each level either facilitates or inhibits the levels above or below it. Nevertheless, it has been suggested that the hypothalamus serves to co-ordinate and adjust the activity of the thermoregulatory systems located at the several lower levels of the neuraxis to ensure appropriate thermoregulatory reflexes[391].

In recent years, doubt has been cast as to whether the "set point" determinant in fact resided within the CNS, i.e., on the pathways connecting temperature sensors to the thermoregulatory effectors[375], or even whether "set point" determinants exist at all[300]. It was also postulated that the "set point" determinant resides in the warm and cold sensors themselves and is not a property of a particular neuronal structure, such as the temperature-insensitive neurones in the POAH area[300,375]. The neuronal organization is assumed to consist of two sensors - to - effector pathways, from the warm sensors to heat loss effectors, and from the cold sensors to heat production effectors, with crossing inhibitory influences between them, i.e., when the warm sensors are excited, thermoregulatory responses which favour heat loss are elicited and, at the same time, the cold sensors are inhibited, and vice versa. Therefore this latter model does not incorporate a reference or set point[300,375]. This view is based on the action of pyrogens on the warm and cold sensitive neurones. Pyrogens were found to decrease

the sensitivity of the warm-sensitive neurones, sometimes even inhibiting them completely[300,394,395]. They either increased the sensitivity of cold-sensitive neurones or did not affect them at all. On the other hand, pyrogens had no effect on the temperature-insensitive neurones in the hypothalamus[300,394,395]. The latter neurones, supposedly, act as the hypothalamic "set-point" or thermostat[370,377-381] and since their sensitivity was not altered by pyrogens it was suggested that these neurones do not provide a reference or "set-point" signal for temperature regulation. According to this view, pyrogens raise the core temperature (cause fever) by increasing the drive to heat production (by decreasing the "gain" or sensitivity of the warm-sensitive neurones) while at the same time inhibiting the drive to heat loss[300,375].

In summary, it is generally believed that warm- and cold-sensitive neurones are present in the POAH area. These neurones are, in turn, influenced by information from receptors situated in lower brain regions, the spinal cord and in the skin. The output from the thermosensitive neurones are compared continuously with a "set point" determinant or reference signal in the temperature insensitive neurones, also present in the POAH area, and appropriate outputs are executed to elicit thermoregulatory responses. However, according to a recent view, no "set point" or reference neurones are present. Instead it is assumed that the "set point" resides in the warm and cold sensors (in the POAH area) themselves with inhibitory connections between them. It is believed that the effect of pyrogens is to decrease the "gain" or sensitivity of warm-sensitive neurones, thus reducing the amount of heat loss and hence cause a rise in body temperature.

2.3.4 Thermoregulatory responses to elevated body temperature

2.3.4.1 Introduction

In man, the tendency of the body to gain heat is counteracted reflexly by vasomotor adjustments and sweating and by behavioural responses. In animals that do not sweat, heat loss is also accomplished by panting. With regard to the autonomic reflexes, the cardiovascular system plays an extremely important role in the adjustment of body temperature.

2.3.4.2 Cardiovascular responses to elevated body temperature

The cardiovascular responses to elevated body temperature (rectal temperature [Tr])as shown in Table 5, include (a) regional redistribution of blood flow, (b) adjustments in the cardiac output, (c) changes in heart rate, and (d) changes in blood pressure.

(a) Regional redistribution of blood flow

The primary cardiovascular adjustment which occurs whenever the body or core temperature becomes elevated is a substantial vasodilatation of cutaneous blood vessels[310,396-401,404-406,410-412], allowing increased blood flow, thus promoting heat loss by convection and radiation. However, the increased cutaneous blood flow poses a major problem to the cardiovascular system, namely, the maintenance of an adequate cardiac filling pressure and arterial blood pressure. The increased cutaneous blood flow in animals and man during heat-stress is accompanied by a substantial decrease in blood flow to the splanchnic regions including the kidneys, and in some cases, to non-respiratory skeletal muscles as well, in

Table 5: Cardiovascular responses to elevated core or rectal temperature (Tr)

Cardiovascular responses	Sheep	Dog	Baboon	Man
Redistribution of blood flow:				
Skin	Increased (396-399)	As for sheep (400,401)	As for sheep (404-406)	As for sheep (410-412)
Splanchnic	Decreased (396-398)	As for sheep (400,401)	As for sheep (404,406,407)	As for sheep (412-420)
Renal	Decreased (397)	-	Decreased (407,408)	Decreased (412,417,420)
Cardiac output:	No change (396,397)	Increased (400) or initial increase followed by a decrease at Tr above 41°C (401) or 43,5°C (402,403)	No change (405,407)	Increased (412,415-417) or decreased at high core temperature (415,420)
Heart rate	Increased (397)	Increased gradually until 42-43°C and then a rapid rise (402,403)	Linear relationship between heart rate and core temperature (405,406,409)	Immediate increase (412,415-417,420,421)
Arterial blood pressure	Initial decrease, followed by increase towards normal and decline at 41-42°C (397)	Similar to sheep (99,401-403)	Little change - decreased and recovered or increased (405, 408,409)	Decreased (412,417,421) or decreased and followed by increase to normal (416,422)
Central venous pressure	-	Increased at Tr 43°C (401)	-	Decreased (416,418, 419) or increased (65)

order to maintain a normal or near - normal blood pressure and cardiac filling pressure[310,396-398,400,401,404,406,407,412-420]. Knochel[68] emphasized that hard work in a hot environment could lead to a serious deficit in arterial blood volume due to increased blood flow to muscles and skin, and profound shock would occur were it not for the intense splanchnic vasoconstriction. In dogs[400] and in man[412] the redistribution of blood flow was also accompanied by an increased cardiac output. It was estimated that the blood flow to the splanchnic regions during hyperthermia decreased by 30 to 35%[404,407,412,419]. The decreased blood flow to the superior mesenteric artery was brought about by an increase in intestinal vascular resistance[404,412,419]. Proppe[404] also noticed that the increase in superior mesenteric artery resistance was a progressive one, parallel to the rise in core temperature (from 37 to 39°C). Rowell[412,419] found a similar relationship in heat-stressed man, where the rise in renal and splanchnic vascular resistance and the fall in blood flow to these regions were directly related to the rise in core temperature. The major significance of the elevated splanchnic vascular resistance during heating is the potentially important role it plays in redistributing blood volume to a dilated capacious cutaneous venous bed in order to maintain the mean arterial blood pressure as close as possible to normal. The constriction of the splanchnic vessels causes a massive reduction in splanchnic venous volume, displacing blood volume towards the heart to increase blood flow to the skin. Splanchnic blood flow also decreases with an increase in exercise intensity[413]. At any given level of exercise, heat exposure causes a further substantial reduction in splanchnic blood flow[414].

Splanchnic vasoconstriction during heat stress is not due to a reflex activation of arterial baroreceptors by the

falling mean arterial pressure or pulse pressure. In man it occurs independent of arterial pressure changes[423]. Furthermore, skin blood flow increases during moderate and strong heating of the spinal cord and hypothalamic preoptic regions and decreases during cooling of these areas[424]. Conversely, intestinal blood flow decreases during heating and increases during cooling of these areas[393,424].

It has been assumed that the antagonistic changes in blood flow in cutaneous and intestinal vascular beds are induced by antagonistic changes of sympathetic vasoconstrictor activity[393,424,425]. Directly measured electrical activity of sympathetic efferents in anaesthetized animals correlated inversely with blood flow[425,426]. Warming the skin or the hypothalamus or the spinal cord caused a decrease in sympathetic activity to the skin (increased blood flow) while concurrently, splanchnic, splenic and renal sympathetic activity increased (blood flow decreased)[425-429]. Proppe[404] showed that in the baboon, the intestinal vasoconstriction during heat-stress was due to an increase in α-adrenergic activity.

Rowell et al.[415,416] found that men working at high ambient temperatures exhibited severe nausea, abdominal pain, dizziness, vomiting and impaired vision followed soon after by exhaustion. Nausea and abdominal pain seemed to represent symptomatic awareness of visceral ischaemia or hyperthermia[416]. Asmussen[420] attributed the vomiting and watery diarrhoea which occur immediately after work in humid heat to an insufficient blood supply to the gastrointestinal organs. The major significance of the reduced splanchnic blood flow is that it may lead to ischaemic or hypoxic damage to the intestinal mucosa and the liver. Moreover, damage to these regions (discussed below) has been observed in heat-stressed humans and animals.

In additon to the decreased blood blow to the splanchnic regions, blood flow to the kidney[397,407,408,412,417,420] and to the brain[397,400,430] was also decreased.

(b) Cardiac output

The cardiac output either increased[400,412,415-417] or showed no change[396,397,405,407] during heat-stress in man and animals (Table 5).

In supine resting subjects, heated to the limits of their thermal tolerance, the cardiac output increased by 6,6 l/minute, i.e., it more than doubled[419]. Bynum et al.[402] observed a substantial increase in cardiac output in heat-stressed dogs as the rectal temperature increased to 42°C. Thereafter the cardiac output remained stable until a temperature of 43,5°C after which it decreased rapidly. Daily and Harrison[63] noticed that in dogs the cardiac output approximately doubled as the body temperature rose from 37 to 42°C and it declined beyond 42°C. On the other hand, in sheep and baboons, very little or no change in cardiac output was observed[396,397,405-407]. It is presumed that in these animals the increased cutaneous blood flow is compensated for only by a decreased splanchnic blood flow.

c) Heart rate

The heart rate generally increases during heat-stress and hyperthermia (Table 5). In the baboon, the heart rate increased progressively during external heating[405,406,409]. The magnitude of the increase was 22-30 beats/minute.°C as the blood temperature increased from 37 to 39,6°C. Similar results were obtained in man (18-43 beats/minute.°C)[416]. In heat-stressed dogs (in an environmental temperature of 42-46°C) the heart rate

increased gradually as the rectal temperature rose, but the heart rate increased very rapidly at the same time as the rectal temperature reached 42-43^{o}C[402,403].

d) Blood pressure

Variable changes in the mean arterial pressure have been reported during heat-stress and hyperthermia (Table 5). In most studies the blood pressure usually showed an initial decrease, then an increase, after which it remained stable[419]. In heat-stressed human subjects, lying in the supine position, the mean arterial pressure decreased initially as the blood temperature rose and then it recovered, but the central venous pressure always decreased[419]. In baboons, the mean arterial pressure decreased slowly, increased or remained unchanged[405,408,409]. As the rectal temperature in sheep[397], dogs[401-403] and rats[431] increased, blood pressure decreased initially, and then reverted to normal levels. It remained at this level until the rectal temperature was 41-42^{o}C when it began to decline. However, a similar decline in blood pressure was not observed in baboons and men probably because they were not heat-stressed to a sufficiently high core temperature.

To recapitulate briefly, a specific pattern of circulatory events occurs during heat-stress; in humans and certain animals a marked increase in skin blood flow occurs and this is brought about by a doubling of the cardiac output; there is a simultaneous redistribution of blood flow to the cutaneous bed and away from splanchnic, renal and non-respiratory muscle beds. The decrease in splanchnic blood flow is independent of the baroreceptor reflex mechanism. As core temperature rises, the mean arterial pressure decreases slightly and then recovers, but the central venous pressure always decreases markedly. However, at very high rectal temperatures (greater than 42-43^{o}C) the mean arterial pressure and cardiac output shows a marked decline and the heart rate a marked increase.

2.3.4.3 Sweating

As the heat load acting on the surface of the body is increased, the proportion of body heat that can be dissipated by convection diminishes and the responses to heat loss shift towards evaporation of sweat[432]. In man, baboons, horses and donkeys evaporation of sweat is the main method of losing body heat when environmental temperature approaches body temperature[342,433]. The evaporation of 1 litre of sweat rids the body of 2428 kJ of heat. In man the maximum rate of sweating is in the order of 2 litres/hour[434] or equivalent heat loss of 4856 kJ/hour.

Sweat secretion on the general body surface in man and higher primates is not a continuous function, but is a cyclical discharge[433]. Sweat is formed and secreted by the basal cells of the eccrine sweat glands which are innervated by sympathetic cholinergic nerve fibres and is controlled by the hypothalamic centres for temperature regulation[433]. The heat required for the evaporation of sweat is provided in the blood flowing to the skin. The blood returning from the skin is cooled and, hence, helps to maintain the core temperature at more or less a constant level. An individual's capacity to secrete sweat depends on the rate of blood flow through the skin; the rate increases with cutaneous vasodilatation[342,434]. Thus a reduction in cutaneous blood flow during exposure to a warm environment will result in a fall in cutaneous evaporative heat loss.

A high rate of sweat production could impose a severe strain on the water content of the body. If the deficit is

not made good, e.g., by drinking, it can result in dehydration which, in turn, could lead to a partial or complete breakdown of homeothermy during exercise in the heat[435,436]. Man is particularly susceptible to high heat loads on account of his relatively poorly developed mechanism for water homeostasis and is known to undergo "voluntary dehydration"[435,436] - (defined as the delay in complete rehydration following water loss through sweating).

Sweat is hypotonic with respect to blood[432]. Since the loss of water is greater than the loss of electrolytes, the osmolarity of the body fluid rises during sweating[432]. In other words, sweating produces hyperosmolar hypovolaemia.

In order to increase both the convective and evaporative heat loss from the skin it is essential that cutaneous blood flow is increased[432].

Thus the thermoregulatory responses to heat-stress place specific demands on the circulatory system, which are met by increasing and/or redistributing the cardiac output. The redistribution consists principally of increased perfusion of the skin, while that of the splanchnic, renal and possibly muscle and brain blood flow is decreased. The decrease in blood flow to the splanchnic regions, especially to the intestine and liver, could in principle lead to ischaemic damage of the gut mucosa and the liver[36,150,320-323,437].

2.3.5 Fever and Hyperthermia

Fever is recognised as a pathological process by which a

regulated increase in core temperature occurs during an infection[147,159]. This increase in core temperature is generally ascribed to a shift in the hypothalamic "set point"[147,159,214,217]. Hyperthermia, on the other hand, is defined as the elevation of body temperature above the normal hypothalamic "set point" and occurs when the peripheral heat-dissipating mechanisms have been either impaired by drugs or diseases or overwhelmed by environmental temperature[147]. The physiological responses in these two conditions, fever and hyperthermia, are, therefore quite different.

Fever results from a rise in the hypothalamic "set point" ("thermostat")[147,149,214,217] and involves mechanisms regulating heat loss and/or heat production[147]. Substances that cause fever are called pyrogens[217]. Pyrogens may be divided into two categories: exogenous pyrogens which come from outside the body, and endogenous pyrogens which are produced and released by the host's cells. Once released into the general circulation, endogenous pyrogens are carried from the peripheral sites of infection, inflammation or trauma to the brain, where they act on the thermoregulatory centre of the hypothalamus to initiate fever[147]. Endotoxins, gram-negative bacteria and viruses are amongst the most frequently encountered exogenous pyrogens[217]. The pattern of fever due to endogenous pyrogens is always monophasic[149].

On the other hand, intravascular administration of moderate doses of endotoxins produce biphasic fevers, whereas minimal doses elicit monophasic fevers in most animal species[159,177,298] (except in rhesus monkeys where monophasic fever is produced when very high doses of endotoxins are administered[243]). Endotoxins produce fever by inducing the release of endogenous pyrogens mainly from

mononuclear phagocytes (monocytes, alveolar macrophages, Kupffer cells, peritoneal macrophages) and granulocytes, or by acting directly on the hypothalamic thermoregulatory centre[147,299]. Endotoxin induces the release of at least three endogenous pyrogens - interleukin-1, cachectin (tumour necrosis factor) and interferon[147] (discussed previously).

The mode of action of these endogenous pyrogens in the production of fever is not clear. It is assumed that the endogenous pyrogens either act directly on the hypothalamic thermoregulatory control centres or indirectly via the release of prostaglandins[147,298]. Recent evidence shows that endotoxin, IL-1 and cachectin may act directly on the hypothalamus, inducing PGE_2 release[146,147,160,177]. Furthermore IL-1 and cachectin activated endothelial cells, in vitro, to synthesize and release PGE_2[166]. Prostangladin E_2 is thought to raise the hypothalamic thermostat to a higher level, but its exact mechanism of action is not known[147]. The new thermostatic setting then signals various neural responses to initiate vasoconstriction of cutaneous blood vessels in order to conserve heat. In addition, the subjective feeling of coldness causes shivering and hence increased metabolic heat production and behavioural changes, such as the seeking of warm environment or wearing of insulated clothing. Once the body temperature has risen to the new "set point", heat gain and heat loss are once again balanced but at a higher core temperature. Aspirin and other antipyretics reduce fever by blocking brain cyclo-oxygenase[147], leading to a reduction in the concentration of PGE_2 which alters the hypothalamic "set-point". This implies that the hypothalamic thermostat can be reset back toward normal if the concentration of endogenous pyrogens falls.

Many investigators have postulated that fever results when

the hypothalamic neuronal "set point" around which the body temperature is usually maintained, is altered by, for example, endogenous pyrogens or prostagladin E_2 or neurotransmitters[147,298]. As discussed in the previous section, some investigators believe that the endogenous pyrogens alter the gain or sensitivity of the thermosensitive neuronal system of the hypothalamus during episodes of fever[300]. However, whatever the mechanism, it is firmly established that endotoxins cause fever. It not only produces fever, but, it also, by inducing the release of the endogenous pyrogens, IL-1 and cachectin and other vasoactive agents, causes various haematological, biochemical, cardiovascular and gastrointestinal disturbances such as intravascular coagulation, metabolic acidosis, diarrhoea and hypotension (discussed in section 2.3.1).

2.3.5.1 Factors predisposing to hyperthermia and heat stroke

Any factor which reduces heat loss or increases heat gain will predispose an individual to hyperthermia[438]. These factors could be environmental and physiological.

Even in a healthy individual progressive heat-stress ultimately results in heat stroke, a medical emergency which could lead to death in the absence of immediate recognition and proper treatment[88,438,439].

From an environmental point of view the criterion used as an indicator of heat illness appears to be the wet bulb temperature because it is the resultant of the external temperature and the relative humidity[434]. The wet bulb temperature is based on the principle of evaporative cooling which, in turn, depends on the amount of water

vapour (humidity) in the atmosphere. The higher the humidity the lower the evaporative cooling process, hence the smaller the difference between the dry bulb and wet bulb temperatures. According to Kew[343], the risk of heat stroke increases as a hyperbolic function of wet bulb temperature, with a sharp rise above 32°C.

From a physiological point of view, the incidence of heat stroke is higher in the unacclimatized rather than in the acclimatized individual because acclimatization improves the physiological responses to heat dissipation[432,440]. Acclimatization is a physiological process whereby an individual develops tolerance to heat-stress[68,360,432,440]. This is accomplished by repeated exposure to heat-stress over a period of 10 to 14 days[432]. An acclimatized individual, as compared with an unacclimatized individual, has a more efficient heat-dissipating machanism, consisting of increased sweat rate and improved cardiovascular responses to heat exposure[432]. Following acclimatization, therefore, an individual's rectal temperature will be lower for any given heat-load than it would be prior to acclimatization. Other physiological factors predisposing to heat stroke are: (a) age - children in the age group 0-10 years[352,441] and the aged are more susceptible than the other age groups[68,82,86,442,443], (b) ingestion of alcholic beverages[82,86,442,444], (c) lack of sleep[81], (d) obesity[81,445], (e) chronic illness[69,86,359,360,446,447] and (f) fatigue[445,446,447] - possibly because of a less efficient heat-dissipating mechanism in such individuals.

2.3.6 Clinical signs of heat stroke

2.3.6.1 Introduction

Heat stroke may be defined as heat illness manifesting

itself as a severe disturbance of the central nervous system with a markedly raised body temperature[68,82,83,342,448]. Heat stroke victims usually present themselves with a typical complex clinical picture[63,67-69,342,440,448]. The disturbances may range from severe confusion, through successive levels of loss of consciousness or delirium, mania and coma[346,448], with "fixed" or widely dilated pupils[360]. Because heat stroke presents itself in different ways, physicians may fail to recognize and treat it promptly[342].

The onset is sudden in about 80% of the patients. There are usually no prodromal symptoms or signs but in cases where prodromal symptoms are present, they may last for minutes to hours[342,431]. These symptoms may include dizziness, weakness, confusion, headache, numbness, uncoordinated movements, nausea, anorexia and a staring and apprehensive facial expression[342,449]. Table 6 shows changes in rectal temperature (Tr) and some of the gastrointestinal, and cardiovascular alterations and changes in central nervous system, and sweating mechanisms in heat stroke patients and experimental animals.

2.3.6.2 Body temperature in heat stroke

The body temperature during heat stroke becomes elevated to levels not commonly seen in other conditions[342]. It is generally accepted that in man heat stroke occurs when the rectal temperature becomes elevated to greater than 40,6 to 41,1^{o}C[68,79,440] It is also characterised by a self-perpetuating hyperthermia probably because of failure of the heat-dissipating mechanism. It is known, however, that whilst some patients with rectal temperatures of 40^{o}C[438] may die, others with temperatures as high as 47^{o}C[87] may well survive. Thus it can be seen that the temperatures which are sufficient to produce heat stroke and death are

Table 6: Changes in certain parameters in man and experimental animals during heat stroke.

SUBJECTS	CRITICAL Tr*(°C) AT WHICH HEAT STROKE SYNDROMES OCCUR	Tr IN HEAT STROKE	GASTRO-INTESTINAL CHANGES % vomiting	% diarrhoea	% MORTALITY RATE	SWEATING	CARDIOVASCULAR CHANGES Cardiac output	Arterial blood pressure	Central venous pressure	CNS RESPONSES
Man: Heat-stress without exertion	40,6-41,1 (68,79,440)	40-44°C (79,84-86,88 355,450,451)	22-74 (85,451)	36-74 (451,85)	5-39 (85-87, 429,452)	Absent (86,429, 451) or present in 25% (75)	High and low output (84)	Normal to hypotensive (84-86,451)	Low (68,79,84,440,451) or elevated (84)	Drowsiness, coma to delirius (86,87,352 429,451)
Heat-stress with exertion	40,6-41,1 (68,79,440)	40-43,3°C (68,79-81)	46-54 (81,479)	34-100 (81,351)	0-100% (80,81, 83,351,429)	Lack of sweating (80), reduced (479) or present (81,351)	High output (65,80) or low output (80)	Normal to hypotensive (68,69,79,80,81,351)	Elevated (80)	Disorientation, to coma (80,429,451) and seizures (85,342)
Dog: Heat-stress without exertion	43-43,3 (402,452)	41°C (63,401	-	-	-	-	Declines (401)	Gradual decline (63, 403)	-	-
		42-43,5°C (63,401,402)	-	-	-		Sharp drop (63,402)	Sharp drop (63,401, 402)	Rise (401)	
		43-44,5°C (402,452)	-	66 (452)	33-80 (452)	-	-	-	-	Ataxia (452),
		44-44,6°C (452)	50 (452)	100 (452)	100 (452)	-	-	-	-	Convulsions and ataxia (452)
Heat-stress with exertion		43-44°C (452)	43 (452)	57 (452)	50 (452)					
		44-45°C (452)	90 (452)	100 (452)	100 (452)	-	-	-	-	Convulsions and ataxia (452)
Sheep: Heat-stress with exertion	43,5 (354)	43,7-44,9°C (354)	-	-	100%	-	-	-	-	Coma (354)
Rat: Heat-stress without exertion	40,4 (66,453)	42,4°C (66,453)	-	-	50 (66,453)	-	-	-	-	-
Heat-stress with exertion		41,7°C (453)	-	-	50 (453)	-	-	-	-	-
Rabbits	-	43,3 (454)	-	-	100 (454)	-	-	decreased (454)	-	Coma and dilirium (454)

* Tr = Rectal temperature

not identical.

Al Khwashki et al.[355] noticed that in the majority of their heat stroke cases the initial rectal temperature was above 42°C while a few had rectal temperatures in the range 40-41°C. Similar figures have been reported by other researchers[79,81,450,451]. A core temperature of 42°C and above invariably produces death[68,69]. It is important to note that in most heat stroke cases, rectal temperatures were not measured immediately at the onset of the problem and the variations noted in many cases would probably have been due to the time lapse between the commencement of the disorder and the time measurements were made.

Shapiro and his co-workers[452] found, in their unanaesthetized dog heat stroke model, that the critical temperature at which heat stroke occurred was 43°C (Table 6). They also noticed that dogs whose rectal temperature (Tr) exceeded 43°C showed clinical, haematological, biochemical and anatomo-pathological manifestations like those of people with heat stroke. Similar observations were reported by Bynum et al.,[402] who noticed that the critical thermal maximum above which lethal injury occurred and survival time became altered was 43,3°C. On the other hand, in the rat the critical rectal temperature was 40,4°C (Table 6) above which the mortality rate increased and a rectal temperature of 42,4°C was equivalent to an LD_{50}[66,453].

In the case of exercise coupled with heat stress, Shapiro and his co-workers[452] detected that half of the dogs died at rectal temperatures 43-44°C compared to fatalities amounting to one-third in non-exercised resting heat-stressed dogs. Similar effects were noticed in exercising and non-exercising heat-stressed rats[453]. When heat-stress was coupled with physical exertion a rectal temperature of

41,7°C was equivalent to an LD_{50} as compared to 42,4°C in sedentary, heat-stressed rats[453]. We may, therefore, infer that exercise plays a role in the development of heat stroke. If direct thermal injury to tissues, per se, is the primary factor in the pathogenesis of heat stroke, then exercise coupled with heat-stress should not have been more lethal than equivalent heat loads in the absence of physical effort[453]. Shapiro et al.[452] found that all the dogs died if their rectal temperature exceeded 44°C, irrespective of exercising or not. The severity of the disease depended on two factors: the elevation in body temperature and the length of time the body was subjected to temperatures above 43°C (or critical temperature). This was expressed as thermal area = degrees x minutes over 43°C. The greater the product (the thermal area) the greater the number of deaths[66,402,452,453]. In sheep the core temperature at which heat stroke death occurred ranged from 43,7 to 44,9°C[354] - temperatures higher than those for rats and dogs. In sheep, the failure of temperature regulating system was accompanied by convulsions and decreased rate of panting before the onset of coma.

In view of the above, it is not surprising that reviewers[68,69] have had difficulty in defining exactly when body temperature is "too high" and what degree and duration of hyperthermia produces tissue injury.

According to Shibolet et al.,[69] the mechanism by which death results from high body temperature is not well understood. However, it is known that extremes of temperature may alter enzyme activity or denature enzymes[69,456,457] or "liquify" membrane lipids[457,458]. Heat damage to mammalian tissues occurred at temperatures of 42°C or above[69,459]. Cabanac et al.[460] and Caputa[456] concluded that 40,5°C is the highest temperature that the

brain can tolerate.

2.3.6.3 Disturbances of the central nervous system

Disturbances of the central nervous system, because the brain cells are thought to be vulnerable to thermal injury, are common clinical findings in patients with heat stroke[81,342,343,360,440]. The level of consciousness is often depressed, accompanied by coma, stupor, delirium, faintness, staggering, thirst or disorientation[78,81,85,344,440]. Patients in the comatose group ran the greatest risk of dying[85]. The majority of early deaths, and even some of the later deaths, in heat stroke victims were thought to be due to irreversible brain damage. Patients who manage to survive may be left with permanent cerebellar or cerebral dysfunctions[78,343]. Malamud et al.,[78] in studying the pathology in 125 fatal heat stroke cases, found that damage to the central nervous system was manifest from the onset and persisted until death, and in some cases (those surviving 24 hours or longer) permanent damage occurred. Similar observations were made by many others[64,81,88,342,352,359,440,448,461]. Damage to the central nervous system may diminish or abolish the thermoregulatory mechanisms.

2.3.6.4 Disturbances of the cardiovascular system

Sinus tachycardia, with heart rates exceeding 140 beats/minute and shallow pulse is the rule in heat stroke cases[78,81,84]. In most patients admitted into hospital the arterial pressure was low and only in few cases normal pressures were recorded[69,79,84,85,88,449-451]. In many of these cases the blood pressure returned to normal levels after the body was cooled. In fatal cases hypotension was found to be prolonged and resistant to therapy[81,86].

Of 13 patients seen by Costrini et al.,[79] 7 were initially hypotensive. While Malamud et al.[78] estimated that in the 55 cases where blood pressure measurements were taken, 60% of them had systolic pressure of less than 100 mm Hg. This could be either due to a decreased venous return, owing to peripheral pooling of blood, or cardiac failure. Austin and Berry[85] reported that in 27 out of 44 patients the systolic pressure was less than 100 mm Hg and 9 of these died of left ventricular failure. Hypotension was observed in 16 out of the 36 heat stroke cases admitted into the Tel-Hashomer Hospital in Israel[81]. According to Leithead and Lind[440] cyanosis of the lips and face is a common feature in heat stroke victims. Such changes are indicative of reduced oxygenation of blood probably due to cardiac failure or to reduced cardiac output due to peripheral pooling. Other investigators have also reported peripheral circulatory failure[80,84,85]; high[65,80,84] and low[80,84] cardiac output failures in heat stroke patients.

Austin and Berry[85] believed that in heat stroke, hypotension carries a poor prognostic sign, and according to Shibolet et al.[69] and Knochel,[68] the normal functioning of the cardiovascular system is a prerequisite for effective heat dissipation.

In most studies, blood pressure and other haemodynamic measurements were carried out after some time had lapsed from the onset of heat stroke. It is therefore not possible to extrapolate the cardiovascular status at the onset of heat stroke or soon after it from those measurements. Furthermore, the prodromal changes which occur during heat stroke and factors which may precipitate heat stroke are not evident from such studies. Hence, investigators have resorted to the use of animal models.

Unanaesthetized mice and rats subjected to an environment temperature of 45 to 50°C showed markedly flushed skin at rectal temperature of 40,5°C indicating increased cutaneous blood flow[63]. At a rectal temperature of 42°C, the skin became violently flushed, and petechiae appeared, and at about 43°C the animals exhibited convulsions followed by coma[63]. In a parallel study similar changes were observed in dogs with rectal temperatures of between 42 and 44°C[63]. Daily and Harrison[63] measured the arterial and venous pressures and cardiac output in heat-stressed dogs. They found that the arterial pressure remained close to base line levels until a rectal temperature of about 41°C, when it began to decline gradually. Beyond rectal temperatures of 42°C, i.e., in the "grey" stage (when the colour of the skin changed from dusky red to pallid grey) the arterial pressure fell sharply. No significant changes in venous pressure were detected[63]. The cardiac output in these dogs, on the other hand, almost doubled as the rectal temperature rose from 37 to 42°C, and beyond 42°C it followed a similar time course as the mean arterial pressure: it declined sharply, indicating circulatory failure. According to Daily and Harrison[63], the mean arterial pressure showed a sharp decline beyond 42°C because the cardiac output was not sufficient to supply the vasodilated skin. The sharp decline in blood pressure indicated circulatory collapse[63]. Furthermore, Daily and Harrison[63] postulated that the colour of the skin goes through a dusky red to pallid grey as vascular collapse sets in. Similar changes in mean arterial pressure and cardiac output were observed by Bynum et al.[402] and, more recently, by Yang et al.,[401] also in a dog heat stroke model. The stroke volume, cardiac output and central venous pressure showed significant declines, commencing at rectal temperature of 41°C[401,402]. In addition, the central venous pressure rose at 42°C and remained above control levels at 43°C[401]. The fall in stroke volume,

cardiac output and mean arterial pressure and the rise in central venous pressure indicated cardiac failure[401]. Moreover, injection of naloxone (which, as discussed previously, blocks the action of endorphins) at a rectal temperature of 40,5°C caused the stroke volume and cardiac output to increase significantly at 41°C[401].

Similar changes in mean arterial pressure were also observed in rabbits[454] and rats[431]. In rats, according to Kielblock et al.[431] a greater fall in systolic than in diastolic pressures, coupled with a fall in ratio of pre-ejection phase to ejection time (PEP/ETc), indicated left ventricular failure. Kielbock et al. suggested that the trigger mechanism for left ventricular failure was excessive reduction in total vascular resistance following the abolishment of the compensatory splanchnic vasoconstriction.

2.3.6.5 Disturbances in respiratory system

During heat-stress the respiratory rate is often found to be rapid, usually more than 32 per minute[82,342,360,440]. It has often been described that respiration is laboured, shallow, gasping and panting in nature[440]. Hyperpnoea is sometimes extreme, with 60 or more per minute, and in the terminal stages of fatal heat stroke, breathing may become cyclical or it may resemble the Cheyne-Stokes type[440]. Such alterations in respiration could possibly decrease oxygenation of blood and could be a component of the cyanosis of lips and face observed by Leithead and Lind in heat stroke victims[440].

2.3.6.6 Renal changes

In almost all heat stroke cases, proteinuria - the presence of cellular and granular casts in urine - and raised blood urea levels were seen [82,462-466]. In severe heat stroke cases, oliguria often occurred and renal failure was a common complication[78,81,85,463,464]. The urine volume in many cases was less than 500 ml/day, dark brown - resembing "machine oil"[81,82,462,464,466]. The urine in an anaesthetized dog heat stroke model was described as "turbid, scanty, and brownish, analagous in some respects, to human heat stroke cases"[402]. In addition, haematuria (trace), proteinuria and alkalinuria were also present[402].

During the acute stage of heat stroke varying degrees of renal damage often occurred[462]. This was reversible in the majority of heat stroke victims. According to Kew et al.[462] the pathogenesis of renal lesions may be due to a direct thermal injury and factors such as hypotension, acidosis, hyperuricaemia and particularly renal ischaemia, which may aggravate the noxious action of heat.

2.3.6.7 Gastrointestinal disturbances and liver damage

Gastrointestinal symptoms such as vomiting and diarrhoea, tinged with blood (Table 6), were a common occurrence in heat stroke victims[69,81,342,360,440,451,465]. Diarrhoea and vomiting may occur shortly before heat stroke. In some patients diarrhoea was reported to be bloody[81]. It was suggested that diarrhoea may have been due to an outpouring of fluid into the small intestine and it

probably was a manifestation of gross disturbances in vasomotor control[82] and/or loss of sphincter control[88] of the gastrointestinal tract.

Toraason and his co-workers[467] observed damage to the superficial intestinal mucosal layer and a decreased rate of glucose metabolism in heat-stressed rats. Bloody, mucinous stools were noticed in heat-stressed dogs by Bynum et al.[402] and, in addition, they found that dogs with heat stroke had ecchymosis of the intestinal wall with occasional punctate ulcerations of the intestinal mucosae. Shapiro et al.[452] noticed that when the rectal temperature of experimentally heat-stressed dogs reached 43,0 to 43,9^{o}C, about 50% of the dogs began to vomit and more than 60% of them had diarrhoea (Table 6). Furthermore, when the dogs were heat-stressed to 44 to 45,1^{o}C all of them had diarrhoea[452]. Similar gastrointestinal disturbances have been described in experimental animals following endotoxin administration (discussed previously).

Pathological changes of the liver were among the most consistent findings in humans after heat-stress[468]. Liver damage, as evidenced by elevations of serum bilirubin, iron, glutamic oxalacetic transaminase (SGOT), and fall in prothrombin and fibrinogen levels, were reported by Shibolet et al.[81]

Biopsy specimens of livers taken from South African mine workers revealed mild pathological changes such as the widening of central veins and adjacent sinusoids, the pooling of blood and varying degrees of hepatocellular degeneration[83]. The latter was characterised by a disarray of liver cell plates, alterations in size and staining properties of hepatocytes, and small foci of cellular

necrosis. In keeping with the above pathological changes, the concentrations of serum enzymes SGOT, glutamate-pyruvate transaminase (SGPT) and lactate dehydrogenase (LDH) were also elevated[83].

Many of the above pathological changes of the liver were also noticed in experimental heat stroke animals[402,467,452]. The liver from rats kept restrained in an environmental temperature of 41,5°C, or when run to exhaustion at 26°C, showed congestion, vacuolization and necrosis of individual hepatocytes (rectal temperature of these rats ranged between 42 and 42,8°C)[468]. In addition, electron microscopy revealed signs of injury: loss of endothelial cells and microvilli from the parenchymal cells, and fragmentation of the endoplasmic reticulum[468]. Similar changes in serum enzymes and liver damage were also observed in heat-stressed dogs[402,452].

Heating the liver to about 43°C in dogs by extracorporeal circulation caused a decrease in indocyanin green clearance by the liver (indicating hypoxia) and the mortality rate rose at a rectal temperature above 43°C[469]. Furthermore, according to Rowell et al.,[414] physical exercise in man, in a high ambient temperature, caused, in addition to elevating the core temperature to above 40°C and heart rate to above 160 beats/minute, a 20% reduction in liver function, which was due to a diversion of blood from the splanchnic region to the skin.

The pathophysiological changes of the gastrointestinal tract and the liver might also have been caused by ischaemia[468] and/or hypoxia[83] in addition to the direct thermal injury. A consequence of local ischaemia would be

damage to the intestinal mucosa. In such case endotoxins may be permitted to enter the circulation and, especially in the presence of a damaged liver, the endotoxins may spill over into the systemic circulation.

2.3.6.8 Haematological disturbances

Haematological disturbances in heat stroke victims are common, frequently leading to haemorrhagic and sometimes to clotting tendencies[470]. Changes in blood coagulation mechanisms, including hypoprothrombinaemia, thrombocytopenia and prolonged bleeding time, appear to be well-recognized complications of heat stroke[69,351,360,451,470-473] and, it is thought, are responsible for widespread haemorrhage and death from heat stroke[81,473]. Other investigators are of the view that increased capillary permeability may also be implicated in haemorrhage seen in heat stroke[473], yet others[351,471] felt that damage to the vascular endothelium could initiate platelet aggregation and disseminated intravascular coagulation (DIC). Electron microscopic evidence seems to indicate that significant endothelial cell damage occurs in heat stroke victims[471].

According to Anderson et al.,[360] disseminated intravascular coagulation, defined by thrombocytopenia, elevated fibrin split products, hypofibrinogenaemia, usually appeared 24-72 hours after the admission of the victim to hospital. In contrast to the above findings Beard and his co-workers[474] were of the view that disseminated intravascular coagulation only became evident if heat stroke was very severe.

Neutrophil leucocytosis is also a prominent feature in heat stroke victims[78,81]. A similar blood picture is observed during endotoxaemia (discussed previously). Fine[475] proposed that haemorrhagic diathesis and disseminated intravascular coagulation, which occur in heat stroke, could be due to endotoxaemia of intestinal origin.

2.3.6.9 Serum enzymes

Serum enzyme levels are widely used in clinical medicine to indicate the degree and extent of tissue damage[476]. Heat stroke, depending on its severity, may be characterized by widespread tissue damage[69,78,476]. Therefore, one would expect elevated concentrations of the enzymes - SGOT, SGPT, LDH and creatine kinase (CPK) in the serum[81,476,477]. Nevertheless, serum enzyme levels of blood samples taken at the point of collapse of heat stroke victims were not of diagnostic value. These showed very small changes, if any, but those taken a few hours later appeared to give a fair indication of the extent of tissue damage[79,81,477]. Costrini et al.[79] found that blood samples taken at zero time from heat stroke patients, showed no changes in CPK-MB isoenzymes, CPK, LDH, SGOT and SGPT - whereas twelve hours later, these enzymes were significantly elevated except for CPK-isoenzymes. Similar changes in serum enzymes were detected by others, in human heat stroke patients[358,450,478] and in animal heat stroke models[452,479]. Kew et al.[477] noticed that in heat stroke patients the level of SGOT was invariably elevated within 24 hours whilst in patients with elevated temperature due to infections, the levels of serum enzymes were normal. Kew et al.[477] and Shibolet et al.[81] agreed that elevations

in SGOT levels from a normal of 32 units to a level in excess of 1000 units indicated both severe heat stroke and a poor prognostic sign.

2.3.6.10 Sweating

Whilst many reports indicate that patients with heat stroke normally present themselves with hot, dry skin[65,85,86,451,470], others have reported patients with profuse sweating[68,76,79,81,342,351-353,373]. Therefore, absence of sweating is not an indicator of heat stroke.

2.3.7 Pathological changes and post-mortem findings

2.3.7.1 Introduction

Since the temperature of all organs is elevated during heat stroke, the pathological picture usually shows widespread tissue damage except in cases where the patient dies within a few hours after the onset of heat stroke[69,342,343]. In the latter case, pathological changes are limited to the central nervous system[343]. Generally, the pathologic picture reveals swelling and degenerative changes of cells and tissues and haemorrhages in most organs[69].

2.3.7.2 The central nervous system

The brain is often congested, oedematous and multiple

haemorrhage occurs[69,78,82,351,454]. In addition, chromatolysis, necrosis of cerebral and cerebellar neurones was reported[78,455]. However, no evidence of hypothalamic (the centre for thermoregulation) damage was detected and if present, was thought to be a rare occurrence[78,454].

2.3.7.3 The cardiac muscle

Haemorrhages, either diffuse and petechiae or more severe and localized, and necrosis were noticed in the myocardium[78,81,351,480]. Furthermore, subendothelial petechiae were observed in the interventricular septum. According to Kew,[480] "although the heart was affected in the majority of the patients, the damage was not sufficiently severe for overt cardiac failure to occur".

2.3.7.4 The gastrointestinal tract and liver

Diffuse mucosal haemorrhage occurred in the stomach, duodenum and small intestine[69,78,351,465] and the blood vessels in the gastrointestinal tract were virtually engorged[78] suggesting reduced splanchnic blood flow.

It was consistently found that pathological changes in the liver in man, subsequent to heat overload, occurred[481]. These changes, in the first few hours after heat stroke, included sinusoidal widening and congestion, portal vein dilatation, centrilobular hepatocellular necrosis, fatty changes and non-fatty vacuolization and congestion[81,83,481]. According to Rubel and Ishok[481], the changes in the liver were similar to those in bacterial

and endotoxin shock.

2.3.7.5 The kidney

Malamud et al.[78] found that in cases where death occurred within 24 hours the kidneys were congested and above normal in weight, with macroscopic and microscopic haemorrhages being present in about 20% of the cases. However, when the duration of illness was longer than 24 hours, pigmented casts were present in the distal convoluted tubules and pigmented casts and necrosis in the lower nephron. Similar changes were also observed by other investigators[81,462-466]. In addition, chronic interstitial nephritis was observed in some patients[466]. It was postulated that circulating fibrin polymers, lodged in small blood vessels, especially the glomerular capillaries, led to impairment of renal blood supply and chronic interstitial nephritis[482]. Endotoxin, as discussed earlier, also causes fibrin plugs in renal vessels and renal damage, similar to the above.

Malamud et al.[78] believed that most of the lesions, apart from those in the brain, may be attributed to anoxia and circulatory collapse, as evident in shock.

2.3.8 Pathophysiology of heat stroke

Hill[483], as early as in 1920, suggested that the direct cause of heat stroke was exhaustion of the sweating mechanism. He also postulated that heat stroke resulted from a rise in body temperature to a level: "incompatible with the maintenance of equilibrium of the physico-

chemical reaction in the cell on which life depends". However, as discussed above, sweating has been observed in heat-stroke victims.

Whilst Adolph and Fulton[484] were of the view that circulatory failure was the precipitating factor in the breakdown of heat regulation, Ferris et al.,[86] in their investigations in heat stroke patients, did not find evidence of definite congestive cardiac failure, since peripheral oedema, and venous engorgement were absent, and venous pressure and arterial oxygen saturation were relatively normal. Moreover, cessation of sweating occurred just prior to the actual heat stroke[86]. Ferris et al.,[86] however, were unable to explain the mechanism involved in the sudden cessation of sweating, but they ruled out loss of chlorides, dehydration and circulatory failure as possible factors.

Malamud and his co-workers[78] observed that heat stroke patients have certain symptoms which were in all respects similar to those seen during shock, the most important of these being pallor or slight cyanosis, vomiting, fall in blood pressure, a rapid thready pulse and shallow, sighing respiration. They agreed with Hill's[483] initial postulate that the precipitating cause of heat stroke was the excessive body temperature itself which incapacitated the central heat-dissipating mechanism leading to cessation of sweating. The latter, in turn, caused a greater accumulation of body heat which became compounded by the increased metabolism and sooner or later shock ensued; moreover, owing to a subsequent vasoconstriction, body temperature continued to increase[78]. However, Malamud et al.[78] were unable to find significant pathological changes in the hypothalamus, to account for the suggested failure

of the thermoregulatory mechanism.

Shih et al.[454] were of the view like other investigators, that neurological damage occurs in heat stroke, but they differed from them in so far as the course of events in heat stroke pathophysiology is concerned[78]. They regarded shock as a "...secondary manifestation and therefore non-specific and unessential to the fundamental pathogenesis of the disorder"[454]. They postulated that the reduction in cerebral perfusion pressure from 80 mm Hg to below the autoregulating level (19 mm Hg) caused cerebral ischaemia resulting in neurological damage and brought on heat stroke[454]. They also stated that cerebral ischaemia or anoxia, coupled with hyperthermia, intracranial hypertension and brain oedema, were the main causes of heat stroke.

The hypothesis put forward by Malamud and his co-workers[78] was at variance with that of Adolph and Fulton[484] but similar to that of Ferris et al.[86] Adolph and Fulton[484] believed that circulatory failure in heat stroke was due to the peripheral pooling of blood, hence a reduced venous return. They assumed that the heart attempted to compensate for the reduced venous return by increasing its rate, the failure of which ultimately led to circulatory failure and shock ensued.

The significant point in both hypotheses, however, is that shock occurs. Apart from slight modifications, the failure of the central and the cardiovascular system have been generally regarded as the main factors causing heat stroke.

