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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled

A PHARMACODYNAMIC APPROACH TO SOME CARDIOVASCULAR EFFECTS OF ENDOTOXIN IN CATS

submitted by Vishnubhakta Shrinivas Murthy

in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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Date 12th Sept. 1972

Dedicated to

Dr. I. R. Innes

...a token of our regard and gratitude...

ACKNOWLEDGEMENTS

For those, whose interest made this study possible and whose assistance in countless ways eased my work immeasurably, my gratitude is expressed most inadequately in the words that follow.

It is my proud privilege to acknowledge the great favour Dr. C.V. Greenway has done by accepting me for training in his laboratory. He has been a friendly teacher. For all the lively and constructive criticism, able guidance, inexhaustible moment to moment encouragement, I received during my stay in Winnipeg, I am most grateful to him.

I wish to express my appreciation to Dr. Deepak Bose for all the concern he furnished during this training. Many hours of discussion and argument with him have been both interesting and educative.

Working in the laboratory, I received technical assistance from Mrs. Anne E. Greenway, Mr. Gary Scott and Mr. Bill O'Neil. I am most thankful to them. I also wish to express my appreciation to Mr. George Lister for helping me with some of the experiments.

I am thankful to Mrs. M. McFee, Miss N. Pohorecky and Miss P. Lewis for all their valuable help in the library. I also wish to acknowledge the help I received from Mr. J. Kiekush and the other members of the animal house staff.

With great pleasure I extend my heartfelt thanks to the staff and graduate students for their kindness. My association

with them has been very rewarding in many ways. To Dr. George Oshiro goes a special commendation for the wit I enjoyed during my association with him. To express my pleasure will be but a disparagement.

I am glad to have the opportunity of recording the kindness and concern shown to me by Mrs. Marge Turner. She is one shoulder any graduate student can lean on, during his hours of disappointment and depression.

Preparation of this manuscript has come to an end because of the valuable assistance I received from many friends. With great pleasure I wish to thank Mrs. N. Beveridge for typing the first draft and Mrs. Joyce Reid for the final typing of the manuscript in such a short time. To Mr. Bill O'Neil I wish to express my deep appreciation for his efficient diligence and gracious acceptance of the task of proof reading. Miss Gail Doane has been very helpful in the preparation of this manuscript in many ways. It is difficult to convey adequately in words my appreciation for her kind concern. I also wish to thank Mr. Roy Simpson for his assistance in the preparation of the illustrations.

I gratefully acknowledge the financial support from the Medical Research Council of Canada during this training.

May I now take the liberty to join my wife Vani and our little Renu to convey this to the members in the Department of Pharmacology.

..... You all have been very good to us.

We owe you a gratitude .....

ABSTRACT

In the past, investigations on the pathogenic mechanism of endotoxin were primarily concerned with the early phase of hypotension. Although hemodynamic, biochemical and histopathological changes taking place during the late phases of cardiovascular failure have been described, no attempt was made to investigate whether these changes were secondary consequences of the prolonged hypotension or some more fundamental effects of endotoxin per se. The present study was designed to investigate whether the early phase of hypotension could be blocked by acetylsalicylic acid and if so, to study the late hemodynamic changes in the absence of this initial hypotensive response.

The anesthetised cat responds to a lethal dose of endotoxin by an initial hypotension and an increase in the right atrial pressure. These changes are due to acute pulmonary vasoconstriction, and hemorrhagic lesions of the lungs are visible after death. Intravenous pretreatment with acetylsalicylic acid prevented the initial hypotension, the increase in right atrial pressure and the pulmonary lesions. Possible mechanisms of this protective effect of acetylsalicylic acid are discussed. However, such pretreatment had no effect on the lethality of endotoxin. These results confirmed the hypothesis that the delayed lethal effect of endotoxin is an independent response and is not a secondary consequence of the initial pulmonary vascular response.

In these cats pretreated with acetylsalicylic acid, endotoxin produced extensive hemorrhagic lesions in the intestinal mucosa. Therefore, superior mesenteric arterial flow was recorded by a non-cannulating electromagnetic flowmeter probe. After pretreatment with acetylsalicylic

acid, endotoxin caused marked mesenteric vasoconstriction reducing the blood flow to 20-30 per cent of the control value in the absence of any change in arterial pressure. Such intense and prolonged mesenteric ischemia is known to be fatal and it could have been the cause of death in these endotoxin-treated cats.

The nature of this mesenteric vasoconstriction was investigated. Study of the fractional distribution of superior mesenteric arterial blood flow by injecting radioactive microspheres revealed that the vasoconstriction occurred uniformly in the intestine, colon and mesenteric lymph nodes. The mechanism of mesenteric vasoconstriction was investigated by removal of known neurohumoral vasoconstrictor influences. Endotoxin was administered to cats which had been subjected to bilateral nephrectomy, hypophysectomy and administration of phenoxybenzamine. The mesenteric vasoconstriction was slightly but significantly reduced. Administration of phenoxybenzamine alone before endotoxin did not affect the mesenteric vasoconstriction. It was concluded that catecholamines, vasopressin and angiotensin play at most a minor role in the mechanism of this vasoconstriction and that other unknown factors are predominant.

In these studies, acetylsalicylic acid was used to prevent the initial hypotensive response to endotoxin in order to allow studies on the late phase. It was possible that the acetylsalicylic acid itself modified the delayed phase. This possibility was excluded by later studies which showed that if endotoxin is administered by very slow intravenous infusion in cats given no pretreatment, the initial

phase of hypotension is absent but marked mesenteric vasoconstriction occurs. Thus very slow infusion of endotoxin provides another method by which the delayed phase can be studied in the absence of the severe initial hypotensive response and confirms that the intense mesenteric vasoconstriction is an important phenomenon during the delayed phase in the cats.

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INTRODUCTION

Endotoxins possess an intrinsic fascination that is nothing less than fabulous. They seem to have been endowed by nature with virtues and vices in the exact and glamorous proportions needed to render them irresistible to any investigator who comes to know them.

Ivan L. Bennett, Jr., M.D.
1964.

PRELUDE

From the time that prehistoric man first understood life, the phenomenon of death has been a constant source of pathos to him and his associates. While human ingenuity has triumphed in its success in modulating the genesis of life, failure has always greeted him at the first steps in his strides to conquer death. This failure and the fascinating dreams of eternal life have motivated him to explore every possible mode of death in order to find a breakthrough. Of the innumerable agencies which end this glorious life, fulminating gram-negative septicemia is one that has stood the test of time. Like every other challenge, man has endlessly tried to fight it and more often than not, has failed.

During the pre-antibiotic era, gram-positive septicemia dominated the scene. However, in recent years, septicemia with gram-negative organisms has shown a constant increase (McCabe, 1970) and despite mammoth attempts, mortality has been very high (Blair, Wise and MacKay, 1969; Bryant, Hood, Hood and Koenig, 1971). Today, considerable information is available on the host, man, on the gram-negative bacteria and on the changes which take place in man during gram-negative septicemia. Surprisingly enough, we know very little about the chain-reaction which connects the organisms to the ultimate outcome, death. One of the changes which has often been implicated as a major cause of death is an irreversible cardiovascular insufficiency that develops during the course of septicemia in many of the unfortunate patients. If the above mentioned insufficiency is the major cause, it is very

likely that one would be able to save many lives if one could stop the events which lead to it. The unfortunate part of the saga is the ignorance we all share when we come forward to explain how the above events take place in man.

Human life has been considered too precious to use for the study of the effects of septicemia in the experimental laboratory. To gain an understanding of the phenomenon of cardiovascular insufficiency produced by gram-negative bacteria, attempts have been made to develop a suitable animal model. Though results have been far from satisfactory, animal experimentation has given us much of the information we have used to understand the changes seen in the patients of septicemia. In animal experiments live bacteria have only recently been used (Hinshaw, Solomon, Holmes and Greenfield, 1968; Guenter, Fiorica and Hinshaw, 1969; Buckberg, Cohn and Darling, 1971). Most of the work done in this discipline has been with the use of the toxins isolated from the cells of gram-negative bacteria. It is generally agreed that the major bacterial factor causing gross hemodynamic changes and ultimate cardiovascular insufficiency is the cell wall component of the gram-negative bacteria, a macro-molecular protein-lipopolysaccharide complex called endotoxin. There is considerable evidence that most of the signs and symptoms seen in clinical gram-negative septicemia including cardiovascular insufficiency can be duplicated in animals using endotoxins.

Intravenous infusions of contaminated blood containing non-virulent bacteria and endotoxins produce the same type of clinical picture seen in gram-negative septicemia (Stevens, Legg, Henry, Dille,

Kirby and Finch, 1953). This is further support for the hypothesis that endotoxins contribute to many if not all of the changes seen in septicemia.

In the experimental laboratory endotoxins have been investigated extensively and research has permeated almost all the branches into which biology and biologists can be classified. Complexity is often a chosen target for a curious mind. Endotoxins produce effects which are very complex in nature, often indirect and always full of unexpected outcomes. In the following section an attempt has been made to review some properties of endotoxins, as explored and revealed by many a curious mind.

HISTORICAL DEVELOPMENT

In 1893 Pfeiffer observed that the intraperitoneal injection of the cholera vibrio into mice resulted in the lysis of the injected bacteria by the host defense mechanisms. After this lysis the mice became sick and died. This led Pfeiffer to postulate that on lysis of the bacteria, a toxin is released. Thus he first coined the term endotoxin. The term has lived a long time though many have forgotten Pfeiffer (Rosen, 1961a). Todd in 1903 started his studies on bacterial toxins, after Shiga discovered the dysentery bacillus in an epidemic in Japan. In the epidemics of dysentery it was noticed that lesions occurred only in the lungs and intestine, while the bacilli were present in the intestine and the mesenteric lymph nodes. This led Todd (1903) to think that possibly a toxin is released which gives rise to the typical clinical picture. He then extracted the filtrate of killed

dysentery bacilli, injected it into rabbits and produced the typical effects of dysentery infection. The intestine showed congestion, lungs showed patches of congestion and often small hemorrhages.

In the following year he (Todd, 1904) precipitated the toxin from the filtrate and demonstrated that the precipitate produced all the effects of live or killed bacilli. Flexner and Sweet (1906) confirmed the observations of Todd and added that the toxin does not produce any effects when introduced into the intestine. Before 1920, various reports appeared in literature describing the effects of toxins from Bacillus dysentery Shiga (Shigella dysenteriae). Some investigators studied the filtrate of broth in which the bacteria were grown while others used the extract obtained after the lysis of the bacteria, thus obtaining the toxins as well as other cell constituents.

In the available literature, Olitsky and Kligler (1920) were the first to describe the toxins as exotoxins and endotoxins. They obtained the endotoxins by autolysis with resultant liberation of the intracellular constituents. They studied the effects of the endotoxin on the rabbits and found it to produce edema, hemorrhages, necrosis and ulceration of the intestinal tract.

In the winter of 1924 Menten and Manning found their rabbits to be sick. During this season a greater mortality was seen. The pathological lesions were seen mainly in the liver, kidney, pancreas and spleen. They could isolate organisms of the enteritidis paratyphoid Group B, from the lesions (Menten and Manning, 1924a). During the course of the infection they found the rabbits to be hyperglycemic, and

when they injected killed organisms obtained from these lesions into healthy rabbits, these healthy rabbits showed a rapid rise in the blood sugar levels which returned to normal levels within a few hours. Lethal doses resulted in early hyperglycemia followed by an intense hypoglycemia and death. This led them to the conclusion that the bacterial bodies contained the toxic substance which is responsible for these toxic effects (Menten and Manning, 1924b).

Following the report of Menten and Manning, considerable interest was shown in the chemical effects produced by bacterial toxins. Zeckwear and Goodell (1925) studied the effects of suspensions of various species of killed bacteria in the rabbits after intravenous injection. They found a rapid rise in the blood sugar levels after intravenous injections of Bacillus proteus (Proteus), Bacillus coli (Escherichia coli), and Bacillus paratyphosus A (Salmonella paratyphi). A less pronounced effect was seen on injection of killed Bacillus paratyphosus A and Bacillus enteritidis (Salmonella enteritidis). They observed these animals to develop copious diarrhea, vasoconstriction in the ears, and in some cases a marked hypotension. The animals showed a marked decrease in the white cell counts and a marked prostration in 2 to 3 hours. Injection of Streptococcus hemolyticus, viridans and Staphylococcus aureus produced no symptoms. Later Menten and King (1930) reported that toxin extracted by precipitation also produced both hyperglycemia and the toxic effects. The following year Delafield (1931) reported that the toxin present in the bacterial cell is resistant to heat and its toxic effects are not destroyed by treating the bacterial cells with alcohol,

acetone, steaming or autoclaving. They also showed that the diphtheria toxin failed to produce similar responses. In the year 1933, André Boivin and his co-workers (Boivin, Mesrobeanu and Mesrobeanu, 1933) of the Pasteur institute in Paris, extracted endotoxin and described it as made up of lipid, protein and polysaccharide.

It is not surprising that the investigators who followed the research on gram-negative septicemia used endotoxins in their experiments since the effects of endotoxins were similar to those seen in gram-negative bacteremia and were very different from those seen with gram-positive bacteria and their toxins. Use of the extract (endotoxin) allowed precise, reproducible doses without the need to maintain and count cultures of the live bacteria.

ENDOTOXINS AND THEIR SOURCE - THE GRAM-NEGATIVE BACTERIA

In all gram-negative bacteria, the general architecture of the cell is similar. The protoplasm is surrounded by a thin plasma membrane and a rigid cell wall made up of peptides and sugar residues. The cell wall also contains some lipoproteins. Covering the cell wall is another thin and loose membrane called the outer membrane (Mergenhagen, Bladen and Hsu, 1966). Although the toxins from gram-negative bacteria were extracted in the beginning of this century, it was in the last two decades that the cell wall of the gram-negative bacteria was studied extensively. Salton isolated pure bacterial cell wall and studied the differences in the chemistry of gram-positive and gram-negative cell walls (Salton and Horne, 1951; Salton, 1953, 1957 and 1960). Fukushi and co-workers extracted endotoxins from purified cell walls

(Fukushi, Anacker, Haskins, Landy, Milner and Ribi, 1964). Two years later Mergenhagen and co-workers (1966) demonstrated that treatment of cells of Veillonella with phenol-water, a method commonly used to extract endotoxins, removed only the trilaminar outer membrane leaving the cell wall intact. This suggested that the outer membrane of the gram-negative bacteria contains endotoxins. Further evidence was presented by Shands (1966), who used ferritin-conjugated antibody to the somatic antigen (endotoxin) of the gram-negative bacteria, and localized the antigen on the outer membrane of the cells of Salmonella typhimurium. Thus endotoxins are part of the cell wall and are especially localized on the outer membrane of the wall. At present it is difficult to state the significance of this distribution in the life cycle of the bacteria.

ISOLATION OF ENDOTOXIN

Following the isolation of endotoxins by Boivin and co-workers (1933) many methods have been used by other investigators for isolation and purification. Boivin and co-workers (1933) used trichloroacetic acid for extraction. Acid-soluble endotoxin went into solution while the protein and nucleic acid fractions remained in the debris. The acid soluble fraction was then dialysed after neutralization of the acid. This fraction was a polymacromolecular complex consisting of polysaccharide lipid A - protein - lipid B. This was further purified by ethanol and ammonium sulphate fractionation. Polysaccharide accounted for the greater part of the complex. The following year Raistrick and Topley (1934) used trypsin digestion of the bacteria for the isolation of endotoxin. Morgan (1937) reported use of diethyleneglycol as a

solvent for endotoxin. Trichloroacetic acid and water dissolved some proteins from the bacterial cells while an anhydrous solvent like diethyleneglycol leaves these substances in the cell residue. The endotoxin extracted by this method was found to be similar to endotoxins extracted by Boivin. Approximately 60% of the material was the polysaccharide. Fuller (1938) used formamide, and the following year Walker (1940) used urea for the extraction of the toxins from bacteria.

Palmer and Gerlough (1940) found a combination of phenol and water to be suitable agent for the extraction of endotoxin. This method was further modified by Westphal and Luderitz (1954). The method employs use of 45% phenol at 60°C. After stirring the cell suspension in the solvent for a predetermined length of time, the suspension is cooled to 0°C in order to separate the phenolic and aqueous phases. The phenolic phase contains most of the bacterial protein and overlies a sediment of bacterial debris and deoxyribonucleic acid. The aqueous phase contains soluble polysaccharide and ribonucleic acid. In this procedure, the protein and lipid B components are dissociated and the aqueous phase contains only the polysaccharide and the lipid A components: the lipopolysaccharide. The lipopolysaccharide prepared in this way demonstrated the serological specificity as well as the toxic properties of endotoxin.

Ribi and co-workers (Ribi, Milner and Perrine, 1959) used water saturated with ethyl ether for the extraction of endotoxin. This method was further improved and is now used commonly to obtain highly purified endotoxin fractions (Fukushi et al. 1964). Nowotny and co-

workers used a detergent, cetyltrimethylammonium bromide (CTB) in 0.5% solution to liberate cell wall endotoxins (Nowotny, Thomas, Duron and Nowotny, 1963). Other less common methods of extraction of endotoxin use pyridine-water (Goebel, Binkley and Perlman, 1945), hot water (Roberts, 1949) and saturated ammonium sulphate solution (Prigge and Helmert, 1947).

Of all the methods described above the three commonly used ones are the trichloroacetic acid method of Boivin, phenol-water method of Westphal and aqueous-ether method of Ribi. The endotoxins extracted by these methods are sometimes referred to as Boivin endotoxin, Westphal endotoxin and Ribi endotoxin.

PHYSICAL AND CHEMICAL NATURE OF ENDOTOXIN

Under the electron microscope endotoxin appears in a variety of shapes. At neutral pH E. coli endotoxin appears as droplets of 100 Å diameter, while at alkaline pH it appears as long ribbons (Schramm, Westphal and Luderitz, 1952). Similar structural features were described by Milner and co-workers in endotoxins obtained from B. pertussis (Milner, Anacker, Fukushi, Haskins, Landy, Malmgren and Ribi, 1963). In a detailed investigation, Beer, Braude and Brinton (1966) found snake-like, donut-like, flat sheet-like and slender rod-like structures. Shands and co-workers studied rough and smooth strains of Salmonella typhimurium and proposed the biomolecular leaflet model. This bi-molecular leaflet structure appeared to be a consistent pattern and was seen in endotoxins from certain mutants differing in chemical composition (Shands, Graham and Nath, 1967). Recently, Shands (1971) produced evi-

dence that the bilayer structure was necessary for toxicity. Splitting the endotoxins into monolayered filaments by alkaline hydrolysis resulted in loss of toxicity. Although attempts are being made to relate the chemical composition to the morphological features of endotoxins, it is too early to derive any definite conclusions from the available literature.

Chemically, endotoxins are macromolecules of protein lipopolysaccharide complex. The large particle size of lipopolysaccharides, in the order of several millions is probably due to aggregation as a result of Van der Waals attraction of lipid groups (long chain fatty acid esters). These can be split off by alkali, leaving lipid and polysaccharides bound to each other. The molecular weight of endotoxin is about 200,000 and is made up of a core polymer, antigenically specific polysaccharide side chains with a molecular weight of approximately 20,000, and a lipid component with a molecular weight of approximately 2,000 (Luderitz, Staub and Westphal, 1966).

An approximate chemical composition of endotoxin has been reported based on the investigations of Morgan (1936, 1937), and Morgan and Partridge (1940, 1941). The lipid fraction contributed 9 to 12%, protein fractions, 17 to 20% while polysaccharide components formed the remaining 55 to 60% of the total. The lipid component is made up of palmitic acid, oleic acid, alphaglycerophosphoric acid and a non-nitrogenous fraction. Nowotny (1963) studied the lipid fraction obtained from Boivin-type endotoxin after acid hydrolysis. He found a lipid yield of 11 to 33% depending on the acid used for hydrolysis. The lipid

fraction contained 12 to 46% fatty acids. After acid hydrolysis of the polysaccharide fraction, the products of hydrolysis were N-acetyl glucosamine, d-glucose, l-rhamnose and some other sugars in small quantities. It contained about one percent phosphorus. Davis (1955) examined the polysaccharide fraction of endotoxins obtained from various species of Salmonella obtained by Boivin's method. Chromatographically-identified sugars included pentoses (Xylose and arabinose), methyl pentoses (rhamnose and fucose) hexoses (glucose, galactose and mannose) and in some cases heptoses and other uncommon sugars. Polysaccharide and protein components differed according to the method used for their separation. Acid hydrolysis resulted in a partially degraded polysaccharide fraction while the protein fraction remained intact. On the other hand dissociation in phenol gave an undegraded polysaccharide and a degraded protein. The protein fraction of the endotoxin contained 11 to 14% nitrogen and less than one percent phosphorus (Burrows, 1951).

To summarize, Endotoxins are macromolecules containing polysaccharides, lipids and proteins. The aggregate forms a trilaminar structure which seems to be essential for toxic actions. Endotoxin can be cleaved into its major constituents and the nature of the dissociated fraction depends on the method used.

ABSORPTION, DISTRIBUTION AND DETOXIFICATION

Endotoxins can enter circulation from various sources. The most common route is probably a localized focus of infection which can either release lysed bacteria per se or intact gram-negative bacteria which, on subsequent lysis, will liberate the toxins.

Absorption of endotoxin from the gut has been reported (Ravin, Rowley, Jenkins and Fine, 1960) using ^{51}Cr-labelled endotoxin. However, Sanford and Noyes (1958) could not find any absorption of endotoxin from the gastro-intestinal tract. It is likely that under normal or pathological conditions the intestinal mucosa may be invaded by gram-negative bacteria which, on lysis, liberate endotoxin and this in turn may enter the portal circulation. This may not, under normal circumstances, result in fulminating endotoxemia since the liver can remove a considerable amount of foreign substances including endotoxin, (Greene, Wiznitzer, Rutenburg, Frank and Fine, 1961, Rutenburg, Skarnes, Palmerio and Fine, 1967). Endotoxemia from intravenous administration of endotoxin is common in experimental studies. Occasionally transfusions of blood contaminated with gram-negative bacteria may result in endotoxemia (Borden and Hall, 1951; Braude, Williams, Siemienski and Murphy, 1953; Stevens et al. 1953). The fate of intravenously injected endotoxins has been investigated by many workers. Quantitative estimations were done either by a bioassay or by using endotoxin labelled with a radioactive isotope or a fluorescent substance. Blood levels, vascular clearance and tissue distribution of injected endotoxin were investigated in rats using 131-Iodine (Barnes, Lupfer and Henry, 1952) and in mice and guinea pigs using 32-phosphorus as the label (Rowley, Howard and Jenkins, 1956). More extensive studies have been carried out in rabbits and mice by Braude and co-workers (Braude, Carey and Zalesky, 1955; Carey, Braude and Zalesky, 1958) and in dogs by Starzecki, Reddin, Gran and Spink (1967) using 51-Chromium as the label. Chromium was found to be a more stable tag

for in-vivo studies.

Distribution of radioactive endotoxin in the blood was qualitatively similar in rabbits, mice, and dogs in that endotoxin was confined to plasma and the buffy coat. No endotoxin was detectable in the red blood cells. Vascular clearance in dogs (Starzecki et al. 1967) showed an initial rapid phase followed by a slower phase and a similar clearance was noticed by Di Luzio and Crafton (1969) in rats. In dogs, within 5 minutes of an intravenous bolus injection, 63 to 66% of the endotoxin disappeared from the circulation. In one hour 75 to 87% and in 6 hours 92% endotoxin left the circulation. At 24 hours, only 3 to 4% of the injected radioactive endotoxin was still circulating in the blood. Qualitatively similar vascular clearance was seen by Braude and co-workers (Braude, Carey and Zalesky, 1955; Carey, Braude and Zalesky, 1958) in rabbits and mice.

Tissue distribution of endotoxin shows marked selectivity. Within 5 minutes of administration, the highest levels are found in liver and spleen. Significant amounts are detected in mesenteric lymph nodes and lungs, while much smaller amounts are found in adrenals and kidneys. No significant radioactivity was detected in central nervous system, cardiac and skeletal muscle, pancreas, stomach, duodenum, ileum and colon.

The reticuloendothelial system was first implicated in the host response to endotoxin by Beeson (1947a, 1947b). Circulating endotoxin was cleared by the reticuloendothelial cells of the liver, spleen and other organs rich in reticuloendothelial cells. Di Luzio and Crafton (1969) showed that in rats, alterations in reticuloendothelial function resulted in marked alterations in the vascular clearance of endotoxins.

Golub, Groschel and Nowotny (1968) studied the factors which influence the uptake of endotoxins by reticuloendothelial cells. They showed that endotoxoids are phagocytosed poorly compared to the toxic parent material. (Endotoxoids-detoxified endotoxins-were prepared by treatment of endotoxin with potassium methylate and 0.1 N sodium hydroxide for 24 hours at 25° followed by neutralization.) Phagocytosis of endotoxin by the reticuloendothelial cells requires plasma components. Antibodies, complement and opsonins may be playing an important role in the phagocytosis of endotoxins.

Enzymatic degradation of endotoxin in plasma was first reported by Rowley et al. (1956). They found a phosphatase-like enzyme in the plasma of mice and guinea pigs which released phosphates from the endotoxin molecule. This system required calcium for its action and was inactivated on heating to 56°C for 30 minutes. More evidence has been produced that plasma plays an important role in the detoxification of endotoxin (Schultz and Becker, 1967).

While studying the fate of ^{51}Cr-tagged endotoxin, Chedid, Parant, Boyer and Skarnes (1964) found that endotoxin, after sequestration by the liver and the spleen, loses its potency. Saline extracts of liver and spleen containing radioactivity equivalent to LD_{100} doses had no lethal effect in adrenalectomized mice. Corwin and Farrar (1964) and Farrar (1965) demonstrated that tissue homogenates of organs rich in reticuloendothelial cells can inactivate endotoxin. Recently Filkins (1971) has shown that hepatic macrophages play an important role in the detoxification of endotoxin while the hepatic parenchymal cells are in-

active in this respect. He found lysosomal enzymes to be the inactivators of endotoxins.

Starzecki and co-workers (1967) showed some loss of non-dialysable endotoxin into bile. About 1% of the radioactivity appeared in urine during the first hour and about 12% was lost in 14 hours. This radioactivity was dialysable and was probably associated with a smaller dialysable molecule, probably a split part of the parent endotoxin molecule. More work is needed to unfold the specific enzymatic process involved in detoxification and various metabolic products of endotoxins.

In brief then, endotoxins are rapidly cleared from circulation, major fraction being taken up by the reticuloendothelial cells of the liver and spleen. Although a small amount of injected endotoxin may be inactivated by plasma, liver and spleen seem to play an important role in the host defense against endotoxins.

BIOLOGICAL EFFECTS OF ENDOTOXINS

An extremely remarkable range of fauna, extending from invertebrates (Levin, 1967) to primates (Gilbert, 1960), respond to endotoxins by an extraordinary array of biological effects. These effects often seem to be causally related, at least very superficially. Since the basic mechanism of action of these bacterial endotoxins is still not clear the postulated interrelations between observed responses make the picture very complex and confusing. In the following description, only the hemodynamic effects will be considered in detail and a brief reference will be made to the other biological effects.

HEMODYNAMIC EFFECTS

In the commonly used experimental animals (monkeys, dogs, cats and rabbits) an intravenous administration of endotoxin produces a decrease in arterial pressure (Gilbert, 1960). The time of onset, magnitude, and duration of this hypotensive effect depends on the dose of endotoxin, the mode of administration and species of experimental animal under study. Under suitable experimental conditions, either this hypotension per se or some subsequent hemodynamic or biochemical mechanism, as yet poorly understood, leads to progressive deterioration of cardiovascular function. Such deterioration of cardiovascular function results in reduced tissue perfusion and generalized cellular hypoxia and vital organ damage. Since in experimental animals, death due to endotoxemia is invariably associated with hypotension and signs of reduced tissue perfusion, one is tempted to assume a causal relation between the two. In the absence of evidence to the contrary such an assumption is justified. A similar deterioration of cardiovascular function is seen in some patients suffering from gram-negative bacteremia, often referred to as septic shock or bacteremic shock. The relationship between the cardiovascular insufficiency and death seen in clinical state of septic shock and that seen after endotoxemia in experimental animals has often led biologists to consider that the two syndromes are similar if not identical. Needless to say, the hemodynamic effects of endotoxin have been investigated in experimental animals in an attempt to understand the pathophysiology of cardiovascular insufficiency seen during the state of clinical septic shock.

In man and monkey, rapid injection of endotoxin produces a gradual fall in arterial pressure (Man- Bradley, Chasis, Goldring and Smith, 1945; Moser, Perry and Luchsinger, 1963. Monkey- Kuida, Gilbert, Hinshaw, Brunson and Visscher, 1961; Gilbert, 1962; Hinshaw, Emerson and Reins, 1966a; Brockman, Thomas and Vasco, 1967). In contrast to this, other species (dogs, cats and rabbits) react more dramatically. Within 1-2 minutes after an injection of a lethal dose of endotoxin, a precipitous fall in arterial pressure occurs. In some animals no recovery of arterial pressure is seen after this initial drop. In others, this initial phase of hypotension is followed by a phase of partial or complete recovery towards normal arterial pressure. This is then followed by a second phase of hypotension which develops gradually.

In the following discussion the hemodynamic changes seen in various species will be described in two phases: the initial phase lasting for first 10 to 15 minutes during which the initial phase of hypotension develops and partial recovery occurs, and the late phase during which the progressive fall in arterial pressure is seen (Gilbert, 1960).

