COMBINED ADRENERGIC BLOCKADE IN CANINE ENDOTOXIN SHOCK

An experimental study

Proefschrift

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Aan mijn ouders

The search for truth is in one way hard and
in another easy,
For it is evident that no one can master it
fully, nor miss it wholly

- Aristotle

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CONTENTS

LIST OF ABBREVIATIONS

i.a.	: inter alia
i.v.	: intravenously
L.D.	: lethal dose
M.B.P.	: mean of systolic and diastolic blood pressure
M.P.F.S.	: maximum peak flow in stationary state
n	: number (of experiments)
N.S.T.	: no significant trend
P.F.S.	: peak flow in stationary state
P.S.R.H.	: peak systolic reactive hyperaemia
R.E.S.	: reticulo-endothelial system
R.H.	: reactive hyperaemia
S.B.P.	: systolic blood pressure
S.D.	: standard deviation
S.D.T.	: significantly decreasing trend
S.I.T.	: significantly increasing trend
T	: time in min. in relation to endotoxin injection
T.P.R.	: total peripheral resistance

A. INTRODUCTION

A. 1 Septic shock in man

Septic shock in man often presents a dramatic clinical picture. The cause and mechanisms of this type of shock are still not fully clarified.

The circulatory failure in patients with septic shock can have two different patterns:

In one form, the circulation is hyperdynamic with an increased cardiac output. The patient is alert though often restless and has a warm, flushed skin.The pulse is full, urinary output is maintained, but the blood pressure is low.

In the other form of septic shock, the circulation is hypodynamic, with a reduced cardiac output. The patient is pale and has cold extremities. A feeble pulse,severe hypotension and oliguria are characteristics of this hypodynamic state of shock (39,40,70,97, 109,110,113).

These two forms of septic shock can and often do occur subsequently in the same patient.

Peripheral resistances can be found high in the hypodynamic state and low in the hyperdynamic state, but most authors agree that the peripheral resistance is mostly low in human septic shock (25,41,70,99).

The combination of a hyperdynamic circulatory state, a decreased peripheral resistance and a narrowed arteriovenous oxygen difference, as is often found in human septic shock, suggests the opening of arteriovenous shunts (12,23,27).

Arteriovenous shunting and vasoconstriction may be operational at the same time, leading to a redistribution of the circulating volume, in such a way that some areas have an increased blood flow while other areas have a reduced flow.

The causative bacteria in septic shock are practically always gram-negative: Escherichia Coli in about 50% of cases, Klebsiella in 22-25% and Proteus in 15-20% (8,95,109).

Literature data indicate that it are not the intact bacteria, but the endotoxins from their cell walls which provoke the circulatory derangement of gram-negative bacterial shock (8,49,76).
For this reason the descriptive terms septic shock, gram-negative bacterial shock and endotoxin shock can be considered synonyms.
The mortality rate of conventionally treated gram-negative shock in man remains high at 50% or more, according to several authors (22,60,78,98).

A. 2 Why adrenergic blockade in experimental endotoxin shock?

Impressed by the high mortality rate of septic shock in man and the complexity of the syndrome, a literature survey on cause and mechanisms of this type of shock was made.
The numerous publications often prove confusing and contradictory. Hypotension and hypoperfusion characterize shock of any aetiology, but in septic shock no blood disappears from the vascular compartment as in haemorrhagic shock and the heart is initially not impaired as in cardiogenic shock (33,42).
The main question to be answered is therefore: Where is the blood that is not effectively circulating?
Apart from arteriovenous shunting, the redistribution of blood in the vascular bed could be explained by vasodilatation and/or pooling in some areas at the expense of other areas, where vasoconstriction predominates (8).
In septic shock endotoxins lead to the circulatory disturbances via release of vasoactive intermediaries (8,54).
The question which of the possible vasoactive intermediaries may cause shock in gram-negative sepsis, was studied with the help of reports on basic research in this field, done by Lillehei (68,69) Fine (28,29,30,49) and others (11,51,57).
Although the evidence on the subject is confusing, it appeared likely nevertheless, that catecholamines play an important, maybe even the primary role as vasoactive intermediary in endotoxin shock: The catecholamine hypothesis.
It may seem paradoxical that compounds hitherto considered to be mainly vasoconstrictive, could be instrumental in provoking a state of shock that is usually thought of as being a vasodilatory phenomenon.
In view of Ahlquist's concept (2) however, it might be advocated that this is less contradictory than originally thought, because in this concept adrenergic effects are distinguished in alpha-receptor functions which are vasoconstrictive and beta-receptor functions which are vasodilatory.
As vasodilatation as well as vasoconstriction probably occur in

septic shock, both phenomena can be explained as catecholamine induced.
At this point in the literature survey it seemed that the catecholamine hypothesis, Ahlquist's concept and the probability of vasodilatory as well as vasoconstrictive phenomena in human septic shock, should lead to a treatment in which not only one but both adrenergic effects would be blocked (7).
Such a treatment should first be investigated in the animal experiment.
Separate adrenergic receptor blockades in the canine endotoxin shock model are documented in literature:
alpha-blockade by Lillehei (68,69), Morris (77), Abrams (1) and others; beta-blockade by Berk (9,10).
Combined adrenergic blockade in haemorrhagic shock is described by Halmahagyi (38).
Documentation of such a combined adrenergic receptor blockade in experimental endotoxin shock could not be found in literature.
Therefore the study of combined adrenergic receptor blockade in an experimental endotoxin shock model seemed justified.

A. 3 Choice of the experimental animal

Though the best model for the study of a clinical condition is man, debatable forms of therapy should obviously first be tried out in the animal model.
Subhuman primates probably resemble man most with regard to circulation physiology. The "next best" experimental animal is the dog, big enough to get reliable shock parameters and relatively easy to breed and handle.
When for practical reasons the dog was chosen as experimental animal, it was realized that there are differences in haemodynamics with the subhuman primate and therefore most likely also with man, that should be reckoned with (27).
Pooling of blood in endotoxin shock occurs in dogs as well as in subhuman primates and therefore probably also in man (19,20,89). The areas of pooling however, are different.

A. 4 Comparison between endotoxin- and viable bacteria shock

Endotoxin is a lipopolysaccharide from the gram-negative bacterial cell wall and has since long been considered the agent responsible for producing the shock associated with gram-negative bacteraemia (8,60,76).
Borden (17) first suggested that endotoxin was the shock inducing part of the bacteria, by producing hypotension in the experimental animal with injection of this endotoxin. Hinshaw (45) found no difference in a canine forelimb preparation in septic shock, whether produced by i.v. endotoxin or live bacteria and states that endotoxin is the active component following injection of live Escherichia Coli organisms.
Hinshaw (47) compared the endotoxin shock model with the viable bacteria model in dogs, using an E. Coli strain and endotoxins derived from E. Coli, both in a 95% lethal dose (L.D. 95%).
After injection of live bacteria, the fall in arterial blood pressure was more gradual, but of the same magnitude as the hypotension after the injection of endotoxin.
Motsay (79) and Wright (112) confirm the similarity between the viable bacteria- and endotoxin shock model, also pointing out that the bacteria model has a more gradual shock pattern than the endotoxin model.
In the viable bacteria model, the circulatory responses are related to the concentration of bacteria injected, necessitating exact counting of the amounts of culturable units of E. Coli to be injected (107).
Also the supernatant of the bacterial suspension has to be tested on free endotoxins.
In the endotoxin shock model a purified, dried endotoxin preparation derived from bacterial cultures is used.
The preparation is provided by Standardized Industrial Methods, using the trichloric acid extraction method, as described by Boivin (15,16).
The lethality of the endotoxin preparation is verified in mice.
Quantification of the purified and standardized endotoxin pre-

paration seems more reliable than assessing the lethality of bacterial cultures.
Even when constantly the same number of culturable units of bacteria are injected, the viability of the bacteria is difficult to assess, and the bacterial supernatant may still contain endotoxins. For these reasons the endotoxin shock model was preferred.
The strongest argument in favour of the endotoxin shock model rather than the live bacteria model is however the fact that in man also, not the intact bacteria but the endotoxins from their cell walls are considered the agents responsible for producing the shock associated with gram-negative bacteraemia (17).

A. 5 Content of the investigation

After a further literature survey on endotoxin shock, experiments were carried out in a canine endotoxin shock model, first in a pilot study and subsequently in the form of a main study.
In the pilot study, the effects of several dosages of dibenzyline (phenoxybenzamine) as alpha-blockade and inderal (propranolol) and trasicor (oxprenolol) as beta-blockade on experimental endotoxin shock were analyzed.
Some estimations of circulating endotoxin and plasma catecholamine levels were performed.
In the main study, "biphasic" adrenergic blockade was the most important topic of interest, applying dibenzyline as premedicative alpha-blockade before inducing endotoxin shock and trasicor as medicative beta-blockade after endotoxin injection.
Alternative forms of adrenergic blockade were also investigated.
Results have been statistically analyzed.

B. LITERATURE SURVEY

B. 1 Canine endotoxin shock

B. 1-1 Materials and methods:

B. 1-1-1 Endotoxin

Endotoxin from Difco Laboratories Detroit, is used by most investigators in the endotoxin shock model. Several dosages and several strains of endotoxin are mentioned. Mostly, 5 mgr/kg is considered a L.D. 95%, although different batches of endotoxin are used, as demonstrated by the following codenumbers:
055:B4 : Blattberg (13); 055:B5 : Park (82) and Guntheroth (36);
011:B4 : Rety (92) and 0127:B8 : Brockman (18) and Thomas (106).

B. 1-1-2 Influence of route of endotoxin administration

Jacobson (52) found only small quantitative differences in the early haemodynamic pattern of experimental endotoxin shock after administration of endotoxin along different routes.
The overall effects on portal venous hypertension, systemic arterial hypotension and the increase in small intestinal vascular resistance were the same, whether the injection of endotoxin was either in the portal circulation, in a femoral vein or in the mesenteric artery.
The femoral vein is thus usually chosen as route of endotoxin administration.

B. 1-1-3 Influence of injection time

In the patient, endotoxaemia develops gradually, probably over several hours. For this reason the common method of producing experimental endotoxaemia in the dog by rapid injection of a lethal dose may not represent accurately the clinical situation in man. Rety (92) therefore studied the effects of slow infusion of a lethal dose of endotoxin in the dog and compared these with the effects after rapid injection.
Haemodynamics deteriorated progressively and in the same magnitude, but more gradually than after rapid injection. The pattern of shock at the end of two hours was identical with that produced by rapid injection. The conclusion is that the rapid injection technique seems to be as good as the slow infusion technique and gives better opportunity to compare haemodynamic data.

B. 1-1-4 Endotoxin detection

Since not the intact bacteria, but the endotoxins of their cell walls cause shock, a reliable test that quantifies the endotoxins rather than the bacteria itself, would be an improvement in dealing with this type of shock in clinical as well as in experimental situations.
Levin (61,62) and Reinhold (91) proposed the use of such a test in vitro: the Limulus lysate assay for circulating endotoxins in gram-negative sepsis.
The assay depends on the gelation by endotoxin of an amoebocytes lysate,derived from the blood of limulus polyphemus, the horseshoe crab (62,91).
The Limulus lysate is incubated in pyrogen-free glassware with the plasma to be tested on endotoxin content.
To avoid possible protein interference with the gelation process, the samples can either be diluted or deproteinized by chloroform extraction (26,61,62,91).
Levin (62) and Reinhold (91) claim a good correlation between positive tests and proven bacteraemia.

Others report more false positive and false negative results with the test (26).

Another test is based on a sharp increase of sensitivity to endotoxins in mice after pretreatment with actinomycin D (87): the Actinomycin D-test for endotoxins. This test is more time consuming than the Limulus lysate test.
The test is performed by mixing a constant dose of actinomycin D with dilutions of the endotoxin containing preparation, and injecting the mixtures into the peritoneal cavities of groups of mice. In 48 hrs., the number of deaths in each group of mice is tabulated and the L.D. 50% is calculated. This L.D. 50% value reflects the endotoxin content of the preparation to be tested (87).

B. 1-2 Symptomatology of canine endotoxin shock

Haemodynamics:

Brockman (18) and Thomas (106) injected 5 mgr/kg bodyweight Escherichia Coli endotoxin i.v. in dogs (127:B8 Difco Laboratries, Detroit, Boivin extraction method) and registrated the following haemodynamic changes: immediately after injection, arterial pressure and cardiac output fell abruptly to levels averaging 29 and 17% of the control values respectively (the first phase of shock). In all experiments, within 10 min. there followed a rise in blood pressure and cardiac output to a mean value of 52 and 50% of controls. Thereafter, blood pressure and cardiac output declined again, taking a final downward course (the second phase of shock), resulting in death of the experimental animal.
Portal vein pressure rose initially with an average of 28% of control. This elevation continued approximately for 15 min. before returning to control values.
Lillehei also describes this biphasic pattern of endotoxin shock in dogs, using an average of 7,5 mgr/kg of a crude endotoxin preparation (68,69).
In these experiments the mean blood pressure fell also precipitously to shock levels after endotoxin administration and returned to normal within 5 min.
Sixty to 90 min. later, a second, more gradual fall in blood pressure began and continued until death, in most cases within 12 hrs.
Others confirm this biphasic shock pattern in canine endotoxin shock (13,71,77).
Brockman (18) showed that when the liver was bypassed with a portocaval shunt, a more gradual fall in systemic arterial blood pressure after endotoxin was noted.
The rise of portalvenous pressure, and the prevention of severe shock after bypassing the liver suggests hepatosplanchnic pooling of blood in the first phase of endotoxin shock in dogs.
McLean (71) performed experiments, designed to determine the relative importance of the liver and intestine in producing the immediate hypotension, observed following endotoxin injection in dogs.

In experiments after evisceration and hepatectomy, injection of endotoxin did not result in the immediate and precipitous blood pressure drop, as seen in controls after endotoxin injection. Only in the second phase, after about 20 min., a slow progressive decline in blood pressure occurred.
When the animals were eviscerated, but the liver and its blood-supply from the hepatic artery were preserved, the response to endotoxin was indistinguishable from controls, with an immediate blood pressure drop after endotoxin injection.
These results suggest that the hepatosplanchnic pooling of blood in the first phase of endotoxin shock in dogs occurs mainly in the liver.
Froneck (32) demonstrated a hepatic outflow block in dogs, subjected to endotoxin shock.
Contraction of the muscular sphincters in the hepatic outflow tract could lead to the trapping or pooling of blood in the liver of dogs in early endotoxin shock.
Blattberg (13,14) found indications that early pooling in response to endotoxin might also occur outside the hepatosplanchnic area: when portalvenous pressure in canine endotoxin shock was kept constant by means of a rollerpump connected to the jugular vein, the arterial pressure decreased nevertheless in the first phase of shock.
Occlusion in the arterial inflow in the splanchnic area did not prevent an early blood pressure drop either in Blattberg's experiments (13,14).
Some of the evidence of early hepatic and probably also extra-hepatic pooling has thus been presented and gives most likely an adequate explanation for the first phase of endotoxin shock in the dog. The variability and the differences in set up of the experiments described, make it however difficult to draw firm conclusions. To understand the cause of the second progressive phase of endotoxin shock is still more difficult.
Venous pooling in the lungs, the intestinal venous system and other systemic veins is thought to occur in later phases of endotoxin shock (13). The increase in venous capacity is supposedly so much diffuse, that it might not be accurately observed in a given vascular system (18).

Arteriovenous shunting may occur in the splanchnic and renal circulation, and possibly also in other areas. (5,6,24).
Hinshaw (44) claims to have demonstrated that pooling in the splanchnic area may not only be operative in the first phase, but also in the second phase of endotoxin shock.
In another study, Hinshaw (45) found no weight increase in canine forelimb preparations after endotoxin and thus no signs of tissue oedema or loss of blood in the leg, muscles or skin.
This seems to exclude these areas as possible sites of pooling
Hermreck (41), Isakson (50) and Passmore (84) measured increased renal inflow in late sepsis attributed to vasodilatory substances from the septic areas, indicating the kidneys as potential pooling sites.
Findings by Hinshaw (45) suggest the absence of a direct toxic action of endotoxin on the myocard in dogs.
Kondo (58) found in a transplantation model no myocardial depression by endotoxin either.
More recent work by Hinshaw and co-workers however, emphasizes that in later stages of canine endotoxin shock, catecholamine-induced myocardial dysfunction might occur (4, 43, 74, 85).
Haemodynamics in the second progressive phase of endotoxin shock are furthermore probably influenced by the profound hypoglycaemia that characterizes this terminal stage (46,72,86).
It can be concluded that the problem of vasodilatation or vasoconstriction in late pooling and the question of pooling sites in the second shock phase seems not yet clearly solved.

Intestinal manifestations:

Lillehei (66,68) describes severe changes in the microcirculation of the mesentery in canine endotoxin shock.
Shortly after endotoxin administration there is a "wave of vasoconstriction" in the small vessels of the mesentery.
This is followed by a progressive dilatation of the arteriolae and venulae and congestion in the capillary bed.
In 4 → 5 hrs., mucosal congestion and eventual necrosis,due to stagnant anoxia, follows. Prior to death, a watery diarrhea , changing to haemorrhagic diarrhea is a common occurrence.

At autopsy, extensive haemorrhagic necrosis of the mucosa is seen, most severely in the small intestine. This bowel necrosis must result from critical ischaemia due to vasoconstriction and oligaemia, because it could be prevented by adequate perfusion of the superior mesenteric artery.
Others also described vasoconstriction in the microcirculation of the mesentery in canine endotoxin shock (88,93).

B. 2 Vasoconstriction in the hepatosplanchnic area

B. 2-1 Final common pathway theory

When an experimental animal is bled into a reservoir,the ensuing hypotension is reversible and can be corrected by re-infusing the blood from the reservoir back into the animal as long as a certain amount of exsanguination is not exceeded.
A point of irreversibility can be reached after which re-infusion does not restore the animal from its shock.
Lillehei (65,67) demonstrated that the severe bowel necrosis characteristic for endotoxin shock also occurs in the late phase of haemorrhagic shock.
The explanation would be that the intestinal wall after prolonged ischaemia and anoxia loses its barrier function against the passage of the intestinal bacteria into the bloodstream.
The result is that the "shocked" gut leaks bacteria and therefore endotoxins into the circulation and the hitherto haemorrhagic shock becomes endotoxic in nature.
Fine (49) endorses this theory by measuring elevated levels of endotoxins in late haemorrhagic shock, applying the Limulus lysate test, but Mee (73) could not duplicate this finding.
Mesenteric vasoconstriction resulting in critical ischaemia of the bowel and leakage of endotoxin would therefore be the common dominator in the endphase of both haemorrhagic and endotoxin shock in dogs: the intestine as final common pathway.
Fine (28,29) and Palmerio (81) thus believe that the state of irreversible shock in exsanguination is due to endotoxaemia.
Additional evidence for the final common pathway theory is found in the fact that after ligature occlusion of the superior mesenteric artery in dogs on release of the ligature, a full blown syndrome of shock develops, indistinguishable from endotoxin shock (28,81).
Furthermore,other studies showed that oral administration of massive amounts of antibiotics and cross perfusion with fresh blood could prevent lethal irreversibility in experimental haemorrhagic shock (29,81).

Finally, Fine demonstrated that animals made resistant to endotoxin by injection of increasing amounts of endotoxin were also resistant to otherwise lethal degrees of haemorrhagic shock (28, 29,81).
Endotoxin shock is thus seen as a self perpetuating condition, in which the basic haemodynamic mechanism is vasoconstriction.
Swan (102) however, challenges the final common pathway theory pointing out that in dogs the mesenteric vasoconstriction after injection of endogenous catecholamines is followed by a secondary vasodilatation.
Others state that endotoxin is a vasodilator or initiates release of vasodilating substances, and that vasoconstriction is a compensatory mechanism, impairing the circulation in a later phase by an overresponse (94).In summary however,one can presume in view of the above mentioned studies, contradictory as they may be, that in any case vasoactive intermediaries can be postulated to play an important role in endotoxin shock.

B. 2-2 R.E.S. and detoxification

Normal spleen and liver, which together contain the major part of the R.E.S. (reticulo-endothelial system), extract and detoxify circulating endotoxins (29).
In the laboratory, an enzyme preparation from a normal spleen detoxifies 50 times its own weight of endotoxin.
The same preparation from the spleen of an animal in shock is inactive, whereas that from an animal with completely denervated spleen in shock remains as active as the preparation from normal spleen (30).
Fine (30) found such a detoxifying function in the liver as well.
Other evidence of endotoxin detoxification in the liver and spleen of dogs in endotoxin shock is described by Fine (29,30): implanting a donor liver or spleen in the animal in endotoxin shock improved haemodynamics.
Cross circulation through a freshly excised homologous liver gave

comparable results. Splenectomy prior to the shock procedure resulted in death within a few hours.
Treatment of the shocked dog with fresh splenic extract intravenously improved survival. Liver and spleen therefore seem to have the same endotoxin detoxifying potential.
Since the only function shared by the spleen and the liver is the reticulo-endothelial function, surviving seems to be dependent on the integrity of the R.E.S. in the liver and spleen and more especially on the accessability of this system to the circulation for clearance of endotoxin.
Impaired flow through liver and spleen,caused by vasoconstriction in the hepatosplanchnic area, as might occur in endotoxin shock, thus could prevent endotoxin detoxification.

B. 3 Possible vasoactive mediators in endotoxin shock

Introduction

All sorts of compounds have been implicated as vasoactive intermediaries in gram-negative shock: the catecholamines, histamine, serotonine and kinins (49).
If a vasoactive substance would be responsible for shock in gram-negative sepsis, it should fulfill the following conditions:

1. Infusion of the compound in the experimental animal should lead to a circulation pattern comparable to septic shock.
2. The level of the compound should be elevated in endotoxin shock (51,53,54,57).

While reports on the subject in literature are conflicting, some of the data on possible vasoactive intermediaries are given here, taking into acount the above mentioned criteria.

B. 3-1 Catecholamines

Lillehei (67) produced a lethal form of shock in the dog by the intravenous administration of 17 µg/kg/min. of epinephrine for 90 minutes. This produced initially an intense ischaemic anoxia within the visceral organs, followed by congestion and stagnation of blood.
The effect on blood flow and vascular resistance in visceral organs was similar to that found in endotoxin shock. The animals all died (average survival time: 20 hrs.) and autopsy findings also were similar to that described for endotoxin shock.
As early as 1940, Freeman already produced shock in dogs by infusion of epinephrine (31).
Yard (114) described shock in dogs produced by norepinephrine infusion.
Berk (9) found a decrease in blood flow and blood pressure during epinephrine infusion in dogs with an increased portal flow and

pooling in the liver.
When the infusion was discontinued,the animals gradually relapsed into a state of irreversible hypotension.
Jacobsen (54) found only a late fall of blood pressure after catecholamine infusion in dogs.