In spite of the great amount of work done and the number of papers published, no consensus has yet been reached on the pathophysiology of heat stroke: it is still not known for certain whether the mechanism is central (nervous system) or peripheral (cardiovascular).

With respect to the failure of the central mechanism or of the thermoregulatory centres, it was reported that the presence and absence of sweating were observed in the heat stroke patients discussed previously, that is, in section 2.3.6.10. In an editorial review in Lancet of July, 1968, the controversy regarding sweating was highlighted. The editor stated that one of the unsolved problems regarding the onset of heat stroke is whether cessation of sweating precedes or follows hyperpyrexia. With respect to the failure of the peripheral mechanism (cardiovascular), firstly, it is not certain whether circulatory failure is a consistent finding in heat stroke and secondly, if present, there is no unanimity as to its cause: whether it is due to cardiac failure or to peripheral failure[470].

Apart from opinion being divided as to the failure of the central and circulatory mechanisms in heat stroke, opinion is also divided amongst circulatory mechanism as the cause of heat stroke, that is to say, whether heat stroke is due to cardiac or peripheral failure.

Daily and Harrison[63] concluded, from their investigation into heat-stressed experimental animals, that the circulatory failure which causes heat stroke is due to vascular collapse. They assumed that, during hyperthermia, there is initially a compensatory splanchnic vasoconstriction, followed by splanchnic vasodilatation due to vascular collapse. Hence they suggested that the circulatory failure was of peripheral rather than cardiac origin because no increase in venous pressure was detected

and, furthermore, the cardiac output showed a drastic decline. Although they accepted the peripheral origin of circulatory failure they nevertheless were of the opinion that "the reserve power of the heart is impaired" and that heart failure may be induced if sufficient fluid is administered at a rapid rate to heat stroke patients.

According to Gold,[485] "the cardiovascular system apparently takes the brunt of heat exposure". In contrast to Daily and Harrison[63], Gold[65] believed that the primary event in circulatory collapse was high-output cardiac failure. He explained the mechanism of cardiac failure which led to circulatory collapse as follows: "The greatly diminished peripheral resistance allows for an abundantly increased and rapid venous return, which in turn increases the cardiac output. Assuming the left side of the heart cannot keep pace with the right, this increase in venous return results in an elevation of venous pressure". He postulated that the increase in venous pressure led to a cessation of sweating. However, he (Gold) failed to explain how an elevated venous pressure could cause the cessation of sweating.

The contradictions in these findings could be explained by species variations. Daily and Harrison[63] made use of mice, rats and dogs whilst Gold[65] made use of human volunteers. The contradictions could also be the result of heat-load variations. Daily and Harrison[63] heat-stressed their animals to a rectal temperature of 43^{o}C or more, whereas Gold[65] heat-stressed his subjects to about 40^{o}C.

O'Donnell and Clowes[80] also believed, like Gold,[65] that "survival from heat stroke depended upon an adequate response of the cardiovascular system", but they found, like Gold,[65] that in most heat stroke patients the central venous pressure was elevated. Most of the eight patients studied by O'Donnell and Clowes[80] exhibited a hyperdynamic

circulatory state with a decreased total peripheral resistance (similar to post-traumatic, post-shock, and septic states); only one case was diagnosed as hypodynamic circulatory failure. They suggested that the disorder leading to heat stroke could be due to right-sided cardiac failure because of the elevated venous pressure. They also felt that vasoactive substances, observed in post-traumatic, post-shock and septic states, could also be involved in heat stroke pathophysiology. Nevertheless, they were unable to cite references in literature, apart from the fact that endotoxin was present in one heat stroke patient seen by Graber et al[44].

Like Daily and Harrison,[63] Sprung[84], in a recent study, was unable to find evidence of cardiac failure in elderly heat stroke patients and suggested that the circulatory failure was due either to a peripheral pooling of blood or to hypovolaemia. He maintained, however, that the factors responsible for heat stroke could be multiple. Similarly, Yang et al.[401] and Shapiro and his co-workers[452] recently postulated that the mechanism of heat stroke was also complex and multiple factors were involved. Shapiro and his co-workers[452] felt that excessive body temperature produced "profound damage in many systems and organs, such as the central nervous system, kidney, liver and the blood clotting mechanism, which results in the clinical laboratory and anatomopathological picture of heat stroke".

On the other hand, Hubbard[479] argued that "...if direct thermal injury to a target tissue such as the thermoregulatory centers of the brain, is the primary factor in the pathogenesis of heat stroke, then the exercise-induced hyperthermia of running rats should not be more lethal than equivalent heat loads in the absence of physical effort". Hubbard further argued that "...if hyperthermia alone were the predominant cause of heat

stroke mortality, then one might have expected a slightly higher mortality at equivalent core temperatures in the older population with cardiovascular disease". However, he found that the latter was not the case, and in fact 30% of heat stroke deaths occurred in young, presumably healthy labourers, whilst no deaths were observed in the older, sedentary part of the population. In addition, exercise to the point of exhaustion resulted in increased rates of cellular injury, and heat stroke mortality at low thermal loads, than heat-stress on its own[453,486]. Hubbard[479], therefore, believed that his findings lent a renewed support for a cardiovascular origin of heat stroke.

In the opinion of Hales,[67,439] Khogali[342] and Robertshaw,[432] peripheral vasoconstriction, in attempting to increase the central venous pressure, plays a major role in heat stroke pathophysiology.

According to Hales[439] and Robertshaw[432], severe heat-stress leads to a competition between skin blood flow and the cardiovascular system, with the former attempting to maintain body temperature and the latter the blood pressure and blood flow to the body. Hales[439] assumed that at the onset of heat stroke, there is a reduced central venous pressure (although a raised central venous pressure was also detected in heat stroke[65,401]) due to reduced cardiac filling. The reduced cardiac filling is detected by the low-pressure receptors, and via the baroreceptor reflex, vasoconstriction of cutaneous vessels is initiated to correct the reduced central venous pressure[432,439]. The cutaneous vasoconstriction leads to a reduced heat loss with a consequential further rise in core temperature. The raised core temperature presumably evokes critical changes in the central nervous system which results in heat stroke[439]. Thus both Hales[439] and Robertshaw[432] proposed that the cardiac filling pressure is the limiting factor in adjusting to heat-stress.

Apart from the two widely held views, namely, that heat stroke is the result of the failure of the central nevous mechanism and circulatory failure, it has been also suggested that endotoxins may also be implicated in heat stroke pathophysiology.

Bynum et al.[62] pointed out that "...the metabolic abnormalities, coagulopathies, cardiovascular collapse, hepato-renal dysfunction and autopsy findings evident in human and animal heat stroke victims and in animal heat stroke models parallel the findings associated with the shock state of endotoxemia". Graber et al.[44] and Caridis et al.[43] reported fatal heat stroke cases that were accompanied by endotoxaemia. These researchers suggested that the cause of death could be endotoxin shock resulting from the severe loss of hepatic function. In the case reported by Graber et al.,[44] the patient had presented himself with hypotension, gastrointestinal bleeding, coagulation abnormalities, bilateral pneumonitis and recurrent ventricular fibrillation. These symptoms were similar to those of patients with massive non-septic injury[44]. The lesions in the lungs and gastrointestinal tract noted at post-mortem were similar to injury caused by endotoxaemia[43]. In addition, reduction of the gut flora with bowel evacuation and antibiotic pre-treatment increased the incidence of 18 hour survival rate in heat stroke in dogs[62]. However, no assay of blood samples for endotoxin were made in the latter study.

Butkow and his co-workers[42] found that rabbits subjected to heat-stress developed endotoxaemia at a rectal temperature of about 41^{o}C - a temperature which is unlikely to cause liver injury. Furthermore, rabbits which were pre-treated with oral antibiotics had a slower rise in rectal temperature and a reduced prevalence of endotoxaemia than control untreated rabbits[42].

On the other hand, DuBose and his co-workers[487,488] did not believe that endotoxins played a direct role in mortality following heat-stress, as they did not find any significant increase in the incidence of invasion by gram-negative bacteria or their endotoxins (using the old and highly suspect LAL gelation test) in blood or extraintestinal tissues of heat-stressed rats, except the lungs. They did find, however, that endotoxin tolerant rats were significantly more resistant to heat-stress mortality than normal control rats. Rats rendered more sensitive to endotoxin by zymosan pre-treatment did not have altered mortality rates[487]. In the cases of extreme heat-stress, even the endotoxin tolerant rats were not significantly resistant. The blockading of the RES with gelatin significantly increased heat-stress mortality[487]. DuBose and his co-workers[488] concluded that the RES may play a fundamental role in the pathogenesis of, and tolerance to, experimental heat-stress, similar to the role of RES in other types of shock[488].

Lind[449] recently commented that "the pathophysiology of heat stroke remains elusive and there are many confusing and even conflicting observations".

CHAPTER 3

MATERIALS AND METHODS

3.0 Introduction

In Part I of this chapter the materials and methods to investigate the role of gut-derived endotoxin during and following a temporary ischaemia by clamping of the superior mesenteric artery are discussed, and those used to investigate the role of gut-derived endotoxins in heat-stress and heat stroke are discussed in Part II.

Part I: INTESTINAL ISCHAEMIA

3.1 Experimental animals

Adolescent cats or vervet monkeys (Cercopithecus aethiops) of either sex were used. The animals were maintained in the animal unit facility of the Medical School of the University of Natal. The cats were housed in cages (3-4 per cage) with natural lighting. They were fed on cat pellets supplemented by meat and provided with tap water, *ad libitum*. The monkeys were housed in large cages, about 5 per cage, with natural lighting. They were fed fresh vegetables and fruit and provided with water, *ad libitum*.

3.2 Anaesthesia

(a) Cats

Cats were anaesthetized with phenobarbitone sodium (30

mg/kg, intraperitonially). Anaesthesia was maintained for the duration of each experiment with phenobarbitone sodium (2 mg/kg), intravenously, as and when required.

b) Monkeys

Monkeys were anaesthetized with ketamine (10 mg/kg) intramuscularly. Anaesthesia was maintained with intravenous ketamine (5-10 mg/kg) as and when required.

3.3 Rectal temperature

The rectal temperature was measured using a thermorectal probe, inserted about 8-10 cm into the rectum, and a telethermometer (Yellow Springs Instrument 46-TUC). The telethermometer was calibrated against a Hewlett Packard Quartz Thermometer type 2804A with an accuracy of 0,001^{o}C.

3.4 Arterial blood pressure

A Statham (B188) transducer connected either to a Honeywell CM 130 patient monitor system or a Beckman recorder was used to record the arterial blood pressure. The recorders were calibrated using a mercury manometer. Recordings were made at 5-minute intervals. The Honeywell CM 130 patient monitor system is fitted with a computer which calculates the mean arterial pressure (MAP) electronically by integrating the area under the arterial pressure curve and the results are displayed as a print-out together with the arterial pressure recording. When the Beckman recorder was used, the mean arterial pressure was calculated using the equation: MAP = 1/3 systolic + 2/3 diastolic pressures[214].

3.5 LPS assay

The quantitative chromogenic substrate modification of the

Limulus amoebocyte lysate (LAL) technique was used for the determination of LPS concentrations in the plasma samples[60,61]. These analyses were carried out by Michelle T Wells (research assistant of Professor SL Gaffin, Department of Physiology, University of Natal Medical School) using the method outlined below.

The plasma was diluted 1:4 with sterile pyrogen-free water, heated for 10 minutes at 75°C, cooled on ice, and centrifuged at 23 000 x g[60,61]. Duplicate samples of 50 ul heat treated plasma and 50 ul of chromogenic LAL were incubated for 10 minutes in sterile depyrogenated glass tubes at 37°C. Thereafter 100 ul aliquots of chromogen (1:1 solution of buffer and chromogen substrate - MA Bioproducts, Walkersville, MD) was added and mixed. The tubes were incubated for a further 3 minutes at 37°C. When adequate colour had developed, the reaction was stopped with the addition of 100 ul of 25% acetic acid. Plasma blanks were run for each sample (50 ul of heat-treated 1:4 dilution plasma plus 150 ul sterile water plus 100 ul 25% acetic acid) in order to account for the absorbance due to plasma alone. Optical density was read at 405 nm using a Titertek Multiskan, Flow Labs. - McLean plate reader. Values (ng/ml) were determined from standard calibration curves employing a reference LPS *E.coli* 0111:B4. The calibration curves were sensitive to LPS from 0,02 ng/ml and were linear from 0,02-0,2 ng/ml LPS (r=0,990). This highly reproducible technique was shown to overcome objections reported for the LAL gelation test[61]. A 95,1% recovery of LPS from spiked plasma samples (shown in Table 7) was obtained using this method (procedure is discussed below in section 3.5.1). This technique had proved to be more sensitive than the previously used gelation test[489].

3.5.1 Recovery of LPS in normal blood samples

In order to test the efficacy of recovery of LPS from plasma samples, by means of the chromogenic LAL technique, blood samples were removed aseptically from healthy persons and placed into sterile heparinised tubes. The samples were centrifuged at 16 000 x g and the clarified plasma was then spiked with different concentrations of a reference LPS *E.coli* 0111:B4. Controls were set up employing sterile saline in place of plasma. The results of the recovery test are shown in Table 7.

TABLE 7: Recovery of LPS in saline and normal plasma by the Chromogenic LAL assay.

	*LPS recovered ng/ml (%)	
Added LPS ng/ml	SALINE	PLASMA
0	0,0	-
0,02	0,019 (95.0%)	0,018 (90.0%)
0,05	0,042 (84.0%)	0,046 (92.0%)
0,08	0,078 (97.0%)	0,079 (98.0%)
0,12	0,121 (100.8%)	0,118 (98,0%)
0,16	0,148 (97,4%)	0,156 (97.0%)
0,20	0,189 (94,5%)	0,180 (90.0%)
0,50	0,492 (98.0%)	0,505 (101.0%)
1,00	0,978 (97.0%)	0,955 (95.0%)
OVERALL MEAN	95.5% ± 1,73%	95,12% ± 1,45%

* Mean value of 2 readings done in duplicate

3.6 Surgical procedure for superior mesenteric artery occlusion and collection of blood samples for LPS analysis

All animals were fasted overnight. The cats were anaesthetized with phenobarbitone sodium (30 mg/kg), intraperitonially, and the monkeys with ketamine (10 mg/kg), intramuscularly. The fur from the inner surfaces of the thighs and from the abdominal surface was shaven. The femoral areas and the abdominal surface were cleansed with topical chlorhexidine gluconate (5% aqueous solution). Catheters were inserted into a femoral vein and artery. Before the blood pressures were recorded, both femoral arteries were catheterized. The catheter to the femoral vein was used for the infusion of normal saline and the administration of anaesthetics and drugs. Blood samples for LPS analysis were drawn from the catheterized, femoral artery. A thermorectal probe was inserted into the rectum for the monitoring of body temperature.

An incision was made in the linea alba to open the abdominal cavity. The superior mesenteric artery was exposed and freed from the surrounding connective tissue. A loop of a sterile piece of cotton was then positioned around the superior mesenteric artery to make rapid identification easy during application of a clamp or, in some cases, it was used for total occlusion of the superior mesenteric artery, when drawn tight. The abdominal cavity was then closed. The animals were allowed a 30 minute stabilization period before the commencement of each experiment. Thereafter, 1 ml arterial blood samples for baseline LPS analyses was taken from the femoral artery at 20 minute intervals for 1 hour. At the end of this period the superior mesenteric artery was clamped for 1 hour[31] and the abdominal cavity was kept closed. Blood samples were collected at 20 minute

intervals from the femoral artery. The abdominal cavity was reopened and the occlusion removed. Blood samples were collected at 5-20 minute intervals for 2 hours during the reperfusion period. At the termination of the experiment the animal was euthanazed with phenobarbitone sodium.

All blood samples were collected in heparinized, sterile, pyrogen-free plastic tubes (Falcon) and stored on melting ice for the duration of the experiment. After each withdrawal of blood sample an equal volume of pyrogen-free saline was infused back into the animal. Sterile procedures were adopted throughout the experiment. The blood samples were centrifuged and the plasma removed under sterile conditions in a laminar flow hood. The samples were either analysed on the day of the experiment or frozen at -20^{o}C for a period of up to one week until analyzed.

3.7 Experimental procedures

The experimental procedures for the various experiments are given below.

3.7.1 Time course of changes in plasma LPS concentration and the effects of corticosteroid prophylaxis (see Appendix 1)

Fourteen cats were used in this experiment. They were anaesthetized with phenobarbitone sodium (30 mg/kg), intraperitonially, and catheters were inserted into a femoral vein and artery. The cats were then divided at random into two groups. The steroid group (n = 6) received a 30 mg/kg methylprednisolone sodium succinate (MPSS) (Upjohn Laboratories) infusion over a 15 minute period via the femoral vein. The other cats (n = 8) acted as controls. The abdominal cavity was opened and the superior mesenteric artery was identified and cleared of

surrounding tissues (as discussed above). After the animals had stabilized, blood samples were withdrawn at 20 minute intervals for one hour. The superior mesenteric artery was then occluded with a bulldog clamp for 60 minutes[31] and blood samples were collected at 20 minute intervals. Blood samples were withdrawn at 5-20 minute intervals during the reperfusion period. At the end of the experiment the animals were euthanazed.

The concentrations of LPS in the plasma samples were determined as described in section 3.5.

3.7.2 The effects of antilipopolysaccharide (anti-LPS) antibodies on plasma LPS levels (see Appendix 2)

Twenty nine anaesthetized cats of both sexes were used. They were at random divided into five groups: groups A-D received an intravenous infusion over a 2 minute period of 1,0 ml/kg of anti-LPS hyperimmune equine plasma (ATOX Pharmaceutical Co., Gillits, South Africa). This dose was selected on the basis of previous studies in this department[53].

Anti-LPS IgG rich equine plasma was administered 1,5 hours prior to occlusion of the superior mesenteric artery to cats in group A (n = 6), immediately after release of the occlusion to cats in group B (n = 5), 10 minutes after release of the occlusion to cats in group C (n = 5) and 20 minutes after release of the occlusion to cats in group D (n=5). The other eight cats (group E), which received no anti-LPS hyperimmune plasma, acted as controls.

The surgical procedures, superior mesenteric artery occlusion and the collection of blood samples were as described under 3.6. and 3.7.1.

The LPS-precipitable IgG in the anti-LPS preparation had a

concentration of 1200 ug/ml according to an ELISA that had been calibrated by an immunoprecipitation reaction[117]. Specific IgG antibodies present could bind to LPS prepared from a wide range of gram-negative bacteria, including *E.coli, Shigella* spp., *Klebsiella* spp., *Pseudomonas* spp., *Salmonella* spp., and *Proteus* spp. and could destroy these bacteria within minutes by means of complement activation[116].

3.7.3 Effect of prophylactic oral, non-absorbable antibiotic on plasma LPS concentrations and cardiovascular parameters (see Appendix 3)

3.7.3.1 Experimental animals and procedure

A total of eight monkeys of both sexes, weighing between 2,6 and 6,8 kg, were used. They were randomly divided into two groups.

(i) Control superior mesenteric artery (n = 4): those primates which were subjected to an 1 hour occlusion of the superior mesenteric artery and had received no treatment.

(ii) Superior mesenteric artery + kanamycin (n = 4): those primates which were pretreated with kanamycin (15 mg/kg) ("Kantrexil" suspension - The B-M group Pty, Ltd). The animals were anaesthetized with ketamine (10 mg/kg,intramuscularly) before antibiotic therapy. The antibiotic was administered every 12 hours over 5 consecutive days via a nasogastric tube. They were subjected to a superior mesenteric artery occlusion shock on the 6th day.

The animals were anaesthetized with intramuscular ketamine (10 mg/kg). Thereafter, incremental bolus doses (5-10 mg/kg) of ketamine were administered intravenously, to

maintain anaesthesia for the duration of the experiment. Following anaesthesia a rectal swab was taken for bacteriological examination. After cleansing both the femoral areas with 5% aqueous chlorhexidine gluconate, catheters were introduced into both femoral arteries and a peripheral vein. One of the arterial catheters was connected to a B188 Statham transducer for the recording of arterial blood pressure (as described in section 3.4), while blood was withdrawn from the opposite catheter (as described under section 3.6). Surgical procedures, superior mesenteric artery occlusion and the collection of blood samples were carried out according to the description in section 3.6 and 3.7.1.

Rectal temperatures were recorded. Arterial blood pressure was monitored continuously and recorded at 5-minute intervals, using a Honeywell CM 130 patient monitor system during the entire 4 hour experimental period. At the end of the experiment the animal was euthanazed with an intravenous overdose of phenobarbitone sodium. Immediately thereafter, a 6 - 7 cm section of the transverse colon was tied off at each end and removed for bacteriologic examination of the faeces.

3.7.3.2 Bacteriology

Bacteriological examination of rectal swabs and faecal samples were carried out by Michelle T Wells (research assistant of Professor SL Gaffin).

A. Bacterial characterisation and identification

Rectal swabs specimens for bacteriological examinations were obtained from all the animals after induction of anaesthesia but before experimental superior mesenteric artery occlusion. The sterile swabs were initially moistened with sterile saline and care was taken to avoid

peri-anal contamination. After inoculation and incubation overnight in nutrient broth, the broth culture was subcultured on desoxycholate citrate agar (DCA), MacConkey's agar, and nutrient agar (Difco, Detroit). As discussed at length below, these agars each are selective for different microorganisms. The plates were then incubated aerobically for 18-24 hours at 37°C. Twenty four hours later each plate was examined for bacterial colonies. The nature of the different colonies was noted, namely, was it entire, irregular, crenated or rhizoid in shape. In addition, pigment formation both on top of and under the surface of the colony was observed and pigment diffusion into the medium was also noted. Each different colony was then further subcultured for 24 hours at 37°C on the respective agars in order to obtain pure cultures.

Identification

A gram stain was made on presumed 'pure' cultures to ensure the homogeneity of the bacterial culture. Gram-negative organisms were then identified using the API 20E[490,491]), or API 20NE[492,493] system. Gram-positive staphylococci[494] and streptococci[495] were identified using similar kits (Path Ident, Johannesburg).

MacConkey's Medium

MacConkey's broth or agar was used to differentiate intestinal organisms into lactose- and non-lactose fermenting organisms. In MacConkey's agar, peptone and agar constitute the nutrient base[496]. Sodium taurocholate inhibits many gram-positive organisms, and lactose and an indicator (neutral red) differentiate the lactose- and non-lactose fermenting organisms. The lactose-fermenting organisms produce lactic acid, resulting in a pH change. Those organisms which do not ferment lactose give an alkaline reaction, and produce colourless colonies[496].

MacConkey's agar

Sodium taurocholate (5 g), peptone (20 g) and sodium chloride (5 g) were dissolved in 1 000 ml distilled water by heating. The reaction was allowed to cool and the pH was adjusted to pH 7,8. Fifteen grams of bactoagar (Difco, Detroit) was added, and autoclaved at 115°C for 20 minutes. The solution was filtered and the pH was adjusted to pH 7,5 and ten grams of lactose was added. An aqueous neutral red solution (7 ml) was added to give a reddish-brown colour. The mixture was then distributed into bottles and autoclaved again at 115°C for 15 minutes. When pouring plates, the melted medium was cooled to 45-50°C prior to pouring.

Desoxycholate citrate medium

A selective medium used for the isolation of the *Salmonella* spp. and especially the dysentery organisms[496]. Sodium desoxycholate (bile salt) will inhibit the growth of many gram-posisitive organisms, while favouring the growth of the intestinal gram-negative organisms. Neutral red, included as an indicator, is toxic in the presence of sodium desoxycholate to some bacterial growth pattern. Sodium citrate and sodium thiosulphate are toxic for the coliforms and to a certain extent the salmonellae. Ferric citrate is added to neutralize this toxicity for the salmonellae without interfering too much with the toxicity for the coliforms[496].

Solution A

Sodium citrate (AR) ($Na_2C_6H_5O_7.2H_2O$)	17 g
Sodium thiosulphate (AR) ($Na_2S_2O_3.5H_2O$)	17 g
Ferric citrate	2 g
Distilled water	100 ml

Solution was sterilized in a water-bath for 2 hours at 60°C.

Solution B
10% solution of sodium desoxycholate in distilled water. The solution was sterilized in a 60°C water-bath for 2 hours.

20 g of meat extract (Difco, Detroit) was dissolved in 200 ml water and made alkaline to phenolphthalein with 50% sodium hydroxide solution. The solution was brought to the boil and filtered. The pH was adjusted to pH 7,4. Twenty grams of proteose peptone was added and the volume was made up to 200 ml. Ninety grams of agar was dissolved in 3 700 ml distilled water by steaming for 1 hour. The agar solution was filtered and the meat extract-peptone solution was added. Ten millilitres of an aqueous 1:1 solution of neutral red and 40 g of lactose was added and mixed well. The mixture was bottled in 100 ml amounts and sterilized by autoclaving at 115°C for 15 minutes.

Prior to pouring plates, 5 ml of solution A and 5 ml of solution B was added to 100 ml of molten agar and mixed well.

Nutrient agar
Nutrient agar is a basic medium able to support the growth of many microorganisms[496].
Nutrient broth 8 g/l, pH 7,4 was added to 1,5% Bacto Agar (Difco, Detroit). The medium was gently heated until the agar had dissolved and then autoclaved while still molten at 121°C and 15 psi for 20 minutes.

Gram Stain
A single bacterial colony was removed aseptically using a sterile inoculating loop and placed into a drop of sterile water, in the centre of a clean glass slide. Glass slides had been rinsed in alcohol and flamed prior to use. The colony was then dispensed into constituent bacterial cells

with an inoculating loop, and allowed to dry. It was fixed with gentle heat. The slide was then flooded with 0,5% crystal violet for 30 seconds. This stain was replaced by Lugols iodine (Iodine 1 g, potassium iodide 2 g, distilled water - 300 ml) and allowed to act for 1 minute. The slide was then decolourized in acetone until no further stain appeared to flow from the preparation and then washed with double distilled water. A counterstain, neutral red (neutral red 1 g, glacial acetic acid [1%] 2 ml, distilled water 100 ml) was then applied for 2 minutes. The slide was rinsed well in water, blotted carefull and air dried. Slides were then examined microscopically using the oil immersion objective. Gram-positive organisms stained violet whilst gram-negative organisms stained red[496].

API 20E System

This system is a standardized miniaturized version of conventional procedures for the identification of Enterobacteriaceae and other gram-negative bacteria[490,491]. It consists of a ready to use microtube system designed for the performance of 23 standard biochemical tests from a single colony of bacteria on a plating medium. The strip consists of 20 microtubes containing deyhdrated substrates. The bacterial suspension distributed into the tube dissolves the substrates. The metabolites produced are revealed by spontaneous coloured reactions or by addition of reagents. The tests include: 6-nitrophenyl-D-galactosidase (ONPG), arginine dihydrolase, lysine and ornithine decarboxylase, citrate utilization, H_2S production, acetoin production, gelatinase and fermentation of glucose, rhamnose, sucrose, melibiose, amygdaline and arabinose. After 18 - 24 hours incubation the results were recorded utilizing the supplied colour charts and identification was made.

API 20 NE

This is also a micromethod, for the identification of

Gram-negative rods not belonging to the Enterobacteriaceae family i.e. *Pseudomonas* spp., *Acinetobacter* spp., *Flavobacterium* spp., *Moraxella* spp., *Vibrio* spp., and *Aeromonas* spp.,[492,493]. The conventional tests were inoculated with a bacterial suspension in saline which reconstitutes the media. The assimilation tests were inoculated with a minimal medium and the bacteria only grow if they are able to utilise the corresponding substrate. After incubation the reactions were read according to the supplied interpretation table and identification was obtained by referring to the identification charts.

API Staph

This is a biochemical system for the identification of staphylococci and micrococci based on 19 characters[494]. The microtube tests were reconstituted by adding to each tube an aliquot of API STAPH (Path Ident, Johannesburg) medium that had been inoculated with the strain to be studied. The strip was then incubated for 18 hours at 37°C after which the results were read and interpreted with reference to the information contained in the provided manual. The order of the substrates was: negative control, glucose, fructose, mannose, maltose, lactose, trehalose, mannitol, xylitol, melibiose, nitrate, α-naphthyl phosphate, pyruvate (for acetoin production), raffinose, xylose, sucrose, α-methyl glucoside, N-acetyl glucosamine, arginine and urea.

API 20 Strep

Consists of strip of 20 microtubes containing dehydrated substrates for demonstration of enzymatic activity or the fermentation of sugars[495]. A dense suspension was made from a pure culture and used to rehydrate the enzymatic substrates. The metabolic end products produced during the incubation period are either revealed through spontaneous colour reactions or by the addition of reagents. The fermentation tests were inoculated with an enriched medium which reconstitutes the sugar substrates.

Fermentation of carbohydrates is detected by a shift in the pH indicator. These reactions were read and results recorded. Enzymatic tests included acetoin production, hydrolysis, ß-glucosidase, pyrrolidonyl arylamidase, α- and ß-galactosidase, ß-glucuronidase, alkaline phosphatase, arginine dehydrolase, leucine arylamidase. The ability to ferment the following sugars was also noted: ribose, arabinose, mannitol, sorbitol lactose, trehalose, inulin, raffinose, starch and glycogen.

B. Bacterial Quantification

One gram of faecal contents was emulsified in 100 ml of sterile peptone water under a laminar flow hood. Serial dilutions of 10^2-10^8 were made up using an initial 1/100 dilution. Triplicate pour plates of 1 ml of each in 20 ml molten nutrient agar were made for bacterial enumeration. Plates were allowed to solidify and were incubated inverted at 37°C for 18-24 hours. Bacterial colonies were enumerated and results are reported as the number of colony-forming units per gram faeces.

Peptone water: Peptone water was prepared by dissolving Bacto Peptone (Difco, Detroit) (10 g) and sodium chloride (5 g) in 1000 ml distilled water and adjusting to pH 7,5. This was then filtered, distributed in bottles and sterilized by autoclaving at 121°C for 20 minutes.

3.7.4 Route of entry of endotoxins from the gut lumen into the circulation (see Appendix 4)

Six adolescent monkeys of both sexes, with a mean weight of 4,15 ± 0,42 kg were used. Surgical procedures adopted were as described under section 3.6 above, except that a "Medican" 16-gauge catheter, connected to an extension tube, was inserted into the portal vein for removal of portal blood samples. This catheter was held in place using purse string sutures. The collection of blood

samples was similar to that described in 3.6 above except that blood samples were taken simultaneously from the femoral artery and portal vein and only two sets of samples were taken during the 1 hour baseline period, one at the beginning and the other at the end of the hour.

The arterial blood pressure was recorded as described in section 3.4 but a B188 Statham transducer and a Beckman recorder were used, and recordings were made at 5-10 minute intervals during the 4 hour experimental period.

Part II: CONTROL: HYPERTHERMIA/HEAT-STRESS, HEAT STROKE AND ENDOTOXAEMIA

3.8 Introduction

The experimental animals, induction of anaesthesia recordings of rectal temperature and arterial blood pressure have been described in Part I of this section. However in addition, anti-LPS IgG antibody concentrations were also assayed.

3.9 Anti-LPS IgG assay - ELISA procedure

Anti-LPS IgG determinations were made by Michelle T Wells (research assistant of Professor SL Gaffin) according to the method of Gaffin et al.[497] Plasma was centrifuged for 5 minutes at 23000 x g. Triplicate samples of plasma were diluted 1 in 100 in 0,1M NaCl containing 0,05 M Tris, adjusted to pH 8,0, and 2% each of sheep and bovine plasmas. The LPS-precipitable IgG of equine plasma was then assayed with plates coated with 14 endotoxins at a concentration of 10 ug/ml in a carbonate-bicarbonate buffer, pH 9,6. The 14 endotoxins were obtained from Difco (Detroit, USA); they had been extracted from the parent bacteria by the procedure of Westphal et al.[498] They were from: *S.typhimurium, S.minnesota, S.abortus equi,*

S.enteritidis, S.typhi, Shigella flexneri, Serratia marscens, E.coli 0127:B8,*E.coli* 0111: B4, *E.coli* 026: B6, *E.coli* 055: B5, *E.coli* 0128:B12, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The general ELISA microplate method used here has been previously described for alpha fetoprotein assay and other applications[499,500]. Microtitration plates (Dynatech M129B) were coated with a mixture of endotoxins for 2 hours at room temperature. Conjugate of antibody to equine IgG with horseradish peroxidase (Cappel Laboratories,USA) was used. Results were measured by absorption at 495 nm and calibrated by an immunoprecipitin reaction (Gaffin et al.[117]) with primate anti-LPS IgG standard. With the latter standard, anti-LPS IgG was quantitated as described.

3.10 Heat-stress chamber

A forced draft baby incubator set at 41,0^{o} ± 0,3^{o}C was used as a heat-stress chamber. The temperature in the incubator was monitored continuously using temperature probes connected to the telethermometer and a digital thermometer. The relative humidity in the incubator was maintained close to 100% by means of open trays containing water into which bibulous paper was immersed, and was monitored continuously with a Weather Measure Corporation HM111 relative humidity indicator.

3.11 Induction of heat-stress

All animals were fasted overnight, preceding an experiment. They were anaesthetized with ketamine (10 mg/kg). A thermorectal probe was inserted about 10 cm into the rectum to monitor and record continuously the rectal temperature. The femoral areas were cleansed with topical chlorhexidine gluconate (5% aqueous solution). Catheters were inserted into a femoral vein for maintenance of

anaesthesia and the femoral arteries for the collection of blood samples and the recording of blood pressure. ECG electrodes were inserted and fastened to the skin. Blood pressure and electrocardiogram were monitored continuously and recorded at 5 minute intervals during the experimental period.

The animals were allowed about 30 minutes to attain steady-state conditions at room temperature before commencing the experiments. Before the animals were placed into the incubator baseline blood samples (1 ml), were taken and the rectal temperature, ECG and blood pressure were recorded. The animals were then placed on a wooden board in the incubator and the extremities fixed loosely by the ankles and wrists to the board. Approximately 10 ml of water was sprayed by nebulizing into the pre-equilibrated incubator in order to have a rapid return to 100% relative humidity. The animals remained in the incubator until a desired rectal temperature was reached or until death. Blood samples were withdrawn soon after the animals were placed in the incubator and thereafter at 15-30 minute intervals until the termination of the experiment. An equal volume of pyrogen-free saline was infused back into the animal after each blood withdrawal. The blood samples were centrifuged and plasma removed under sterile conditions in a laminar flow hood, placed into sterile plastic tubes (Falcon) and frozen at -20^{o}C. The plasma samples were analysed for LPS within a week using the method decribed in section 3.5.

3.12 Experimental procedures

The procedures for each of the experiments conducted in Part 2 are described below.

3.12.1 Time course of changes in plasma LPS concentration and cardiovascular parameters (see Appendix 5)

3.12.1.1 Procedure

Five monkeys with a mean weight of 3,61 kg (2,5-5,9 kg) were used. All the animals were fasted during the night preceding the experiment but were allowed water *ad libitum*. They were anaesthetized with intramuscular ketamine (10 mg/kg). The anaesthesia was maintained throughout the experiment with intravenous ketamine as required. The femoral areas were cleared as described above and catheters were inserted into a femoral vein and a femoral artery.

Arterial pressure was measured with a B188 Statham transducer. A Honeywell CM130 patient monitor system was used to record the arterial pressure and the electrocardiogram at 5-minute intervals. After the animals had attained steady-state conditions at room temperature, 1 ml arterial blood sample for LPS analysis was taken and baseline rectal temperature, ECG and blood pressure were recorded. The animal was then heat-stressed as described in section 3.11. The animal remained in the incubator until death. Blood samples were withdrawn (section 3.11) and analysed for plasma LPS concentration as described above (section 3.5).

3.12.2 Effects of prophylactic corticosteroid on plasma LPS levels and cardiovascular parameters (see appendix 6)

3.12.2.1 Experimental animals and procedure

Twelve male and female monkeys, having a mean weight of 5,6 ± 0,41 kg were studied. They were at random divided into two groups, a control group (n = 8) and a steroid group (n = 4). The animals were fasted overnight but were

allowed water *ad libitum* and anaesthetized with ketamine as described previously. After catheterization of the femoral vein and arteries, the steroid group received an intravenous infusion of methylprednisolone sodium succinate (Upjohn Laboratories) (30 mg/kg). An equivalent volume of normal saline was infused into the control group. After a 30 minute stabilization period, a 1 ml blood sample for LPS analysis was collected, and baseline recordings of rectal temperature as well as recordings of blood pressure and electrocardiograms were made. The animals were then heat-stressed. The blood samples were analysed for LPS concentration as described in section 3.5.

3.12.3 Effects of oral non-absorbable antibiotics on plasma LPS concentrations and cardiovascular parameters (see appendix 7)

3.12.3.1 Experimental animals and procedure

Eight monkeys of either sex, weighing between 2,7 and 6,8 kg, were studied. The animals were divided into 2 groups, a control (n = 4) group and a kanamycin (n = 4) group. The kanamycin group had received kanamycin (15 mg/kg) (Kantrexil suspension, the B-M Group Pty Ltd) every 12 hours over 5 consecutive days via a nasogastric tube (described in section 3.7.3.1) prior to heat-stress.

Following catheterization of the femoral vein and arteries and before being heat-stressed the anaesthetized animals were allowed a 30 minute stabilization period, during which rectal swabs for bacteriological examinations were taken. Thereafter blood samples was removed for LPS and anti-LPS IgG analyses and rectal temperature, blood pressure and ECG recordings were made. In addition, a 3 ml blood sample was collected for the determination of serum enzymes, bilirubin, albumin, and total protein concentrations. The animals were then heat-stressed and

blood samples for plasma and anti-LPS, IgG analyses were removed. Blood samples for serum enzymes, bilirubin, albumin and total protein analyses were taken when the rectal temperature reached 40, 42 and 43^{o}C. Immediately after the death of each animal, the abdominal cavity was opened. A 6-7 cm section of the transverse colon was tied off at each end and removed.

3.12.3.2 Plasma LPS and anti-LPS IgG analyses

The plasma LPS concentrations were determined as described in section 3.5. The relative plasma anti-LPS IgG concentrations were determined using the ELISA technique as described in section 3.9. Owing to the lack of a monkey anti-LPS IgG standard, the concentrations of anti-LPS IgG at a rectal temperature of 37,5^{o}C for each animal was taken as 100%.

3.12.3.3 Serum enzymes, bilirubin, albumin and total protein

These analyses were carried out by the Department of Pharmacology, University of Natal Medical School. Boehringer Manning Diagnostic Monotest (Johannesburg) kits were used for determining the enzymatic activities of aspartate aminotransferase (AST) (formerly glutamate oxaloacetate transaminase - GOT) (Kit Cat. no. 124362), alanine aminotransferase (ALT) (formerly glutamate-pyruvate transaminase - GPT) (Kit Cat. no. 161071), L-γ-glutamyltransferase (GGT)(Kit Cat. no. 125938), alkaline phosphatase (Kit Cat. no. 123846). Enzymatic activity was measured spectrophotometrically after allowing the respective reactions to proceed for exactly 1, 2, & 3 minutes. The mean absorbance change per minute was then determined and utilized in the calculation of enzymatic activity in U/litre. In addition, Boehringer Mannheim Diagnostic Test combination kits were utilized in the determination of the concentrations of bilirubin (Kit Cat.

no. 123919), albumin (Kit Cat. no. 263869) and protein (Kit Cat. no. 124281). Total protein kit assays employed the biuret method with protein forming a coloured complex with cupric ions in alkaline medium. The formation of an albumin/bromcresol-green complex at pH 4.2 and the subsequent photometric measurement of absorbance formed the basis of the albumin test assay. In the determination of total bilirubin, bilirubin was coupled with diazotized sulfanilic acid in the presence of caffeine to give an azo dye which was measured and calibrated spectrophotometrically.

3.12.3.4 Bacteriology

Bacteriological examinations of the rectal swabs and faeces were made as described in section 3.7.3.2.

3.12.4 Route of entry of LPS into the circulation (see appendix 8)

In order to determine the route of entry of LPS into the circulation the time course of changes in plasma LPS concentrations in the portal vein and in the systemic artery were determined. These changes in LPS concentrations were correlated with changes in mean arterial pressure and heart rate.

3.12.4.1 Experimental animals and procedure

Five adolescent monkeys of either sex with a mean weight of 2,78 ± 9,14 kg were studied. After anaesthesia, the femoral vein and the portal vein, and femoral arteries were catheterized as described previously. After a 30-minute recovery period to attain steady-state conditions, the animals were heat-stressed, blood samples were collected and arterial pressures, and rectal temperatures were recorded. The mean arterial pressures were recorded using a Statham transducer and a Beckman recorder. The

mean arterial pressure was determined using the equation MAP = 1/3 systolic + 2/3 diastolic pressures[214].

3.12.5 Effects of prophylactic equine anti-LPS IgG plasma on the survival rate of heat stroked primates (see Appendix 9)

3.12.5.1 Preliminary study

Before carrying out this investigation it was necessary to determine the critical rectal temperature above which death was caused by heat-stress. Six monkeys divided into 3 groups were studied. One group (n = 2) was heat-stressed to a rectal temperature of 42,5°C, the second group (n = 2) was heat-stressed to 43°C and the third group (n = 2) to 43,5°C. Both pairs of animals heat-stressed to 42,5 and 43°C survived while one heat-stressed to 43,5°C survived and the other died. Thus a rectal temperature of 43,5°C was taken as the cut-off rectal temperature. This cut off temperature was also used by other workers in rabbits and dog models[42,69,402].

3.12.5.2 Experimental animals and procedure

A total of 19 monkeys, with a mean weight of 3,4 ± 0,3 kg, were used. The animals were fasted overnight but were allowed water *ad libitum* and anaesthetized with ketamine as described in section 3.9. They were divided into two groups, A (n = 11) and B (n = 8). Based on the preliminary study two rectal temperatures were selected: 43,5 and 43,8°C. (Shapiro et al.[452] have shown in dogs that rectal temperatures of 44°C and above were lethal. Hence a rectal temperature of 43,8°C was chosen). Animals in groups A and B were heat-stressed to rectal temperature of 43,5 and 43,8°C, respectively. In addition, 5 animals from group A and 4 from group B received, intravenously, 1 ml/kg equine anti-LPS IgG plasma (Atox Pharmaceutical Co., Gillits, South Africa) a day before induction of heat

stroke and these served as the experimental groups. The remaining animals from group A (n = 6) and B(n = 4) served as controls, but equivalent doses of equine non-immune plasma were administered only to the controls in group A. After catheterization of the femoral vein the animals were heat-stressed.

As soon as the desired rectal temperature was reached (43,5°C or 43,8°C), the animal was removed from the heat-stress chamber and allowed to cool passively at room temperature (about 25°C and 35% relative humidity). The rectal temperature was again recorded after the removal of the animal until a temperature of 38°C was reached.

Blood samples (2 ml) for LPS and anti-LPS IgG analyses were taken from the femoral vein before the animals were placed in the incubator and immediately after the desired rectal temperatures were reached. In the case of the experimental animals, an additional sample was taken prior to administration of anti-LPS IgG antibodies. Changes in plasma LPS concentrations, survival and survival rates of the animals were compared statistically within each group.

3.12.6 Effect of prophylactic corticosteroid - methylprednisolone sodium succinate (MPSS) on survival rate of heat-stroked primates (see Appendix 10)

3.12.6.1 Experimental animals and procedure

Eleven adolescent monkeys of both sexes with a mean weight of 4,08 ± 0,28 kg were used for this experiment. The animals were anaesthetized with intramuscular ketamine (10 mg/kg) and were kept anaesthetized with intravenous ketamine as required during the heat-stress period.

The animals were divided into two groups: a steroid group (n = 5) and a control group (n = 6).

The former received, intravenously, 30 mg/kg (0,48 ml/g) dose of methylprednisolone sodium succinate (Upjohn Laboratories) infusion, via a peripheral vein, over a 15 minute period, within 15 minutes of induction of heat-stress. The control group received an equivalent volume of normal saline.

Soon after the induction of anaesthesia a peripheral vein was catheterised for the removal of blood samples, the administration of either methylprednisolone sodium succinate or normal saline, and for the maintenance of anaesthesia. The rectal temperature was recorded continuously. Venous blood samples were taken for the determination of LPS and anti-LPS Immunoglobulin type G (IgG) concentrations.

The animals were heat-stressed to a rectal temperature of 43,5^{o}C, which required 143 ± 8,7 minutes (110 to 195 minutes). No significant overshoot of the rectal temperature occurred on reaching 43,5^{o}C and after removal of the animal from the heat-stress chamber. The animal was immediately removed from the incubator and allowed to recover at room temperature (about 25^{o}C and 30% relative humidity), without forced draft. The rectal temperature was recorded until it reached 38^{o}C.

Blood samples were taken prior to heat-stress and immediately after a rectal temperature of 43,5^{o}C from all the animals and an additional sample was taken prior to the administration of methylprednisolone sodium succinate from the steroid group.

3.12.7 Statistical Analysis

The results are presented as a mean and ± standard error of the mean (SEM) and compared by means of an unpaired Student's t test, Fisher test, and chi-square test with the Yates small number correction.

The data within a group were compared using the Student's unpaired t test, or analysis of variance (ANOVA) and Duncan's multiple range test. The data between groups were compared using the Student's paired t test, Fisher test and chi-square test with Yates small number correction.

For the purpose of statistical analysis of the data in the heat-stress and heat stroke studies, the rectal temperature range of $37^{o}C$ - $45^{o}C$ was divided into four equal intervals. The data within each of the four $2^{o}C$ temperature intervals was grouped together for a 1-way ANOVA. Then the four groups of data were subjected to Duncan's multiple range test for significance of differences between means.