THE EARLY HEMODYNAMIC EFFECTS IN THE DOG

The initial hypotension

The mechanism of the initial phase of hypotension has been investigated in some detail in dogs and cats, but little is known regarding its mechanism in man and the monkey. Much emphasis has been put on species-dependent variations in the mechanism of initial hypotension but there appears to be a quantitative difference in the responsive-

ness of various species rather than a qualitative difference in the action of endotoxin. Although not investigated as extensively, the monkey might prove to be an exception. In the following section, the mechanism of initial hypotensive response as well as other hemodynamic changes seen during the initial phase in the dog will be considered in detail and the changes seen in other species will be discussed later since much less is known about their responses. In dogs, intravenous injection of a lethal dose of endotoxin produced a dramatic fall in arterial pressure within 5 minutes. Arterial pressure is a product of cardiac output and peripheral arteriolar resistance. Hence a decrease in the arterial pressure could occur due to either a decrease in cardiac output or a decrease in arteriolar resistance. During the initial phase of hypotension, total peripheral resistance either increased (Weil, MacLean, Visscher and Spink, 1956; Tsagaris, Koehler, Kuida and Hecht, 1963; Anas, Neely and Hardy, 1968) or showed no change (Hinshaw, Vick, Wittmers, Worthen, Nelson and Swenson, 1961b). A marked decrease in the cardiac output was recorded by various workers during this phase (Hinshaw et al. 1961b; Tsagaris et al. 1963; Brockman et al. 1967) although cardiac function was normal (Alican, Dalton and Hardy, 1962; Brockman et al. 1967). Hinshaw and co-workers have conclusively shown that endotoxin does not have direct effects on the myocardium (Hinshaw, Archer, Greenfield and Guenter, 1971). The classical studies of MacLean and Weil (1956) and Weil and co-workers (1956) demonstrated a marked decrease in venous return which was due to pooling of blood in the splanchnic vascular bed. These observations have been confirmed by Hinshaw and

co-workers (Hinshaw, Gilbert, Kuida and Visscher, 1958; Iampietro, Hinshaw and Brake, 1963). Total blood volume did not decrease during this hypotension (Gilbert, 1960).

Hepatic outflow block

The acute fall in arterial pressure was preceded by an abrupt rise in portal pressure, and an increase in the weight of the liver (MacLean, Weil, Spink and Visscher, 1956; Hinshaw, Reins and Hill, 1966b). The rise in the portal pressure was due to an increase in the resistance within the hepatic vascular bed. This outflow block led to splanchnic pooling and a marked decrease in venous return. Exclusion of the liver from the circulation and opening of a porto-caval shunt prevented this portal hypertension and the initial hypotension (MacLean and Weil, 1956; Brockman et al. 1967).

Central venous pressure remained unchanged or decreased. Similar changes were seen in hepatic venous pressure (Hinshaw et al. 1966b; Brockman et al. 1967). Hepatic venous wedge pressure increased, indicating an increase in the sinusoidal pressure (Chien, Chang, Dellenback, Usami and Gregersen, 1966a; Bashour and McClelland, 1967). From these observations it could be concluded that the principal site of increased resistance was the intrahepatic venous system. Hinshaw and co-workers demonstrated that this phenomenon was not influenced by adrenalectomy and sympathectomy (Hinshaw, Brake, Emerson, Jordan and Masucci, 1964), and studies on the isolated perfused liver showed that the response did not depend on the release of vasoactive substances from extrahepatic sites (Hinshaw et al. 1966b). Considerable similarities were noticed between

the responses produced by histamine, compound 48/80 (a potent histamine liberator) and endotoxin (Hinshaw, Emerson, Iampietro and Brake, 1962a; Hinshaw et al. 1966b) and it was proposed that intrahepatic release of histamine could initiate the response (Hinshaw, 1964). It must be recalled that the initial hypotensive response has many features of an anaphylactic reaction (Weil and Spink, 1957; Spink, Davis, Potter and Chartrand, 1964; Thomas and Essex, 1949). Mast cell disruption and release of histamine from liver have been shown during anaphylactic reaction in dogs (Ojers, Holmes and Dragstedt, 1941; Akcasu and West, 1960). Weil and Spink (1957) demonstrated a prompt rise in histamine-like substances in hepatic venous blood immediately after injection of endotoxin. Histamine is the only autacoid known to produce hepatic outflow block. Thus histamine release within the liver remains an attractive, though unproven, hypothesis for mechanism of hepatic outflow block (Greenway and Stark, 1971; Greenway and Oshiro, 1972a).

Pulmonary vasoconstriction

Kuida and co-workers studied the effects of endotoxin on pulmonary circulation. In dogs endotoxin caused pulmonary hypertension without a rise in left atrial pressure. Pulmonary artery wedge and small pulmonary vein pressures rose during the initial phase of hypotension. Pulmonary venous resistance increased more than the arterial resistance, leading to pulmonary oedema and a consistent increase in lung weight (Kuida, Hinshaw, Gilbert and Visscher, 1958). Sukhandan and Thal (1965) came to similar conclusions using isolated perfused lungs and Brockman and co-workers (1967) confirmed the increase in pulmonary

arterial pressure in intact dogs during the initial hypotensive phase. A greater rise in pulmonary arterial pressure was observed when venous return was maintained from an extra-corporeal blood reservoir. Since pulmonary hypertension was seen in isolated perfused lungs, the mechanism of this hemodynamic effect of endotoxin does not depend upon release of vasoactive substances from another vascular bed.

Hinshaw and co-workers demonstrated that endotoxin does not have a direct effect on the saline-perfused pulmonary vascular bed. The presence of plasma and the formed elements of blood (white blood cells and/or platelets) were needed to produce the response. This however, does not exclude the participation of various tissue factors (Hinshaw, Kuida, Gilbert and Visscher, 1957). Broncho-constriction was not seen when the dog was made thrombocytopenic. This observation by Stein and Thomas (1967) further supports the hypothesis that an interaction between endotoxin and the platelets is necessary for the initiation of the pulmonary vascular response in dogs. These workers also reported a marked decrease in circulating platelet counts after administration of endotoxin. Using pharmacological agents to block the hemodynamic changes during the initial phase, Kuida and co-workers suggested that serotonin may be the chemical mediator responsible for the pulmonary response (Tsagaris, Koehler, Kuida and Hecht, 1963; Anderson, Kuida and Hecht, 1963; Koehler, Tsagaris, Kuida and Hecht, 1963). A marked decrease in the blood serotonin level occurred during the initial phase of hypotension after endotoxin (Jacobson, Mehlman and Kalas, 1964). Davis, Meeker and McQuarrie (1960a) reported a similar decrease in serum serotonin levels along with

a decrease in the platelet count during the initial 5 minutes after injection of endotoxin. Pulmonary arterial serum serotonin levels were found to be higher than those in the femoral arterial samples for up to 3 minutes and marked platelet aggregation was seen in the pulmonary arterial blood within 1 minute after injection of endotoxin. In dogs, serotonin produced pulmonary vasoconstriction (Douglas, 1970). Sukhandan and Thal have reported striking similarities between the hemodynamic effects of endotoxin and serotonin on the isolated perfused lungs (Sukhandan and Thal, 1965). Thus the local release of serotonin from platelet-aggregates within the lungs appears to be the mechanism responsible for the pulmonary hemodynamic changes seen in dogs after intravenous administration of endotoxin.

Early hemodynamic changes in other vascular beds

Profound changes take place in the mesenteric vascular bed during the initial phase of hypotension. Superior mesenteric arterial blood flow approximately mirrored the decrease in arterial pressure (Lillehei, Longerbeam, Bloch and Manax, 1965; Brobmann, Ulano, Hinshaw and Jacobson, 1970). Mesenteric arterial resistance showed variable changes during this phase (Meyer and Visscher, 1962; Brobmann *et al*. 1970). Studies done on isolated pump-perfused segments of intestine revealed an increase in arterial resistance (Hinshaw and Nelson, 1962; Hinshaw, 1968). Significant venoconstriction was observed in these preparations which resulted in an increase in intestinal weight. It was suggested that mesenteric venoconstriction was a second cause of pooling of blood in the mesenteric vascular bed and it seemed to contribute to

extravasation of protein-rich fluid into the interstitial spaces leading to increased weight as well as an increased lymph flow (Meyer and Visscher, 1962).

The vascular bed of the stomach responded to the injected endotoxin by an increase in arterial resistance. Jacobson and co-workers produced evidence that the increased vascular resistance was due to a chemical mediator released from a distant site. Neurogenic constrictor influences did not seem to play any role in this response (Jacobson, Dooley Scott and Frohlich, 1963).

The contribution of the splenic vascular bed to the early hemodynamic changes seen after endotoxin has been investigated. During the initial phase of hypotension, an increase in the splenic vascular resistance was observed (Frohlich, 1963). This was probably mediated by a reflex release of adrenal medullary hormones since adrenalectomy was shown to abolish this response (Boruchow and Abel, 1966). Contraction of the spleen during the initial phase of hypotension caused release of concentrated red blood cells into the portal circulation, leading to a raised portal hematocrit and later an increased arterial hematocrit (Boruchow and Abel, 1966; Chien, Dellenback, Usami, Treitel, Chang and Gregersen, 1966b).

The renal vascular bed responded to endotoxin-induced acute hypotension by a decrease in renal blood flow and an increased vascular resistance. Impaired renal function seen during the hypotensive phase appeared to be secondary to the hypotension and reduced arterial flow (Hinshaw, Spink, Vick, Mallet and Finstad, 1961c; Shanbour, Lindeman,

Archer, Tung and Hinshaw, 1971). When sublethal doses of endotoxin were given and the profound fall in arterial pressure did not take place, renal blood flow increased. In eviscerated dogs, renal weight decreased very rapidly (Hinshaw and Bradley, 1957). When the arterial pressure in these dogs decreased, renal blood flow also decreased but it increased when the hypotension was prevented (Hinshaw, Bradley and Carlson, 1959). Isolated pump-perfused kidney in a heart-lung circuit responded to endotoxin by a transient increase in resistance which recovered within 10 minutes. From the observations made by the above workers, it appears that renal vascular response to endotoxin is secondary to the more fundamental hemodynamic changes occurring in other vascular beds during the initial phase of hypotension.

Hinshaw and co-workers have investigated the changes in the vascular bed of the isolated forelimb of the dog after endotoxin administration. After intravenous administration of endotoxin to the donor dog, the arterial pressure decreased within 5 minutes. During this period, no changes were seen in the isolated perfused forelimb. After this period the arterial and venous resistances increased (Hinshaw et al. 1962a; Hinshaw, Vick, Jordan and Wittmers, 1962b). Since neural influences were excluded by using an isolated preparation, one can conclude that during the first 5 minutes when the hepatic vascular bed was undergoing dramatic changes leading to the initial fall in arterial pressure, no humoral factors were circulating in concentrations large enough to affect the pump-perfused forelimb. It is also possible that some humoral factors were circulating in blood to which the isolated vascular bed was

not responsive. After the arterial pressure had declined (after 5 minutes) there was an increased arterial resistance which was abolished after adrenalectomy. This suggests that it was due to circulating adrenal medullary hormones released by reflexes initiated by the marked fall in arterial pressure. Since during this phase venoconstriction was not modified after adrenalectomy, the limb gained weight (Iampietro et al. 1963; Hinshaw et al. 1964). Perfusing the dog forelimb from the venous outflow of an isolated perfused liver in a heart-lung preparation, Hinshaw and co-workers (1966b) found no changes in the vascular resistance of the limb during the period when the hepatic vascular bed was showing marked hemodynamic changes.

THE LATE HEMODYNAMIC EFFECTS IN THE DOG

The initial phase of hypotension is followed by a phase of partial recovery of arterial pressure and cardiac output. Portal pressure recovers to normal levels. This phase of recovery reaches its peak within 15 to 30 minutes. The initial hypotension leads to a marked decrease in carotid sinus baroreceptor discharge (Trank and Visscher, 1962). This in turn seems to initiate an increased sympathetic activity. During the phase of initial hypotension a rise in the blood catecholamine levels has been reported by various workers (Nykiel and Glaviano, 1961; Rosenberg, Lillehei, Longerbeam and Zimmerman, 1961; Spink, Reddin, Zak, Peterson, Starzecki and Seljeskog, 1966). The release of catecholamines from the adrenal medulla during the initial hypotension depends on increased central sympathetic activity. Denervation of the adrenals or the cervical cord section prevented the release of catecholamines

(Nykiel and Glaviano, 1961; Spink et al. 1966). Adrenalectomized animals showed marked susceptibility to the initial hypotensive response and little or no recovery was seen (Hinshaw et al. 1964; Spink et al. 1966). Similar hemodynamic changes have been reported by Nykiel and Glaviano (1961) after denervation of the adrenals. From the above observations it can be concluded that the phase of partial recovery seen after the initial hypotension is due to reflexly-mediated release of catecholamines from the adrenal medulla. White, Gold and Vaughn (1967) produced evidence for the participation of the kidneys in the partial recovery of arterial pressure. Recently, Hall and Hodge (1971) showed a rise in circulating angiotensin levels during this phase.

After the partial recovery arterial pressure declined again. This was a more gradual hypotension and was associated with a parallel decrease in cardiac output (Weil et al. 1956; Hinshaw et al. 1961b; Brockman et al. 1967). The decrease in cardiac output was due to venous pooling and resulting decreased venous return. Investigations carried out in eviscerated animals showed a smaller but continued pooling of blood (MacLean and Weil, 1956; Hinshaw et al. 1958). Further evidence for extrahepatic pooling of blood has been produced by Brockman and co-workers (1967) and more recently by Blattberg and Levy (1970).

Individual vascular beds continue to show the same hemodynamic changes which were initiated during the initial phase of hypotension. Superior mesenteric vascular resistance remained elevated during this phase (Lillehei et al. 1965; Vaughn, Bersentes, Kirschbaum and Assali, 1967; Hinshaw, 1968; Brobmann et al. 1970; Wangensteen, Geissinger,

Lovett, Glenn and Lefer, 1971). The mechanism of mesenteric vasoconstriction has not been investigated in detail. Celiac blockade has been shown to prevent mesenteric vasoconstriction and to improve the mesenteric arterial flow (Hauman, 1968; Wangensteen et al. 1971). These studies, however, failed to elucidate the mechanism of mesenteric constriction. It is unlikely that an overactivity of sympathetic vasoconstrictor nerves plays any significant role in this response. An isolated, pump-perfused loop of intestine showed a similar increase in resistance in response to endotoxin (Hinshaw, 1968). Mesenteric venous resistance increased and the intestine gained weight during the late phase (Hinshaw and Nelson, 1962, Meyer and Visscher, 1962; MacLean et al. 1956).

Splenic and renal vascular beds showed continued increase in vascular resistance. The renal function deteriorated due to decreased renal blood flow (Frohlich, 1963; Hinshaw et al. 1961c; Shanbour et al. 1971). The forelimb showed increased vascular resistance. Venoconstriction continued with increased limb weight (Hinshaw et al. 1962b; Iampietro et al. 1963).

Conflicting results have been reported by various investigators regarding changes in total peripheral resistance during the late phase. Weil and co-workers (1956) found either no change or an increase in the total peripheral resistance. Other investigators have found an increase in the total resistance (Lillehei et al. 1965; Chien, Chang, Dellenback, Usami and Gregersen, 1966a; Anas et al. 1968; Wangensteen et al. 1971). Brockmann and co-workers (1967) reported an early increase and a late decline in the total peripheral resistance. Hinshaw and co-workers

(1958, 1961b) reported a decrease in the total peripheral resistance in intact as well as in eviscerated dogs. These conflicting reports are not surprising if one considers the complexity of various biochemical as well as hemodynamic changes taking place in the animals during this period.

During the phase of late hemodynamic changes, the arterial pressure is low and the cardiac output has declined to low levels. This would initiate various compensatory reflexes which would in turn alter the hemodynamic status of various vascular beds. The reduced tissue perfusion due to low perfusion pressure or to an increased vascular resistance can lead to release of various vasoactive substances. Endotoxin itself can cause release of various vasoconstrictor as well as vasodilator substances from tissues as well as from blood. Under such conditions the response of the animal will depend on many mutually antagonistic influences.

In addition, the depth and the type of anesthetic agent used in the study will also play its role since it can influence the compensatory reflexes. It must also be remembered that total peripheral resistance is the sum of the vascular resistances of the various vascular beds and therefore gives no information regarding changes occurring in any individual vascular bed (Gilbert, 1960).

Thus it can be concluded that during the late phase, predominant hemodynamic changes which take place are a progressive decrease in cardiac output due to pooling of blood in various vascular beds and an increased vascular resistance in some of the vital organs like the intestine and the kidneys. Resulting ischemia of vital organs can lead to a fatal outcome. Many metabolic as well as histological derangements have been shown to take place in various vascular beds during the late stages of

cardiovascular insufficiency. It is difficult to conclude whether these changes are mediated by a primary action of endotoxin on the tissue or are due to the ischemic changes induced by some more fundamental effects of endotoxins on cardiovascular system.

EARLY AND LATE HEMODYNAMIC RESPONSES OF THE CAT

Initial hemodynamic effects of endotoxin have been investigated in the past. Gilbert, Kuida, Hinshaw, Vick and Visscher (1957) first described the initial hypotensive response. Within 10 to 30 seconds after the intravenous injection of endotoxin, a precipitous fall in arterial pressure was observed. This was accompanied by a brief period of apnea which was followed by hyperpnea. Pulmonary vascular resistance increased during this phase. Some of these cats died during the initial hypotension. Animals which survived showed a phase of recovery similar to one seen in the dog and subsequently responded by a gradual decline of arterial pressure to severe hypotensive levels.

The following year, Kuida and co-workers investigated the mechanism of pulmonary hypertension and increase in the weight of the lungs seen after endotoxin. During the initial hypotensive response the pulmonary arterial pressure increased. This response always preceded the initial decline in the arterial pressure. Left atrial pressure remained unchanged in these cats. Investigations done on isolated perfused lungs revealed an increase in the precapillary as well as the postcapillary resistances. The relative increase in the venous resistance was consistently greater than the increase in the arteriolar resistance. The resulting increase in the capillary hydrostatic pressure caused pulmonary

oedema and increase in the lung weight (Kuida et al. 1958).

In cats, when cardiac output was maintained by an extra-corporeal blood reservoir, significant pooling of blood was observed but this pooling in the peripheral vascular beds was considerably less than that seen in dogs. Unlike dogs, portal venous pressure remained unchanged or showed a very small and transient increase. No mucosal hemorrhages were seen and the intestine appeared pale. Changes in the weight of the isolated loop of intestine were measured. Either a slight gain or a loss in the weight was seen (Kuida et al. 1961). Isolated denervated foreleg perfused with a constant flow pump interposed between the animal and the foreleg showed a small increase in the vascular resistance. No increase in the weight was observed (Hinshaw et al. 1964).

Humoral mechanisms responsible for the pulmonary response in cats are not known. Gilbert (1959) studied the effects of antihistaminic and antiserotonin drugs on the pulmonary vascular responses. Although these agents partly reduced the response, they failed to block the effects produced by large doses of endotoxin. This does not exclude the possibility of histamine and serotonin mediating the pulmonary responses, since the blocking agents also failed to block the effects of large doses of histamine and serotonin. Further work in this direction will be of great value in understanding the mechanism of action of endotoxin.

Animals which survive the initial hypotensive response show the late phase of prolonged hypotension (Kuida et al. 1961; Brockman et al. 1967; Greenway, Lautt and Stark, 1969). Mechanisms responsible for the phase of recovery and subsequent late phase of hypotension have not been investigated.

HEMODYNAMIC RESPONSES OF THE MONKEY

Phylogenetic proximity of monkey to man has attracted many investigators to study the hemodynamic responses of the monkey to intravenous injection of endotoxin. The initial hypotensive response is slow in onset and in this regard it differs from other species (Kuida et al. 1961; Gilbert, 1962; Hinshaw et al. 1966a; Brockman et al. 1967; Vaughn, Gunter and Stooky, 1968). During this phase, an increase in pulmonary arterial pressure as well as portal pressure have been reported (Kuida et al. 1961; Brockman et al. 1967). The mechanism of the gradual decline in arterial pressure has been investigated by Hinshaw and co-workers (1966a) who found a decrease in venous return during total body perfusion. A gradual decline in cardiac output and an associated decrease in total peripheral resistance led to the decrease in arterial pressure (Gilbert, 1962; Hinshaw et al. 1966a; Guenter et al. 1969).

The effect of endotoxin on the mesenteric vascular bed has been investigated. Hinshaw (1968) and Brobmann and co-workers (1970) found a decrease in the mesenteric vascular resistance during early as well as late phases of hypotension. Kuida and co-workers (1961) found only minor changes in the weight of an isolated pump-perfused loop of intestine after endotoxin. Intestinal mucosal hemorrhages and splanchnic pooling of blood, which characterize the initial phase in dogs was not seen in the monkey.

Isolated pump-perfused foreleg of monkey showed a small increase in the vascular resistance. Unlike the response seen in the dog, the weight of the foreleg decreased after endotoxin (Hinshaw et al. 1964). The renal vascular bed responded to endotoxin by an increase in vascular

resistance and marked reduction in urine flow (Hinshaw, Solomon, Reins and Fiorica, 1967a; Vaughn et al. 1968). The increased renal vascular resistance was due to increased sympathetic activity and was blocked by denervation of the kidney (Hinshaw et al. 1967a).

During the late phase of hypotension, progressive decline in arterial pressure, cardiac output, and peripheral resistance was seen. There was no evidence of cardiac depression (Gilbert, 1962). Some increase in the total peripheral resistance during the late stages of hypotension has been reported (Gilbert, 1962; Guenter et al. 1969). The mechanism responsible for the decrease in venous return is not known. Both histamine (Hinshaw, Jordan and Vick, 1961a) and kinins (Nies, Forsyth, Williams and Melmon, 1968) may be responsible for the decrease in total peripheral resistance seen in this species. Plasma catecholamine levels increased during late stages of hypotension (Hinshaw et al. 1967a; Guenter et al. 1969).

HEMODYNAMIC RESPONSES OF OTHER SPECIES

Responses of the coyote (Hinshaw, Holmes, Smith, Reins and Ogilvie, 1967b) and rat (White, Ross, Barajas and Jacobson, 1966) resemble those of the dog. The initial precipitous fall in arterial pressure is associated with a rise in portal venous pressure. Intestinal mucosal lesions similar to those seen in the dog have been reported. In the rat the initial hypotension appears to be due to a decrease in venous return resulting from hepatosplanchnic pooling. Evisceration of rats prevented the initial fall in arterial pressure (White et al. 1966). Responses of the bear also resembled those of the dog. Some minor differences have

been reported (Solomon, Reins, Holmes and Hinshaw, 1966).

Hemodynamic responses of the sheep have been investigated by Halmagyi, Starzecki and Horner (1963). After injection of endotoxin, a marked increase in pulmonary arterial pressure was seen, associated with a decrease in arterial pressure. Pulmonary arterial wedge pressure increased without any change in left atrial pressure. A decrease in cardiac output was also observed. Thus the responses in sheep were similar to those seen in the cat. Similar responses have been reported in the rabbit (Kuida et al. 1961). Hemodynamic effects of endotoxin in the calf resembled those of the cat. However, the pulmonary hypertension was smaller in magnitude and occurred after a longer latent period. Arterial pressure decreased and showed an inverse relation with pulmonary arterial pressure. Cardiac output and total peripheral resistance decreased after endotoxin (Tikoff, Kuida and Chiga, 1966). Shetland ponies responded to endotoxin by an initial phase of hypotension and a late phase which follows the phase of recovery as seen in the cat. Central venous pressure increased (Burrows and Cannon, 1970).

From the literature reviewed, it appears that endotoxin produces an initial phase of hypotension during which either the hepatic vascular bed or the pulmonary vascular bed is predominantly affected. This leads to decreased venous return to the left ventricle due to pooling of blood either in the splanchnic vascular bed (as in the dog) or in the pulmonary vascular bed and the large systemic veins (as in cats). During the phase of recovery, arterial pressure returns to near normal levels and declines again, this time, more gradually, to marked hypotensive levels. Although in most of the species this later fall in arterial pressure

results from decreased cardiac output, in some species decreased total peripheral resistance also contributes to it. The responses of the monkey are similar in that pulmonary hypertension occurs in this species. Probably it is not of a magnitude to produce any significant pooling in the pulmonary vascular bed, hence the precipitous fall in arterial pressure seen in other species is not seen in the monkey.

OTHER BIOLOGICAL EFFECTS OF ENDOTOXIN

Various biological effects of endotoxin have been investigated in detail and have been reviewed by various investigators. Some of these biological effects will be considered in this section.

The pyrogenic effect of endotoxin is well documented (Bennet and Cluff, 1957; Atkins, 1960). Release of endogenous pyrogen from leucocytes (Wood, 1958) as well as direct cerebral action of endotoxin (Bennet, Petersdorf and Keene, 1957) have been implicated in the mechanism of this febrile response. Tolerance to this effect of endotoxin can be induced in animals and in man by repeated injections of small doses (Beeson, 1947a, 1947b; Morgan, 1948) and by slow intravenous infusions (Greisman and Woodward, 1965).

Endotoxin has profound effects on the formed elements of blood. After an intravenous administration of endotoxin, an initial leucopenia followed by a prolonged leucocytosis has been observed (Atkins, 1960). The mechanism of this leucopenic response is poorly understood. It is likely that lysis of these cells (Dennis and Senekjian, 1939) as well as sequestration into various organs, especially the liver and the lungs may be contributing to it (McKay, Margaretten and Csavossy, 1967; Branemark

and Urbaschek, 1967; Harrison, Beller, Hinshaw, Coalson and Greenfield, 1969). Endotoxin also affects the migration of leucocytes (Mergenhagen, Snyderman, Gewurz and Shin, 1969).

In the experimental animals, an intravenous administration of endotoxin causes marked thrombocytopenia (Shimamoto, Yamazaki, Ohno, Uchida, Konishi and Iwahara, 1958a; Horowitz, Des Prez and Hook, 1962; Cohen, Braunwald and Gardner, 1965). Intravenously administered endotoxin is rapidly taken up by platelets (Braude et al. 1955) which phagocytose the endotoxin and undergo degranulation (Davis, 1966; Spielvogel, 1967). A heat-labile plasma factor is required for endotoxin induced platelet injury (Des Prez, 1967). Such injury results in release of serotonin (Shimamoto, Yamazaki, Sagawa, Iwahara, Konishi and Maezawa, 1958b; Davis et al. 1960a; Davis, Meeker and Bailey, 1961; Des Prez, Horowitz and Hook, 1961; Kobold, Lovell, Katz and Thal, 1964), and platelet factor 3 (Horowitz et al. 1962).

Effects of endotoxin on blood coagulation are well known (McKay and Shapiro, 1958; Gans and Krivit, 1960; McKay, 1969). Immediately following an intravenous administration of endotoxin an initial decrease (Deutsch and Elsner, 1960) and later a prolongation of clotting time occurs (Hardaway, Husni, Geever, Noyes and Burns, 1961). This is associated with a decrease in circulating fibrinogen levels (Gans and Krivit, 1961; Hardaway and Johnson, 1963), as well as the levels of factor V and factor VII (Hardaway and Johnson, 1963). Fibrin deposition has been demonstrated in various organs in the monkey (McKay et al. 1967), the rabbit (Beller, Graeff and Gorstein, 1969), and the rat (Muller-Berghaus

and McKay, 1967). In dogs a simultaneous increase in the plasminogen-activator activity and a decrease in the plasminogen levels of plasma have been seen. This leads to the lysis of clots, and therefore the thromboembolic phenomenon, seen in the rabbit, is not seen in the dog. In rabbits, the activation of plasminogen-plasmin system is not seen (Gans and Krivit, 1961).

Endotoxin-induced local dermal necrosis has been investigated extensively in the past (Thomas, 1954; Rosen, 1961b; Raskova and Vanecek, 1964). Local Shwartzman reaction is a phenomenon of local reactivity leading to dermal necrosis. It is induced by a preparatory intradermal injection of endotoxin followed 18 to 24 hours later by a provoking dose. Dermal necrosis develops at the site of preparatory injection. By giving the preparatory as well as the provoking doses intravenously, one can induce the generalized Shwartzman reaction. This reaction is characterized by occurrence of bilateral renal cortical necrosis.

Endotoxins also have tumor-necrotising activity. Many other biological effects of endotoxin have been adequately reviewed (Burrows, 1951; Thomas, 1954; Rosen, 1961a, 1961b, Raskova and Vanecek, 1964) and will not be discussed. Recently Neter has reviewed the immunological aspects of effects of endotoxin (Neter, 1969).

A PREFATORY LOOK AT THE PRESENT INVESTIGATION

In the preceding section considerable emphasis has been laid on the separation of the hemodynamic changes into an initial response and a more delayed response. Initial hemodynamic changes take place during the first 10-15 minutes after a rapid intravenous injection of endotoxin. Considerable evidence has accumulated which suggests that the initial hemodynamic changes are due to an anaphylactic or anaphylactoid response. In the past, much work has been done to understand the mechanism of the initial hemodynamic responses in various species, but little is known about the nature of delayed hemodynamic changes and the fatal outcome.

A considerable amount of work has been carried out in an attempt to modify the irreversible cardiovascular failure after lethal doses of endotoxin by various pharmacological agents. Although some of these agents, especially corticosteroids and adrenergic blocking agents, have improved survival in some cases, they have not helped in the understanding of the mechanism of delayed hemodynamic changes. Some attempts have been made in the past to dissociate the immediate hypotensive response from the delayed response and the fatal outcome. Hildebrand, Ng, Seys and Madin (1966) first demonstrated that small doses of endotoxin failed to produce the initial hypotensive response in rabbits while the delayed hemodynamic changes were seen. They subjected rabbits to anaphylactic shock and showed that such animals were rendered tachyphylactic to a subsequent challenge of the antigen. They challenged these rabbits with a lethal dose of endotoxin and showed that the initial phase was suppressed while the delayed phase was not modified. On the basis of their observations they proposed that the initial and delayed phases may be the outcome of two independent mechanisms which may not have any causal rela-

tionship. Reddin, Starzecki and Spink (1966) investigated the effects of endotoxin on arterial pressure and mortality in adult dogs and 2 to 4 week old puppies. The puppies did not show the typical initial response but were more susceptible to the lethal effects of endotoxin, while the adult dogs were less susceptible although they responded by a typical initial hypotension.