Measurements of catecholamines during endotoxin shock were done by Lillehei , who measured a 10 → 30 fold rise in the plasma catecholamines, immediately after the injection of endotoxin in dogs (66). This was followed by a return to less abnormal, but still elevated levels. Near the time of death of the experimental animals,the catecholamines rose again to high levels.
Jacobsen (54) also found increases of plasma epinephrine levels after endotoxin injection in dogs.Griffiths finally, found increased epinephrine- and norepinephrine levels in canine gram-negative bacteraemia (35).

B. 3-2 Histamine and serotonine

Weil (111) found a sharp rise of blood histamine immediately after endotoxin injection in dogs.
Vick (108) also measured elevated plasma histamine levels both in early and late endotoxin shock in dogs.
Jacobsen (53,54) however,found no significant increases of histamine levels in canine endotoxin shock. Serotonine concentrations were even decreased after endotoxin.
Histamine and serotonine infusions did not lead to the major early vascular responses as seen after endotoxin injection.
Prior treatment of dogs with chemicals that reduce tissue stores of vasoactive substances (i.a. cortisone acetate and reserpine) failed to prevent the typical haemodynamic events of endotoxaemia.

Miller (75) also states that although histamine, serotonine (or bradykinin) may mimic the vascular response of the early phase of endotoxin shock, the rise in plasma concentrations of these three compounds during endotoxin shock is transient and counteracting drugs (i.a. phenothiazines, methysergide and salicylates)

do not alter the development or course of the vascular reactions to endotoxin.
Lichtenstein (64) indicates that catecholamines,but also some endotoxins could in fact inhibit histamine release.

B. 3-3 Kinin and bradykinin

Shah (96) could not duplicate in dogs findings of authors who measured significantly elevated kinin levels in the first phase of endotoxin shock in monkeys.
Although in dogs after endotoxin the level of bradykinin in the portal blood rose slightly above that in the hepatic vein and femoral artery, bradykinin levels were not statistically different from pre-injection values or from each other.
Carretero (21) found no detectable amounts of kinin after endotoxin.

B. 4 The catecholamine hypothesis

B. 4-1 Alpha- and beta receptors

Ahlquist (2) postulated that there are two different types of receptor sites with which a sympathicomimetic agent can react to elicit a response.
These receptor sites, which he termed"alpha"and"beta",were classified on the basis of their responses to a variety of sympathicomimetic amines.
The hypothesis gained support from the development of specific blocking agents for each receptor type.
The alpha-adrenotropic receptor is associated with most of the excitatory functions (vasoconstriction) and one important inhibitory function (intestinal relaxation).
The beta-adrenergic receptor is associated with most of the inhibitory functions (vasodilatation and inhibition of bronchial musculature) and one important excitatory function (myocardial stimulation).
In the original concept a differentiation was made between the so called "pure adrenergic amines", phenylephrine and isoproterenol, and the "mixed amines", epinephrine and norepinephrine (104).
The pure amines would react with only one type of receptor, giving therefore a receptor specific effect: phenylephrine vasoconstrictive and isoproterenol vasodilatory. The mixed amines would possessthe potential for combined receptor stimulation, with a relative specificity for one of the two.
Thus, in the classical view, norepinephrine is considered predominantly an alpha- and epinephrine a beta stimulant.
Swan (104,105) showed in two critical studies with catecholamine infusion experiments in dogs after alpha- and/or beta-blockade, that the so called "pure" amines phenylepinephrine and isoproterenol also reacted with the alternative receptor.
Thus, they cause a combined receptor stimulation as well, like the naturally occurring mixed amines epinephrine and norepinephrine.
In Swan's studies it was confirmed that norepinephrine had alpha- and beta effects with a slight preponderance of the alpha function.

Epinephrine also displayed both properties, but had a more significant beta effect. The double properties were sequential: norepinephrine and epinephrine had both an initial vasoconstrictive and a secondary vasodilatory effect.

B. 4-2 Circulating or locally released catecholamines?

Palmerio (81) and Fine (29) subjected dogs to haemorrhagic or endotoxin shock after coeliac blockade.
After denervation of the splanchnic area by coeliac blockade, a significant decrease in mortality rate was found. Moreover, a remarkable improvement in systemic haemodynamics and preservation of the functional and structural integrity of the tissues in the denervated area was seen. Specifically, in dogs with all of the intestine except the distal half of the colon denervated, the only part which showed haemorrhagic necrosis was the non-denervated portion.
This is considered as proof that norepinephrines released in the tissues and not the circulating catecholamines are responsible for the tissue injury and the eventual vascular collapse.
As for the reliability of the blockade, authors state that they got complete and sustained blockade by ganglionectomy or with nupercaine hydrochloride in oil, injected under direct vision into the coeliac ganglia (29,81).
The blockade is not secured by readily absorbed local anaesthetics. Proof of effective blockade was obtained by assaying the norepinephrine content of the tissues.
Apart from shock experiments with denervation of the splanchnic area, Fine worked with denervated and half denervated spleen preparations (28,29,30). When half of the exteriorized spleen is denervated and the other half not, the response of the denervated half to haemorrhage is remarkably different from that of the other half. The non-denervated half contracts immediately to about one third of its normal size and remains shrunken.
The denervated half of the spleen on the other hand, does not

change in size or appearance throughout the period of shock.
If one injects 100 µgr. norepinephrine i.v. early in the course of the experiment, that is before haemorrhage, both halves of the spleen contract vigorously.
Fine concludes from both observations, that since the sensibility of the denervated half to circulating catecholamines is at least as much as that of the non-denervated half, it follows that the contraction of the latter in response to haemorrhage is not caused by circulating catecholamines, but by norepinephrine released from its store in that half of the spleen.
These effects of denervation also apply to shock,produced by a lethal dose of endotoxin, given intravenously.
The conclusion is therefore that in endotoxin shock also,locally released catecholamines are responsible for the functional and structural damage in the splanchnic area as well as for the progressive failure of the systemic circulation.

B. 4-3 Alpha-adrenergic blockade

Lillehei (66,68,69) investigated the effect of pretreatment with 0,5 → 2,5 mgr/kg dibenzyline (phenoxybenzamine) in endotoxin shock in dogs. The dose of endotoxin used in these experiments was 7,5 mgr/kg bodyweight of a crude endotoxin preparation.
Dogs given dibenzyline did have the characteristic primary drop in blood pressure, but the secondary fall in 60 → 90 minutes rarely went below 80 mm Hg.
By 4 hrs. following endotoxin, the blood pressure had returned to pre-injection levels. These dogs did not get diarrhea and at sacrifice the bowel was normal.
Nine out of 10 dogs survived permanently, whereas in the control-group the endotoxin proved fatal for 90% of dogs. Other authors could not duplicate Lillehei's findings however, neither as far as survival improvement after alpha-blockade is concerned, nor with regard to the blood pressure in the second shock phase.
Morris (77) for example, injecting 2 mgr/kg endotoxin in 10 dogs

after pretreatment with 0,9 mgr/kg dibenzyline, saw an improvement in the first phase of shock, whereas Lillehei did not. There was no improvement in the second phase and therefore in the ultimate picture of the endotoxin shock in these experiments, whereas Lillehei noted a clear improvement after dibenzyline pretreatment in this later phase.
The dosages however,are different and this factor may have influenced the results.
Abrams (1) states that,if dibenzyline has any effect on endotoxin shock in dogs, it must be related to the additional fluid suppletion.
Iampietro (48) prevented with 10 mgr/kg dibenzyline at least partially the precipitous drop in blood pressure immediately after injection of 2 mgr/kg endotoxin in dogs. Dibenzyline did not prevent a gradual lowering of arterial pressure in the second phase (Scheme, p. 26).

B. 4-4 Beta-adrenergic blockade

Berk (11) investigated the effect of beta-adrenergic blockade with inderal (propranolol) in canine endotoxin shock.
A total of 90 dogs was injected with 0,5 mgr/kg Endotoxin coli (Difco 127:B8). The dogs were divided into the following groups:

Group 1: 36 dogs, untreated, functioning as controlgroup.

Group 2: 32 dogs, treated with an average of 0,05 mgr/kg inderal after the endotoxin injection and also receiving additional i.v. therapy, consisting of Ringer's solution, sodiumbicarbonate, atropin, dextrose and calciumchloride and in some cases digitalin. This additional therapy was necessary to combate early bradycardia and terminal arythmia's.

Group 3: 22 dogs, receiving this additional therapy after endotoxin, buth without inderal.

The survival rate in group 1, the untreated dogs, was 19%.
In group 2 with inderal and additional therapy, the survival rate was 78% and in group 3 with the same regimen, but without inderal,it was 27%.
These differences between all three groups appeared significant with P<0,001.
The dogs that were given endotoxin and then treated with inderal showed haemodynamic patterns that were different from those,given endotoxin only. These dogs did have the early hypotensive phase of endotoxin shock, but the second phase was less severe.

Author	Dibenzyline dose mgr/kg bodyweight	Inderal dose mgr/kg bodyweight	Improvement of first shock phase	Improvement of second shock phase	Improvement of survival
Lillehei (68,69)	0,5 → 2,5	-	-	+	+
Morris (77)	0,3	-	+	-	-
Abrams (1)	1	-	-	-	-
Iampietro (48)	10	-	+	-	-
Berk (11)	-	0,05 (+ additional therapy)	-	+	+

Scheme : Anti-adrenergic effects, found by various authors.

B. 5 Summary

In the canine endotoxin shock model, 5 mgr/kg bodyweight endotoxin (Difco Laboratories, Detroit) i.v. would lead to shock with a mortality rate of 95% of the experimental animals (L.D. 95%).
Circulating endotoxins can be detected with the Limulus lysate- and Actinomycin D-tests.
Endotoxin shock in dogs has a biphasic pattern with regard to blood pressure and blood flow.
In the first phase of this biphasic shock pattern, hepatosplanchnic pooling is the predominant feature.
Vasoconstriction in the hepatic outflow tract could lead to this pooling. In the second phase of canine endotoxin shock, haemodynamics are more difficult to understand.
In the first phase,severe changes in the microcirculation of the mesentery, leading to haemorrhagic necrosis of the mucosa,is also a constant finding.
The resulting ischaemia of the intestinal wall would lead to leakage of the intestinal bacteria into the bloodstream.
Any type of shock leading to hypocirculation in the intestines thus can become endotoxic in nature in its endphase.
Vasoconstriction may prevent the R.E.S. in liver and spleen from detoxifying the endotoxins.
Circumstantial evidence for a role of the catecholamines in canine endotoxin shock is found in the observation that catecholamine infusions lead to circulation disturbances, comparable to that in endotoxin shock.
Plasma catecholamine levels are found elevated in endotoxin shock.
Histamine, serotonine and the kinins might also have some influence on the syndrome. Catecholamine effects are not uniform: alpha- and beta-receptor functions are connected with vasoconstriction and vasodilatation respectively.
The catecholamines epinephrine and nor-epinephrine have a combined receptor-stimulating effect, with some preponderance for one or the other. These double properties are sequential.
Shock experiments after denervation of the splanchnic area and with half denervated spleen preparations implicate the locally released

and not the circulating catecholamines as the vasoactive intermediary.
Lillehei and others (68,69) experimented with alpha-adrenergic blockade in canine endotoxin shock.Berk (11) did the same with beta-adrenergic blockade. The results are contradictory.
Combined alpha- and beta-adrenergic blockade in canine endotoxin shock has not been described in literature.

C THE EXPERIMENTS

C. 1 The working hypothesis

As a result of the literature survey and the considerations described in chapter B , the following working hypothesis was formulated as the basis for the present study:

1. In canine endotoxin shock the first shock phase is for an important part caused by alpha-adrenergic influences, and the second phase by beta-adrenergic influences (Fig.1).

2. Anti-adrenergic treatment can only be effective when alpha-blockade is directed to the first, and beta-blockade to the second shock phase: thus, combined biphasic-adrenergic blockade would be indicated in canine endotoxin shock (7).

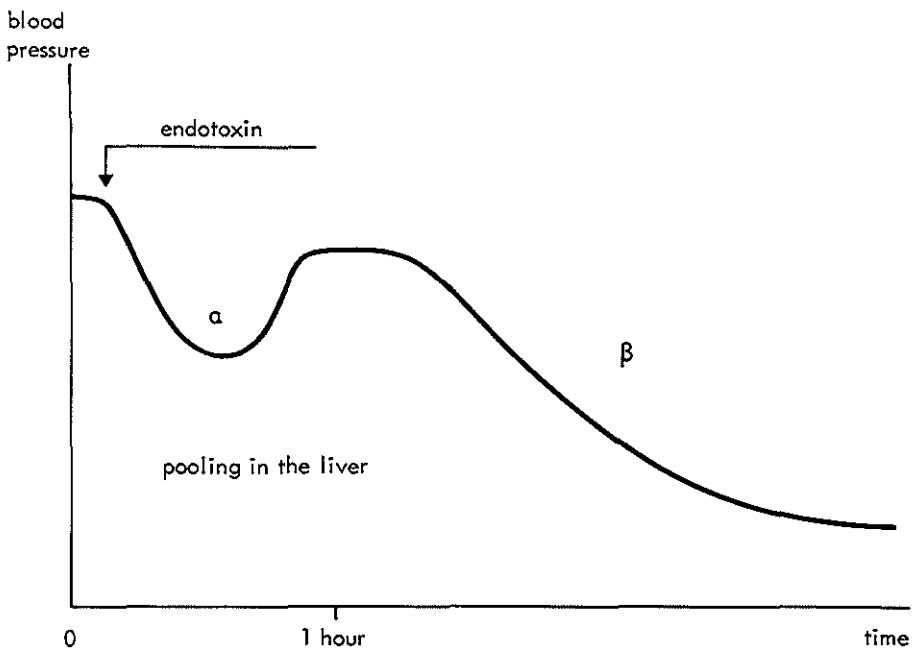

Fig. 1. Schematization of endotoxin shock pattern and working hypothesis.

C. 2 Pilot study

To be able to test the working hypothesis a number of preliminary questions, formulated in the following objectives, had first to be answered in a pilot study, before starting a more extended study.

C. 2-1 Objectives

1. Analysis of influence on survival in canine endotoxin shock of various dosages anti-adrenergics:
   dibenzyline (phenoxybenzamine) as alpha-blockade, inderal (propranolol) and trasicor (oxprenolol) as beta-blockade.
   These anti-adrenergics were given either before endotoxin injection (premedication), after endotoxin injection (medication) or premedicative as well as medicative in a biphasic sequence.

2. Analysis of vasodilatory effects of increasing dosages of dibenzyline and additional trasicor.

3. Estimation of circulating endotoxin in experimental canine endotoxin shock.

4. Estimation of plasma catecholamine levels in experimental canine endotoxin shock.

C. 2-2 Materials and methods

The experimental animal: the experiments were performed in mongrel dogs, weighing from 10 → 25 kg.

Endotoxin: shock was induced by i.v. injection of Endotoxin coli, codenumber E. coli 0127:B8 (Difco Laboratories, Detroit).
In all experiments the same dose of 5 mgr/kg bodyweight was

applied, supposedly a L.D. 95%, thus following the shock model of Brockman (18), Thomas (106) and others (71).
The endotoxin was suspended in 25 ml Ringer's solution and injected i.v. in 1 min. via the femoral vein.
Alpha-blockade was achieved with dibenzyline (phenoxybenzamine), a potent, longworking specific alpha-blocking agent (1,48,66).
Beta-blockade was achieved with inderal (propranolol) or trasicor (oxprenolol). Inderal and trasicor have both a negative chronotropic effect on the heart, but trasicor has a significantly less marked depressive effect on myocardial contractility (less negative inotropic effect)(34,63,80,83).Dibenzyline, inderal and trasicor were applied in a slow (1½ hr.) i.v. infusion, each chemical suspended in 500 ml Ringer's solution. All dogs, controls included, received the same amount of fluid. Premedication meant that the anti-adrenergic compound was infused in 1½ hr. before endotoxin injection. Medication meant that the compound was infused also in 1½ hr., infusion starting after injection of endotoxin.
Biphasic treatment consisted of premedicative infusion of the alpha-blocker and medicative infusion of the beta-blocker.
With regard to the dosages of the anti-adrenergics used, the first series of experiments were performed with dibenzyline doses up to 3,5 mgr/kg bodyweight, which is about the dose considered by several authors to be the maximum dose (1,68,69,77).
Only Iampitero (48) gave dibenzyline dosages up to 10 mgr/kg bodyweight (Scheme,p.26).The inderal dosage in these first experiments was 0,05 mgr/kg bodyweight, according to Berk (11). The dosages of dibenzyline and inderal were increased in later experiments.
Dibenzyline was given in doses up to to 15 mgr/kg bodyweight and in some experiments in even a higher dose.
Inderal was given in doses up to 0,5 mgr/kg and also in some experiments in even a higher dose.
Trasicor was primarily applied in high doses:1,5 mgr/kg bodyweight and once in a dose of 3 mgr/kg bodyweight.
Experiments were performed under a light O_2-N_2O-fluothane-anaesthesia with intubation and with spontaneous respiration.
The analysis of the vasodilatory influence of dibenzyline was performed with blood flow measurements in the femoral artery of the dog .
An electromagnetic flowprobe, Nikotron 372 M, was fitted

around the femoral artery and the blood flow curve registrated on the Elema-recorder with frequent zero-adjustments, using the Nikotron transducer. Endotoxin circulation was estimated in a limited number of experiments, using the Limulus lysate test or the Actinomycin D-test in mice.
Plasma catecholamine determinations were performed in some experiments, using the extraction procedure as described by Anton (3) and the fluorometric assay as described by Laverty (59).

C. 2-3 Analysis of survival with adrenergic blockade

Results:

Table 1 gives the number of dogs in the various experiments, the dosages of anti-adrenergics applied, and in the last column the average survival times in hours with the shortest and longest survivals of that group between brackets.

Exp.	Treatment	Number of dogs	Endotoxin mgr/kg bw	Alpha blockade dibenzyline mgr/kg bw	Beta-blockade inderal mgr/kg bw	Beta-blockade trasicor mgr/kg bw	Average survival in hours
0	Medication	4	0	15	0,5		3 >14 days 1 died
1	No medication	8	5				(2¾) 6 (12)
2	Premedication	7	5	3,5 → 15	0,05→0,5		(1) 9 (35)
3	Medication	4	5	15 → 20	0,5→1,5		(1) 16 (43)
4	Premedication	5	5	3,5	-		(2¾) 8½(19½)
5	Premedication	3	5	15	-		(20) 24 (28)
6	Biph. block.	7	5	15		1,5	(20) 37 (58)
7	Biph. block.	1	5	30		3	14

Table 1. Survival after various dosages anti-adrenergics.

Statistical analysis of survival times:

Applying the Mann-Whitney-U-test at a significance level of alpha=0,05. the differences in survival times between the various experiments were tested (101).
Differences in survival times between the experiments 1,2 and 3 were not significant.
The differences between the experiments 1, 4, 5, and 6 were as follows:

Group 1 versus 4 :	p- value :	0,416	not significant
Group 1 versus 5 :	p- value :	0,006	significant
Group 1 versus 6 :	p- value :	0,001	significant
Group 4 versus 5 :	p- value :	0,018	significant
Group 4 versus 6 :	p- value :	0,002	significant
Group 5 versus 6 :	p- value :	0,058	not significant

Discussion and conclusions:

As far as the relationship between dosage and survival time is concerned, dibenzyline in a dose of 3,5 mgr/kg bodyweight made no difference with no treatment at all (group 1 versus 4), but dibenzyline in a dose of 15 mgr/kg bodyweight did (group 1 versus 5). Even the difference between dibenzyline in a dose of 3,5 mgr/kg bodyweight and 15 mgr/kg bodyweight is significant (group 4 versus 5).
So is the difference between biphasic treatment and no treatment also in favour of the therapy (group 1 versus 6). The difference between dibenzyline in a dose of 15 mgr/kg bodyweight and biphasic treatment with combined dibenzyline and trasicor in high doses (15 mgr/kg and 1,5 mgr/kg resp.) is (just) not significant (group 5 versus 6).
Therefore, alpha-blockade only, with dibenzyline in a high dose (15 mgr/kg bodyweight) as well as biphasic alpha-beta-blockade with dibenzyline (15 mgr/kg bodyweight) and trasicor (1,5 mgr/kg

bodyweight) in high doses, both improved survival significantly. Any combination with inderal did not improve survival.
As a result of these preliminary experiments, the dosages chosen for the further studies were rather arbitrarily: 15 mgr/kg bodyweight dibenzyline and 1,5 mgr/kg bodyweight trasicor.

C. 2-4 Analysis of vasodilatory effects of adrenergic blockade

After release of an occlusion of an artery, the flow through that vessel is under normal circumstances temporarily enhanced. The empty vessel-bed beyond the occlusion is dilated under the impact of the sudden thrust of blood that is liberated after release of the blood passage through the vessel (37).
After some time, the blood flow normalizes again.
This sequence is called "reactive hyperaemia" (R.H.) and depends on the expansibility or compliance of the vessel.
The peak systolic reactive hyperaemia (P.S.R.H.) is the difference between the peak flow in stationary state(P.F.S.) and the maximum peak flow in stationary state(M.P.F.S.) immediately after this desocclusion of the vessel(R.H.= M.P.F.S.-P.F.S.)(Fig.2,p.35).
Occlusion and release of the occlusion is regularly performed in flow measurements by placing a clamp proximal from the probe, which, together with a clamp distal from the probe, makes zero-adjustments possible.
This gives the opportunity to measure the reactive hyperaemia and to analyze as well the vasodilatory effect of alpha-blockade with dibenzyline in various dosages.