CHAPTER 4

RESULTS

4.1 Part I - Intestinal ischaemia and endotoxaemia

4.1.1 Time course of changes in plasma LPS concentration in a feline superior mesenteric artery occlusion model and the effect of prophylactic corticosteroids

Six of eight control cats and all six steroid-treated cats survived the 4-hour experimental period.

As shown in Figure 6, the mean pre-occlusion plasma LPS concentrations in surviving animals from both the control and steroid groups remained stable at low levels of 0,069 ± 0,015 and 0,069 ± 0,030 ng/ml, respectively. During superior mesenteric artery occlusion, the plasma LPS concentration in the control group began to rise after 20 minutes and reached 0,239 ± 0,032 ng/ml ($p<0,01$). In the steroid-treated cats, the mean plasma LPS concentration did not rise significantly from the pre-occlusion level. After release of the bulldog clamp, plasma LPS concentration in the control cats rose rapidly to a mean of 0,825 ± 0,110 ng/ml ($p<0,01$) after 20 minutes and then returned to pre-occlusion levels over the next 60 to 80 minutes. On the other hand, the steroid group showed no significant change in plasma LPS concentration.

The time course of changes in plasma LPS concentration in the two non-surviving control animals remained stable during the 1 hour baseline period, but rose at the end of the occlusion period to 0,28 and 0,36 ng/ml respectively (Figure 7). As soon as the clamp was removed and perfusion resumed, there was a rapid rise in plasma LPS

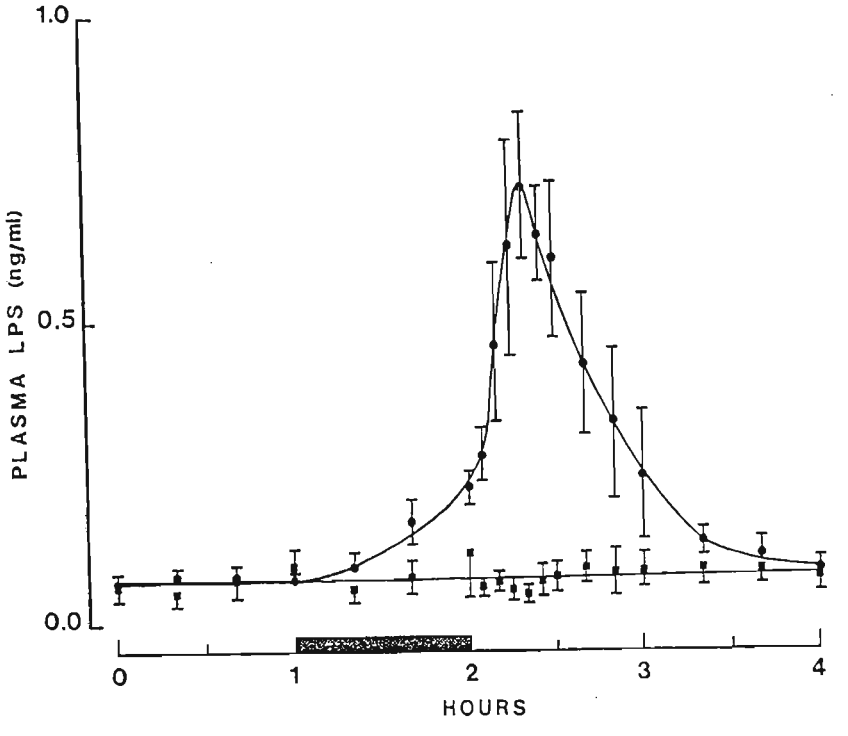

Figure 6: Changes in plasma LPS levels in control (circles) and steroid (squares) groups. The superior mesenteric artery was occluded for 60 minutes (bar). The values given are the mean and ± SEM.

concentration, similar to the control except that the peak concentrations were about twice those of the survivors (1,52 and 1,29 ng/ml compared to 0,825 ng/ml in the survivors). In both cats the plasma LPS concentration returned to baseline levels within an hour and rose a second time immediately before death.

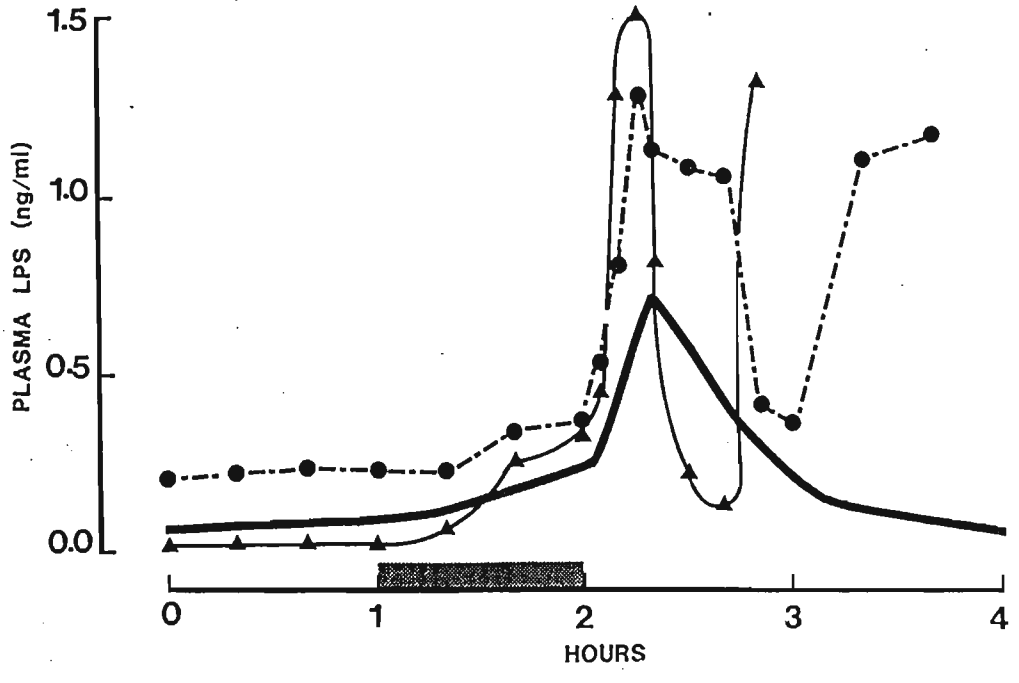

Figure 7: Time course of endotoxaemia after intestinal ischaemia in the two nonsurviving control cats (circles and triangles). The bar represents the superior mesenteric artery occlusion period. The dark line is the data from the six surviving control cats from Figure 6. The non-survivors had elevated peak concentrations of LPS compared to those of the survivors, followed by a decline, and then a second rise in LPS concentration just before death.

4.1.2 Effects of anti-lipopolysaccharide (anti-LPS) IgG antibodies on the time course of changes in plasma LPS concentration

All 29 out of 29 animals in this experiment survived the 1 hour occlusion of the superior mesenteric artery. Figure 8 and Table 8 show the time course of changes in mean plasma LPS concentration of the control group (E) and the prophylactically-treated group A, and Figure 9 and Table 8 show the time course of changes in mean plasma LPS concentrations of groups B, C and D which received anti-LPS IgG antibodies at 0, 10 and 20 minutes of reperfusion, respectively.

4.1.2.1 Baseline period

The plasma LPS concentration remained stable and at low levels in the control (0,075 ± 0,006 ng/ml), prophylactically-treated (0,069 ± 0,008 ng/ml) (Figure 8, Table 8) and in groups B (0,068 ± 0,017), C (0,063 ± 0,006) and D (0,056 ± 0,008) (Figure 9, Table 8) cats during the 1 hour baseline period.

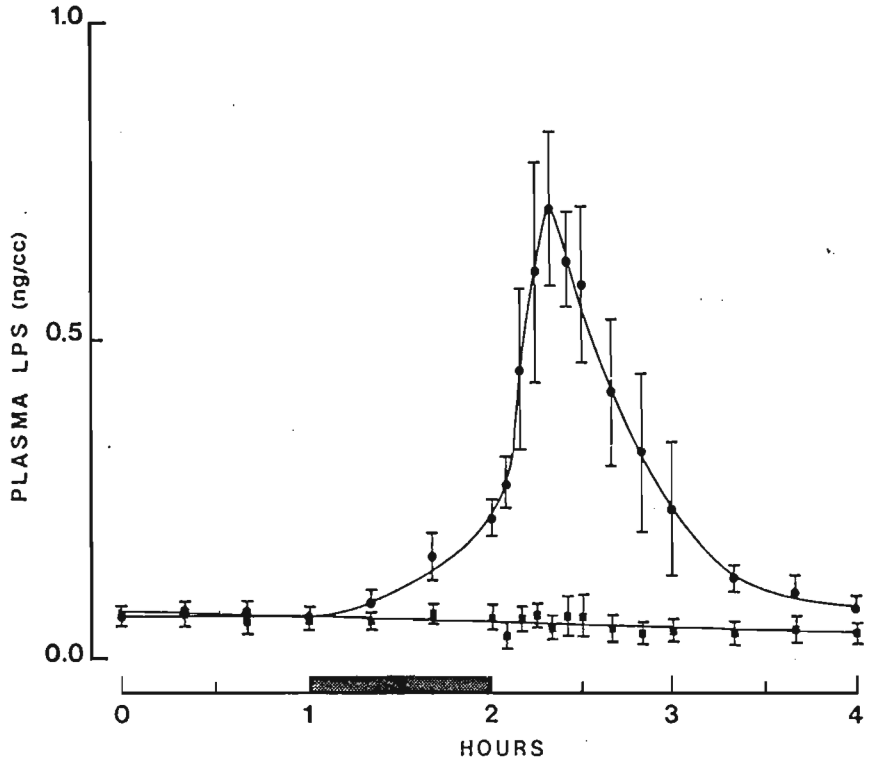

Figure 8: Effect of prophylactic anti-LPS antibodies on superior mesenteric artery occlusion endotoxaemia. Cats received intravenously, 1,0 ml/kg equine hyperimmune plasma 1,5 hours before occlusion of their superior mesenteric arteries for 60 minutes. In the untreated cats (circles), femoral arterial blood samples showed a small rise in LPS concentration during the occlusion period but a larger one peaking 20 minutes after release of the occlusion, which returned to baseline levels after an additional 90-100 minutes. In those cats receiving prophylactic anti-LPS (squares), no rise was seen in blood samples either during or after the occlusion period.

TABLE 8: Plasma LPS concentration (ng/ml) during the baseline, at the end of 1 hour superior mesenteric artery occlusion and during the reperfusion periods in groups A to E cats. Equine anti-LPS IgG hyperimmune plasma was administered prophylactically to group A, and at 0, 10 and 20 minutes of reperfusion to groups B, C and D respectively. Group E served as control. The values are mean and ± S.E.M.

GROUP	ANTI-LPS ADMIN. (MIN. OF REPERFUSION)	MEAN (SEM) LPS CONCENTRATIONS (ng/ml)										
		BASELINE PERIOD	END OF OCCLUSION PERIOD	REPERFUSION PERIOD (MIN) 5	10	15	20	25	30	40	60	120
E	CONTROL NO ANTI-LPS	0,075 ±0,006	0,219 ±0,026	0,276 ±0,044	0,461 ±0,133	0,615 ±0,176	0,716 ±0,122	0,634 ±0,077	0,596 ±0,124	0,425 ±0,119	0,242 ±0,104	0,084 ±0,016
A	PROPHYLACTIC	0,069 ±0,008	0,068 ±0,017	0,041 ±0,019	0,068 ±0,016	0,072 ±0,017	0,054 ±0,019	0,070 ±0,030	0,071 ±0,034	0,052 ±0,020	0,047 ±0,019	0,046 ±0,013
B	0 MIN	0,068 ±0,017	0,241 ±0,042	0,089 ±0,021	0,108 ±0,042	0,097 ±0,050	0,075 ±0,042	0,068 ±0,038	0,066 ±0,035	0,073 ±0,038	0,078 ±0,039	0,048 ±0,028
C	10 MIN	0,063 ±0,006	0,156 ±0,031	0,167 ±0,150	0,241 ±0,073	0,114 ±0,034	0,096 ±0,034	0,087 ±0,026	0,071 ±0,023	0,068 ±0,016	0,064 ±0,017	0,049 ±0,011
D	20 MIN	0,056 ±0,008	0,193 ±0,017	0,217 ±0,029	0,264 ±0,034	0,338 ±0,071	0,455 ±0,072	0,105 ±0,023	0,090 ±0,031	0,084 ±0,027	0,069 ±0,019	0,033 ±0,009

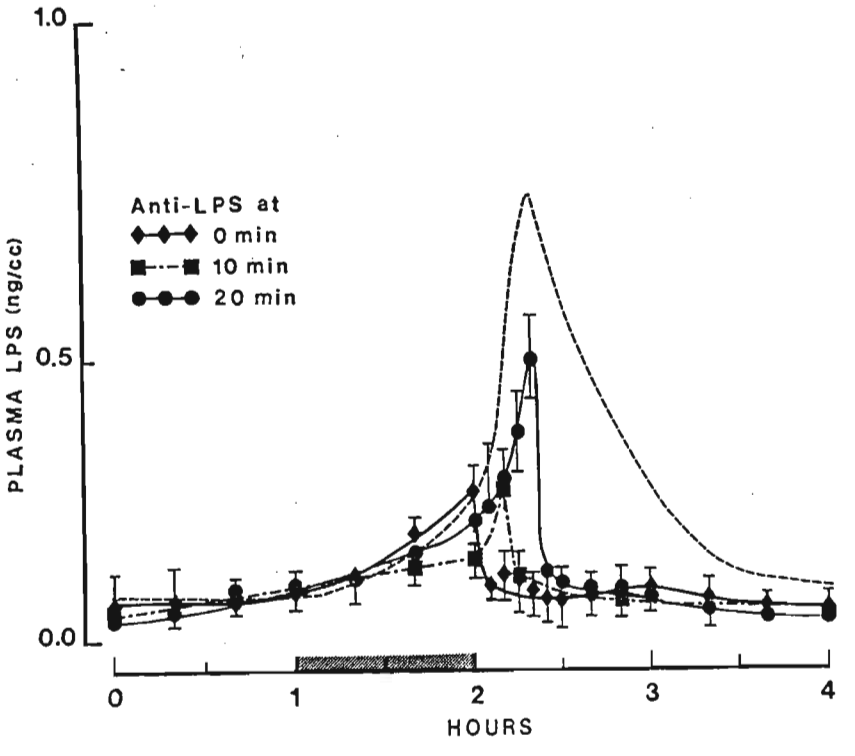

Figure 9: Therapeutic anti-LPS. Cats received 1,0 ml/kg anti-LPS, intravenously, immediately upon release of the clamp occluding the superior mesenteric artery (diamonds). 10 minutes (squares), or 20 minutes (circles) afterwards. The dashed line indicates untreated cats. Note that within 5-10 minutes of anti-LPS administration the plasma LPS concentration fell to near baseline levels.

4.1.2.2 Superior mesenteric artery occlusion period

In the control group E (Figure 8, Table 8) and in groups B-D (Figure 9, Table 8), the plasma LPS levels began to rise after 20 minutes of occlusion to reach final values of 0,219 ± 0,026 ng/ml ($p<0,001$), 0,241 ± 0,042 ng/ml ($p<0,001$), 0,156 ± 0,031 ng/ml ($p<0,001$), and 0,193 ± 0,017 ng/ml ($p<0,001$), respectively. On the other hand, the cats that had received prophylactic anti-LPS antibodies (group A) showed no significant change from the baseline levels (Figure 8, Table 8).

4.1.2.3 Reperfusion Period

In the control cats (Figure 8, Table 8) the plasma LPS level rose rapidly to reach a mean peak value of 0,716 ± 0,122 ng/ml 20 minutes after release of the clamp. Thereafter, the LPS concentration began to decline to reach baseline levels 100-120 minutes following release of the clamp.

On the other hand, in those cats receiving prophylactic anti-LPS (group A), their plasma LPS concentrations remained at or below baseline levels throughout the post-occlusion period (Figure 8, Table 8). In the group B cats (Figure 9), which received anti-LPS immediately after releasing the clamp, the plasma LPS concentration fell (from the raised value during the occlusion period) from 0,241 ng/ml to 0,088 ng/ml ($p<0,01$) within the first 5 minutes. Following this, the plasma LPS concentration continued to fall slowly to a value of 0,048 ng/ml ± 0,028 ng/ml 120 minutes after the release of the clamp.

In the remaining cats, as in the controls, the plasma LPS concentration rose after release of the clamp. However, when anti-LPS was given 10 minutes afterwards (group C), the plasma LPS concentration fell from 0,241 to 0,114 ng/ml ($p<0,02$) within 5 minutes of administration and reached baseline levels during the next 15 minutes (Figure 9, Table8). When anti-LPS was given 20 minutes afterwards (group D), the plasma LPS concentration fell from 0,455 to 0,10 ng/ml ($p<0,005$) within 5 minutes and reached baseline levels over the next 20 minutes (Figure 9, Table 8).

4.1.3 Effects of prophylactic oral antibiotics in a primate superior mesenteric artery occlusion model

4.1.3.1 Effects on plasma LPS concentration

As shown in Figure 10, plasma LPS levels in both the control and kanamycin groups remained at levels of 0,069 ± 0,006 ng/ml and 0,092 ± 0,005 ng/ml, respectively, during the 1 hour baseline period, although the control group had a gradually rising baseline. During the 1 hour superior mesenteric artery occlusion period in the control group plasma LPS concentrations increased slightly to reach a value of 0,090 ± 0,009 ng/ml ($p<0,1$) at the end of the occlusion period but this was not significantly above the expected rise from the increasing baseline. In the kanamycin group the plasma LPS level remained close to their pre-occlusion values (0,082 ± 0,012 ng/ml).

When the superior mesenteric artery occlusion was released, the plasma LPS levels in the control group increased slowly during the initial 10 minutes. Thereafter, it showed a very rapid and significant increase to peak at 0,378 ± 0,103 ng/ml ($p<0,001$) within 20 minutes of reperfusion with oxygenated blood. After this the plasma LPS concentration steadily declined and returned to pre-occlusion levels 80 minutes after the beginning of reperfusion. On the other hand, in the kanamycin group the plasma LPS concentration never rose above the baseline levels throughout the post-occlusion period.

4.1.3.2 Arterial blood pressure, heart rate and rectal temperature

The arterial blood pressure (systolic, diastolic and mean), heart rate and rectal temperature in both groups remained more or less constant throughout the entire

experiment (Figure 10). None of the animals exhibited shock symptoms, and, furthermore, it was necessary to administer only small doses of anaesthetic periodically to the control group but larger doses to the kanamycin group.

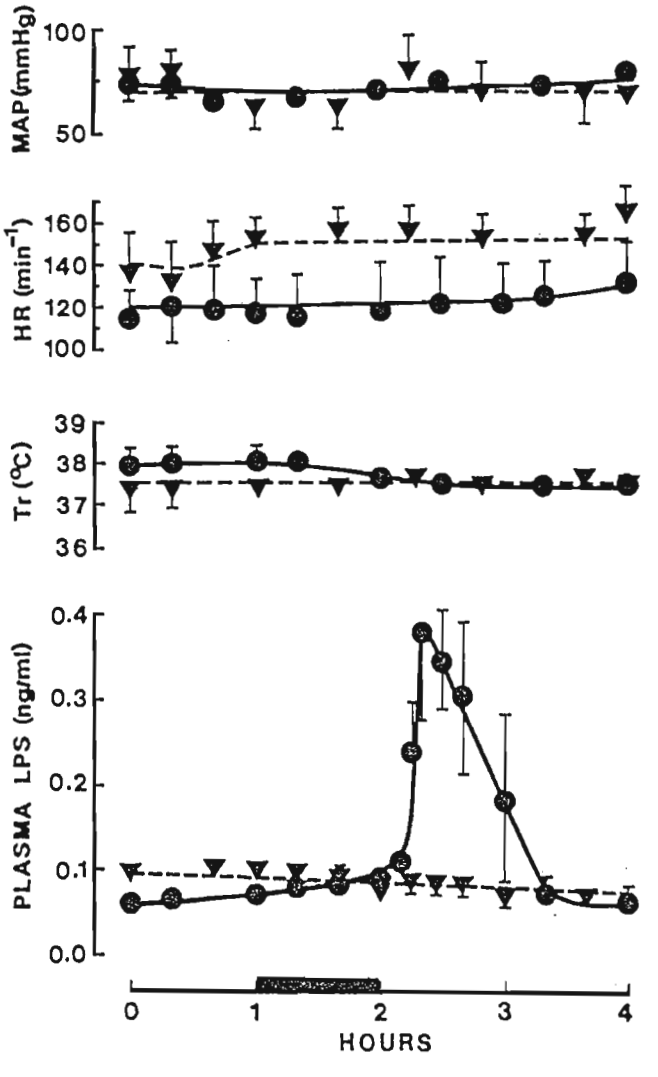

Figure 10: Changes in plasma LPS concentration, mean arterial pressure (MAP), heart rate (HR), and rectal temperature (Tr) before, during and after occlusion of the superior mesenteric artery for 1 hour (bar) in control (circles joined by solid lines) monkeys and in monkeys pre-treated with oral kanamycin (15 mg/kg)(triangles joined by broken lines). The values are mean and ± SEM of 4 monkeys in each group.

4.1.3.3 Bacterial counts

Bacterial examination of rectal swabs from control animals yielded *E.coli, Pseudomonas* spp., coliform bacilli., *Proteus* spp., *Serratia* spp., *Staphylococcus* spp. and *Lactobacillus* spp. In contrast, pretreatment with kanamycin resulted in an overgrowth of the gram-positive *Staphylococcus* spp. and *Lactobacillus* spp., although *E.coli* was encountered in some animals. Plate counts in the controls yielded 21,23 ± 1,75 cfu x 10^9/gram faeces. In the kanamycin group plate counts showed a significant reduction to 0,051 ± 0,007 cfu x 10^9/gram faeces ($p<0,001$).

4.1.4 Route of entry of LPS from the gut lumen into the circulation

Because the results of all monkeys were very similar, and statistical significance was reached only six were used for ethical reasons.

4.1.4.1 Plasma LPS concentration - portal and arterial circulation

As is shown in Figure 11 the LPS concentrations in the hepatic portal and systemic arterial plasmas remained stable during the 1 hour baseline period (0,051 ± 0,009 ng/ml and 0,065 ± 0,011 ng/ml respectively), rising slightly at the end of the occlusion period (0,055 ± 0,016 and 0,073 ± 0,018 respectively (N.S.). Upon release of the occlusion and reperfusion of the splanchnic circulation there was a rapid rise (within 5 minutes) in LPS concentration in the hepatic portal plasma while in the systemic plasma there was a latency of about 10 minutes before a gradual rise. The portal plasma LPS concentration reached a peak of 0,431 ± 0,124 ng/ml ($p<0,01$, ANOVA and

Duncan's multiple range test) after 17,5 ± 1,71 minutes (range 15-25 minutes) of reperfusion (Figure 11, Table 9).

In the systemic circulation the plasma LPS concentration began to increase only after a 10 minute lag period to peak at 0,287 ± 0,126 ng/ml ($p<0,05$, ANOVA and Duncan's multiple range test) (Figure 11) after 32,5 ± 4,23 minutes of reperfusion ($p<0,01$, systemic - portal) (Table 9). That

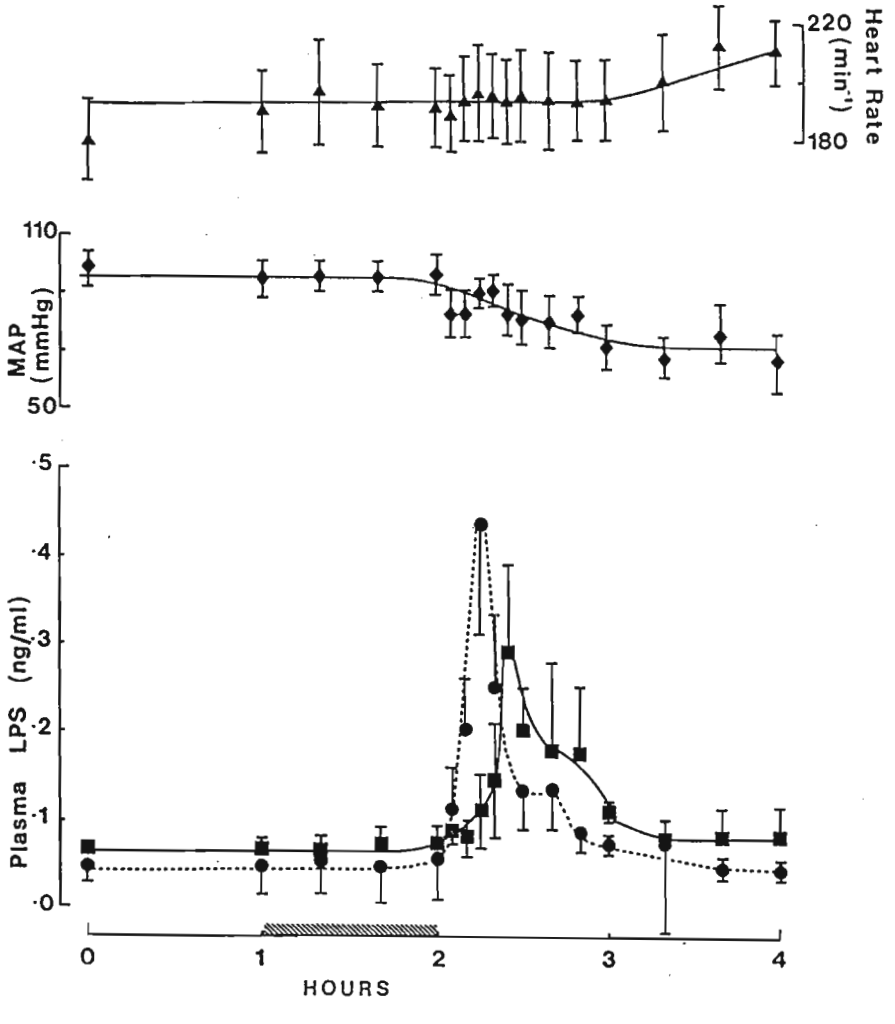

Figure 11: Changes in LPS concentrations in hepatic portal (circles) and systemic arterial (squares) plasma, mean arterial pressure (MAP) (diamonds) and heart rate (triangles) before, during and after occlusion of the superior mesenteric artery for 1 hour (bars).

is this peak occurred 10 (n = 3) to 15 (n = 2) minutes after the peak was reached in the portal circulation in most animals except one in which it occurred 30 minutes later.

After the peak, the portal plasma LPS concentration decreased, returning to baseline after about 80 minutes of reperfusion. On the other hand, in the systemic circulation, the plasma LPS level fell more slowly and returned to baseline in about 100 minutes of reperfusion.

TABLE 9: Peak LPS concentrations and time-to-peak LPS concentrations in hepatic portal and systemic arterial plasma following 1-hour occlusion of the superior mesenteric artery in monkeys (n = 6). Values are mean ± SEM.

Portal LPS peak (ng/ml)	Systemic LPS peak (ng/ml)	Post-reperfusion time to portal LPS peak (min)	Post-reperfusion time to systemic LPS peak (min)
0,431±0,124	0,287±0,126	17,50±1,71#	32,50±4,23*+

15 minutes, n = 4; 20 minutes, n = 1; 25 minutes, n = 1

* 25 minutes, n = 3; 30 minutes, n = 1; 40 minutes, n = 1; 50 minutes, n = 1

+ Time to systemic vs. portal LPS peaks ($p<0,01$)

4.1.4.2 Mean arterial pressure

The mean arterial pressure during the 1 hour baseline period remained more or less constant at 98,60 ± 6,89 mm Hg (Figure 11). During the reperfusion period, the mean arterial pressure declined and reached significance at 60 minutes to 71,3 ± 8,3 mm Hg ($p<0,05$, ANOVA and Duncan's multiple range test). This decline in mean arterial

pressure continued to 65,0 ± 9,5 mm Hg ($p<0,05$) until the termination of the experiment.

4.1.4.3 Heart rate

There was a small but not significant rise in the mean heart rate commencing at about 80 minutes of reperfusion from 183 ± 13,2 beats/minute to 211 ± 12,1 beats/minute at the end of the reperfusion period (Figure 11).

4.2 Part II - Role of plasma LPS in hyperthermia/heat stress and heat stroke

4.2.1 Time course of changes in plasma LPS concentration and cardiovascular parameters.

The general shapes of the curves of rectal temperature, heart rate, arterial pressures (systolic, diastolic and mean) and plasma LPS were similar in all 5 monkeys, but the time to death varied from 240 to 497 minutes in the different animals (mean = 372,8 minutes ± 43,8 SEM). Figure 12 shows typical results of changes in rectal temperature, cardiovascular parameters and plasma LPS levels observed in one of the monkeys. Figure 13, which is discussed below, summarizes the changes in cardiovascular parameters and plasma LPS levels of all 5 monkeys relative to their rectal temperature.

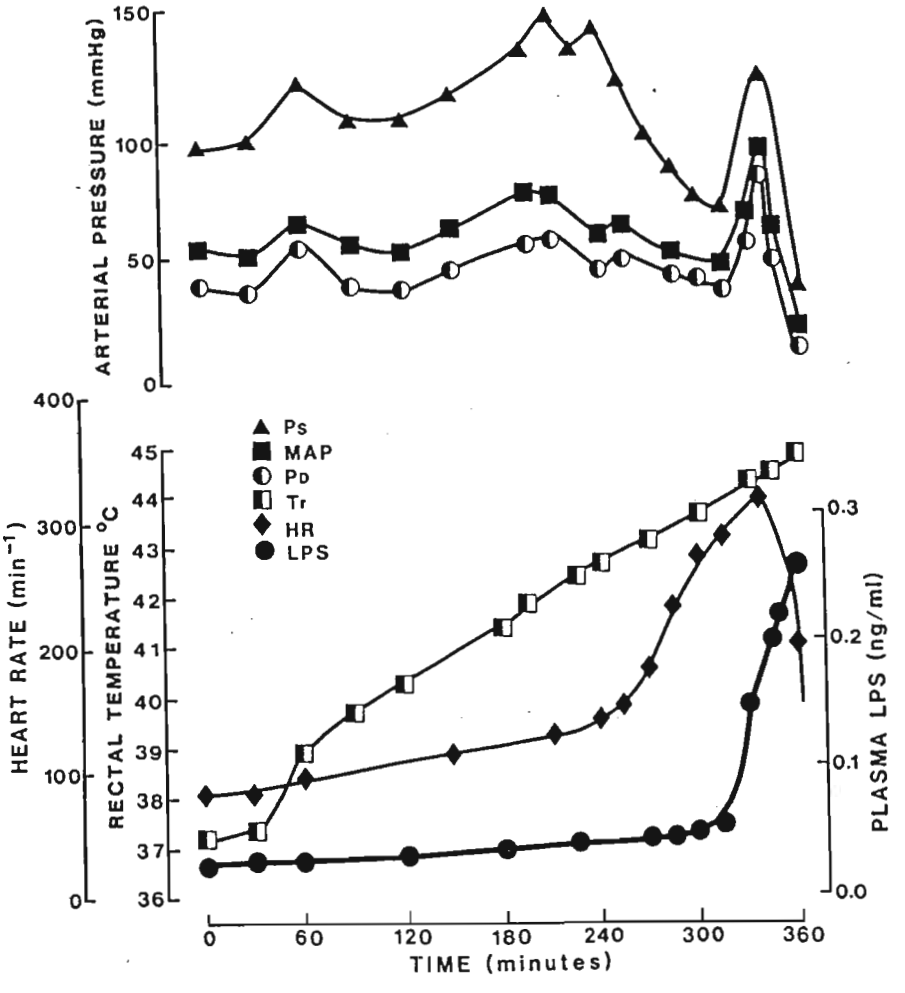

Figure 12: Changes in systolic blood pressure (Ps), diastolic blood pressure (P_D), mean arterial pressure (MAP), rectal temperature (Tr), heart rate (HR) and plasma LPS concentrations in one monkey exposed to an environmental temperature of 41,0 ± 0,3°C and 100% relative humidity. Death occurred within 5 minutes of the last point.

4.2.1.1 Plasma LPS

The plasma LPS concentration remained at stable and low levels (0,071 ± 0,006 ng/ml) until a rectal temperature of 41,5°C was reached. Thereafter there was a gradual increase in plasma LPS concentration until 43°C whereupon the LPS concentration rapidly rose to reach 0,347 ± 0,024 ng/ml, just before death, at a rectal temperature of 44,16 ± 0,22°C.

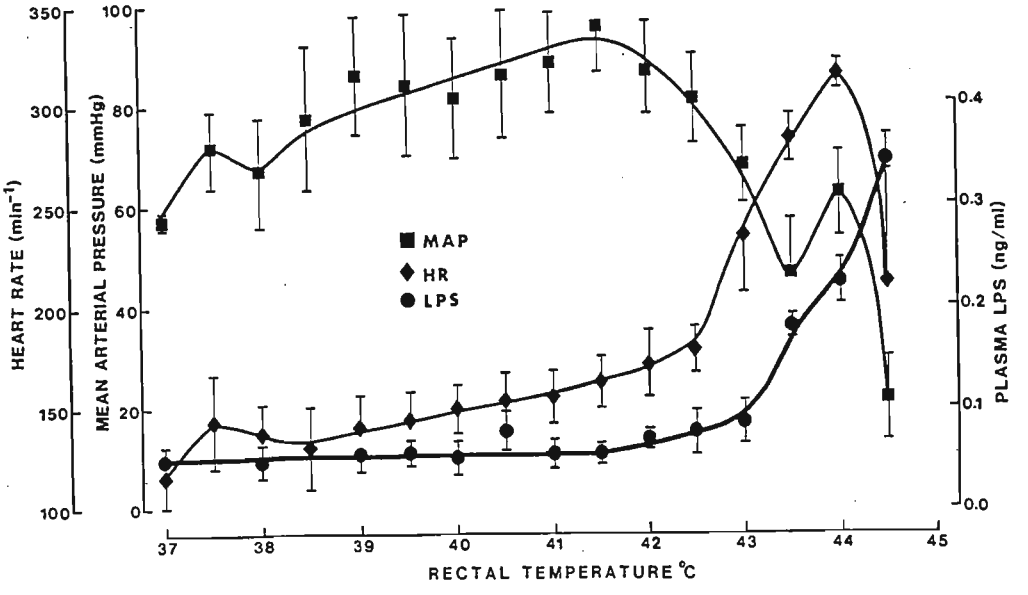

Figure 13: Mean arterial pressure(MAP), heart rate(HR), and plasma LPS concentrations at progressively elevated rectal temperatures in all five monkeys exposed to an environmental temperature of 41,0 ± 0,3°C and 100% relative humidity. The values are mean ± SEM of 5 monkeys.

4.2.1.2 Cardiovascular responses

Initially, at a rectal temperature of 37°C, the mean arterial pressure was 58,2 ± 1,7 mm Hg (Figure 13). As the rectal temperature rose to 41,5°C the mean arterial pressure increased steadily to 96,2 ± 9,0 mm Hg, except for a small but significant "kink" at 38°C. As temperatures rose above 41,5°C the mean arterial pressure declined to 47,8 ± 10,5 mm Hg at a rectal temperature of 43,5°C. This was followed by a consistent rise to 62,8 ± 8,4 mm Hg at a rectal temperature of 44°C (corresponding to a peak in heart rate) before death at 44,5°C. This final phase of falling mean arterial pressure corresponded with a rapid decline in heart rate and a more rapid increase in plasma LPS level.

The mean heart rate for the monkeys at 37°C was 117 ± 16 beats/minute and it increased gradually to 164 ± 13 beats/minute at a rectal temperature of 41,5°C (Figure 13). As temperatures continued to rise, there was a rapid increase in heart rate after 42,5°C peaking at 315 ± 6,7 beats/minute just prior to death.

When the rectal temperature of all animals rose above approximately 42,0°C anaesthesia was no longer deemed necessary.

4.2.2 Effects of prophylactic corticosteroid - methylprednisolone sodium succinate on plasma LPS concentration, mean arterial pressure and heart rate

4.2.2.1 Plasma LPS

Figure 14 shows the changes in plasma LPS concentrations in the control and steroid groups. In the former plasma LPS level remained at 0,060 ± 0,013 ng/ml until a rectal temperature of 41,5°C was reached. Thereafter the plasma LPS concentration increased gradually until 43°C, when there was a rapid rise in plasma LPS level to 0,315 ± 0,030 ng/ml at a rectal temperature of 44,4 ± 0,1°C ($p<0,01$). In contrast, the plasma LPS concentration in the steroid group showed no increase during the entire heat-stress period (Figure 14). Before heat-stress, the plasma LPS concentration in the steroid group was 0,066 ± 0,010 ng/ml and just before death the level was 0,030 ± 0,010 ng/ml. Moreover, the animals in the steroid group succumbed at a significantly higher rectal temperature as compared to controls (44,90 ± 0,14 vs 44,4 ± 0,1°C) ($p<0,02$).

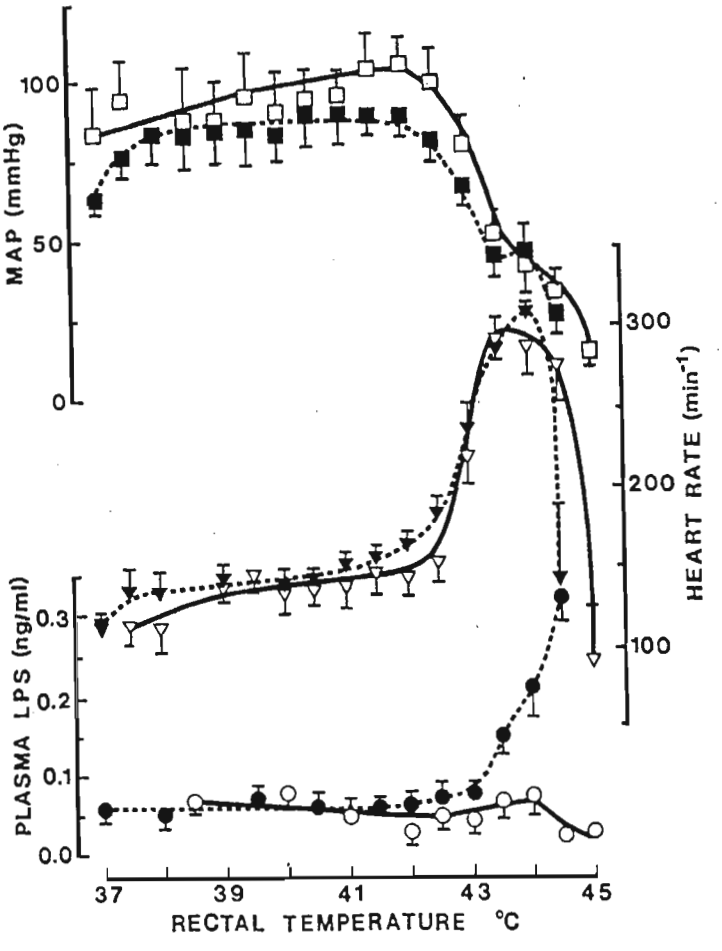

Figure 14: Changes in plasma LPS concentration (circles), heart rate (triangles) and mean arterial blood pressure (squares) at progressively increasing rectal temperature in control monkeys (broken line) and in monkeys pretreated with methylprednisolone sodium succinate (solid lines) during heat-stress in an environmental temperature of 41°C ± 0,3°C and 100% relative humidity.

4.2.2.2 Cardiovascular responses

As is shown in Figure 14 the mean arterial pressure in the control group was 63,8 ± 4,0 mm Hg at a rectal temperature of 37,0°C. As the rectal temperature increased beyond 37°C the mean arterial pressure rose to 89,3 ± 8,4 mm Hg at a rectal temperature of 41°C. The mean arterial pressure began to decline at temperatures greater than 41°C to reach 46,3 ± 7,7 mm Hg at 44°C. The mean arterial pressure in the steroid group was higher than the control group at all rectal temperatures, and between rectal temperature

42-43^{o}C this difference was significant ($p<0,05$). In the steroid group, the mean arterial pressure increased steadily from 83,5 ± 15,5 mm Hg at 37^{o}C to 105 ± 8,4 mm Hg at 42^{o}C, and thereafter declined to 37,0 ± 7,4 mm Hg at 44,5^{o}C.

The heart rate in the control group increased gradually from 112 ± 10,9 beats/minute at 37^{o}C to 141 ± 8 beats/minute at 40,5^{o}C (Figure 14). The heart rate showed a more rapid rise as the rectal temperature increased beyond 40,5^{o}C to reach a peak of 310 ± 5 beats/minute just before the death of the animals. The changes in heart rate in the steroid group showed a similar trend to that of the control group as the rectal temperature increased except that the heart rates at any given rectal temperature were lower than the control group. In the steroid group the rate increased from 113 ± 1,5 at 37^{o}C to a maximum of 292 ± 14,1 beats/minute at 43,5^{o}C. However, there was no significant difference in the heart rate between the control and steroid groups. When the rectal temperature of all animals rose above approximately 42,0^{o}C anaesthesia was no longer deemed necessary.

4.2.3 Effects of prophylactic oral antibiotics on plasma LPS level, mean arterial pressure and heart rate

4.2.3.1 Plasma LPS

As shown in Figure 15, the control group showed an initial plasma LPS concentration of 0,044 ± 0,004 ng/ml, similar to the low values seen above. As the rectal temperature rose there was a small, insignificant elevation in plasma LPS concentration near 39,5^{o}C followed by a large rise at

about 42°C and reaching 0,308 ± 0,038 ng/ml ($p<0,01$) (ANOVA and Duncan's multiple range test) at about 44,5°C. Within a few minutes of reaching this rectal temperature each animal succumbed. In contrast, the group treated with kanamycin showed no significant change in plasma LPS concentrations during the entire heat-stress period.

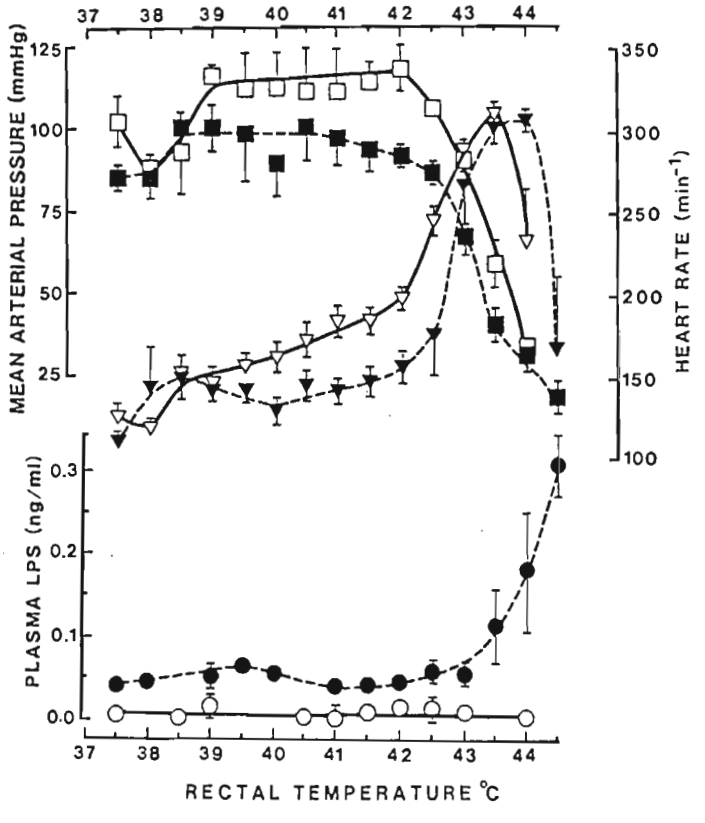

Figure 15: Mean (SEM) changes in plasma LPS concentration (circles), mean arterial pressure (squares) and heart rate (triangles) in control (broken lines) and kanamycin (solid lines) groups at progressively elevated rectal temperature in monkeys exposed to an environmental temperature of 41,0 ± 0,3°C and 100% relative humidity.

Before this latter group was subjected to heat-stress, the plasma LPS concentration (0,007 ± 0,008 ng/ml) was significantly lower than the controls ($p<0,02$) and after heat-stress it never rose significantly above baseline and a concentration of 0,005 ± 0, 002 ng/ml was measured

just before the monkeys died. The primates in the group treated with kanamycin succumbed at a significantly lower rectal temperature than those in the control group (44,1 vs 44,6^{o}C) ($p<0,025$).

4.2.3.2 Cardiovascular parameters

As the core temperature rose, the mean arterial pressure in the control group (Figure 15) increased from 85 ± 4,2 mm Hg at a rectal temperature of 37,5^{o}C to 100 ± 5 mm Hg at rectal temperatures of 39 - 41^{o}C. Above 41^{o}C the mean arterial pressure gradually declined until a rectal temperature of about 43^{o}C when there was a rapid decline. This rapid fall coincided with a rapid rise in heart rate and occurred shortly before the rapid rise in plasma LPS concentration. In the group treated with kanamycin, the mean arterial pressure curve was similar to the controls but at a level of about 10-20 mm Hg higher than the controls throughout the whole temperature range.

As the rectal temperature rose, the heart rate (Figure 15) in the control group increased rapidly from 113 ± 3 beats/minute at a rectal temperature of 37,5^{o} to 152 ± 16 beats/minute at 39^{o}C. This increase was followed by a slight decline in heart rate to 128 ± 9 beats/minute at 40^{o}C before rising steadily to 155 ± 9 beats/minute at 42^{o}C. Thereafter, the heart rate increased rapidly to reach a peak of 303 ± 6 beats/minute ($p<0,001$) at 44,0^{o}C after which it declined rapidly until a temperature of about 44,5^{o}C was recorded. In the group treated with kanamycin, the heart rate increased steadily from 126 ± 7 beats/minute at 37,5^{o}C to 309 ± 7 beats/minute at 43,5^{o}C, with a "dip" at 38,0^{o}C. For any given rectal temperature between 39^{o}C and 43,5^{o}C, both the mean arterial pressure and heart rate were higher in the group treated with kanamycin than those recorded for the controls.