Following this, Greenway and co-workers (1969) reported an interesting observation in cats. They used endotoxin which was subjected to mild alkaline hydrolysis and found that this treatment abolished the initial hypotensive response while the delayed hypotension was still present and the mortality was unaffected. This further supported the concept that the delayed hemodynamic changes have an independent mechanism, and that they are not secondary consequences of the initial hemodynamic changes.

When a lethal dose of endotoxin is injected intravenously into a healthy adult animal the initial response is produced. This response is sometimes of such intensity that it could be fatal. If the animal survives, an array of compensatory mechanisms will come into operation and thus alter the whole hemodynamic status of the animal. Under such circumstances it would be difficult to differentiate the late hemodynamic changes which are secondary consequences of the initial hypotensive response from those which are due to more fundamental actions of endotoxin per se. To investigate delayed hemodynamic changes which are independent of the initial response, it is mandatory that the initial hypotensive response is prevented.

The following investigation is an outcome of an attempt to study the hemodynamic changes which occur in an animal after the initial hypotensive response is abolished. This investigation was carried out in cats which were normotensive after administration of a lethal dose of endotoxin. It is therefore not a study of hemodynamic changes during endotoxin shock.

THE PRESENT INVESTIGATION

SECTION I.

INITIAL HYPOTENSIVE RESPONSE OF CATS TO AN INTRAVENOUS INJECTION OF ENDOTOXIN AND ITS MODIFICATION BY ACETYLSALICYLIC ACID.

INTRODUCTION

The response of the anesthetized cat to a lethal dose of endotoxin consists of an acute and a delayed phase. The acute response is manifested by a decrease in arterial pressure and a rise in right atrial pressure (the initial phase). If the animal survives, the arterial pressure recovers but then declines again after several hours and the animal dies (the late phase). The initial phase could be abolished in a significant proportion (70%) of the cats without abolition of the late phase of hypotension and death if the endotoxin was subjected to mild alkaline hydrolysis. Thus it was shown that in cats, the late phase of hypotension and death are not secondary consequences of the initial hypotensive response (Greenway et al. 1969).

The present investigation is an extension of the above study. Since mild alkaline hydrolysis is likely to cause physico-chemical alterations in the endotoxin molecule (Neter, Westphal, Luderitz, Gorzynski and Eichenberger, 1956; Shands, 1971), the above method was not suitable for further investigations. Search for a better method led to the following considerations.

The initial hypotensive response in the rabbit is due to severe pulmonary hypertension and a resulting decrease in cardiac output (Kuida et al. 1961). It has many characteristics of an anaphylactic response (Gilbert and Braude, 1962; Hildebrand et al. 1966). In cats endotoxin produces similar effects on the pulmonary vascular bed (Kuida et al. 1961; Brockman et al. 1967). Although the pulmonary vascular response of cats to endotoxin has not been investigated in detail, cats have been shown to

respond to anaphylactic stimuli by marked pulmonary emphysema, hemorrhage and oedema (McCusker and Aitken, 1966). In dogs, the initial hypotensive response has been shown to have many features of an anaphylactic reaction (Spink and Vick, 1961; Spink et al. 1964).

We selected acetylsalicylic acid for our experiments to block the initial hypotensive response on the basis of the following reports in the literature. In rabbits acetylsalicylic acid has been shown to prevent anaphylactic reaction (Campbell, 1948; Lepper, Caldwell, Smith and Miller, 1950). The response of pulmonary vascular bed of rabbits depends on the presence of platelets and thrombocytopenic animals do not show the typical response to endotoxin (Evans and Mustard, 1968). Endotoxins are known to cause platelet agglutination and release of vasoactive substances (Des Prez et al. 1961; Des Prez, 1967). Acetylsalicylic acid prevents the platelet aggregation induced by endotoxin (Mustard, Evans, Packham and Nishizawa, 1969), and inhibits the release of serotonin from the platelets (Evans, Nishizawa, Packham and Mustard, 1967). In dogs, endotoxin induced portal hypertension and the initial phase of hypotension was markedly reduced after pretreatment with acetylsalicylic acid (Hinshaw, Solomon, Erdos, Reins and Gunter, 1967c). The effect of acetylsalicylic acid on the hemodynamic effects of endotoxins in the cat has not been investigated.

METHODS

Cats were used for the experiments after a preliminary period of observation lasting 4 to 7 days. Sodium pentobarbital (30 mg/kg Nembutal, Abbott Laboratories Ltd.), was injected intraperitoneally to anesthetize the animals. When reflex limb and eye movements returned, additional doses (7.5 mg) of sodium pentobarbital were given intravenously.

The left femoral artery was cannulated to record arterial pressure. The right external jugular vein was cannulated to record the right atrial pressure. The left forelimb cutaneous vein was cannulated for intravenous injections. Surgery was carried out under clean but not sterile conditions. Arterial and right atrial pressures were recorded on a Beckman type R Dynograph with Statham Series P-23 pressure transducers.

Acetylsalicylic acid (Matheson, Coleman and Bell) was dissolved in warm ammonium acetate solution (1.15 gm/100 ml water). The final solution used for intravenous infusion contained 25 mg/ml of acetylsalicylic acid. In all the experiments endotoxin obtained from Salmonella enteritides (Bactolipopolysaccharide, Difco Laboratories) has been used. This toxin was isolated by Boivin's trichloroacetic acid procedure as modified by Webster, Sagin, Landy and Johnson (1955). The endotoxin was suspended in 5 ml of Ringer-Locke solution. Cats were divided into four groups.

Group I. Six cats were given 4 ml/kg of ammonium acetate solution (solvent) followed after 20 minutes by endotoxin (3 mg/kg).

Group II. Six cats were given acetylsalicylic acid (100 mg/kg) in ammonium acetate solution followed after 20 minutes by endotoxin (3 mg/kg).

Group III. Five cats were given acetylsalicylic acid (100 mg/kg) in ammonium acetate solution followed after 20 minutes by 5 ml of Ringer-Locke solution.

Group IV. When this data was communicated (Murthy and Greenway, 1971), it was pointed out that the dose of acetylsalicylic acid (100 mg/kg) was large in comparison with normal therapeutic doses used in man. Therefore in 3 cats, similar experiments were carried out with smaller doses of acetylsalicylic acid (10 mg/kg).

In all the cats, arterial pressure and right atrial pressure were recorded for at least one hour after the initial treatment. After this period, Penicillin G (100,000 U) and Streptomycin (100 mg) were injected intramuscularly to each cat and 5 ml of 25% glucose in 5 ml Ringer-Locke solution was given intravenously. Wounds were closed and the animals allowed to recover under comfortable conditions. Animals which were alive and active after 48 hours were considered survivors. An autopsy was performed on all the cats which died during the 48 hours of observation. Survivors were sacrificed after 48 hours and an autopsy was performed.

RESULTS

Effects of ammonium acetate and acetylsalicylic acid.

Infusion of ammonium acetate solution produced no marked changes in arterial pressure or right atrial pressure. A transient increase in arterial pressure was observed after administration of acetylsalicylic acid. Arterial pressure recovered to preinjection levels within 20 minutes. No significant changes in right atrial pressure were observed.

Effects of endotoxin

In cats given ammonium acetate alone (Group I), endotoxin produced its typical hemodynamic effects. In 4 cats, an acute hypotension developed within 1 to 2 minutes of the intravenous injection associated with a marked rise in right atrial pressure. The arterial pressure did not recover and the cats died during this initial phase. Respiratory arrest preceded cardiac arrest. A typical response is shown in Fig. 1. In the remaining 2 cats the acute hypotension occurred accompanied by a marked rise in right atrial pressure. However, after a short period, the arterial pressure recovered and right atrial pressure returned to control values as shown in Fig. 2.

Pretreatment of cats with acetylsalicylic acid (100 mg/kg) prevented the initial hemodynamic changes seen in solvent pretreated cats. No initial fall in arterial pressure or increase in right atrial pressure was observed in any cat. A typical response to endotoxin in a cat pretreated with acetylsalicylic acid is shown in Fig. 3. Fig. 4 compares the arterial pressure and right atrial pressure seen after

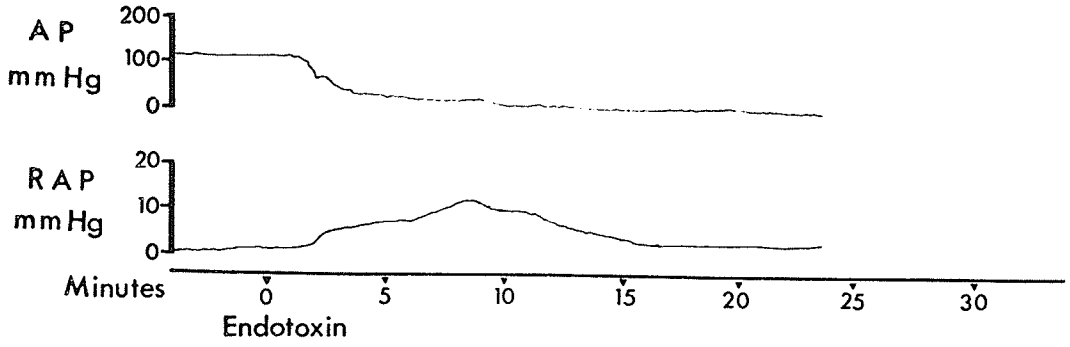


Figure 1: Record of arterial pressure (AP) and right atrial pressure (RAP) showing acute hypotensive response resulting in death after administration of endotoxin.

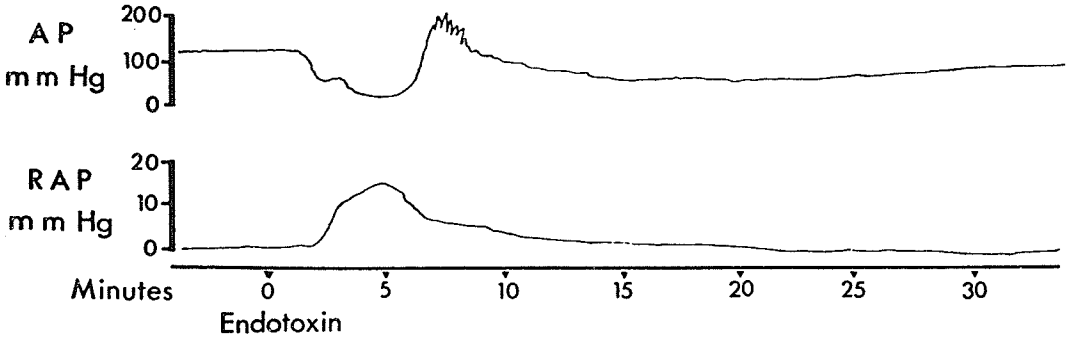


Figure 2: Record of arterial pressure (AP) and right atrial pressure (RAP) showing acute hypotensive response followed by recovery after intravenous administration of endotoxin.

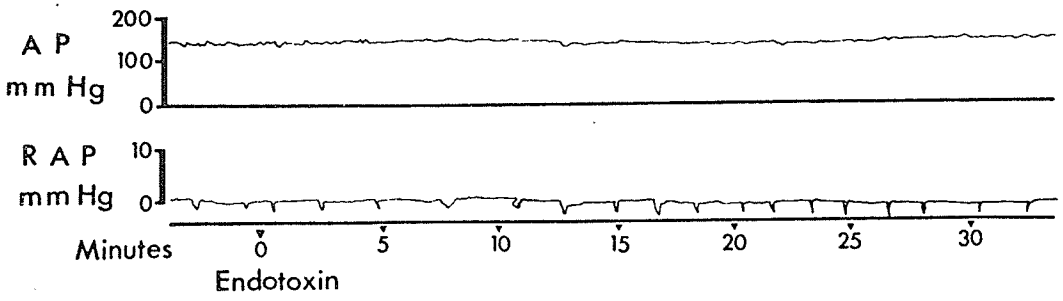


Figure 3: Record of arterial pressure (AP) and right atrial pressure (RAP) showing protection from endotoxin-induced acute hypotensive response in a cat given 100 mg/kg of acetylsalicylic acid 20 minutes prior to challenge with endotoxin.

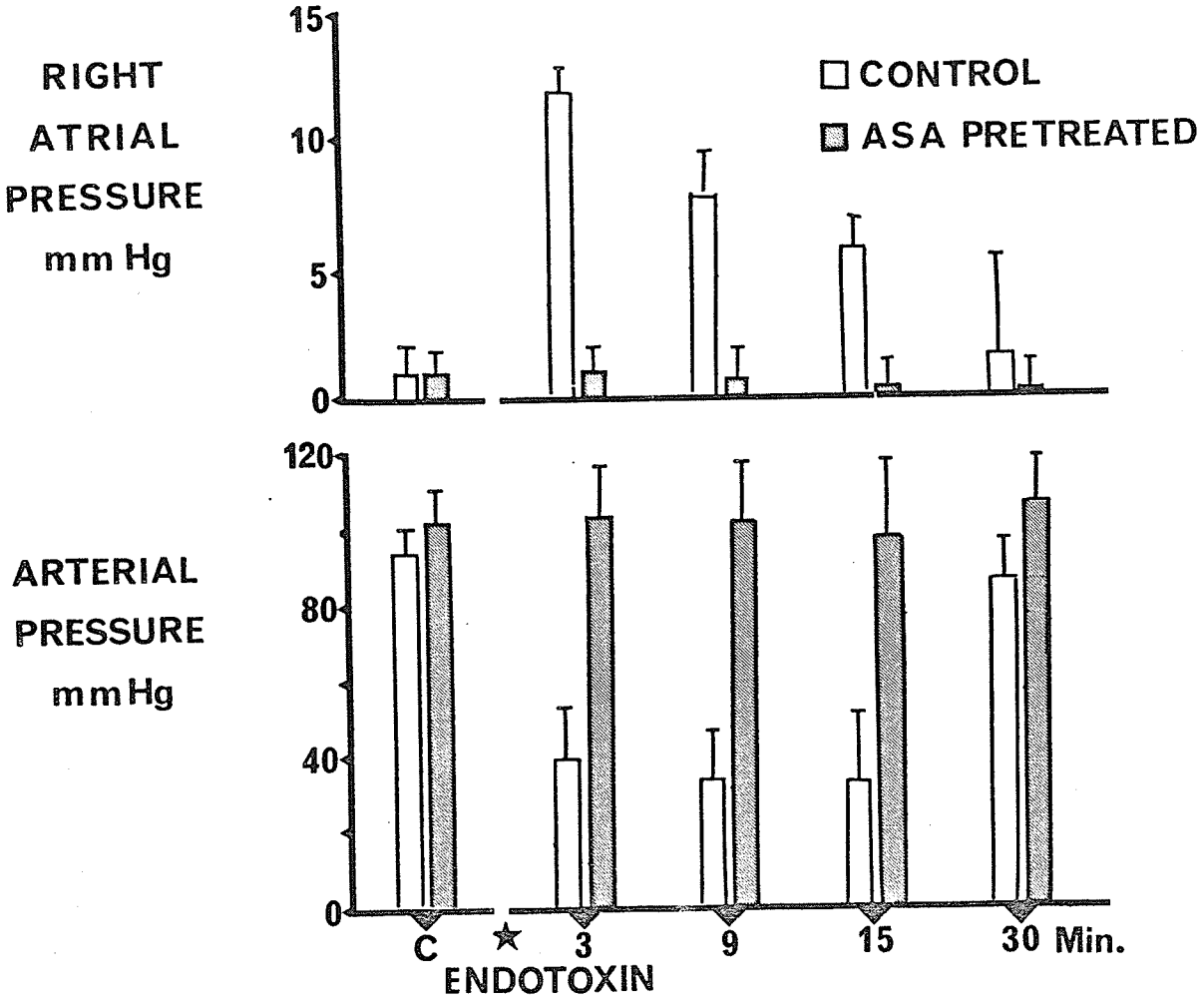


Figure 4: Changes in arterial pressure and right atrial pressure (mean ± SE) induced by endotoxin in control cats and in cats pretreated with acetylsalicylic acid (ASA).

intravenous administration of endotoxin in control (Group I) and acetylsalicylic acid-pretreated cats (Group II).

In cats pretreated with a smaller dose of acetylsalicylic acid (Group IV), right atrial pressure did not change at any time during the period of observation. In 2 cats arterial pressure remained unchanged and in 1 cat it decreased by 30mm of Hg but soon recovered. In 5 cats, treated with acetylsalicylic acid only (Group III), no significant hemodynamic changes were seen during the period of observation.

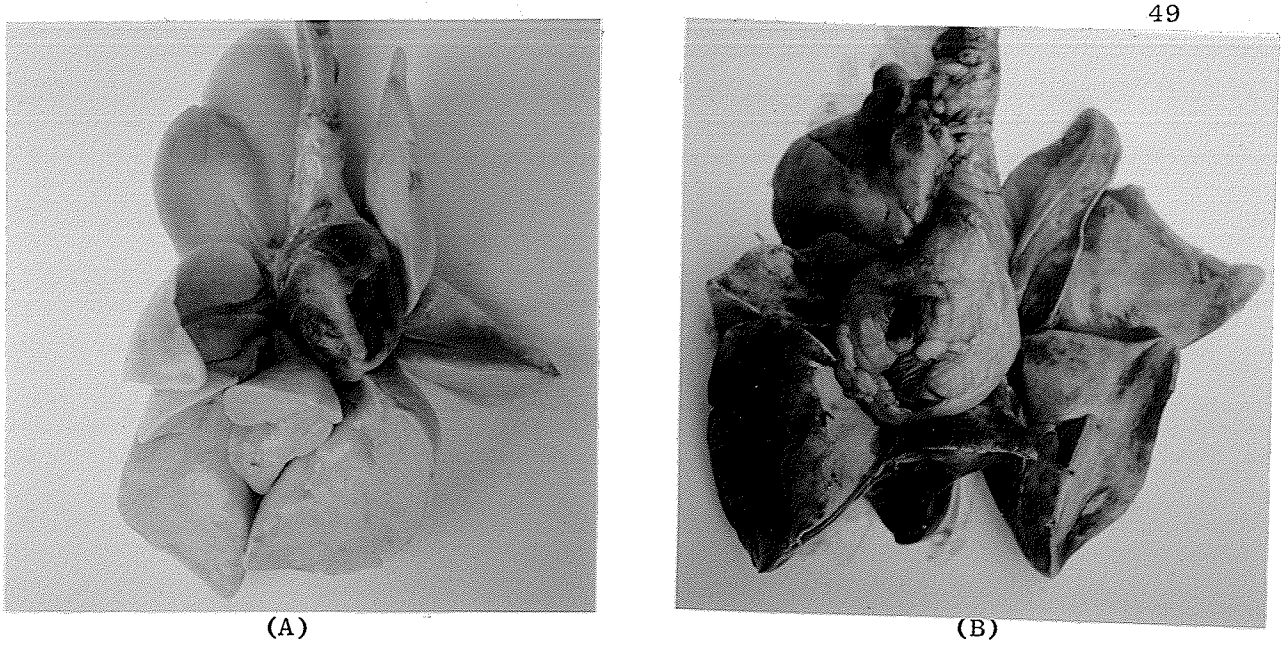
Mortality during the 48 hours following the experiments is presented in Table 1. All the cats which were pretreated with solvent (Group I) died. Four cats died during the initial hypotensive phase and the remaining 2 cats died within the next 24 hours. Five cats given endotoxin after pretreatment with acetylsalicylic acid (100 mg/kg) (Group II), died within 24 hours. Similar pretreatment with a smaller dose of acetylsalicylic acid (10 mg/kg) (Group IV), failed to offer any significant protection from the lethal effects of endotoxin. Two out of the 3 cats died within 24 hours. The third cat survived for 48 hours, during which it suffered from bloody diarrhea. All 5 cats given only acetylsalicylic acid (Group III), survived.

Autopsy was performed. Cats which died of the initial hypotensive response showed marked congestion and hemorrhagic patches in the lungs. Hemorrhages were seen in the endocardial surface of the atria. The intestine was in spasm and the spleen was contracted. No other abnormality was seen. Cats treated with acetylsalicylic acid did not show the pulmonary congestion and hemorrhages. Fig. 5 compares lungs

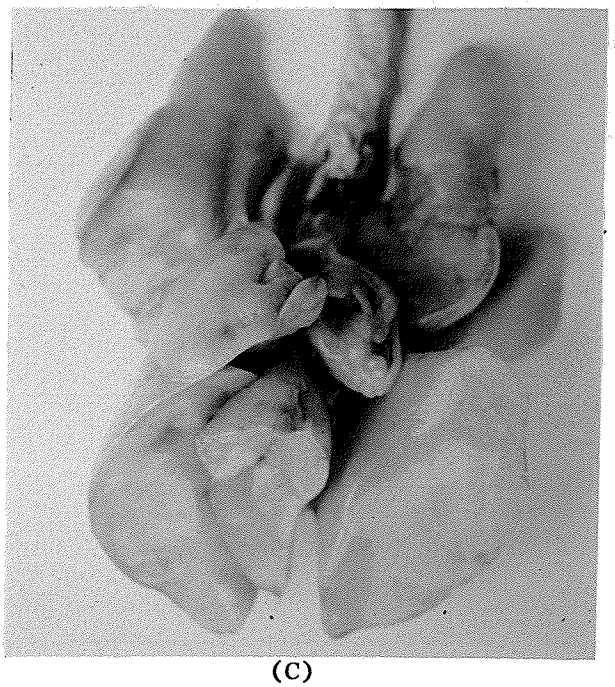
TABLE 1

Summary of the effect of acetylsalicylic acid on the survival of cats injected with 3 mg/kg of endotoxin.

	Pretreatment	Challenge	No. of cats	No. of cats survived
Group I	Solvent	Endotoxin	6	0
Group II	Acetylsalicylic acid 100 mg/kg	Endotoxin	5	0
Group IV	Acetylsalicylic acid 10 mg/kg	Endotoxin	3	1
Group III	Acetylsalicylic acid 100 mg/kg	-	5	5



(A) (B)



(C)

Figure 5: Photographs showing the heart, lungs and trachea from a healthy cat (A), a cat given endotoxin after pretreatment with solvent (B) and a cat given endotoxin after pretreatment with acetylsalicylic acid (c). The endotoxin-induced hemorrhagic lesions in the lungs seen in cats pretreated with solvent are not seen after pretreatment with acetylsalicylic acid.

from an untreated cat (Fig. 5A), a cat given endotoxin after ammonium acetate (Fig. 5B) and a cat given 100 mg/kg of acetylsalicylic acid before challenge with endotoxin (Fig. 5C). In acetylsalicylic acid-pretreated cats, which died due to endotoxin, marked hemorrhagic patches were seen in the intestine and colon. The lumen was filled with mucus, fluid and blood.

Occasional hemorrhages were seen in the mesenteric lymph nodes. The liver was dark and hard in consistency. All the cats which were treated with acetylsalicylic acid only (Group III) showed no abnormality on gross examination.

It was concluded that ammonium acetate does not modify the initial hypotensive response seen in cats challenged with intravenous injection of a lethal dose of endotoxin. Pretreatment with acetylsalicylic acid abolishes the initial hypotension as well as the rise in right atrial pressure. However, such pretreatment does not protect the cats from the lethal effects of endotoxin. In the doses used, acetylsalicylic acid alone does not lead to any morbid or fatal outcome.

SECTION II.

HEMODYNAMIC RESPONSES OF CATS TO INTRAVENOUSLY INJECTED ENDOTOXIN AFTER PRETREATMENT WITH ACETYLSALICYLIC ACID.

INTRODUCTION

In our investigations on the initial hemodynamic effects produced by endotoxin, acetylsalicylic acid was found to be effective in blocking the acute hypotension and the rise in right atrial pressure. However such pretreatment with acetylsalicylic acid did not modify the mortality in cats. It was therefore concluded that the initial hypotension is probably one of the several lethal effects endotoxin exerts on animals. It appears that blockade of the initial hypotensive response with acetylsalicylic acid leaves other effects to play an important role in leading the animal to a fatal end.

Cats which were pretreated with acetylsalicylic acid and which died after endotoxin revealed gross pathological lesions in the intestinal wall, colon and lymph nodes. This was suggestive of involvement of these mesenteric organs in the fatal outcome. Such pathological lesions have been described in dogs after intravenous injection of endotoxin (MacLean and Weil, 1956; Lillehei and MacLean, 1958; Hinshaw, Brake and Emerson, 1965). Since such changes also occur in low flow states (Chiu, McArdle, Brown, Scott and Gurd, 1970), an attempt was made to investigate the hemodynamic changes in the mesenteric vascular bed in cats pretreated with acetylsalicylic acid.

METHODS

Cats of either sex weighing between 1.7 and 3.5 kg (2.7 ± 0.1 kg mean ± S.E.) fasted overnight after an initial period of observation extending from 3 days to one week, and were used for the experiments. Anesthesia was induced by intraperitoneal injection of sodium pentobarbital (30 mg/kg Nembutal, Abbott Laboratories Ltd.). Additional doses of 7.5 mg were injected intravenously when reflex ear and swallowing movements returned. The trachea was cannulated to ensure a free airway. In all experiments cats respired spontaneously. All experiments were performed on warm tables maintained at 38°C. Rectal temperature was recorded. The abdomen was opened by a midline incision. Free edges of the skin, rectus abdominis muscle and the peritoneum, on either side, were sutured.

Arterial pressure was measured from a cannula placed in the left femoral artery. In experiments where right atrial pressure was recorded, a cannula was placed in the right external jugular vein. For measurement of portal venous pressure, a tributary draining the appendix was cannulated. All cannulae were filled with Ringer-Locke solution containing Heparin sulphate (2 mg/ml). Statham Series P-23 pressure transducers were used to measure the arterial and venous pressures.

A 1.5 cm long segment of the superior mesenteric artery was dissected free from surrounding nerve fibers and lymphatic tissue close to its origin from the abdominal aorta (Fig. 6A). Care was taken to prevent any damage to the nerve fibers.

An electromagnetic flowmeter probe (2 mm internal diameter) was tied to a micrometer-controlled screw clamp (Fig. 6B) and placed

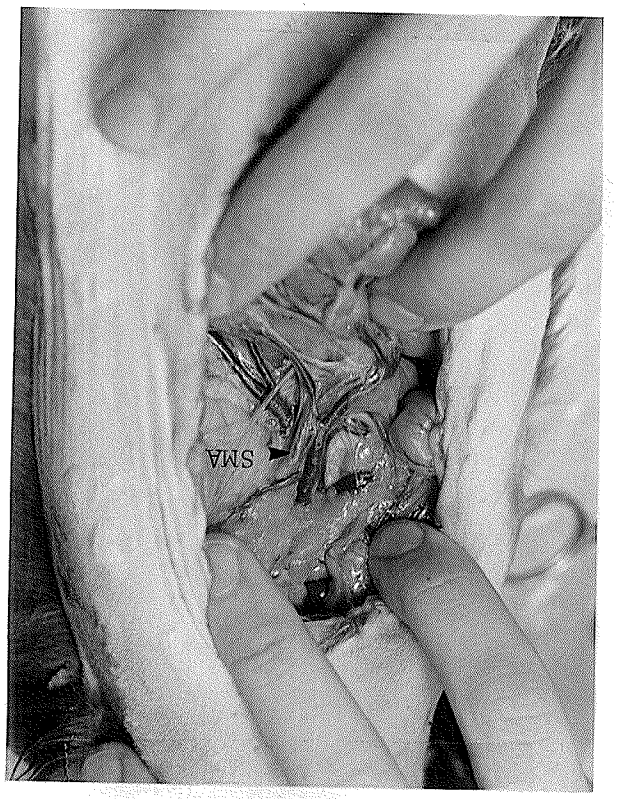


Figure 6 (A)

Photograph showing the origin of superior mesenteric artery (SMA) and the sympathetic nerve fibers around it.

Figure 6 (B)

The lateral view of the micrometer-controlled screw clamp and the flow probe.

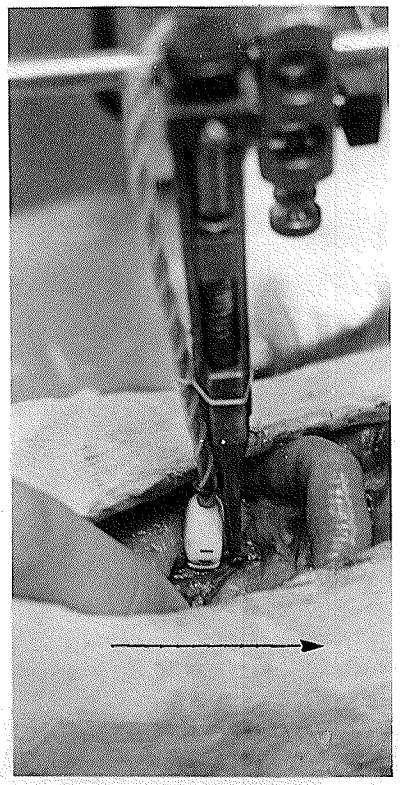


Figure 6 (C)

The position of the micrometer screw clamp and the flow probe during the experiment. The arrow shows the direction of blood flow in the artery.

around the cleaned segment of the artery. The screw clamp was placed downstream from the flowmeter probe (Fig. 6C). This procedure served two purposes. Firstly, the micrometer-controlled screw clamp maintained the flowmeter probe in a steady position during the period of the experiment. Secondly, it was used to occlude the flow in the superior mesenteric artery for the zero calibration of the flowmeter, which was carried out several times during the course of the experiment. The electromagnetic flowmeter probe was coupled to an electromagnetic flowmeter (Nycotron, Oslo, Norway). Signals were fed to Beckman type R Dynograph recorder and monitored for the duration of the experiments. A schematic diagram of the preparation is shown in Fig. 7.