Results:

After infusion of dibenzyline, in all experiments a decrease of reactive hyperaemia was seen. In one experiment (exp. 7) up till 30 mgr/kg was infused before endotoxin (5 mgr/kg) was given.
In this experiment also a high dose of trasicor (3 mgr/kg) was given. Fig. 2 demonstrates the change in reactive hyperaemia

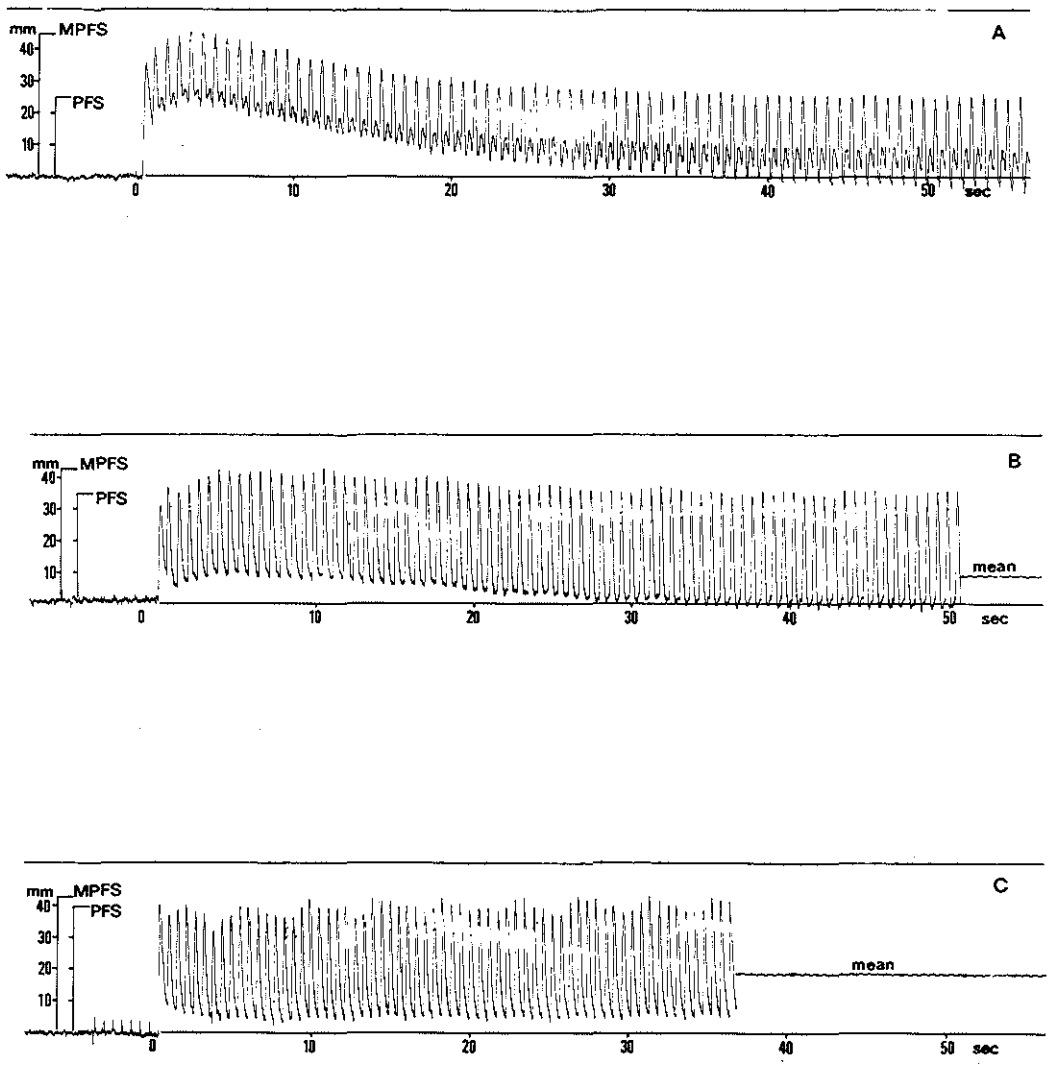

Fig. 2. Reactive hyperaemia after 0 mgr, 15 mgr. and 30 mgr/kg dibenzyline (A,B and C resp.): 10 mm in the registration corresponds with a pulsatile flow of 122 ml/min.

during dibenzyline infusion in this experiment:
A. is the R.H. before dibenzyline infusion, B. illustrates the decreased reactive hyperaemia after infusion of 15 mgr/kg dibenzyline, and C. illustrates that the reactive hyperaemia has practically disappeared after infusion of 30 mgr/kg dibenzyline.
Further analysis of the reactive hyperaemia in this experiment is demonstrated in Fig.3 (p.37). If an increase in the pulsatile flow to twice the original value is taken as 100% reactive hyperaemia, the values were calculated as follows:

I. At 0 min. at the start of the dibenzyline infusion:
P.F.S.: 24 mm M.P.F.S.: 46 mm R.H.$= \frac{46 - 24}{34} . 100 = 91\%$ (in percentages of the original value).

II. At 15 min.: R.H.$= \frac{50 - 34}{34} . 100 = 47\%$

III. Thus, the reactive hyperaemia is reduced to 20% with 15 mgr/kg dibenzyline. Only with twice that amount the reactive hyperaemia is reduced to near zero with 2%.
Further on in this experiment the following values for reactive hyperaemia were calculated:

IV. 10 Min. after E. coli: R.H.$= \frac{20 - 19}{19} . 100 = 5\%$
With decreasing values for the flow and therefore for the M.P.F.S. and P.F.S., the quotient becomes less reliable and thus the R.H. percentage also:

V. 30 Min. after start E. coli: R.H.$= \frac{9 - 8}{8} . 100 = 10\%$

VI. 20 Min. after start trasicor infusion: R.H.$= \frac{15 - 14}{14} . 100 = 7\%$

VII. 45 Min. after start trasicor infusion: R.H.$= \frac{15 - 14}{14} . 100 = 7\%$
1,5 mgr/kg in.

VII. 90 Min. after start trasicor infusion: R.H.$= \frac{15 - 14}{14} . 100 = 7\%$
3,0 mgr/kg in.

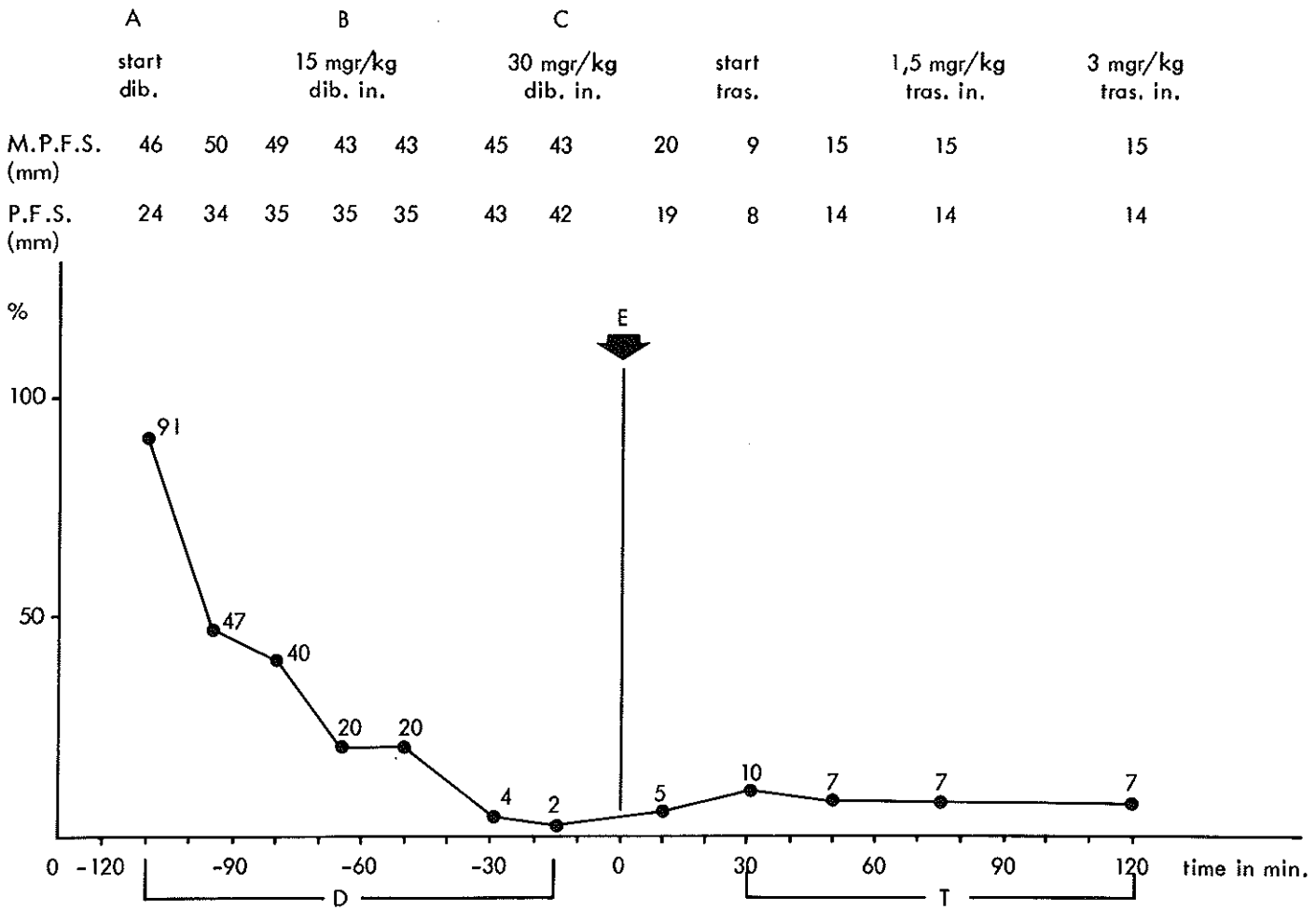

Fig.3. Decrease of reactive hyperaemia during biphasic blockade with 30 mgr/kg dibenzyline and 3 mgr/kg trasicor in endotoxin shock (5 mgr/kg).

Discussion and conclusions:

It can be assumed that the infusion of massive amounts of a vasodilatory substance like dibenzyline, as is the case in this experiment, will lead to a decrease of further expansibility or loss of rest compliance of the vessel, expressed in a decreased reactive hyperaemia.
Fig. 3 illustrates that the reactive hyperaemia before dibenzyline is clear enough, but that it is still there after infusion of 15 mgr/kg.
It is only after another dose, far above the accepted maximum, 30 mgr/kg bodyweight in total, that reactive hyperaemia practically disappears.
These results explain why chosing a high dose of dibenzyline was thought necessary in our further experiments: 15 mgr/kg dibenzyline, although many times the accepted maximum dose of literature, still does not fully abolish the reactive hyperaemia phenomenon and therefore the faculty of the vessels to dilate even further.
If one considers a vasodilatory substance to have a maximum dose, apart from the lethal and toxic effects, it should be the dose beyond which no further dilatation is possible.
Apparently that dose is not yet even reached with 15 mgr/kg, which is much more than the accepted dose of 3,5 mgr/kg (68,69).
After addition of trasicor, the reactive hyperaemia does not increase but is constant at a low level.

C. 2-5 Estimation of circulating endotoxin

Objective: To test the experimental model used, it was thought necessary to verify if endotoxin could be determined in the circulation.

Materials and methods: Two indirect tests, as described in chapter B. 2-1-1-4, were used in a small number of experiments with 5 mgr/kg bodyweight endotoxin injection.

The L i m u l u s l y s a t e t e s t was performed three times in 4 experiments (2 after dibenzyline premedication, 2 without treatment).
Blood sample 1 was taken immediately after endotoxin injection, blood sample 2 in the first phase of shock, 20 min. after endotoxin injection and blood sample 3 in the second phase of shock, 120 min. after endotoxin injection.
In a preliminary test blood samples taken before endotoxin injection, proved endotoxin-free. Results are given as +, ++ and +++ ratings, according to the time needed to give a positive test (formation of a solid gel of the plasma to be tested with the limulus lysate) (Table 2).

The A c t i n o m y c i n D - t e s t was performed in two experiments (1 with biphasic blockade, 1 without treatment).
Blood samples were taken at the same moments during the shock procedure as in the Limulus lysate test.
The activity of a standard preparation endotoxin with a L.D. 50% corresponded arbitrarily with 10 calculation units.
The results are compared with this standard preparation and expressed in these calculation units (Table 2).

A. LIMULUS LYSATE TEST	Sample 1	Sample 2	Sample 3	Survival/ death
Exp. 1 Dibenzyline premedication	+++	+++	++	survived >14 days
Exp. 2 Without treatment	++	++	++	died
Exp. 3 Dibenzyline premedication	++	++	++	survived >14 days
Exp. 4 Without treatment	+++	+++	++	died
B. ACTINOMYCIN D-TEST				
Exp. 1 Biphasic blockade (Dibenzyline and trasicor	40 U	85 U	59 U	died
Exp. 2 Without treatment	8,2 U	25 U	6,2 U	died

Table 2. Results of the Limulus lysate test and the Actinomycin D-test on circulating endotoxin (5 mgr/kg bodyweight) in endotoxin shock experiments.

Discussion and conclusions:

The Limulus lysate test demonstrated, that in these experiments circulating endotoxin could be detected up till 2 hours after injection.This was confirmed in the two experiments with the Actinomycin D-test in mice.
Although the level 20 min. after endotoxin injection was mostly higher than after 2 hrs., the latter level gave no indication of survival prognosis.

C. 2-6 Estimation of plasma catecholamine levels

Objective: To find support for the catecholamine hypothesis, as discussed in chapters B. 2-3 and B. 2-4, it seemed necessary to verify in our experimental set up if an increase of catecholamine levels could be detected after endotoxin injection.

Materials and methods: Catecholamine levels were measured in 4 experiments, according to the techniques as developed by Anton and Sayre (3) and Laverty (59).
The blood samples were taken before endotoxin injection (a), as a control of the normal value in that experiment, and in the second phase of shock, 1½ hr. after endotoxin injection (b).
In all experiments 5 mgr/kg bodyweight endotoxin was injected.
Two experiments were performed with biphasic blockade, one with dibenzyline premedication and one with combined simultaneous blockade, all paired with an untreated control animal (Table 3).

	Epinephrine µgr/l plasma	Norepinephrine µgr/l plasma	Survival/ death
Exp. 1			
Dibenzyline premedication	a: 2,2	4,7	died
	b: 3,3	8,7	
Control animal	a: 1,4	0,8	survived
	b: 2,2	0,5	>14 days
Exp. 2			
Combined biphasic blockade (dibenzyline and trasicor)	a: 0,67	0,75	survived
	b: 1,58	4,94	>14 days
Control animal	a: 0,44	0,94	died
	b: 7,91	4,59	
Exp. 3			
Combined simultaneous blockade (premedication)	a: 0,9	0,6	died
	b: 3,8	8,5	
Control animal	a: 1,1	1,3	survived
	b: 3,4	1,5	>14 days

Table 3. Results of plasma catecholamine determinations in endotoxin shock experiments (5 mgr/kg bodyweight).

Conclusions:

Increased plasma epinephrine levels were detected after endotoxin injection in all animals tested; both in adrenergically blocked and untreated animals.
The two surviving control animals had no increase in norepinephrine levels, the other animals did have an increase in norepinephrine levels.
This small amount of data seems to endorse the validity of the catecholamine hypothesis in our experimental model. In this respect our study is comparable to other experimental studies as described in literature

Summary and conclusions of the pilot study:

Premedication with dibenzyline, as well as biphasic alpha-beta-blockade with dibenzyline and trasicor,both in a high dose, clearly improved survival. No other treatment improved survival.
It was shown that the high dose of dibenzyline used (15 mgr/kg bodyweight) did not even give maximum vasodilatation. It can be concluded that 15 mgr/kg dibenzyline is still not the maximum dose. It could be demonstrated that endotoxin circulated for at least 2 hrs. after injection.
This observation is taken as another argument in favour of high dosage of any therapy to protect the circulation for a prolonged period of time against the endotoxin effects. The increase of epinephrine levels found, might support the catecholamine hypothesis.
The results of the pilot study justified further more systematic analysis of the working hypothesis in the following investigation.

C. 3 Main study

C. 3-1 Objectives:

As a result of the pilot study, the principal objective of the main investigation was to analyse more extensively in a standardized model the effects of biphasic combined alpha-beta-blockade on canine endotoxin shock.
As comparison, alternative treatment schedules would be studied as well. Data on survival, haemodynamics, biochemics and histology were compiled and subjected to statistical analysis.

C. 3-2 Materials and methods

The experimental animal: Experiments were performed on beagles, weighing from 10 to 20 kg.

Endotoxin: This was injected i.v. in 1 min. suspended in 25 ml Ringer's solution. A number of batches of Endotoxin coli was used, codenumber 127:B8, Difco Laboratories, Detroit.

Anaesthesia: After induction with 30 mgr/kg bodyweight Nembutal and endotracheal intubation, a light O_2-N_2O-Fluothane narcosis was maintained throughout the experiments. The testing of various parameters was only started when spontaneous respiration occurred, which was maintained during the whole experiment.

Infusions and timing: All animals, treated and untreated alike, received the same amount of fluid, in the following time schedule:

1. A 500 ml Ringer's solution was given before the injection of endotoxin , in an infusion time of 1½ hrs. In case of pretreatment the anti-adrenergic compound(s) were dissolved in this 500 ml Ringer's solution.

In cases without premedication, the Ringer's solution was given as well, but without adjunctive chemical compounds.

2. Endotoxin injection followed 15 min. after the end of the infusions mentioned above,for both the treated and untreated cases alike.

3. After endotoxin injection, 200 ml Ringer's solution was infused over 30 min. This solution did not contain any adjunctive medication in treated and untreated cases alike.

4. After these 30 min. postendotoxin,infusion was started of 500 ml Ringer's solution with an infusion time of 1½ hrs. When medication was given during this period (in biphasic blockade or monophasic trasicor) the anti-adrenergic compound(s) were dissolved in this 500 ml Ringer's solution.

5. Longer surviving animals received 1500 ml Ringer's solution i.v. with an infusion time of 24 hrs., starting the first day after the experiment, unless oral fluid intake was possible.

Thus, all animals in all experiments received the same amounts of fluid, including the control animals which were injected with endotoxin without anti-adrenergic treatment, and also including the animals which received anti-adrenergic treatment without endotoxin, as a check on the effects of the treatment as such.

In all animals subjected to endotoxin shock, the only variable was therefore the addition or omission of the anti-adrenergic compounds to be tested.

Medications: The anti-adrenergics used were dibenzyline in a dose of 15 mgr/kg bodyweight and trasicor in a dose of 1,5 mgr/kg bodyweight. The choice of the dibenzyline and trasicor dose was based on the results in the pilot study.
Dibenzyline was always given as premedication and was suspended in the first 500 ml Ringer's solution. Trasicor was either given

as medication after endotoxin, being suspended in the last 500 ml Ringer's solution, or given as premedication together with dibenzyline in the first 500 ml Ringer's solution.
All animals received 2.000.000 U penicillin and 1 gr streptomycin/day, for 7 days or until death, starting on the day of the experiment.

Types of experiments: Five different types of experiments were carried out:

1. Combined biphasic adrenergic blockade without endotoxin (C 3-3).

2. Endotoxin shock without treatment (C 3-4).

3. Monophasic adrenergic blockade before or after endotoxin administration (C 3-5).

4. Combined simultaneous adrenergic blockade before endotoxin administration (C 3-6).

5. Combined biphasic adrenergic blockade before and after endotoxin administration (C 3-7).

Survival: Survival was recorded in hours after endotoxin injection. Survival for more than 2 weeks was considered indefinite.

Blood pressure measurements: The pulsatile blood pressure was continuously recorded in all experiments via an intra-arterial catheter, situated in the femoral artery. The pressure recordings were calibrated in such a way that 100 mm Hg corresponded with 2 cm of the record sheets of the Elema-recorder.
Average values of the systolic blood pressures during the shock procedure (S.B.P.) in the experimental animals of the various groups, were calculated at 10 min. intervals. In some experiments the mean of systolic and diastolic blood pressure (M.B.P.) was registrated and here also average values for these experiments were calculated (C 3-7-1).

Trends in the changes of arterial blood pressure were analyzed.

Blood flow measurements: The transflow 600 "Scalar"apparatus was used with the corresponding transflow 600 peri-vascular electromagnetic flowprobes "E.F.M.".
The probes were fitted around the (other) femoral artery of the experimental animal. Both pulsatile and mean flow were registrated. The deflections resulting from changes in blood flow were visualilized on the Elema-recorder. The sensitivity of the recorder was such that an output of 0,3 volt was chosen for the calibration. The pulsatile flow output corresponded with 1,5 cm deflection on the Elema-recorder,the mean flow output with a choice of 2,5 ; 4,5 or 5 cm.All measurements were done with "acute" (not previously implanted) flowprobes. This made frequent 0-controls necessary. Registrations which were unreliable were discarded from the study. Average values of the mean flow during the shock procedure in the experimental animals of the various groups were calculated at 10 min. intervals. Trends in the changes in the mean flow were analyzed.

Peripheral resistance: The formula used for calculating the total peripheral resistance (T.P.R.) was (37,113):

$$T.P.R. = \frac{P - 20 \text{ (mm Hg)}}{F}$$

P = blood pressure
F = blood flow
20 mm Hg = the critical closing pressure in dogs (37)

Duration of the blood flow and -pressure registration:

These parameters were continuously registrated on the Elema-recorder throughout the duration of the experiments up till 2½ hrs. after endotoxin injection.
This period was chosen because at 2 hrs. after endotoxin injection the shock pattern is well into the second phase and usually in a steady state.
The 2½ hrs. registration period was also based on information in literature by Swan (103), who did registrations until death in experimental endotoxin shock and found that parameters in the later shock phase only change in the last 15 min.

In a few experiments (C 3-9) registration was continued up till 6 hrs. after endotoxin, to check whether in this experimental model any changes in the trend of blood pressure and -flow occurred later on.
Registrations longer than approximately 6 hrs. were not considered usefull, because measurements might become inexact.

Heart rates: The influence of adrenergic blockades on the heart rates of the dogs, subjected to endotoxin shock were analyzed (C.3-4 to C.3-8).
Average values of the heart rates were calculated at 10 min. intervals, and trends in increase or decrease of the rates analyzed.

Biochemical data: Serum levels of creatinine, urea, alc.phosphatase, bilirubin, SGOT and SGPT were determined before endotoxin injection, at the end of the period of measurement and, in case of survival, 20 hrs. after endotoxin injection.
On statistical analysis of these biochemical data they were found not to correlate with the severity of shock.
They are for that reason not included in the description of experimental results.
Blood gases were analyzed with the Astrup method, at regular intervals throughout the period of measurement.
Values for pH and HCO_3^- were analyzed for the experiments with combined biphasic blockade and endotoxin shock without treatment.
Results are described in chapter C. 3-7-3.