4.2.3.3 Serum enzymes, bilirubin, albumin, total protein and anti-LPS IgG

Except for a significant increase in serum AST (formerly GOT) concentration ($p<0,01$) which increased from 47,55 ± 3,25 IU/l at 37°C to 75,9 ± 2,9 IU/l at 43°C in the control group, no significant changes in the serum enzymes ALT (formerly GPT), L-γ-glutamyltransferase, and alkaline phosphatase were observed in either groups (Table 10). Albumin, total protein, bilirubin concentrations and anti-LPS IgG titres were also not found to have changed significantly during heat-stress (see Table 10).

4.2.3.4 Bacterial counts

Bacterial examination of rectal swabs from control animals yielded *E.coli, Pseudomonas* spp., coliform bacilli, *Proteus* spp., *Serratia* spp., *Staphylococcus* spp. and *Lactobacillus* spp. In contrast, pretreatment with kanamycin resulted in an overgrowth of the gram-positive *Staphylococcus* spp. and *Lactobacillus* spp., although *E.coli* was encountered in some animals. Plate counts in the controls yielded a total of 12,56 ± 1,83 cfu x 10^9/gram faeces of gram negative and gram positive bacteria. In the kanamycin group plate counts showed a significant reduction to 0,183 ± 0,040 cfu x 10^9/gram faeces ($p<0,001$).

4.2.4 Route of entry LPS into the circulation during heat-stress

As the results reached statistical significance after the use of five monkeys, the study was terminated.

4.2.4.1 Plasma LPS

Prior to heat-stress, the LPS concentrations in the hepatic portal and systemic arterial plasma were 0,088 ±

TABLE 10: Changes in the concentration of serum enzymes, bilirubin, albumin, total protein and anti-LPS (IgG) in control and kanamycin groups at rectal temperature 37,5°C, 40°C, 42°C and 43°C in monkeys heat-stressed in an environmental temperature of 41,0 ± 0,3°C and 100% relative humidity.

	Concentration at rectal temperatures			
	37°C	40°C	42°C	43°C
Control group				
AST (GOT) (IU/l)	47,55±3,25	58,0±17,2	68,2±9,1	75,9±2,9 *
ALT (GPT) (IU/l)	6,2±0	10,9±5,0	40,8±36,55	39,1±34,0
Alkaline phosphatase (IU/l)	376,1±7,8	367,8±31,85	418,4±13,2	406,9±36,0
L-γ-glutamyltransferase (IU/l)	22,5±3,3	21,2±2,8	24,2±3,0	24,3±2,0
Bilirubin (umol/l)	3,5±0,5	3,0±1,0	5,0±2,0	4,5±0,5
Albumin (g/l)	31,25±3,35	29,45±0,55	34,3±0,7	32,2±3,9
Total protein (g/l)	48,9±10,2	57,05±2,15	59,45±0,35	60,25±0,35
Anti-LPS (IgG)%	100	-	61,8±7,2	94,1±28,3
Kanamycin group				
AST (GOT) (IU/l)	34,85±7,85	32,0±11,71	31,4±6,17	31,83±8,12
ALT (GPT) (IU/l)	5,73±2,38	8,68±1,06	10,25±3,86	4,85±1,19
Alkaline phosphatase (IU/l)	431,2±78,8	372,0±64,7	413,1±101,6	437,6±105,9
L-γ-glutamyltransferase (IU/l)	26,53±3,23	26,15±3,65	27,78±4,35	26,38±3,6
Bilirubin (umol/l)	4,18±,14	2,63±0,86	3,5±0,75	4,85±,83
Albumin (g/l)	27,8±1,05	27,68±2,38	27,85±1,65	27,48±1,83
Total Protein (g/l)	54,78±2,57	53,4±3,19	56,48±3,42	54,9±3,74
Anti-LPS (IgG) %	100	-	87,8±14,8	80,9±21,3

* $p < 0,05$

0,017 and 0,078 ± 0,021 ng/ml (N.S.), respectively, shown in Figure 16 (bottom). During the heat-stress period these concentrations remained more or less stable until a rectal temperature of about 42,5^{o}C was reached after approximately 120 minutes of heat-stress. Thereafter the LPS concentration rose first in the portal vein and then, 10-15 minutes later, in the systemic arterial circulation. Beyond rectal temperature of 43,0^{o}C, the LPS concentration in the portal plasma rose rapidly to a maximum of 0,244 ± 0,050 ng/ml ($p<0,01$, ANOVA and Duncan's multiple range test) just before the animals succumbed (about 200 minutes from the beginning of heat-stress). The LPS concentration in the systemic arterial plasma, on the other hand, increased gradually until a temperature of 43,5^{o}C. Thereafter it rose rapidly to a maximum of 0,224 ± 0,060 ng/ml ($p<0,01$). At rectal temperature 43,5^{o}C the concentration of LPS in the portal plasma was significantly greater than in the systemic arterial plasma ($p<0,025$). However, the final concentration of LPS in the portal plasma was not significantly greater than that of the arterial plasma.

4.2.4.2 Plasma anti-LPS IgG antibodies

Before the primates were heat-stressed, the concentrations of anti-LPS IgG antibodies were 20,66 ± 7,35 ug/ml in portal plasma and 22,14 ± 7,43 ug/ml (N.S.) in systemic arterial plasma, shown in Figure 16 (bottom). The concentration of the antibodies in both the above circulatory compartments began to decline at rectal temperatures of 38,0^{o}C and above, and by 40,0^{o}C the concentrations fell to 15,30 ± 4,28 (N.S.) and 11,86 ± 2,80 (N.S.) in the portal and systemic arterial plasmas, respectively. At above 40^{o}C, the concentrations of antibodies in both compartments declined, reaching 5,51 ± 1,28 ug/ml (portal) ($p<0,05$) and 4,60 ± 1,69 ug/ml (arterial) ($p<0,05$) just prior to death.

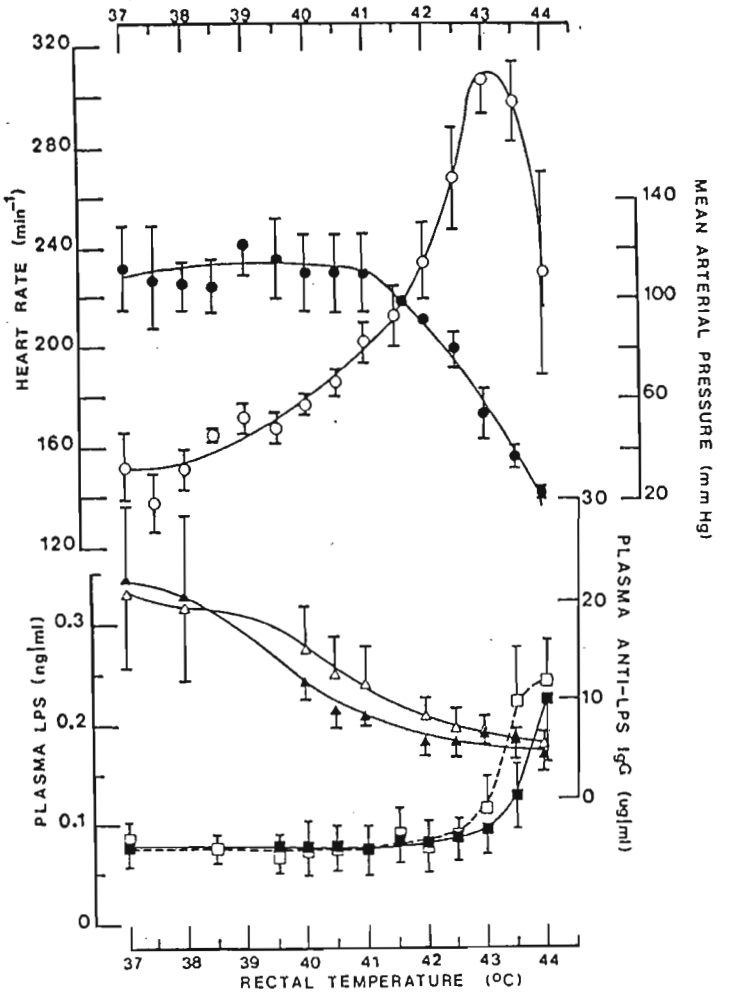

Figure 16: Changes in LPS concentrations in hepatic portal plasma (open squares) and systemic arterial plasma (solid squares), and changes in concentrations of anti-LPS antibodies in hepatic portal (open triangles) and systemic arterial plasmas (solid triangles) heart rate (open circles), and mean arterial pressure (solid circles) in monkeys heat-stressed in an environmental temperature of 41 ± 0,3°C and 100% relative humidity.

4.2.4.3 Mean arterial pressure

As shown in Figure 16 (top) the mean arterial pressure remained more or less at a constant level of 112,10 ± 17,03 mm Hg until rectal temperature 41,0°C and then began to decline rapidly and continuously to 23,33 ± 2,64 mm Hg just prior to death ($p<0,01$, ANOVA and Duncan's multiple

range test). This decline in mean arterial pressure commenced slightly before the plasma LPS concentration began to rise.

4.2.4.4 Heart rate

Except for a small dip at 37,5°C, the heart rate increased gradually from a pre-heat-stress level of 154 ± 14 beats/minute to 214 ± 12 beats/minute at 41,5°C ($p<0,01$, ANOVA and Duncan's multiple range test) (Figure 16, top). Thereafter, there was a rapid rise to 307 ± 13 beats/minute ($p<0,01$, ANOVA and Duncan's multiple range test) at 43,0°C followed by a rapid decline until death.

4.2.5 Effects of anti-LPS IgG antibodies on the survival rate in heat stroke

As the rectal temperature of the primates rose to 42°C in both Groups A and B, the control primates not treated with anti-LPS required less and less ketamine, and above 42°C no ketamine was required. The animals pretreated with anti-LPS plasma needed no anaesthetic above 42,5°C. Just before these temperatures were reached (42 and 42,5°C), the animals became restless, were unresponsive to further infusion of ketamine, and it was considered that, together with tachypnoea, heat stroke was established[454].

4.2.5.1 Heat-stress to rectal temperature 43,5°C (Group A): Survival

As is shown in Table 11 only 16,7% (1 out of 6) of the control animals survived heat-stress to 43,5°C and passive cooling in this group. The survival time of those animals who died ranged between 55 and 155 minutes. By contrast, in the experimental group, prophylactic anti-LPS plasma resulted in 100% (5 out of 5) survival after greater than 72 hours ($p<0,025$).

TABLE 11: Effect of heat-stress to rectal temperatures 43,5 and 43,8°C on survival and survival time (minutes) (min) in control and anti-LPS groups.

	RECTAL TEMPERATURE	PERCENT SURVIVAL (no)	SURVIVAL TIME (mins)
GROUP A:			
Control (n = 6)	43,5°C	16,7% (1/6)	108,6 ±24,1
Anti-LPS (n = 5)	43,5	100% (5/5)	72 hrs
GROUP B:			
Control (n = 4)	43,8	nil (0/4)	81,25 ±33,94
Anti-LPS (n = 4)	43,8	nil (0/4)	427,50 ±61,39

4.2.5.2 Heat-Stress to rectal temperature 43,5°C (Group A) : Plasma LPS concentration

As a result of heat-stress to 43,5°C the plasma LPS concentration in the control group increased significantly from 0,080 ± 0,010 ng/ml to 0,346 ± 0,094 ng/ml ($p<0,02$) (Figure 17). The single control animal that survived the heat-stress showed only a small increase in plasma LPS concentration (from 0,066 to 0,110 ng/ml). Moreover, this survivor also had very high "natural" anti-LPS IgG concentration - about 144% more than the mean level measured in 18 of the monkeys in this study. On the other hand, in the experimental animals, the plasma LPS concentration remained at the pre-heat-stress level(0,104 ± 0,005 ng/ml prior to heat-stress and 0,104 ± 0,024 ng/ml after heat-stress (Figure 17). Furthermore, at a rectal temperature of 43,5°C, there was a significant difference in the plasma LPS concentration between the control and experimental groups ($p<0,025$). Heat-stress to 43,5°C also caused vomiting and bloody diarrhoea during the passive

cooling period in all the animals that eventually died.

4.2.5.3 Heat-stress to rectal temperature 43,8°C (Group B): Survival

None of the animals subjected to a rectal temperature of 43,8°C survived from the heat-stress; however, the administration of prophylactic anti-LPS plasma increased the survival time significantly to 427,50 ± 61,39 minutes compared with the control group, which had a mean survival time of 81,25 ± 33,94 minutes ($p<0,05$) (Table 11).

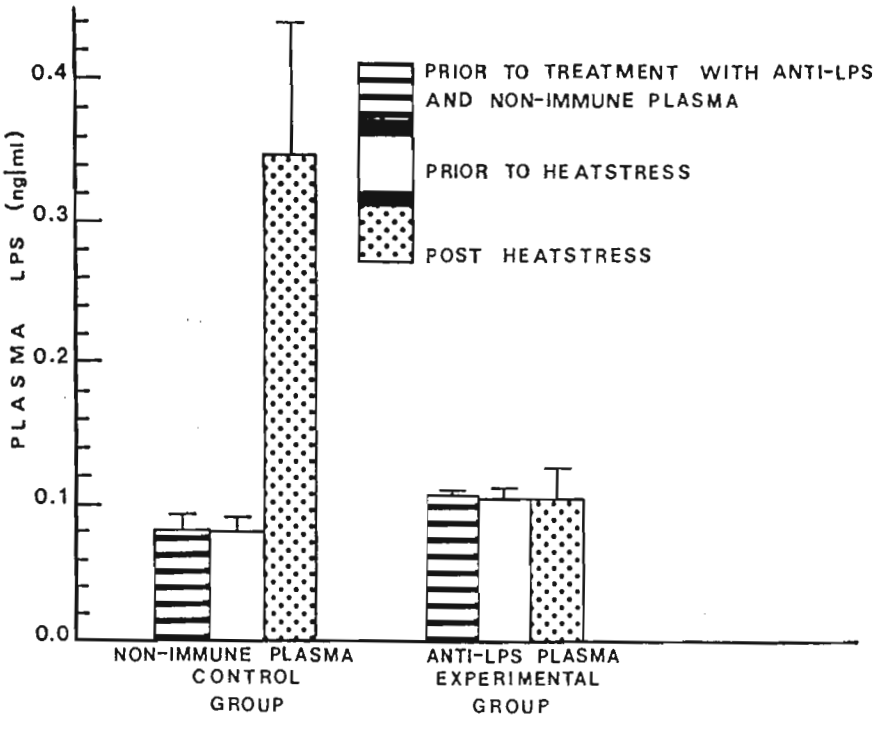

Figure 17: Plasma LPS concentrations in control and anti-LPS antibody pre-heated primates (experimental) before treatment, prior to being heat-stressed and after being heat-stressed to 43,5°C. The post heat-stress value for the control group represent the concentration in non-survivors. The values are the mean ± SEM for 6 monkeys in the control group and 4 in the experimental group.

4.2.5.4 Heat-Stress to rectal temperature 43,8°C (Group B): Plasma LPS concentration

The plasma LPS concentration rose in the control group from 0,107 ± 0,008 ng/ml to 0,251 ± 0,028 ng/ml ($p<0,005$). In contrast, the plasma LPS concentration in the experimental group remained close to pre-heat-stress levels (0,091 ± 0,010 ng/ml prior to heat-stress and 0,097 ± 0.010 ng/ml post-heat-stress) (Figure 18).

Except for one animal (from the experimental group) none regained consciousness after they were removed from the heat-stress chamber. All animals vomited and had diarrhoea. Interestingly, the diarrhoea was bloody in the

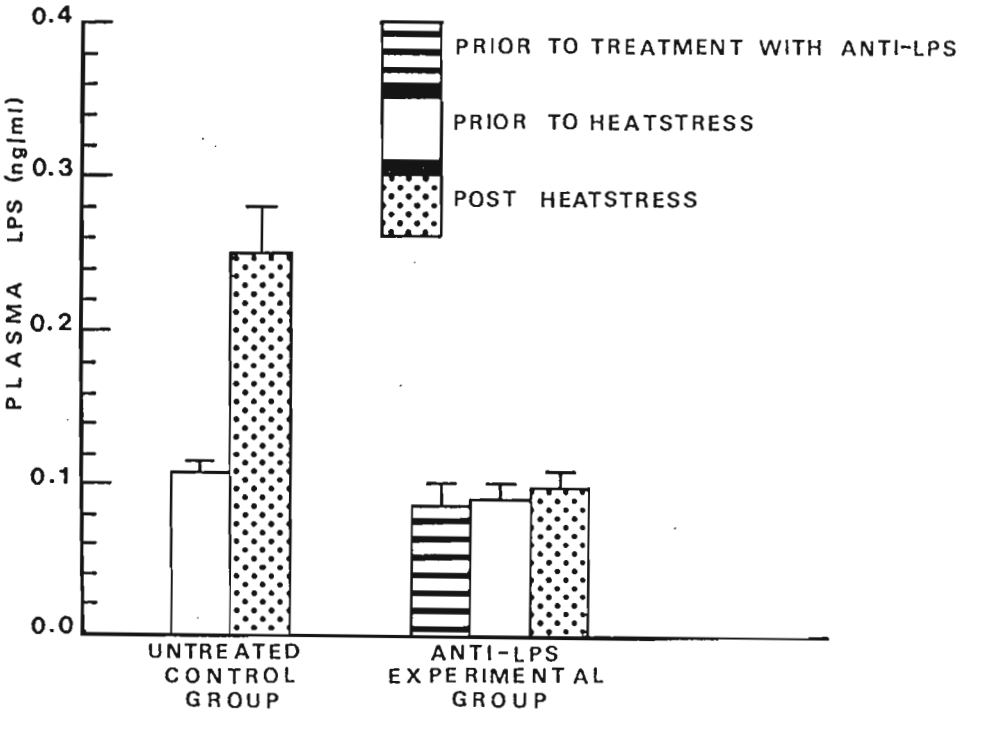

Figure 18: Plasma LPS concentrations in control monkeys before and after being heat-stressed to 43,8°C and in experimental monkeys before administration of anti-LPS plasma, before and after being heat-stressed to 43,8°C. The values are the mean and ± SEM for 4 monkeys in each group.

case of all the control animals, but in only one of the experimental animals.

4.2.6 Effects of prophylactic methylprednisolone sodium succinate on survival during heat stroke

4.2.6.1 Effect on survival rate and survival time

When the animals were removed from the incubator, they were all unconscious and hyperventilating. As shown in Table 12, only 33% (2/6) of the control animals survived (>3 months) from the induced heat stroke. On the other hand pretreatment with a high dose of methylprednisolone sodium succinate (MPSS) increased the incidence of survival (>3 months) significantly to 100% (5/5) (X^2 = 2,753) ($p<0,05$). Of the four control animals that died, only two regained consciousness before their deaths. The time-to-death of these animals ranged from 55 to 480 minutes. The two surviving control animals, on the other hand, were conscious within 50 to 60 minutes, which compares favourably with the steroid group which took an average time of 48 minutes to regain consciousness. Furthermore, no differences in the heating rates between the control survivors (0,054 ± 0,003^{o}C/minute) and non-survivors (0,054 ± 0,005^{o}C/minute) were noticed (Table 13). The heating rate in the steroid or experimental group was found to be 0,048 ± 0,008^{o}C/minute) (Table 13).

All the dying control animals vomited and had bloody diarrhoea, whereas the two surviving control animals, although they vomited, had diarrhoea without blood. In contrast to the control group, no animals from the steroid group had diarrhoea, although two vomited.

TABLE 12: Survival (%) and survival time (minutes) of control and methylprednisolone sodium succinate pretreated monkeys after being heat-stressed to rectal temperature of 43,5°C.

Group	Survival (%) (number in brackets)	Survival time (min)
Control (n = 6)	33,3 (2)	223,8 ± 90,9 (range 55-480)
Steroid (MPSS pretreated) (n = 5)	100 (5)	3 months

TABLE 13: Heating rates (°C/minute) and duration of heat-stress (minutes) of monkeys in control (survivors and non-survivors) and steroid groups heat-stressed to a rectal temperature of 43,5°C (values are mean ± S.E.M.).

	Heating rate (°C/minute)	Duration of heat-stress (minutes)
Control Group:		
Survivors (n = 2)	0,054 ± 0,003	127,5 ± 10,5
Non-survivors (n = 3)	0,054 ± 0,005	124,5 ± 11,3
Steroid Group (n = 5)	0,048 ± 0,008	154,4 ± 11,6

4.2.6.2 Plasma LPS

From Figure 19 it can be seen that prior to heat-stress the plasma LPS concentration in the control group, including the two survivors, was 0,089 ± 0,007 ng/ml, similar to the steroid group (0,099 ± 0,006 ng/ml). However, after the induction of heat stroke there was a significant increase in plasma LPS concentration to 0,257 ± 0,031 ng/ml in the non-surviving control animals ($p<0,005$). On the other hand, plasma LPS concentration showed very little or no change in the two control animals that survived and in the five which were pretreated with

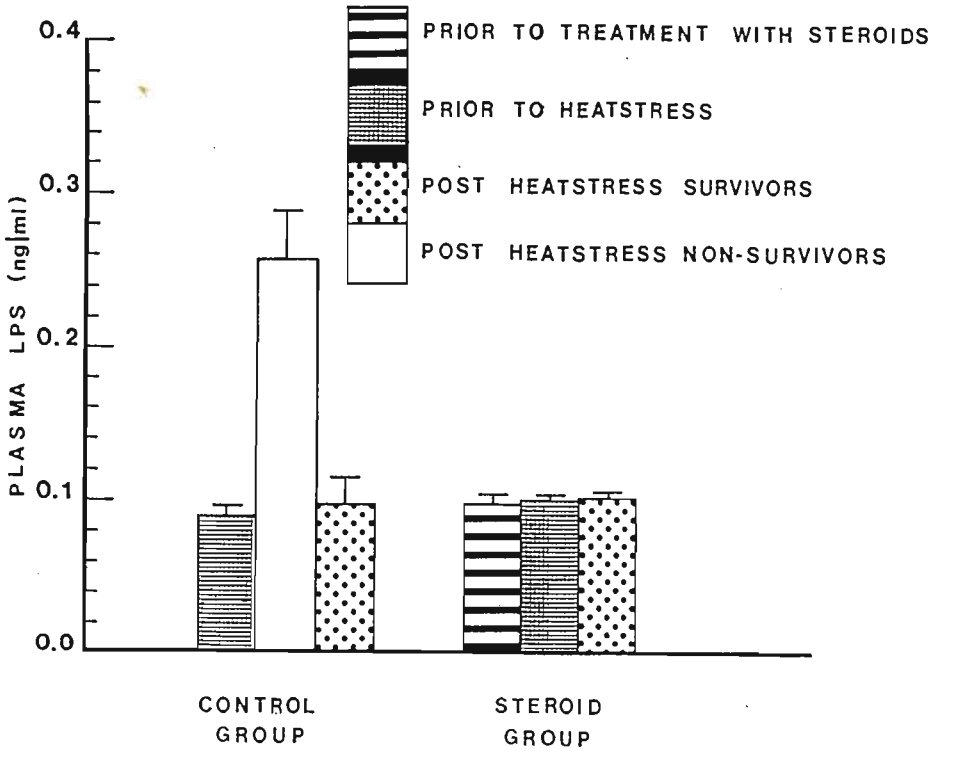

Figure 19: Plasma LPS concentration of primates in the control (survivors and non-survivors) and steroid groups prior to treatment with methlyprednisolone sodium succinate (MPSS) (only the steroid group) and before and after being heat-stressed to a rectal temperature of 43,5°C. The values are the mean and ± SEM.

the corticosteroid. In the two surviving control animals, the plasma LPS concentration increased to 0,098 ± 0,014 ng/ml and in the steroid group to 0,102 ± 0,004 ng/ml. The plasma anti-LPS IgG concentration (%) decreased in both groups after heat-stress.

Interestingly, the two surviving control animals had a relatively very high basal anti-LPS IgG levels compared to those that died, with a mean of 325,1 ± 64,0% more than the mean basal levels measured in 28 normal monkeys (Table 14). In contrast to those in the controls, the plasma LPS concentrations of the steroid group did not change significantly during the heat stress experiment, and were significantly less than those of the controls after heat stroke ($p < 0,001$) (Figure 19).

TABLE 14: Plasma anti-LPS IgG levels (%) in control (survivors and non-survivors) and steroid groups before and after heat stroke (values are mean ± S.E.M. and are expressed as a percentage of the basal levels estimated in 28 normal monkeys).

	ANTI-LPS IgG (%)	
	Before heat stroke	After heat stroke
Control Group		
Non-survivors (n = 3)	112,4 ± 53,1	67,1 ± 30,5
Survivors (n = 2)	325,1 ± 64,0	244,2 ± 43,9
Steroid Group (n = 5)	117,8 ± 39,7	81,8 ± 25,6

CHAPTER 5

DISCUSSION

5.1. PART I - INTESTINAL ISCHAEMIA AND ENDOTOXAEMIA

LPS, but not whole bacteria, was previously shown to pass through the wall of ischaemic dog bowel in less than 30 minutes,[35] and enter the systemic circulation within a few minutes of intestinal ischaemia[31]. However, systematic studies have not previously been carried out with frequent sampling capable of showing rapid changes in plasma LPS concentrations during and after insults to the intestines.

This study showed that in primates during the occlusion period the concentration of LPS in both the systemic and portal plasma remained unchanged. Also the mean arterial pressure and the heart rate were more or less constant (Figure 11). When the clamp was removed from the superior mesenteric artery, and oxygenated blood once again flowed through the gut, it "rinsed" out LPS which had diffused through the gut wall.

However, in the systemic arterial plasma LPS concentration began to rise slowly after a latent period of about ten minutes as compared with the immediate and rapid rise in LPS concentration in the portal plasma. This latency in the increase of systemic plasma LPS concentration represents, at least in part, the time required for the blood to perfuse the liver and the time required for increased portal LPS levels to overwhelm the liver's RES function. The increase in LPS concentration in the systemic arterial plasma is, therefore, the "spill-over" from elevated portal LPS blood levels which temporarily overwhelmed the liver's RES. There may be an additional contribution of LPS from the thoracic duct, but this

remains to be investigated. Furthermore, the time-to-peak systemic arterial plasma LPS concentration was significantly greater than the time-to-peak portal plasma LPS concentration ($p<0,01$) (Table 9), that is, this peak occurred 10 ($n = 3$) to 15 ($n = 2$) minutes after the peak was reached in the portal circulation in all the animals but one, in which case it occurred 30 minutes later.

Ultrastructural changes due to ischaemia were seen by other investigators, 10 minutes after clamping the superior mesenteric artery, in the rough endoplasmic reticulum, mitochondria and the nuclear membrane of ileal mucosal epithelial cells[336-341]. At 30 minutes post-occlusion, subepithelial spaces and lifting of the columnar cells occurred and the columnar cells had a "washed-out" appearance[341].

After reperfusion the "leaky" intestinal wall presumably "self-repaired" leading to a reduced permeability to LPS (indicated by the decline in portal plasma LPS concentration). This, coupled with an improved RES function, resulted in a subsequent slow decline in plasma LPS concentration in the systemic circulation.

A biphasic clearance of LPS from the vascular compartment was observed by Mathison and Ulevitch[49] during intravenous injection of LPS in rabbits. An initial rapid clearance of LPS was due to a rapid uptake by the RES and to a lesser extent by granulocytes and adrenal glands[49,92-94]. An additional slower rate of disappearance of LPS was due to the binding of LPS to high-density lipoproteins in plasma[49,93].

Cuevas and Fine[329] (using the relatively insensitive LAL "clot" test) also found that the portal venous plasma contained little or no LPS one hour after occlusion of the superior mesenteric artery in rabbits, whereas the blood

sample taken at the same time from the systemic venous circulation had elevated LPS levels. They attributed the latter increase to be due to leakage of LPS from the damaged gut via the peritoneal cavity. However, in contrast to the findings in this study, Cuevas and Fine reported that, 60 minutes after reperfusion, LPS levels in the plasma taken from both the portal and systemic venous blood had risen to high levels in the animals which had been alive. The discrepancy between the findings in the present study and that of Cuevas and Fine could be due to species differences.

The opening of the abdominal cavity - to remove the clamp from the superior mesenteric artery - as well as the peaking of LPS concentration in the systemic arterial plasma were accompanied by a decline in the mean arterial pressure ($p<0,05$, Figure 11). Similar changes in mean arterial pressure were reported by others following the release of occlusion of the superior mesenteric artery[31,34,50,317,320,323]. We believe that the decline in mean arterial pressure noticed in this study was due to the toxic actions of LPS. The latter either directly[216,217] or by inducing the release of cachectin,[148,172-176,501] vasoactive agents[127-129,213] and oxygen free radicals from PMN leucocytes[218,255,256] damaged the permeability properties of the capillary endothelium and caused extravasation of plasma[219,220], leading to a reduction in blood volume and venous return. It was shown recently that a bolus injection of endotoxin into human volunteers resulted in an increase in plasma cachectin levels within 60 to 90 minutes[172]. Furthermore, elevations in cachectin levels, and not endotoxin, were associated with "classic" endotoxic responses such as chills, headaches and nausea, which became most severe when plasma cachectin levels were at their maximum[172]. In keeping with these findings a significant reduction in mean arterial pressure in this study occurred about 60

minutes after removal of the occlusion. In addition, the heart rate in the primates showed an ascending trend following the drop in the mean arterial pressure.

Prophylactic administration of anti-LPS IgG antibodies (Figure 8) and a corticosteroid (methylprednisolone sodium succinate) (Figure 6) to cats and oral non-absorbable antibiotics (kanamycin) to monkeys (Figure 10) suppressed any rise in plasma LPS concentration during occlusion of the superior mesenteric artery and protected all animals from death. On the other hand, 2 out of 16 control cats failed to survive the one hour occlusion of the superior mesenteric artery.

In the control cats, unlike the primates (Figures 10 and 11), we found that the plasma LPS concentration was significantly elevated at the end of the occlusion period (Figures 6 and 8). It is possible that during the occlusion period, the liver was able to clear most LPS which may have entered the circulation, hence the low concentration. However, as there was very little or no blood flow to carry the LPS into the portal vein, the small amount of systemic plasma LPS observed presumably had by-passed the liver by means of collateral circulation[329,502] and/or might have entered it by passing directly into the peritoneal cavity and into the lymphatics[102]. Alternatively, ischaemia of the splanchnic regions might have impaired liver function[28,503,504].

The reason for the species difference noted in this study is not known. However, it was reported that meat diets, as in the case of cats, tended to increase intestinal gram-negative bacterial flora[306,307], hence LPS concentration, compared to the case of monkeys, which had a mixed diet. Alternatively, it may be possible that very little LPS is carried via collateral and/or lymphatic vessels in the monkey or that the monkey has a more efficient RES to

detoxify the LPS. Nevertheless, this study shows that an endotoxaemia does occur in monkeys due to a non-septic etiology.

When the superior mesenteric artery of control cats was reperfused with oxygenated blood upon release of the clamp, the liver's RES apparently removed much of the LPS which had leaked into the portal blood from the intestinal vascular system. The peak systemic LPS concentrations of 0,825 ± 0,110 ng/ml (Figure 6) and 0,717 ± 0,122 ng/ml (Figure 8) at about 20 minutes of reperfusion may represent part of a bolus of LPS which had accumulated in the intestinal intravascular space during occlusion but could not flow to the liver due to zero blood flow. Upon reperfusion, the liver RES commenced to remove this LPS from the circulation but the bolus temporarily overwhelmed the capacity of the RES. Gradually, RES function improved and the raised intestinal permeability to LPS might have been reduced upon reperfusion with oxygenated blood, leading to the subsequent decline in plasma LPS concentration. During the reperfusion period in cats the changes in systemic arterial plasma LPS concentration were similar to those in the primates except that the peak levels were about twice those of the primates (more than 0,717 ng/ml in cats compared to 0,378 ng/ml in primates). The higher peak plasma LPS concentration in the cats could be due to species differences and to dietary factors.

Schoenberg and his co-workers[339] recently reported that:

1) during an ischaemic period xanthine dehydrogenase is converted into the oxidase and

2) the adenine monophosphate (AMP) which accumulates is converted into adenosine, which moves extracellularly where it is further metabolized into hypoxanthine, and upon reperfusion of the

tissues with fresh oxygenated blood, xanthine oxidase was thought to act on the hypoxanthine present, producing a mixture of toxic products including superoxides, peroxides, and oxygen free radicals. Schoenberg and his co-workers reported some damage to cat intestinal mucosa immediately after one hour of ischaemia, but which increased during one hour of reperfusion with oxygenated blood.

They suggested that the additional damage was caused by free radicals. Similar observations were made by others[337,338,340]. However, we are not certain whether such a mechanism was operational during the occlusion period in this study, since other investigators have suggested that during total occlusion of the superior mesenteric artery, factors not dependant on oxygen free radicals may play a more important role in structural alteration of the intestinal mucosa[336]. Nevertheless, it is possible that during the reperfusion period the slow decline in plasma LPS concentration in the control group was due to LPS that might have leaked into the circulation due to damage of intestinal mucosa by free radicals. The two non-surviving control cats appeared to have higher peak plasma LPS concentrations (1,52 and 1,29 ng/ml - Figure 7) compared to 0,815 ng/ml in the survivors (Figure 6). Interestingly, both cats had a second stage of severe endotoxaemia (second LPS peak) immediately before death. The source of this second peak is uncertain but might have been the intestines in which the oxygen free radical mediated damage was excessive.

In contrast to the results of the control groups, prophylactic methylprednisolone sodium succinate completely inhibited the post-occlusion LPS peak (Figure 6). The mechanism of steroid action in this case is unknown. However, steroids exert a stabilizing effect on

lysosomal[505] and other cell membranes[194], so methylprednisolone sodium succinate may be protecting the mucosa of the gut, hence preventing a leakage of LPS into the portal vein. In other studies it was observed that corticosteroids protected dogs against haemorrhagic necrosis of the intestines following *E.coli* infusion[20,208], and prevented intestinal mucosal lesions in cats[323]. It was also found that corticosteroids transiently increased blood flow to the splanchnic region, including the liver[506], and by so doing, protected the small intestinal capillary bed in endotoxin shock[507], and also protected the liver during ischaemia[508]. It is suggested here that corticosteroids, by protecting the gut mucosal membrane and increasing splanchnic and liver blood flow, reduced the "leakage" of LPS into the hepatic portal circulation, thereby increasing the efficiency of the liver in removing LPS from the blood during occlusion of the superior mesenteric artery. This effect of methylprednisolone sodium succinate on plasma LPS levels could also explain its occasional beneficial effect in shock[509]. Furthermore, in light of these findings, it is suggested that prophylactic methylprednisolone might be of benefit for abdominal surgery requiring the temporary occlusion of some arteries, or before the release of volvulus or torsions, or any treatment likely to cause a temporary ischaemia of any part of the bowel.

Patients continue to die from gram-negative bacterial shock and endotoxaemia despite the development of potent antimicrobial agents. This is due, in part, to the presence of LPS in the circulation of patients whose RES is not capable of detoxifying it[50]. LPS in the systemic circulation could come from the cell walls of the destroyed bacteria[16,21,22] and/or from the gut as a result of damage of its wall owing to many insults[16,31-47,50].

Currently, the basic approach to the treatment of septic

shock usually includes potent broad-spectrum antimicrobial therapy, control of the source of infection, attempts to ameliorate predisposing factors, the replacement of blood volume, the correction of electrolyte imbalance, and careful management of complications, such as cardiac failure[11,12]. However, all these have no effect in reducing the concentration or activity of circulating LPS. It is believed that treatment should also be directed specifically against the LPS[4].

The administration of various preparation of anti-LPS antibodies reduces mortality and morbidity in septic and non-septic shock in animals and humans[7,14,35,52,53,106-121]. The anti-LPS antibody preparation of Gaffin inactivates and opsonizes the LPS present in the circulation, and in the presence of a complement can rapidly destroy a wide range of gram-negative bacteria[115,116].

It was observed in this study that unless anti-LPS antibodies were present, the plasma LPS concentration in all groups of cats increased significantly after 20-30 minutes of occlusion of the superior mesenteric artery (Figures 6 to 9). On the other hand, in the presence of prophylactic anti-LPS antibodies, the plasma LPS concentration remained at or even below the control baseline levels throughout the entire 4-hour experimental period. Specific antibodies in anti-LPS at the concentrations employed do not inhibit the LAL assay[116] and therefore the low LPS levels observed are not an artifact.

In cats receiving anti-LPS antibodies prophylactically or immediately upon release of the clamp (Figures 8 and 9), the specific antibodies prevented any rise in plasma LPS concentration during the reperfusion period. In other cases, when the plasma LPS concentration had risen (groups

C and D - Figure 9), anti-LPS antibody administration reduced the LPS value to baseline levels within 20 minutes.

Studies by Gaffin and his co-workers have shown that anti-LPS antibodies bound to the surface of gram-negative bacteria stimulate granulocytes to ingest these bacteria[115]. Earlier studies showed that normal nonimmune plasma or serum did not have a protective effect in septicaemia or endotoxaemia[14]. The equine anti-LPS plasma which was used in the present study was found; (a) to protect pregnant rats against *E.coli*-induced abortion[121], (b) to protect mice against X-irradiation death[39,120], and (c) to control pseudomonas keratitis in rabbits[510].

The presence of anti-LPS antibodies bound to the surface of "free" LPS probably facilitates the liver Kupffer cells to remove LPS from the circulation more rapidly. This study shows the rapidity with which anti-LPS antibodies reduce high concentrations of LPS from the systemic circulation, thereby improving survival in septic shock.

In control primates, not pre-treated with kanamycin but subjected to one hour occlusion of the superior mesenteric artery, it was found that when the flow of oxygenated blood was resumed through the gut, the plasma LPS concentration rose, at first slowly and then rapidly to peak at 0,378 ng/ml within 20 minutes (Figure 10). Thereafter, it declined, at first rapidly and then slowly to reach a baseline similar to that seen in cats.

In contrast to the case of the control group, prophylaxis with the non-absorbable antibiotic prevented the rise in LPS concentration in the kanamycin group (Figure 10). This is consistent with previous experiments in which injection of kanamycin into rabbit intestines prevented shock due to burns[34] or superior mesenteric artery occlusion[33]. The

active ingredients in "Kantrexil" suspension used here are kanamycin and kaolin. Kanamycin is non-absorbable and bactericidal against aerobic gram-negative bacteria and, therefore, may leave the anaerobic bacteria intact. The latter, incidently, make up a large proportion of the gut flora but lack certain characteristics of classic LPS (as discussed previously), and as such are less toxic[18,302,511]. In view of this no attempts were made to either suppress the levels of the anaerobic gram-negatives in the gut or determine their count in the faeces. Furthermore, prophylactic treatment with kanamycin had been used for sterilization of the gut prior to surgery[308,309]. Excessive destruction of anaerobic gram-negative gut flora may reduce or eliminate the "interference phenomenon" and encourage invasion by pathogenic bacteria[302,303,511]. While the prophylactic treatment with kanamycin reduced the aerobic gram-negative bacterial count, by so doing it also promoted an overgrowth of gram-positives, particularly staphylococci and lactobacilli, and possibly anaerobic gram-negatives as well. The lactobacilli population adheres to the epithelium of the gastrointestinal tract, thus having a protective effect[304,305]. Furthermore, an overgrowth of the anaerobic gram-negatives might also have been beneficial by controlling the colonization by pathogenic organisms[303]. This pre-treatment can be seen as a possible positive step toward the prevention of, or reduction of, an endotoxaemia in either elective or emergency bowel surgery.

In the control primate during the reperfusion period, no changes in mean arterial pressure and heart rate were noticed even though the circulating plasma LPS concentration reached high values (Figure 10). These data differed from those described earlier in primates (Figure 11) where a significant drop in mean arterial pressure was noticed. The fact that the mean arterial pressure did not

fall could be due to ketamine, the anaesthetic agent which is an α-receptor agonist[512] whereas in the experiment described previously (Figure 11) phenobarbitone was the anaesthetic. The heart rates in the kanamycin group were, however, consistently higher than the control group throughout the experiment. This could be due to the anticholinergic properties of aminopentamide, one of the constituents of "Kantrexil" suspension. However, lack of changes in mean arterial pressure and increase in the heart rate were observed by others in canines which survived after the release of occlusion of the superior mesenteric artery[326,327]. On the other hand, in the case of rabbits the same insult caused progressive hypotension, hypovolaemia and death within 1 to 2 hours of reperfusion[33,34]. Moreover, monkeys and baboons, unlike rabbits, horses or man[15] were reported to be highly resistant to the acute actions of LPS. Furthermore, variable changes in mean arterial pressure[186,206-209] and either no change or bradycardia were observed in monkeys following intravenous injection of endotoxin[207].

The suppression of increases in plasma LPS concentration by prophylactic oral kanamycin in this study strongly suggests that the origin of the increased plasma LPS concentration as observed after ischaemia of the gut is mainly derived from the gut. Furthermore, gut-derived LPS may be important in contributing to the severity of illness in a variety of shock states caused by traumatic injuries, or bowel surgery.

To recapitulate, if intestinal ischaemia is allowed to prevail for a period of time (for example, for one hour in as this study), then an endotoxaemia may develop owing to leakage of LPS from the gut lumen, first into the portal circulation and then into the systemic circulation. The changes in plasma LPS concentration in primates during the occlusion or ischaemic period are a little different from

those in felines. In primates, virtually no rise in plasma LPS concentration were noticed during this period while in cats plasma LPS levels became elevated as early as 20 minutes of occlusion.

If upon reperfusion of the gut, the concentration of LPS in the systemic circulation rises to a sufficiently high level, a fall in blood pressure may result. This endotoxaemia during intestinal ischaemia may be prevented by destroying the gut gram-negative flora (using "Kantrexil" suspension), or by the use of steroids, which possibly stabilize the gut wall or by the use of anti-LPS IgG antibodies in order to "consume" the LPS entering the circulation.

As a control to the superior mesenteric artery occlusion studies it was shown in Part II that gut-derived endotoxins gained access to the intravascular compartment during heat-stress and played a role in the pathophysiology of heat stroke.

5.2. Part II - HYPERTHERMIA/HEAT STRESS, HEAT STROKE AND ENDOTOXAEMIA.

Heat stroke is characterised by hyperthermia, sudden loss of consciousness, circulatory failure, evidence of widespread tissue damage and failure of haemostasis[62,69,81,351,364,402,412-422]. Many of these features parallel the findings associated with the shock state of endotoxaemia[4,43,62,475].

There have been a number of suggestions that endotoxaemia is also involved in the pathogenesis of heat stroke[4,41-44,475]. No detailed investigations relating changes in plasma LPS concentration with cardiovascular parameters have previously been undertaken in heat-stressed experimental animals.

Hyperthermia or heat-stress leads to an increase in cutaneous blood flow in order to increase dissipation of heat from the core and concomitantly results in a decrease in splanchnic blood flow in animals and man[310,396-398,400,401,404,406,407,412-420]. The reduced splanchnic blood flow, as demonstrated by the previously described superior mesenteric artery occlusion experiments (Part I) leads to an increased vascular permeability with leakage of endotoxins into the systemic circulation.

In this study, a rise in temperature to above 41,5°C was accompanied by a rapid decline in the mean arterial pressure and a rapid rise in the heart rate. Significantly, the cardiovascular changes noticed here occurred prior to and at a lower rectal temperature than the rise in plasma LPS concentrations as determined directly by the LAL assay.

The rise in LPS concentration commenced approximately 15 minutes earlier and at a slightly lower rectal temperature in the hepatic portal than in the systemic arterial plasma (Figure 16). The baseline concentration of systemic arterial plasma LPS observed here was similar to those measured in other experiments (discussed below) in which the abdominal cavity was not opened.

It was anticipated that the decline in plasma anti-LPS IgG concentration (due to antigen-antibody consumption) would mirror the rise in plasma LPS concentration. However, this was in contrast to what was observed. The parallel decreases in LPS specific IgG antibody concentrations in both portal and arterial plasmas, commenced at a rectal temperature of 40,0°C (Figure 16). This reduction in antibody concentration appears to be an indirect estimation of the presence of LPS. These results, therefore, suggest that LPS "leaks" out of the gut at the

lower rectal temperature of 40°C and NOT at a rectal temperature above 42,0 to 42,5°C, where the plasma LPS concentration rises according to the LAL test. It was previously shown in the laboratory used for these experiments that the plasma of normal blood donors contains anti-LPS IgG antibodies which can reduce the concentration of "free" LPS in vitro[116,513].

At a rectal temperature of 43,5°C, the higher concentration of LPS in the portal plasma indicates that LPS now enters the circulation mainly via the portal vein. Unexpectedly, there was no significant difference in plasma LPS concentrations between the hepatic portal and systemic arterial compartments at rectal temperatures 42,0 to 43,0°C. A substantially reduced LPS concentration was expected in the systemic plasma due to the passage of the portal blood and LPS through the liver recticuloendothelial system and its dilution with the systemic blood. This may indicate that at these temperatures, LPS may also enter the circulation from the gut lumen by routes other than the portal vessels such as the lymphatic vessels.