At the end of each experiment, the flowmeter was calibrated in the following manner. Heparin sulphate (5 mg/kg) was injected intravenously. The superior mesenteric artery was occluded with the micrometer screw clamp and cannulated downstream from the flow probe. The screw clamp was released and timed volumes of blood were collected in graduated cylinders. Several such collections were made. From these timed volumes of blood and their corresponding flowmeter records, superior mesenteric arterial flow was calculated and the mean of these values was used to calibrate the flow record obtained during the experiment.

Acetylsalicylic acid was dissolved in warm ammonium acetate solution (1.15 gm in 100 ml distilled water). The final solution contained 25 mg/ml of acetylsalicylic acid. Endotoxin was suspended in 5 ml of Ringer-Locke solution. In 8 cats 100 mg/kg of acetylsalicylic acid was infused intravenously followed 20 to 30 minutes later by endo-

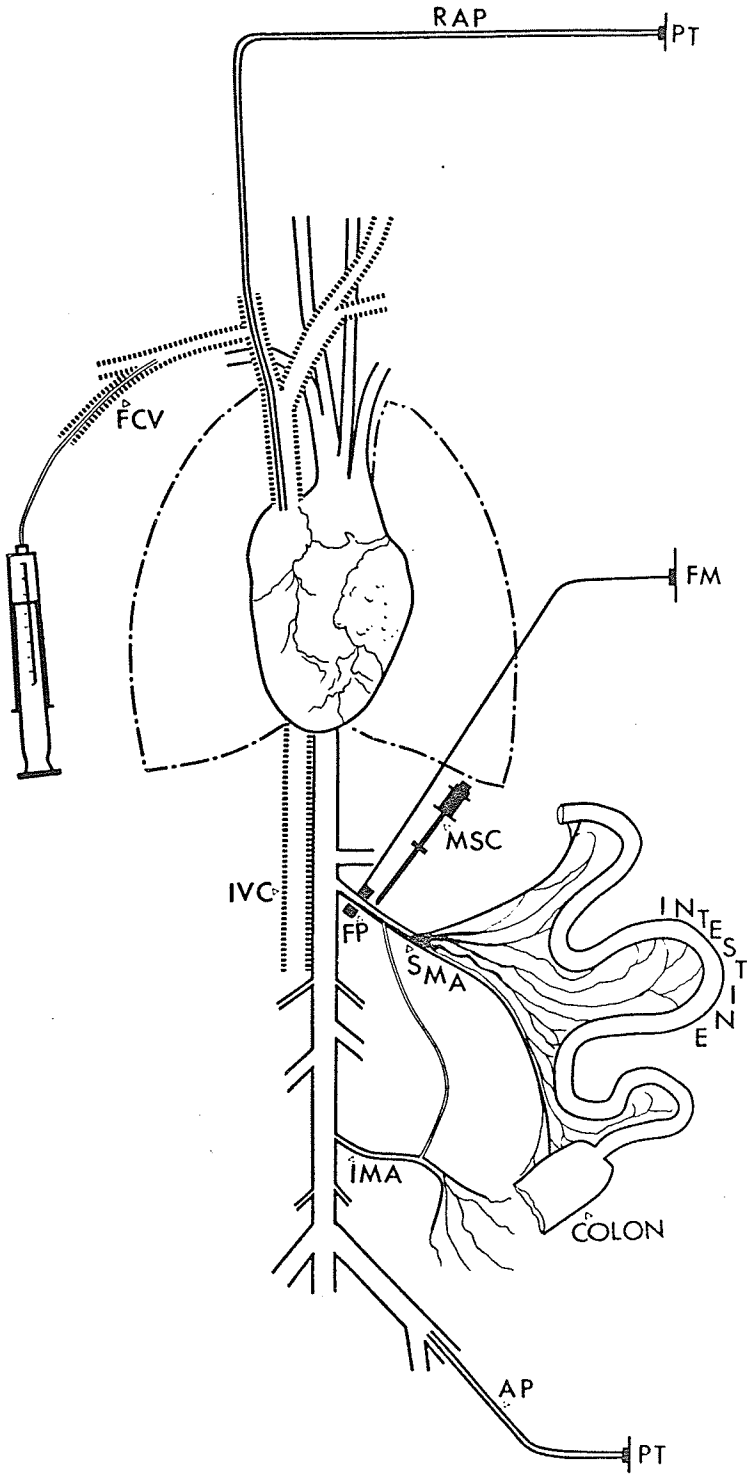


Figure 7: Schematic presentation of the preparation used to investigate the effects of endotoxin on arterial pressure (AP), right atrial pressure (RAP) and superior mesenteric arterial (SMA) flow. (FP- flowmeter probe, FM- flowmeter, MSC- micrometer screw clamp, PT- pressure transducer, IMA- inferior mesenteric artery, IVC- inferior vena cava, FCV- forearm cutaneous vein.)

toxin (3 mg/kg). In 3 cats 100 mg/kg of acetylsalicylic acid was infused but no endotoxin was injected and these cats served as controls.

In another set of 5 cats a smaller dose of acetylsalicylic acid (10 mg/kg) was infused, followed 20 to 30 minutes later by endotoxin. One cat which served as control experiments was given 10 mg/kg of acetylsalicylic acid and no endotoxin was injected into these cats.

At the end of the experiment the cats were sacrificed and lungs, heart and the abdominal viscera were examined.

Mesenteric vascular conductance (Stark, 1968) was calculated as the superior mesenteric arterial blood flow divided by the pressure gradient across the mesenteric vascular bed (arterial pressure minus portal venous pressure) and values during the experimental procedure were expressed as a per cent of resting value. Changes in superior mesenteric blood flow are expressed as a per cent of resting flow.

RESULTS

Effects of acetylsalicylic acid and ammonium acetate.

In 11 cats acetylsalicylic acid was injected intravenously in doses of 100 mg/kg. This dose of acetylsalicylic acid produced a transient rise in arterial pressure. Within 3 minutes arterial pressure increased from 125 $\pm$ 6 mm of Hg (mean $\pm$ S.E.) to 143 $\pm$ 4 mm of Hg, and thereafter declined to 135 $\pm$ 8 mm of Hg at 15 minutes. Within 20 to 30 minutes arterial pressure returned to control values. In cats injected with 10 mg/kg of acetylsalicylic acid, no significant changes in the arterial pressure were observed.

A transient increase in the superior mesenteric arterial flow was seen when acetylsalicylic acid was injected in doses of 100 mg/kg. The blood flow returned to control values within 9 minutes. No change in the blood flow was observed when a smaller dose (10 mg/kg) of acetylsalicylic acid was injected. No significant change was seen in the calculated superior mesenteric arterial conductance with either 10 mg/kg or 100 mg/kg of acetylsalicylic acid.

In 12 cats, right atrial pressure was measured. Acetylsalicylic acid in doses of 100 mg/kg (5 cats) and 10 mg/kg (7 cats) produced no changes in the right atrial pressure. In 5 cats portal venous pressure was measured. Acetylsalicylic acid in either dosage had no effect on portal venous pressure.

It was concluded that pretreatment with acetylsalicylic acid did not have any significant hemodynamic effects on the mesenteric vascular bed. The transient increase in the blood flow was due to the rise in arterial pressure.

Superior mesenteric arterial flow responses.

Superior mesenteric arterial flow was recorded in 20 cats. Mean superior mesenteric arterial flow (resting level) in this group was 84 ± 13 ml per minute or 33 ± 5 ml min^{-1} kg^{-1}. Mean arterial pressure (resting level) was 129 ± 5 mm of Hg.

Endotoxin (3 mg/kg) was injected intravenously in 8 cats after pretreatment with 100 mg/kg of acetylsalicylic acid. A typical response is shown in Fig. 8. Calculated results from these 8 experiments are presented in Fig. 9. Arterial pressure decreased within 3 minutes reaching 81.2% of the resting value within 9 minutes. After this period the pressure recovered reaching 97.2% of the resting values within 30 minutes. No significant change was seen after this period. Superior mesenteric arterial flow showed no change during the first 9 minutes and then started declining, reaching 55% of the resting level by 30 minutes and 29% of the resting level by 60 minutes. After this period flow remained at 20-30% of the resting level. The time of onset of the decrease in flow varied in different animals. In 4 cats, the flow began to decrease by 9 minutes after the endotoxin. In the other 4 cats, the decrease was preceded by an increase in flow which lasted for 15-30 minutes. These variations account for the large standard errors during first 30 minutes after endotoxin in Fig. 9. In all the experiments arterial pressure remained above 100 mm of Hg and flow remained low for 180-550 minutes (mean, 280 minutes). After this, the experiments were either terminated or the arterial pressure slowly decreased and the animal died.

Calculated conductance reflects the state of the resistance

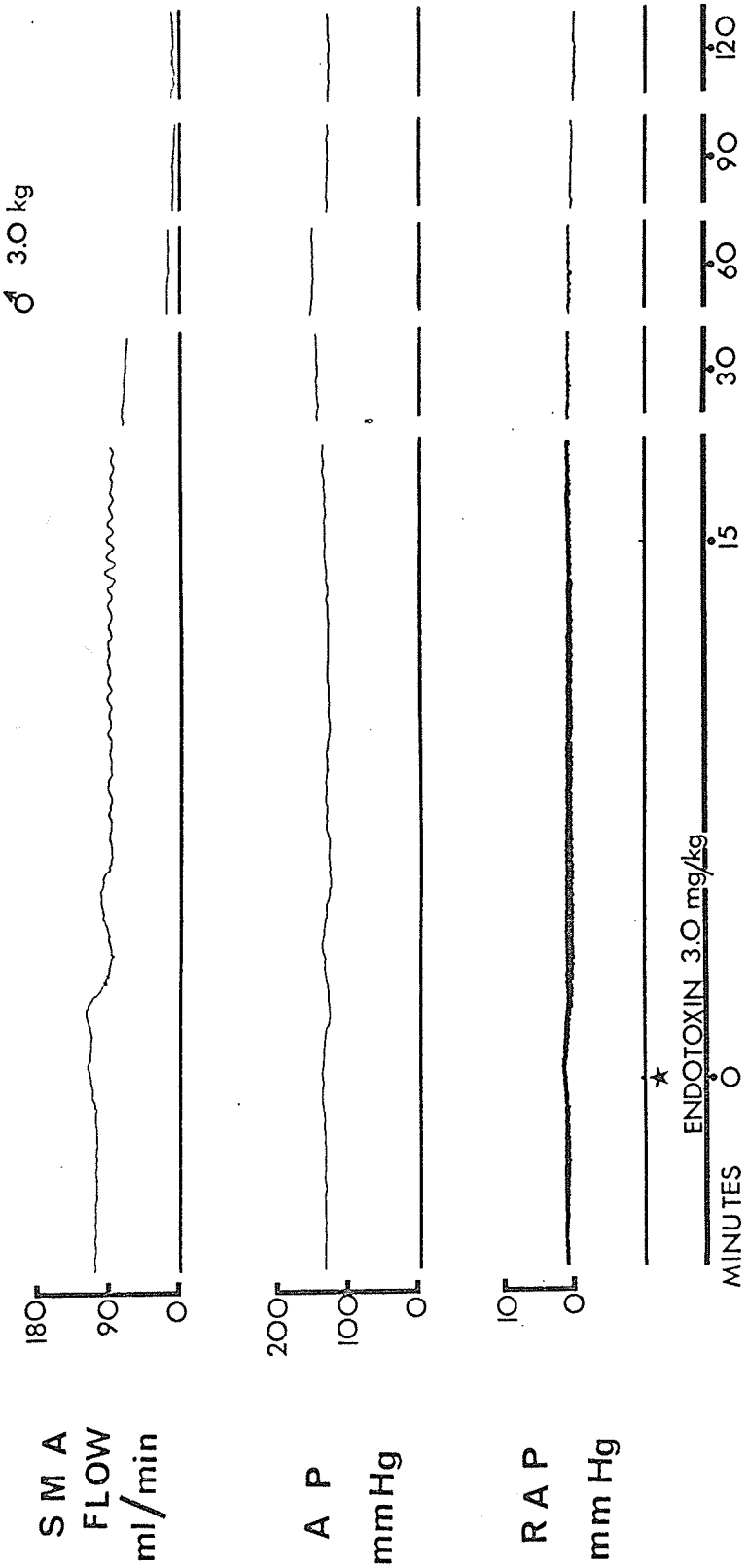


Figure 8: Record of arterial pressure (AP), superior mesenteric arterial flow (SMA FLOW) and right atrial pressure (RAP) after intravenous administration of endotoxin showing development of mesenteric vasoconstriction without hypotension or rise in right atrial pressure. The cat was given pretreatment with acetylsalicylic acid (100 mg/kg) 20 minutes prior to the administration of endotoxin.

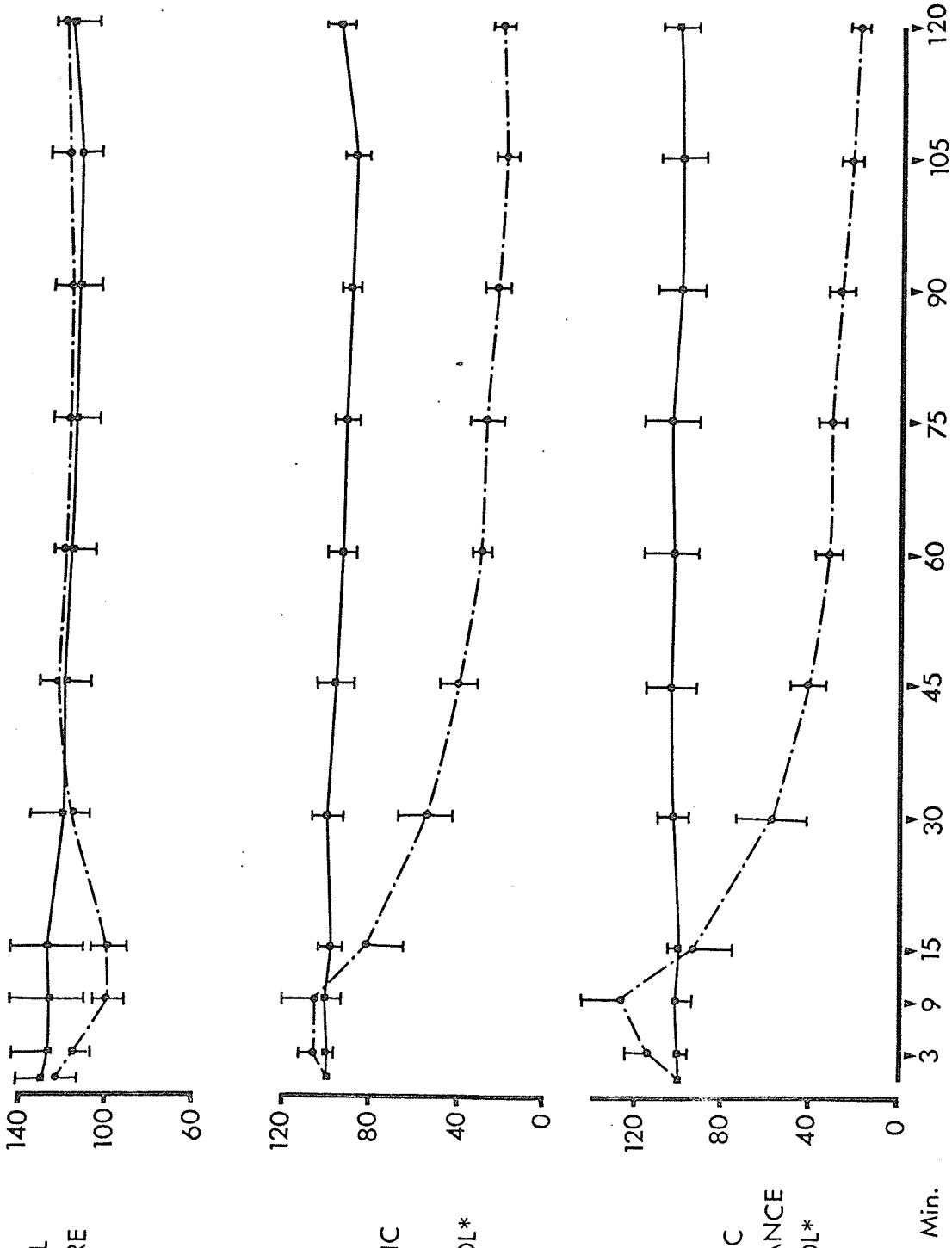


Figure 9: Graph showing changes (mean ± SE) in arterial pressure, superior mesenteric arterial flow and calculated superior mesenteric arterial conductance in endotoxin-treated cats (●—·—·—●) and Ringer-Locke solution-treated control cats (■——■). All cats were given pretreatment with acetylsalicylic acid. (* resting value)

vessels. Increase in conductance reflects a dilation of the resistance vessels while a decrease in conductance means constriction. From Fig. 9 it can be seen that endotoxin caused an initial dilation of the resistance vessels followed by an intense constriction which lasted for the rest of the period of observation.

In 5 cats a smaller dose (10 mg/kg) of acetylsalicylic acid was used for pretreatment. 3 mg/kg of endotoxin was injected intravenously 20-30 minutes after pretreatment. In this group of cats, endotoxin produced hemodynamic changes similar to those seen in the preceding group. The results are shown in Fig. 10. There was no difference between the two groups in the arterial pressure, superior mesenteric arterial flow and conductance during different time intervals after endotoxin (Fig. 9 and 10).

In 6 cats portal venous pressure was recorded. Three of these cats received 100 mg/kg of acetylsalicylic acid while the others received only 10 mg/kg of acetylsalicylic acid as pretreatment. In all the cats endotoxin was injected in doses of 3 mg/kg. Since the response was not different in the two groups given different doses of acetylsalicylic acid, data are pooled for presentation (Fig. 11). Within 9 minutes after intravenous injection of endotoxin, portal venous pressure rose from a control value of 8.2 ± 0.5 mm of Hg (mean $\pm$ S.E.) to 9.0 ± 0.6 mm of Hg. After this period pressure gradually declined and reached a lower level of 6.9 ± 1.0 mm of Hg by 120 minutes. These changes in portal venous pressure were too small to be of any biological significance.

A set of 4 cats was used for control experiments. In 3 of these

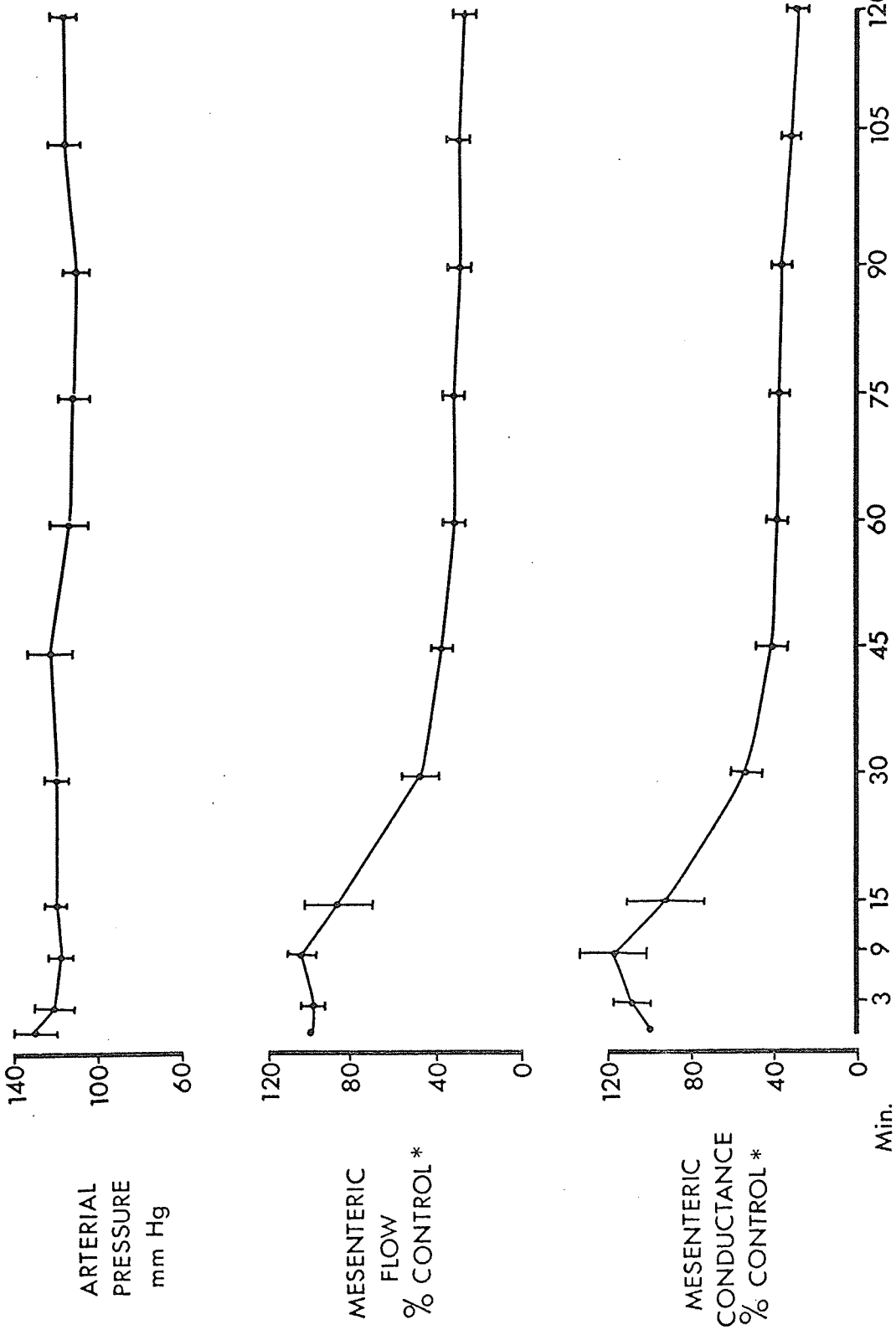


Figure 10: Graph showing effects (mean ± SE) of endotoxin on arterial pressure, superior mesenteric arterial flow and calculated superior mesenteric arterial conductance in cats pretreated with a small dose (10 mg/kg) of acetylsalicylic acid. (* resting value)

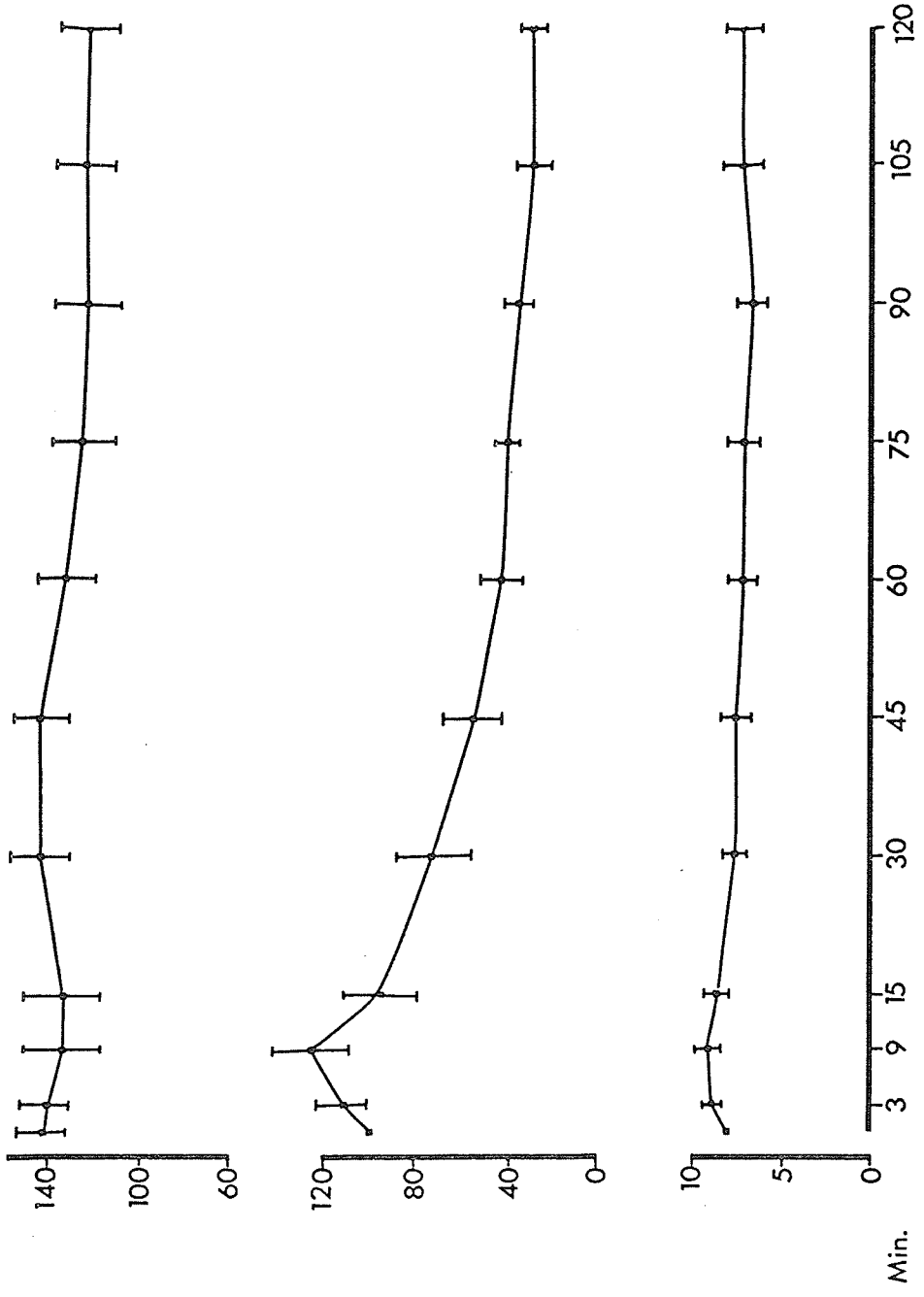


Figure 11: Graph showing effects (mean ± SE) of endotoxin on arterial pressure, calculated superior mesenteric arterial conductance and portal venous pressure in cats pretreated with acetylsalicylic acid. (* resting value)

cats acetylsalicylic acid was injected in doses of 100 mg/kg while one cat was given 10 mg/kg of acetylsalicylic acid. 3-5 ml of Ringer-Locke solution was injected 20 to 30 minutes after pretreatment with acetylsalicylic acid. No endotoxin was injected in these cats. No significant hemodynamic changes were seen during the period of observation (120 minutes). Data are presented in Fig. 9.

Postmortem examination of cats injected with endotoxin after pretreatment with acetylsalicylic acid showed hemorrhages in intestinal mucosa as well as colonic mucosa. The lumen of the intestine was filled with mucus and occasionally with blood mixed with mucous. In some cats hemorrhagic lesions were seen in the mesenteric lymph nodes. The lungs looked healthy and no hemorrhages were seen.

Control animals which were given pretreatment with acetylsalicylic acid only showed no abnormal changes in the abdominal viscera or lungs.

From these experiments it was concluded that a pretreatment with acetylsalicylic acid protects the cats from the acute hypotensive response. Cats protected in this manner respond to endotoxin by a transient mesenteric vasodilation followed by an intense mesenteric constriction which lasts for more than 2 hours. Since mesenteric ischemia of this magnitude can lead to a severe mucosal damage in the intestine (Chiu et al. 1970) and significant hemodynamic changes in other organs in the body (Vyden, 1971), it is likely that this intense mesenteric ischemia is one of the mechanisms by which endotoxin causes death in cats.

SECTION III.

HEMODYNAMIC RESPONSES OF CATS TO A SLOW INTRAVENOUS INFUSION OF ENDOTOXIN.

INTRODUCTION

In 1903, Todd suggested that certain clinical signs seen in dysentery were due to bacterial toxins. Since then many investigators have studied the effects of endotoxin in experimental animals. From time to time clinicians brought to light new pathophysiological findings in the clinical state of shock associated with gram-negative septicemia. Many of these were remarkably similar. In the last decade however, use of endotoxin in experimental animals to simulate and evaluate clinical septic shock has been questioned (Waisbren, 1964). To solve the problem Hinshaw and co-workers (Hinshaw et al. 1968) investigated the effects of intravenously-injected live E. coli in dogs and compared it with responses produced by intravenously injected endotoxin. A similar investigation was carried out in the monkey (Guenter et al. 1969). The responses were qualitatively similar; however, differences were seen in the magnitude of response as well as their time of onset. Rety and Couves (1969) investigated the effects of slow intravenous infusions of endotoxins and found it qualitatively different from the rapid injection of endotoxin. They proposed that slow infusions make a better method of introducing endotoxemia, since in gram-negative bacterial infections endotoxemia probably develops more gradually with progressive lysis of bacterial cells.

In our previous investigation (Section II) we found that endotoxemia produced by rapid injection leads to development of intense mesenteric vasoconstriction when cats are pretreated with acetylsalicylic acid. It was therefore planned to investigate the effects of slow infusion of endotoxin on the mesenteric vascular bed.

METHODS

Cats weighing 2.5 ± 0.2 kg (mean ± S.E.) were anesthetized with intraperitoneal sodium pentobarbital and used for the experiments. Mean arterial pressure was recorded from the femoral artery. Right atrial pressure was recorded by cannulating the external jugular vein. The abdomen was opened by a midline incision and the superior mesenteric arterial flow was measured.

In 5 cats, endotoxin suspended in Ringer-Locke solutions (1 mg/ml), was infused intravenously into the femoral vein with a Harvard infusion pump at the rate of about 0.1 ml/minute until the total dose was 3 mg/kg. This required an average duration of 86 minutes for infusion. In 4 cats only Ringer-Locke solution (0.1 ml/min) was infused instead of endotoxin (control) and the measurements were made over the 4 hours period after the onset of infusion. Since the effect of endotoxin on mesenteric vascular bed had not been studied in cats without pre-treatment with acetylsalicylic acid, 4 cats received a bolus dose of 3 mg/kg of endotoxin intravenously.

At the end of the experiment animals were heparinized and the blood flow was calibrated.

RESULTS

Mean arterial pressure in this series of cats was 141 ± 8 mm of Hg. Superior mesenteric arterial flow was 63.4 ± 5.1 ml per minute or 24.6 ± 2.9 ml kg^{-1} min^{-1}. Mean right atrial pressure was 1.0 ± 0.4 mm of Hg.