Autopsy findings:

In the non-surviving animals autopsy was performed.

M a c r o s c o p i c a l l y, the characteristic haemorrhagic necrosis of the bowel mucosa (68,69) and pulmonary oedema were rated as follows:

0 = no changes
+ = light
++ = moderate
+++ = severe changes

M i c r o s c o p i c a l l y , the lungs, the small bowel, the colon and the kidney were specifically examined.
The changes found were rated as follows:

Lung: 0 = normal
+ = mild congestion
++ = severe congestion
+++ = congestion + thrombus
++++ = congestion + haemorrhage + tissue damage

Small bowel:
0 = normal
+ = mild congestion
++ = severe congestion
+++ = congestion + superficial mucosal damage
++++ = congestion + severe mucosal loss

Colon: as small bowel

Kidney: 0, +, ++, +++. ++++: increasing severity of congestion

The macroscopic findings will be described in the sub-chapters on the various groups of experiments.
The microscopic findings were compiled for all groups and will be described in chapter C. 3-7-3.

Statistical analysis of parameters: Shock parameters of the experimental animals in the various groups were compiled. The averages with standard deviation (S.D.) of (systolic) blood pressure, (mean) blood flow and peripheral resistance were calculated for the whole group at fixed moments during the shock procedure.
Reliable flow measurements could not be obtained in all experiments. Most parameters were statistically analyzed, using one of the following tests: the matched-pairs signed Rank test, the Mann-Whitney-U test, the Chi-square test , the test against trend in

related samples, the Binomial test and the Yates-Cochran test (55,56,100,101).
All tests were performed at a significance level of alpha=0,05. Most tests were two sidedly performed.
For this further analysis, some groups of experiments were combined and the effect of various dosages of endotoxin on the shock pattern were compared.
Survival differences in treated and untreated animals were compared for the various dosages of endotoxin and the various forms of anti-adrenergic treatment.
The influence on blood flow, blood pressure and heart rates of anti-adrenergic blockades was analyzed.
In the combined groups, effects of adrenergic blockade on biochemical data and autopsy findings were estimated.

C. 3-3 Biphasic adrenergic blockade without endotoxin

Objective: To check the effect of dibenzyline and trasicor on haemodynamics and survival in the experimental animal without endotoxin.

Materials and methods: Seven dogs were treated with a biphasic blockade, applying 15 mgr/kg bodyweight dibenzyline and 1,5 mgr/kg trasicor, given in the same time relations as used in the experiments with endotoxin.
Infusions and timing in these and all following experiments were described in chapter C. 3-2.

Results:

1. Survival: All 7 dogs survived >14 days.
2. Systolic blood pressure (S.B.P.): Table 4 gives the average values of the systolic blood pressures with S.D.
   This is graphically illustrated in Fig. 4.
   During dibenzyline infusion the (average) S.B.P. decreased from 176 mm Hg → 109 mm Hg. Trasicor infusion gave an initial increase of the S.B.P. to 130 mm Hg at 35 min., followed by a decrease to 102 mm Hg at 40 min. and 106 mm Hg at 130 min.
3. Mean blood flow: Table 5 gives the average values of the mean blood flow in this group. This is graphically illustrated in Fig. 5. During dibenzyline infusion the (average) blood flow increased from 101 ml/min. → 115 ml/min. Trasicor leads to some flow decrease: from 115 ml/min. → 92 ml/min.
4. Peripheral resistance (P.R.): Table 6 gives the values and Fig. 6 is the graphic illustration of the P.R. in this group. During dibenzyline infusion the (average) P.R. decreased from 1,26 mm Hg/min./ml → 0,48 mm Hg/min./ml. During trasicor infusion it increased again to 0,82 mm Hg/min./ml.
5. Autopsy findings: No autopsies were performed, all animals surviving the adrenergic blockade without endotoxin.

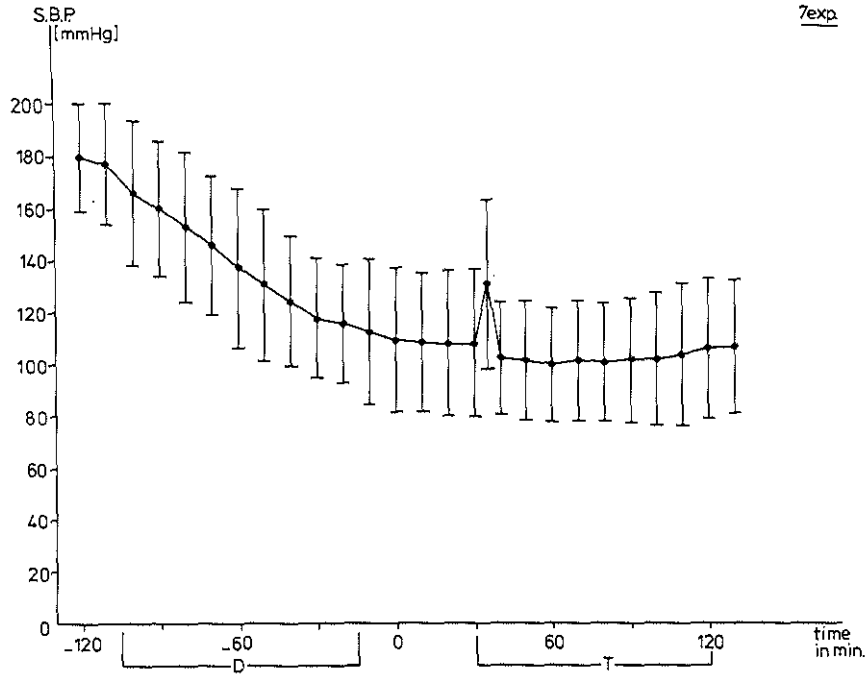

Fig. 4. Biphasic adrenergic blockade without endotoxin.
Systolic blood pressure ± S.D.

	DIBENZYLINE INFUSION				TRASICOR INFUSION		
	Time in min.	S.B.P.	S.D.		Time in min.	S.B.P.	S.D
Start dibenzyline →	- 110	176,14	22,35		0	109,42	27,26
	- 100	165,85	27,90		10	108,71	26,49
	- 90	159,85	25,70		20	108,00	27,83
	- 80	152,57	28,80	Start trasicor →	30	108,00	27,83
	- 70	145,14	26,39		35	130,14	32,59
	- 60	137,00	30,03		40	102,14	21,55
	- 50	130,85	26,06		50	101,42	22,52
	- 40	124,42	24,72		60	99,85	21,73
	- 30	117,85	22,13		70	101,00	23,05
End dibenzyline →	- 20	115,28	22,96		80	101,14	22,56
	- 10	112,71	27,21		90	101,71	23,29
	0	109,42	27,26		100	102,00	25,61
					110	103,18	27,59
				End trasicor →	120	105,42	26,17
					130	106,28	25,43

Table 4. Biphasic adrenergic blockade without endotoxin.
n = 7. S.B.P. in mm Hg.

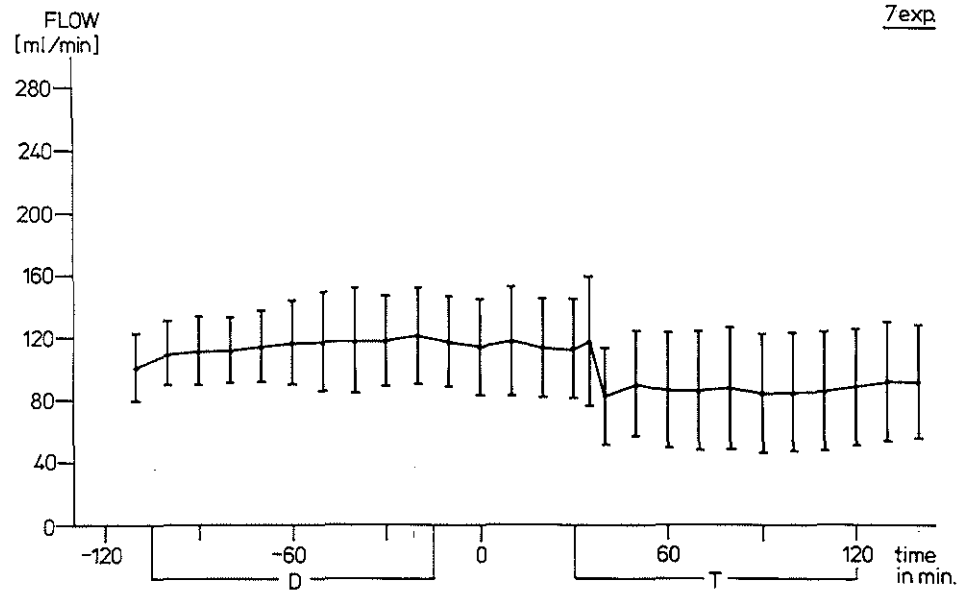

Fig. 5. Biphasic adrenergic blockade without endotoxin.
Blood flow ± S.D.

	DIBENZYLINE INFUSION				TRASICOR INFUSION		
	Time in min.	Blood flow	S.D.		Time in min.	Blood flow	S.D.
Start dibenzyline →	- 110	101,24	22,24		0	115,25	30,41
	- 100	101,43	21,25		10	119,57	35,71
	- 90	112,40	22,31		20	115,56	32,68
	- 80	112,91	21,33	Start trasicor →	30	113,41	32,67
	- 70	115,16	23,46		35	118,89	42,35
	- 60	118,62	27,59		40	83,71	31,50
	- 50	118,53	32,78		50	90,41	34,10
	- 40	119,77	34,47		60	87,55	37,76
	- 30	119,56	29,13		70	87,84	38,24
End dibenzyline →	- 20	122,25	30,51		80	85,77	38,42
	- 10	118,96	29,31		90	86,37	38,38
	0	115,25	30,41		100	87,60	38,81
					110	89,68	37,09
				End trasicor →	120	92,80	38,84
					130	92,37	36,67

Table 5. Biphasic adrenergic blockade without endotoxin.
n = 7. Blood flow in ml/min.

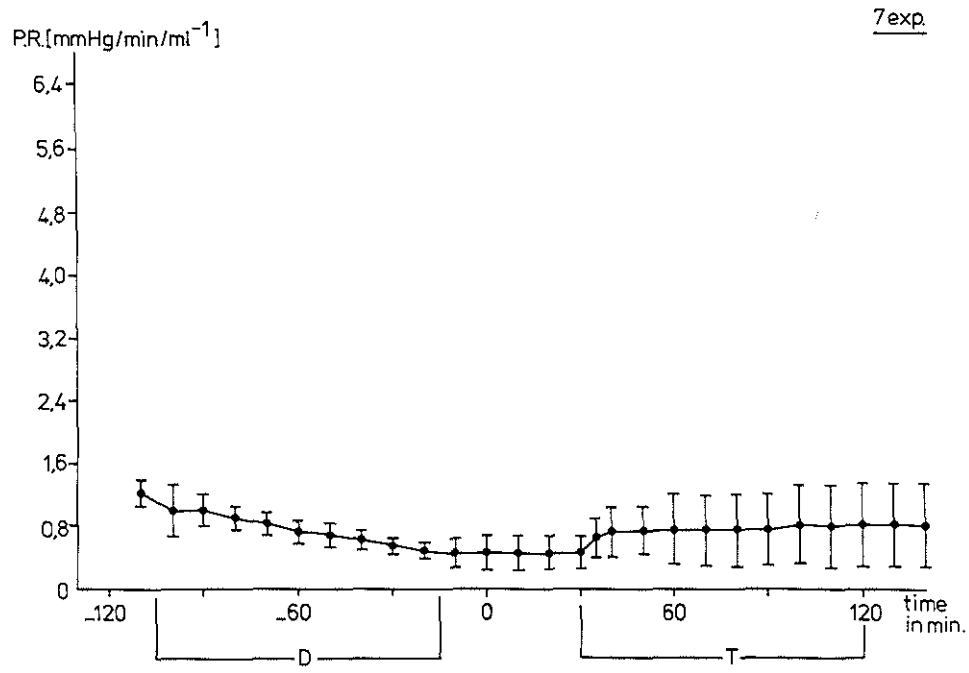

Fig. 6. Biphasic adrenergic blockade without endotoxin.
Peripheral resistance ± S.D.

	DIBENZYLINE INFUSION				TRASICOR INFUSION		
	Time in min.	P.R.	S.D.		Time in min.	P.R.	S.D.
Start dibenzyline →	- 110	1,26	0,17		0	0,48	0,21
	- 100	0,97	0,41		10	0,46	0,21
	- 90	1,00	0,14		20	0,49	0,21
	- 80	0,94	0,14	Start trasicor →	30	0,62	0,21
	- 70	0,85	0,14		35	0,74	0,24
	- 60	0,76	0,13		40	0,73	0,32
	- 50	0,72	0,14		50	0,77	0,31
	- 40	0,64	0,12		60	0,76	0,45
	- 30	0,58	0,11		70	0,76	0,47
End dibenzyline →	- 20	0,50	0,11		80	0,76	0,46
	- 10	0,47	0,18		90	0,78	0,50
	0	0,48	0,21		100	0,81	0,53
					110	0,79	0,53
				End trasicor →	120	0,81	0,53
					130	0,81	0,53
					140	0,82	0,52

Table 6. Biphasic adrenergic blockade without endotoxin.
n = 7. P.R. in mm Hg/min./ml.

Discussion and conclusions:

Blood pressure and peripheral resistance decrease and blood flow increases during dibenzyline infusion.
In the trasicor phase the flow is slightly lower and the peripheral resistance slightly higher in comparison with the dibenzyline phase.
Anti-adrenergic medication in high dose in itself was not harmful to the experimental animal.

C. 3-4 Endotoxin shock without treatment

Objective: Evaluation of shock parameters in untreated animals, subjected to endotoxin shock. Verification in our model of the biphasic shock pattern, as described in literature. Subsequent comparison of these parameters with the shock pattern of treated animals.

Materials and methods: Average values of systolic blood pressure with standard deviation (S.D.) was calculated in a group of 25 experiments, in which 5 mgr/kg bodyweight endotoxin was given. The trends (increasing or decreasing) of the average systolic blood pressure after endotoxin was investigated in the following intervals: 0 → 5 min., 5 → 10 min., 10 → 15 min. and 60 → 140 min. after endotoxin injection. In the intervals 0 → 5 min, and 5 → 10 min., the Wilcoxon matched-pairs signed ranks test was used (56). In the intervals after 10 min. a test in related samples was used (55), all two sidedly performed at a level of significance of alpha=0,05. Reliable flow measurements could be obtained in a group of 14 of these experiments. Average values of the mean blood flow in this group were calculated. The trend of the blood flow after endotoxin injection was investigated in the same time relations as the blood pressure trends, applying the same statistical tests (alpha=0,05). Peripheral resistance was also calculated for these experiments. The average values of the heart rates were calculated at 10 min. intervals with S.D. Shock parameters of surviving and non-surviving experimental animals were taken together.

Results:

1. Survival: Of the 25 dogs, 16 died and 9 survived >14 days.
2. Systolic blood pressure (S.B.P.): Table 7 gives the average values for the systolic blood pressure of the 25 cases with S.D. Those values are graphically illustrated in Fig. 7. After endotoxin the S.B.P. decreased within 10 min. from 174 mm Hg → 66 mm Hg. A recovery to 102 mm Hg at 50 min. was followed by a further decrease to 68 mm Hg at 140 min.

Trends of the blood pressure: from 0 → 5 min. after endotoxin injection, a significantly decreasing trend (S.D.T.) was found. From 5 → 10 min. after endotoxin injection no significant trend(N.S.T.) was found.The trend in blood pressure from 10 → 50 min. was significantly increasing (S.I.T.) and from 60 → 140 min. significantly decreasing (S.D.T.).(Fig. 28,p. 104).

3. Mean blood flow: Table 8 gives the average values of the mean blood flow in the group of 14 experiments in which reliable flow measurements were obtained during the whole procedure. Fig. 8 is the graphic illustration of the same.
   After endotoxin injection the flow decreased within 10 min. from 124 ml/min. → 14 ml/min. A recovery to 61 ml/min. at 50 min. was followed by a further decrease to 32 ml/min. at 140 min.
   Trends in mean blood flow: from 0 → 5 min after endotoxin injection: S.D.T.; 5 → 10 min.: N.S.T.; 10 → 50 min.: S.I.T. and from 50 → 130 min. after endotoxin injection S.D.T. (Fig.32).

4. Peripheral resistance: Table 9 gives the values in numbers and Fig. 9 in graphic form.
   After endotoxin injection the P.R. increases within 10 min. from 1,05 mm Hg/min./ml → 5,09 mm Hg/min./ml.
   A decrease to 1,54 mm Hg/min./ml at 50 min. was followed by another increase to 1,66 mm Hg/min./ml at 140 min.

5. Heart rates: The following average values with S.D. are relevant: at T =-120: 161 beats/min. (S.D.:20); at T = 0: 164 beats/min. (S.D.:29); at T = 120: 156 beats/min. (S.D.:29).

6. Autopsy findings: All non-surviving animals in this group showed intestinal haemorrhagic necrosis, mostly rated as severe lesions. Four animals had light pulmonary changes.

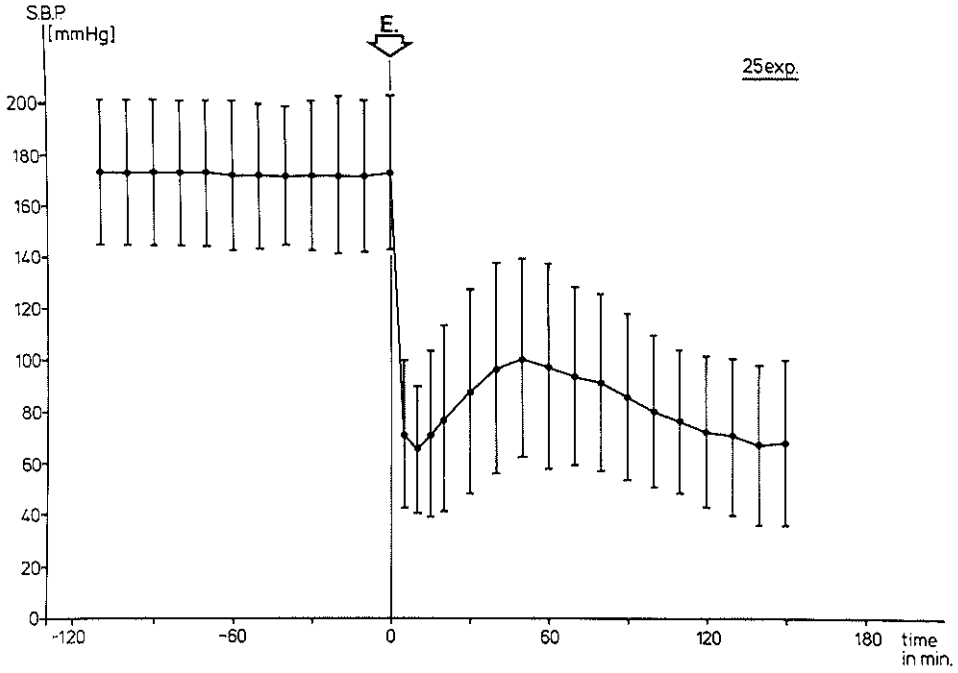

Fig. 7. Endotoxin shock (5 mgr/kg) without treatment.
Systolic blood pressure ± S.D.

	PRE-ENDOTOXIN				POSTENDOTOXIN		
	Time in min.	S.B.P.	S.D.		Time in min.	S.B.P.	S.D.
	- 110	174,65	27,81	Endotoxin injection →	0	174,34	29,58
	- 100	174,11	28,58		5	73,03	30,29
	- 90	174,19	28,50		10	66,57	25,09
	- 80	174,46	29,01		15	72,76	32,53
	- 70	174,26	28,91		20	78,19	35,82
	- 60	173,53	29,11		30	89,11	40,56
	- 50	173,38	28,60		40	98,42	40,25
	- 40	172,88	28,62		50	102,53	38,61
	- 30	173,38	29,38		60	98,73	35,85
	- 20	173,84	29,38		70	94,80	34,83
Endotoxin injection →	- 10	173,42	29,44		80	92,11	34,23
	0	174,34	29,58		90	87,23	31,88
					100	81,73	29,13
					110	77,34	28,27
					120	73,20	29,49
					130	71,63	30,14
					140	68,65	31,50
					150	69,73	32,78

Table 7. Endotoxin shock (5 mgr/kg) without treatment.
n = 25. S.B.P. in mm Hg.

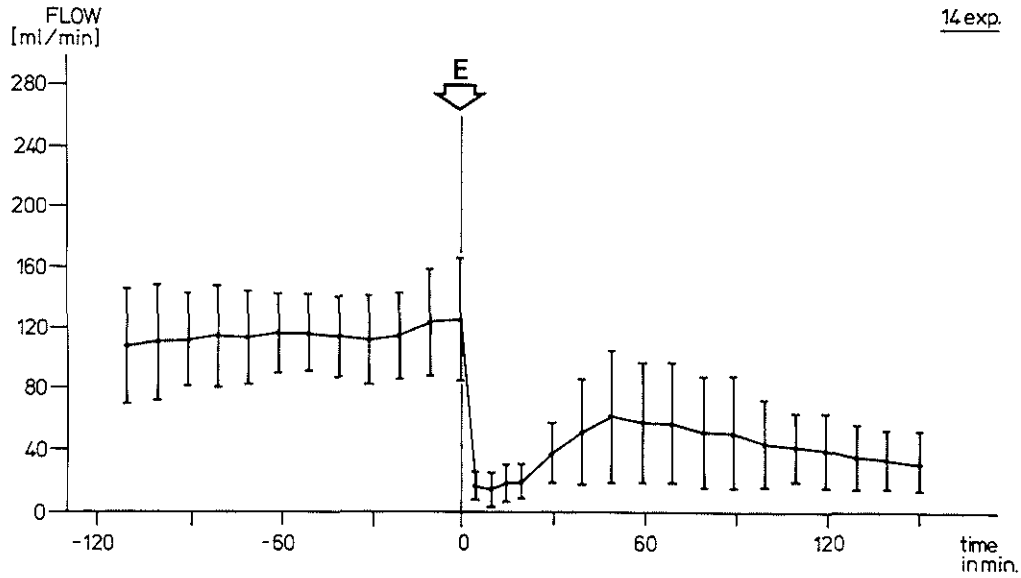

Fig. 8. Endotoxin shock (5 mgr/kg) without treatment.
Blood flow ± S.D.