This experiment suggests that during heat-stress, at lower temperatures (e.g. 40°C) the reduced splanchnic blood flow[310,396-398,400,401,404,406,407,412-420] minimally damages the permeability properties of the gut mucosa and allows a slow leakage of LPS into the interstitial spaces and then into the circulating blood. Once in the circulation, some LPS rapidly complexes with anti-LPS IgG antibodies[4] and high density lipoproteins[48,49] while others are taken up by the recticuloendothelial system[28-30,93,94,96-101] and are therefore not detected by the LAL technique. However, at rectal temperatures greater than 42,0°C, the reduction in blood flow to the splanchnic regions is made more severe by the decline in mean arterial pressure commencing at 41,5°C. (Figure 16). This,

plus direct thermal injury, may further damage the gut mucosa and, possibly, also the capillary endothelium. In support of the phenomenon of gut wall damage, ecchymosis of the intestinal wall, ulceration of the intestinal mucosa, necrosis, and sloughing of epithelial cells have been observed in dogs heat-stressed to a rectal temperature of 43-44,5°C[402].

In the superior mesenteric artery occlusion model in primates, upon release of the occlusion and reperfusion of the gut with fresh oxygenated blood, LPS concentration rose in the portal vein about 10 minutes prior to that in the systemic artery and peaked significantly earlier ($p<0,01$ - Table 9 and Figure 11). In addition, the mean arterial pressure declined significantly ($p<0,05$), soon after the LPS concentration had peaked (0,30 ng/ml) in the arterial plasma (Figure 11). The peak plasma LPS concentration in the gut ischaemia study is similar to that observed here in heat stroke and, therefore, the amount of LPS observed in heat stroke should be considered as "toxic". High core temperature is known to damage cells of the reticuloendothelial system[69,486] and, probably contributes to the rise in LPS concentration seen in the systemic arterial compartment.

Other studies have indicated liver tissue damage at 42-43°C[81,83,402,452,469]. The rapid rise in systemic plasma LPS concentration in this experiment was found at a rectal temperature of about 43°C and might therefore be due to increasing rates of entry of LPS into the portal circulation, overwhelming an already compromised liver which may no longer be able to remove LPS effectively from the portal circulation. It is of interest that liver damaged by heat stroke has been described to be morphologically similar to liver damaged from diseases associated with circulating bacteria and LPS[481].

Other studies could be interpreted in a manner consistent with the concept that gut-derived LPS is responsible for part of the pathophysiology of heat stroke. A high concentration of LPS was reported in a heat stroke patient whose condition was complicated by shock, septicaemia, anuria and consumption coagulopathy[44].

As in the superior mesenteric artery occlusion studies prophylactic administration of anti-LPS IgG antibodies (Figure 17), corticosteroids (methylprednisolone sodium succinate) (Figures 14 and 19) and oral, non-absorbable antibiotics (Figure 15) suppressed any rise in plasma LPS concentration in heat-stressed primates. In addition, prophylactic anti-LPS IgG antibodies (Table 11) and corticosteroids (Table 12) increased survival in heat-stroked primates.

The mean arterial pressure in most control animals during heat-stress rose gradually at first until a rectal temperature of about 41,5°C was reached (Figures 13 and 15) when it began to drop rapidly (at about 210 minutes after the beginning of heat-stress, Figure 12). This rapid drop in mean arterial pressure occurred just before the plasma LPS concentration began a gradual increase.

The heart rate increased gradually at first until a rectal temperature of 42,5°C was reached (Figures 13 and 15) (or at about 240 minutes after the beginning of heat-stress - Figure 12). The rapid rise in heart rate occurred just prior to the rapid increase in plasma LPS concentration (Figures 13 and 15). Similar changes in mean arterial pressure and heart rate were reported in heat-stressed dogs[401-403], baboons[409] and heat stroked patients[65,79,80,84].

Most published investigations into the pathogenesis of heat stroke had been carried out in a situation where the

rectal temperature of about 41°C was reached. In the present investigation, it was however, found that the major pathophysiological changes in mean arterial pressure, heart rate and plasma endotoxin levels, during heat-stress occurred around a rectal temperature of 43°C, and may be related to an endotoxaemia. Clearly, one should not extrapolate the observed changes at 41°C to that during a heat stroke episode.

In conditions such as prophylaxis with anti-LPS IgG antibodies and corticosteroid, which prevent a rise in plasma LPS concentrations and increase survival, it appears that endotoxaemia may be a contributing or even a major factor in the pathogenesis of heat stroke. Most studies are highly suggestive but not exhaustive, so it is possible that LPS might rather be a marker for another unidentified toxin released at the same time from a damaged gut.

Prophylactic methylprednisolone sodium succinate prevented any rise in plasma LPS concentration in our primates during the entire heat-stress period (Figure 14) - similar to that noticed in the feline superior mesenteric artery occlusion model (Figure 6). The mechanism of action of methylprednisolone sodium succinate in suppressing plasma LPS levels in both these shock models is not known.

The mean arterial pressure, in both the control and the steroid groups, increased as the rectal temperature rose to 41°- 42°C (Figure 14). Thereafter, the mean arterial pressure began to drop rapidly as the rectal temperature increased beyond 41°C in the control group and 42°C in the steroid group. At most temperatures, the mean arterial pressures in the steroid group were higher than the control group, though not highly significant, except between rectal temperatures 42-43°C ($p<0,05$) and appeared more stable than the control group.

On the other hand, the heart rates in the steroid group were lower than the control group for any given rectal temperature until a temperature of 43,5°C was reached. Moreover, the heart rate in the former peaked at a slightly lower rectal temperature than in the control group (43,62° vs 43,88°C).

Corticosteroids have been shown to increase cardiac contractility[514] and cardiac output[515] in normal subjects and patients in shock, to inhibit the vasoconstrictor action of endogenous vasoactive mediators[516], to inhibit the release of epinephrine and norepinephrine[132], to cause vasodilatation of peripheral vessels, and to augment the systemic arterial pressure[517] during shock. In addition, recent research findings show that corticosteroids also inhibit the release of cytokines such as cachectin[173] and interleukin-1 (IL-1)[160] from macrophages. Therefore, the differential behaviour of the control and the steroid groups could be related to the "leakage" of LPS into the hepatic portal blood (control group) or to methylprednisolone sodium succinate in the steroid group.

On the other hand, cardiovascular parameters also deteriorated in the steroid group but at a higher rectal temperature (42°C). It is likely that the high core temperature results in multiple tissue damage and cardiovascular collapse[63,65,69,484,485] over and above that due to LPS.

In this experiment, the primates in the steroid group died at a significantly higher rectal temperature than the control group (44,9 ± 0,14 vs. 44,4 ± 0,01°C) ($p<0,01$).

In addition, pre-treatment with the oral, non-absorbable antibiotic, kanamycin, also completely prevented any significant increase in plasma LPS concentration during

heat-stress (Figure 15). As descussed previously, the active ingredients of "Kantrexil" suspension are kanamycin and kaolin which prevent a rise in plasma LPS concentrations. These agents also inhibited any rise in plasma LPS concentration in the primate superior mesenteric artery occlusion model (Figure 10). Furthermore, pre-treatment with oral kanamycin has been shown to reduce the incidence of persisting endotoxaemia, lung lesions, and mortality rate from 90% to 32% during a temporary occlusion of the superior mesenteric artery, intravenous injection of endotoxin or bradykinin and intraperitoneal injection of *E.coli* in rabbits[32]. Whilst the prophylactic treatment with kanamycin used in this experiment significantly reduced the total bacterial count ($p<0.001$), it promoted an overgrowth of the gram-positive bacteria similar to that observed in the primate superior mesenteric artery occlusion model (Section 4.1.3). It would appear, therefore, that the reduction in count from 12,56 ± 1,83 cfu x 10^9/gram faeces to 0,183 ± 0,04 cfu x 10^9/gram faeces of total bacterial flora may not be sufficient to cause the reduction in the plasma LPS level noted in this study. It should, however, be taken into account that the count would have been much lower had it not been for the overgrowth of the gram-positives.

In contrast to what transpired in the experimental or kanamycin group, the plasma LPS concentration in the control group began to increase when the rectal temperature reached 42^{o}C (Figure 15). Initially this increase was gradual until a temperature of 43^{o}C was reached, when a rapid rise occurred, reaching 0,308 ± 0,038 ng/ml ($p<0,001$) just before the death of the animal.

As the rectal temperature increased, the mean arterial pressure followed similar trends in both the control and kanamycin groups (Figure 15). In the control group, not only was the mean arterial pressure lower for any given

rectal temperature than in the kanamycin group, but the mean arterial pressure also began a downward trend at a lower rectal temperature than in the kanamycin group (40,5°C vs 42°C) ($p<0,05$). As stated previously, the anti-cholinergic properties of aminopentamide, the other constituent of "Kantrexil" suspension which also contains kanamycin, might have caused the heart rates to be higher in the kanamycin group and possibly accounted for their death at a lower rectal temperature[360,518]. Gorman and Proppe[409] also found that the heart rates for any given blood temperature were higher in heat-stressed baboons which had received a cholinergic blocking agent than those which were not treated.

Significantly, it was found by some researchers that the reduction of gut flora with antibiotics and enemas increases survival in dogs subjected to heat-stress from 20% to 70,6%[62]. Furthermore, rabbits pre-treated with oral antibiotics had a reduced prevalence in developing endotoxaemia than untreated, heat-stressed rabbits[42].

Serum enzyme levels are widely used in clinical medicine to indicate the degree and extent of tissue damage[476]. However, from the findings in this study we are not able to conclude that any significant tissue damage had occurred up to a rectal temperature of 43°C. Except for a significant increase in serum AST (GOT) concentration from 47,6 IU/l at 37°C to 75,9 IU/l at 43°C in the control group, there were no significant changes in the serum enzymes; ALT (GPT) , L-γ-glutamyltransferase, and alkaline phosphatase in both groups (control or kanamycin) (Table 10). Blood samples taken from heat stroke victims at the point of collapse also showed very small changes, if any, in enzyme levels, while those taken a few hours later had elevated levels[79,81,477]. Costrini et al.[79] found that blood samples taken from heat stroke patients at zero time showed no changes in CPK-MB isoenzymes, CPK, LDH, SGOT,

and SGPT, whereas 12 hours later the concentrations of these enzymes were significantly elevated except for CPK-isoenzyme. Similar changes were observed in other heat stroke patients[358,450,478] and in experimental heat stroke animal models[452,479].

The standard therapy for heat stroke is rapid cooling, correction of fluid and electrolyte disturbances and treatment of shock[69,88,451]. The prophylactic administration of anti-LPS plasma in this study decreased the mortality rate significantly from 83,3% to nil ($p<0,025$) in primates heat-stressed to rectal temperature 43,5°C (Group A) (Table 11). Furthermore, the plasma LPS concentration of all the primates who survived the heat-stress, including the one from the control group, remained very close to the initial concentrations (Figure 17). The plasma LPS concentration in the surviving control primate was prevented from rising, presumably by the presence of a high level of "natural" anti-LPS IgG antibody which was about 144% more than the basal levels measured in these primates. On the other hand, the primates that died, owing to the high core temperature, had significantly elevated plasma LPS levels (Figure 17). Moreover, the prophylactic administration of non-immune equine plasma was not found to suppress the plasma LPS concentration in the control group.

However, prophylactic administration of equine anti-LPS IgG plasma did not alter the survival rate of animals which were subjected to a higher rectal temperature of 43,8°C. All the animals in this group (Group B) died, although the plasma LPS concentrations of the treated animals remained low and those of the controls had increased significantly ($p<0,005$) (Figure 8). Nevertheless, prophylactic anti-LPS plasma did increase the survival time significantly in this group ($p<0,05$) (Table 11). Similar changes in survival rates were

observed by DuBose et al.[487] in control rats and rats rendered tolerant to endotoxins. DuBose et al.[487] showed that endotoxin tolerant rats had reduced mortality to moderate heat-stress (rectal temperature 42,23 to 42,69^{o}C - total thermal area <60^{o}C.minute) but not to extreme heat-stress (thermal area - >60^{o}C.minute). However, their LPS data differed from those in this experiment in that they found that less than 20% of the non-survivors had positive LAL test in their blood samples. They also observed that heat-stress mortality in rats rendered more sensitive to endotoxin with zymosan (a lipopolysaccharide protein complex derived from yeast cell walls and which supposedly stimulate the RES to increase their phagocytic activity) treatment were not different from untreated controls. There is evidence from the present study using prophylactic anti-LPS IgG antibodies to support the findings of Bynum et al.,[62] who showed that destruction of the intestinal gram-negative flora by pre-treatment with oral antibiotics, cathartics and enemas, increased the incidence of 18-hour survival rate of heat-stressed dogs. Rabbits pre-treated with oral antibiotics and then heat-stressed to 43,5^{o}C also had a significantly reduced mortality and a slower rise in rectal temperature than control rabbits[42]. The failure of animals to survive after being subjected to extreme heat-stress, in this study and that reported by DuBose et al.,[488] even though they were pre-treated with anti-LPS antibodies and made tolerant to LPS, respectively, may simply be due to irreversible damage caused by hyperthermia to critical tissues, e.g. the brain[78,343,454,459].

Similar to the above findings using prophylactic anti-LPS antibodies, prophylactic administration of methylprednisolone sodium succinate also significantly increased the survival following heat stroke ($p<0,05$) (Table 12). Four out of six control animals that died had substantially increased plasma LPS concentrations (from

0,089 ± 0,007 to 0,257 ± 0,031 ng/ml) ($p<0,005$) (Figure 19). In contrast to the control group, the plasma LPS levels in the surviving steroid group (5 out of 5) remained close to baseline ($p<0,001$). The two control animals (not treated with steroid) that survived also showed very little change in the plasma LPS concentration. This may have been due to the high titre of "naturally" occurring plasma anti-LPS IgG levels in these animals prior to heat-stress (Table 14). The survival of, and lack of increase in plasma LPS concentration, in the two control and five experimental steroid treated animals cannot be ascribed to differences in the degree of heat-stress because there was no significant difference in the rate of heating between the survivors and non-survivors in the control group and between the non-survivors and the steroid group (Table 13).

What was also important, as observed in this study, was the decrease in relative plasma anti-LPS IgG concentration after heat-stress in the control and steroid groups (Table 14). This decrease could be attributed largely to its binding to LPS.

Corticosteroids were also shown to increase the survival rate in septic and endotoxin shock[20,509,519-521]. Hinshaw and his co-workers[20] noticed that dogs treated with methylprednisolone sodium succinate and gentamycin sulphate after a lethal *E.coli* shock had normal blood glucose concentration and pH, were free from intestinal haemorrhagic necrosis, had no loss of intestinal intimal lining, and had recovered completely from the shock. On the other hand, the control dogs not treated with the steroid died within 24 hours after variable periods of hypotension, intestinal haemorrhage and diarrhoea. It was observed here that the monkeys pre-treated with methylprednisolone sodium succinate were also free from diarrhoea, whereas the control animals which died had

bloody diarrhoea. The administration of corticosteroids to a few victims of heat stroke, however, has not been found to be beneficial[85,88,442], the reasons for which could have been inappropriate doseage and/or time of administration[20,505,509,521], especially the latter, since corticosteroid therapy was only used as a last resort. The reason could also have been that the patient's core temperature might have exceeded the "critical" temperature of survival, which in this study was found to be 43,5^{o}C in monkeys.

Braude et al.[106] suggested that the susceptibility of rabbits to a lethal challenge by the LPS molecule could be prevented by the presence of "natural" antibodies against the LPS molecule. Moreover, high serum anti-LPS titres were associated with increased survival from bacteraemia[109,110]. Furthermore, according to Schade et al.,[22] repeated injections of sub-lethal amounts of LPS to experimental animals and humans render them less responsive to a second dose of LPS challenge. However, other researchers were of the opinion that the phenomenon of tolerance was not dependent on the formation of specific antibodies but to the increased phagocytic activity of the RES,[22,97-101] since the latter could also be induced by using zymosan, a non-immunological agent. It is shown in this study, in two separate experiments, that animals having a high "natural" anti-LPS IgG antibody concentrations prior to heat-stress survived the induced heat stroke and had lower plasma LPS concentrations than those that died. The increased survival of rats made tolerant to endotoxin could, therefore, be possibly due to increased anti-LPS IgG antibodies and not, as explained, to increased phagocytic activity of RES cells[126,513].

The results of the current studies suggest that the endotoxaemia occurring during heat stroke could be contributing to the pathophysiology of heat stroke and

that the use of prophylactic anti-LPS antibodies and methylprednisolone sodium succinate may be protective.

Despite the wealth of information available so far, the primary pathophysiological cause initiating heat stroke is still uncertain[449,453]. Two opposing views regarding the pathophysiology of heat stroke, have appeared in scientific literature, (viz., peripheral vs. central mechanisms). According to the former hypothesis, proposed by Adolph and Fulton[484] heat stroke is caused by a failure of the circulatory system which ultimately leads to shock. Malamud et al.,[78] however, proposed a central mechanism in which elevated temperature causes direct thermal injury to target tissues, in particular, the thermoregulatory centres of the brain, and this, in turn, results in failures of sweating and thermoregulatory controls, eventually leading to shock. Although there is evidence that a failure of sweating may precipitate heat stroke[65,85,86,451,468], there are numerous reports of heat stroke accompanied by profuse sweating[68,79,81,342,351-353,373] (Table 6).

We believe that the seriously reduced intestinal blood flow coupled with the raised core temperature damages the permeability properties of the gut wall which then permits the leakage of LPS into the portal circulation. Furthermore, the reduced splanchnic blood flow and the high core temperature may also compromise the liver which may no longer be able to remove the LPS effectively from the circulating blood, thus leading to an elevated plasma LPS concentration. The latter, in turn, may exacerbate the deleterious effects of hyperthermia, either directly or by inducing the release of vasoactive or toxic agents, such as cachectin, which act on the cardiovascular system.

The results in the present studies suggest that both mechanisms operating together - circulatory (reduced

splanchnic blood flow) and thermal damage (gut wall and liver) - would lead to an endotoxaemia. It is possible that the changes in haemodynamic events and plasma LPS concentrations, noticed in the present study, are unrelated phenomena. However, it is felt that the presence of low concentrations of gut-derived endotoxins in the circulating blood could exacerbate the changes in the haemodynamic events which were initially caused by elevated core temperatures.

Results reported in these heat-stress experiments are consistent with the suggestion of Bynum et al.[62] that the plasma LPS concentration may initially contribute to the pathophysiological events in heat stroke. While these studies do not show by direct methods the movement of LPS across the gut wall, the rise in LPS concentration earlier in the portal vein than in the systemic artery, and the reduction of LPS concentration by prophylactic oral non-absorbable antibiotics, strongly suggest that the gut is the source of this LPS.

Thus the findings in the control heat-stress hyperthermia model corroborates the intestinal ischaemia model, which means that whenever the blood flow to the intestine is reduced it may damage the permeability properties of the gut mucosa and lead to leakage of LPS into the circulating blood. In addition, this study supports Fine's contention that blood flow to the splanchnic region becomes reduced in all forms of shock, causing endotoxaemia. It is possible, therefore, that had we used any other shock model (such as haemorrhage, or burn), as a control, we would also have induced an endotoxaemia in the subjects. It could be hypothesized that any agent which is capable of damaging the permeability properties of the gut mucosa would also give rise to the transmural migration of LPS from the gut lumen into the vascular compartment. Chemotherapy, x-irradiation, gastro-enteritis, intestinal

strangulation and obstruction, and volvulus are possible examples of such agents.

To summarize, as the body temperature rises above the normal level during heat-stress, the thermoregulatory effectors cause cutaneous vasodilation in order to dissipate heat and concomitant sphanchnic vasoconstriction to compensate for the increased blood flow to the skin[310,396-398,401]. We suggest that during hyperthermia, when the body temperature approaches 41°C-42°C the reduced blood flow to the intestines, especially the mucosa, causes ischaemic damage, and alters its permeability properties. This, in turn, allows the leakage of large amounts of LPS, first into the portal circulation, through the liver where some is removed, and then into the systemic. It is demonstrated in this study that a temporary ischaemia of the gut (by clamping of the superior mesenteric artery) results in an elevated plasma LPS concentration. Circulating LPS is known to induce the release of endogenous pyrogens and other vasoactive substances from the host's cells. The former acts on the hypothalamic thermoregulatory centres which then attempts, by causing cutaneous vasoconstriction and decreased sweating, to minimize heat loss from the surface. The actions of LPS on the thermoregulatory system opposes the effects of hyperthermia[384]. In addition, LPS by inducing the release of cachectin and IL-1[146,147,160,171,299] and other vasoactive agents[127,143] also causes other pathophysiological changes, such as hypotension, DIC, and diarrhoea. These effects of LPS exacerbate the deleterious effects of the high temperature on the tissues and organs of the body, and results in heat-stroke and, possibly death. Therefore, inhibition of the rise in plasma LPS concentration during heat-stress (by administering prophylactic anti-LPS antibodies or methylprednisolone sodium succinate) improves the survival rate and survival time of animals subjected to heat

stroke.

Recent studies, as discussed above, show that endotoxin-induced cachectin synthesis and release from macrophages may play an important role in the pathophysiology of endotoxin shock[148,501]. Hence, further work, using the models as used in this thesis but measuring simultaneously plasma cachectin and LPS concentrations and cardiovascular parameters appears to be indicated. Should there be an increase in plasma cachectin levels, it would be interesting to see the effects of administrations of anti-LPS IgG antibodies or corticosteroids on the changes in cachectin levels.

CHAPTER 6

CONCLUSION

From the results of the experiments presented in this thesis it may be concluded that:

i) a temporary occlusion of blood flow to the splanchnic region leads to a leakage of LPS from the gut lumen into, first, the portal and then, about ten minutes later, into the systemic circulation,

ii) felines respond differently from primates to a temporary occlusion of the superior mesenteric artery in that their plasma LPS concentration rises during both the occlusion and reperfusion periods; in primates it rises only during the reperfusion period. This is important in evaluating data from different species,

iii) the rise in plasma LPS concentration during or following a temporary occlusion of the superior mesenteric artery can be prevented by prophylactic administration of corticosteroids, anti-LPS antibodies and oral, non-absorbable antibiotics; procedures which appear to stabilize membranes, neutralize LPS and destroy intestinal aerobic gram-negative bacteria, respectively

iv) Administration of anti-LPS IgG antibodies during a period of endotoxaemia caused by ischaemia lowers the plasma LPS concentration to baseline levels within 20 minutes,

v) in the control heat-stress/stroke model, the plasma LPS concentration appears to rise at rectal

temperatures only above 41,5°C when using a direct assay. However, using an indirect assay LPS from the gut lumen appears to leak into the circulation at rectal temperatures as low as 40°C,

vi) a rise in plasma LPS concentration during heat-stress can be prevented by the use of anti-LPS IgG antibodies, corticosteroids and oral non-absorbable antibiotics,

vii) animals pretreated with those agents which stabilize membranes (corticosteroids) and neutralize LPS (anti-LPS IgG antibodies) have much improved survival rates following heat stroke. Since suppression of endotoxins improves the survival rate of heat stroke in primates, gut-derived endotoxins contribute to the pathophysiology of heat stroke,

viii) since the plasma LPS concentration rises in both the splanchnic ischaemia and heat-stress models, it is possible that elevations in plasma LPS concentration also occur in other pathophysiological conditions which compromise the integrity of the intestinal wall such as haemorrhagic and burn shocks and congestive cardiac failure and

ix) because of the successes in improving the survival in these LPS mediated disorders corticosteroids and anti-LPS IgG antibodies might beneficially be used for the treatment of a variety of other endotoxin-related disorders.

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CRITICAL CARE MEDICINE

APPENDIX 1

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Effect of corticosteroid prophylaxis on lipopolysaccharide levels associated with intestinal ischemia in cats

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Ischemia of the intestines damages the permeability of the intestinal wall, allowing lipopolysaccharide (LPS) (endotoxin) to leak from the gut lumen into the blood circulation, causing shock and death. We measured LPS levels associated with corticosteroid treatment vs. no treatment in cats whose superior mesenteric artery had been occluded for 60 min. In untreated cats, the preocclusion mean plasma LPS concentration remained stable at 0.069 ± 0.015 ng/ml. Toward the end of the occlusion period, mean plasma LPS rose to 0.239 ± 0.032 ng/ml ($p < .01$). Release of the clamp and reperfusion with oxygenated blood was followed within 20 min by a large rise in plasma LPS concentration to 0.825 ± 0.11 ng/ml ($p < .01$), which had returned to preocclusion levels about 80 min later. Methylprednisolone (30 mg/kg) was infused into a second group of cats 1.5 h before SMA occlusion. In these cats there was a complete inhibition of the LPS rise both during and after occlusion. These data suggest that the reported beneficial effect of corticosteroids in the treatment of septic shock may be mediated, in part, by reducing LPS leakage from the gut.

The intestines of mammals contain large amounts of highly toxic Gram-negative bacterial lipopolysaccharide (LPS). If a small amount of LPS leaks into the blood circulation, it is detoxified by the reticuloendothelial system (RES) (1, 2). However, if a large amount of LPS rapidly enters the circulation, it may overwhelm the detoxifying action of the RES and lead to endotoxemia, shock, and death (2, 3). This could occur if the intestinal wall is damaged by almost any means, including ischemia, trauma, hyperthermia, vasoactive agents, or ionizing radiation (2–6). Clinically, ischemia of the intestine could occur by volvulus, torsion, intussusception, or thrombosis of a splanchnic artery.

Temporary occlusion of the superior mesenteric artery (SMA) is a well-studied model for producing intestinal ischemia, shock, and death. However, the precise temporal relationship between intestinal ischemia and the presence of elevated LPS levels in the systemic blood has not been reported, mainly because the limulus amebocyte lysate (LAL) clot method for determining LPS is only semiquantitative and not easily reproducible (7). Such a determination would be useful for establishing the relative pathophysiologic importance of various agents (kinins, prostanoids, catecholamines, enkephalins) produced in excess by LPS challenge (8–11). Here we used the recently described quantitative and highly reproducible chromogenic substrate modification of the LAL method (12) to determine the time course of plasma LPS concentration in cats subjected to a one-hour occlusion of the SMA. The possible beneficial effect of the administration of prophylactic corticosteroid was also investigated (13).

MATERIALS AND METHODS

Fourteen cats were anesthetized with sodium pentobarbital (30 mg/kg) and catheters were inserted into a femoral vein and artery. The cats were then randomly divided into two groups. The steroid group (n = 6) received a 30-mg/kg methylprednisolone infusion over a 15-min period via the femoral vein. The other cats (n = 8) acted as controls. An incision was made in the linea alba and the abdominal cavity was opened. The SMA was identified and cleared of surrounding tissues. After a one-hour period for baseline measurements, the SMA was occluded in all cats with a bulldog clamp for 60 min (14). At the end of the experiment the animals were killed.

Arterial blood was collected at 5- to 20-min intervals throughout the 4-h study, using heparinized sterile, pyrogen-free plastic tubes. The volume of blood removed was replaced with an equal amount of normal pyrogen-free saline. LPS concentrations were determined by the chromogenic substrate method as previously described (12, 15). The standard curve was calibrated with LPS obtained from *Escherichia coli* strain 0111:B4.

Success in these studies required a highly reproducible and accurate LPS assay. The previously widely used LAL clot test provided only semi-quantitative data (16) which were not adequate for a study of this kind. The

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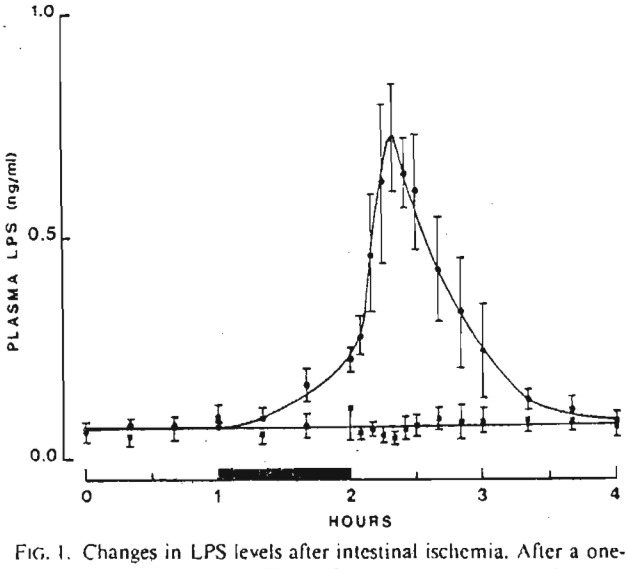

FIG. 1. Changes in LPS levels after intestinal ischemia. After a one-hour baseline period, the SMA of each control cat (*circles*) was occluded for 60 min (*bar*). The plasma LPS concentration remained at a low level during the baseline period, but rose slightly after 20 min of occlusion. Upon release of the clamp, there was a rapid rise in plasma LPS concentration to 0.825 ± 0.100 ng/ml ($p < .001$) after 20 min, followed by a return to baseline (preocclusion) levels within 60 to 80 min. Prophylaxis with a 30-mg/kg methylprednisolone infusion completely inhibited the rise in plasma levels (*squares*) both during and after occlusion.

development of the chromogenic substrate modification of the LAL test in combination with rapid-reading, rapid-recording photometers (Titertek Multiskan, Flow Labs, McLean, VA) makes feasible a wide range of in vivo endotoxin studies.

RESULTS

Six of eight control cats and all six steroid-treated cats survived the 4-h experimental period.

As shown in Figure 1, the mean preocclusion plasma LPS concentrations in surviving animals from both the control and steroid groups remained stable at low levels of 0.069 ± 0.015 and 0.069 ± 0.030 ng/ml, respectively. During SMA occlusion, the plasma LPS concentration in the control group began to rise after 20 min and reached 0.239 ± 0.032 ng/ml ($p < .01$). In the steroid-treated cats, the mean plasma LPS concentration did not rise significantly from the preocclusion level. After release of the bulldog clamp, plasma LPS concentration in the control cats rose rapidly to a mean of 0.825 ± 0.110 ng/ml ($p < .01$) after 20 min and then returned to preocclusion levels over the next 60 to 80 min. On the other hand, the steroid group showed no significant change in LPS concentration.

DISCUSSION

LPS, but not whole bacteria, reportedly can pass through the wall of ischemic dog bowel in less than 30 min (17), and LPS can enter the systemic circulation within a few minutes of intestinal ischemia (14). However, changes in LPS concentrations during endotoxemia have not previously been documented.

During the occlusion period ischemia may have damaged the intestinal walls of our control cats, increasing permeability to LPS, but little LPS was detected in the blood. Possibly, the liver was able to clear most of it; the small amount of systemic plasma LPS observed presumably bypassed the liver by collateral circulation and/or by directly passing into the peritoneal cavity and into the lymphatics. Alternatively, ischemia of the splanchnic regions may have impaired liver function (18).

When the SMA of control cats was reperfused with oxygenated blood upon release of the clamp, the liver's RES apparently removed much of the LPS which had leaked into the portal blood from the intestinal vascular system. The peak LPS concentrations at 10 to 30 min postocclusion (Fig. 1) may represent cumulative blood levels which temporarily overwhelmed the RES. Gradually RES function improved and the intestinal permeability to LPS may have been reduced upon reperfusion with oxygenated blood, leading to the subsequent decline in plasma LPS concentration.

Schoenberg and coworkers (19) recently reported that during an ischemic period, intracellular xanthine dehydrogenase is converted into the oxidase. Upon reperfusion of the tissues with fresh oxygenated blood, this oxidase was thought to act on hypoxanthine present in high concentrations in ischemic tissue to produce a mixture of toxic products including superoxides, peroxides, and oxygen-free radicals. They reported some damage to cat intestinal mucosa immediately after one hour of ischemia, which significantly increased during one hour of reperfusion with oxygenated blood. They suggested that the additional damage was caused by free radicals. In our study, this mechanism could ex-

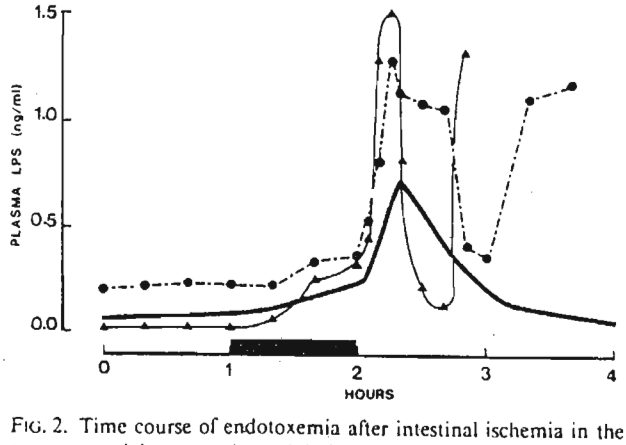

FIG. 2. Time course of endotoxemia after intestinal ischemia in the two nonsurviving control cats (*circles* and *triangles*). *Bar* represents the intestinal occlusion period. *Dark line* is the mean of six surviving control cats from Figure 1. The nonsurvivors had elevated peak concentrations of LPS compared to the survivors, followed by a decline and then a second rise in LPS concentration just before death.

plain the slow LPS decline in the control group; additional LPS might have leaked into the circulation due to damage of intestinal mucosa by free radicals. The two nonsurviving control cats both appeared to have higher peak plasma LPS concentrations (1.52 and 1.49 ng/ml, compared to 0.716 ng/ml in the survivors; Fig. 2). Interestingly, both cats had a second stage of severe endotoxemia (second LPS peak) immediately before death. The source of this second peak is uncertain, but might have been intestines in which the peroxide-mediated damage was excessive.

Prophylactic methylprednisolone completely inhibited the postocclusion LPS peak. The mechanism of steroid action in this case is unknown; however, these results may explain the reported beneficial effect of methylprednisolone in patients suffering from septic shock (13). Furthermore, prophylactic methylprednisolone might be of benefit for abdominal surgery requiring the temporary occlusion of some arteries, or before the release of volvulus or torsions, or any treatment likely to cause a temporary ischemia of any part of the bowel.

ACKNOWLEDGMENTS

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APPENDIX 2

Circulatory Shock 19:231-237 (1986)

Superior Mesenteric Artery Occlusion Shock in Cats: Modification of the Endotoxemia by Antilipopolysaccharide Antibodies (Anti-LPS)

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We measured the time course of elevated plasma LPS concentration caused by a temporary intestinal ischemia using the superior mesenteric artery (SMA) occlusion shock model in anesthetized cats. The systemic plasma LPS increased from 0.075 ± 0.006 ng/cc to 0.219 ± 0.026 ng/cc ($P < 0.001$) during the occlusion period. On release of the clamp, the plasma LPS concentration rose rapidly to 0.716 ± 0.122 ng/cc ($P < 0.001$) within 20 min. Thereafter, it declined to reach baseline levels after 100–120 min reperfusion. A total of 21 animals received IV 1.0 cc/kg antilipopolysaccharide hyperimmune equine plasma (anti-LPS) either 1.5 hr before the occlusion or at 0, 10, or 20 min after release of the occlusion. Prophylactic anti-LPS prevented any rise in plasma LPS both during and after release of the occlusion. The administration of anti-LPS during the reperfusion period completely reversed the endotoxemia caused by intestinal ischemia within 5–10 min. This rapidity of response to anti-LPS may be important in the previously reported therapeutic benefit of anti-LPS.

Key words: LPS, endotoxin, ischemia, shock, sepsis, trauma, anti-LPS

INTRODUCTION

Septic shock is a serious and life-threatening complication of gram-negative bacterial infection. Conventional antibiotic therapy is unsuccessful in about 50% of patients, in part because a stable and highly toxic lipopolysaccharide (LPS, endotoxin) is released from the surface of the killed bacteria [1,2]. LPS may also enter the systemic circulation from the lumen of the gut if its wall is damaged by almost any means, including ischemia, trauma, hyperthermia, vasoactive agents, ionizing radiation, burns, and viral gastroenteritis [3–8]. Once in the circulation, it is ordinarily

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removed by the reticuloendothelial system (RES) [9]. However, it can persist in the circulation if the RES is inadequate to remove it, eventually leading to vascular collapse and death [3].

A new approach to therapy for septic shock is the administration of antibodies directed against the endotoxins, in addition to conventional antibiotic and supportive therapy. Human anti-LPS has been successfully used to treat gram-negative bacterial infections in humans [10,11]. Equine anti-LPS prophylactically protected rabbits against superior mesenteric artery (SMA) occlusion shock [8] and mice from X-irradiation overdose [12].

We recently determined the time course of plasma LPS concentration in cats subjected to a 1 hr occlusion of their superior mesenteric arteries (Gaffin et al. submitted). The present paper examines the possible beneficial effects of administering anti-LPS hyperimmune plasma on this system.

MATERIALS AND METHODS

Twenty-nine cats of both sexes used in this study were obtained from the university animal colonies. They were fasted overnight and anesthetized with phenobarbitone sodium (30 mg/kg IP). Catheters were inserted into a femoral vein and artery, an incision was made in the linea alba, and the abdominal cavity was opened. The SMA was identified and cleared of surrounding tissues. After surgery was completed, the cats were allowed to stabilize for 30 min before commencement of the experiment. The cats were divided into five groups: groups A–D received an IV infusion over a 2 min period of 1.0 cc/kg of anti-LPS hyperimmune plasma (ATOX Pharmaceutical Co., Gillitts, South Africa). This dose was selected on the basis of a variety of clinical studies [13]. The anti-LPS was administered 1.5 hr prior to occlusion of the SMA to cats in group A ($n = 6$), immediately after release of the occlusion to cats in group B ($n = 5$), 10 min after release of the occlusion to cats in group C ($n = 5$), and 20 min after release of the occlusion to cats in group D ($n = 5$). The other eight cats (group E), which received no anti-LPS hyperimmune plasma, acted as controls.

The LPS-precipitable IgG in the anti-LPS preparation had a concentration of 1,200 μg/ml according to an ELISA that had been calibrated by an immunoprecipitation reaction [14]. Specific IgG antibodies present could bind to LPS prepared from a wide range of gram-negative bacteria, including *Escherichia coli*, *Shigella*, *Klebsiella*, *Pseudomonas*, *Salmonella*, *Proteus*, etc, and could destroy these bacteria within seconds to minutes by means of complement activation (Wells et al, submitted). Recent investigations indicate that >99% of the specific antibodies are directed against the "O" antigens (Wells et al, submitted).

Baseline measurements were taken for 1 hr. The SMA was then occluded by placing a bulldog clamp on it for 60 min and then releasing it. After an additional 2 hr of collecting blood samples, the animals were euthanized with pentobarbital. One milliliter femoral arterial blood samples were collected for LPS determinations at 5–20 min intervals into heparinized sterile pyrogen-free plastic tubes (Falcon) and stored on melting ice during the 4 hr experimental period. An equal volume of pyrogen-free saline was administered back into the cat at each blood collection. The blood was centrifuged, and the plasma was removed under sterile conditions in a laminar flow hood and either analyzed immediately or placed into plastic tubes and frozen at

−20°C for up to 1 week until analyzed. The recently described chromogenic substrate modification of the *Limulus* amebocyte lysate (LAL) technique was employed to determine plasma LPS concentration [15,16]. This highly reproducible technique has been shown to overcome objections reported for the LAL gel test [17]. We previously found a 95.1% recovery of LPS from spiked plasma samples using this method, calibrating the standard curve with LPS prepared from *E. coli* 0111:B4 (Wells et al, submitted).

RESULTS

Baseline Period

As is shown in Figure 1, the plasma LPS concentration remained stable and at low levels (0.075 ± 0.006 ng/cc) in both the control and prophylactically treated cats (groups E and A) during the 1 hr baseline period.

SMA Occlusion Period

In the control group E (Fig. 1) and in groups B–D (Fig. 2), the plasma LPS levels began to rise after 20 min of occlusion to reach final values of 0.219 ± 0.026

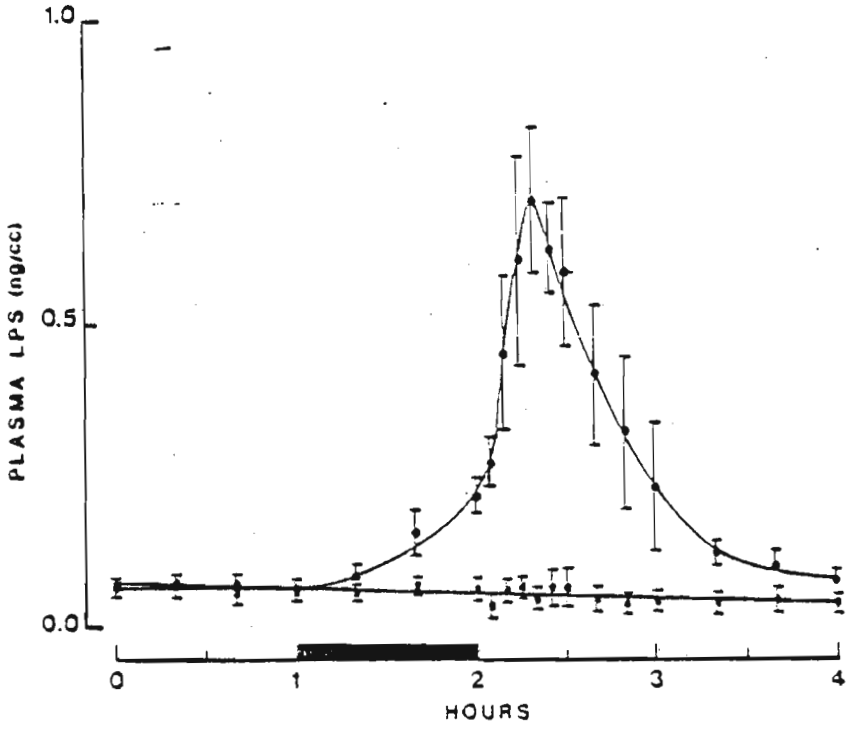

Fig. 1. Effect of prophylactic anti-LPS on SMA occlusion endotoxemia. Cats received 1.0 cc/kg IV equine hyperimmune plasma 1.5 hr before occlusion of their superior mesenteric arteries for 60 min. In the untreated cats (circles), femoral arterial blood samples showed a small rise in LPS concentration during the occlusion period but a larger one peaking 20 min after release of the occlusion, which returned to baseline levels after an additional 90–100 min. In those cats receiving prophylactic anti-LPS (squares), no rise was seen in blood samples either during or after the occlusion period.

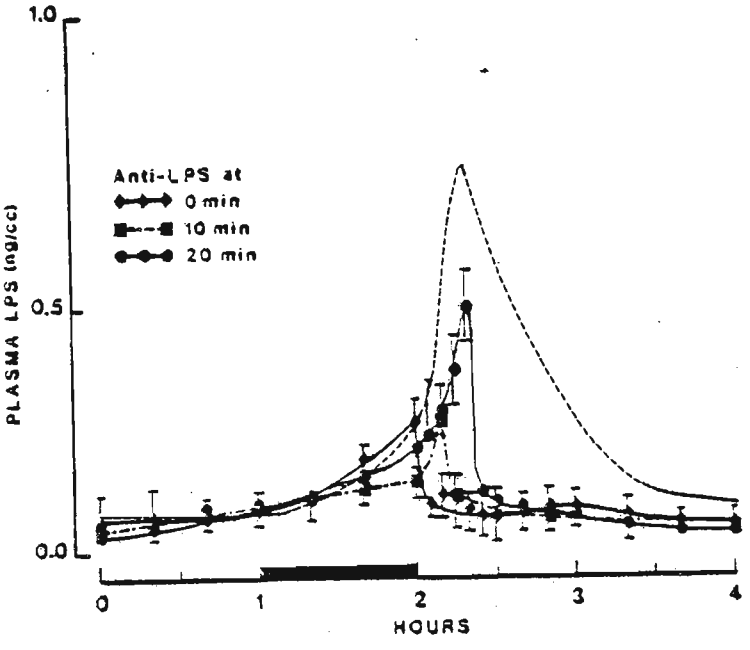

Fig. 2. Therapeutic anti-LPS. Cats received 1.0 cc anti-LPS IV either immediately upon release of the clamp occluding the superior mesenteric artery (diamonds), 10 min afterwards (squares), or 20 min afterwards (circles). The dashed line is for untreated cats. Note that within 5–10 min of anti-LPS administration the plasma LPS concentration fell to near baseline levels.

ng/cc ($P < 0.001$), 0.241 ± 0.042 ng/cc ($P < 0.001$), 0.156 ± 0.031 ng/cc ($P < 0.001$), and 0.193 ± 0.017 ng/cc ($P < .001$), respectively. On the other hand, the cats that had received prophylactic anti-LPS (group A) showed no significant change from the baseline levels (Fig. 1).

Reperfusion Period

In the control cats (Fig. 1) the plasma LPS level rose rapidly to reach a mean peak value of 0.716 ± 0.122 ng/cc 20 min after release of the clamp. Thereafter, the LPS concentration began to decline to reach baseline levels 100–120 min following release of the clamp.

On the other hand, in those cats receiving prophylactic anti-LPS (group A), their plasma LPS concentrations remained at or below baseline levels throughout the postocclusion period. In the group B cats (Fig. 2), which received anti-LPS immediately after releasing the clamp, the plasma LPS concentration *fell* (from the raised value during the occlusion period) from 0.241 ng/cc to 0.088 ng/cc ($P < 0.01$) within the first 5 min. Following this, the plasma LPS concentration continued to fall slowly to a value of 0.048 ng/cc ± 0.028 ng/cc 120 min after release of the clamp.

In the remaining cats, as in the controls, the plasma LPS rose after release of the clamp. However, when anti-LPS was given 10 min afterwards (group C), the plasma LPS concentrations fell from 0.241 to 0.114 ng/cc ($P < 0.02$) within 5 min of administration and reached baseline levels during the next 15 min. When anti-LPS was given 20 min afterwards (group D), the LPS concentration fell from 0.455 to

0.10 ng/cc ($P < 0.005$) within 5 min and reached baseline levels over the next 20 min.