In 5 cats, endotoxin (3 mg/kg) was given by slow intravenous infusion. Within 10 to 20 minutes after the onset of the infusion, the arterial pressure began to decrease gradually and reached a low level of 84 mm Hg in 80 minutes. After this period, arterial pressure slowly returned to pre-infusion levels. By 180 minutes arterial pressure recovered to 93% of the pre-infusion level. Superior mesenteric arterial flow decreased within 10 minutes to 81% of the pre-infusion value and showed a continuous decrease reaching 42% of pre-infusion value in 60 minutes and 33% of pre-infusion value in 180 minutes. Flow remained at this low level for the rest of the period of observation. No change in right atrial pressure was seen. A typical experiment is shown in Fig. 12.

Superior mesenteric arterial conductance decreased gradually starting within 10 minutes of the onset of infusion and continued to decrease for 180 minutes, reaching a low value of 36% of the conductance seen during pre-infusion period. After this period conductance remained low for the period of observation (Fig. 13). In 3 of these cats, at the end of the 4 hour period of observation a bolus of endotoxin (3 mg/kg) was injected intravenously over 30 seconds. This dose of endotoxin which normally produced an immediate hypotensive response failed to produce any change in arterial pressure or right atrial pressure. A typical experiment is presented in Fig. 14.

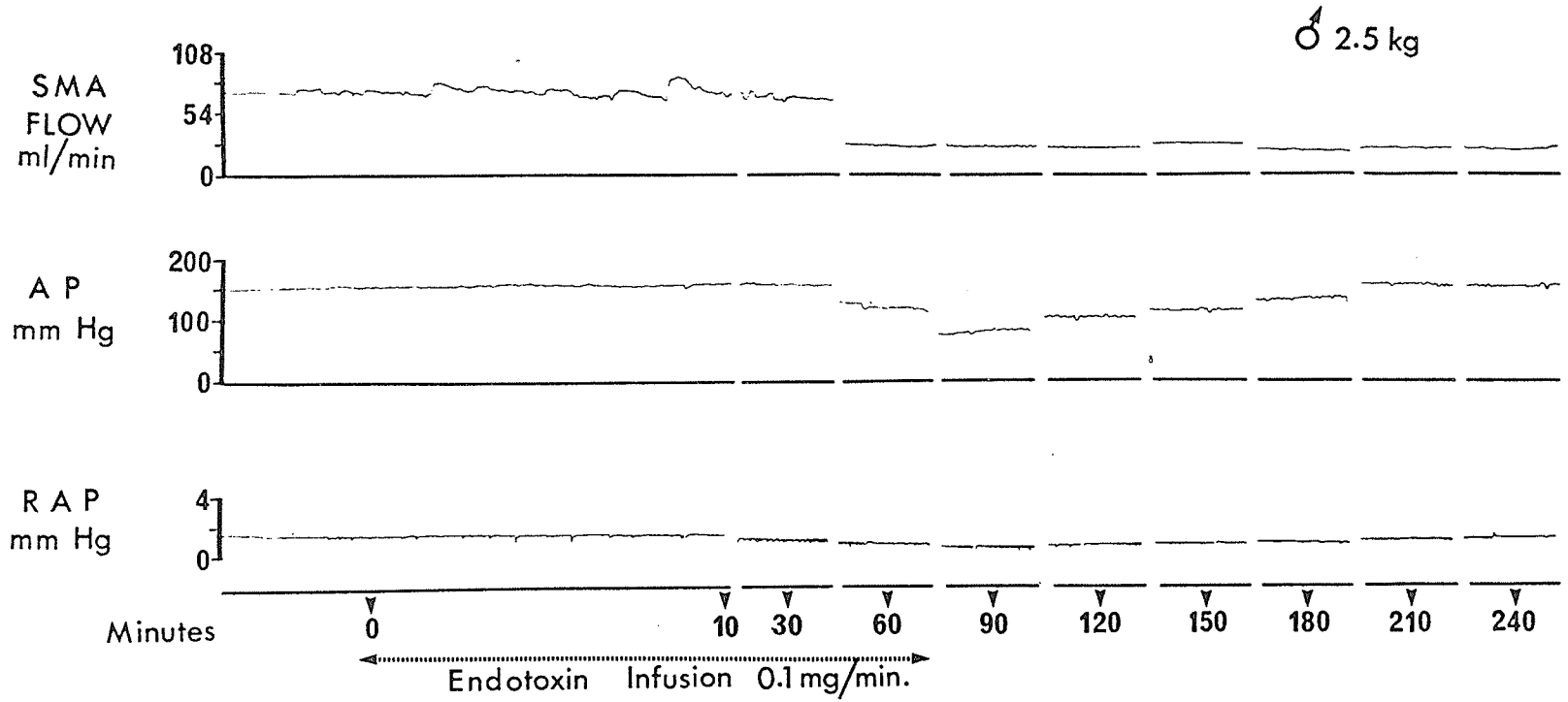


Figure 12: Record of arterial pressure (AP), superior mesenteric arterial flow (SMA FLOW) and right atrial pressure (RAP) showing effects of slow intravenous infusion of endotoxin (3.0 mg/kg).

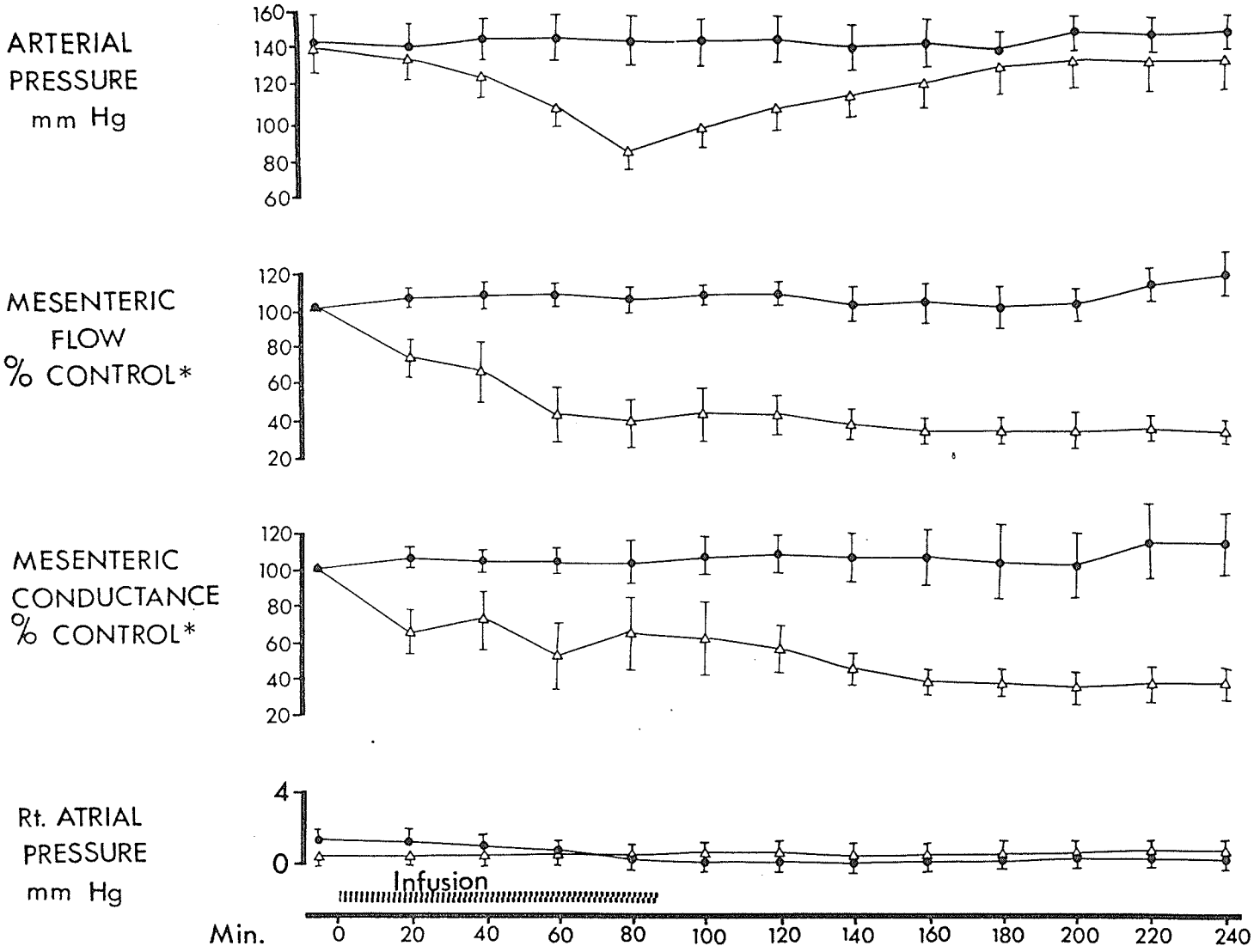


Figure 13: Graph showing changes (mean ± SE) in arterial pressure, superior mesenteric arterial flow, calculated superior mesenteric arterial conductance and right atrial pressure during and after a slow intravenous infusion of endotoxin (△——△) and Ringer-Locke solution (●——●). (* resting value)

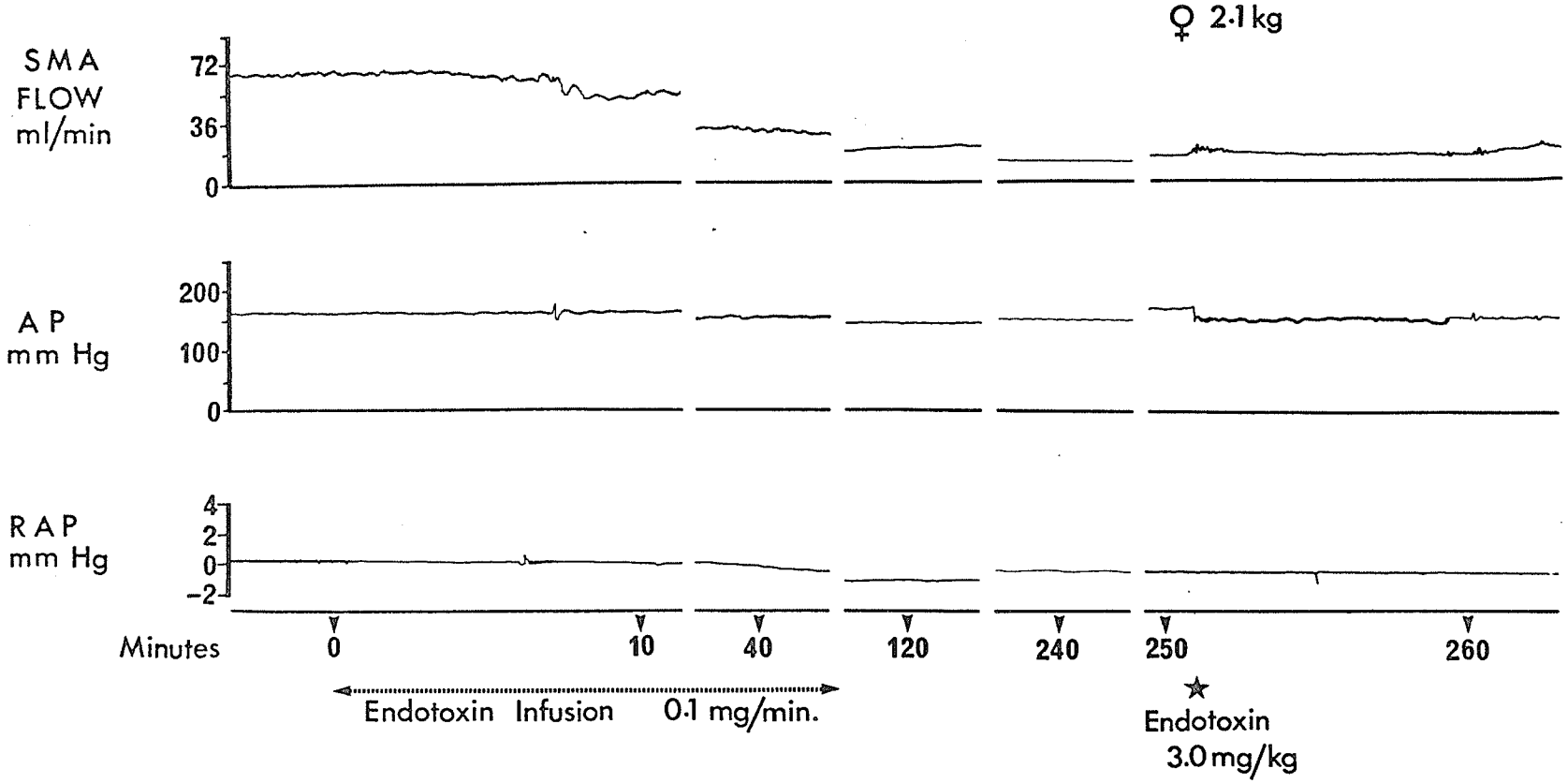


Figure 14: Record of arterial pressure (AP), superior mesenteric arterial flow (SMA FLOW) and right atrial pressure showing refractoriness to a second dose of endotoxin given by rapid intravenous injection after a slow infusion of endotoxin.

In 4 control cats Ringer-Locke solution was infused. No endotoxin was given. No significant hemodynamic changes were observed over the 240 minutes of observation (Fig. 13).

In cats given a bolus of endotoxin (3 mg/kg) by rapid intravenous injection, very dramatic hemodynamic changes were seen within 1 to 2 minutes. A precipitous fall in arterial pressure occurred, associated with a rise in right atrial pressure. Superior mesenteric arterial flow also decreased precipitously within 2 minutes to very low values. Two cats died during the acute hypotensive response. In the other 2 cats the arterial pressure and right atrial pressure recovered very slowly with a small recovery in arterial flow. After this phase the superior mesenteric arterial flow decreased and right atrial pressure remained unchanged. A typical experiment is presented (Fig. 15) for comparison.

After the experiment, the cats were sacrificed and the lungs and mesenteric organs were examined. The lungs of the cats given a slow infusion of endotoxin as well as those given slow infusions of Ringer-Locke solution showed no congestion or hemorrhagic patches. Occasional areas of hemorrhage were seen in the intestinal mucosa in cats given a slow infusion of endotoxin but not in cats infused with Ringer-Locke solution. The cats which were injected with only a bolus injection of endotoxin (3 mg/kg) showed the typical pulmonary congestion and hemorrhages similar to those reported in Section I.

From these experiments it was concluded that slow intravenous infusion of endotoxin does not produce the severe initial hypotensive response associated with rise in right atrial pressure. A hypotension

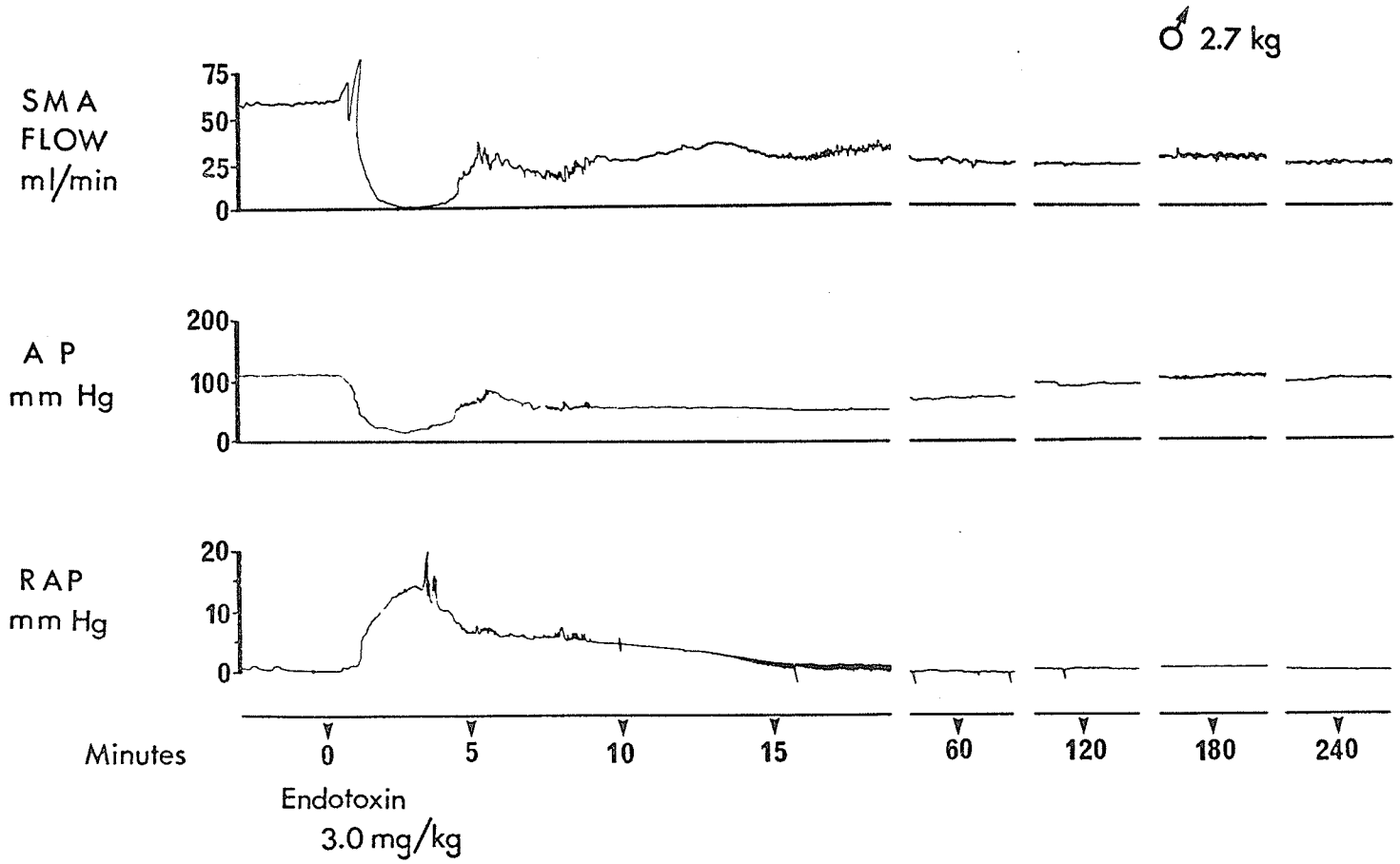


Figure 15: Record of arterial pressure (AP), superior mesenteric arterial flow (SMA FLOW) and right atrial pressure (RAP) of a cat given 3.0 mg/kg of endotoxin by rapid intravenous injection.

develops which is slow in onset and recovery invariably occurs in all the cats. During this phase of hypotension the right atrial pressure does not rise. Mesenteric vasoconstriction of the same magnitude as seen in cats given pretreatment with acetylsalicylic acid (Section II) was observed in all the cats infused with endotoxin. Although no rise in right atrial pressure occurred during the slow infusion, it rendered the cat refractory to the subsequent bolus injection of endotoxin.

SECTION IV.

DISTRIBUTION OF SUPERIOR MESENTERIC ARTERIAL FLOW IN CONTROL ANIMALS AND IN ANIMALS AFTER MESENTERIC VASOCONSTRICTION INDUCED BY ENDOTOXIN.

INTRODUCTION

The following investigation was designed to study the distribution of superior mesenteric arterial blood flow into various mesenteric organs and the possible changes which might be taking place during the intense mesenteric vasoconstriction seen after endotoxin.

Rayner, MacLean and Grim (1960) used deuterium oxide to study the distribution of blood flow in the intestinal wall of the dog after endotoxin. They found marked reduction in the mucosal perfusion without a change in total blood flow. It was suggested that, during endotoxemia, a shift of capillary blood flow towards arterio-venous anastomoses might lead to reduced tissue perfusion of the mucosa and to mucosal damage. Participation of the submucosal vessels in the arterio-venous shunting of blood flow during autoregulatory escape (see appendix) has been postulated (Folkow, Lewis, Lundgren, Mellander and Wallentin, 1964a, 1964b). The availability of microspheres labelled with radioactive isotopes provided us with a method to study the distribution of blood flow in the various layers of intestinal wall as well as in other tissues supplied by the superior mesenteric artery. While investigating the validity of use of microspheres for such studies, it was found that the vascular beds of the mucosa and submucosa are coupled in series and submucosal shunts do not exist. A redistribution from mucosa to submucosa could not occur. Details of this investigation (Greenway and Murthy, 1972) are presented in the appendix. In this section, effects of endotoxin-induced mesenteric vasoconstriction on the distribution of blood flow to various mesenteric organs has been investigated.

METHODS

Healthy cats of either sex weighing 2.5 ± 0.2 kg (mean ± S.E.) were anesthetized by intraperitoneal injection of sodium pentobarbital. Arterial pressure and superior mesenteric arterial flow were measured. The anastomotic branch of the superior mesenteric artery was identified. This is the first branch of the superior mesenteric artery and it anastomoses with the inferior mesenteric artery. Since its ligation does not deprive any area of its arterial blood supply, it was cannulated for injection of radioactive microspheres into the superior mesenteric artery.

Carbonized microspheres (17 ± 0.16μ diameter; mean ± S.E.), labelled with ^{51}Cr were suspended in 10% dextran solution (3M Nuclear Products, Minnesota) and a dose of approximately 220,000 microspheres was injected into the superior mesenteric artery in each experiment.

In 6 cats, 10 mg/kg of acetylsalicylic acid was injected intravenously followed 30 minutes later by 5 ml of Ringer-Locke solution. Two hours later microspheres were injected. These experiments served as control. In another set of 7 cats an intravenous injection of 10 mg/kg of acetylsalicylic acid was followed 30 minutes later by endotoxin (3 mg/kg). Two hours after injection of endotoxin, microspheres were injected. Heparin (5 mg/kg) was injected and blood flow was calibrated, as described in Section II.

In each experiment, the abdominal viscera, lungs and liver were removed and the following procedure was carried out. The pancreas, lymph nodes, mesentery, colon, mesocolon, lungs and liver were cut into small

pieces and placed in plastic tubes. The intestine was opened along the mesenteric border and cut into 7 cm lengths.

Tissue samples were weighed and the radioactivity was counted in a two channel auto-gamma spectrometer (Packard Instrument Co.). Fractional blood flow to each piece of tissue was calculated from the principle of Stewart-Hamilton as described by Wagner, Rhodes, Sasaki and Ryan (1969).

$$f = F \frac{q}{Q}$$

where f is the fractional blood flow to the tissue, F is the total blood flow in the superior mesenteric artery at the time when microspheres were injected, q is the radioactivity in the piece of tissue and Q is the total injected radioactivity, obtained by summation of the counts in all the samples. Since the tissue weight was measured, flow/100 g tissue could also be calculated.

RESULTS

Hemodynamic changes.

In the control group consisting of 6 cats, pretreated with acetylsalicylic acid (10 mg/kg) and injected with 5 ml of Ringer-Locke solution, no significant hemodynamic changes were seen. The resting arterial pressure was 139 ± 8 mm of Hg (mean ± S.E.) and 2 hours later, when microspheres were injected, arterial pressure was 135 ± 6 mm of Hg. Resting superior mesenteric arterial flow was 72.1 ± 11 ml/min or 29.6 ± 5 $ml/min^{-1}\ kg^{-1}$ or 80.3 ± 17 $ml\ min^{-1}\ 100\ g^{-1}$. Two hours later the arterial flow was 102 ± 4.6% of resting value.

In the endotoxin-treated group, resting arterial pressure was 121 ± 6 mm of Hg and superior mesenteric arterial flow was 74.6 ± 8 ml/min or 28.5 ± 4 $ml\ min^{-1}\ kg^{-1}$ or 75.7 ± 7 $ml\ min^{-1}\ 100\ g^{-1}$. After administration of endotoxin (3 mg/kg), mesenteric vasoconstriction developed. Two hours after the administration of endotoxin, microspheres were injected. Arterial pressure at this time was 111 ± 6 mm of Hg, and superior mesenteric arterial flow had decreased to 26.9 ± 3 ml/min (or 10.2 ± 1.5 $ml\ min^{-1}\ kg^{-1}$ or 26.9 ± 2.7 $ml\ min^{-1}\ 100\ g^{-1}$), a level of 34.1 ± 3.0% of the resting value. Calculated superior mesenteric vascular conductance before endotoxin administration was 0.62 ± 0.04 $ml\ min^{-1}\ 100\ g^{-1}\ mm\ Hg^{-1}$ and when microspheres were injected after endotoxin it had decreased to 39.3 ± 3.3% of the above (Fig. 16).

Distribution of superior mesenteric arterial flow.

The anatomical distribution of superior mesenteric arterial flow observed in the present series of experiments is presented in Fig. 17.

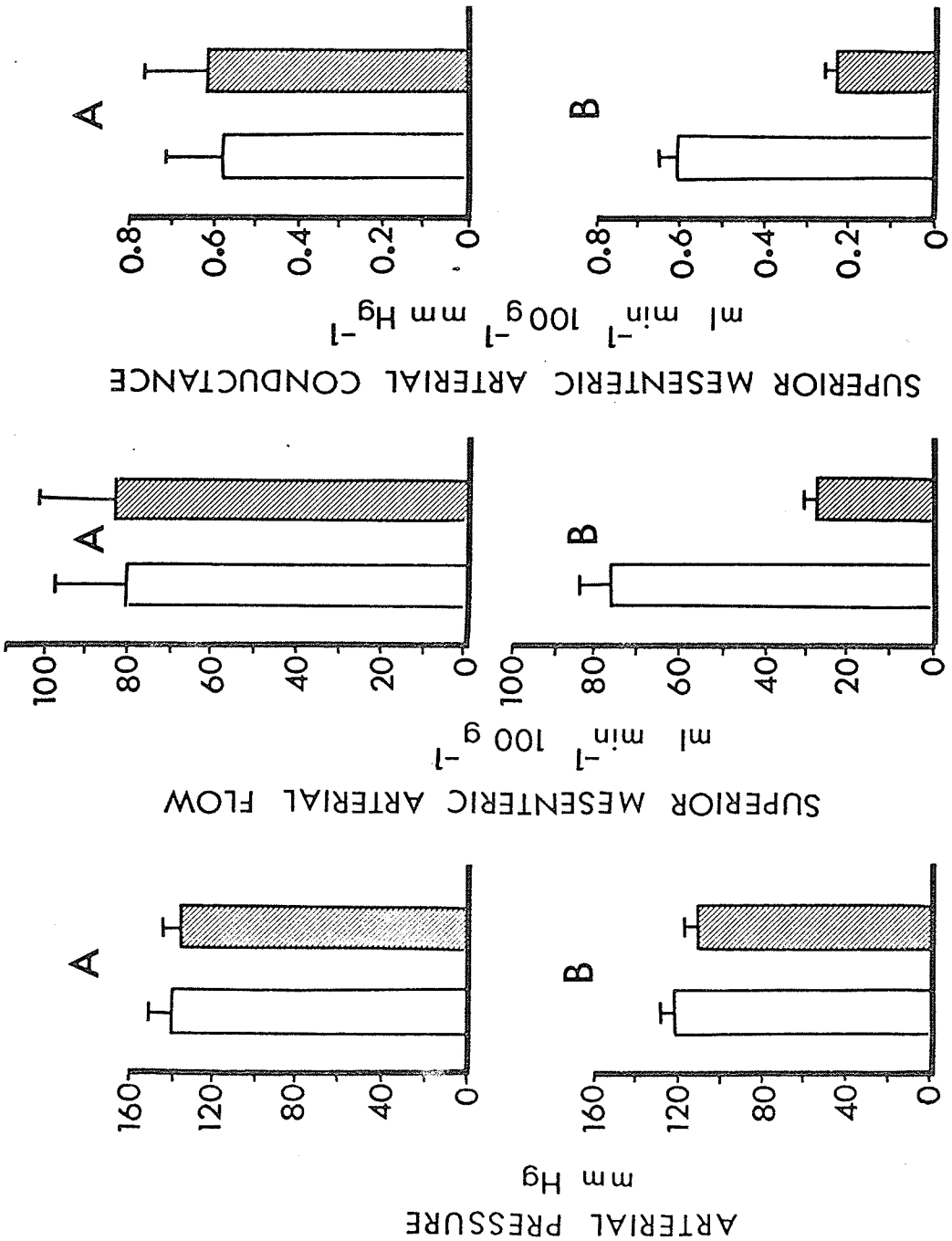


Figure 16: Graph showing (mean ± SE) various hemodynamic parameters in control (A) and endotoxin-treated groups (B). Open bars represent the measurements after administration of acetylsalicylic acid and the hatched bars represent the measurements made two hours later at the time of injection of microspheres.