	PRE-ENDOTOXIN				POSTENDOTOXIN		
	Time in min.	Blood flow	S.D.		Time in min.	Blood flow	S.D.
	- 110	105,90	36,84	Endotoxin injection →	0	124,03	39,38
	- 100	109,22	36,11		5	15,09	9,51
	- 90	110,63	29,14		10	14,13	11,64
	- 80	113,68	33,65		15	18,23	12,04
	- 70	112,74	29,10		20	19,08	11,43
	- 60	115,68	24,60		30	37,64	20,91
	- 50	115,79	24,30		40	52,44	33,38
	- 40	112,38	26,61		50	61,59	42,35
	- 30	110,76	28,66		60	57,31	38,85
	- 20	114,49	28,19		70	57,34	38,57
Endotoxin injection →	- 10	122,79	35,53		80	51,43	35,82
	0	124,03	39,38		90	51,32	35,50
					100	44,59	27,07
					110	41,16	21,81
					120	39,09	23,47
					130	35,73	20,49
					140	32,36	19,95

Table 8. Endotoxin shock (5 mgr/kg) without treatment.
n = 14. Blood flow in ml/min.

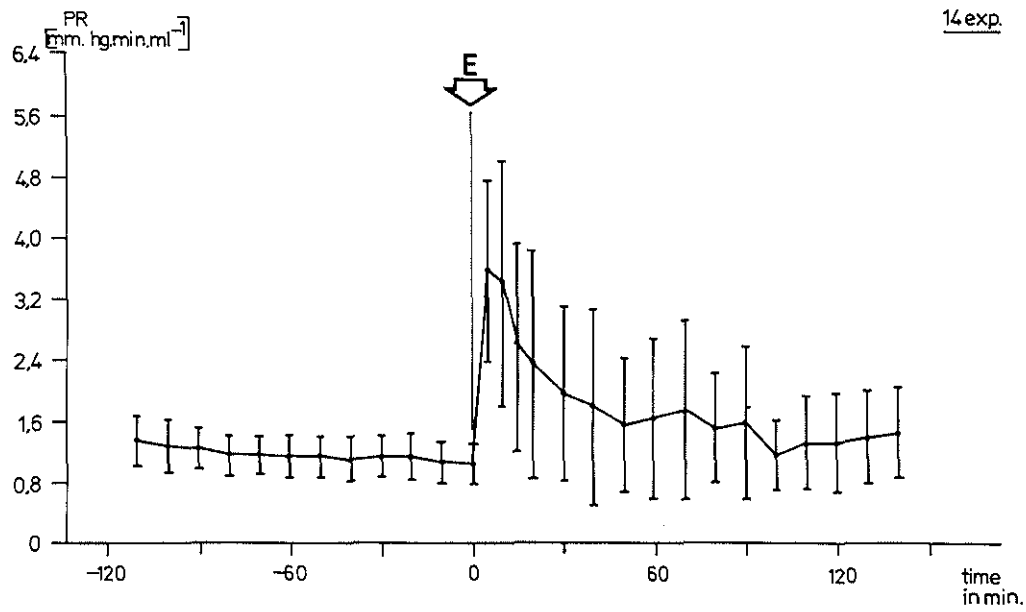

Fig. 9. Endotoxin shock (5 mgr/kg) without treatment.
Peripheral resistance ± S.D.

	PRE-ENDOTOXIN				POSTENDOTOXIN		
	Time in min.	P.R.	S.D.		Time in min.	P.R.	S.D.
	- 110	1,33	0,33	Endotoxin injection →	0	1,05	0,26
	- 100	1,28	0,34		5	3,54	1,39
	- 90	1,24	0,25		10	5,09	3,56
	- 80	1,14	0,25		15	2,54	1,36
	- 70	1,17	0,25		20	2,87	2,79
	- 60	1,15	0,29		30	1,96	1,13
	- 50	1,14	0,26		40	1,86	1,42
	- 40	1,10	0,29		50	1,54	0,86
	- 30	1,14	0,28		60	1,64	1,03
	- 20	1,15	0,30		70	1,75	1,18
Endotoxin injection →	- 10	1,08	0,25		80	1,51	0,69
	0	1,05	0,26		90	1,58	1,00
					100	1,17	0,47
					110	1,31	0,61
					120	1,31	0,66
					130	1,61	1,18
					140	1,66	1,17

Table 9. Endotoxin shock (5 mgr/kg) without treatment.
n = 14. P.R. in mm Hg/min./ml.

Discussion:

5 Mgr/kg bodyweight endotoxin appeared not to be a L.D. 95%, as suggested in literature (18,106).
The biphasic pattern of blood pressure in experimental endotoxin shock was confirmed, as illustrated in Fig. 7.
Fig. 8 demonstrates what happens to blood flow in the femoral artery: the decrease immediately after endotoxin injection (the first phase) is steep.
A recovery follows and then the slower progressive decrease of the second phase sets in.
Blood flow in the femoral artery therefore also follows a biphasic pattern and has an inverse relationship with peripheral resistance (Fig. 9).
At autopsy the intestinal haemorrhagic necrosis, described in literature, was confirmed (66,67).

Conclusion:

Endotoxin shock in dogs has a biphasic pattern: immediately after endotoxin injection a steep decrease of blood pressure and blood flow is seen, a recovery phase setting in after 5 to 10 min.
A second blood pressure- and blood flow decrease starts approximately 60 min. after endotoxin injection.
These trends in the biphasic pattern of blood pressure and blood flow proved statistically significant.
The L.D. 95% of a shock model in which 5 mgr/kg bodyweight Endotoxin coli (0127:B8 Difco) is applied, appears questionable (C. 3-8).

C. 3-5 Monophasic blockade

C. 3-5-1 Dibenzyline premedication

Objective: To test the influence of premedication with dibenzyline in a high dose on the pattern of endotoxin shock.

Materials and methods: Five paired experiments were done with endotoxin shock, applying 5 mgr/kg bodyweight endotoxin, batchnumber 581374-2.
In each paired experiment, one animal was premedicated with 15 mgr/kg bodyweight dibenzyline and the other, which functioned as control, was not.
All other experimental conditions (endotoxin, infusions, timing etc.) were the same in these paired experiments.
Shock parametersof surviving and non-surviving animals in each group, treated and untreated alike, were taken together.
Blood pressure, blood flow and peripheral resistance are presented in this chapter as far as the dibenzyline premedicated group is concerned. The data of the untreated animals in these experiments were combined with those of other untreated series (C. 3-5-2, C. 3-6 etc.) to form a compiled controlgroup (C 3-4 and C. 3-7-3).
By applying the Mann-Whitney-U test, it was investigated whether there were moments when the blood flow of treated dogs showed a statistically significant difference from the flow of untreated dogs (5 dogs with dibenzyline pretreatment against the compiled group of 14 untreated dogs).
The trends of the heart rates in the dibenzyline infusion period (T= - 120 → T = 0 min.) and in the period after endotoxin (T = 0→ T = 120 min.)were analyzed with a test against trend in related samples (alpha=0,05).
The heart rates of the dibenzyline premedicated animals at T = 0 and T = 120 were compared with those in a compiled controlgroup of 25 untreated dogs, applying the Mann-Whitney-U test (two sidedly performed, alpha = 0,05).

R e s u l t s :

1. Survival: In the treated and untreated group alike, 3 out of 5 dogs survived >14 days.

2. Systolic blood pressure: Table 10 gives the average values for the systolic blood pressure with S.D. of the treated group. Fig. 10 graphically illustrates these values.
The S.B.P. at - 110 min. was 181 mm Hg, at 0 min. 119 mm Hg, at 5 min. after endotoxin injection 86 mm Hg, at 50 min. 101 mm Hg and at 130 min. it was 74 mm Hg.

3. Blood flow: Table 11 and Fig. 11 give the data. The flow increased from 105 ml/min. at T = - 110 → 129 ml/min. at T = 0, at T = 130 it was 87 ml/min.
In the statistical analysis, at the moments T = 0 and T = 50 min. after endotoxin injection, no significant difference in flow was found. At all other moments the flow was significantly higher in the treated dogs.

4. Peripheral resistance: Table 12 and Fig. 12 give the data. The P.R. decreased from 1,28 mm Hg/min./ml at T = - 110 → 0,45 mm Hg/min./ml at T = 0. At T = 130 it was 0,38 mm Hg/min/ml .

5. Heart rates: The following average values with S.D. are relevant: at T = - 120: 168 beats/min. (S.D.: 36); at T = 0: 202 beats/ min. (S.D.: 43); at T = 120 : 186 beats/min.(S.D. 27).
In a compiled controlgroup these values were: at T = - 120 : 161 beats/min. (S.D. : 20), at T = 0 : 164 beats/min. (S.D.: 29); at T = 120 : 156 beats/min (S.D. : 29).
In the T =-120 → T = 0 range, a significantly increasing trend was found in the dibenzyline premedicated group.
In the T= 0 → T= 120 range no significant trend was found.
On comparison of the heart rates in this group at T = 0 and T = 120 with the controlgroup, it was found that at both moments the heart rate in dibenzyline premedicated animals was significantly higher than in the controlgroup.

6. Autopsy findings: At autopsy, the 2 non-surviving untreated animals showed severe intestinal lesions with haemorrhagic sludging of the mucosa, but no important pulmonary changes.
One of the dibenzyline pretreated, non-surviving animals could

not be autopsied. The other had pulmonary atelectasis and no clear signs of intestinal lesions.

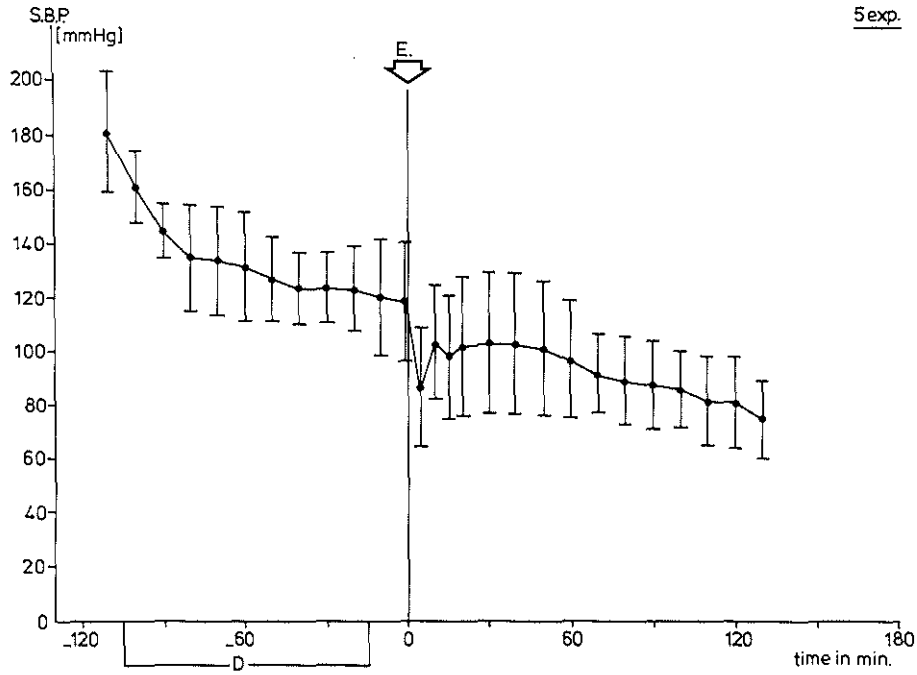

Fig. 10. Endotoxin shock (5 mgr/kg). Dibenzyline premedication. Systolic blood pressure ± S.D.

	PRE-ENDOTOXIN				POSTENDOTOXIN			
	Time in min.	S.B.P.	S.D.			Time in min.	S.B.P.	S.D.
Start dibenzyline →	- 110	181,20	21,92		Endotoxin injection →	0	119,60	22,16
	- 100	161,80	13,14			5	86,60	22,24
	- 90	145,20	10,30			10	103,80	21,08
	- 80	135,20	19,27			15	98,80	23,54
	- 70	134,40	19,15			20	102,40	26,23
	- 60	131,60	19,98			30	103,00	26,73
	- 50	127,00	15,77			40	103,60	26,65
	- 40	123,60	13,06			50	101,00	25,37
	- 30	124,60	13,08			60	97,80	21,64
End dibenzyline →	- 20	123,20	15,94			70	91,46	14,89
	- 10	120,80	21,27			80	89,80	15,46
Endotoxin injection →	0	119,60	22,16			90	87,80	16,48
						100	86,00	14,10
						110	81,60	16,89
						120	81,00	16,91
						130	74,75	14,81

Table 10. Endotoxin shock (5 mgr/kg). Dibenzyline premedication. n = 5. S.B.P. in mm Hg.

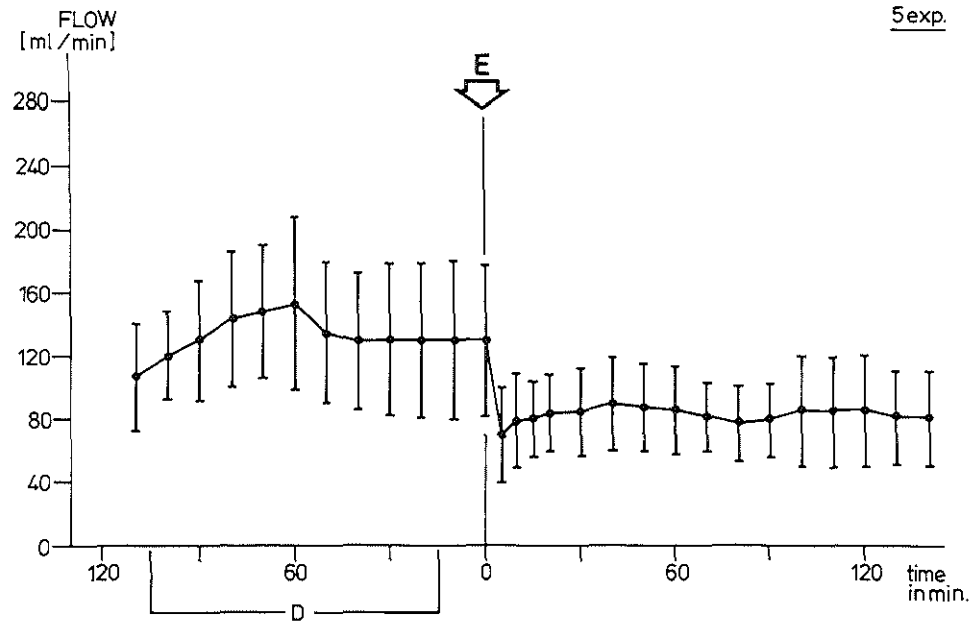

Fig. 11. Endotoxin shock (5 mgr/kg). Dibenzyline premedication. Blood flow ± S.D.

	PRE-ENDOTOXIN				POSTENDOTOXIN			
	Time in min.	Blood flow	S.D.			Time in min.	Blood flow	S.D.
Start dibenzyline →	- 110	105,68	27,16		Endotoxin injection →	0	129,02	47,94
	- 100	118,57	31,50			5	82,07	30,23
	- 90	126,85	32,69			10	79,16	30,80
	- 80	137,75	38,85			15	79,63	24,07
	- 70	148,79	41,85			20	84,18	24,17
	- 60	154,16	48,04			30	85,74	28,82
	- 50	138,83	48,49			40	89,55	29,51
	- 40	130,68	44,34			50	88,11	28,41
	- 30	130,39	46,04			60	85,20	26,26
End dibenzyline →	- 20	130,39	46,64			70	81,35	22,42
	- 10	131,03	50,88			80	78,28	22,72
Endotoxin injection →	0	129,02	47,94			90	81,57	28,30
						100	88,88	36,12
						110	90,33	39,49
						120	90,91	44,40
						130	87,36	37,72

Table 11. Endotoxin shock (5 mgr/kg). Dibenzyline premedication. n = 5. Blood flow in ml/min.

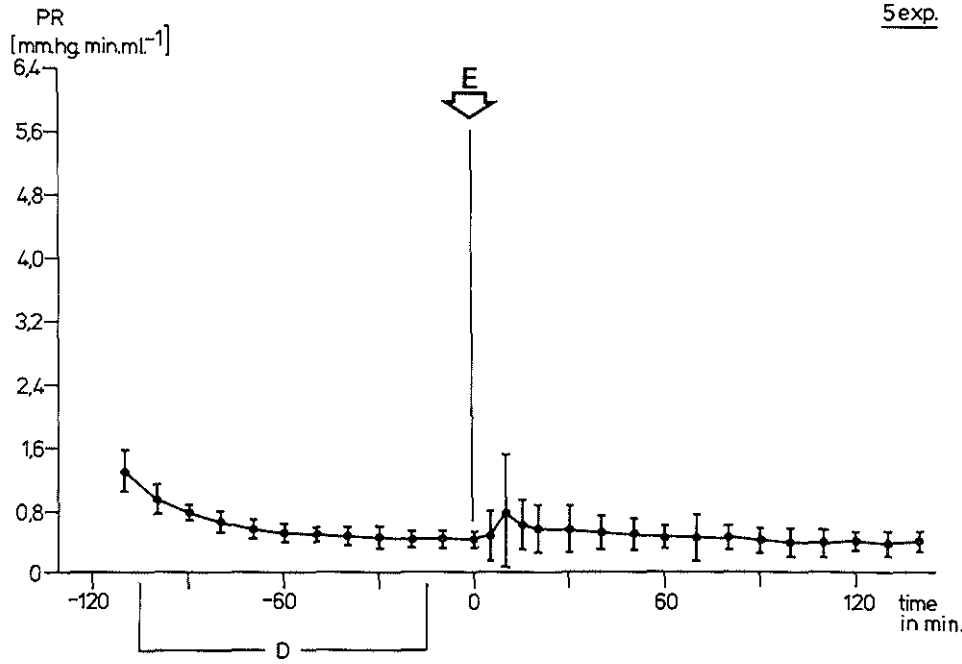

Fig. 12. Endotoxin shock (5 mgr/kg). Dibenzyline premedication. Peripheral resistance ± S.D.

	PRE-ENDOTOXIN				POSTENDOTOXIN		
	Time in min.	P.R.	S.D.		Time in min.	P.R.	S.D.
Start dibenzyline →	- 110	1,28	0,27	Endotoxin injection →	0	0,45	0,12
	- 100	0,99	0,28		5	0,48	0,33
	- 90	0,80	0,16		10	0,79	0,72
	- 80	0,67	0,17		15	0,62	0,33
	- 70	0,60	0,17		20	0,57	0,28
	- 60	0,55	0,17		30	0,55	0,29
	- 50	0,52	0,13		40	0,51	0,20
	- 40	0,49	0,12		50	0,51	0,19
	- 30	0,48	0,13		60	0,47	0,16
End dibenzyline →	- 20	0,45	0,12		70	0,46	0,16
	- 10	0,45	0,11		80	0,46	0,18
Endotoxin injection →	0	0,45	0,12		90	0,41	0,17
					100	0,38	0,17
					110	0,38	0,18
					120	0,39	0,17
					130	0,38	0,16

Table 12. Endotoxin shock (5 mgr/kg). Dibenzyline premedication. n = 5. P.R. in mm Hg/min./ml.

Discussion:

The difference in survival between dibenzyline pretreated and untreated cases was found not to be significant.
The impact of the endotoxin shock procedure with regard to lethality was small in this series.
The potency of the endotoxin batch used might have been at fault.
Survival data of control experiments (untreated animals) for all batches used are analyzed in chapter C. 3-8.
During dibenzyline premedication, the blood pressure decreased while the blood flow increased. This means that the peripheral resistance decreases at the same time.
After endotoxin, some decrease of blood pressure and - flow is seen , concomitant with some increase in peripheral resistance.
On comparison with the pattern of untreated cases (C. 3-4, Fig. 7, 8 and 9), the first shock phase seems to be less severe.
In these dibenzyline premedicated animals no distinct secondary drop in blood flow was registrated after endotoxin. The flow remained in a steady statewith no significant increase or decrease from the level shortly after endotoxin injection.
The higher flow after endotoxin in this group as compared with controls was statistically significant for all moments of registration, with the exception of T = 0 and T = 50 min. after endotoxin injection.
During dibenzyline infusion the heart rate increased significantly and remained significantly higher after endotoxin injection.

Conclusion:

Dibenzyline premedication with 15 mgr/kg bodyweight prevented for a large part the severe drop in blood flow during the first, but also during the second phase of endotoxin shock.
This blood flow was maintained with a high heart rate.

C. 3-5-2 Trasicor medication

Objective: To be informed about the influence on survival and shock parameters of beta-blockade with trasicor, starting later on in the first, and directed to the second phase of shock, without premedicative alpha-blockade.

Materials and methods: Four paired experiments were done with endotoxin shock, applying 5 mgr/kg bodyweight endotoxin, batchnumber 582495-1.
1,5 Mgr/kg bodyweight trasicor was given in the treated group, infusion starting as medication 30 min. after endotoxin injection.
As in the preceding chapter, statistical comparisons were made of differences in mean flow at fixed moments between the trasicor medicated animals and a compiled group of 14 untreated dogs (Mann-Whitney-U test, one sidedly performed: alpha = 0,05). The trends and average values of the heart rates were also statistically analyzed.

Results:

1. Survival: In the treated group 3 out of 4 dogs survived >14 days and in the untreated group 2 out of 4 dogs.
   The difference in survival is not significant.

2. Blood pressure: Table 13 presents the values for average systolic blood pressures in the treated group, with S.D.
   Fig. 13 is the graphic illustration of these values.
   The S.B.P. drops from 166 mm Hg to 99 mm Hg at 10 min. after endotoxin injection.
   A secondary increase to 120 mm Hg at 50 min. is followed by a decrease to 82 mm Hg at 140 min.

3. Blood flow: Table 14 and Fig. 14 give the data. The flow in the treated dogs was lower from T = 15 min. after endotoxin injection, as compared with the untreated dogs, but differences were not statistically significant. It has to be taken into account that the treated group is small.
   The flow decreases from 78 ml/min. to 11 ml/min. at 15 min. after endotoxin injection. There is only a small secondary increase to a maximum level of 27 ml/min. at 50 min. At 130 min. the level is approximately the same with 26 ml/min.

4. Peripheral resistance: Table 15 and Fig. 15 give the data. The P.R. increases after endotoxin injection from 1,4 mm Hg/min./ml → 4,0 mm Hg/min./ml at 10 min., and remains at a high level of >2,7 mm Hg/min./ml.