DISCUSSION

Patients continue to die from gram-negative bacterial shock and endotoxemia despite the development of potent antimicrobial agents. This is due in part to the presence of LPS in the circulation of patients whose RES is not capable of detoxifying it [3]. LPS in the systemic circulation could come from the cell walls of the killed bacteria [1,2] and/or from the gut as a result of damage of its wall owing to many insults [3–8].

The basic approach to the treatment of septic shock usually includes potent broad-spectrum antimicrobial therapy, control of the source of infection, attempts to ameliorate predisposing factors, replacement of blood volume, correction of electrolyte imbalance, and careful management of complications (such as cardiac failure) [17]. However, these have no effect in reducing the concentration or activity of circulating LPS. We believe that treatment should also be directed specifically against the LPS [18].

The administration of anti-LPS antibodies is known to reduce mortality and morbidity in septic and nonseptic shock in animals and humans [11,13,19–22]. Anti-LPS antibodies inactivate and opsonize the LPS present in the circulation, and in the presence of a complement can kill a wide range of gram-negative bacteria [23,24]. In this study, we observed that unless anti-LPS was present the plasma LPS concentration in all groups increased significantly after 20–30 min of occlusion of the SMA (Figs. 1 and 2). On the other hand, in the presence of prophylactic anti-LPS, the plasma LPS concentration remained at or even below the control baseline levels throughout the entire 4 hr experimental period. Specific antibodies in anti-LPS at the concentrations employed do not inhibit the LAL assay (Wells et al, submitted).

The ischemia probably damaged the integrity of the intestinal walls, leading to an elevated LPS permeability. However, because of the occlusion, there was very little or no blood flow to carry the LPS into the portal vein. The increased plasma LPS detected during the occlusion period might have entered the circulatory system by bypassing the liver and spleen via collateral and/or lymphatic vessels.

Once the clamp was released, the SMA was reperfused with oxygenated blood. The plasma LPS concentration rose steeply in the control cats to reach a peak 20 min postreperfusion and thereafter declined initially rapidly and then slowly to reach baseline 100–120 min after removal of the clamp.

A biphasic clearance of LPS from the vascular compartment was observed by Mathison and Ulevitch [25] during IV injection of LPS in rabbits. An initial rapid clearance of LPS was due to a rapid uptake by the RES and to a lesser extent by granulocytes and adrenal glands [25–27]. An additional slower rate of disappearance of LPS was due to the binding of LPS to high-density lipoprotein in plasma [25,27]. In cats receiving anti-LPS prophylactically or immediately upon release of the clamp, the specific antibodies prevented any rise in plasma LPS concentration during the reperfusion period. Furthermore, in other cases, when the plasma LPS concentration had risen (groups C and D), anti-LPS administration reduced the LPS value to baseline levels within 20 min. IgGs present in anti-LPS are known to bind to LPS prepared from a wide range of gram-negative bacteria in a matter of seconds and to

destroy homologous and heterologous gram-negative bacteria by complement activation in a time scale of seconds to minutes (Wells et al, submitted).

Other studies in this laboratory have shown that anti-LPS antibodies bound to the surface of gram-negative bacteria stimulate granulocytes to ingest these bacteria [23]. In previous studies, we did not observe any protective effect of normal nonimmune plasma or serum in septicemia or endotoxemia [11]. The equine anti-LPS plasma used in this study was found to protect pregnant rats against *E. coli*-induced abortion [28], to protect mice against X-irradiation death [12], and to control *Pseudomonas* keratitis in rabbits, whereas normal serum in these studies had either no effect or actually increased the mortality [29]. Possibly, the presence of anti-LPS antibodies bound to the surface of "free" LPS facilitates the liver Kupffer cells to remove LPS from the circulation more rapidly. This study shows the rapidity with which anti-LPS antibodies reduce high concentrations of LPS from the systemic circulation and might thereby improve survival in septic shock.

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In conducting the experiments described in this report the investigators adhered to the NIH guidelines for the use of experimental animals.

APPENDIX 3

JOURNAL OF SURGICAL RESEARCH 45, 187–193 (1988)

Oral Administered Nonabsorbable Antibiotics Prevent Endotoxemia in Primates following Intestinal Ischemia

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Submitted for publication March 12, 1987

Plasma lipopolysaccharide (LPS) concentrations have been found to increase during a temporary occlusion of the superior mesenteric artery (SMA). We have attempted to show, by a prophylactic oral administration of a nonabsorbable antibiotic to monkeys subjected to an SMA occlusion shock, that the increased LPS is intestinal in origin. A total of eight monkeys were subjected to a temporary occlusion of the SMA. Four monkeys received prophylactic oral administration of a nonabsorbable antibiotic, while the rest acted as controls. The plasma LPS concentrations before occlusion in the control and the kanamycin group were 0.069 ± 0.006 and 0.092 ± 0.005 ng/ml, respectively. At the end of the 1-hr occlusion period the plasma LPS concentration in the controls increased to 0.09 ± 0.009 ng/ml ($P < 0.1$) and peaked to 0.378 ± 0.103 ng/ml ($P < .001$) within 20 min of reperfusion. Thereafter, the plasma LPS concentration returned slowly to baseline. In the kanamycin group the plasma LPS concentration remained at baseline throughout both the occlusion and reperfusion periods. These data suggest that the origin of the increased plasma LPS concentration seen following temporary occlusion of the SMA is from the gut, and is information of possible importance in patients about to undergo intestinal surgery.

INTRODUCTION

The management of gram-negative sepsis continues to be a major problem with unacceptably high mortality rates of 40–70% [2, 20] despite the administration of modern antibiotic therapy together with fluid resuscitation and respiratory assistance. Antimicrobial drugs may destroy the gram-negative organisms in the blood but leave intact a highly toxic component of their outer membrane, endotoxin (lipopolysaccharides, LPS), which is believed to be responsible for much of the high mortality [17, 39]. Furthermore, the lumen of the gut always contains gram-negative bacteria and, hence, LPS. Normally, the gut wall is impermeable or slightly permeable to LPS. The small amounts of LPS that may leak into the portal circulation are detoxified by the reticuloendothelial system (RES) [6, 26]. However, damage to the gut wall, by, e.g., ischemia, trauma, hyperthermia, vasoactive agents, ionizing radiation, and viral gastroenteritis, may permit LPS to leak rapidly into the portal circulation [4, 6, 15, 30, 43]. Should the RES of the liver and spleen become overwhelmed by the rapidly rising blood LPS levels, or should the RES function be inadequate to remove the LPS, then, the blood LPS concentration in the systemic circulation would rise and persist in the circulation—eventually causing vascular collapse, shock, and death [6, 7, 36].

Recently, we determined the time course of changes in circulating plasma LPS levels in two shock models: superior mesenteric artery occlusion (SMAO) shock in cats [12, 15] and heat stroke in primates [16]. In addition, in the SMAO shock model, we found that prophylactic administration of either anti-LPS antibodies [15] or methylprednisolone sodium succinate [12] completely prevented a rise in plasma LPS concentrations. In this study we have attempted to prove the intes-

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tinal origin of LPS in SMA occlusion in primates. The amount of endogenous gram-negative flora and, hence, LPS in the gut were reduced by administering orally a non-absorbable antibiotic prior to the experimental insult.

MATERIALS AND METHODS

Experimental Animals and Surgical Procedures

A total of eight monkeys (*Cercopithecus aethiops*) of either sex, weighing between 2.7 and 6.8 kg, were used. They were anesthetized with ketamine (10 mg/kg, im). Thereafter, incremental bolus doses (5–10 mg/kg iv) of ketamine were used to maintain anesthesia.

After cleansing both the femoral areas with chlorhexidine gluconate, catheters were introduced into both femoral arteries and a peripheral vein. One of the arterial catheters was connected to a B188 Statham transducer for recording of blood pressure, while blood samples were collected at various times from the opposite catheter. Only pyrogen-free equipment was used and each heparinized plastic tube into which the blood was collected was stored on ice until centrifuged for LPS and anti-LPS IgG analyses. Arterial pressure was recorded, using a Honeywell CM 130 patient monitor system, at 5-min intervals during the experiment. Heart rate, systolic, diastolic, and mean arterial pressures were calculated electronically and presented as a print-out with the blood pressure recording. The mean arterial pressure (MAP) was measured automatically from integration of the area under the arterial pressure curve.

Rectal temperatures (T_r) were recorded continuously using a rectal probe inserted about 10 cm into the rectum, and a telethermometer (Yellow Springs Instrument 46 - TUC) was connected to a chart recorder.

Experimental Protocol

The subjects were assigned to one of the following groups:

1. Control SMAO (n = 4). Those primates subjected to a 1-hr occlusion of the superior mesenteric artery (SMA) according to [15].

2. SMAO + kanamycin (n = 4). Those primates pretreated with a standard antibiotic therapy before being subjected to a 1-hr occlusion of the SMA.

Antibiotic therapy. The four treatment primates were anesthetized with ketamine (10 mg/kg, im) before antibiotic therapy. Thereafter kanamycin (15 mg/kg) (Kantrexil suspension—The B-M Group (Pty), Ltd.) was administered every 12 hr over 5 consecutive days via a nasogastric tube. They were subjected to an SMAO shock on the sixth day after kanamycin.

Experimental Procedure

SMAO shock. Following catheterization of the femoral arteries and a peripheral vein each primate assigned to either the control SMAO or the SMAO + kanamycin group had the abdominal cavity exposed through a linea alba incision. The SMA was exposed and freed from surrounding connective tissue. A loop of a sterile piece of cotton was then positioned around the SMA to ensure that total occlusion would occur when this material was drawn tight. The abdominal cavity was closed using sterile nylon sutures. Following a 30-min stabilization period, femoral arterial blood samples (1 ml) were collected at 20-min intervals for 1 hr. After this initial period of gathering baseline data, the SMA was occluded for 1 hr and blood samples were collected at 20-min intervals. The vascular occlusion was then released and blood samples were obtained at 5- to 20-min intervals during the 2-hr reperfusion period. Following each blood withdrawal an equal volume of pyrogen-free saline was infused back into the animal. Arterial blood pressure was monitored continuously and recorded at 5-min intervals, while the T_r was recorded continuously during the entire 4-hr experimental period. At the end of the experiment the animal was euthanized with an intravenous overdose of phenobarbitone.

The blood samples for LPS and anti-LPS IgG assay were stored in ice until the end of each experiment. After centrifugation of the samples the plasma was removed under sterile conditions in a laminar flow hood. The samples were either analyzed immediately or stored at −20°C for up to a week until analyzed. Plasma LPS concentrations were determined using the chromogenic substrate modification of the Limulus amebocyte lysate (LAL) technique [3, 9] (MA Bioproducts). We previously found a 95.1% recovery from spiked plasma samples using this method, calibrating the standard curve with LPS prepared from *Escherichia coli* 0111: B4 [44].

Bacteriology

Rectal swabs for bacteriological examination were taken from all animals after induction of anesthesia but before experimental SMAO. These sterile rectal swabs were initially moistened with sterile saline, and care was taken to avoid peri-anal contamination. After storing in nutrient broth, the swabs were individually plated out on deoxycholate citrate agar (DCA), MacConkey agar, and nutrient agar. These plates were then incubated aerobically for 18–24 hr at 37°C and further subcultured on to DCA. Twenty-four hours later each plate was examined for non-lactose-fermenting colonies which were then isolated and identified by gram stain, biochemical reactions, and colony form and pigmentation.

Immediately after the termination of each experiment the abdomen of each animal was opened. A 6- to 7-cm section of the transverse colon was tied off at each end and removed. One gram of fecal contents was then emulsified in 100 ml of sterile peptone water under a laminar flow hood. Serial dilutions of 10^2–10^8 were made up using an initial 1/100 dilution. Triplicate pour plates of 1 ml of each in nutrient agar were made for bacterial enumeration. Plates were incubated inverted at 37°C for 18–24 hr. Bacterial colonies were enumerated and results are reported as the number of colony-forming units per gram feces.

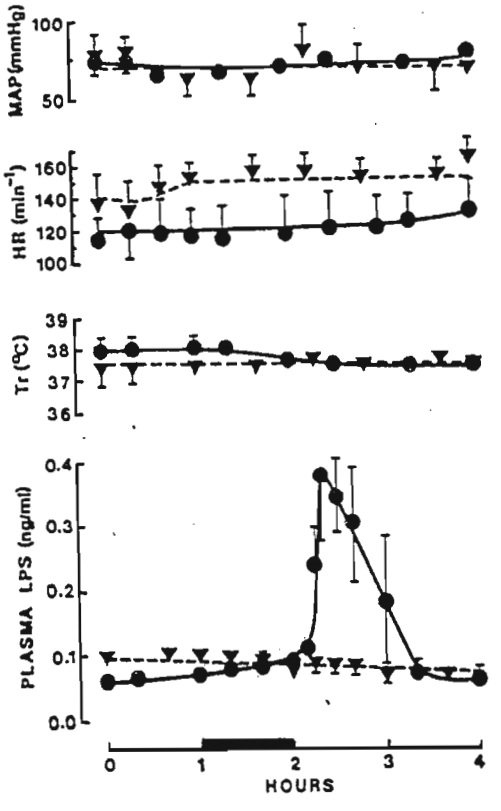

FIG. 1. Changes in plasma LPS concentration, mean arterial pressure (MAP), heart rate, and rectal temperature (T_r) before, during, and after occlusion of the SMA for 1 hr (bars) in control (circles joined by solid lines) monkeys and in monkeys pretreated with oral kanamycin (15 mg/kg) (triangles joined by broken lines). The values are means ± SEM of four monkeys in each group.

Statistical Analysis

The data are presented as means ± SEM and compared using the Student t test, the analysis of variance (ANOVA), and Duncan's multiple range test. A P value of <0.05 was taken as significant.

RESULTS

As shown in Fig. 1, plasma LPS levels in both the control and kanamycin groups remained at levels of 0.069 ± 0.006 and 0.092 ± 0.005 ng/ml, respectively, during the 1-hr baseline period, although the control group

had a gradually rising baseline. During the 1-hr SMA occlusion period in the control group plasma LPS concentrations increased slightly to reach a value of 0.09 ± 0.009 ng/ml ($P < 0.1$) at the end of the occlusion period but this was not significantly above the expected rise from the increasing baseline. In the kanamycin group the plasma LPS level remained close to their preocclusion values (0.082 ± 0.012 ng/ml).

When the SMAO was released, the plasma LPS levels in the control group increased slowly during the initial 10 min. Thereafter it showed a very rapid and significant increase to peak at 0.378 ± 0.103 ng/ml ($P < 0.001$) within 20-min of reperfusion with oxygenated blood. After this the plasma LPS concentration steadily declined and returned to preocclusion levels 80 min after the beginning of reperfusion. On the other hand, in the kanamycin group the plasma LPS concentration never rose above the baseline levels throughout the postocclusion period.

The arterial blood pressure (systolic, diastolic, and mean), heart rate, and T_r in both groups remained more or less constant throughout the entire experiment (Fig. 1). None of the subjects exhibited shock symptoms, and furthermore, it was necessary to administer only small doses of anesthetic periodically to the control group but larger doses to the kanamycin group.

Bacterial Counts

Bacterial examination of rectal swabs from control animals yielded *E. coli, Pseudomonas, coliform bacilli, Proteus, Serratia, Staphylococcus,* and *Lactobacilli.* In contrast, pretreatment with kanamycin resulted in an overgrowth of the gram-positive *Staphylococcus* spp. and *Lactobacilli,* although *E. coli* was encountered in some animals. Plate counts in the controls yielded 21.23 ± 1.75 CFU × 10^9/gram feces. In the kanamycin group plate counts showed a significant reduction to 0.051 ± 0.007 CFU × 10^9/gram feces ($P < 0.001$).

DISCUSSION

Abdominal surgery, extensive soft tissue trauma, large burns, and certain viral and hemorrhagic enteric diseases are examples of problems which can give rise to endotoxin shock [15, 19, 43, 46, 47]. Therapeutic regimens for these conditions include restoration of the cardiovascular failure, counteraction of other pathophysiological processes, and elimination of the causative microorganisms [22, 38]. In spite of the above it would appear that a mortality rate of up to 80% worldwide is still experienced in patients with endotoxin shock [2, 20]. Recent works from this and other laboratories have shown that anti-LPS antibodies can be of benefit in patients and experimental animals suffering from a variety of LPS-mediated disorders [5, 11, 13, 14, 25, 46, 47]. In this study we observed that during a nonseptic onslaught, namely, the SMAO, a concurrent endotoxemia was present and that the endotoxins were gut-derived.

In the primates, in the control SMAO group of this study, there was no significant increase in plasma LPS concentration during the 1-hr occlusion period (Fig. 1). This is in contrast to similar experimental studies in cats [12, 15] where significant increases did occur. Meat diets, as in the cats, are reported to increase intestinal gram-negative bacterial flora [24] compared to the monkeys' mixed diet. Nevertheless, this study shows that an endotoxemia does occur in monkeys due to a nonseptic etiology. Furthermore, observations in dogs in this laboratory, have shown that LPS and not whole bacteria pass through the ischemic bowel in less than 30 min [32].

It is known that intestinal ischemia can produce the necessary damage to the mucosal barrier which in turn will allow endogenous LPS to migrate intravascularly [12, 15, 19, 41]. LPS is destroyed by specific IgGs and the macrophage–phagocytic system [28, 45, 49]. However, if these defences are not functioning significantly, LPS may persist in tissues for up to 2 weeks [8].

When the clamp was removed from the SMA in the control group, in this study, and flow of oxygenated blood was resumed through the gut, the plasma LPS concentration rose, initially slowly and then rapidly to peak at 0.378 ± 0.103 ng/ml ($P < 0.001$) within 20 min. Thereafter it declined, initially rapidly then slowly to reach baseline (Fig. 1). Presumably, ischemia per se or possibly coupled with oxygenated free radicals generated during the reperfusion period may have caused the mucosal barrier to LPS to be breached, resulting in its leakage and the elevated LPS levels in the plasma. It has been shown that significant gut mucosal damage occurs not only during ischemia but also during the posthypotensive period in cats subjected to a partial occlusion of the SMA [33, 37]. The additional damage which occurs during the posthypotensive period has been attributed to the cytotoxic effects of oxygen-free radicals [33, 37]. However, during complete occlusion of the SMA, (as in this study) factors not dependent on the generation of oxygen-free radicals have been suggested to contribute to the damage [18, 29, 34]. In contrast to the control group, prophylaxis with the nonabsorbable antibiotic prevented the occurrence of the endotoxemia in the kanamycin group (Fig. 1). This is consistent with previous reports in which injection of kanamycin into rabbit intestines prevented shock due to burns [19] or SMA occlusion [41]. The active ingredients in "Kantrexil" suspension used here are kanamycin and kaolin. While kanamycin is bactericidal against the gram-negative bacteria which, incidently, make up a large proportion of gut flora, lack certain characteristics of classic LPS, and as such are less toxic [21, 35]. In view of this no attempts were made to either suppress the levels of the anaerobic gram-negatives in the gut or determine their count in the feces. Furthermore, prophylactic treatment with kanamycin only has been advocated and used for sterilization of the gut during surgery [1, 23]. Moreover, excessive and indiscriminate use of antimicrobes active against the anaerobic gram-negative gut flora may reduce or eliminate the interference phenomenon and encourage invasion by pathogenic bacteria [35, 42]. While the prophylactic treatment with kanamycin reduced the aerobic gram-negatives it at the same time promoted an overgrowth of gram-positives, particularly Lactobacilli, and possibly anaerobic gram-negatives as well. The Lactobacilli population may have adhered to the epithelium of the gastrointestinal tract and in doing so may have provided a protective effect [10, 40]. Furthermore, an overgrowth of the anaerobic gram-negatives may have been beneficial as well by controlling the colonization by pathogenic organisms [42]. Also, this pretreatment can be seen as a possible positive step toward the prevention of, or reduction of, an endotoxemia in either elective or emergency bowel surgery.

During the reperfusion period in the control group when the circulating plasma LPS concentration reached high values, no changes in MAP and heart rate were noticed (Fig. 1). The heart rates in the kanamycin pretreated group were, however, persistently higher than those in the control group. This could possibly be due to the anticholinergic properties of aminopentamide, one of the constituents of "Kantrexil" suspension. Similar findings on MAP and heart rate were observed by others in canines which survived after release of occlusion of the SMA [27, 31]. In contrast, in the case of rabbits the same insult caused progressive hypotension, hypovolemia, and death within 1 to 2 hr of reperfusion [19, 41]. In keeping with this, monkey and baboons were reported to be highly resistant to the acute actions of LPS unlike rabbits, horses, and man [48].

The present studies suggest that the origin of the increased plasma LPS concentration seen following ischemia of the gut is mainly derived from the gut. Furthermore, gut-derived LPS may be important in contributing to the severity of illness in a variety of shock states caused by traumatic injuries, bowel surgery, or heat stroke. The models described here may be of value in studying the possible benefits of other prophylactic antimicrobial

therapy for these potentially lethal conditions. Furthermore, clinical trials in patients could be done to also look at morbidity, length of hospital stay, and mortality.

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APPENDIX 4

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Changes in Lipopolysaccharide Concentrations in Hepatic Portal and Systemic Arterial Plasma During Intestinal Ischemia in Monkeys

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The time course of changes in the level of plasma lipopolysaccharides (LPS) in both the hepatic portal and the systemic arterial circulations, together with changes in cardiovascular parameters, was ascertained during a 1 hr occlusion of the superior mesenteric artery (SMA) in six primates. The LPS concentrations before occlusion of the SMA in the hepatic portal and systemic arterial circulation were 0.051 ± 0.009 and 0.065 ± 0.011 ng/ml, respectively (NS). At the end of the occlusion period, there was no significant increase in either the hepatic portal or systemic arterial plasma LPS concentrations. Immediately on removal of the occlusion, however, the LPS concentration in the *portal* plasma increased and peaked at 0.431 ± 0.124 ng/ml ($P < 0.01$) within 17.5 ± 1.71 min, whereas in the *systemic* arterial circulation the LPS concentration began to rise but only after a delay of approximately 10 min to peak at 0.287 ± 0.126 ng/ml ($P < 0.05$) within 32.5 ± 4.23 min of reperfusion. The mean arterial pressure (MAP) declined during the reperfusion period from 98.6 ± 6.89 to 65.0 ± 9.5 mm Hg ($P < 0.05$). The heart rate showed a small but not significant increase ($P > 0.2$) after about 80 min of reperfusion. These data indicate that the gut is the source of the increased plasma LPS concentration following occlusion of the SMA.

Key words: LPS, endotoxin, superior mesenteric artery occlusion, arterial pressure, plasma LPS

INTRODUCTION

Lipopolysaccharides (LPS), also known as endotoxins or pyrogen, are highly toxic components of the outer cell membrane of gram-negative bacteria and are always found in mammalian intestines. The permeability properties of the gut wall normally prevent the leakage of this toxic LPS into the circulation. Nevertheless, small amounts appear to migrate through and are cleared by the reticuloendothelial system (RES) [1–3]. However, if large amounts of LPS rapidly enter the circulation,

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it may overwhelm the detoxifying action of the RES and lead to hypotension, vascular collapse, renal failure, shock, and, if it is not promptly treated, death [3,4]. The intestinal mucosal barrier to endotoxin can be broken down by a wide variety of diseases and physical onslaught such as trauma, ischemia, hyperthermia, vasoactive agents, ionizing radiation, hypoxia, and hemorrhagic enteritis [4–12]. In man, the mortality as a result of endotoxemia is still at an unacceptably high rate of 30–80% [13,14].

Intestinal ischemia produced by the occlusion of the superior mesenteric artery (SMA) is a useful model for studying the pathophysiological changes associated with endotoxic shock. In recent studies using feline [5,6] and primate [7] SMA occlusion (SMAO) shock models, we determined the time course of changes in LPS concentration in the systemic arterial plasma. The elevated plasma LPS levels found in these studies have been suggested to originate from the gut. However, the precise quantitative temporal relationships between intestinal insult and hepatic portal and systemic arterial plasma LPS concentrations have not yet been reported. We have carried out such studies and furthermore relate LPS concentrations to alterations in heart rate and mean arterial pressure.

MATERIALS AND METHODS

Six adolescent monkeys (*Cercopithecus aethiops*) of either sex, with mean weights of 4.15 ± 0.42 kg, were used in this study. The animals were anesthetized with ketamine (10 mg kg^{-1}, i.m.), and anesthesia was maintained for the duration of the experiment with phenobarbitone sodium (10 mg kg^{-1}, i.v.) as required. Catheters were inserted into a femoral vein (for the administration of anesthetic) and into both femoral arteries. A Statham pressure transducer was connected to one of the arteries, and arterial blood pressure was recorded at 5–10 min intervals for the duration of the experiment using a Beckman recorder. The other femoral artery was used for removing 1 ml blood samples during the experiment. A thermorectal probe was inserted about 10 cm into the rectum, and the rectal temperature was monitored continuously during the experiment using a telethermometer (Yellow Springs Instrument 46-TUC). Using sterile surgical procedures, a midline incision was made in the linea alba, and the abdominal cavity was opened. The SMA was identified and cleared of surrounding tissues. A "Medican" 16-gauge catheter, connected to an extension tube, was inserted into the hepatic portal vein for removal of blood samples. The catheter was held in place using pursestring sutures. After the surgical procedures were complete, the animal was allowed a 30 min recovery period to attain steady-state conditions. Thereafter, two sets of blood samples (one from the femoral artery and the other from the hepatic portal vein) were taken at the onset and at the end of a 1 hr baseline period. The SMA was then clamped using a bulldog clamp for 1 hr, and blood samples were taken simultaneously from the femoral artery and portal vein at 20 min intervals. Blood samples were taken at 5–20 min intervals during the 2 hr reperfusion period. All blood samples were collected in heparinized, pyrogen-free plastic tubes and kept in melting ice. After each withdrawal of blood sample, an equal volume of normal, pyrogen-free saline was infused into the animal. At the end of the 4 hr experimental period, the animal was euthanized with phenobarbitone sodium.

The blood samples were centrifuged, and the plasma was removed, under sterile conditions, in a laminar flow hood and then stored at −20°C. The plasma samples

were analyzed within 7 days for LPS and anti-LPS IgG concentrations. The chromogenic substrate modification of the limulus amebocyte lysate (LAL) test was used for the determination of plasma LPS concentration [15,16]. The mean arterial pressure (MAP) was determined from the values of the systolic and diastolic pressures. The results are expressed as mean ± SEM and are compared using the paired Student's t test, ANOVA, and Duncan's multiple range test.

RESULTS

Because the results for all monkeys were very similar and statistical significance was reached, only six were used for ethical reasons.

Plasma LPS Concentration: Portal and Arterial Circulation

The LPS concentrations in the hepatic portal and systemic arterial plasma remained stable during the 1 hr baseline period (0.051 ± 0.009 ng/ml and 0.065 ± 0.011 ng/ml, respectively), rising slightly at the end of the occlusion period (0.055 ± 0.016 and 0.073 ± 0.018, respectively; N.S.) (Fig. 1). Upon release of the occlusion and reperfusion of the splanchnic circulation, there was a rapid rise (< 5 min) in LPS concentration in the hepatic portal plasma; in the systemic plasma, there was a

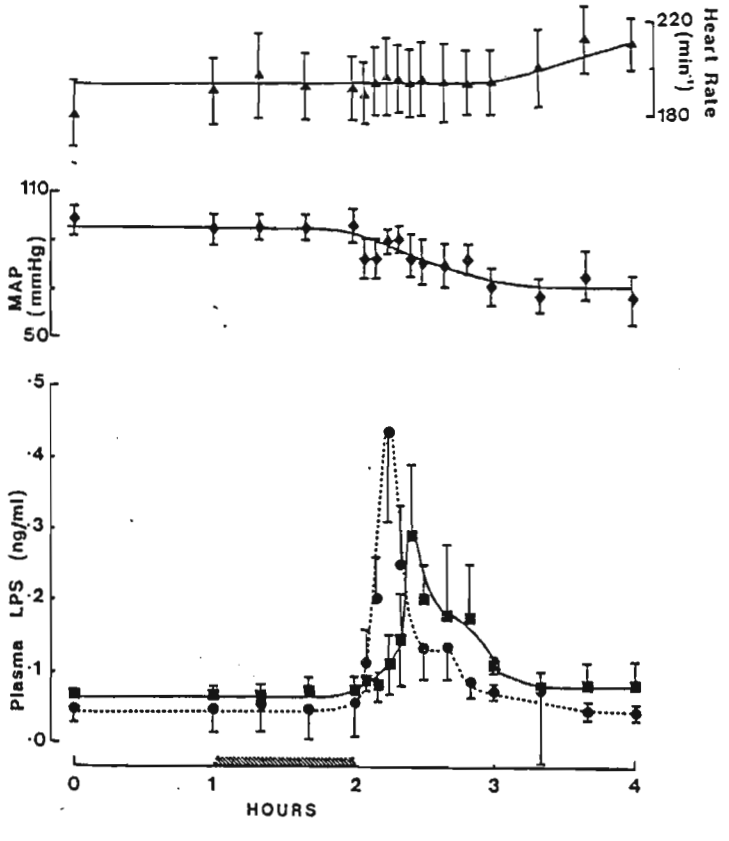

Fig. 1. LPS concentrations in hepatic portal (●) and systemic arterial (■) plasma, mean arterial pressure (MAP) (♦), and heart rate (▲) before, during, and after occlusion of the superior mesenteric artery for 1 hr (//////) in monkeys (n = 6). Values are mean ± SEM.

latency of about 10 min before a gradual rise. The portal plasma LPS concentration reached a peak of 0.431 ± 0.124 ng/ml ($P < 0.01$; ANOVA and Duncan's multiple range test) after 17.5 ± 1.71 min (range 15–25 min) reperfusion (Table I).

In the systemic circulation, the plasma LPS concentration began to increase only after a 10 min lag period to peak at 0.287 ± 0.126 ng/ml ($P < 0.05$; ANOVA and Duncan's multiple range test) (Fig. 1) after 32.5 ± 4.23 min (range 25–50 min) reperfusion (Table I) ($P < 0.01$ systemic-portal). That is, this peak occurred 10 min (n = 3) to 15 (n = 2) min after the peak was reached in the *portal* circulation in most animals, except in one, in which it occurred 30 min later.

After the peak, the portal plasma LPS concentration decreased, returning to baseline after about 80 min of reperfusion. On the other hand, in the systemic circulation, the plasma LPS level fell more slowly and returned to baseline after about 100 min reperfusion.

MAP

The MAP during the 1 hr baseline period remained more or less constant at 98.6 ± 6.89 mm Hg (Fig. 1). During the reperfusion period, the MAP declined and reached significance at 60 min to 71.3 ± 8.3 mm Hg ($P < 0.05$; ANOVA and Duncan's multiple range test). This decline in MAP continued to 65.0 ± 9.5 mm Hg ($P < 0.05$) until the termination of the experiment.

Heart Rate

There was a small but not significant rise in the mean heart rate commencing at about 80 min of reperfusion from 183 ± 13.2 min^{-1} to 211 ± 12.1 min^{-1} at the end of the reperfusion period.

DISCUSSION

Despite the use of intensive therapeutic agents for restoration after cardiovascular failure and the counteraction of other pathological processes, a mortality rate of up to 80% is still experienced in endotoxin and septic shock [13,14]. Abdominal surgery, extensive soft tissue trauma, large burns, hyperthermia, ionizing radiation, vasoactive agents, and certain viral and hemorrhagic enteric diseases are examples of conditions that can give rise to endotoxin shock [4–12].

It has been shown in this laboratory and in others that LPS can enter the systemic circulation within a few minutes of intestinal ischemia in cats and rabbits

TABLE I. Peak LPS Concentrations and Time to Peak LPS Concentrations in Hepatic Portal and Systemic Arterial Plasma Following 1 hr Occlusion of the SMA in Monkeys (n = 6) (mean ± SEM)

Portal LPS peak (ng/ml)	Systemic LPS peak (ng/ml)	Postreperfusion time to portal LPS peak (min)	Postreperfusion time to systemic LPS peak (min)
0.431 ± 0.124	0.287 ± 0.126	17.5 ± 1.71[a]	32.5 ± 4.23[b]*

[a]15 min, n = 4; 20 min, n = 1; 25 min, n = 1.
[b]25 min, n = 3; 30 min, n = 1; 40 min, n = 1; 50 min, n = 1.
*Time to systemic vs. portal LPS peaks $P < 0.01$.

[5,6,17]. Furthermore, observations in dogs have shown that LPS, but not whole bacteria, passes through the ischemic bowel in less than 30 min of occlusion [18]. The increased levels of plasma LPS concentrations in the systemic circulation previously seen during the occlusion period in the cat, rabbit, and dog, but not in monkey, were attributed to the leakage of LPS from the damaged gut into the peritoneal cavity and via collateral and/or lymphatic vessels into the systemic circulation [5,17–19]. However, the findings of this study and a previous study [7] show that in primates the concentration of plasma LPS in the systemic circulation increases only slightly, if at all (increasing, here, from 0.065 ± 0.011 to 0.073 ± 0.018 ng/ml), during the occlusion period. We are not in a position, at present, to advance a reason for this species difference. The possible causes, however, include that in primates, during the occlusion period, very little LPS enters the systemic circulation via collateral and/or lymphatic vessels or that the primate's RES is able to clear most of the LPS at a faster rate than those in cats, rabbits, and dogs. On the other hand, as was expected, the concentration of LPS in portal plasma remained unchanged during the occlusion period.

When the clamp was removed from the SMA, and oxygenated blood once again flowed through the gut, it "rinsed" out the LPS that had diffused through the gut wall. There was an immediate and a rapid rise in portal plasma LPS concentration, reaching a peak at about 17 min of reperfusion. Thereafter, it declined, initially rapidly and then gradually, to reach baseline level after about 80 min of reperfusion. At present, the role of oxygen free radical generation in damaging the barrier to LPS in this model is not clear [20–22]. On the other hand, in the systemic arterial plasma, the LPS concentration only *began* to rise after a latency of about 10 min. The liver's RES removed much of the early LPS in the portal circulation. This latency in rise of systemic LPS concentration represents, at least, the time required for the blood to perfuse the liver and the time required to overwhelm the liver's RES to detoxify the LPS present in the reperfused blood. The increase in systemic arterial plasma LPS concentration is, therefore, the "spillover" from elevated portal LPS blood levels that temporarily overwhelmed the liver RES. There may be an additional contribution of LPS from the thoracic duct [23], but this remains to be investigated.

After reperfusion, the "leaky" intestinal wall presumably "self-repaired," leading to a reduced permeability to LPS. This, coupled with an improved RES function, resulted in a subsequent decline in plasma LPS concentration in the systemic circulation.

Cuevas and Fine [19], by the relatively insensitive LAL "clot" test, also found that the portal venous plasma contained little or no LPS 1 hr after occlusion of the SMA in rabbits, whereas the plasma taken at the same time from the systemic venous circulation had elevated levels. They attributed the latter increase to leakage of LPS from the damaged gut via the peritoneal cavity. However, in contrast to the findings in this study, Cuevas and Fine reported that, 60 min after reperfusion, LPS levels in the plasma taken from both the portal and systemic venous blood had risen to high levels in the animals that had been alive.

Both the opening of the abdominal cavity, to remove the clamp from the SMA and the peaking LPS concentration in the systemic arterial plasma were accompanied by a decline in the MAP, which continued until termination of the experiment. We believe that this decline was due, at least in part, to the toxic action initiated by the previously elevated plasma LPS concentration [24,25]. Recent studies show that

most, if not all, of the major manifestations of endotoxin shock are due to the production and release of cachectin from macrophages following stimulation by LPS [26,27]. Other groups found that plasma cachectin concentrations rose 60–90 min after i.v. administration of endotoxin to human volunteers [27]. Consistent with our study, these elevations of plasma cachectin concentrations were associated with "classic" endotoxic responses, such as chills, headaches, myalgias, and nausea. In conclusion, the model described here may be of value in studying the possible benefits of antimicrobial and LPS-specific therapy administered by various routes for endotoxin-mediated diseases.

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In conducting the experiments described in this report the investigators adhered to the NIH guidelines for the use of experimental animals.

APPENDIX 5

Time Course of Endotoxemia and Cardiovascular Changes in Heat-Stressed Primates

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GATHIRAM P, GAFFIN SL, BROCK-UTNE JG, WELLS MT. *Time course of endotoxemia and cardiovascular changes in heat-stressed primates.* Aviat. Space Environ. Med. 1987; 58:1071–4.

Heat stress causes a marked reduction in splanchnic blood flow in order to compensate for the increased flow to the skin. Splanchnic ischemia causes a leakage of endotoxins from the gut lumen into the portal circulation and, especially in the presence of a compromised reticuloendothelial system, may cause severe systemic endotoxemia. Since many of the pathological features of heat stroke are similar to the shock state produced by LPS, we examined whether heat-stress causes endotoxemia. Five anesthetized monkeys were subjected to an environmental temperature of 41° ± 0.3°C and relative humidity of 100%, until death. Rectal temperatures were recorded continuously, blood pressure and ECG were recorded at 5-min intervals, and arterial blood samples were taken at 15–30 min intervals. A decline in mean arterial pressure and rapid rise in heart rate occurred at about 42°C. Plasma LPS remained at 0.071 ± 0.006 ng·ml^{-1} until a rectal temperature of ±42°C. Thereafter, it increased slowly until beyond 43°C when it rose rapidly to 0.347 ± 0.024 prior to death. Endotoxemia may have been a contributing factor in the pathogenesis of heat stroke. If so, then the use of anti-LPS antibodies may be expected to be beneficial.

HEAT STROKE, a serious clinical problem encountered in sports, military, occupational, and civilian medicine, is caused by extreme elevation of body temperature and is prevalent in hot, humid climates (11,31,35,41,44). Without prompt recognition and immediate treatment, mortality can be as high as 80% (1,11,41,45) and, at times, even with optimum therapy, heat stroke may cause death or permanent damage (16).

It is well known that the primary cardiovascular adjustment to heat stress is an increase in skin blood flow to promote heat loss and reduce the rate of heat gain. Current experimental evidence in man and animals shows that the increased skin blood flow is accompanied by a concomitant, substantial fall in splanchnic blood flow in order to maintain a normal or increased blood pressure (3,27,38,39,44).

The importance of this finding may be that a seriously reduced intestinal blood flow can damage the permeability properties of the gut wall. The lumen of the intestines always contains large amounts of gram-negative bacteria and its highly toxic cell wall component lipopolysaccharide (LPS). Insufficient intestinal blood flow permits LPS to leak at high rates into the portal circulation with detrimental effects (12,17,21,23,26,37). Furthermore, if the reticuloendothelial system (RES) is also compromised, then the systemic LPS will reach higher levels, persist in the circulation longer and, if in sufficient concentration, may lead to vascular collapse, shock and death (13,17). Some of the pathological features of heat stroke seem similar to gram-negative bacteremia and, therefore, also possibly endotoxemia (6,8,18,22,40). Furthermore, endotoxins have been occasionally detected in the plasma of patients and experimental animals with heat stroke (5,8,25,34). However, no systematic studies have, to our knowledge, been undertaken to correlate cardiovascular parameters with plasma LPS levels during experimental heat-stress.

This study was undertaken to determine the time course of systemic endotoxemia in heat-stressed primates, and to correlate the changes in plasma LPS levels with changes in heart rate and mean arterial pressure (MAP).

MATERIALS AND METHODS

Five adolescent monkeys (Cercopithecus aethiops) of both sexes, with a mean weight of 3.6 kg (2.5–5.9 kg) were

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used in this study. All the animals were fasted during the night preceding the experiment, then anesthetised with ketamine (10 mg•kg^{-1}, i.m.). The anesthesia was maintained throughout the experiment with i.v. ketamine as required.

Rectal temperature (T_r) was recorded continuously using a probe inserted about 10 cm into the rectum and a telethermometer (Yellow Springs Instrument 46-TUC) connected to a chart recorder. The femoral areas were cleaned with topical chlorohexidine gluconate. Catheters were inserted into a femoral vein for maintenance of anesthesia and a femoral artery for recording arterial pressure and collecting blood samples.

Arterial pressure was measured with a B188 Statham transducer. Electrocardiograms were recorded from subcutaneous needle electrodes placed across the chest. A Honeywell CM130 patient monitor was used to record the arterial pressure and the electrocardiogram at 5-min intervals. Heart rate, systolic, diastolic and mean arterial pressures were calculated electronically and presented as a printout with the blood pressure and ECG recordings. The MAP was measured automatically by integrating the area under the arterial pressure curve.

A 1-ml arterial blood sample for LPS analysis was taken before heat-stress and subsequently, at 15–30 min intervals during the heat-stressed period. Blood samples were collected into heparinized sterile, pyrogen-free plastic tubes (Falcon) and stored on melting ice during the experimental period. An equal volume of pyrogen-free saline was administered back into the monkeys at each blood collection. The blood was centrifuged and the plasma removed under sterile conditions in a laminar flow hood, placed into plastic tubes, and frozen at −20°C. The plasma was analyzed for LPS within 1 week. The chromogenic substrate modification of the Limulus amebocyte lysate (LAL) technique was employed to determine plasma LPS concentration (10,20) (M A Bioproducts). We previously found a 95.1% recovery of LPS from spiked plasma samples using this method, calibrating the standard curve with LPS prepared from E. coli 0111:B4 (Wells *et al.*, submitted).

A forced draft baby incubator set at 41.0° ± 0.3°C was used as a heat-stress chamber. The relative humidity in the incubator was maintained close to 100% by means of open trays containing water into which bibulous paper was immersed, and was monitored continuously with a Weather Measure Corporation HM111 relative humidity indicator.

Experimental Procedure

After anesthesia, the femoral vein and artery were catheterised and the temperature probe inserted into the rectum. The catheter to the femoral artery was connected to the pressure transducer. ECG electrodes were inserted and fastened to the skin. The animals were allowed about 30 min to attain steady-state conditions at room temperature before commencing the experiment. Thereafter the animal was placed on a wooden board in the incubator and the extremities fixed loosely by the ankles and wrists to the board. Approximately 10 ml of water was sprayed by nebulizer into the pre-equilibrated incubator in order to have a rapid return to 100% relative humidity. The animals remained in the incubator until death. Blood samples were withdrawn soon after the animals were placed in the incubator and thereafter at 15–30 min intervals until their demise. The data are presented as mean and ± S.E.M. and compared by Students *t* test.

RESULTS

The general shapes of the curves of T_r, heart rate, arterial pressures (systolic, diastolic, and mean) and plasma LPS were similar in all five monkeys, but the time to death varied from 240–497 min in the animals (mean = 372.8 min ± 43.8 S.E.M.). Fig. 1 shows typical results of changes in T_r, cardiovascular parameters, and plasma LPS levels in one of the monkeys. Fig. 2 summarizes the changes in cardiovascular parameters and plasma LPS levels of all five monkeys relative to their T_r.

Cardiovascular Responses

Initially, at a T_r of 37°C, the MAP was 58.2 ± 1.7 mm Hg. As the T_r rose, the MAP increased steadily to 96.2 ± 9.0 mm Hg at a T_r of 41.5°C, except for a small but significant "kink" at 38°C. As temperatures rose above 41.5°C the MAP declined to 47.8 ± 10.5 mm Hg at a T_r of 43.5°C. This was followed by a consistent rise to 62.8 ± 8.4 mm Hg at a T_r of 44°C, corresponding to a peak in heart rate, before death at 44.5°C. This final phase of falling MAP corresponded with a rapid decline in heart rate and a more-rapid increase in plasma LPS level.

The mean heart rate for the monkeys at 37°C was 117 ± 16 b•min^{-1} and it increased gradually to 164 ± 13 b•min^{-1} at a T_r of 41.5°C. As temperatures continued to rise, there was a rapid increase in heart rate peaking at 315 ± 6.7 b•min^{-1} just prior to death.

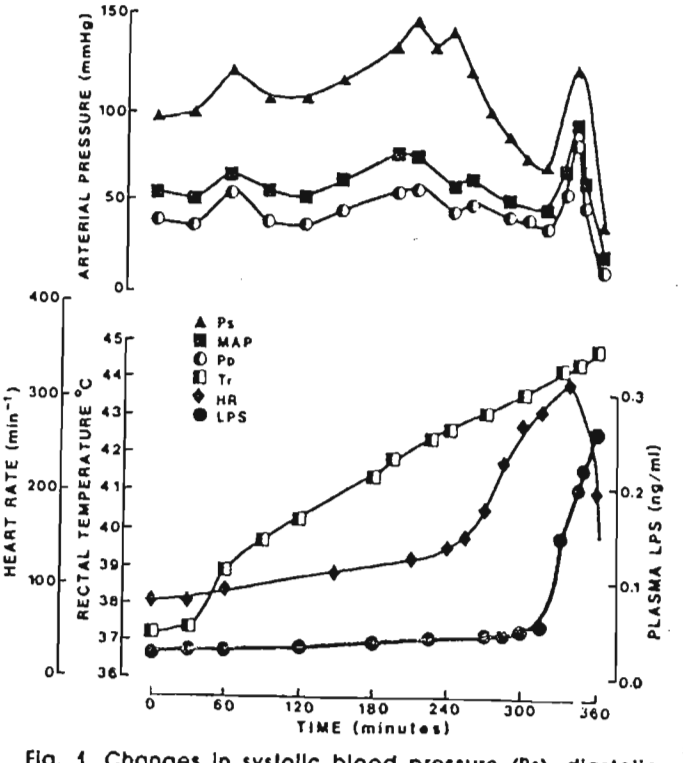

Fig. 1. Changes in systolic blood pressure (Ps), diastolic blood pressure (Pd), mean arterial pressure (MAP), rectal temperature (T_r), heart rate (HR), and plasma LPS concentrations (LPS) in a monkey exposed to an environmental temperature of 41.0 ± 0.3°C and 100% relative humidity. Death occurred within 5 min of the last point.