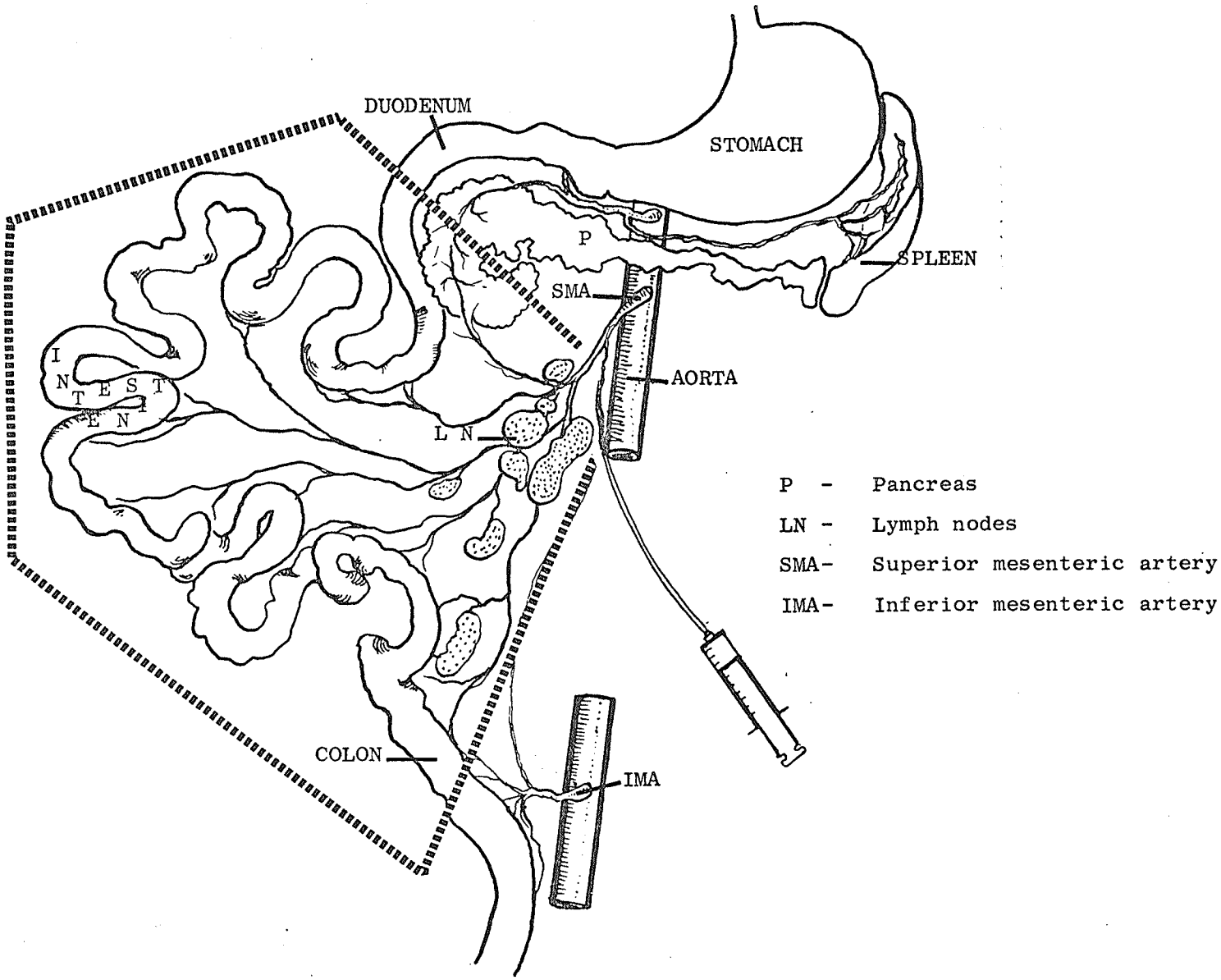


Figure 17: Schematic presentation of the anatomical distribution of superior mesenteric arterial flow (demarcated by interrupted line). Anastomotic branch of the superior mesenteric artery was cannulated for the injection of microspheres.

The intestinal wall with the exception of the first 2-3 cm of the duodenum, the mesentery, the mesenteric lymph nodes, the proximal half of the colon with its attached mesocolon, and the head but not the body or tail of the pancreas were supplied by the superior mesenteric artery. No significant difference was observed in the relative weights of various tissues, perfused by the superior mesenteric artery, in the control and the endotoxin-treated group. Relative weights, proportions of superior mesenteric arterial flow and calculated flows to various tissues of the control group are presented in Table 2. The intestine received 85.5 $\pm$ 1.0% of the total flow. Fractional flows to colon and lymph nodes were 8.9 $\pm$ 0.9% and 3.3 $\pm$ 0.3% respectively. Mesentery, mesocolon and pancreas received the remaining small fraction of the flow.

The differences between treated and control animals in the fractional flows to the mesentery, mesocolon and pancreas were too small relative to the variability to permit any meaningful comparison and these tissues are not discussed further.

In endotoxin-treated animals, the superior mesenteric arterial flow decreased to 34.13 $\pm$ 3.0% of the control value while in the solvent-treated control group blood flow did not show any significant change (102.0 $\pm$ 4.6% of control value). Despite a marked decrease in the total superior mesenteric arterial flow in endotoxin-treated animals, the fractional blood flows to the various major tissues remained unchanged. Comparison of fractional distribution of blood flow to intestine, colon and lymph nodes expressed as a percent of total flow and as flow per 100 g of tissue have been presented in Fig. 18 and 19 respectively. A marked

TABLE 2

The weights (drained of blood) and blood flows (means ± S.E.) of the splanchnic organs perfused by the superior mesenteric artery (SMA).

	Wt of perfused tissue (g/kg body wt)	% of SMA flow	Flow (ml/min)/ 100 g tissue
Intestine	29.0 ± 4.3	85.5 ± 1.0	100.9 ± 21.9
Mesenteric lymph nodes	1.7 ± 0.3	3.3 ± 0.3	76.9 ± 22.1
Mesentery	4.3 ± 0.6	0.8 ± 0.1	7.5 ± 2.6
Head of pancreas	1.0 ± 0.3	1.4 ± 0.8	31.4 ± 11.0
Proximal colon	3.0 ± 0.5	8.9 ± 0.9	105.7 ± 28.6
Proximal mesocolon	1.2 ± 0.6	0.4 ± 0.1	14.4 ± 9.1

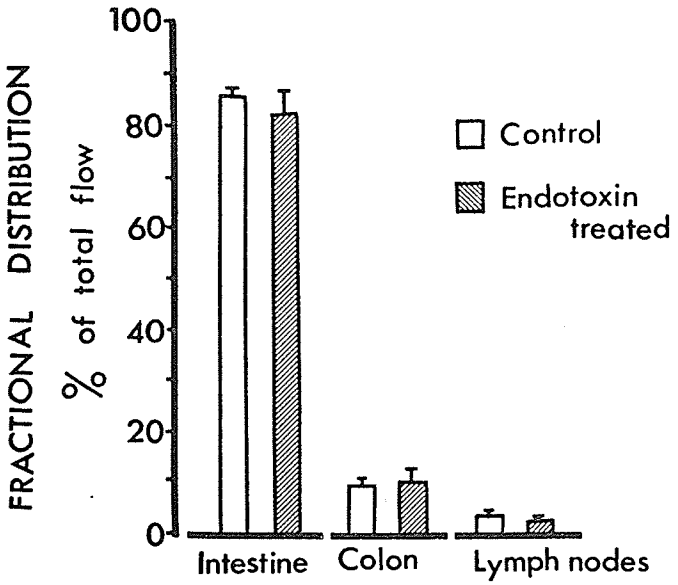


Figure 18: Graph showing fractional distribution of superior mesenteric arterial flow (mean ± SE) in control and endotoxin-treated cats.

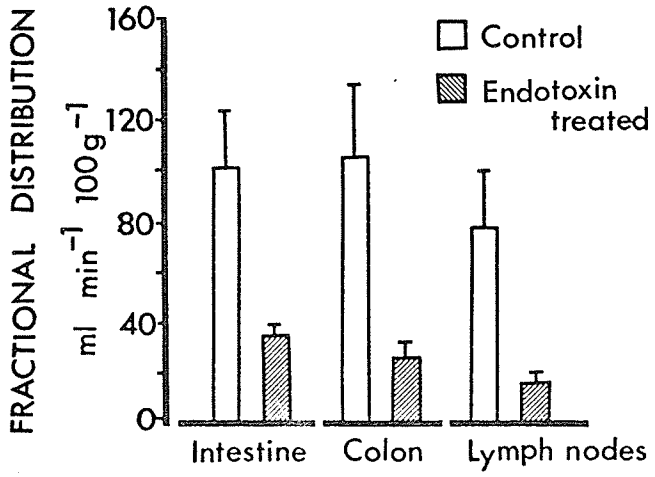


Figure 19: Graph showing perfusion of intestine, colon and mesenteric lymph nodes expressed as ml/minute per 100 g of tissue in control and endotoxin-treated cats.

decrease in tissue perfusion ranging from 70% in intestine to 80% in lymph nodes was observed.

In these experiments the amount of the injected radioactivity detected in the liver was 0.20 $\pm$ 0.005% (mean $\pm$ S.E.) in control and 0.16 $\pm$ 0.002% in the endotoxin-treated group. The lungs did not show any significant radioactivity.

From these observations it is concluded that endotoxin induces intense mesenteric vasoconstriction. In spite of a significant reduction in total flow, the fractional distribution of superior mesenteric arterial flow to intestine, colon and lymph nodes did not reveal any significant change. The magnitude of vasoconstriction in the above vascular beds was found to be similar. A very small proportion of the injected radioactivity was detected in the liver; therefore, it was concluded that less than 1% of the total superior mesenteric arterial blood flows through vessels larger than 17μ in diameter.

SECTION V.

AN INVESTIGATION INTO THE MECHANISM OF MESENTERIC VASOCONSTRICTION INDUCED BY ENDOTOXIN IN CATS PRETREATED WITH ACETYLSALICYLIC ACID.

INTRODUCTION

In dogs mesenteric vasoconstriction after intravenous administration of endotoxin has been reported by various investigators (Lillehei et al. 1965; Hinshaw, 1968; Brobmann et al. 1970; Wangensteen et al. 1971). The mechanism of this vasoconstriction has not been investigated. Hauman (1968) reported partial reversal of mesenteric constriction after celiac blockade. Similar observations have been made by Wangensteen and co-workers (1971). However, these studies have not elucidated the mechanism by which celiac blockade modified the mesenteric vasoconstriction.

In cats, the mesenteric as well as the splenic vascular beds responded to hemorrhage by vasoconstriction. In mesenteric vasoconstriction, vasopressin and angiotensin play a major role and the sympathetic nervous system does not contribute to it. However, the splenic response is mediated by an overlapping participation of angiotensin, vasopressin and the sympathetic nervous system (Greenway, Lawson and Stark, 1967; Greenway and Stark, 1969; McNeill, Stark and Greenway, 1970; Stark, McNeill and Greenway, 1971). In cats, the mechanism of mesenteric vasoconstriction seen after endotoxin has not been investigated. Recently Hall and Hodge (1971) reported increased levels of circulating catecholamines and angiotensin in cats after intravenous administration of endotoxin.

In the present investigation the role of the kidney, pituitary and sympathetic nervous system in the development of mesenteric vasoconstriction after endotoxin has been evaluated in cats pretreated with acetylsalicylic acid.

METHODS

In this series of experiments, cats weighing 2.5 ± 0.1 kg (mean ± S.E.) were used under pentobarbital anesthesia. Arterial pressure, right atrial pressure and superior mesenteric arterial flow were measured as described in Section II.

In these cats, bilateral adrenalectomy was performed by ligating the vascular connections followed by excision of the gland. Hydrocortisone (5 mg/kg, Solucortef, Upjohn Co.) was injected intramuscularly (Greenway and Stark, 1969; McNeill et al. 1970). Denervation of mesenteric organs was accomplished in the following manner. Bundles of nerve fibers running along the superior mesenteric artery were dissected free. Cotton wool soaked with 1.0% procaine was placed around the artery. The nerve bundles were ligated at two places one cm apart and sectioned between the ligatures. Care was taken not to leave any nerve fibre bundles intact. Bilateral nephrectomy was performed by ligation of the renal pedicles followed by excision of the kidneys.

Hypophysectomy was performed in the following manner. A midline incision was made through the soft palate and the mucosal lining covering the sphenoid was scraped with gauze. The pterygoid hamuli were identified. Between the hamuli and on the saggital midline, a small foramen was located which made the topographic landmark for the sella turcica. A trephine (6 mm diameter) was used to penetrate the sphenoid. Bleeding from the bone was controlled with bone wax. Meninges covering the pituitary were divided with a sharp needle and the gland was removed by suction. Bleeding was controlled with Surgicel (Johnson and Johnson).

Blood loss during hypophysectomy was seldom more than 2-3 ml. After the experiment, the skull was opened and the removal of the pituitary was confirmed in each experiment.

Phenoxybenzamine hydrochloride (Dibenzyline, SKF) was dissolved in propylene glycol (250 mg/25 ml) acidified with 4 drops of 10 N hydrochloric acid. Prior to injection the required dose (5 mg/kg) was diluted with 5 volumes of Ringer-Locke solution and infused intravenously over 7-10 minutes. All the cats were pretreated with 100 mg/kg of acetylsalicylic acid as described in Section II.

Blood from donor cats was obtained after cannulating the jugular vein under ether anesthesia. Heparin (10 mg/100 ml) was added. Fresh blood was used in each experiment.

RESULTS

In 4 cats, mesenteric organs were denervated and bilateral adrenalectomy was performed to eliminate sympathetic neurohumoral influences. Bilateral nephrectomy and hypophysectomy removed the constrictor influences of vasopressin and angiotensin. All the cats were pretreated with acetylsalicylic acid (100 mg/kg). Endotoxin (3 mg/kg) was injected 30 minutes after administration of acetylsalicylic acid.

In these cats endotoxin produced a progressive and severe hypotensive response. This hypotensive response was not associated with rise in right atrial pressure. Within 3 minutes the arterial pressure decreased from a control level of 104 $\pm$ 9 mm of Hg to 88 $\pm$ 9 mm of Hg. Within 60 minutes arterial pressure dropped to 44 $\pm$ 16 mm of Hg and by 120 minutes to 37 $\pm$ 13 mm of Hg. Similar hypotensive responses were seen when cats were subjected to bilateral adrenalectomy and denervation of mesenteric organs and endotoxin was administered. It was therefore concluded that endotoxins produce a hypotensive response in cats which have undergone adrenalectomy and denervation of mesenteric organs even when pretreated with acetylsalicylic acid. This hypotensive response is effectively counteracted by the adrenals and other reflexes. In absence of these mechanisms, the hypotensive response was intense and unopposed. During such low levels of arterial pressure, no meaningful conclusions could be derived regarding the responses of the mesenteric vascular bed to endotoxin.

In another set of 4 cats, phenoxybenzamine (5 mg/kg) was administered intravenously. Hypotension produced by this procedure was pre-

vented by infusion of 12 ± 1.9 ml of blood per kg of body weight. After 40 minutes, acetylsalicylic acid (100 mg/kg) was administered as before, followed 20 minutes later by endotoxin (3 mg/kg). In these experiments arterial pressure tended to decrease after endotoxin and therefore a further infusion of 7.7 ± 2.8 ml of blood per kg was given to maintain the arterial pressure. The responses are shown in Fig. 20. The superior mesenteric arterial flow before endotoxin was 39 ± 15 ml min^{-1} kg^{-1}. After endotoxin, flow and conductance increased initially and then decreased gradually after 15 minutes. Within 60 minutes, superior mesenteric arterial conductance decreased to 44 ± 9.2% of control and by 120 minutes to 25 ± 4.6% of the control level. Changes seen in the conductance were not different from those in cats which were not given phenoxybenzamine (t-test for unpaired data, $p > 0.1$) (Fig. 20).

In 7 cats, bilateral nephrectomy was performed followed by hypophysectomy. Phenoxybenzamine was given as before in doses of 5 mg/kg. To maintain arterial pressure as the phenoxybenzamine was given, 16 ± 1.9 ml of blood per kg was infused intravenously. After 40 minutes, acetylsalicylic acid (100 mg/kg) was administered followed 20 minutes later by endotoxin. After administration of endotoxin, arterial pressure was maintained by infusion of 12 ± 1.9 ml/kg during the first hour and 7.4 ± 2.0 ml/kg in the second hour. The results are shown in Fig. 21. The superior mesenteric arterial flow before endotoxin was 35 ± 7.1 ml min^{-1} kg^{-1}. After endotoxin the conductance increased transiently followed by a gradual decrease. After 60 minutes of endotoxin administration, conductance declined to 78 ± 15% of the pre-endotoxin level. By the end of

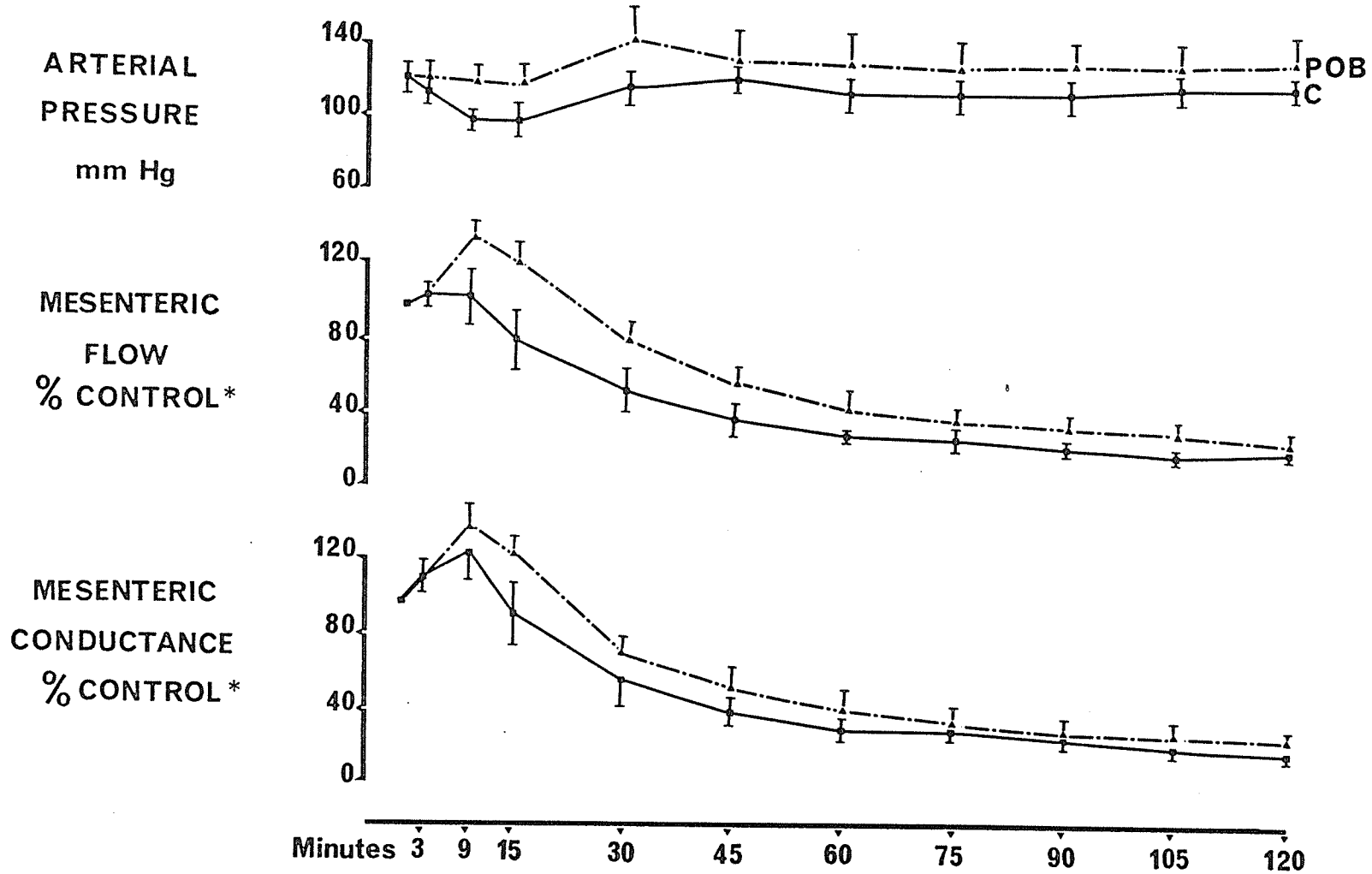


Figure 20: Graph showing effects (mean ± SE) of endotoxin on arterial pressure, superior mesenteric arterial flow and calculated superior mesenteric arterial conductance in control (C) and phenoxybenzamine-treated cats (POB). All cats received pretreatment with acetylsalicylic acid (100 mg/kg) 20-30 minutes prior to the administration of endotoxin. (* resting value)

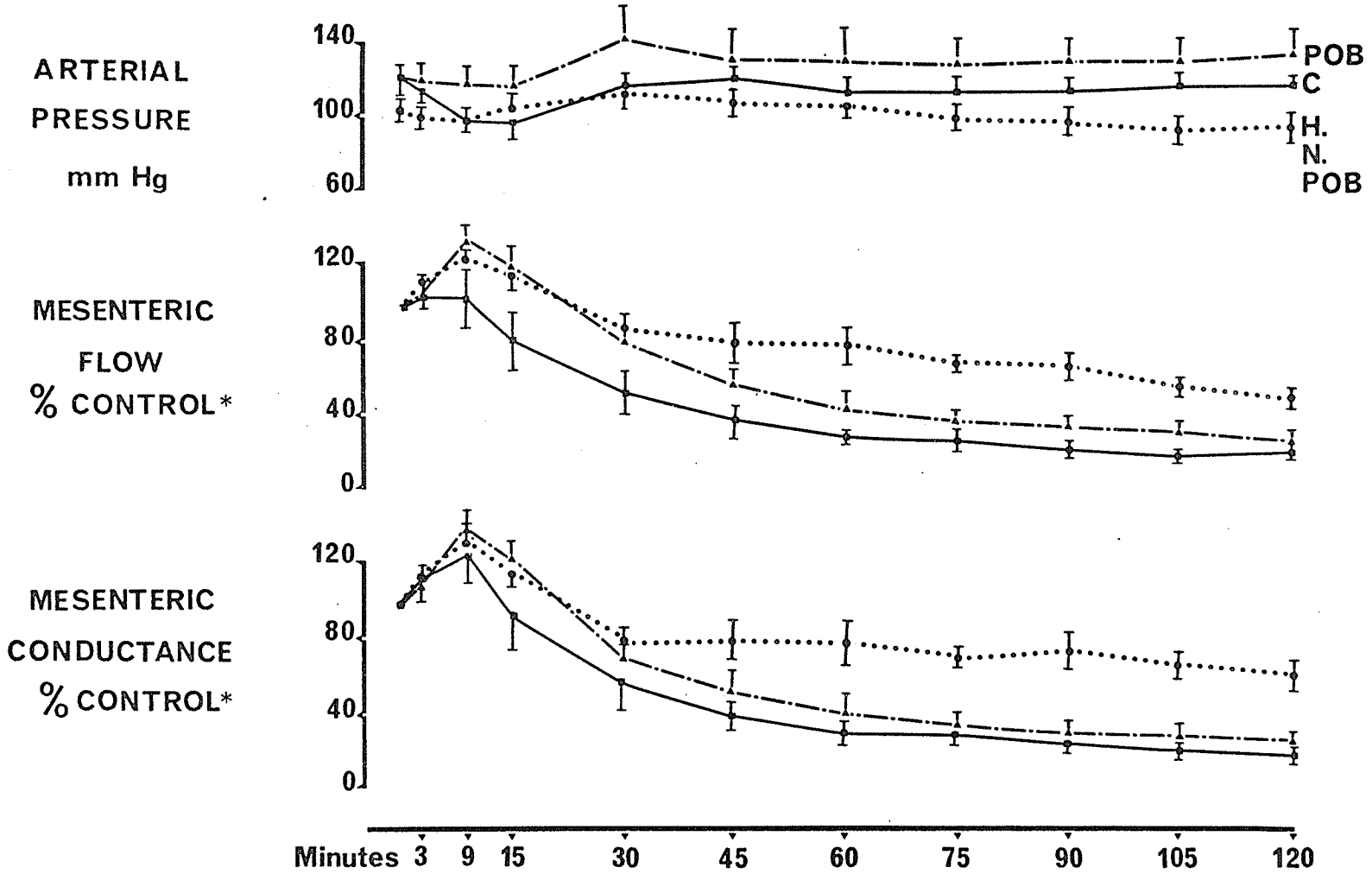


Figure 21: Graph showing effects (mean ± SE) of endotoxin on arterial pressure, superior mesenteric arterial flow and calculated superior mesenteric arterial conductance in control cats (C), phenoxybenzamine treated cats (POB) and in cats subjected to hypophysectomy, bilateral nephrectomy and treated with phenoxybenzamine (H.N.POB). All cats received pretreatment with acetylsalicylic acid (100 mg/kg) 20-30 minutes prior to the administration of endotoxin. (* resting value)

the 120 minutes of observation, conductance decreased to 56 $\pm$ 9% of the pre-endotoxin level.

In these experiments, the decrease in conductance 60 minutes after endotoxin administration was significantly smaller than that in the control experiments (unpaired t-test, $p < 0.001$) and in cats given phenoxybenzamine alone ($p < 0.02$). However, the decrease in conductance was still highly significant when compared with the control pre-endotoxin level (paired t-test, $p < 0.001$). For comparison please refer to Fig. 21.

In all the experiments arterial pressure was maintained above 100 mm of Hg.

From these experiments it was concluded that administration of phenoxybenzamine alone did not significantly alter the intestinal vasoconstriction after endotoxin in cats pretreated with acetylsalicylic acid. Bilateral nephrectomy and hypophysectomy in addition to phenoxybenzamine significantly decreased the vasoconstriction; however the vasoconstriction was not completely abolished.

DISCUSSION

In the cat, intravenous injection of a lethal dose of endotoxin produced an immediate hypotensive response associated with a rise in right atrial pressure. This initial hemodynamic effect has been shown to be due to pulmonary hypertension (Kuida et al. 1961). During the past decade many attempts have been made to prevent the initial hypotensive response in cats. Antihistamine and antiserotonin agents were found to modify the acute hypotensive response to small doses of endotoxin. These pharmacological agents were ineffective against the responses produced by larger doses of endotoxin (Gilbert, 1959). Greenway and co-workers (1969) used endotoxin which was subjected to mild alkaline hydrolysis and found no initial hypotensive response in two-thirds of the cats. However the lethal effects were not modified by this treatment. This procedure seemed unsatisfactory for the study of the late hemodynamic changes since it would involve use of a modified endotoxin and one third of the cats would show the initial phase of hypotension. Kadowitz and Yard (1970) reported a significant reduction in the initial hypotensive response after pretreatment with hydrocortisone. However this pretreatment failed to provide complete protection. In addition to this, the lethal effects of endotoxin were also modified.

In this series of experiments, pretreatment with 10 mg/kg or 100 mg/kg of acetylsalicylic acid was consistently effective in blocking the initial hypotensive response and preventing the hemorrhagic lesions in the lungs. Although the initial hypotensive response was blocked, pretreatment with acetylsalicylic acid failed to modify the lethal effects of endotoxin. These results confirm the hypothesis put forward by Greenway and co-workers (1969) that the late lethal response is an independent

effect of endotoxin and not a secondary consequence of the acute response.

The mechanism of this protective action of acetylsalicylic acid is not clear. In rabbits and dogs, the pulmonary vascular responses to endotoxin seem to depend on the presence of platelets in the blood (Hinshaw et al. 1957; Stein and Thomas, 1967; Evans and Mustard, 1968). The possible role of platelets in the development of the pulmonary response in cats has not been investigated but it is likely that the mechanism of pulmonary vascular response seen in cats is similar to that seen in other species. Intravenous administration of endotoxin produces marked thrombocytopenia in a variety of species (Rabbit- Shimamoto et al. 1958a; Cohen et al. 1965; Dog- Davis et al. 1960a; Rat- Roy, Djerassi, Neitlich and Farber, 1962). Recently, a similar decrease in the circulating platelet count has been reported in cats after administration of endotoxin (Kitzmiller, Lucas and Yelenosky, 1972). Endotoxin is known to produce platelet damage and to release various vasoactive substances including serotonin (Shimamoto et al. 1958b; Davis et al. 1960a, 1961). Since serotonin is a potent pulmonary vasoconstrictor (Gilbert, 1959) and large doses of serotonin injected intravenously produce hemodynamic changes resembling the initial hypotensive response to endotoxin (Greenway, unpublished observations), one might implicate endotoxin-induced platelet injury and the resulting release of serotonin in the development of pulmonary hypertension in cats.

It is likely that acetylsalicylic acid prevents the platelet injury produced by endotoxin and thus prevents the release of serotonin. Though this has not been shown in the cat, acetylsalicylic acid has been

shown to inhibit endotoxin-induced platelet aggregation in rats (Renaud and Godu, 1970) and rabbits (Evans and Mustard, 1968). Collier (1969) has reviewed the pharmacology of acetylsalicylic acid and suggests that it antagonizes some of the humoral mediators by a local action which does not involve a receptor antagonism. Acetylsalicylic acid also inhibits histamine release (Haining, 1956) and anaphylactic shock (Campbell, 1948). Recently, acetylsalicylic acid as well as other anti-inflammatory agents have been found to prevent release of prostaglandins from the platelets and lungs subjected to anaphylactic reaction (Vane, 1971; Smith and Willis, 1971). Since neither the basic mechanism involved in the release of chemical mediators nor the nature of the chemical mediators responsible for the initial hypotensive response in the cat is known, any one of the above mentioned, or even some action not yet known to us, might be responsible for such protection. From the available information it is difficult to implicate any one mechanism though some are more tempting than others.

Although the initial hypotensive response to endotoxin is abolished, the cats still show a delayed phase of hypotension which preceeds death. Since the lethal effects were seen in the absence of initial hypotension, it can be concluded that a lesion other than the initial hypotension now plays a major role in the lethal effects of endotoxin. Hinshaw and co-workers (1967c) showed similar prevention of the initial hypotensive response in the dog after pretreatment with acetylsalicylic acid. However, such prevention of the initial hypotensive response was associated with an increased survival of the endotoxin-treated animals.

When acetylsalicylic acid was administered after the onset of the hypotension, no improvement in the survival rate was observed. These observations indicate that, at least in the dog, the initial hypotensive response can lead to death and therefore prevention of acute hypotension has been associated with increased survival. This however does not eliminate the presence of an independent lethal effect which might have been very transient at the doses used by the above investigators. It is possible that a higher dose of endotoxin might have revealed the delayed lethal effect. In the cat, however, acetylsalicylic acid does not appear to have any protective effect from the delayed lethal effects of endotoxin.