5. Heart rates: The following average values with S.D. are relevant. At T =-120: 177 beats/min.(S.D.: 17); at T = 0:167 beats/min. (S.D.: 11); at T = 120: 109 beats/min. (S.D.: 9).
On statistical analysis in the T = 0 → T = 120 range no significant trend was found. At T = 0 no significant difference between the heart rate of treated and untreated dogs was found (C. 3-4). At T = 120 the heart rate of treated dogs was significantly lower than the heart rate of untreated ones.

6. Autopsy findings: At autopsy the non-surviving treated animal had no intestinal changes and a light pulmonary atelectasis. One of the non-surviving untreated animals showed severe, and the other light intestinal haemorrhagic lesions; no pulmonary changes were seen.

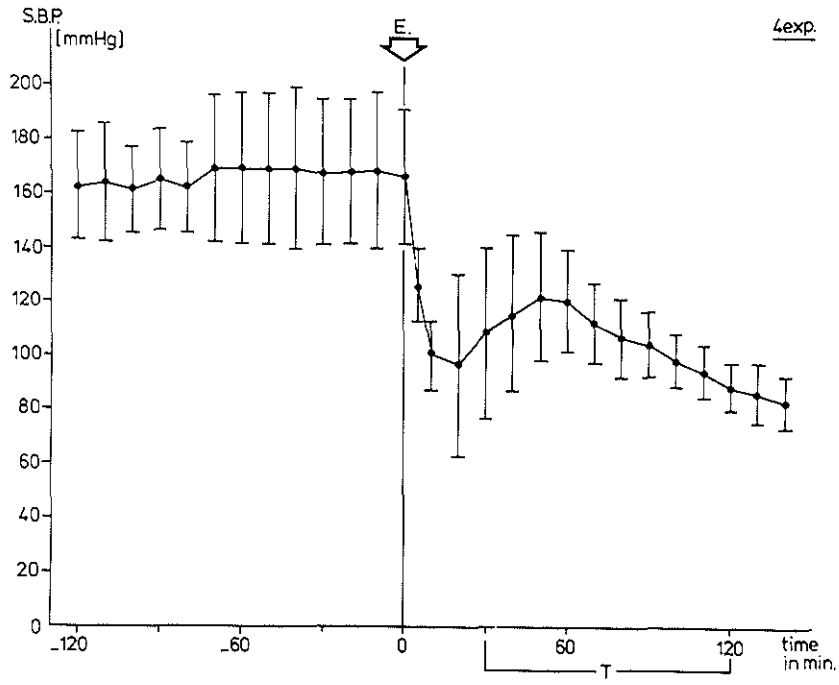

Fig. 13. Endotoxin shock (5 mgr/kg). Trasicor medication.
Systolic blood pressure ± S.D.

	PRE-ENDOTOXIN				POSTENDOTOXIN		
	Time in min.	S.B.P.	S.D.		Time in min.	S.B.P.	S.D.
	- 120	162,00	19,66	Endotoxin injection →	0	166,25	24,52
	- 110	163,25	21,89		5	126,00	13,44
	- 100	161,00	15,34		10	99,75	12,99
	- 90	164,25	18,78		15	99,75	12,99
	- 80	161,25	16,31		20	95,75	33,51
	- 70	168,50	26,40	Start trasicor →	30	108,00	31,14
	- 60	168,75	27,14		40	114,50	28,43
	- 50	168,75	27,26		50	120,50	23,47
	- 40	169,00	29,56		60	119,75	18,57
	- 30	167,25	26,27		70	111,25	14,66
	- 20	167,00	25,59		80	106,50	14,24
	- 10	168,00	28,01		90	103,25	11,95
Endotoxin injection →	0	166,25	24,52		100	97,75	9,74
					110	93,25	9,91
				End trasicor →	120	88,00	8,90
					130	85,50	10,24
					140	82,75	9,77

Table 13. Endotoxin shock (5 mgr/kg). Trasicor medication.
n = 4. S.B.P. in mm Hg.

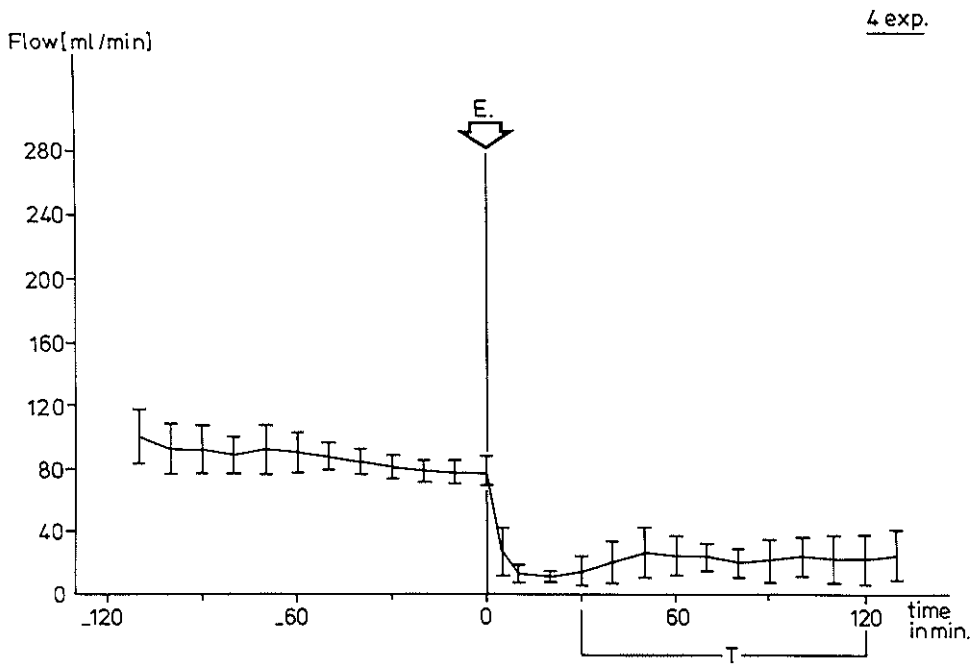

Fig. 14. Endotoxin shock (5 mgr/kg). Trasicor medication. Blood flow ± S.D.

	PRE-ENDOTOXIN Time in min.	Blood flow	S.D.		POSTENDOTOXIN Time in min.	Blood flow	S.D.
	- 110	90,84	16,46	Endotoxin injection →	0	78,95	9,36
	- 100	93,14	16,01		5	27,89	15,68
	- 90	92,78	14,89		10	13,78	4,63
	- 80	89,68	11,53		15	11,32	2,64
	- 70	92,97	14,38		20	11,32	2,64
	- 60	90,65	12,27	Start trasicor →	30	15,29	8,35
	- 50	88,50	8,87		40	21,56	14,00
	- 40	84,83	7,75		50	27,06	15,91
	- 30	81,98	6,69		60	25,39	12,61
	- 20	79,68	6,29		70	24,19	8,90
Endotoxin injection →	- 10	78,04	6,82		80	21,92	9,91
	0	78,95	9,36		90	23,67	13,16
					100	24,69	12,86
					110	23,21	14,60
				End trasicor →	120	23,40	15,00
					130	26,05	15,62

Table 14. Endotoxin shock (5 mgr/kg). Trasicor medication. n = 4. Blood flowin ml/min.

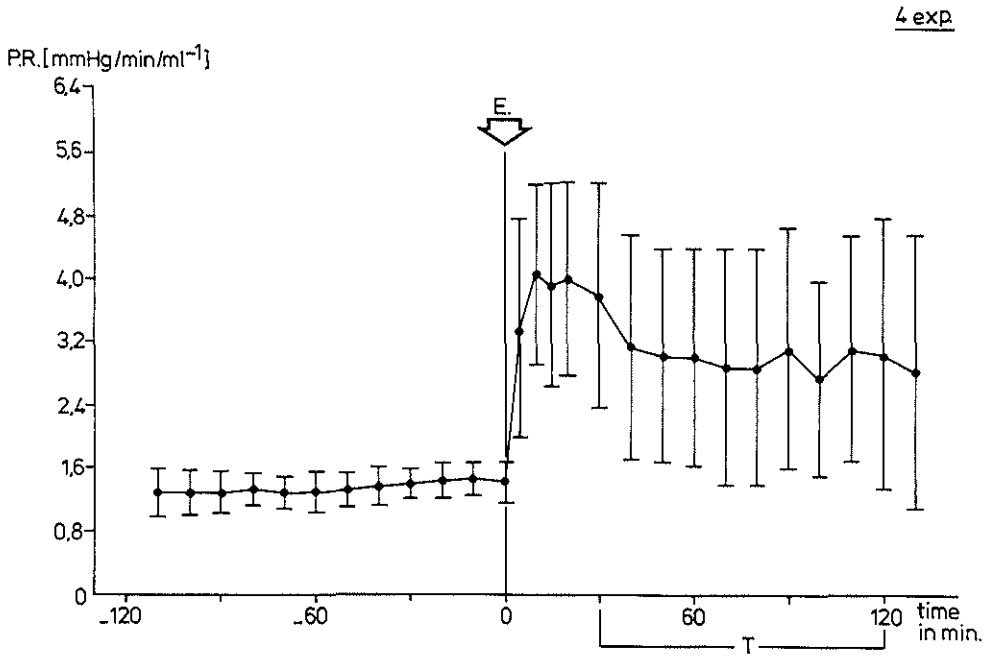

Fig. 15. Endotoxin shock (5 mgr/kg). Trasicor medication. Peripheral resistance ± S.D.

	PRE-ENDOTOXIN			POSTENDOTOXIN			
	Time in min.	P.R.	S.D.		Time in min.	P.R.	S.D.
	- 110	1,25	0,31	Endotoxin injection →	0	1,40	0,25
	- 100	1,25	0,28		5	3,37	1,37
	- 90	1,26	0,26		10	4,04	1,13
	- 80	1,29	0,20		15	3,89	1,29
	- 70	1,25	0,20		20	3,97	1,22
	- 60	1,27	0,25	Start trasicor →	30	3,77	1,43
	- 50	1,29	0,21		40	3,09	1,42
	- 40	1,35	0,24		50	3,00	1,35
	- 30	1,37	0,19		60	2,99	1,38
	- 20	1,41	0,23		70	2,87	1,49
Endotoxin injection →	- 10	1,44	0,20		80	2,85	1,49
	0	1,40	0,25		90	3,10	1,52
					100	2,70	1,23
				End trasicor →	110	3,08	1,42
					120	3,01	1,76
					130	3,22	2,54

Table 15. Endotoxin shock (5 mgr/kg). Trasicor medication. n = 4. P.R. in mm Hg/min./ml.

Discussion:

Differences in survival between trasicor treated and untreated animals are not statistically significant.
Blood pressure is not influenced by trasicor in the first, nor in the second shock phase; the curve has the same biphasic pattern as in animals with untreated endotoxin shock.
Trasicor medication, started later on in the first phase and directed to the second phase of shock does not prevent the severe blood flow decline , characteristic for cases without dibenzyline premedication.
The peripheral resistance increased after endotoxin injection and remained at a high level during trasicor medication.
This is different from the situation without trasicor in untreated endotoxin shock, where the peripheral resistance after the initial sharp increase immediately after endotoxin injection has a tendency to decrease again later on (C. 3-4).
Beta-blockade decreased heart rates during endotoxin shock, to a significantly lower level as compared with untreated cases.

Conclusion:

Beta-blockade "in itself" gave no favourable change of the shock pattern. One reason for this lack of beneficial influence of monophasic beta-blockade directed to the second phase seems to be that the peripheral resistance increases after endotoxin injection and remains high during trasicor infusion.

C. 3- 6 Combined simultaneous blockade (premedication)

Objective: To investigate combined adrenergic blockade in which dibenzyline and trasicor were not given biphasically, but at the same time as premedication.

Materials and methods: Ten paired experiments were done with endotoxin shock, applying 5 mgr/kg bodyweight endotoxin, batchnumbers 573093-2, 581374-1 and 581374-2. 15 Mgr/kg bodyweight dibenzyline and 1,5 mgr/kg bodyweight trasicor were given together as premedication in the treated groups.

Results:

1. Survival: In the treated group 4 out of 10 dogs survived >14 days. In the untreated group 1 dog died during intubation. Five out of the other 9 untreated dogs survived >14 days. Survival differences between treated and untreated animals were not statistically significant.
2. Systolic blood pressure: The data of the S.B.P. are given in Table 16 and Fig. 16. The S.B.P. decreases from 106 mm Hg → 57 mm Hg at 20 min. after endotoxin injection. At 60 min. the S.B.P. is 84 mm Hg and at 130 min. 62 mm Hg.
3. Blood flow: The data of the blood flow are given in Table 17 and Fig. 17. The blood flow has a low level at all moments after endotoxin injection, not surpassing 34 ml/min.
4. Peripheral resistance: The data of the P.R. are given in Table 18 and Fig. 18. The P.R. increases from 1,02 mm Hg/min./ml → 2,83 mm Hg/min./ml at 5 min. after endotoxin injection and remains thereafter at a higher level than before endotoxin injection.
5. Heart rates: The following average values with S.D. are relevant: At T = 0: 125 beats/min (S.D.: 26); at T = 120 : 124 beats/min. (S.D. : 25). Therefore, in the T = 0 → T = 120 min. range no trend was found.
6. Autopsy findings: Of the 4 non-surviving animals in the control-group, three had signs of haemorrhagic intestinal necrosis at

autopsy, twice rated as moderate and once as severe.
The animal without intestinal changes showed some atelectasis, the others had no pulmonary changes.
Three of the 6 non-surviving treated animals presented intestinal lesions at autopsy, twice moderate and once severe. In 4 cases, pulmonary oedema and/or atelectasis was seen, twice rated as severe.

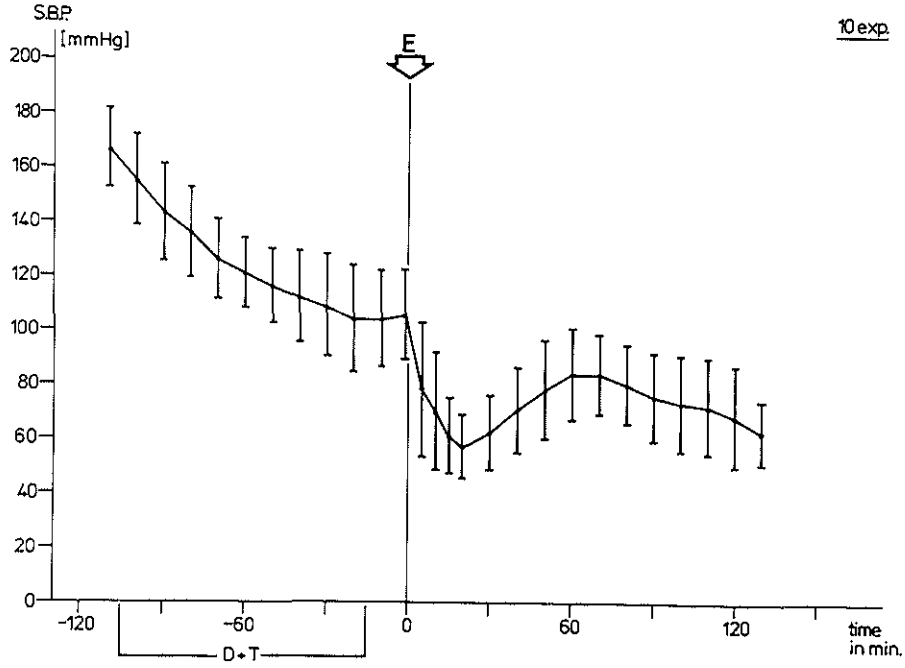

Fig. 16. Endotoxin shock (5 mgr/kg).
Combined simultaneous blockade (premedication).
Systolic blood pressure ± S.D.

	PRE-ENDOTOXIN				POSTENDOTOXIN		
	Time in min.	S.B.P.	S.D.		Time in min.	S.B.P.	S.D.
Start dibenzyline + trasicor →	- 110	167,50	14,30	Endotoxin injection →	0	106,30	16,33
	- 100	155,20	16,28		5	78,80	24,68
	- 90	143,80	17,53		10	76,50	21,40
	- 80	136,00	16,46		15	61,20	13,95
	- 70	126,70	14,65		20	57,50	11,61
	- 60	121,70	12,80		30	62,50	13,51
	- 50	116,60	13,50		40	70,70	15,69
	- 40	112,80	16,24		50	78,90	17,99
	- 30	109,80	18,94		60	84,30	16,64
End dibenzyline + trasicor →	- 20	104,50	19,85		70	84,30	14,07
	- 10	104,10	17,66		80	80,70	14,11
Endotoxin injection →	0	106,30	16,33		90	76,00	16,15
					100	73,90	17,59
					110	72,30	17,82
					120	69,10	18,86
					130	62,55	11,35

Table 16. Endotoxin shock (5 mgr/kg).
Combined simultaneous blockade (premedication).
n = 10. S.B.P. in mm Hg.

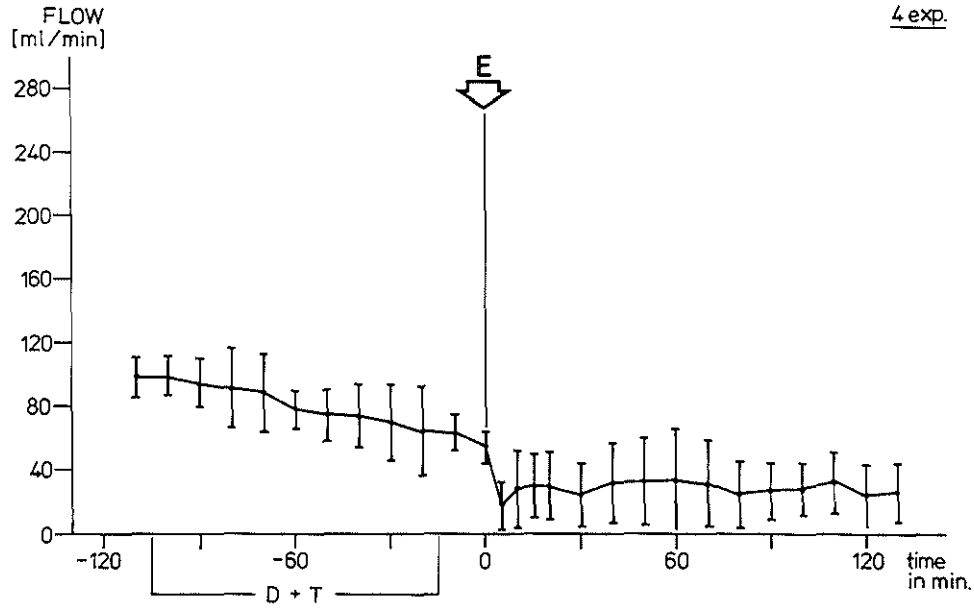

Fig. 17. Endotoxin shock (5 mgr/kg).
Combined simultaneous blockade (premedication).
Blood flow ± S.D.

PRE-ENDOTOXIN				POSTENDOTOXIN			
	Time in min.	Blood flow	S.D.		Time in min.	Blood flow	S.D.
Start dibenzyline + trasicor	- 110	98,10	7,32	Endotoxin injection	0	55,70	9,48
	- 100	99,30	7,04		5	17,83	14,40
	- 90	94,33	15,61		10	28,38	24,51
	- 80	91,34	25,42		15	30,48	19,38
	- 70	88,84	23,53		20	30,40	21,53
	- 60	77,86	16,06		30	25,20	19,48
	- 50	75,20	16,07		40	32,38	25,14
	- 40	74,64	19,43		50	33,94	26,45
	- 30	70,78	23,62		60	34,52	31,74
End dibenzyline + trasicor	- 20	64,94	18,57		70	32,30	26,74
	- 10	63,18	11,32		80	25,20	20,38
Endotoxin injection	0	55,70	9,48		90	27,04	17,69
					100	28,68	16,43
					110	22,82	18,54
					120	24,38	19,68

Table 17. Endotoxin shock (5 mgr/kg).
Combined simultaneous blockade (premedication).
n = 4. Blood flow in ml/min.

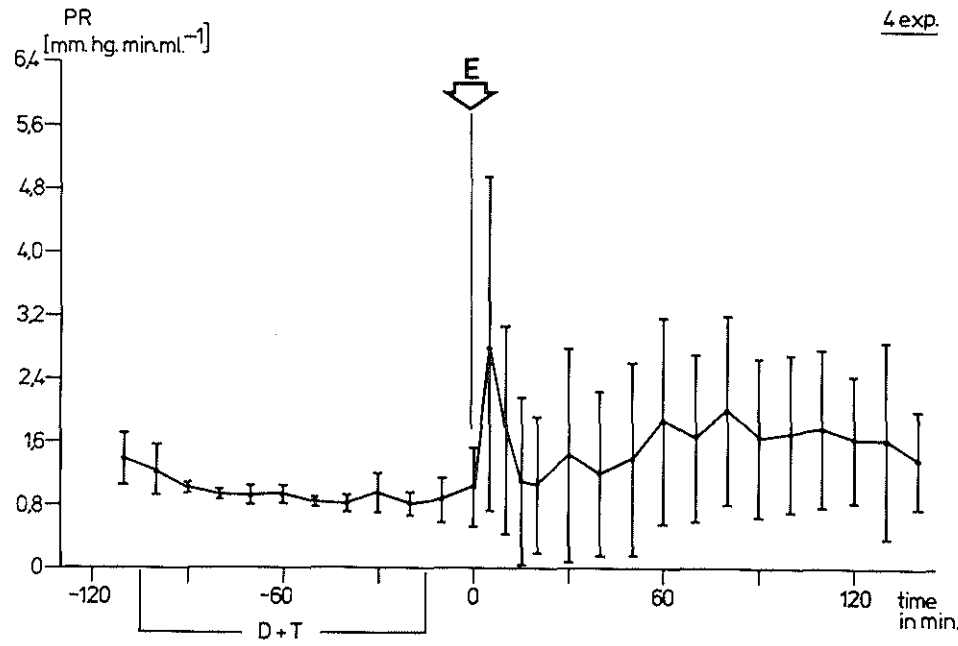

Fig. 18. Endotoxin shock (5 mgr/kg).
Combined simultaneous blockade (premedication).
Peripheral resistance ± S.D.