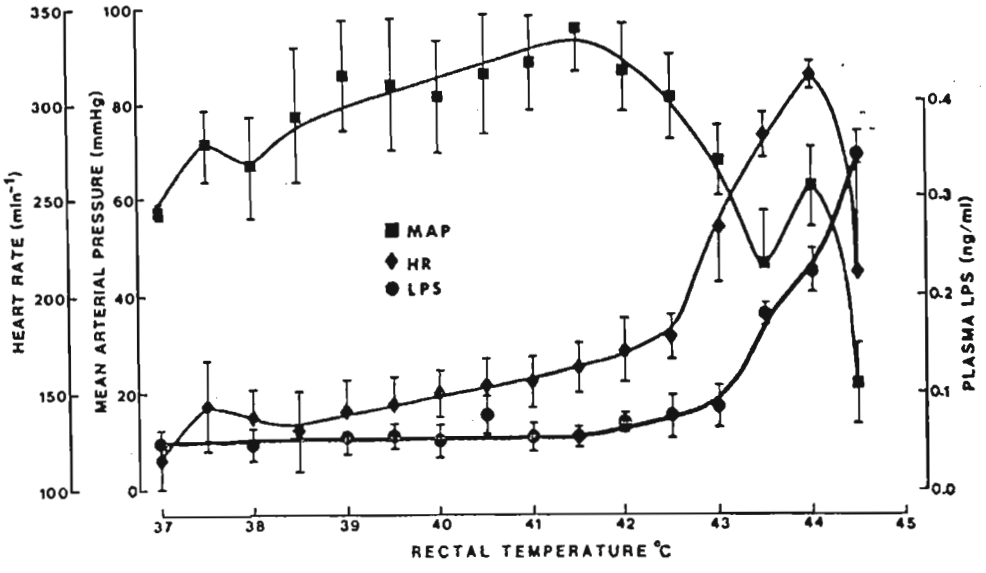

Fig. 2. Mean arterial pressure (MAP), heart rate (HR), and plasma LPS (LPS) concentrations at progressively elevated rectal temperatures in monkeys exposed to an environmental temperature of 41.0° ± 0.3°C and 100% relative humidity. The values are mean ± S.D. of five monkeys.

Plasma LPS

The plasma LPS remained at stable and low levels (mean = 0.071 ± 0.006 ng·ml^{-1}) until a T_r of 41.5°C was reached. Thereafter, there was a gradual increase in plasma LPS concentration until 43°C, whereupon the LPS concentration rapidly rose to reach 0.347 ± 0.024, just before death at a T_r of 44.16 ± 0.22°C. When the rectal temperature of all animals rose above approximately 42.0°C, anesthesia was no longer deemed necessary.

DISCUSSION

The changes in cardiovascular parameters (systolic, diastolic and mean arterial pressures) observed in this study were similar to those previously reported in the literature (7,11,19,24,30,35,45) for patients and experimental animals during heat-stress.

Heat stroke is characterised by hyperthermia, sudden loss of consciousness, circulatory failure, and evidence of widespread tissue damage and failure of hemostasis (7,9,32,36,39,41–43). Many of these features parallel the findings associated with the shock state of endotoxemia (6,8,18,22,25).

There have been a number of suggestions that endotoxemia is also involved in the pathogenesis of heat stroke (5,6,8,18,22,25,34). To our knowledge, no detailed investigations relating changes in plasma LPS with cardiac parameters have previously been undertaken in heat experiments.

It is well described that hypoxia leads to a temporary reduction in splanchnic blood flow followed by vasodilation (21). The reduced splanchnic blood flow leads to an increased vascular permeability with leakage of endotoxins into the systemic circulation (12,17,26,37).

Hyperthermia also leads to a decrease in splanchnic blood flow in both animals and man (3,27,38,39,44). It is, therefore, of considerable importance that, in this study, the plasma LPS rose when T_r was about 42°C; markedly increased levels were found at a T_r above 43°C. Initially, the LPS leaving the gut would seem to be detoxified by the liver (28, 33), thereby accounting for the initial slow increase in plasma LPS observed at a T_r of about 42°C.

Other studies have indicated liver tissue damage at 42–43°C (4,9,29,40,43). The rapid rise in systemic LPS in this study was found at a T_r of about 43°C and might, therefore, be due to increasing rates of entry of LPS into the portal circulation overwhelming an already compromised liver which might no longer be able to remove LPS effectively from the portal circulation. Liver damaged by heat stroke has been described as morphologically similar to liver damaged from diseases associated with circulating bacteria and LPS (40).

Other studies could be interpreted in a manner consistent with the concept that gut-derived LPS is responsible for part of the pathophysiology of heat stroke. A high concentration of LPS has been reported in a heat stroke patient whose condition was complicated by shock, septicemia, anuria, and consumption coagulopathy (25). A reduction of intestinal stool and bacterial contents with antibiotics, and enemas has also been seen to protect dogs against heat stroke (6). Rabbits pretreated with oral antibiotics and then exposed to heat had a slower rise in rectal temperature and a reduced prevalence of endotoxemia than control rabbits (5).

A review of the literature shows that most investigations into the pathogenesis of heat stroke have been carried out to a T_r of about 41°C. Since we show here that the major pathophysiological changes during heat stroke occur around a T_r of 43°C, and may be related to an endotoxemia, we feel that it is not possible to extrapolate the observed changes at 41°C to the temperature during a heat stroke episode.

From this study alone we can only conclude that endotoxemia may be a contributing factor in the pathogenesis of heat stroke. However, it is possible that LPS may be simply a marker for other toxins released at the same time from a damaged gut. Further studies are in progress where the possible beneficial effect of prophylactic anti-LPS hyperimmune plasma for heat stroke will be investigated. Core temperatures of long-distance runners, football and soccer players, and other sportsmen may rise to 42°C (46) without the development of fulminating heat stroke. However, they may have developed a low level of systemic endotoxemia which may have affected their performance.

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APPENDIX 6

Prophylactic Corticosteroid Suppresses Endotoxemia in Heat-Stressed Primates

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GATHIRAM P, GAFFIN SL, BROCK-UTNE JG, WELLS MT. *Prophylactic cortiscosteroid suppresses endotoxemia in heart-stressed primates.* Aviat. Space Environ. Med. 1988;59:142–5.

We previously found that lipopolysaccharides (LPS) leak from the gut lumen into the hepatic portal vein during heat stroke. Furthermore, we found that prophylactic corticosteroid administration could prevent a rise in plasma LPS concentration in superior mesenteric artery occlusion shock. In this study, we found that treatment prior to heat-stress with corticosteroids could prevent any rise in plasma LPS concentration in heat-stressed primates. Two groups of primates, one of which received a prophylactic dose of methylprednisolone sodium succinate (MPSS) (n = 4) were subjected to heat-stress (41 ± 0.3°C). Their arterial blood pressure, heart rate and rectal temperature (T_r) were continuously recorded. In the untreated control group (n = 8), the plasma LPS concentration tended to increase slowly at a T_r of 41.5°C from an initial 0.06 ± 0.013 ng•ml^{-1}. Above a T_r of 43°C, the plasma LPS level rose rapidly until at a T_r of 44.4 ± 0.1°C, the mean LPS level was 0.315 ± 0.03 ng•ml^{-1} ($p < 0.001$). Prophylactic treatment with MPSS suppressed the increase in plasma LPS levels to 0.066 ± 0.01 ng•ml^{-1} before heat-stress and 0.03 ± 0.01 ng•ml^{-1} at T_r 44.4°C just before primate demise. The mean arterial pressure of the control group was lower than the treated group for any given T_r; between T_r 42–43° this difference was significant ($p < 0.05$). Moreover, the cardiovascular parameters began to deteriorate at a lower T_r in the control group.

DESPITE THE VAST amount of published data, the mechanism by which death occurs during heat-stroke is not well understood (28). Knochel (19) emphasized that hard physical work in a hot environment could lead to a serious deficit of effective arterial blood volume, and profound shock would occur were it not for the intense splanchnic vasoconstriction. This reduced blood flow to the gut, together with the high core temperature, may damage the permeability properties of the gut mucosa and cause elevated leakage of endotoxins (lipopolysaccharides, LPS) into the hepatic portal circulation. When small amounts of LPS enter the portal circulation, some form complexes with plasma high-density lipoproteins (10), while the rest are cleared by the reticuloendothelial system (RES) (9). However, if the RES is compromised, or when excess amounts of LPS enter the portal circulation, then the concentration of LPS in the systemic circulation tends to rise (9).

Elevated circulating LPS concentration causes hypotension and the release of vasoactive mediators, such as catecholamines (24), and vasoactive intestinal polypeptide (VIP) (5). LPS also leads to disseminated intravascular coagulation (DIC) (21) and alterations in metabolism (18,25). Many of these pathophysiological changes occur during heat stroke as well (20,28). Moreover, increased concentrations of LPS have been detected in the circulating blood during heat stroke (13,14).

A large dose of corticosteroids is commonly used to treat septic and endotoxic shock, although its clinical effectiveness remains controversial (4,15–17,23,26,27). The precise mechanism of steroid action in shock remains unclear (27). We recently found that prophylactic methylprednisolone sodium succinate (MPSS) prevented a rise in plasma LPS concentration in a feline superior mesenteric artery occlusion (SMAO) shock model (12). The experiments were designed to investigate whether prophylactic MPSS can prevent a rise in plasma LPS concentrations caused by heat stroke in primates.

MATERIALS AND METHODS

Studied were 12 male and female adolescent monkeys (Cercopithecus aethiops), having a mean weight of 5.16 ± 0.41 kg. They were randomly divided into a control (n = 8) and a steroid (n = 4) group. The subjects were anesthetised with 10 mg•kg^{-1} I.M. ketamine, and anesthesia was maintained throughout the experimental period as required.

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Rectal temperature (T_r) was recorded continuously during the duration of the experiment using a rectal probe inserted approximately 10 cm into the rectum, and a telethermometer (Yellow Springs Instrument 46—TUC) connected to a chart recorder. Following anesthesia, a femoral vein and both femoral arteries were catheterised. A Statham transducer (B188) was then connected to one of the femoral arteries. A Honeywell CM130 patient monitor was used to monitor continuously and record at 5-min intervals the arterial blood pressure for the duration of the experiment. MPSS at 30 mg·kg^{-1} was infused via a femoral vein into the subjects in the steroid group and an equivalent volume of normal saline into the subjects in the control group. Each animal was allowed about 30 min after surgery to attain steady-state conditions at room temperature. Thereafter a 1-ml arterial blood sample for LPS analysis was collected in a heparinized, pyrogen-free plastic tube, and base line recordings of blood pressure and T_r were taken. An equal volume of saline was reinfused. The animal was then placed in a forced-draft baby incubator, with the temperature maintained at 41 ± 0.3°C and the relative humidity at 100%. Arterial blood samples of 1 ml were collected at 15–30 min intervals until the demise of the animal. In all, 12–13 samples were taken. All the blood samples collected were stored in melted ice.

At the termination of the experiment, the blood samples were centrifuged, and the plasma was removed under sterile conditions in a laminar flow hood and placed into plastic tubes. The samples were analyzed for LPS either immediately or stored at −20°C and analyzed within a week.

The chromogenic substrate modification of the Limulus amebocyte lysate (LAL) technique (M.A. Bioproducts) (11) was used to determine the concentration of LPS in the plasma samples.

A detailed description of the methodology used in this experiment has been described elsewhere (13). The data are presented as means and ± S.E.M. and compared by Student's *t* test, analysis of variance (ANOVA), and Duncan's multiple range test (13).

RESULTS

Plasma LPS: Fig. 1 shows the changes in plasma LPS concentrations in the control and steroid groups. In the control group, the plasma LPS level remained at 0.06 ± 0.013 ng·ml^{-1} until a T_r of 41.5°C was reached. Thereafter, the plasma LPS concentration increased gradually until 43°C, when there was a rapid rise to 0.315 ± 0.03 ng·ml^{-1} at a T_r of 44.4 ± 0.1°C ($p < 0.01$). In contrast, the plasma LPS concentration in the steroid group showed no increase during the entire heat-stress period (Fig. 1). Before heat-stress, the plasma LPS concentration in the latter group was 0.066 ± 0.01 ng·ml^{-1}, and just before death the level was 0.03 ± 0.01 ng·ml^{-1}. Moreover, the animals in the steroid group succumbed at a significantly higher T_r compared to controls (44.9 ± 0.14 vs. 44.4 ± 0.1°C) ($p < 0.02$).

Cardiovascular responses: As shown in Fig. 1, the MAP in the control group was 63.8 ± 4.0 mm Hg at a T_r of 37.0°C. As the T_r increased beyond 37°C, the MAP rose to 89.3 ± 8.4 mm Hg at a T_r of 41°C. The MAP began to decline at temperatures greater than 41°C to reach 46.3 ± 7.7 mm Hg at 44°C. The MAP in the steroid group was higher than the control group at all rectal temperatures, and

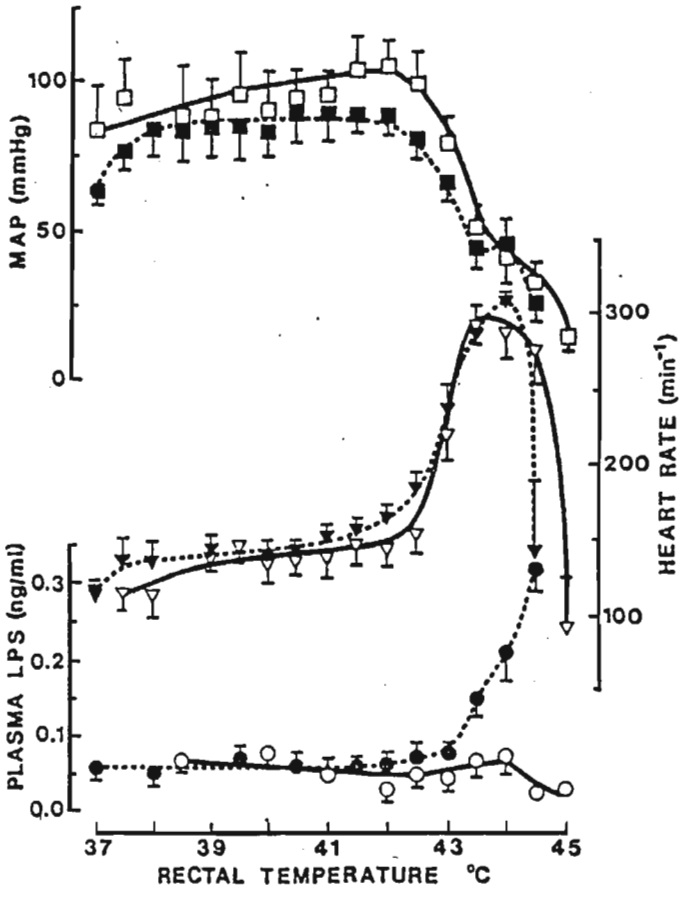

Fig. 1. Changes in plasma LPS concentration (circles), heart rate (triangles), and mean arterial blood pressure (squares) at progressively increasing T_r in control monkeys (broken line) and in monkeys pretreated with MPSS (solid lines) during heat-stress in an environmental temperature of 41°C ± 0.3°C and 100% relative humidity.

between T_r 42–43°C this difference was significant ($p < 0.05$). In the steroid group, the MAP increased steadily from 83.5 ± 15.5 mm Hg at 37°C to 105 ± 8.4 mm Hg at 42°C, and thereafter declined to 37.0 ± 7.4 mm Hg at 44.5°C.

The HR in the control group increased gradually from 112 ± 10.9 beats·min^{-1} at 37°C to 141 ± 8 beats·min^{-1} at 40.5°C (Fig. 1). The heart rate showed a more rapid rise as the T_r increased beyond 40.5°C, to reach a peak of 310 ± 5 beats·min^{-1} just before the demise of the animals. The changes in heart rate in the steroid group showed a similar trend to the control group as the T_r increased, except that the heart rates at any given T_r were lower than the control group. The steroid group HR increased from 113 ± 1.5 at 37°C to a maximum of 292 ± 14.1 beats·min^{-1} at 43.5°C. When the rectal temperature of all animals rose above approximately 42.0°C, anesthesia was no longer deemed necessary.

DISCUSSION

In the control group, the plasma LPS concentration began to rise at a T_r of about 41°C. This increase was initially gradual until a T_r of about 43.5°C, when there was a rapid rise to 0.315 ± 0.03 ng·ml^{-1} (Fig. 1) ($p < 0.01$). These changes in plasma LPS concentrations were similar to those of a previous, similar study (13).

In contrast, prophylactic MPSS prevented any rise in plasma LPS concentration in the steroid group during the entire heat-stress period. A similar effect of prophylactic MPSS was also noticed in a feline SMAO shock model (12). The mechanism of action of MPSS in suppressing plasma LPS levels in both these shock models is not known. However, MPSS exerts a stabilizing effect on lysosomal (7) and other cell membranes (17), so MPSS could be protecting the mucosa of the gut and, hence, preventing a leakage of LPS into the portal vein. Corticosteroids appeared to protect dogs from intestinal hemorrhagic necrosis following *E. coli* infusion (16). Corticosteroids have also been found to transiently improve blood flow to the splanchnic region including the liver (30), to protect the small intestinal capillary bed in endotoxin shock (23), and to protect the liver during ischemia (6). We suggest that corticosteroids, by protecting the gut mucosal membrane and increasing splanchnic and liver blood flow, reduce the "leakage" of LPS into the hepatic portal circulation and thereby increase the efficiency of the liver in removing LPS from the blood during heat stroke. This effect of MPSS on plasma LPS levels could also explain its sometime-beneficial effect in shock.

The changes in cardiovascular variables in the control group of this study were similar to those observed in our previous study (13) and to those noticed by other investigators (20,28). The MAP in both the control and the steroid groups increased as the T_r rose to 41–42°C. Thereafter the MAP began to drop rapidly as the T_r increased beyond 41°C in the control group and 42°C in the steroid group. The mean arterial pressures in the steroid group were higher than the control group, though not highly significant except between T_r 42–43°C ($p < 0.05$), and appeared more stable than the control group.

On the other hand, the heart rates in the steroid group were lower than the control group for any given T_r until 43.5°C. Moreover, the HR in the steroids peaked at a slightly lower T_r than in the controls (43.62 vs. 43.88°C).

Endotoxins cause hypotension in septic and nonseptic shock by initiating the release of vasoactive mediators, such as catecholamines (24) and VIP (5). Furthermore, this and previous studies (13) have shown conclusively that plasma LPS levels increase during heat stress. Certain corticosteroids, on the other hand, are known to increase cardiac contractility (1) and cardiac output (26) in normal subjects and patients in shock, to inhibit the vasoconstrictor action of endogenous vasoactive mediators (2), to inhibit the release of epinephrine and norepinephrine (24), to cause vasodilatation of peripheral vessels, and to augment the systemic arterial pressure (22) during shock. In addition, certain steroids have been shown to have a positive inotropic action on isolated cat cardiac muscles (29). Therefore, the differential behaviour of control and steroid groups could be related to the "leakage" of LPS into the hepatic portal blood in the control group or to MPSS in the steroid group.

However, in a recent study we observed that the MAP in heat-stressed primates pretreated with nonabsorbable antibiotics behaved in a similar fashion to those of the steroid group in this study (Gathiram *et al.*, unpublished). On the other hand, cardiovascular parameters also deteriorated in the steroid group but at a higher T_r (42°C). It is likely that the high core temperature results in multiple tissue damage and cardiovascular collapse (28) over and above that due to LPS. The administration of corticosteroids has not been found to improve the survival of heat stroke victims (3,8). The probable reasons for this could be inappropriate dosage and time of administration (15,27), especially the latter since, in most of these cases, corticosteroid therapy was used only as a last resort.

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In conducting the experiments described in this report, the investigators adhered to the NIH guidelines for the use of experimental animals.

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APPENDIX 7

Prevention of endotoxaemia by non-absorbable antibiotics in heat stress

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SUMMARY Four anaesthetised monkeys were given oral kanamycin (15 mg 1 kg 12 hourly) over five consecutive days before being heat stressed. Four other anaesthetised monkeys served as controls. The plasma lipopolysaccharide concentration in control primates increased initially from 0·044 (SEM 0·004) ng/ml to 0·062 (0·006) ng/ml as the rectal temperature increased from 37·5 to 39·5°C. A second increase in lipopolysaccharides started at 42°C and reached 0·308 (0·038) ng/ml ($p < 0·01$) at 44·5°C. Before heat stress the plasma lipopolysaccharide concentration in the primates who had been pretreated with kanamycin was 0·007 (0·006) ng/ml, and despite heating these animals to 44·5°C no increase in plasma lipopolysaccharide concentrations were seen in this group. The cardiovascular variable during heat stress were more unstable in the control group and began to deteriorate at a lower temperature than in the group receiving antibiotic. These data suggest that the increased plasma lipopolysaccharide concentration during heat stress originates mainly from the gut.

Recent evidence shows that endotoxins (lipopolysaccharides or LPS), the highly toxic components of the outer cell membrane of Gram negative bacteria, may have a role in heat stroke pathophysiology.[1-6] The lumen of the mammalian gut always contains Gram negative bacteria, and hence LPS. Normally, the gut wall is impermeable or slightly permeable to LPS. Small amounts of LPS that may leak into the portal circulation are detoxified by the reticuloendothelial system.[7] Damage to the gut wall by ischaemia, trauma, hyperthermia, vasoactive agents, ionising radiation, hypoxia, and viral gastroenteritis, however, enables LPS to leak rapidly into the portal and systemic circulations.[3 8-13] Should the reticuloendothelial system of the liver and spleen become overwhelmed by the rapidly rising blood LPS concentrations or should the reticuloendothelial system function be inadequate to remove the LPS, then the plasma LPS concentration in the systemic circulation would rise and persist in the circulation, eventually causing vascular collapse, shock, and death.[9 14]

In a previous study we determined the time course of changes in circulating plasma LPS concentrations and cardiovascular variables in heat stressed monkeys.[1] Furthermore, high concentrations of plasma LPS have been detected in a few cases of fatal heat stroke.[2 5] In addition, reduction of gut flora with antibiotics has been found to increase an 18 hour survival in heat stressed dogs[4] and to reduce the prevalence of endotoxaemia in heat stressed rabbits.[6] We believe that during heat stress the reduced blood flow to the visceral regions[15 16] coupled with the high core temperature leads to damage to the permeability properties of the gut mucosa and results in leakage of LPS into the portal and lymphatic circulation.

In this study an attempt has been made to show that the increase in LPS that occurs during heat stress is intestinal. The amount of endogenous Gram negative flora and hence LPS in the gut can be reduced by the oral administration of a non-absorbable antibiotic before heat stress.

Material and methods

Eight monkeys (Cercopithecus aethiops) of either sex, weighing between 2·7 and 6·8 kg were used. They were anaesthetised with ketamine (10 mg/kg, given intramuscularly). Thereafter, incremental intravenous bolus doses (5–10 mg/kg intravenously) of ketamine were used to maintain anaesthesia. The animals were divided into two groups. Four of them received kanamycin (15 mg/kg) (Kantrexil suspension, the B-M Group Ltd) every 12 hours over five consecutive days via a nasogastric tube prior to heat stress; four others served as controls.

After cleansing both the femoral areas with chlor-

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hexidine gluconate catheters were introduced into both femoral arteries and a peripheral vein. One of the arterial catheters was connected to a B188 Statham transducer for recording of blood pressure, while blood samples were collected at various times from the opposite catheter. Only equipment without pyrogen was used, and each heparinised plastic tube into which the blood was collected was stored on ice until centrifuged for LPS and anti-LPS IgG analyses. After each withdrawal of blood sample an equal volume of physiological saline was reinfused. Arterial pressure was recorded using a Honeywell CM 130 patient monitor system at five minute intervals during the experiment. Heart rate, systolic, diastolic, and mean arterial pressures were calculated electronically and presented as a printout with the blood pressure recording. The mean arterial pressure (MAP) was measured automatically from integration of the area under the arterial pressure curve.

Rectal temperatures were recorded continuously using a rectal probe inserted about 10 cm into the rectum, and a telethermometer (Yellow Springs Instrument 46-TUC) connected to a chart recorder.

After surgery a 30 minute stabilisation period was allowed and then baseline rectal temperature, arterial blood pressures, and room temperature were recorded, and 1 ml blood sample for LPS and anti-LPS analyses and 3 ml for serum enzyme analyses were collected in heparinised and non-heparinised tubes, respectively. Each animal was then positioned in a forced draft incubator, where the environmental temperature was maintained at 41·0 (0·3)°C and relative humidity close to 100%. During heat stress, blood samples for LPS and anti-LPS IgG analyses were taken at 15–30 minute intervals and for serum enzyme analyses when the rectal temperature reached 40, 42, and 43°C.

The blood samples for LPS and anti-LPS IgG assay were stored in ice until the end of each experiment. After centrifugation of the samples the plasma was removed under sterile conditions in a laminar flow hood. The samples were either analysed immediately or stored at −20°C for up to a week until analysed. Plasma LPS concentrations were determined using the chromogenic substrate modification of the limulus amebocyte lysate (LAL) technique (MA Bioproducts).[17,18] We previously found a 95·1% recovery from spiked plasma samples using this method, calibrating the standard curve with LPS prepared from *Escherichia coli* 0111:B4.[19] Relative plasma anti-LPS IgG concentrations were determined using an enzyme linked immunoabsorbent assay (ELISA).[20] The concentration of anti-LPS IgG at a rectal temperature of 37·5°C for each animal was taken as 100%. A detailed description of the procedure adopted has already been described.[1] Boehringer kits were used for determining the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), L-γ-glutamyl-transferase, alkaline phosphatase and the concentrations of bilirubin, albumin, and total protein in the serum.

Rectal swabs for bacteriological examination were taken from all animals after anaesthesia but before experimental heat stress. These sterile rectal swabs were initially moistened with sterile saline and care was taken to avoid perianal contamination. After storing in nutrient broth the swabs were individually plated out on deoxycholate citrate (DCA) MacConkey, and nutrient agars. These plates were then incubated aerobically for 18–24 hours at 37°C and further subcultured on to DCA. Twenty four hours later each plate was examined for non-lactose fermenting colonies which were then isolated and identified by Gram stain, biochemical reactions, colony form and pigmentation.

Immediately after the termination of each experiment the abdomen of each animal was opened. A 6–7 cm section of the transverse colon was tied off at each end and removed. One gram of fecal contents was then emulsified in 100 ml of sterile peptone water under a laminar flow hood. Serial dilutions of 10^2–10^8 were made up using an initial 1/100 dilution. Triplicate pour plates of 1 ml of each in nutrient agar were made for bacterial enumeration. Plates were incubated inverted at 37°C for 18–24 hours. Bacterial colonies were enumerated and results were reported as the number of colony forming units/g faeces.

All data were presented as a mean and (SEM) and compared using Student's *t* test, the analysis of variance (ANOVA), and Duncan's multiple range test[21]: a p value of $<0·05$ was taken as significant.

Results

The figure shows an initial plasma LPS concentration of 0·044 (0·004) ng/ml in the control group similar to the low values we have previously seen.[21] As the rectal temperature rose there was a small but insignificant increase in plasma LPS concentration near 39·5°C followed by a large rise at about 42°C and reaching 0·308 (0·038) ng/ml ($p < 0·01$) (ANOVA and Duncan's multiple range test) at about 44·5°C. Within a few minutes of reaching this rectal temperature each animal succumbed. In contrast, the group treated with kanamycin showed no significant change in plasma LPS concentrations during the entire heat stress period. Before this latter group was subjected to heat stress the plasma LPS concentration 0·007 (SEM 0·008) ng/ml was significantly lower than that of the controls ($p < 0·02$) and after heat stress it never rose significantly above baseline, and a concentration of 0·005 (0·002) ng/ml was measured just

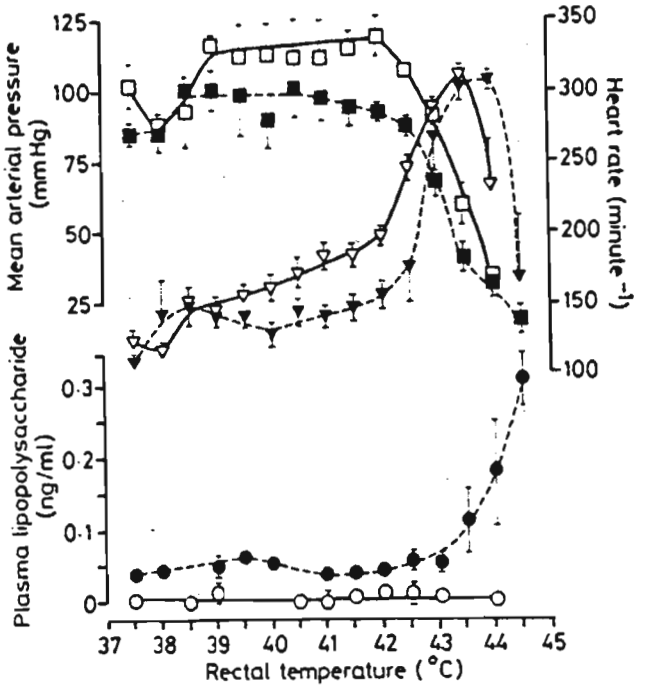

Figure *Mean (SEM) changes in plasma LPS concentration (circles), mean arterial pressure (squares), and heart rate (triangles) in control (broken lines) and treated (solid lines) groups at progressively raised rectal temperature in monkeys exposed to environmental temperature of 41·0 (0·3) °C and 100% relative humidity.*

before the monkeys died. The primates in the group treated with kanamycin succumbed at a significantly lower rectal temperature than those in the control group (44·1 *v* 44·6°C) ($p < 0·025$).

As the core temperature rose the MAP in the control group (figure) increased from 85 (SEM 4·2) mm Hg at a rectal temperature of 37·5°C to 100 (5) mm Hg at 39–41 C. Above 41°C the MAP gradually declined until a rectal temperature of about 43°C when there was a rapid decline. This rapid fall in MAP coincided with a rapid rise in heart rate and shortly before the rapid rise in plasma LPS. In the group treated with kanamycin the MAP curve was similar to that of the controls but at a level about 10–20 mm Hg higher than the controls throughout the whole temperature range.

As the rectal temperature rose the heart rate (figure) in the control group increased rapidly from 113 (SEM 3) beats/minute at a rectal temperature of 37·5°C to 152 (SEM 16) beats/minute at 39°C. This increase was followed by a slight decline in heart rate to 128 (SEM 9) beats/minute at 40°C before rising steadily to 155 (SEM 9) beats/minute at 42°C. Thereafter, the heart rate increased rapidly to reach a peak of 303 (SEM 6) beats/minute ($p < 0·001$) at 44°C after which it declined rapidly until a temperature of about 44·5°C was recorded. In the group treated with kanamycin the heart rate increased steadily from 126 (SEM 7) beats/minute at 37·5°C to 309 (SEM 7) beats/minute at 43·5°C, with a dip at 38°C. For any given rectal temperature between 39°C and 43·5°C, both the MAP and heart rate were higher than those recorded for the controls.

Except for a significant increase in serum AST concentration ($p < 0·01$) which increased from 47·55 (SEM 3·25) IU/l at 37°C to 75·9 (SEM 2·9) IU/l at 43°C in the control group, no significant changes in the serum enzymes ALT, L-γ-glutamyltransferase, and alkaline phosphatase were observed in either groups (table). Albumin, total protein, bilirubin concentrations and anti-LPS IgG titres were also not found to have changed significantly during heat stress (table).

Bacterial examination of rectal swabs from control animals yielded *Escherichia coli*, *Pseudomonas* sp, *Coliform bacilli*, *Proteus* sp, *Serratia* sp, *Staphylococcus* sp and *Lactobacilli* sp. In contrast, pretreatment with kanamycin resulted in an overgrowth of the Gram positive *Staphyloccus* sp and *Lactobacillus*, although *E coli* was found in some animals. Plate counts in the controls yielded a total of 12·56 (SEM 1·83) colony forming units × 10^9/g faeces of Gram negative and Gram positive bacteria. In the group treated with kanamycin plate counts showed a significant reduction to 0·183 (SEM 0·04) colony forming units × 10^9/g faeces ($p < 0·001$). This count would have been much lower had it not been for the overgrowth of Gram positive bacteria.

Discussion

Heat stroke is characterised by high body temperature, loss of consciousness, irritability, diarrhoea, vomiting and other symptoms.[21] These symptoms are similar to those in endotoxic shock caused by high plasma concentrations of LPS.[22–24] Hence LPS has been postulated to play a part in the pathophysiology of heat stroke.[1–6] We have recently shown the time course of endotoxaemia in heat stressed monkeys.[1]

In the control group of this study the plasma LPS concentration began to increase when the rectal temperature reached 42°C (figure). Initially this increase was gradual up to 43°C when a rapid rise occurred, reaching 0·308 (0·038) ng/ml ($p < 0·001$) just before death. The changes in plasma LPS concentration seen here were similar to those previously reported.[1] On the other hand, in the experimental group pretreatment with the oral non-absorbable antibiotic completely prevented any significant increase in plasma LPS concentration. The active ingredients of Kantrexil suspension are kanamycin and kaolin. Although kanamycin is sensitive for most Gram negative bacteria and aerobic bacteria, together with kaolin, it suppresses the plasma LPS concentration.

Table *Changes in concentration of serum enzymes, bilirubin, albumin, total protein and anti-LPS (IgG) in control and treated groups*

	Concentration at rectal temperatures of:			
	37°C	*40°C*	*42°C*	*43°C*
Control group:				
AST (IU/l)	47·55 (3·25)	58·0 (17·2)	68·2 (9·1)	75·9 (2·9)*
ALT (IU/l)	6·2 (0)	10·9 (5·0)	40·8 (36·55)	39·1 (34·0)
Alkaline phosphatase (IU/l)	376·1 (7·8)	367·8 (31·85)	418·4 (13·2)	406·9 (36·0)
L-γ-glutamyltransferase (IU/l)	22·5 (3·3)	21·2 (2·8)	24·2 (3·0)	24·3 (2·0)
Bilirubin (μmol/l)	3·5 (0·5)	3·0 (1·0)	5·0 (2·0)	4·5 (0·5)
Albumin (g/l)	31·25 (3·35)	29·45 (0·55)	34·3 (0·7)	32·2 (3·9)
Total protein (g/l)	48·9 (10·2)	57·05 (2·15)	59·45 (0·35)	60·25 (0·35)
Anti-LPS (IgG)%	100	—	61·8 (7·2)	94·1 (28·3)
Treated group:				
AST (IU/l)	34·85 (7·85)	32·0 (11·71)	31·4 (6·17)	31·83 (8·12)
ALT (IU/l)	5·73 (2·38)	8·68 (1·06)	10·25 (3·86)	4·85 (1·19)
Alkaline phosphatase (IU/l)	431·2 (78·8)	372·0 (64·7)	413·1 (101·6)	437·6 (105·9)
L-γ-glutamyltransferase (IU/l)	26·53 (3·23)	26·15 (3·65)	27·78 (4·35)	26·38 (3·6)
Bilirubin (μmol/l)	4·18 (0·14)	2·63 (0·86)	3·5 (0·75)	4·85 (0·83)
Albumin (g/l)	27·8 (1·05)	27·68 (2·38)	27·85 (1·65)	27·48 (1·83)
Total protein (g/l)	54·78 (2·57)	53·4 (3·19)	56·48 (3·42)	54·9 (3·74)
Anti-LPS (IgG)%	100	—	87·8 (14·8)	80·9 (21·3)

*$p < 0.05$.

The LPS of anaerobic Gram negative bacteria which, incidentally, make up a large proportion of the gut flora, lack certain characteristics of classic endotoxins and as such are less toxic.[25 26] In view of this no attempts were made either to suppress their activity in the gut or to determine their count in the faeces. Moreover, prophylaxis with kanamycin only has been advocated and used for sterilisation of the gut during surgery.[27 28] Furthermore, pretreatment with oral kanamycin has been shown to reduce the incidence of persisting endotoxaemia, lung lesions, and mortality from 90% to 32% during temporary occlusion of the superior mesenteric artery, intravenous injection of endotoxin or bradykinin, and intraperitoneal injection of *E coli* in rabbits.[29] Excessive and indiscriminate use of antimicrobes active against the anaerobic Gram negative gut flora may also reduce or eliminate the "interference phenomenon" and encourage invasion by pathogenic bacteria.[26] Although prophylaxis with kanamycin used in this study significantly reduced the total bacterial count ($p < 0.001$), at the same time it promoted an overgrowth of the Gram positive bacteria. The reduction in count from 12·56 (1·83) colony forming units $\times 10^9$/g faeces to 0·183 (0·04) colony forming units $\times 10^9$/g faeces of total bacterial flora might, therefore, not be sufficient to have caused the reduction in the plasma LPS concentration observed in this study. The count shown would have been much lower had it not been for the overgrowth of the Gram positive bacteria.

In this study as the rectal temperature increased so did the MAP in both the groups. In the control group not only were the MAPs lower for any given rectal temperature but the MAP also began a downward trend at a lower rectal temperature than the group treated with kanamycin (40·5°C v 42°C) ($p < 0.05$). Gorman and Proppe[30] also noticed a similar decline in MAP in the early period of heat stress in baboons. The higher MAP in the group treated with kanamycin may be partly explained by the anticholinergic properties of aminopentamide, one of the constituents of Kantrexil suspension[31]; and this could also explain why the heart rates were higher in those primates pretreated with kanamycin, and also possibly why they succumbed at a lower rectal temperature.[31 32] Gorman and Proppe also found that the heart rates for any given blood temperature were higher in heat stressed baboons who had received a cholinergic blocking agent than those who were not treated.[30]

Hyperthermia not only causes an increased blood flow to the skin but also a decreased visceral blood flow.[15 16] These could result in an ischaemic gut wall and hence raised plasma LPS.[1 13] High concentrations of plasma LPS have been reported in patients with fatal heat stroke[2 5] and a reduction of gut flora with antibiotics and enemas have been found to increase the incidence of an 18 hour survival in dogs subjected to heat stress from 20% to 70·6%.[4] Furthermore, rabbits pretreated with oral antibiotics have a reduced tendency toward developing endotoxaemia than untreated heat stressed rabbits.[6] The standard treatment for heat stroke is rapid cooling, correction of fluid and electrolyte disturbances, and treatment of shock.[21 33]

Our study suggests that the origin of the increased plasma LPS concentration seen during heat stress is mainly derived from the gut. Furthermore, gut derived LPS may be an important contribution to the

pathophysiology of heat stroke, and the use of anti-LPS antibodies may prove beneficial in heat stroke.

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APPENDIX 8

Portal and Systemic Plasma Lipopolysaccharide Concentrations in Heat-Stressed Primates

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Lipopolysaccharide (LPS) concentrations in hepatic portal and systemic arterial plasma were determined in five anesthetised monkeys heat-stressed by an environmental temperature of 41.0 ± 0.3°C and 100% relative humidity. As the rectal temperature (Tr) rose, the LPS concentrations in both the portal and systemic arterial plasma remained at the pre-heat-stress levels of 0.088 ± 0.017 and 0.078 ± 0.021 ng/ml (N.S.), respectively, until a Tr of 42.5–43.0°C, when the LPS concentration increased slowly, first in the portal plasma and then in the systemic plasma. On the other hand, the concentration of plasma anti-LPS IgG antibodies began to decline at temperatures as low as 40°C from 20.66 ± 7.35 μg/ml (portal) and 22.14 ± 7.43 μg/ml (arterial) to 5.51 ± 1.28 μg/ml (portal) ($P < .05$) and 4.6 ± 1.69 μg/ml (arterial) ($P < .05$) just prior to death. Above a Tr of 43°C, the LPS concentration increased rapidly to a maximum of 0.244 ± 0.05 ng/ml (portal) ($P < .01$) and 0.224 ± 0.06 ng/ml (arterial) ($P < .01$). The mean arterial pressure remained more or less constant at 112 ± 17.03 mm Hg until a Tr of 41.5°C and then rapidly declined as Tr rose ($P < .01$). The heart rate rose gradually from 154 ± 14 min^{-1} as Tr increased and then rapidly after a Tr of 41.5°C to a maximum of 307 ± 13 min^{-1} at 43.0°C. Thereafter it declined rapidly until death. This study supports previous suggestions that the endotoxemia developed during heat stroke is gut derived; however, LPS appears to enter the circulation at temperatures as low as 40°C.

Key words: endotoxin, heat stress, sepsis, trauma, heat stroke

INTRODUCTION

The primary cardiovascular adjustments to hyperthermia are an increased blood flow to the skin to facilitate heat dissipation and decreased blood flow to the splanchnic region [1–6] to maintain normotension. The lumen of mammalian gut normally contains gram-negative bacteria and its highly toxic outer cell membrane component—lipopolysaccharide (LPS), also known as endotoxin or pyrogen. Many of the clinical and pathological abnormalities that occur during heat stroke resemble the syndrome of endotoxin shock, viz.,

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hypotension, diarrhea, vomiting, renal cortical necrosis, and intravascular coagulation [1,4,6–11].

Possibly, the reduced blood flow to the intestines coupled with the high core temperature causes loss of the mucosal integrity, thus allowing transmural migration of LPS from the lumen of the gut into the hepatic portal vein. Under normal circumstances, small amounts of LPS leaking into the circulating blood would be detoxified by the reticuloendothelial system (RES) of the liver [12,13]. However, if the liver is overwhelmed by large amounts of LPS or is compromised, such as by elevated temperature or reduced blood flow, then the concentration of LPS in the systemic plasma would be expected to rise [13]. In heat-stressed primates, we have recently found a rise in LPS concentrations in the systemic arterial plasma when the rectal temperature (Tr) is elevated above 41.5°C [14–16] and a parallel decline in mean arterial pressure (MAP) and a rise in heart rate. However, these experiments could not detect the Tr at which LPS in the intestinal lumen could gain access to the circulation.

In the present investigation, we measured simultaneously in vervet monkeys the concentrations of LPS and anti-LPS IgG antibodies in the hepatic portal and systemic arterial plasma in order to determine whether LPS first is elevated in the portal blood and then subsequently in the systemic arterial blood. Furthermore, we have also attempted to correlate the changes in LPS concentration in the above two circulatory compartments with changes in cardiovascular parameters.

MATERIALS AND METHODS

Five adolescent monkeys (*Cercopithecus aethiops*) of either sex with a mean weight of 2.78 ± 0.14 kg were used in this study. The animals were anesthetized with ketamine (10 mg kg^{-1}, i.m.), and anesthesia was maintained with ketamine (5–10 mg kg^{-1}, i.v.) as required. Catheters were inserted into a femoral vein (for the administration of anesthetics) and both femoral arteries. A Statham pressure transducer was connected to one of the arterial catheters for the monitoring and recording of arterial blood pressure. The other femoral artery was used for removing 1-ml blood samples during the experiment. A thermorectal probe was inserted about 10 cm into the rectum, and the rectal temperatures were recorded continuously by using a telethermometer (Yellow Springs Instruments 46-TUC) connected to a chart recorder.

Adopting sterile surgical procedures, a midline incision was made in the linea alba and the abdominal cavity opened. A "Medican" 16-gauge catheter, connected to an extension tube, was inserted into the hepatic portal vein for removal of blood samples. The catheter was held in place by using purse-string sutures, and the abdominal incision was closed. After the surgical procedures were complete the animal was allowed a 30-min recovery period to attain steady-state conditions. Thereafter baseline recordings of Tr and arterial blood pressure were taken, and 1-ml blood samples were withdrawn from the femoral artery and hepatic portal vein and placed in heparinized, pyrogen-free plastic tubes.

Each animal was then positioned in a forced-draft incubator, where the environmental temperature was maintained at 41.0 ± 0.3°C and relative humidity was kept close to 100%. The procedures adopted for heat stress have been described in detail elsewhere [14]. During the heat-stress period blood samples were withdrawn at 15–30-min intervals. Tr was recorded continuously and arterial blood pressure was monitored continuously and recorded at 5–10-min intervals. The blood samples were kept in melting ice for the duration of the experiment.

At the end of the experiment the blood samples were centrifuged and the plasma was removed under sterile conditions in a laminar flow hood. The samples were either analysed for LPS immediately or stored at −20°C for up to a week until analysed. The chromogenic substrate modification of the Limulus amebocyte lysate (LAL) (MA Bioproducts) technique was used for the determination of LPS concentration in the samples [17,18]. This highly reproducible technique has been shown to overcome objections reported for the LAL gel test [18]. We previously found a 95.1% recovery from spiked plasma by using this technique, calibrating the standard curve with LPS prepared from *E. coli* 0111:B4 [19]. Plasma anti-LPS antibody concentrations were analysed by using an ELISA technique of Gaffin and colleagues [20], except that a monkey anti-LPS IgG standard and an antimonkey IgG peroxidase conjugate were employed.

The mean arterial pressure was determined by using the equation MAP = 1/3 systolic + 2/3 diastolic pressures.

Statistical Analysis

The data are presented as means ± SEM and compared by using the Student's paired and unpaired t-test, analysis of variance (ANOVA), and Duncan's multiple range test [14]. A P value of $< .05$ was taken as being significant.

RESULTS

The results reached statistical significance after the use of five monkeys, and therefore the study was terminated.