The pathological lesions seen in the cats which died after endotoxin directed us to investigate the effects of endotoxin on the mesenteric vascular bed. After pretreatment with acetylsalicylic acid, endotoxin causes a marked mesenteric vasoconstriction. The superior mesenteric arterial flow decreased to 30% of the control level. This intense mesenteric constriction persisted for the remaining period of observation. The degree of mesenteric ischemia was similar to that seen after hemorrhage (McNeill et al. 1970) in cats. However, the mesenteric vasoconstriction was greater after endotoxin than after hemorrhage since arterial pressure was not reduced and the decrease in arterial flow was due entirely to vasoconstriction. Mesenteric ischemia of this magnitude could be fatal to the animal. Mesenteric ischemia is known to have detrimental effects on cats (Glenn and Lefer, 1970; Haglund and Lundgren, 1972) dogs (Selkurt, 1959; Williams, Anastasia, Hasiotis, Bosniak and

Byrne, 1968; Chiu et al. 1970) and man (Ottinger and Austen, 1967; Jordan, Boulafendis and Guinn, 1970) so this may be one cause of the subsequent hypotension and death of the cats. Intravenous infusions of vasopressin (10-20 mU min^{-1} kg^{-1}) for 3-4 hours caused death within 48 hours in cats (Greenway, unpublished observations). Such doses have been shown to produce marked intestinal and splenic vasoconstriction with only small to moderate increases in arterial pressure (Cohen, Sitar, McNeill and Greenway, 1970). Unless this mesenteric constriction which occurs after endotoxin administration is blocked, it is not possible to state whether mesenteric ischemia or some other factor leads to death.

Mesenteric vasoconstriction has been reported in dogs after endotoxin (Lillehei et al. 1965; Hinshaw, 1968; Brobmann et al. 1970). In these experiments the animal responded to endotoxin by an initial hypotensive response, which was followed by recovery to near normal levels, then a late hypotension. Under such experimental circumstances, many factors can lead to mesenteric vasoconstriction. Initial hypotension leads to a reflex increase in the sympathetic activity, which leads in turn to renal vasoconstriction. Renal blood flow undergoes further decrease due to the decrease in arterial pressure. An ischemic kidney is known to release renin and to lead to formation of angiotensin, a potent vasoconstrictor. Release of angiotensin has been demonstrated in response to endotoxin in dogs (Hall and Hodge, 1971). Hypotension resulting from hemorrhage has been shown to cause release of vasopressin (Weinstein, Berne and Sachs, 1960). Share and Levy (1962) reported that a decrease in the carotid sinus pressure results in marked increase in the blood levels of vasopressin. These factors can cause intense mesenteric

vasoconstriction. Furthermore the intestinal vascular bed shows the phenomenon of autoregulatory venous-arteriolar response. Selkurt and Johnson (1958), and Johnson (1959) showed that an increase in portal venous pressure is always associated with an increase in the mesenteric vascular resistance. During the initial phase of hypotension, portal venous pressure rises dramatically, and during later phases pressure in the small mesenteric veins remains elevated even though portal venous pressure returns to normal levels (Hinshaw and Nelson, 1962; Meyer and Visscher, 1962). Although this phenomenon has not been investigated during endotoxin-induced mesenteric constriction, it is possible that such an increase in venous pressure might at least in part contribute to increased mesenteric vascular resistance. Measurements of resistance during low perfusion pressure are further complicated by a passive decrease in vessel diameter. Such passive geometric factors increase the resistance to flow at low arterial pressures. Under these circumstances it is difficult to state whether vasoconstriction is due to a direct effect of endotoxin or to one or more of these secondary consequences.

In the present series of experiments, the arterial pressure remains near normal levels throughout the period of observation. This eliminates the possible involvement of the hypotensive phase in the development of mesenteric constriction. The role of other known humoral factors will be discussed later.

Since acetylsalicylic acid has many diverse effects in the body, one can not exclude the possibility that the delayed hemodynamic effects of endotoxin are also modified in part or completely. Experiments with

slow infusion of endotoxin reveal that the pattern of arterial hypotension seen during slowly developing endotoxemia is different from that seen after a rapid injection. Thus we found that without pretreatment with acetylsalicylic acid, the same lethal dose (3 mg/kg) of endotoxin does not produce the initial hypotension; however, it produces mesenteric vasoconstriction of similar magnitude. Although a slowly developing hypotension was observed in all the cats, arterial pressure invariably recovered. None of them showed any increase in right atrial pressure. Though the mechanism of this slowly developing hypotension is not known, it is unlikely that it is due to pulmonary hypertension.

Four hours after the infusion of endotoxin, the mesenteric vasoconstriction was intense while arterial pressure was at pre-endotoxin levels. It can be concluded that not only do the cats pre-treated with acetylsalicylic acid show vasoconstriction, but also the cats which were given a slow infusion of endotoxin show the same hemodynamic response. It appears from these observations that mesenteric vasoconstriction is one of the hemodynamic effects of endotoxin which is not dependent on the initial hypotensive response.

Investigations into the fractional distribution of the superior mesenteric arterial blood flow into various mesenteric organs revealed that this vasoconstriction is not selective, but affects the major mesenteric organs. The intestine, the colon, and the lymph nodes show vasoconstriction. Less than 1% of the superior mesenteric arterial blood flows through arterio-venous channels larger than 17μ in diameter. This fraction of the flow does not change after endotoxin-induced mesenteric

vasoconstriction. Thus it can be concluded that no anatomical shunts open to permit arterio-venous shunting of blood in cats. In a similar investigation carried out in the dog, Motsay, Alho, Zachman, Dietzman and Lillehei (1970) found no arterio-venous anastomoses exceeding 25μ in diameter in the superior mesenteric vascular bed of the dog 2 hours after injection of endotoxin. Whatever mechanism is postulated to explain the mesenteric vasoconstriction, it must account for the uniform constriction in the intestine, colon and lymph nodes.

We attempted to analyse the mechanism of this mesenteric vasoconstriction by techniques previously used to study the intestinal and splenic vasoconstriction after hemorrhage (McNeill et al. 1970; Stark et al. 1971; Greenway and Stark, 1971). However, after removal of the animals' compensatory mechanisms, endotoxin caused severe hypotension. Similar responses have been reported by Hinshaw and co-workers (1964) and Spink and co-workers (1966) in adrenalectomized animals. In our experiments this hypotension was not due to cardiac depression or to pulmonary vasoconstriction since right atrial pressure did not increase and intravenous infusion of blood could be used to maintain arterial pressure without increasing right atrial pressure. It was not due to hepatic venoconstriction such as occurs in the dog, since no increase in portal pressure was seen. Thus a primary event after administration of endotoxin appeared to be venodilatation and pooling of blood induced either by endotoxin per se or by release of a chemical mediator. This response was unmasked only when the compensatory vasoconstrictor mechanisms were removed. This observation requires further investigation.

In the present experiments, it was overcome by infusion of donor blood to maintain arterial pressure.

The mesenteric vasoconstriction was not modified after phenoxybenzamine was given in doses known to block mesenteric vascular responses to sympathetic nerve stimulation, and infusions of noradrenaline in amounts likely to be released by the adrenal medullae (McNeill et al. 1970). Thus the sympathetic innervation of the intestine and circulating noradrenaline appear to play no part in this vasoconstriction after endotoxin. A similar conclusion was reached in regard to intestinal vasoconstriction after hemorrhage in the cat (McNeill et al. 1970). These conclusions are at variance with previous reports. It has been claimed that intestinal vasoconstriction after hemorrhage and endotoxin in the dog was blocked by phenoxybenzamine (Lillehei et al. 1965). In other reports, the vasoconstriction was blocked after infiltration of lidocaine into the coeliac ganglion (Hauman, 1968; Wangensteen et al. 1971). It is difficult to explain these observations on the basis that sympathetic nerves play a major role since mesenteric vasoconstriction has been shown to occur in isolated pump-perfused loops of intestine (Hinshaw, 1968).

Removal of the pituitary and kidneys significantly reduced the intestinal vasoconstriction seen after endotoxin. It appears from these observations that angiotensin and vasopressin play some role in the vasoconstriction but their contribution to mesenteric vasoconstriction seems to be less after endotoxin than after hemorrhage (McNeill et al. 1970; Stark et al. 1971). This involvement of renal and hypophyseal humoral

factors reveals another interesting fact: the release of these substances must be mediated by some mechanism other than hypotension. The role other reflexogenic areas of cardiovascular system play in this response during endotoxemia, is not known. It is unlikely that endotoxin per se causes release of these humoral agents, since no direct effects of endotoxin are known.

A substantial vasoconstriction remained after removal of these compensatory mechanisms and this residual response was much greater than the residual response after hemorrhage. This suggests that vasoconstrictor factors other than the humoral factors of the pituitary and kidney are involved in this vasoconstriction. This phenomenon needs further investigation. Various known vasoactive substances must be taken into consideration. Serotonin is one of the agents known to be released after administration of endotoxin (Davis, Ausman, Meeker, Gemmill and Aust, 1960b). Endotoxin initiates intravascular coagulation in many species (McKay and Shapiro, 1958; Gans and Krivit, 1960; Hardaway and Johnson, 1963; Rodriguez-Erdmann, 1965). The peptides released in the process of formation of fibrin are potent vasoconstrictors (Osbahr, Morris and Colman, 1967; Bayley, Clements and Osbahr, 1967). In cats, endotoxin has been shown to increase the levels of myocardial depressant factor in the blood (Glenn and Lefer, 1970). Recently Glucksman and Lefer (1971) reported that the myocardial depressant factor has vasoconstrictor effects on the isolated strip of superior mesenteric artery. During the period when mesenteric ischemia occurred in the cats, no evidence of myocardial depression was seen. Similar reports have appeared from various laboratories (Alican et al. 1962; Londe, Massie, Monafo and Bernard, 1967; Brockman

et al. 1967; Hinshaw et al. 1971). Thus it is unlikely that the mesenteric vasoconstriction seen in these cats was due to the myocardial depressant factor. It is likely that prolonged ischemia of the mesenteric organs may lead to formation of the myocardial depressant factor in the late stages but it is difficult to implicate this substance as the mediator for any of the early hemodynamic changes seen after endotoxemia. Evisceration of dogs removes the source of the myocardial depressant factor, however the deterioration of the cardiovascular function still takes place (MacLean and Weil, 1956; Blattberg and Levy, 1970). Recently Wangensteen and co-workers (1971) have shown that celiac blockade prevents mesenteric ischemia after endotoxin and also prevents the formation of the myocardial depressant factor. However, the arterial pressure as well as the cardiac output remained low and no difference in the lethal effects of endotoxin could be seen whether the myocardial depressant factor was formed or not.

Numerous other vasoactive substances are released into the circulation during endotoxemia (Kobold et al. 1964). In addition, mesenteric ischemia can release a variety of chemical substances, with cardiovascular and respiratory depressant actions (Bergan, Gilliland, Troop and Anderson, 1964; Rangel, Dinbar, Stevens, Byfield and Fonkalsrud, 1970). These vasoactive substances could lead to progressive cardiovascular insufficiency and death.

From the present investigation it can be concluded that the initial hypotensive response can be blocked by pretreating the cats with acetylsalicylic acid. The lethal effects of endotoxin are not blocked,

indicating no causal relationship between the lethal effects and the initial hypotension. An intense mesenteric vasoconstriction developed in these cats. When the same dose of endotoxin was infused slowly, the initial hypotension and the rise in right atrial pressure were not seen. However, mesenteric vasoconstriction was seen in these cats. It is therefore concluded that mesenteric vasoconstriction is an independent action of endotoxin and does not depend on the initial hypotension. Since mesenteric vasoconstriction was seen in cats which did not receive acetylsalicylic acid before a slow infusion of endotoxin, this response is not an experimental artifact induced by administration of acetylsalicylic acid. The observations made with slow infusion of endotoxin further underscore the importance of the mode of induction of endotoxemia in experimental animals, since it is likely that under more natural circumstances, endotoxemia develops very slowly and therefore initial hypotensive response may not be seen.

The mesenteric vasoconstriction affects all the major mesenteric organs in a similar manner. No evidence for arterio-venous shunting of blood could be obtained within the mesenteric vascular bed. The mechanism of mesenteric vasoconstriction was investigated. Sympathetic vasoconstrictor influences seem to play no part in this response. Humoral factors from the kidney and the pituitary contribute significantly to it. Some residual constriction persisted after removal of the vasoconstrictor influences of the kidney, the pituitary and the sympathetic nervous system. It is likely that some other locally-released or circulating vasoconstrictor substance plays an important role in this vasoconstriction.

Although the mesenteric ischemia seen after endotoxin is severe enough to be fatal, it is difficult to exclude the possibility of other factors contributing to it. Recently, it has been shown that mesenteric vasoconstriction does not occur in primates after endotoxin (Hinshaw, 1968; Wyler, Forsyth, Nies, Neutze and Melmon, 1969; Brobmann et al. 1970). It is likely that human responses to endotoxin are different from those of the cat, yet proper understanding of the basic pharmacological properties of endotoxin will be of great value in the future evaluation of the role of endotoxin in the pathogenesis of progressive cardiovascular insufficiency seen in some patients with gram-negative bacteremia.

APPENDIX

EFFECTS OF VASOPRESSIN AND ISOPRENALINE INFUSIONS ON THE DISTRIBUTION OF BLOOD FLOW IN THE INTESTINE.

Criteria for the validity of microsphere studies

INTRODUCTION

This study was begun as an attempt to compare the effects of a variety of vasoactive agents on the distribution of blood flow within the wall of the small intestine. A variety of effects on total intestinal blood flow have been reported in the literature and some of these effects have been explained on the basis of complex changes in flow distribution within the intestinal wall (Dresel and Wallentin, 1966; Greenway and Lawson, 1966; Ross, 1971a; Shanbour and Jacobson, 1971; Shehadeh, Price and Jacobson, 1969; Swan and Reynolds, 1971a, 1971b). The following is a brief outline of the development of the concept of parallel-coupled vascular circuits in the intestinal wall.

In 1964, Folkow, Lewis, Lundgren, Mellander and Wallentin showed that stimulation of the sympathetic nerves to the intestinal vascular bed in cats produced an initial vasoconstriction followed by a recovery of the flow towards the control level in spite of maintained nerve stimulation. They termed this recovery 'autoregulatory escape'. The capillary filtration coefficient (a measure of capillary surface area) decreased for the duration of nerve stimulation, there was a post-stimulatory hyperaemia and India ink was not distributed to the mucosa to the same extent during stimulation as compared with the control period. These observations were interpreted on the hypothesis that there was a neurogenic redistribution of blood flow from the mucosa towards the submucosa (Folkow, Lewis, Lundgren, Mellander and Wallentin, 1964a, 1964b; Dresel and Wallentin, 1966).

Further studies on the arterio-venous extraction of rubidium excluded the involvement of true arterio-venous shunts and led to the concept of a dense plexus of thin-walled, small vessels which allowed exchange across their walls in the submucosa (Dresel, Folkow and Wallentin, 1966). This concept was elaborated further by Lundgren (1967) from studies on the wash-out of intra-arterially injected ^{85}Kr. Three parallel-coupled circuits were postulated: the muscle layer with a flow of (10-15 ml/min) /100 g at rest, a well-vascularised area located in the submucosa and adjacent mucosa with a flow of (400-600 ml/min)/100 g and the mucosa with a flow of (40-60 ml/min)/100 g. During isoprenaline infusion, the flow in the submucosal area increased to 50-60% of the total flow - (800-1000 ml/min)/100 g. The situation was further complicated by evidence for a countercurrent exchange of material between the ascending and descending limbs of mucosal vascular loops mainly located in the villi (Folkow, 1967; Lundgren, 1967).

Recently evidence has been accumulating against this concept of three parallel-coupled sections and a redistribution from mucosa to submucosa during autoregulatory escape. This evidence has been reviewed by Ross (1971b) who also presented the most convincing evidence against the hypothesis. He showed from the uptake of ^{86}Rb that the distribution of flow between mucosa, submucosa and muscle during auto-regulatory escape was not significantly different from that in a control group of animals. This recent work has been interpreted to suggest that escape was due to relaxation of the same vascular elements which were originally constricted by noradrenaline (Ross, 1971c). In addition,

^{86}Rb uptake by the submucosa was only 12% of the total suggesting that an extensive capillary network was not present in the submucosa.

The recent availability of radioactive microspheres suggested a new approach to this question of parallel-coupled circuits in the intestine. Grim and Lindseth (1958) studied the distribution of radioactive microspheres in the layers of the intestinal wall and although the distribution between mucosa and submucosa was markedly different when different sized microspheres were used, they interpreted this distribution as a valid measure of flow in each layer and calculated mucosal, submucosal and muscle flows. We began to use this method to determine whether the distribution between the layers was altered during infusions of vasopressin, isoprenaline and noradrenaline but as the work progressed, it became clear that the distribution of the spheres between the mucosa and submucosa was not a measure of the distribution of flow. The data and our interpretation of them are described in this section.

METHODS

Cats were anaesthetized by intraperitoneal injection of sodium pentobarbitone (Abbott Laboratories, 30 mg/kg body weight). Supplementary doses (2 mg/kg) were given through a cannula in a forelimb cutaneous vein when reflex ear, eye and swallowing movements returned. The trachea was cannulated and arterial pressure was recorded from a femoral artery. The abdomen was opened by a mid-line incision and the anastomotic branch of the superior mesenteric artery was identified. This is the first branch of the superior mesenteric artery and it anastomoses with the inferior mesenteric artery. Its ligation does not deprive any area of flow and it was cannulated for injection of radioactive microspheres into the superior mesenteric artery (Fig. 22). The total flow in the superior mesenteric artery was measured by a 2 mm diameter non-cannulating flow probe of an electromagnetic flowmeter (Nycotron, Oslo). The probe was set up, zero flow was determined and the probe was calibrated as previously described (Section II).

Microspheres (15 $\pm$ 5μ diameter), labelled with ^{141}Ce or ^{51}Cr, were suspended in 10% dextran solution (3M Nuclear Products, Minnesota) and a dose of approximately 220,000 microspheres was given into the superior mesenteric artery in each experiment.

Isoprenaline HCl (B.D.H.) was dissolved in 0.9% w/v NaCl containing ascorbic acid (0.2 mg/ml) and vasopressin (Pitressin, Parke Davis Co.) was diluted in 0.9% NaCl. The isoprenaline was infused into the superior mesenteric artery through the cannula in the anastomotic branch while the vasopressin was infused intravenously in doses which produced a relatively specific intestinal vasoconstriction (Cohen, Sitar,

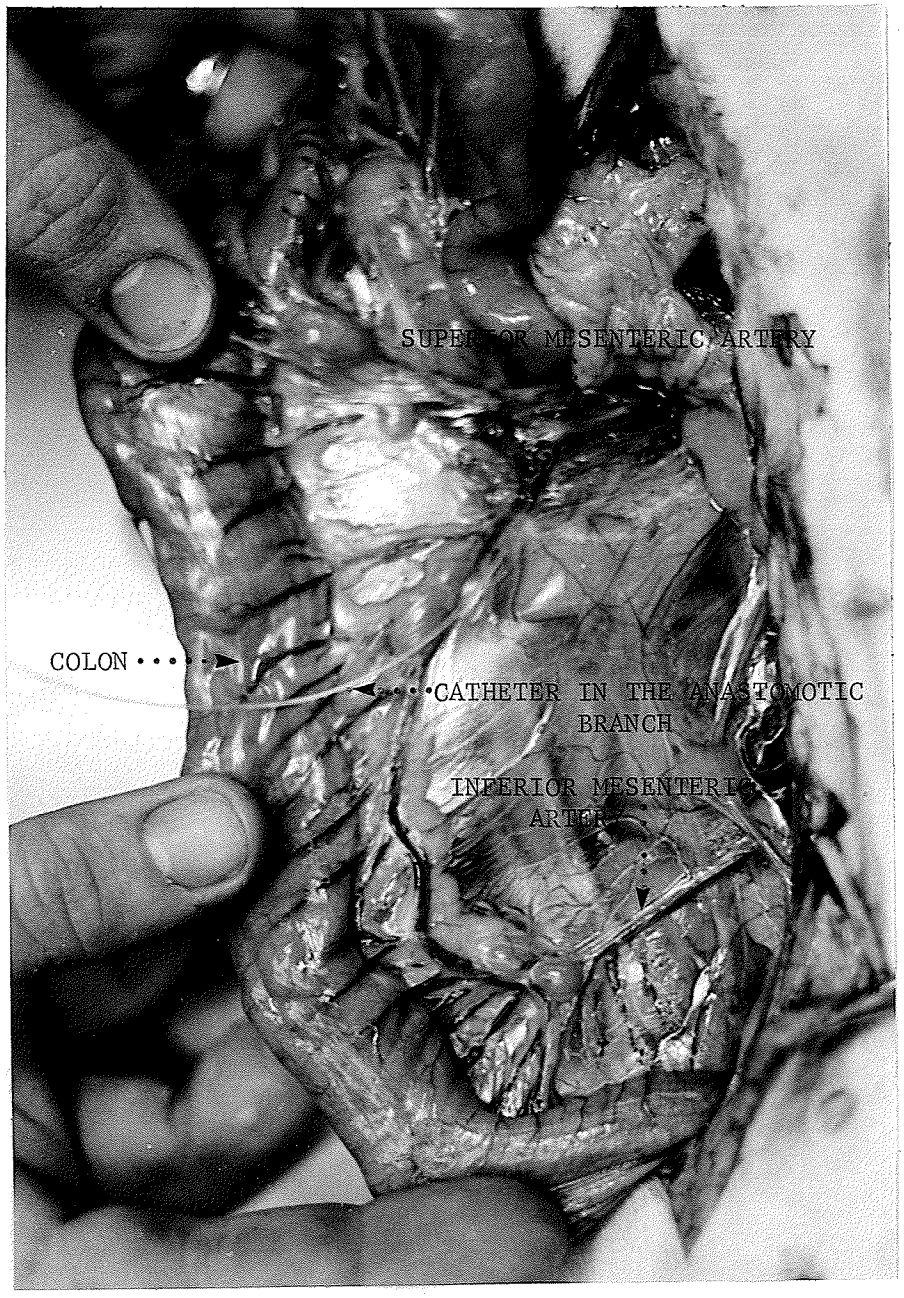


Figure 22: Photograph showing the anatomical relationship of the anastomotic branch to superior mesenteric artery.

McNeill and Greenway, 1970).

At the end of each experiment, the abdominal viscera, lungs and liver were removed and the following procedures were carried out. The pancreas, lymph nodes, mesentery, colon, mesocolon, lungs and liver were cut into small pieces and placed in plastic tubes. The intestine was opened along the mesenteric border and cut into 7 cm lengths. Each length was laid on a paper towel with the mucosal surface in contact with the paper (Fig. 23A). The cleavage between submucosa and muscle layer was identified and the two layers were separated (Fig. 23B). The muscular layer was then stripped from the submucosa and placed in a plastic tube (Fig. 23C). Care was taken to avoid damaging the mucosa by pressing on it. The remaining tissue was then spread on another piece of paper with the mucosa uppermost (Fig. 24A) and the mucosa was stripped from the submucosa (Fig. 24B). The two layers were then placed in separate plastic tubes. This was done for the whole length of the intestine. In 3 experiments, the papers on which this dissection was done were placed in plastic tubes to estimate losses of radioactivity during the dissection.

The tissue samples were weighed and the radioactivity was counted in a two channel auto-gamma spectrometer (Packard Instrument Co.). Corrections for overlap of radioactivity between the two channels were made (Greenway and Oshiro, 1972b). Fractional flow to each piece of tissue was calculated from the principle of Stewart-Hamilton as described by Wagner, Rhodes, Sasaki and Ryan (1969):

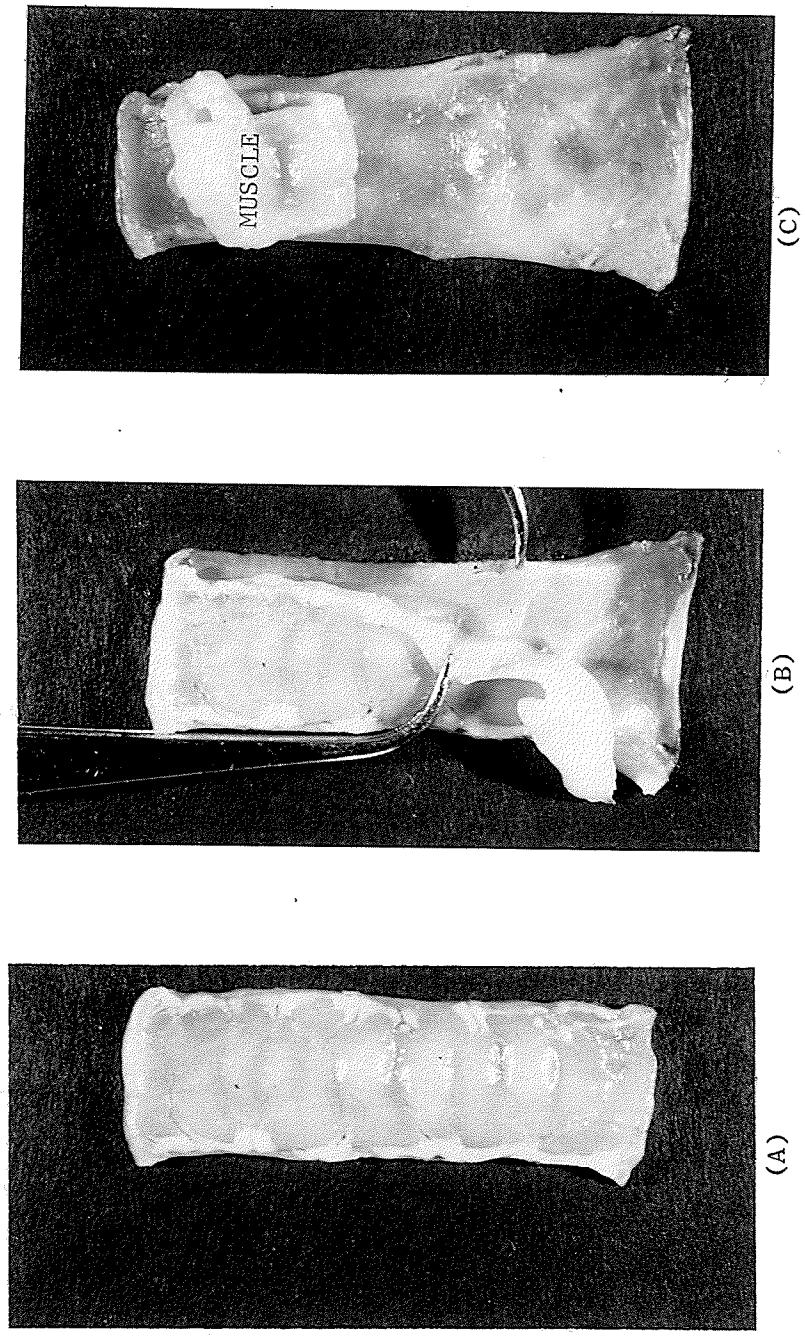


Figure 23: Photographs illustrating the steps in the separation of the outer layer of smooth muscle from the inner layer containing mucosa and submucosa.

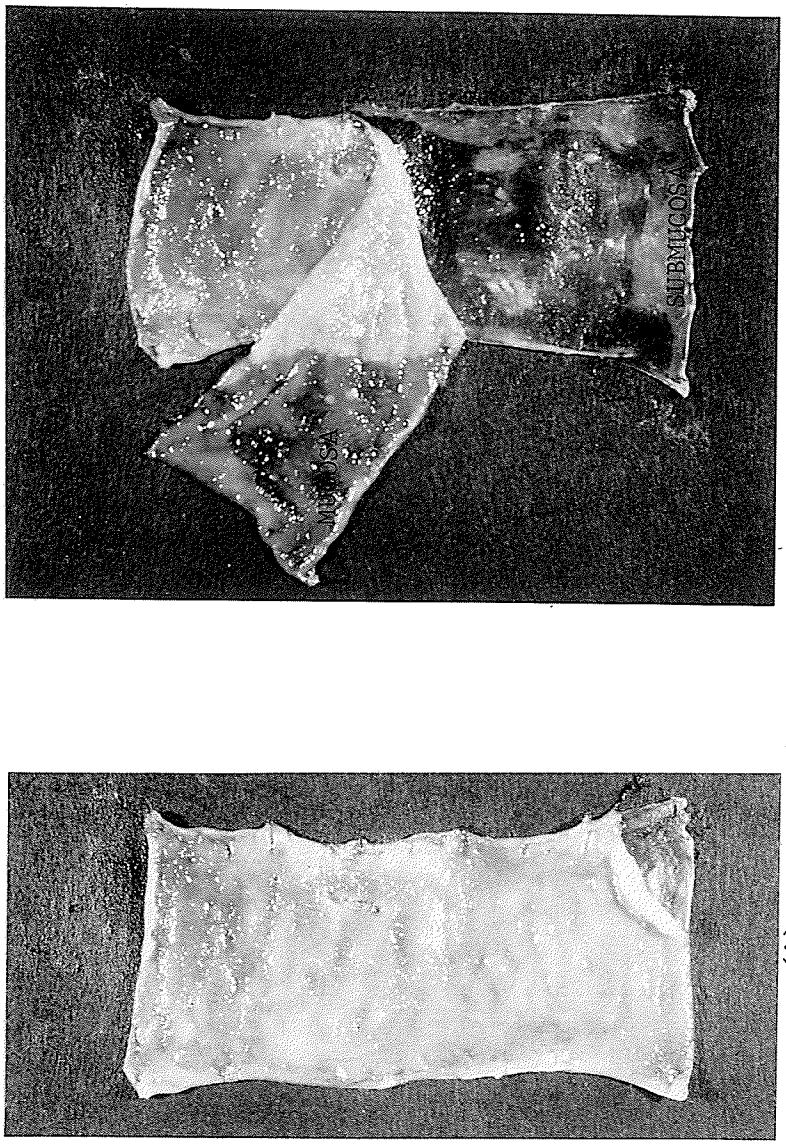


Figure 24: Photographs illustrating the steps in the separation of the mucosal layer from the submucosal layer.

$$f = F.\frac{q}{Q}$$

where f is the fractional blood flow to the tissue, F is the total blood flow in the superior mesenteric artery at the time the microspheres were injected. q is the radioactivity in the piece of tissue and Q is the total injected radioactivity obtained by summation of the counts in all the samples. Since tissue weight was measured, flow/100 g tissue could also be calculated.