	PRE-ENDOTOXIN				POSTENDOTOXIN		
	Time in min.	P.R.	S.D.		Time in min.	P.R.	S.D.
Start dibenzyline + trasicor →	- 110	1,34	0,31	Endotoxin injection →	0	1,02	0,48
	- 100	1,23	0,32		5	2,83	2,18
	- 90	1,00	0,06		10	1,74	1,34
	- 80	0,91	0,05		15	1,10	1,07
	- 70	0,90	0,12		20	1,05	0,88
	- 60	0,92	0,11		30	1,53	1,31
	- 50	0,82	0,05		40	1,20	1,03
	- 40	0,81	0,12		50	1,38	1,33
	- 30	0,95	0,25		60	1,87	1,30
End dibenzyline + trasicor →	- 20	0,81	0,14		70	1,65	1,06
Endotoxin injection →	- 10	0,86	0,29		80	1,99	1,18
	0	1,02	0,48		90	1,65	0,98
					100	1,71	0,94
					110	1,65	0,91
					120	1,62	0,82

Table 18. Endotoxin shock (5 mgr/kg).
Combined simultaneous blockade (premedication).
n = 4. P.R. in mm Hg/min./ml.

Discussion:

Differences in survival are not statistically significant. It seems that batchnumber 581374-2 had a diminished lethality. In the part of the series in which this batch was used, the animals did survive the impact of the endotoxin when no treatment was given, but not anymore after complete adrenergic blockade as premedication.
This could mean that in the first phase of shock the animal "needs" only the alpha-blockade and is worse of when the blockade is complete, and that beta-adrenergic functions in the first phase still have a beneficial role.
On comparison of Fig. 10 (monophasic dibenzyline) with Fig. 16,it is seen that completing the blockade by addition of beta-blocking makes no difference in blood pressure decline.
Endotoxin leads to a small, temporary additional decrease of the systolic pressure in the first phase of shock, when no beta-blockade is added to the dibenzyline pretreatment (Fig. 10).
When beta-blockade is added to the pretreatment (Fig. 16), the blood pressure decrease in the first phase is more progressive.
The blood flow curve (Fig. 17) illustrates that the flow declines during simultaneous blockade pre-endotoxin, whereas it rises during infusion of solely dibenzyline (Fig. 11).
Endotoxin subsequently decreases the blood flow even more to a level far below that of animals pretreated with solely dibenzyline. The blood flow has no tendency to rise later on. The possible explanation is given in Fig. 18: the peripheral resistance, deducted from blood pressure and blood flow, appears to be increased after endotoxin in this group and is,at nearly all times, during the registrated shock periods above the P.R. in animals pretreated with solely dibenzyline (Fig. 12).
No further use of a statistical test for differences in the blood flow was considered necessary, because in these experiments the levels of the mean blood flow after endotoxin were at all times lower than these levels after premedication with solely dibenzyline (Fig. 11) and in the range of untreated animals (Fig. 8).

Conclusions:

Addition of trasicor to premedication with dibenzyline did not improve the shock pattern after endotoxin.
The first phase of shock is more severe than after pretreatment with solely dibenzyline.
This might indicate that the first phase is a pure alpha-adrenergic phenomenon, because shock parameters deteriorate further upon additional beta-blockade.

C. 3-7 Combined biphasic blockade

C. 3-7-1 Combined biphasic blockade 5 mgr/kg endotoxin

Objective: To investigate the influence of combined biphasic adrenergic blockade on survival and haemodynamics in endotoxin shock.

Materials and methods: The endotoxin dose was 5 mgr/kg bodyweight, batchnumbers used: 260253, 572818 and 581374-1. The biphasic blockade consisted of premedication with 15 mgr/kg bodyweight dibenzyline and medication after endotoxin injection with 1,5 mgr/kg bodyweight trasicor.

Twelve paired experiments (treated versus untreated animals) were performed. Some other experiments were non-paired. In total 18 animals received biphasic blockade and 16 animals were not treated. Blood pressure, -flow and peripheral resistance are presented in this chapter, as far as the experiments with biphasic blockade are concerned.

The data of the untreated group were combined with those of other untreated series to form a compiled control group (C. 3-4 and C. 3-7-3).Systolic blood pressure was measured in all experiments. In 9 experiments with biphasic blockade, mean values of systolic and diastolic blood pressures were registrated to compare mean blood pressure with systolic blood pressure. The trend of the systolic blood pressures of the 18 biphasically treated dogs was considered in the following intervals:

0 → 5 min.; 5 → 10 min.; 10 → 30 min.; 30 → 90 min. and 90 → 120 min. after endotoxin injection. Trends were analyzed with the Wilcoxon-test, the test in related samples and the Mann-Whitney-U test (55,56) with a significance level of alpha=0,05 .

These trends were also compared with the blood pressure trends in the compiled controlgroup of 25 untreated animals, described in chapter C. 3-4.

In 9 experiments with biphasic blockade, reliable blood flow curves and thus peripheral resistance curves were obtained. By means of the Mann-Whitney-U test it was checked at which moments after endotoxin injection, the mean flow in these experiments showed a significant difference from the mean flow in the 14 experiments

with endotoxin shock without treatment, described in chapter C. 3-4 (alpha=0,05).The test was one sidedly performe d against the alternative that the flow in untreated dogs is on the average smaller than the flow in the treated dogs (55,56).
Trends in the changes of the mean blood flow were analyzed, applying the same statistical tests as in the analysis of blood pressure trends (alpha= 0,05).
The heart rates of the biphasically treated and untreated animals were compared at T = 0 and T = 120 after endotoxin injection, applying the Mann-Whitney-U test (two sidedly performed), with a significance of alpha= 0,05).

Results:

1. Survival: In the treated group, 15 out of 18 dogs survived >14 days. In the untreated group, 4 out of 16 dogs survived >14 days:

	Number of dogs	Dibenzyline mgr/kg bw	Endotoxin mgr/kg bw	Trasicor mgr/kg bw	Survival/ death
Biphasic blockade	18	15	5	1,5	15 survived >14 days 3 died
Control-group	16	-	5	-	4 survived >14 days 12 died

Applying the Binomial test for the statistical analysis of the survival data in the paired experiments, the percentage of definitive survivors appears to be significantly higher in the treated group than in the untreated group, with a p-value <0,035.
Applying the Chi-square-test for the same statistical analysis of survival of all treated and untreated animals (paired as well as unpaired experiments), the differences in survival are significant with a p-value <0,0002.

2. Blood pressure: Calculations of average values of mean blood pressure and systolic blood pressure are given in Table 19 and 20. Fig. 19 and 20 are the graphic illustrations of the same. The average value of systolic blood pressures in the experimental animals of this group decreases from 176 mm Hg → 101 mm Hg, during dibenzyline infusion. In the first 5 min. after endotoxin injection, a further decrease to 85 mm Hg, followed by an increase to 95 mm Hg at 10 min. is seen. Both the decreasing and increasing trend proved statistically significant. From 10 → 30 min. no significant trend is found. The decrease in systolic blood pressure from 98 mm Hg at 30 min. after endotoxin injection to 73 mm Hg at 120 min. after endotoxin injection,also represents a significant trend. In comparison with the trends in blood pressure of the compiled controlgroup in chapter C. 3-4, it can be seen that the pressure in the biphasically treated dogs increases already significantly after 5 min., whereas in the untreated dogs the recovery of the blood pressure starts only at 10 min. after endotoxin.
Comparison of Fig. 19 and 20 shows that the average value of mean blood pressure also follows a biphasic pattern.

3. Blood flow: Table 21 gives the calculations of mean blood flow, which are graphically illustrated in Fig. 21.
The flow increased from 120 ml/min. → 180 ml/min., during dibenzyline infusion. Endotoxin injection gave a decrease from 175 ml/min. → 125 ml/min. at 5 min. after the injection, followed by an increase to 134 ml/min., at 10 min. after the injection. A further increase in flow to 151 ml/min. at the start of the trasicor infusion was followed by a decrease to 82 ml/min. at 70 min. and a later increase to 105 ml/min., at 140 min. after endotoxin injection.
Trends in mean blood flow: from 0 → 5 min. after endotoxin injection: S.D.T.; 5 → 10 min.: N.S.T.; 10 → 30 min.: N.S.T.; 30 → 70 min.:S.D.T. and 70 → 130 min.: N.S.T. (Fig.32.,p.111).
The statistical analysis of the differences in blood flow between these biphasically treated and untreated dogs, described in chapter C. 3-4, gave the following results:

At all moments after the injection of endotoxin, the flow in the treated dogs was significantly higher than in the untreated dogs.

4. Peripheral resistance: Table 22 gives the values of the peripheral resistance and Fig. 22 is the graphical illustration. The P.R. decreases under the influence of dibenzyline: from 1,27 mm Hg/min.ml → 0,25 mm Hg/min./ml.
   Endotoxin injection thereafter gives no increase (0,29 mm Hg/min./ml at 5 min. after endotoxin injection). The P.R. remains low during trasicor infusion.

5. Heart rates: The following average values with S.D. are relevant: At T = 0 : 229 beats/min (S.D. : 38); at T = 120 : 130 beats/min. (S.D. : 28).
   Applying the Mann-Whitney-U-Test, at T = 0 the heart rate in the biphasically treated group was found to be significantly higher than the heart rate in the untreated group (164 beats/min. (S.D. : 29). At T = 120 the heart rate in the treated group was significantly lower than the heart rate in the untreated group (156 beats/min (S.D.: 29).

6. Autopsy findings: All non-surviving animals in the untreated group showed at autopsy a more or less severe degree of the characteristic intestinal haemorrhagic necrosis. Three untreated animals had light pulmonary changes. In the non-surviving, biphasically treated animals, pulmonary oedema was the predominant feature at autopsy. None of these animals had haemorrhagic lesions in the intestines.

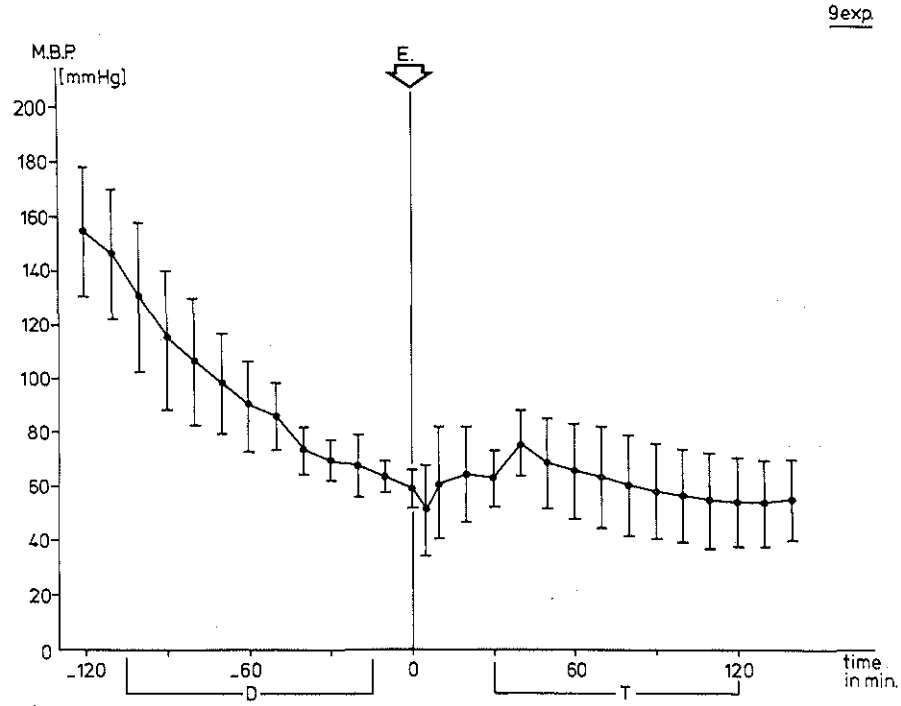

Fig. 19. Endotoxin shock (5 mgr/kg).Biphasic adrenergic blockade. Mean blood pressure ± S.D.

	PRE-ENDOTOXIN				POSTENDOTOXIN		
	Time in min.	M.B.P.	S.D.		Time in min.	M.B.P.	S.D.
Start dibenzyline →	- 120	153,66	23,04	Endotoxin injection →	0	58,66	7,00
	- 110	145,44	23,67		5	50,88	16,82
	- 100	129,11	27,03		10	60,77	20,27
	- 90	113,88	25,34		20	63,44	17,70
	- 80	105,88	23,87	Start trasicor →	30	62,66	10,02
	- 70	97,55	18,81		40	75,11	12,83
	- 60	89,88	16,22		50	67,77	16,96
	- 50	85,11	12,46		60	65,00	17,38
	- 40	72,88	8,23		70	62,66	18,94
	- 30	68,66	7,36		80	59,77	18,29
End dibenzyline →	- 20	67,00	11,67		90	57,88	17,94
	- 10	63,77	5,47		100	56,00	17,57
Endotoxin injection →	0	58,66	7,00	End trasicor →	110	54,33	17,05
					120	53,88	16,24

Table. 19. Endotoxin shock (5 mgr/kg).Biphasic adrenergic blockade. n = 9. M.B.P. in mm Hg.

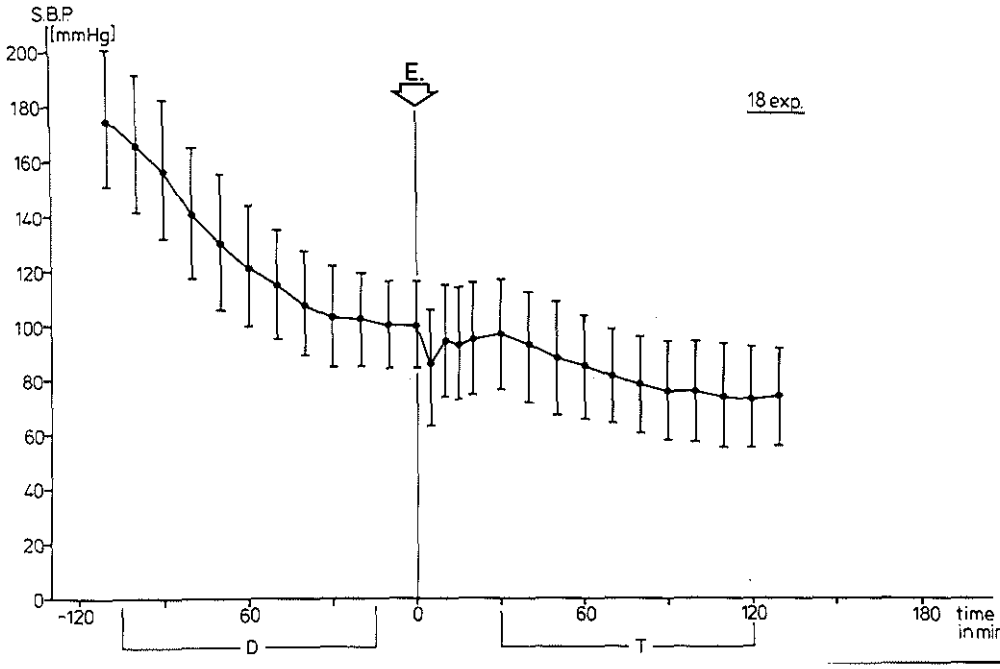

Fig. 20. Endotoxin shock (5 mgr/kg). Biphasic adrenergic blockade. Systolic blood pressure ± S.D.

	PRE-ENDOTOXIN				POSTENDOTOXIN		
	Time in min.	S.B.P.	S.D.		Time in min.	S.B.P.	S.D.
Start dibenzyline →	- 110	176,13	25,50	Endotoxin injection →	0	101,16	17,81
	- 100	169,56	30,51		5	85,48	22,68
	- 90	156,36	35,39		10	95,48	23,88
	- 80	141,28	29,27		15	94,52	20,80
	- 70	130,20	24,62		20	96,04	21,81
	- 60	121,48	20,25	Start trasicor →	30	98,88	20,45
	- 50	115,68	19,90		40	94,76	21,76
	- 40	109,24	19,66		50	89,44	21,13
	- 30	104,32	18,75		60	86,16	18,24
End dibenzyline →	- 20	103,32	17,64		70	82,00	17,32
	- 10	101,40	16,63		80	79,56	18,66
Endotoxin injection →	0	101,16	17,81		90	76,92	18,58
					100	76,28	19,11
					110	74,80	19,10
				End trasicor →	120	73,92	19,71
					130	74,32	18,88

Table 20. Endotoxin shock (5 mgr/kg). Biphasic adrenergic blockade. n = 18. S.B.P. in mm Hg.

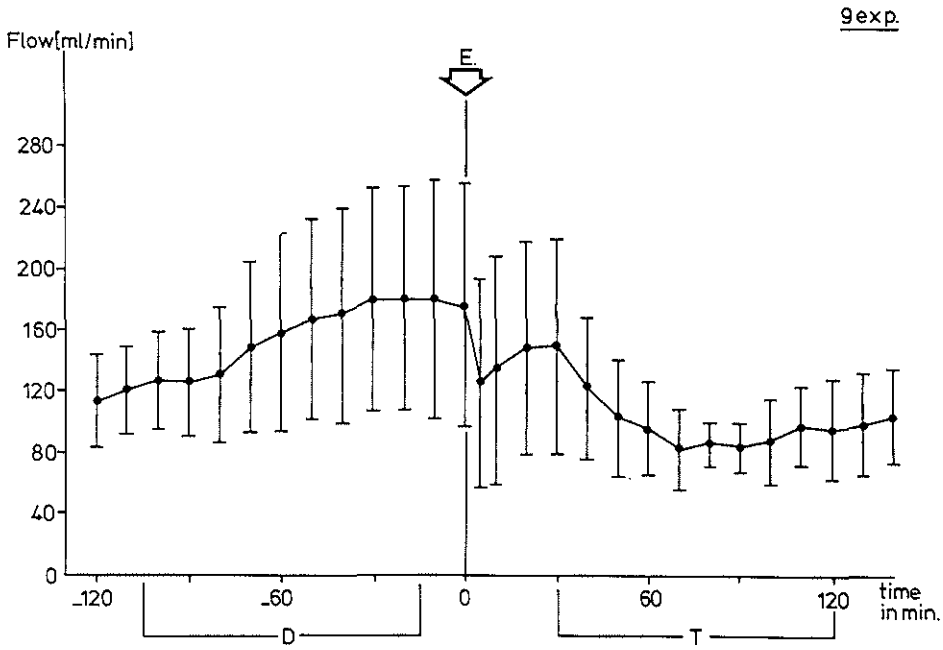

Fig. 21. Endotoxin shock (5 mgr/kg). Biphasic adrenergic blockade. Blood flow ± S.D.

	PRE-ENDOTOXIN				POSTENDOTOXIN		
	Time in min.	Blood flow	S.D.		Time in min.	Blood flow	S.D
Start dibenzyline →	- 120	113,00	29,94	Endotoxin injection →	0	175,00	79,54
	- 110	120,33	28,52		5	125,55	22,99
	- 100	126,44	32,72		10	134,66	25,88
	- 90	125,33	35,19		20	148,88	23,95
	- 80	130,11	44,36	Start trasicor →	30	151,44	23,02
	- 70	148,11	56,15		40	122,66	15,43
	- 60	158,44	65,20		50	103,33	12,59
	- 50	167,44	65,53		60	96,77	10,58
	- 40	169,44	71,46		70	82,44	8,46
	- 30	180,22	73,11		80	86,00	4,95
End dibenzyline →	- 20	181,33	73,74		90	85,33	5,46
	- 10	180,44	77,96		100	87,77	9,35
Endotoxin injection →	0	175,00	79,54		110	98,55	8,88
				End trasicor →	120	98,00	11,07
					130	99,66	11,03
					140	105,00	10,63

Table 21. Endotoxin shock (5 mgr/kg). Biphasic adrenergic blockade. n = 9. Blood flow in ml/min.

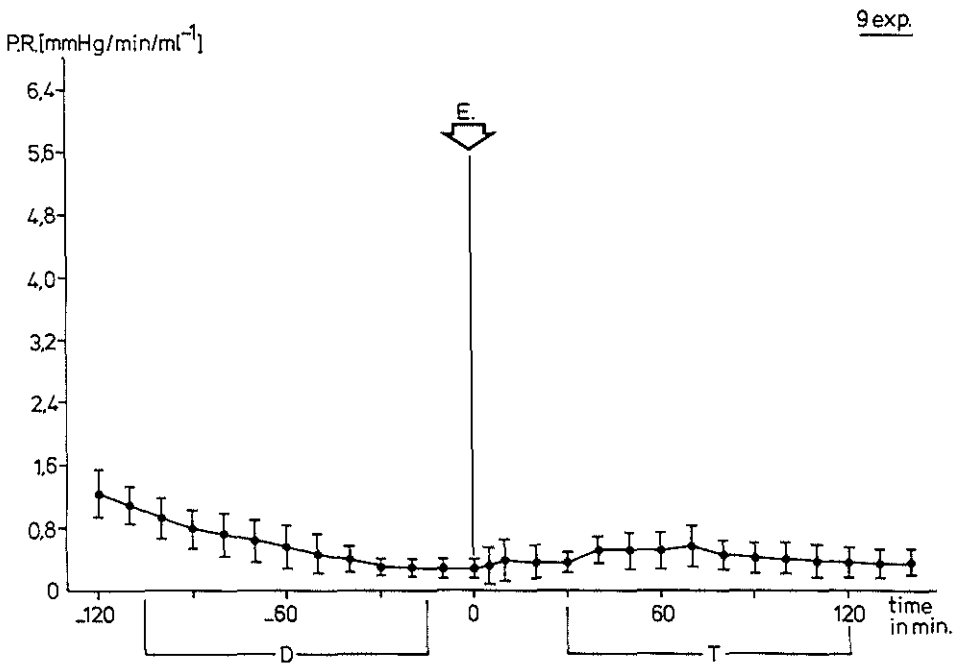

Fig. 22. Endotoxin shock (5 mgr/kg). Biphasic adrenergic blockade. Peripheral resistance ± S.D.