Plasma LPS

Prior to heat stress, the LPS concentrations in the hepatic portal and systemic arterial plasma were 0.088 ± 0.017 and 0.078 ± 0.021 ng/ml (N.S.), respectively, shown in Figure 1 (bottom). During the heat-stress period these concentrations remained more or less stable until a Tr of about 42.5°C was reached after approximately 120 min of heat stress. Thereafter the LPS concentration rose first in the portal vein and then, 10–15 min later, in the systemic arterial circulation. Beyond Tr of 43.0°C, the LPS concentration in the portal plasma rose rapidly to a maximum of 0.244 ± 0.05 ng/ml ($P < .01$, ANOVA and Duncan's multiple range test) just before the animals succumbed (about 200 min from the beginning of heat stress). The LPS concentration in the systemic arterial plasma, on the other hand, increased gradually until a temperature of 43.5°C. Thereafter it rose rapidly to a maximum of 0.224 ± 0.06 ng/ml ($P < .01$). At Tr of 43.5°C the concentration of LPS in portal plasma was significantly more than in the systemic arterial plasma ($P < .025$). However, the final concentration of LPS in the portal plasma was not significantly greater than that of the arterial plasma.

Plasma Anti-LPS IgG Antibodies

Before the primates were heat stressed, the concentrations of anti-LPS IgG antibodies were 20.66 ± 7.35 μg/ml in portal plasma and 22.14 ± 7.43 μg/ml (N.S.) in systemic arterial plasma, shown in Figure 1 (bottom). The concentration of the antibodies in both the above circulatory compartments began to decline above Tr's of 38.0°C, and by 40.0°C the concentrations had fallen to 15.30 ± 4.28 (N.S., ANOVA) and 11.86 ± 2.80 (N.S., ANOVA) in the portal and systemic arterial plasmas, respectively. Above 40°C, the concentrations of the antibodies in both compartments declined, reaching 5.51 ± 1.28 μg/ml

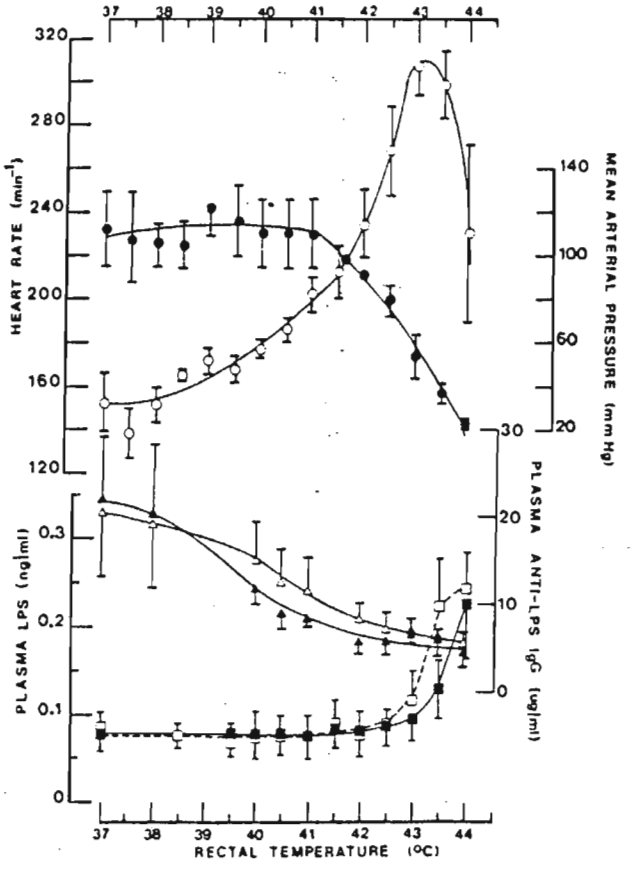

Fig. 1. Changes in LPS concentrations in hepatic portal plasma (open squares) and systemic arterial plasma (solid squares), and changes in concentrations of anti-LPS antibodies in hepatic portal (open triangles) and systemic arterial plasmas (solid triangles), heart rate (open circles), and mean arterial pressure (solid circles) in monkeys heat-stressed in an environmental temperature of 41 ± 0.3°C and 100% relative humidity.

(portal) ($P < .05$, ANOVA and Duncan's multiple range test) and 4.6 ± 1.69 μg/ml (arterial) ($P < .05$, ANOVA and Duncan's multiple range test) just prior to death.

Mean Arterial Pressure

As shown in Figure 1 (top) the MAP remained more or less at a constant level of 112.1 ± 17.03 mm Hg until Tr 41.5°C and then began to decline rapidly and continuously to 23.33 ± 2.64 mm Hg just prior to death ($P < .01$, ANOVA and Duncan's multiple range test). This decline in MAP commenced slightly before the plasma LPS concentration began to rise.

Heart Rate

Except for a small dip at 37.5°C, the heart rate increased gradually from a pre-heat-stress level of 154 ± 14 min^{-1}, to 214 ± 12 min^{-1} at 41.5°C ($P < .01$, ANOVA and Duncan's multiple range test) (Fig. 1, top). Thereafter, there was a rapid rise to 307 ± 13

min^{-1} ($P < .01$, ANOVA and Duncan's multiple range test) at 43.0°C followed by a rapid decline until death.

DISCUSSION

Heat stroke is a complex disorder produced initially by an excessive rise in body temperature. In many cases, heat stroke has a sudden onset, without prodromal signs, and when not promptly recognized and treated has a mortality rate as high as 80% [21]. At times, even with optimum therapy, heat stroke may cause death or permanent damage to the central nervous system or kidneys [22,23].

In this and previous studies [14–16] we and others [24,25] found in heat-stressed experimental animals that as the Tr rose above 41.5°C, the MAP began to decline rapidly and at the same time the heart rate rose rapidly. Significantly, the cardiovascular changes noticed here and elsewhere [14–16] occurred prior to and at slightly lower temperatures than the rise in plasma LPS concentrations as determined directly by the LAL assay. The rise in LPS concentration commenced approximately 15 min earlier and at a slightly lower Tr in the hepatic portal than in the systemic arterial plasmas (Fig. 1). The baseline concentration of systemic arterial plasma LPS observed here was similar to those measured in other studies in which the abdominal cavity was not opened [14–16,26].

On the other hand, the LPS-specific IgG concentration in the systemic circulation began to decline much earlier and at a lower temperature of 40°C. This decline is probably due to the presence of LPS in circulation and represents LPS-specific antibody "consumption." Since the LPS was consumed, it was therefore not detected by the LAL assay. We have already shown that the plasma of normal blood donors contains anti-LPS IgG and that anti-LPS IgG can reduce the concentration of "free" LPS in vitro [27,28]. The parallel decrease in anti-LPS IgG concentrations in both portal and arterial plasmas commences at a Tr of 40.0°C (Fig. 1). This indirect estimation therefore suggests that LPS "leaks" out of the gut at these lower Tr's and not—as previously proposed—at a Tr only above 42.0–42.5°C, where the LPS concentration rises according to the LAL test.

At Tr 43.5°C the higher concentration of LPS measured in the portal plasma indicates that LPS now enters the circulation mainly via the hepatic portal vein. Unexpectedly, there was no significant difference in plasma LPS concentrations between the hepatic portal and systemic arterial compartments at Tr's 42.0 to 43.0°C despite the passage of the portal blood and LPS through the liver RES as well as its dilution with the systemic blood. This may possibly indicate that at these temperatures, LPS from the gut lumen enters the circulating blood by routes other than the portal vessels, such as through the lymphatic vessels. In contrast to our findings, in primates, DuBose and co-workers [29] did not observe increased incidence of invasion by gram-negative bacteria or their endotoxins in blood or extraintestinal tissues in heat-stressed rats although the incidence of gram-negative bacterial invasion was increased in the duodenum of these rats. However, they did notice that the incidence of survival was increased if the rats were rendered tolerant to endotoxin or if they had received sublethal heat treatment prior to heat stress while pretreatment with zymosan had no effect and blockade of RES function had increased the mortality rate [29,30]. They attributed the increased incidence of survival associated with endotoxin tolerance to factors other than protection against endotoxemia [29].

We suggest that during heat stress, at lower temperatures (e.g., 40°C) the reduced splanchnic blood flow [1–6] minimally damaged the permeability properties of the gut mucosa and allowed the slow leakage of LPS into the interstitial spaces and then into the

circulating blood. Once in the circulation, some of the LPS formed complexes with anti-LPS antibodies [11] and high-density lipoproteins [31] while others were taken up by the RES [12,13] and were not detected by the LAL technique. However, at Tr greater than 42.0 the reduction in blood flow to the splanchnic regions was made more severe by the decline in MAP at 41.5°C (Fig. 1). This, plus direct thermal injury, may further damage the gut mucosa and possibly also the capillary endothelium. In support of the concept of gut wall damage, ecchymosis of the intestinal wall, ulceration of the intestinal mucosa, necrosis, and sloughing of epithelial cells have been observed in dogs heat-stressed to a rectal temperature of 43–44.5°C [24].

Endotoxins are known to induce in humans and experimental animals a variety of pathophysiological reactions such as fever, neutropenia and thrombocytopenia, hypotension, disseminated intravascular coagulation (DIC), and metabolic changes [32]. It is believed that LPS exerts its toxic effects via vasoactive and other mediators released from target cells—in particular, macrophages—as a result of interaction between LPS and the target cells [32].

In a study of superior mesenteric artery occlusion shock in primates, upon release of the occlusion and reperfusion of the gut with fresh oxygenated blood, the LPS concentration rose in the portal vein 10–15 min prior to that in the systemic artery and peaked earlier (Gathiram et al., submitted). In addition, the mean arterial pressure began to decline soon after the LPS concentration had peaked (0.30 ng/ml) in the arterial plasma. The peak LPS in the gut ischemia study is similar to that observed here in heat stroke, and therefore the amount of LPS observed in heat stroke should be considered as "toxic." High core temperature is known to damage cells of the RES function [6,10] and therefore probably contributes to the rise seen in the systemic arterial compartment.

Despite the wealth of information, the primary pathophysiological cause initiating heat stroke is still uncertain [33,34]. Two opposing views regarding the pathophysiology of heat stroke have appeared in the literature, viz., peripheral vs. central mechanisms. According to the former hypothesis, proposed by Adolph and Fulton [35], heat stroke is caused by a failure in the *circulatory* system which ultimately leads to shock. Malamud et al. [36] proposed a central mechanism in which elevated temperature causes direct thermal injury to target tissues, in particular, to the thermoregulatory centers of the brain, which, in turn, results in failures of sweating and thermoregulatory controls, eventually also leading to shock. Although there is evidence that a failure of sweating may precipitate heat stroke [37,38], there are numerous reports of heat stroke accompanied by profuse sweating [1, 38–40]. Our results suggest that both mechanisms operating together—circulatory (reduced splanchnic blood flow) and thermal damage (gut wall and liver)—would lead to an endotoxemia which may exacerbate other damage. It may be possible that the changes in hemodynamic events and plasma LPS concentrations, noticed in this study, are unrelated phenomena. However, we feel that the presence of low concentrations of gut-derived endotoxins in circulation could exacerbate the changes in the hemodynamic events which were initially caused by elevated core temperatures.

Agents which appeared to reduce intestinal flora (nonabsorbable antibiotics) or stabilize the intestinal wall (corticosteroids) or bind LPS (Anti-LPS IgG) were previously found to suppress any rise in plasma LPS concentration and increased the survival rate of experimental heat stroke in primates [15,16,26]. Results reported here are consistent with the suggestion of Bynum et al. [7] that the plasma LPS concentration may initially contribute to the pathophysiological events in heat stroke. While these studies do not show by direct methods the movement of LPS across the gut wall, the rise in LPS concentration

earlier in the portal vein than in the systemic artery and the reduction of LPS concentration by prophylactic oral nonabsorbable antibiotics strongly suggest that the gut is the source of this LPS.

Anti-LPS could not prevent death when the core temperature rose above a "critical" temperature of 43.5°C [26]. Therefore, it appears that LPS is a major component causing death up to 43.5°C, but above this temperature other mechanisms become more important —e.g., direct thermal damage to brain tissue.

The model described here may be of value in studying the possible benefits of antimicrobial and anti-LPS specific therapy for these potentially lethal conditions.

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The experiments described in this paper were conducted in adherence to the NIH guidelines for the use of experimental animals.

APPENDIX 9

Circulatory Shock 23:157–164 (1987)

AntiLipopolysaccharide Improves Survival in Primates Subjected to Heat Stroke

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Recent investigations have suggested that endotoxins or lipopolysaccharides (LPS) may play a role in heat stroke pathophysiology. In this study we wish to investigate whether prophylactic administration of anti-LPS hyperimmune plasma improves survival of experimental heat stroke in primates.

Eleven monkeys were anesthetised and heat-stressed to a rectal temperature of 43.5°C (group A) and then allowed to recover at room temperature. Five had received a prophylactic i.v. dose of equine anti-LPS (experimental group), while the other six (control group) received an equivalent i.v. dose of nonimmune equine plasma. All the experimental monkeys survived, while only one out of the six controls survived ($\chi^2 = 4.65$, $p < 0.025$). All the control animals that succumbed had significantly elevated plasma LPS levels ($p < 0.05$) as compared to the experimental group and the single surviving control. The latter showed very little or no change in plasma LPS levels.

A further eight monkeys (group B) were heat-stressed to a rectal temperature of 43.8°C. Of these eight, four had received a prophylactic i.v. dose of equine anti-LPS plasma. Although all eight animals died, the four which were pretreated with anti-LPS plasma had a significantly longer survival time (427.5 ± 61.39 min) than the untreated group (81.25 ± 33.94 min) ($p < 0.05$). Furthermore, the plasma LPS levels in the treated monkeys remained unchanged whereas in the untreated group, a significantly elevated plasma LPS level was noticed ($p < 0.005$).

We conclude that LPS may have a role in heat stroke pathophysiology and that prophylactic treatment with anti-LPS antibodies would seem to offer protection against the effects of heat stress.

Key words: LPS, endotoxin, trauma, hyperimmune anti-LPS plasma

INTRODUCTION

The pathogenesis of heat stroke is thought to be multifactorial [1–3]; however, recent findings indicate that endotoxins (lipopolysaccharides [LPS]) found in mammalian gut lumen may also play a contributory role [4–10]. Furthermore, some of the

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pathological features observed during heat stroke (hypotension, hyperkalemia, acidosis, disseminated intravascular coagulation, diarrhea, hemorrhage) are also common to endotoxin shock [1–12]. Recently we found that pretreatment with either oral, nonabsorbable antibiotics [13] or with intravenous corticosteroids prevented a rise in plasma LPS concentration in heat-stressed primates [14].

A new approach to therapy for septic and endotoxin shock has been the use of antibodies directed against the endotoxins in addition to other supportive therapy [12,15,16]. Moreover, we showed recently that prophylactic equine antilipopolysaccharide antibodies (anti-LPS) plasma prevented a rise in plasma LPS concentration in both cats subjected to a 1-h occlusion of the superior mesenteric artery [17] and primates subjected to hypoxia [18].

The purpose of the present investigation was to determine whether prophylactic administration of equine anti-LPS hyperimmune plasma can improve the survival of primates subjected to experimental heat stroke.

MATERIALS AND METHODS

A total of 19 adolescent monkeys (*Cercopithecus aethiops*) of either sex with a mean weight of 3.4 ± 0.3 kg were used in this study. The animals were fasted overnight and anesthetised with ketamine (10 mg/kg, i.m.) During the heat-stress period ketamine was given as required (10 mg/kg, i.v). A forced-draft incubator was used as a heat-stress chamber and was maintained at 41°C ± 0.3°C and relative humidity close to 100%. The technique used for heat-stress has been described elsewhere [4]. The rectal temperature (Tr) was recorded continuously for the duration of the experiment using a rectal probe inserted about 10 cm into the rectum and a telethermometer (Yellow Springs Instrument 46-TUC) connected to a chart recorder.

The monkeys were divided into two groups: A (n = 11) and B (n = 8). Based on pilot studies two different temperatures were selected: 43.5°C and 43.8°C. Animals in groups A and B were heat-stressed to 43.5°C and 43.8°C, respectively. In addition, five animals from group A and four from group B had received 1 ml/kg i.v. equine anti-LPS hyperimmune plasma (Detoxin-Stega Pharmaceutical Co., Vienna, Austria) a day before induction of heat stroke, and these served as the experimental groups. The remaining animals from group A (n = 6) received equivalent doses of equine nonimmune plasma, while those from group B (n = 4) received no treatment and served as controls.

As soon as the desired Tr was reached (43.5°C or 43.8°C), the animal was removed from the heat-stress chamber and allowed to cool passively at room temperature (about 25°C and 35% relative humidity). The Tr was continued to be recorded after removal of the animal until a temperature of 38°C was reached.

Blood samples (2 ml) for LPS and anti-LPS IgG analyses were taken from the femoral vein before the animals were placed in the incubator and just after their removal when the desired Tr's were reached. In the case of the experimental animals, an additional sample was taken prior to administration of anti-LPS antibodies. The blood samples were collected in heparinized, sterile, pyrogen-free plastic tubes. The sample tubes were stored on melting ice and centrifuged in a clinical centrifuge at the end of the experiment. The plasma was removed under sterile conditions in a laminar flow hood, placed in pyrogen-free plastic tubes and stored at −20°C. The samples were analyzed within a week. The chromogenic substrate modification of the Limulus

Amebocyte Lysate (LAL) technique (MA Bioproducts, Walkersville, MD) was employed for LPS analysis [19,20]. The relative plasma anti-LPS IgG concentration was determined using an enzyme-linked immunoassay (ELISA) except that rabbit anti-monkey IgG peroxidase conjugate was employed [21]. Owing to the lack of the appropriate monkey anti-LPS IgG standard, it was not possible to quantitate the reaction by weight, and the concentrations as reported are relative concentrations. The concentration of anti-LPS IgG in the initial plasma taken from each animal was regarded as 100%.

Statistical Analysis

The results are presented as a mean and ± standard error of the mean (SEM) and compared by means of an unpaired Student's t-test, Fisher test, and chi-square test with the Yates small number correction.

RESULTS

As the Tr of the primates rose to 42°C in both groups A and B, the control primates not treated with anti-LPS required less and less ketamine, and above 42°C no ketamine was required. The animals pretreated with anti-LPS plasma needed no anesthetic above 42.5°C. Just before these temperatures were reached (42°C and 42.5°C), the animals became restless, were unresponsive to further infusion of ketamine, and together with tachypnoea, heat stroke was considered established [21].

Heat-Stress to Tr 43.5°C (Group A): Survival

As shown in Table I, only 16.7% (one out of six) of the control animals survived heat-stress to 43.5°C and passive cooling in this group. The survival time of those animals who died ranged between 55 and 155 min. By contrast, in the experimental group, prophylactic anti-LPS plasma resulted in 100% survival after greater than 72 h ($p < 0.025$).

Heat-Stress to Tr 43.5°C (Group A): Plasma LPS Concentration

As a result of heat-stress to 43.5°C the plasma LPS concentration in the control group increased significantly from 0.08 ± 0.01 ng/ml to 0.346 ± 0.094 ng/ml ($p < 0.02$) (Fig. 1). The single control animal that survived the heat-stress showed

TABLE I. Effect of Heat-Stress to Tr's 43.5°C and 43.8°C on Survival (%) and Survival Time (min) in Control and Anti-LPS Groups

		Rectal temperature (°C)	% survival (No.)	Survival time (min)
Group A	Control (n = 6)	43.5	16.7 (1/6)	108.6 ± 24.1
	Anti-LPS (n = 5)	43.5	100 (5/5)	72 h
Group B	Control (n = 4)	43.8	Nil (0/4)	81.25 ± 33.94
	Anti-LPS (n = 4)	43.8	Nil (0/4)	427.5 ± 61.39

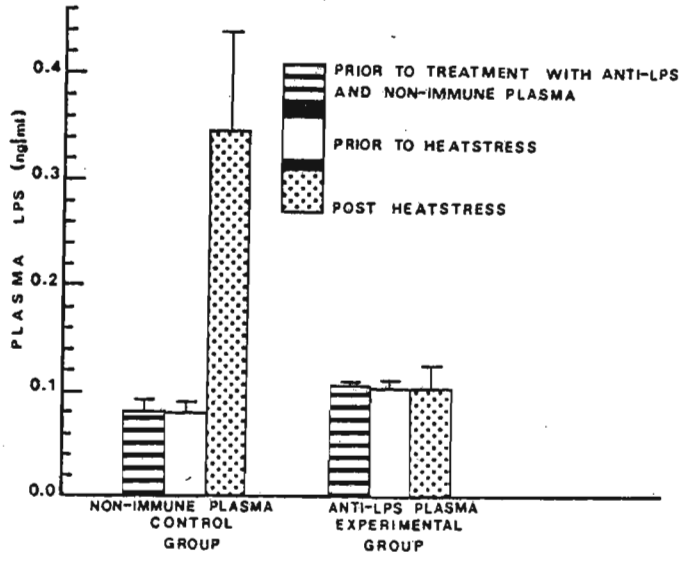

Fig. 1. Plasma LPS concentrations in control and experimental monkeys before treatment, prior to being heat-stressed, and after being heat-stressed to 43.5°C. The post-heat-stress value for the control group represents the concentration in nonsurvivors. The values are the mean ± SEM for six monkeys in the control group and four in the experimental group.

only a small increase in plasma LPS concentration (from 0.066 to 0.110 ng/ml.) Moreover, this survivor also had very high "natural" anti-LPS IgG concentration–about 144% more than the mean level measured in 18 of the monkeys in this study. On the other hand, in the experimental animals, the plasma LPS concentration remained at the pre-heat-stress level (0.104 ± 0.005 ng/ml prior to heat-stress and 0.104 ± 0.024 ng/ml after heat-stress) (Fig. 1). Heat-stress to 43.5°C also caused vomiting and bloody diarrhea during the passive cooling period in all the animals that eventually died.

Heat-Stress to Tr 43.8°C (Group B): Survival

None of the animals subjected to a Tr of 43.8°C survived the heat-stress; however, the administration of prophylactic anti-LPS plasma increased the survival time significantly to 427.5 ± 61.39 min compared with the control group, which had a mean survival time of 81.25 ± 33.94 min ($p < 0.05$) (Table I).

Heat-Stress to Tr 43.8°C (Group B): Plasma LPS Concentration

The plasma LPS concentration rose in the control group from 0.107 ± 0.008 ng/ml to 0.251 ± 0.028 ng/ml ($p < 0.005$). In contrast, in the experimental group the plasma LPS concentration remained close to pre-heat-stress levels (0.091 ± 0.01 ng/ml prior to heat-stress and 0.097 ± 0.01 ng/ml after heat-stress) (Fig 2).

Except for one animal (from the experimental group) none regained consciousness after they were removed from the heat-stress chamber. All animals vomited and had diarrhea. Interestingly, the diarrhea was bloody in the case of all the control animals, but in only one of the experimental animals.

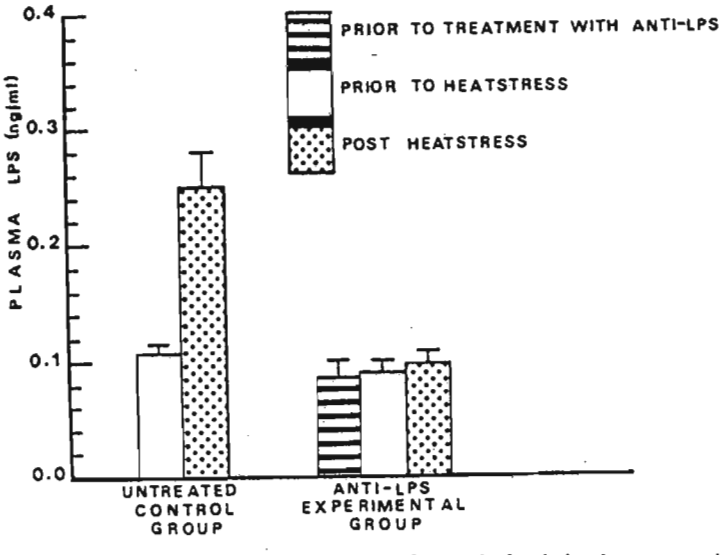

Fig. 2. Plasma LPS concentrations in control monkeys before and after being heat-stressed to 43.8°C and in experimental monkeys before administration of anti-LPS plasma, before and after being heat-stressed to 43.8°C. The values are the mean ± SEM for four monkeys in each group.

DISCUSSION

Heat stroke is a serious clinical problem, encountered in sports, military, occupational, and civilian medicine and is prevalent in hot, humid climates [2,22–24]. Without prompt recognition and immediate treatment, mortality from heat stroke may be higher than 50% [2,25,26] and at times even with optimum therapy, heat stroke may cause death or permanent damage [27]. The standard therapy for heat stroke is rapid cooling, correction of fluid and electrolyte disturbances, and treatment of shock [2,27,28].

In previous studies in monkeys, we found that heat-stress caused a rise in mean arterial pressure (MAP) until a Tr of about 41°C was reached. Thereafter the MAP showed a rapid drop. In addition, the plasma LPS began to rise at a Tr of between 41°C and 42°C. This rise in plasma LPS concentration was completely inhibited by administration of a nonabsorbable antibiotic [13] or by prophylactic administration of steroids in heat-stressed monkeys [14]. Furthermore, in the steroid treated monkeys, the cardiovascular parameters appeared more stable and began to deteriorate at a higher Tr (42°C) than control monkeys subjected to the same heat-stress [14]. On the basis of the above findings we had concluded that gut-derived LPS may be one of the contributory factors in the pathophysiology of heat stroke and that the administration of anti-LPS antibodies may be beneficial.

In this study the prophylactic administration of anti-LPS plasma decreased the mortality rate significantly from 83.3% to nil ($p < 0.025$) in primates heat-stressed to Tr 43.5°C. Furthermore, the plasma LPS concentration of all the primates who survived the heat-stress, including one from the control group, remained very close to the initial concentrations. The plasma LPS concentration in the surviving control primate was prevented from rising, presumably by the presence of a high level of "natural" anti-LPS IgG antibodies which was about 144% more than the basal levels measured in these primates. On the other hand, the primates that succumbed to the

high core temperature had significantly elevated plasma LPS levels (0.346 ± 0.094 ng/ml). Moreover, the prophylactic administration of nonimmune equine plasma was not found to suppress the plasma LPS concentration in the control group (which increased from 0.082 ± 0.01 ng/ml (prior to heat-stress) to 0.306 ± 0.086 ng/ml (after heat stress)].

The administration of anti-LPS plasma did not alter survival of the animals which were subjected to a higher Tr of 43.8°C. All the animals in this group (group B) died, although the plasma LPS concentrations of the treated animals remained low and those of the controls had increased significantly ($p < 0.005$). But, interestingly, prophylactic anti-LPS plasma increased the survival time significantly in this group ($p < 0.05$). Similar changes in survival rate were observed by Du Bose et al [29] in control rats and rats rendered tolerant to endotoxins. The latter workers found that endotoxin-tolerant rats were significantly more resistant as compared to non-endotoxin-tolerant rats to moderate heat-stress but not to extreme heat-stress; however, these researchers did not detect either the development of endotoxemia using the LAL test or the presence of gram-negative bacteria.

Bynum et al [6] found that pretreatment with oral antibiotics, cathartics, and enemas, to destroy the intestinal gram-negative flora, increased the incidence of 18-h survival rates of dogs heat-stressed to Tr in the range 43.3°C ± 0.4°C from 20% to 70.6%, but plasma LPS levels were not measured. We noticed in a recent similar study that pretreatment of monkeys with oral antibiotics suppressed the rise in plasma LPS concentration [13]. Other workers have also observed that the mortality rate of heat-stressed animals is reduced significantly if the gram-negative flora in the gut are destroyed [5].

The failure of animals to survive after being subjected to extreme heat-stress, in the case of this study to a Tr of 43.8°C, even though being pretreated with anti-LPS antibodies, may simply be due to excess irreversible damage caused by hyperthermia to critical tissues—e.g., brain [30,31].

It is known that hyperthermia causes increased blood flow to the skin to promote heat loss and a concomitantly decreased splanchnic blood flow [32,33]. We believe that the seriously reduced intestinal blood flow coupled with the raised core temperature damages the permeability property of the gut wall which then permits the leakage of LPS into the hepatic portal circulation. Furthermore, the reduced splanchnic blood flow and the high temperature may also compromise the liver, which may no longer be able to remove the LPS effectively from the circulating blood, thus leading to an elevated plasma LPS concentration. The latter, in turn, may exacerbate the deleterious effect of hyperthermia on the cardiovascular system. The suppression of plasma LPS concentration with prophylactic anti-LPS antibodies could possibly explain the increased survival rate and survival time of the primates subjected to elevated Tr of 43.5°C and 43.8°C, respectively, in this study. Further studies are in progress attempting to show any beneficial effect of anti-LPS IgG therapy as compared to prophylaxis.

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In conducting the experiments described in this report the investigators adhered to the NIH guidelines for the use of experimental animals.

APPENDIX 10

Prophylactic Corticosteroid Increases Survival in Experimental Heat Stroke in Primates

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It has been suggested that endotoxins or lipopolysaccharides (LPS), may contribute to heat stroke pathophysiology. In this study, 11 anesthetised monkeys were divided into 2 groups. The steroid group (n = 5) had received a dose of MPSS (30 mg·kg^{-1}, i.v.) before being heat-stressed and the control animals (n = 6) received saline equivolumetrically. The animals were heat-stressed to a rectal temperature of 43.5°C in an environmental temperature of 41 ± 0.3°C and 100% relative humidity and then allowed to recover at room temperature. Blood samples for LPS and anti-LPS IgG analyses were taken both before treatment and before and after heat-stress. The administration of prophylactic MPSS increased the survival rate significantly from 33% to 100% ($p < 0.05$). The plasma LPS level in the steroid group showed very little change after heat-stress, whereas in the non-surviving controls there was a significant increase in plasma LPS level (from 0.089 ± 0.007 to 0.257 ± 0.031 ng·ml^{-1}) ($p < 0.005$). The control animals that survived showed very little increase in plasma LPS levels, but had about 300% greater plasma Anti-LPS IgG levels. We conclude that pretreatment with MPSS improves the survival rate during heat stroke, possibly by suppressing the rise in plasma LPS concentration.

HEAT STROKE is a medical emergency, and without prompt recognition and immediate treatment its mortality rate can be higher than 50% (1,20). At times, even with optimum treatment, heat stroke may cause death or permanent damage (9). It may be characterised by high body temperature, loss of consciousness, irritability, diarrhea, vomiting, and other symptoms (30). These symptoms are similar to those seen in endotoxic shock (11). Furthermore, endotoxins have been detected in the plasma of isolated heat stroke cases (4,17). In a recent study we determined the time course of endotoxemia and cardiovascular changes in heat-stressed primates (14). In a subsequent, similar study, we showed that the endotoxemia could be prevented by administering a prophylactic dose of corticosteroid (15). In the latter study, we also observed that suppression of the systemic plasma LPS concentration was accompanied by an improvement of the mean arterial pressure which appeared more stable and only began to deteriorate at higher rectal temperatures (T_r). Due to these encouraging results we have here studied the effect of prophylactic administration of a large dose of methylprednisolone sodium succinate (MPSS) on the survival of primates undergoing experimental heat stroke.

MATERIALS AND METHODS

We used 11 adolescent monkeys (Cerecopithecus aethiops) of either sex with a mean weight of 4.08 ± 0.28 kg in this study. The animals were anesthetised with ketamine (10 mg·kg^{-1}, i.m.) and were kept anesthetised with ketamine (i.v.) as required during the heat-stress period.

The animals were divided into two groups: a steroid group (n = 5) and a control group (n = 6). The former had received a 30 mg·kg^{-1} (0.48 ml·g^{-1}) dose of MPSS infusion, via a peripheral vein, over a 15-min period, within 15 min of induction of heat-stress. The control group had received an equivalent volume of normal saline (15).

A forced-draft incubator, the temperature of which was maintained at 41.0 ± 0.3°C and relative humidity at about 100%, was used as a heat-stress chamber. A detailed method of induction of heat-stress has been described elsewhere (14).

Soon after induction of anesthesia a peripheral vein was catheterised for the removal of blood samples, administration of either MPSS or normal saline, and for the mainte-

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nance of anesthesia. A thermo-rectal probe was inserted about 10 cm into the rectum and the rectal temperature (T_r) was recorded continuously using a telethermometer (Yellow Springs Instruments, 46-TUC) connected to a chart recorder. Venous blood samples were taken for the determination of LPS and "natural" anti-LPS Immunoglobulin G (IgG) concentrations. The blood samples were collected in heparinized, sterile, pyrogen-free plastic tubes and were stored on melting ice for the duration of the experiment.

The animal was placed in the incubator and heat-stressed to a T_r of 43.5°C, which usually required 143 ± 8.7 min (110 to 195 min). No significant overshoot of the T_r occurred on reaching 43.5°C and after removal of the animal from the heat-stress chamber. The animal was immediately removed from the incubator and allowed to recover at room temperature (about 25°C and 30% relative humidity), without forced draft. The T_r was recorded until it reached 38°C. Heat-stress to a T_r of 43.5°C was chosen because it appeared to be a "critical temperature" for survival in a previous study (16).

Blood samples were taken prior to heat-stress and immediately after a T_r of 43.5°C from all the animals and an additional sample was taken prior to the administration of MPSS from the steroid group. The samples were centrifuged, the plasma removed under sterile conditions in a laminar flow hood and stored at −20°C. The samples were analysed within 7 d. The chromogenic substrate modification of the Limulus amoebocyte lysate (LAL) (MA Bioproducts) technique was employed for the determination of LPS concentration in the plasma (5, 10). An enzyme linked immunoassay (ELISA) technique (12) was used for measuring the relative plasma anti-LPS IgG, concentrations. Due to the lack of a known IgG standard, the concentrations of IgG are expressed as a percentage of the mean concentration measured in 28 normal monkeys. Hence the concentrations shown are relative and not absolute concentrations.

Statistical Analyses: The results are expressed as a mean ± standard error of the mean (± S.E.M.) and compared using the unpaired Student's *t*-test and the Fisher test.

RESULTS

When the animals were removed from the incubator, they were all unconscious and hyperventilating. As shown in Table I, only 33% (2/6) of the control animals survived (>3 months) from the induced heat-stress. On the other hand, pretreatment with a high dose of MPSS increased the incidence of survival (>3 months) significantly to 100% (5/5) ($\chi^2 = 2.753$) ($p < 0.05$). Of the four control animals that died, only two regained consciousness before their demise. The time-to-death of these animals ranged from 55 to 480 min. The two surviving control animals on the other hand, were conscious within 50 to 60 min which compares favourably with the steroid group which took an average time of 48 min to regain consciousness. Furthermore, no differences in the heating rates between the control survivors (0.054 ± 0.003°C·min^{-1}) and non-survivors (0.054 ± 0.005°C·min^{-1}) were noticed. The heating rate in the steroid or experimental group was found to be 0.048 ± 0.008°C·min^{-1}) (Table II).

All the dying control animals vomited and had bloody diarrhea, whereas the two surviving control animals, although vomiting, had diarrhea without blood. In contrast to the control group, no animals from the steroid group had diarrhea, although two vomited.

TABLE I. SURVIVAL (%) AND SURVIVAL TIME (min) OF CONTROL AND MPSS PRETREATED MONKEYS AFTER BEING HEAT-STRESSED TO Tr of 43.5°C.

Group	Survival (%) (number in brackets)	Survival time (min)
Control (n = 6)	33.3 (2)	223.8 ± 90.93 (range 55–480)
Steroid (MPSS pretreated) (n = 5)	100 (5)	3 months

TABLE II. HEATING RATES (°C·min^{-1}) AND DURATION OF HEAT-STRESS (min) OF MONKEYS IN CONTROL (SURVIVORS AND NON-SURVIVORS) AND STEROID GROUPS HEAT-STRESSED TO A Tr OF 43.5°C (VALUES ARE MEAN ± S.E.M.).

	Heating rate (°C·min^{-1})	Duration of heat-stress (min)
Control Group		
Survivors (n = 2)	0.054 ± 0.003	127.5 ± 10.5
Non-survivors (n = 3)	0.054 ± 0.005	124.5 ± 11.3
Steroid Group (n = 5)	0.048 ± 0.008	154.4 ± 11.6

From Fig. 1 it can be seen that prior to heat-stress the plasma LPS concentration in the control group, including the two survivors, was 0.089 ± 0.007 ng·ml^{-1}, similar to the steroid group (0.099 ± 0.006 ng·ml^{-1}). However, after the induction of heat stroke there was a significant increase in plasma LPS concentration to 0.257 ± 0.031 ng·ml^{-1} in the non-surviving control animals ($p < 0.005$). On the other hand, plasma LPS concentration showed very little or no change in the two control animals that survived and in the five which were pretreated with the corticosteroid. In two surviving controls, the plasma LPS concentration increased to 0.098 ± 0.014 ng·ml^{-1} and in the steroid group to 0.102 ± 0.004 ng·ml^{-1}. The plasma anti-LPS concentration (%) decreased in both groups after heat stress. Interestingly, the two surviving control animals had very high basal anti-LPS IgG levels compared to those that died, with a mean of 325.1 ± 64.0% more than the mean basal levels measured in 28 normal monkeys (Table III). In contrast to the controls, the plasma LPS concentrations of the steroid group did not change significantly during the heat stress experiment, and were significantly less than those of the controls after heat stroke ($p < 0.001$) (Fig. 1).

DISCUSSION

Using a total of 11 monkeys, we found that prophylactic administration of MPSS significantly increased survival following heat stroke ($p < 0.05$). For obvious reasons, large numbers of animals were not used once 5% significance was reached. The present study is in keeping with our previous results (15), that prophylactic administration of MPSS suppresses a rise in plasma LPS concentration during heat-stress. In the control animals that died, there was a substantial increase in plasma LPS concentration (from 0.089 ± 0.007 to 0.257 ± 0.031 ng·ml^{-1}) ($p < 0.005$). In contrast to the control group, the plasma LPS levels in the steroid group remained close to baseline and were signifi-

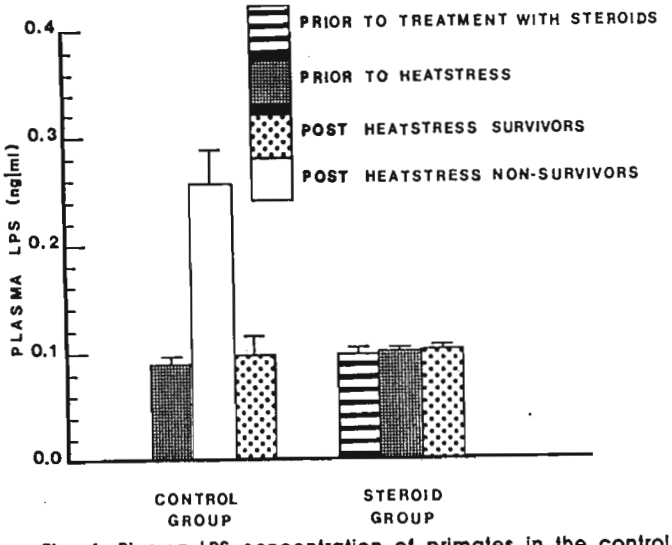

Fig. 1. Plasma LPS concentration of primates in the control (survivors and non-survivors) and steroid groups prior to treatment with MPSS (only the steroid group), and before and after being heat-stressed to a T_r of 43.5°C. The values are the mean and ± S.E.M.

TABLE III. PLASMA ANTI-LPS IgG LEVELS (%) IN CONTROL (SURVIVORS AND NON-SURVIVORS) AND STEROID GROUPS BEFORE AND AFTER HEATSTROKE (VALUES ARE MEAN ± S.E.M. AND ARE EXPRESSED AS A PERCENTAGE OF THE BASAL LEVELS ESTIMATED IN 28 NORMAL MONKEYS).

	ANTI-LPS IgG (%)	
	Before heat stroke	After heat stroke
Control Group		
Non-survivors (n = 3)	112.4 ± 53.1	
Survivors (n = 2)	325.1 ± 64.0	244.2 ± 43.9
Steroid Group (n = 5)	117.8 ± 39.7	81.8 ± 25.6

cantly less than the control group ($p < 0.001$). In the previous study (15), the plasma LPS concentration in control heat-stressed primates, remained close to baseline levels until a T_r of about 41.0°C. Thereafter there was an initial slow rise in plasma LPS concentration until beyond 43.0°C when there was a rapid rise to a maximum LPS concentration of 0.315 ± 0.03 ng•ml^{-1} just before the demise of the animals. In the present study, the two surviving control animals, like the steroid group, also showed very little change in the plasma LPS concentration. This may have been due to the high titre of naturally occurring plasma anti-LPS IgG levels in these control animals prior to heat-stress (Table III).

The decrease in relative plasma anti-LPS IgG concentration seen after heat-stress is attributed mainly to binding to LPS. The survival of, and lack of increase in plasma LPS concentration in the two control and five experimental animals cannot be ascribed to differences in the degree of heat-stress because there was no significant difference in the rate of heating between the survivors and non-survivors in the control group and between the non-survivors and the steroid group (Table II). Furthermore, in a recent, similar study (16), we found that pretreatment of monkeys with anti-LPS IgG hyperimmune plasma before being heat-stressed to a T_r of 43.5°C also suppressed the rise in plasma LPS concentration and improved the survival rate significantly compared to animals pretreated with normal non-immune plasma. In addition, we observed that while prophylactic anti-LPS hyperimmune plasma improved the survival of the primates subjected to moderate heat-stress (43.5°C), it did not have the same effect on primates subjected to extreme heat-stress ($T_r \geq 43.8$°C).

The primary cardiovascular adjustment to heat-stress is an increased blood flow to the skin, to promote heat loss, and a concomitant, decreased blood flow to the splanchnic regions, especially the intestines (26,30). The reduced blood flow to the gut may, in combination with the elevated core temperature, have damaged the permeability properties of the gut wall causing a leakage of LPS, which is always found in the lumen, into the portal circulation. Once in the circulation, some LPS may bind with the high density lipoprotein (32) or anti-LPS IgG (11) and others are taken up by the reticuloendothelial system (RES) (25). However, LPS may persist in the circulation if the RES becomes overwhelmed or compromised. Elevated levels of LPS in the circulation can cause vomiting, diarrhea, disseminated intravascular coagulation, hypotension, vascular collapse, shock and death (19,22). Similar symptoms have been observed in heat stroke (20).

The mechanism by which corticosteroids prevents a rise in plasma LPS concentration in heat stroke (15) and experimental intestinal ischemia (13) is not known. However, corticosteroids, in addition to increasing the survival rate in septic and endotoxic shock (18,21,22,28,29) have been known to have a membrane stabilizing effect (7,23), to increase splanchnic circulation (31) and cardiac output (27) and to protect the liver during ischemia (6). Furthermore, Hinshaw and co-workers (22) noticed that dogs treated with MPSS and gentamycin sulphate after a lethal *E. coli* shock had normal blood glucose concentration and pH, were free from intestinal hemorrhagic necrosis and had no loss of intestinal intimal lining and had recovered completely from the shock. On the other hand, their control dogs died within 24 hrs, after variable periods of hypotension, intestinal hemorrhage and diarrhea. We observed here that the monkeys pretreated with MPSS were free from diarrhea, whereas the control animals who died had bloody diarrhea. It is therefore possible that corticosteroids, by protecting the intestinal intimal lining, may have prevented leakage of LPS into the circulation. This could account for the low plasma LPS concentration and increased survival rate of the steroid group.

In keeping with our observations, others have shown that treatment of dogs (3) and rabbits (2) with oral antibiotics or rendering rats resistant to endotoxin (8) before being subjected to heat-stress improves the survival rate significantly. Bynum and co-workers (3) found that dogs which were pretreated with antibiotics, cathartics and enemas before they were heat-stressed to a T_r of about 43.5°C in an environmental temperature of 42–46°C for 2 h had a significantly increased incidence of 18 h survival compared to untreated control animals. Rabbits pretreated with oral antibiotics and then heat-stressed to 43.5°C also had a significantly reduced mortality and a slower rise in T_r than control rabbits (2). DuBose *et al.* (8) showed that endotoxin tolerant rats had reduced mortality to moderate heat-stress (T_r 42.23 to 42.69°C—total thermal area <60°C•min^{-1}) but not to extreme heat-stress (thermal area > 60°C•min^{-1}). However, their LPS data differed from ours in that they

found that less than 20% of the non-survivors had positive LAL test in blood samples. They also observed that heat-stress mortality in rats rendered more sensitive to endotoxin with zymosan treatment were not different from untreated controls. Furthermore, they suggested that endotoxins may not be responsible for the shock state noted in heat-stressed animals. This discrepancy in LPS data could possibly be due to species differences or to the more sensitive chromogenic substrate modification of the LAL technique used in our studies.

Administrations of corticosteroids to a few victims of heat stroke have not been found to be beneficial (1,9,24). The reasons for this could have been inappropriate dose and/or time of administration (18,21,22), especially the latter, since corticosteroid therapy was only used as a last resort, or the patients' core temperature may have exceeded the "critical" temperature of survival, which we found to be 43.5°C in monkeys (16).

The results of the current study suggest that the endotoxemia occurring during heat stroke could be contributing to the pathophysiology of heat stroke and that the use of prophylactic MPSS may be protective. Of interest, the two control animals that survived had high anti-LPS IgG concentrations. This points to a possible therapeutic benefit of anti-LPS IgG, especially since prophylactic anti-LPS IgG has been shown to be beneficial in heat stroke (16). We are currently investigating this possibility.

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In conducting the experiments described in this report the investigators adhered to the NIH guidelines for the use of experimental animals.

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