To study the architecture of the intestinal vascular bed, India ink was injected into the superior mesenteric artery in 3 experiments. Paraffin sections (15 and 30μ) of the whole intestinal wall were stained with eosin and serial sections were examined. In addition, separated sheets of submucosa were cleared with glycerine and examined (Boulter and Parks, 1960; Reynolds, Brim and Sheehy, 1969).

RESULTS

Distribution of superior mesenteric arterial flow

Thirty-five cats were used (2.8 ± 0.3 kg body weight: mean ± S.E.). Mean arterial pressure was 134 ± 7 mm Hg and superior mesenteric arterial flow was (25 ± 4 ml/min)/kg body weight or (67 ± 9 ml/min)/100 g tissue at the time the microspheres were injected.

In 17 cats, ^{141}Ce-microspheres were injected into the superior mesenteric artery during the control period. The organs in which significant radioactivity was found are shown in Table 3. The small intestine with the exception of the first 2-3 cm of the duodenum, the mesentery, the lymph nodes, the proximal half of the colon and its mesocolon and the head but not the body of the pancreas were supplied by the superior mesenteric artery. The relative weights, proportions of the superior mesenteric arterial flow and the calculated flows to these regions are shown in Table 3. The validity of the calculations is discussed later.

Distribution of microspheres in the layers of the intestine

Histological sections of the separated layers were made to determine the contamination between layers and examples are shown in Fig. 25. The loss of radioactivity onto paper was 0.35 ± 0.08% (mean ± S.E.) during separation of the muscle layer and 1.72 ± 0.31% during removal of the mucosa. It is concluded that the separation procedure did cleanly separate the layers and that the loss of radioactivity during these procedures was small.

In 11 cats, the mean length of the small intestine was 90 ± 6.5 cm and the weight was 0.74 ± 0.05 g/cm. The mucosa formed 37 ± 2.0%,

TABLE 3

The weights (drained of blood) and blood flows (means ± S.E.) of the splanchnic organs perfused by the superior mesenteric artery (SMA).

	Wt of perfused tissue (g/kg body wt)	% of SMA flow	Flow (ml/min)/kg body wt	Flow (ml/min)/ 100 g tissue
Intestine	26.00 ± 2.5	83.00 ± 1.20	21.00 ± 3.10	85.0 ± 13.0
Mesenteric lymph nodes	1.60 ± 0.17	3.30 ± 0.19	0.87 ± 0.15	59.0 ± 12.0
Mesentery	5.20 ± 0.70	1.40 ± 0.06	0.32 ± 0.05	8.9 ± 2.7
Head of pancreas	0.97 ± 0.13	1.00 ± 0.22	0.23 ± 0.08	25.0 ± 10.0
Proximal colon	3.20 ± 0.50	11.00 ± 1.20	2.70 ± 0.51	92.0 ± 22.0
Proximal mesocolon	1.20 ± 0.18	0.56 ± 0.42	0.13 ± 0.04	12.0 ± 5.5

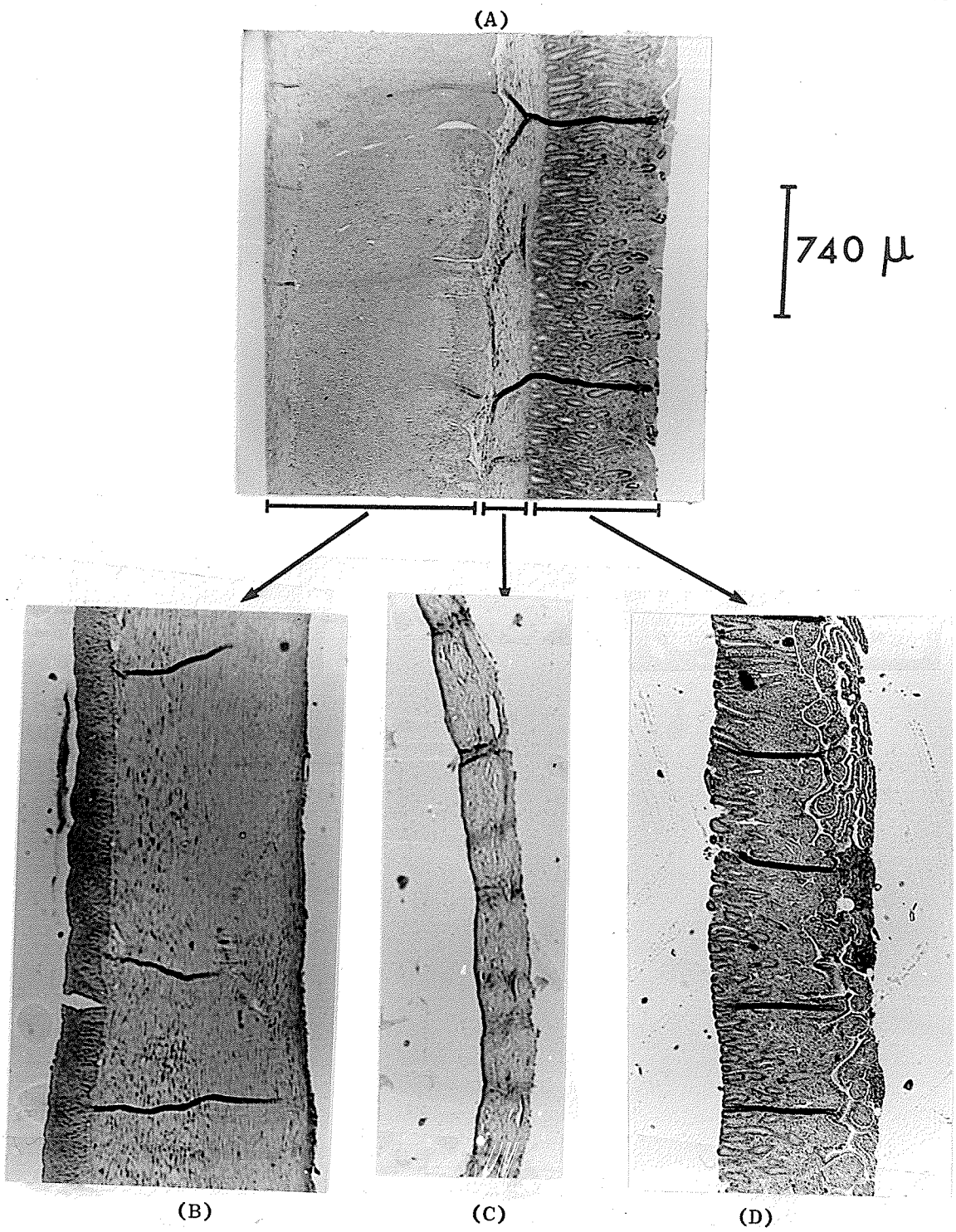


Figure 25: Histological sections of the intestinal wall (A) and of the separated muscular (B), submucosal (C), and mucosal layer (D).

the submucosa 13 ± 0.4% and the muscle 50 ± 2.3% of the total intestinal weight. There was no significant difference in the weight/unit length or in the relative proportions of mucosa, submucosa and muscle between segments of duodenum, jejunum or ileum ($p > 0.1$, unpaired t-test).

The small intestine received 83 ± 1.2% (mean ± S.E.) of the total injected radioactivity (Table 4). The mucosa received 47 ± 4.6%, the submucosa 28 ± 1.8% and the muscle 7.8 ± 1.4% of the total radioactivity. This distribution cannot be interpreted in terms of flow for reasons to be discussed later. The proportion of the total injected in each 7 cm length of intestine and the proportion in each layer were not significantly different ($p > 0.1$, unpaired t-test) between duodenum, jejunum and ileum, except for the first 2-3 cm of duodenum which was not perfused by the superior mesenteric artery.

Injection of two types of microspheres

At this stage in the experiments, we began the use of two microspheres labelled with ^{141}Ce and ^{51}Cr, with the aim of obtaining the control distribution of flow and the distribution during infusion of a drug in each animal. To test the validity of this procedure and the effect of time on the flow distribution the following experiments were done. The ^{141}Ce- and ^{51}Cr-spheres were mixed and the mixture was injected (2 experiments), the ^{141}Ce-spheres were given 10 minutes before the ^{51}Cr-spheres (3 experiments), the ^{141}Ce-spheres were given 2 hours before the ^{51}Cr-spheres (3 experiments) and a ten times larger dose of ^{141}Ce-spheres was given 10 minutes before the ^{51}Cr-spheres (3 experiments). The results in each of these series of control experiments were

TABLE 4

The relative distribution of ^{141}Ce and ^{51}Cr microspheres in 11 cats.

	^{141}Ce as % of total injected	^{51}Cr as % of total injected	Paired S.E.
Mucosa	47.4	26.3*	(1.72)
Submucosa	27.5	50.0*	(2.08)
Mucosa + Submucosa	74.7	76.3	(1.39)
Muscle Layer	7.8	7.7	(0.52)
Total Intestine	82.6	84.0	(1.22)
Mesentery	1.37	1.05	(0.06)
Lymph nodes	3.33	2.92	(0.19)
Head of pancreas	1.02	0.79	(0.22)
Colon	11.2	10.9	(1.22)
Mesocolon	0.56	0.52	(0.42)

(* $p < 0.0001$, paired t-test)

not significantly different ($p > 0.2$, unpaired t-test) and for presentation, the results are pooled and shown in Table 4.

The proportion of the ^{51}Cr-spheres in the mucosa was always lower and the proportion in the submucosa higher than that for the ^{141}Ce-spheres ($p < 0.0001$, paired t-test). The sum of the proportions in the mucosa and submucosa for the ^{141}Ce-spheres was not significantly different from that for the ^{51}Cr-spheres. In the muscle layer and in the other tissues, the distribution of the two types of spheres was not significantly different.

Since Grim and Lindseth (1958) had shown that the distribution between mucosa and submucosa depended on microsphere size, it seemed possible that the ^{141}Ce-spheres were smaller than the ^{51}Cr-spheres even though they were both purchased as $15 \pm 5\mu$ diameter. The size was determined in 2 ways: by direct measurement under a microscope with an eyepiece graticule and by measurement of photomicrographs of known magnification. The ^{141}Ce-spheres were $12 \pm 0.15\mu$ diameter (mean $\pm$ S.E.) and the ^{51}Cr-spheres were $17 \pm 0.16\mu$ diameter. This size difference was highly significant ($p < 0.0001$, unpaired t-test).

Thus the distribution of microspheres between the mucosa and submucosa depended on the size of the microspheres, while the distribution in all other areas and in the combined mucosa plus submucosa did not depend on the microsphere size. This suggests that the mucosa and submucosa are not parallel-coupled sections but are in series, with the mucosal section being of smaller diameter than the submucosal section. Thus larger spheres are trapped in the submucosa while smaller ones pass

further into the mucosa. On this hypothesis, we predicted that if the microspheres were given when the vessels were constricted, subsequent dilation of the vessels should cause some microspheres to move from the submucosa into the mucosa. This prediction was tested.

Effects of vasopressin followed by isoprenaline infusions

In 7 cats, superior mesenteric arterial flow was recorded and vasopressin was infused intravenously. During infusions of (10 mU/min)/kg, the flow decreased from (31 ml/min)/kg to (10 ml/min)/kg (paired S.E. $\pm$ 2.6; $p < 0.001$, paired t-test). When the flow was steady at this low level, ^{141}Ce-spheres were injected into the superior mesenteric artery followed 2-3 minutes later by ^{51}Cr-spheres. A 40-50 cm length of intestine was then removed while the infusion of vasopressin was continued. The position of the excised piece was not critical since the proportional weights and microsphere distributions were found to be similar in all segments of small intestine. The vasopressin was then stopped and isoprenaline was infused into the superior mesenteric artery. The dose was increased until maximal vasodilation was obtained. Flow increased to (49 $\pm$ 5.5 ml/min)/kg but this cannot be compared to the control flow since half of the small intestine had been removed. After the flow had been steady for 5-10 minutes, the animal was killed and the remaining portions of the intestine were removed. The distribution of the two types of microspheres in the mucosa and submucosa during vasopressin infusion and after subsequent vasodilation by isoprenaline are shown in Table 5. It can be seen that isoprenaline caused a highly significant movement of both microspheres from the submucosa into the mucosa.

TABLE 5

Relative distributions of ^{141}Ce and ^{51}Cr-microspheres injected during vasopressin infusion into the superior mesenteric artery. Part of the intestine was removed during vasopressin infusion and the remainder after subsequent vasodilation by isoprenaline. The mean radioactivity in the mucosa and submucosa for 7 cats is expressed as a % of the sum in the two layers.

	During vasopressin	After subsequent isoprenaline	Paired S.E.
^{141}Ce in mucosa	51.0	63.1*	(1.13)
^{141}Ce in submucosa	49.0	36.9*	(1.13)
^{51}Cr in mucosa	20.9	44.4*	(1.30)
^{51}Cr in submucosa	79.1	55.6*	(1.30)

(* $p < 0.0001$, paired t-test)

Microspheres in the liver and lungs

In all the experiments, the liver and lungs were counted to determine the proportion of microspheres which passed through the mesenteric organs and were trapped in the liver or lungs. The proportion of the injected dose which was found in the liver was 0.20 ± 0.005% for ^{141}Ce and 0.03 ± 0.001% for ^{51}Cr-spheres. No detectable radioactivity was present in the lungs. It is concluded that less than 1% of the blood flow passed through vessels larger than the ^{141}Ce microspheres (12μ) in control experiments or after maximal vasodilation of the mesenteric organs.

Histological studies

The most notable feature of the sections of intestine and sheets of submucosa after India ink injection was the almost complete absence of capillary-sized vessels in the submucosa except close to the mucosal border. These small vessels close to the mucosal border appeared to pass into the mucosa when serial sections were examined. An example of the type of picture seen is shown in Fig. 26. No vessels appeared to pass from the submucosa to the muscle layer and the branches to the muscle arose from the arteries on the peritoneal surface of the muscle. The sheets of submucosa showed a dense plexus of vessels. These observations confirm those of other workers (Barlow, 1952; Boulter and Parks, 1960). The submucosal vessels were larger than 30μ diameter except for the small branches to the mucosa. No arterio-venous anastomoses were seen (confirming Jacobson and Noer, 1952) and this is confirmed by the small proportion of radioactivity in the liver. If any of these

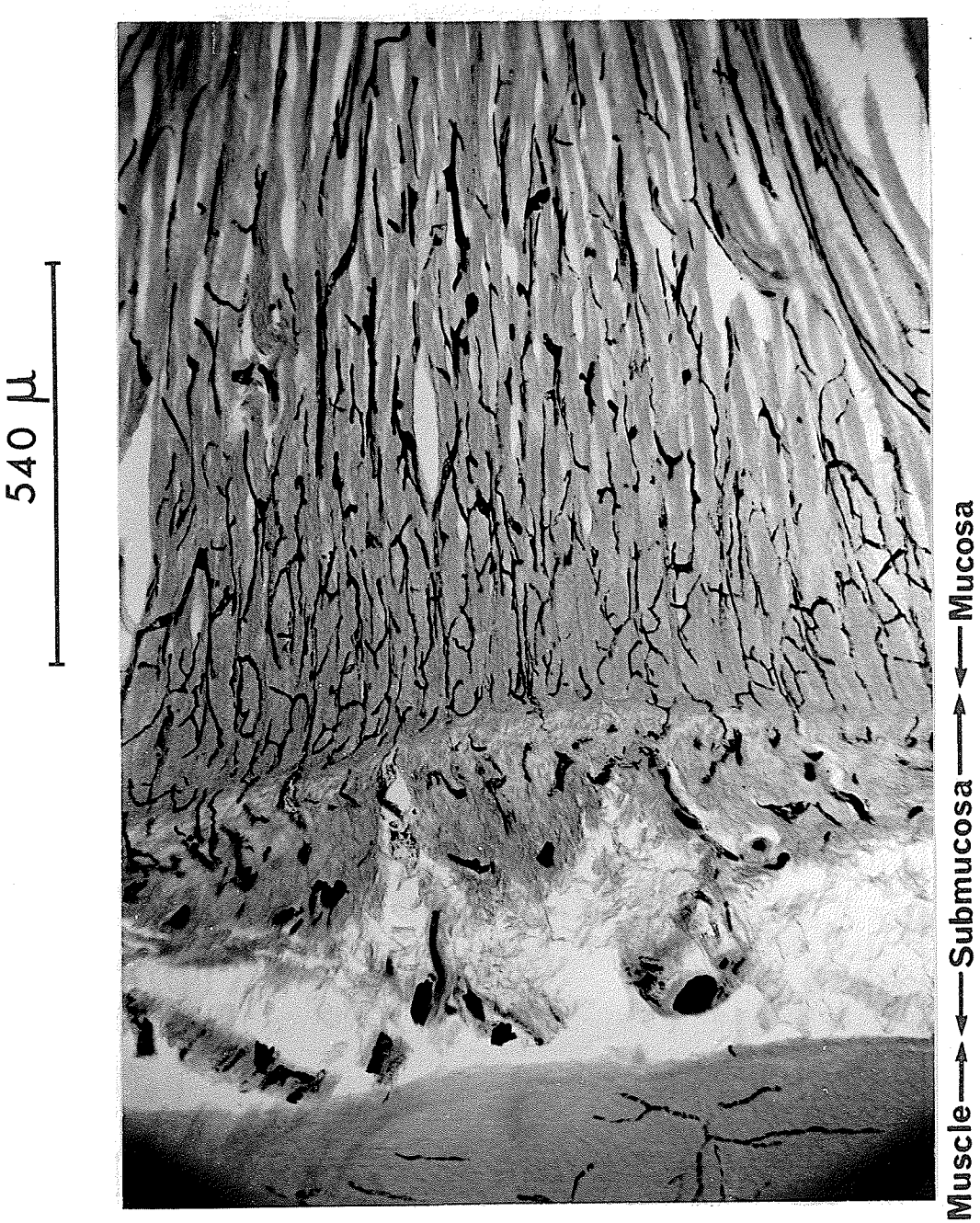


Figure 26: Histological section showing the submucosa and the adjoining mucosal and smooth muscle layers, after injection of India ink. Note the relative absence of capillary-sized vessels in the submucosa except close to the mucosal border.

vessels (larger than 30 μ) were arterio-venous anastomoses, microspheres would be expected to pass through them.

DISCUSSION

The use of radioactive microspheres to study fractional distribution of cardiac output or blood flow to some region is based on the assumption that the spheres are uniformly mixed with the blood, that they have the same rheological properties as the red cells, that they become impacted in some section of the pre-capillary bed depending on their size and thus that they are distributed proportionally to the blood flow in each of the parallel-coupled sections of the vascular bed (Wagner, Rhodes, Sasaki and Ryan, 1969). If a series of determinations of flow distribution are to be made by repeated injections of several types of microspheres, it must be shown that any one measurement is not modified by the previously administered spheres. In some situations, these assumptions are clearly not valid; for example, portal flow to the liver cannot be measured by intra-arterial injection of microspheres since the liver is in series and not in parallel with the other mesenteric organs. Also if the spheres are small enough to pass through any part of the bed, the distribution will not accurately reflect flow. In most studies, some indication of the validity of the assumptions can be gained by administration of microspheres labelled with two different isotopes and of two different sizes. The spheres should be given simultaneously in some control experiments and one after the other in other control experiments. If the distribution of these two types is closely similar in the areas being studied and if no significant radioactivity passes through the bed to be trapped in the next series-coupled capillary bed (liver or lungs), the assumptions are mainly justified.

In the mesenteric vascular bed, these criteria are fulfilled in the intestine as a whole, the mesentery, lymph nodes, pancreas, colon and mesocolon and our data confirm and extend earlier work with microspheres varying in size from 15 to 80μ diameter (Delaney, 1969; Kaihara, Van Heerden, Migita and Wagner, 1968). Therefore it appears reasonable to suggest that the distribution of spheres in these regions is a valid measure of blood flows (Table 3). These values are in substantial agreement with data obtained by other methods (Delaney, 1969; Ericsson, 1971; Folkow and Neil, 1971; Goodhead, 1969; Lundgren and Wallentin, 1964; Ross, 1971c). The values for pancreatic blood flow reflect only the proportion supplied by the superior mesenteric artery and the contribution from the celiac artery was not measured. The criteria were also fulfilled for the muscle layer of the intestine and for the combined mucosa plus submucosa. The muscle received 7.8% of the total radioactivity giving a mean flow of (2.0 ml/min)/kg body weight, (8.0 ml/min)/100 g intestine or (16 ml/min)/100 g muscle. The combined mucosa and submucosa received 74% of the total radioactivity giving a mean flow of (19 ml/min)/kg body weight, (77 ml/min)/100 g intestine or (154 ml/min)/100 g mucosa plus submucosa. These values are in agreement with those obtained from the ^{85}Kr wash-out studies in intestine (Kampp and Lundgren, 1968) and colon (Hultén, 1969) but much lower than the value for muscle flow reported by Grim and Lindseth (1958). No significant radioactivity was found in the liver or lungs and this suggests that less than 1% of the superior mesenteric arterial flow passes through arterio-venous shunts larger than 12μ . This proportion

is even smaller than that reported by Delaney (1969) and is substantially smaller than that reported by Grim and Lindseth (1958). In the latter experiments the authors suggest some leaching of the isotope from the spheres occurred.

The distribution of microspheres between the mucosa and submucosa did not meet the criteria set out above in that the distribution was dependent on the size of the spheres. Grim and Lindseth (1958) also observed this but no explanation was suggested. If the microspheres in the submucosa were impacted in some part of a parallel-coupled vascular bed in the submucosa, it is extremely unlikely that they would move into the mucosa when the vascular bed was subsequently dilated. On the other hand, if the submucosal vessels were in series with the mucosal vessels, the site of impaction of the spheres would depend on the relative sizes of the spheres and vessels as they passed from submucosa to mucosa. This would explain the greater proportion of ^{141}Ce-spheres (12μ) compared to ^{51}Cr-spheres (17 μ) in the mucosa and the movement of both types of spheres when the vascular bed was dilated.

We conclude from these arguments that a portion of the microspheres in the submucosa were trapped in vessels which subsequently passed into the mucosa. However, some microspheres were trapped in the submucosa even during maximal vasodilation of the intestine (Table 5). This could be due either to a true parallel-coupled submucosal section or to the fact that 37% of the vessels passing from the submucosa to mucosa are smaller than 12μ diameter even when maximally dilated. The second

possibility seems more likely for several reasons. Ross (1971c) showed in his ^{86}Rb uptake studies that only 7-12% of the superior mesenteric arterial flow passed through the submucosa. If no contamination occurred during separation of the intestinal layers, this would represent true nutritional flow to the submucosa. When Grim and Lindseth (1958) used 12μ spheres, only 7% were trapped in the submucosa; this value is lower than ours but their experiments were on dogs. The histological observations show very few capillary-sized vessels within the submucosa, except close to the mucosal border where a large number of small vessels arise and run into the mucosa.

Thus these observations, together with those by other workers discussed above, suggest that the vascular architecture in the intestine involves only two parallel-coupled sections, one to the muscle and the other through the submucosa to the mucosa. The vessels in the submucosa are in series with those in the mucosa and submucosal shunts do not exist. This is illustrated diagrammatically in Fig. 27. If this hypothesis is correct, redistribution from mucosa to submucosa during autoregulatory escape or infusions of vasoactive drugs cannot occur. This does not exclude the possibility of a redistribution within the mucosa, for example from villous to non-villous mucosa, but at present there is little evidence for this except the prolonged decrease in capillary filtration coefficient during sympathetic nerve stimulation (Folkow *et al.* 1964, see introduction). Adrenaline cannot be dilating the intestine by opening submucosal shunts as previously suggested (Greenway and Lawson, 1966) and it seems likely that α - and β -adrenoceptors have

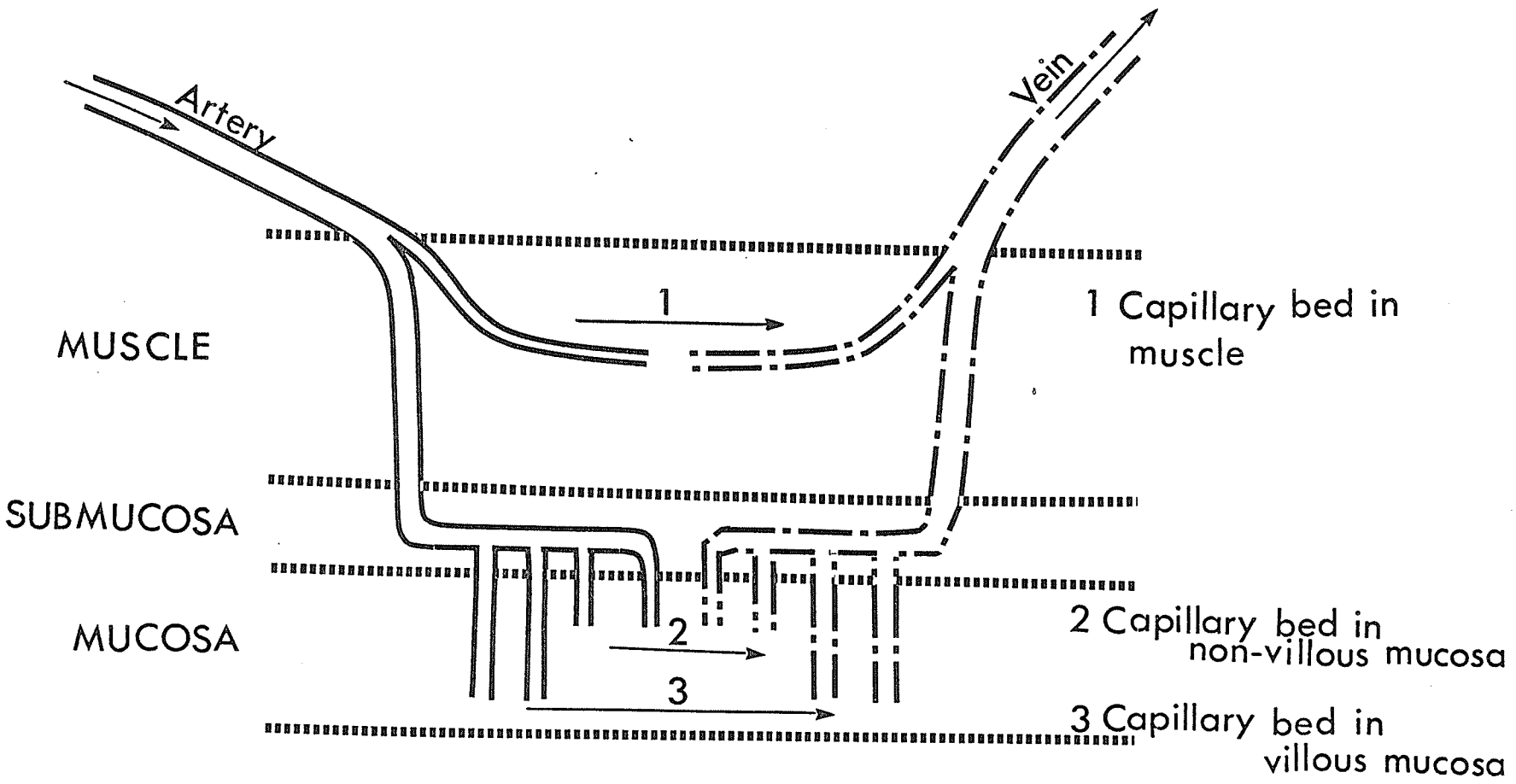


Figure 27: Diagrammatic representation of the two parallel-coupled vascular circuits in the intestinal wall and the series-coupled sections between the submucosa and mucosa.

opposite effects on the same vascular smooth muscle as was shown to be the case in the spleen (Greenway and Stark, 1970).

On this hypothesis that there are no submucosal shunts, the sizes of the vessels between the submucosa and mucosa can be calculated from the microsphere data (Table 5). In the constricted vascular bed, 21% of these vessels are $> 17\mu$, 30% are between 12 and 17μ and 49% are $< 12\mu$ diameter while in the dilated bed, 44% are $> 17\mu$, 19% are between 12 and 17μ and 37% are $< 12\mu$ diameter.

When microspheres are used in pharmacological investigations on distribution of blood flow in organs, controls to validate the method for the particular areas being studied are essential. If the blood vessels in the areas studied are in series rather than in parallel, the method is invalid. We have shown that this is true in the intestinal wall.

SUMMARY

1. The distribution of superior mesenteric arterial flow was investigated by radioactive microspheres. The small intestine received 83% of the flow (85 ml/min)/100 g intestine) and flow was uniform along the length of the small intestine.

2. The intestinal wall was separated into 3 layers - muscle, submucosa and mucosa. The muscle received (8 ml/min)/100 g intestine and the combined submucosa and mucosa (77 ml/min)/100 g intestine.

3. The distribution of microspheres between the mucosa and submucosa depended on the size of the microspheres; the smaller the spheres, the more were found in the mucosa. It also depended on the state of the vascular bed; if microspheres were given during an infusion of vasopressin, a subsequent infusion of isoprenaline resulted in movement of some of the spheres from the submucosa into the mucosa.

4. Histological studies after India ink injection showed few capillary-sized vessels but many large vessels in the submucosa. Capillary-sized vessels arose close to the junction with the mucosa and passed into the mucosa.

5. These and other data suggest that the intestine consists of two parallel-coupled sections, one to the muscle and the other through the submucosa to the mucosa. The vessels in the submucosa are in series with those in the mucosa and submucosal shunts do not exist. Redistributions of flow between mucosa and submucosa cannot therefore occur during stimulation of the sympathetic nerves or infusions of drugs such as noradrenaline or adrenaline.

6. When microspheres are used in pharmacological investigations on distribution of blood flow in organs, controls to validate the method for the particular areas being studied are essential. If the vessels in the areas studied are in series rather than in parallel, the method is invalid.

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