	PRE-ENDOTOXIN				POSTENDOTOXIN		
	Time in min.	P.R.	S.D.		Time in min.	P.R.	S.D.
Start dibenzyline →	- 120	1,27	0,29	Endotoxin injection →	0	0,25	0,11
	- 110	1,09	0,24		5	0,29	0,22
	- 100	0,95	0,26		10	0,37	0,27
	- 90	0,78	0,24		20	0,35	0,20
	- 80	0,69	0,28	Start trasicor →	30	0,33	0,15
	- 70	0,62	0,26		40	0,49	0,16
	- 60	0,53	0,27		50	0,51	0,21
	- 50	0,46	0,24		60	0,49	0,23
	- 40	0,37	0,17		70	0,55	0,26
	- 30	0,31	0,11		80	0,44	0,18
End dibenzyline →	- 20	0,29	0,10		90	0,43	0,19
Endotoxin injection →	- 10	0,27	0,10		100	0,40	0,20
	0	0,25	0,11		110	0,36	0,20
				End trasicor →	120	0,35	0,18
					130	0,34	0,18
					140	0,34	0,17

Table. 22. Endotoxin shock (5 mgr/kg). Biphasic adrenergic blockade. n = 9. P.R. in mm Hg/min./ml.

Discussion:

As stated in chapter C. 3-4 on endotoxin shock without treatment, 5 mgr/kg Endotoxin coli (0127:B8 Difco Laboratories, Detroit) proved not to be a L.D. 95%.

The question if biphasic adrenergic blockade improved survival in canine endotoxin shock,could therefore not be answered by comparison of the survival percentage after such a treatment with the 5 percent of animals, which supposedly could survive without such a treatment. It was therefore necessary to set up our own untreated control experiments.

Thus, statistical comparison of the percentage of survivors in the biphasically treated and untreated experiments showed that biphasic adrenergic blockade significantly improved survival.

Dibenzyline premedication led to a significantly earlier recovery of blood pressure after endotoxin injection, as compared with experiments without this premedication.

Although the blood flow after endotoxin injection in the treated animals also followed a biphasic pattern,it was at all times higher than in untreated animals and increased again in the second phase of shock.

One of the main reasons for the beneficial influence of dibenzyline is probably that, after dibenzyline premedication, endotoxin has virtually no influence on the peripheral resistance, which remains low throughout the period of measurement.

During dibenzyline infusion, the heart rate increases significantly. After addition of trasicor, the rate decreases again significantly.

Without addition of trasicor, as in the experiments with monophasic dibenzyline (C.3-5-1), the heart rate remains high.

With or without additional trasicor medication,the blood flow after dibenzyline premedication is significantly higher than in untreated cases. Therefore, the addition of trasicor leads to the maintenance of a high blood flow with a much lower heart rate and thus more effective heart action.

Conclusions:

Biphasic adrenergic blockade effectively improved survival.
With biphasic blockade, blood pressure recovered earlier in the first phase of shock.
Biphasic blockade resulted in a significantly higher blood flow at all moments after endotoxin injection.
This higher blood flow, apparently caused by the alpha-blockade, could be maintained with a much lower heart rate with additional beta-blockade.

C. 3-7-2 Combined biphasic blockade 6 and 7 mgr/kg endotoxin

Objective: To increase the endotoxin dose in an effort to work out a L.D. 95% endotoxin shock model. This objective resulted from foregoing experiments in which 5 mgr/kg endotoxin proved not to be a L.D. 95%, but in all control animals together a L.D. 60% (C. 3-8).

Materials and methods: Eight paired experiments were done with 6 mgr/kg bodyweight endotoxin, batchnumber used: 568487. Five paired experiments were done with 7 mgr/kg bodyweight endotoxin, batchnumber used: 573093-1.
In the treated animals, 15 mgr/kg bodyweight dibenzyline was given as premedication and 1,5 mgr/kg bodyweight trasicor postendotoxin as medication. The data on survival, blood pressure, -flow, peripheral resistance and heart rates of the experiments with 6 and 7 mgr/kg endotoxin were calculated together.
In all experiments systolic blood pressure was measured. Flow measurements were obtained in 5 experiments with biphasic blockade and 6 mgr/kg endotoxin and in 3 experiments with biphasic blockade and 7 mgr/kg endotoxin.
Average values of these data in the 6 and 7 mgr/kg groups were calculated separately as well as together.
It was checked whether the flow of 14 untreated dogs, injected with 5 mgr/kg endotoxin (C.3-4), showed a significant difference from the flow of 8 biphasically treated dogs, which were injected with 6 and 7 mgr/kg endotoxin.
The Mann-Whitney-U test was used to compare the blood flow in the two groups at the moments T= 0, 5, 10, 15 min. etc., after endotoxin injection. Survival differences were analyzed with the Binomial test.
Results:

1. Survival: In the 6 mgr/kg endotoxin experiments, 5 out of 8 dogs, treated with biphasic blockade and 2 out of 8 dogs without treatment survived >14 days.
   In the 7 mgr/kg endotoxin experiments, 3 out of 5 dogs, treated with biphasic blockade survived >14 days, but still 1 out of 5 dogs without treatment survived after this higher dose of

endotoxin:

	Number of dogs	Dibenzyline mgr/kg bw	Endotoxin mgr/kg bw	Trasicor mgr/kg bw	Survival >14 days
Biphasic blockade	8	15	6	1,5	5
Control-group	8	-	6	-	2
Biphasic blockade	5	15	7	1,5	3
Control-group	5	-	7	-	1

The amount of definitive survivors in the treated groups taken together (8 out of 13), is significantly higher in comparison with the untreated group (3 out of 13), with a p-value $<0,031$.

2. Blood pressure: Table 23 gives the average values of the systolic bloodpressures with S.D. in the untreated group and Table 24 for the same parameter in the group treated with biphasic blockade (both 13 experiments). Fig. 23 and 24 are the graphic illustrations of these average values.
In the untreated group the S.B.P. drops from 184 mm Hg → 79 mm Hg, after endotoxin injection. Here also the shock pattern was biphasic with an increase of the S.B.P. to 104 mm Hg,at 60 min, and a subsequent decrease to 76 mm Hg, at 140 min.
In the treated group the S.B.P. decreases from 98 mm Hg→ 75 mm Hg at 5 min. after endotoxin injection and increases again to 83 mm Hg at 10 min. after endotoxin injection.
The S.B.P. curve is comparable with the pattern after injection of 5 mgr/kg endotoxin (Fig.20).

3. Blood flow: The average values of the treated groups with S.D. are given in Table 25,26 and 27. Fig. 25,26 and 27 are the graphic illustrations of the same.
In the experiments with 6 mgr/kg endotoxin, the flow recovered between 5 and 10 min. after endotoxin injection. In the experiments with 7 mgr/kg endotoxin, it did not (anymore). The average values of both groups together do show a recovery in this phase.
The flow decreased in both groups after 30 min. and in the group with 7 mgr/kg endotoxin, it did already before trasicor. In the group with 6 mgr/kg endotoxin (and in the summation of the experiments with 6 and 7 mgr/kg endotoxin), the blood flow subsequently has a tendency to rise during and after the trasicor phase. In the experiments with 7 mgr/kg endotoxin, it has not. The flow in the treated animals is comparable with the pattern after 5 mgr/kg endotoxin (Table 21 and Fig. 21), but the average level is lower. For example, 5 min. after endotoxin injection, the mean flow with S.D. in the 6 mgr/kg group is below the values in the 5 mgr/kg group: the 6 mgr/kg flow is 56,38 ml/min. with S.D. 29,75 ml/min., the 5 mgr/kg flow is 125,55 ml/min. with S.D. of 22,90 ml/min.
At 130 min. after endotoxin injection, the values are 57,38 ml/min. with S.D. 11,88 ml/min. and 99,66 with S.D. 11,03 ml/min. respectively.
On statistical comparison of the flow in the treated dogs with the controlgroup (C. 3-4), it was found that at the moments T = 0, 40, 50, 60, 70, 80, 90 and 100, the difference in flow was not significant.
At the moments T = 5, 10, 15, 20 and 30 and also at the moments T = 110, 120 and 130, the flow was significantly higher in the treated dogs.

4. Peripheral resistance: The average values of the peripheral resistances of the 6 and 7 mgr/kg endotoxin group with biphasic blockade were not essentially different from the P.R.'s in the 5 mgr/kg endotoxin experiments with biphasic blockade and were for that reason calculated together with the 5 mgr/kg endotoxin group (Table 22 and Fig. 22). Results are given in

chapter C. 3-7-3.
In short, the P.R. in the 6 and 7 mgr/kg endotoxin experiments with biphasic blockade decreased from a level > 1,0 mm Hg/min./ml → <0,5 mm Hg/min./ml and after endotoxin injection never surpassed a level of 0,75 mm Hg/min./ml.

5. Heart rates: The following average values with S.D. are relevant: In the treated group at T = 0 : 220 beats/min (S.D.: 36); at T = 120: 118 beats/min. (S.D. : 22).
In the untreated group at T = 0 : 157 beats/min. (S.D : 16); at T = 120:140 beats/min. (S.D. : 33).
The heart rate at T = 0 in the treated group was found to be significantly higher than in the untreated group.
The difference was not significant at T = 120. Differences in heart rate between these experiments with biphasic blockade after 6 → 7 mgr/kg endotoxin and those after 5 mgr/kg endotoxin were not significant at T = 0, nor at T = 120 (C.3-7-1).

6. Autopsy findings: In the 6 mgr/kg experiments the autopsy findings in the 3 non-surviving treated animals were as follows: one had a light pulmonary oedema, one a severe pulmonary oedema with both showing very light intestinal changes. In the third autopsy there were no pulmonary changes, but light intestinal lesions were found.
Five out of the 6 non-surviving untreated animals had intestinal damage, rating from well recognizable to severe haemorrhagic necrosis. In the sixth case, the cause of death was an invagination.
Pulmonary changes occurred once. In the 7 mgr/kg experiments, three out of the 4 non-surviving non-treated animals showed a severe intestinal haemorrhagic necrosis, in the fourth case the haemorrhagic changes were rated light.
No pulmonary changes occurred. The 2 non-surviving treated animals presented a distinct pulmonary oedema and no gastrointestinal changes.

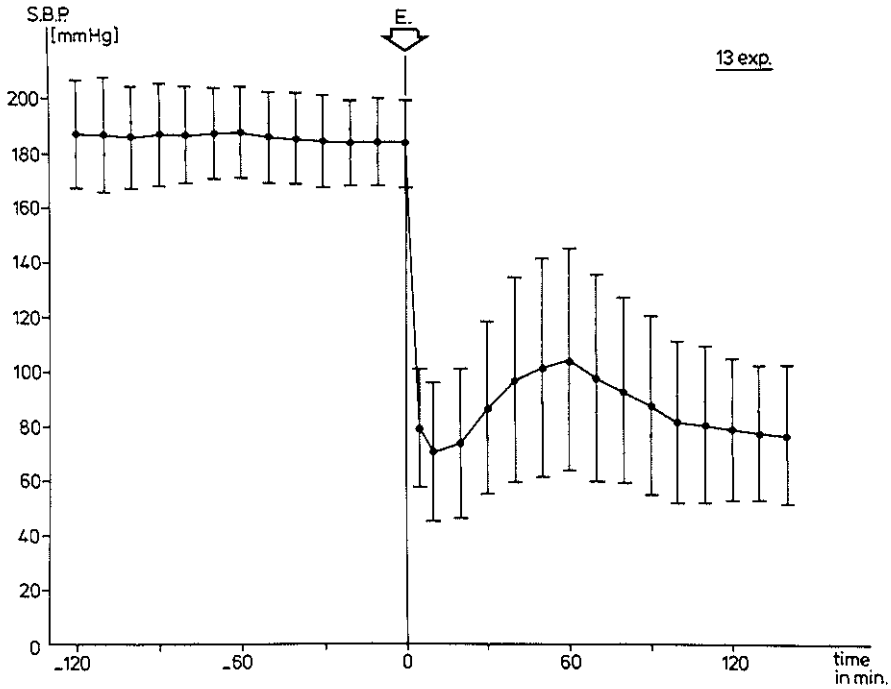

Fig. 23. Endotoxin shock (6→7 mgr/kg). No adrenergic blockade
Systolic blood pressure ± S.D.

	PRE-ENDOTOXIN				POSTENDOTOXIN		
	Time in min.	S.B.P.	S.D.		Time in min.	S.B.P.	S.D.
	- 120	187,00	19,09	Endotoxin injection →	0	184,00	15,86
	- 110	186,30	20,01		5	79,10	21,08
	- 100	185,90	18,58		10	70,20	25,35
	- 90	186,20	18,12		20	73,70	27,80
	- 80	186,60	17,51		30	86,20	31,53
	- 70	187,20	16,91		40	97,00	37,41
	- 60	187,10	16,76		50	101,20	39,82
	- 50	186,00	16,65		60	104,40	40,23
	- 40	185,50	16,40		70	97,40	37,43
	- 30	184,90	16,33		80	93,00	33,92
	- 20	184,00	15,93		90	87,20	32,79
Endotoxin injection →	- 10	184,30	15,77		100	81,60	29,12
	0	184,00	15,86		110	80,50	28,29
					120	78,50	26,00
					130	77,40	24,78
					140	76,60	25,13

Fig. 23. Endotoxin shock (6→7 mgr/kg).No adrenergic blockade.
n = 13 S.B.P. in mm Hg.

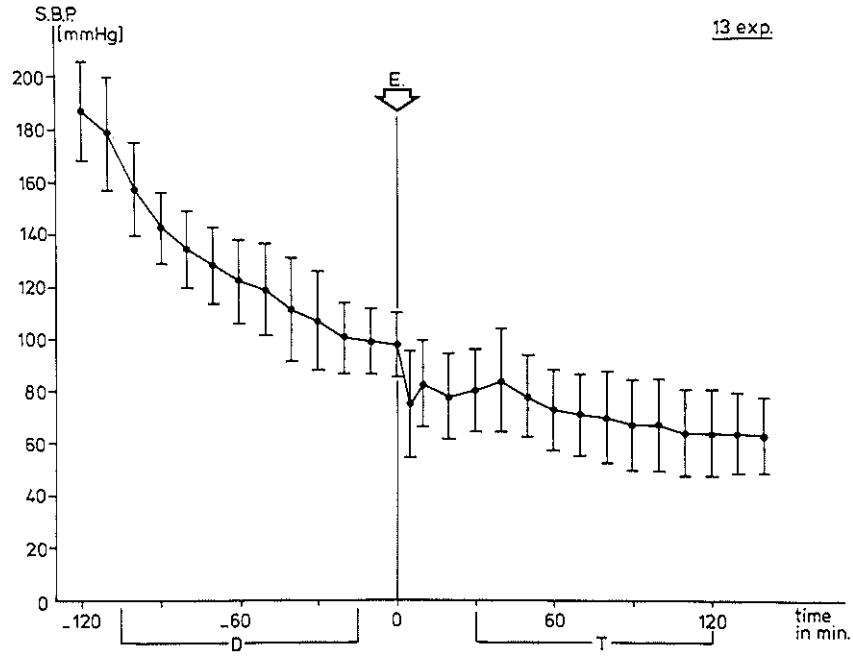

Fig. 24. Endotoxin shock (6→7 mgr/kg). Biphasic adrenergic blockade. Systolic blood pressure ± S.D.

	PRE-ENDOTOXIN				POSTENDOTOXIN			
	Time in min.	S.B.P.	S.D.			Time in min.	S.B.P.	S.D.
Start dibenzyline →	- 120	186,45	18,40	Endotoxin injection →	0	98,63	12,21
	- 110	178,36	21,38		5	75,36	20,63
	- 100	157,90	17,76		10	83,09	16,99
	- 90	142,81	13,21		20	78,90	16,81
	- 80	134,81	14,66	Start trasicor →	30	80,36	15,43
	- 70	128,63	14,27		40	84,54	19,75
	- 60	122,36	15,42		50	78,63	15,66
	- 50	119,09	17,14		60	73,81	15,04
	- 40	111,54	19,08		70	71,63	15,96
	- 30	107,27	18,54		80	70,18	17,61
End dibenzyline →	- 20	101,36	13,74		90	67,27	17,95
	- 10	100,00	12,41		100	67,36	17,08
Endotoxin injection →	0	98,63	12,21		110	64,90	16,84
				End trasicor →	120	64,36	16,74
					130	64,27	15,70
					140	63,27	14,96

Table 24. Endotoxin shock (6→7 mgr/kg). Biphasic adrenergic blockade. n = 13. S.B.P. in mm Hg.

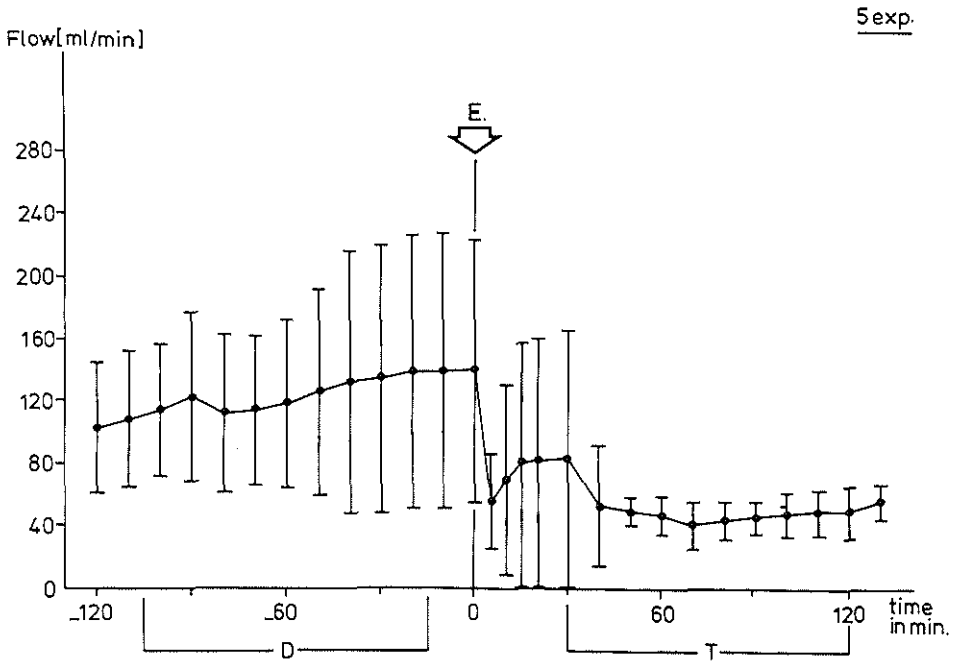

Fig. 25. Endotoxin shock (6 mgr/kg). Biphasic adrenergic blockade. Blood flow ± S.D.

	PRE-ENDOTOXIN Time in min.	Blood flow	S.D.		POSTENDOTOXIN Time in min.	Blood flow	S.D.
Start dibenzyline →	- 120	102,54	41,82	Endotoxin injection →	0	139,03	84,83
	- 110	108,89	43,51		5	56,38	29,75
	- 100	113,43	42,73		10	68,98	60,83
	- 90	122,05	54,05		15	79,54	82,60
	- 80	112,00	49,69		20	82,39	84,83
	- 70	114,88	47,19	Start trasicor →	30	83,21	83,90
	- 60	117,33	53,52		40	53,83	39,52
	- 50	126,26	66,36		50	49,99	8,67
	- 40	132,64	84,39		60	48,00	12,44
	- 30	133,41	85,94		70	41,72	14,95
End dibenzyline →	- 20	138,66	87,32		80	43,97	12,42
Endotoxin injection →	- 10	139,84	87,41		90	46,82	10,09
	0	139,03	84,83		100	47,64	13,19
					110	51,31	13,36
				End trasicor →	120	49,68	16,37
					130	57,38	11,88

Table 25. Endotoxin shock (6 mgr/kg). Biphasic adrenergic blockade. n = 5 . Blood flow in ml./min.

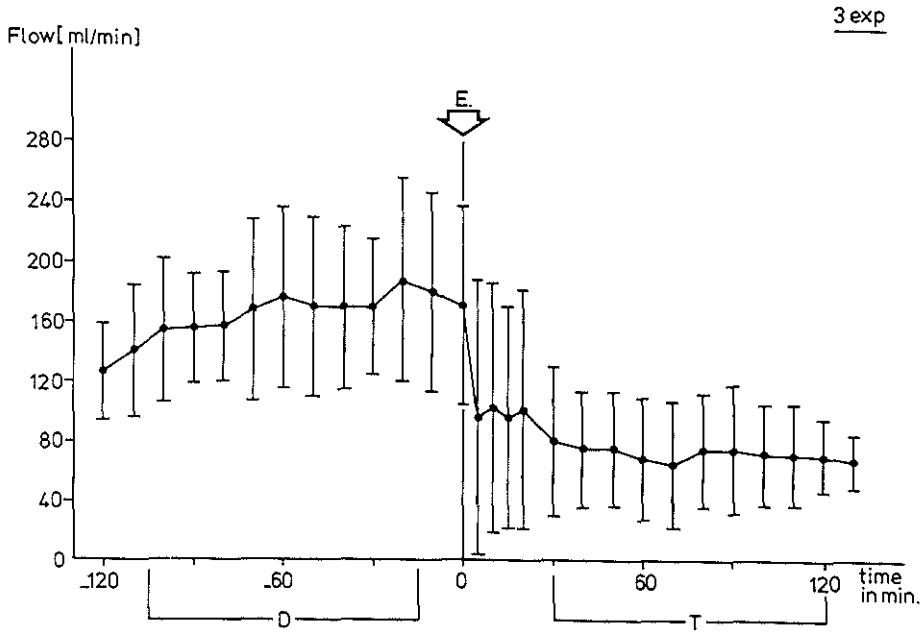

Fig. 26. Endotoxin shock (7 mgr/kg). Biphasic adrenergic blockade. Blood flow ± S.D.

	PRE-ENDOTOXIN				POSTENDOTOXIN		
	Time in min.	Blood flow	S.D.		Time in min.	Blood flow	S.D.
Start dibenzyline →	- 120	127,77	32,27	Endotoxin injection →	0	170,85	66,04
	- 110	140,04	44,28		5	96,96	92,83
	- 100	153,40	47,12		10	102,05	83,41
	- 90	155,56	35,09		15	95,25	78,20
	- 80	156,16	35,85		20	101,63	79,57
	- 70	167,01	59,03	Start trasicor →	30	79,05	49,11
	- 60	175,40	60,16		40	74,05	37,64
	- 50	170,32	60,06		50	75,49	37,69
	- 40	170,66	53,68		60	67,69	41,18
	- 30	170,58	44,56		70	63,37	42,95
End dibenzyline →	- 20	188,16	68,00		80	74,24	37,72
	- 10	179,50	66,98		90	74,04	43,07
Endotoxin injection →	0	170,85	66,04		100	71,77	35,94
					110	71,77	35,94
				End trasicor →	120	69,65	23,50
					130	65,92	17,55

Table 26. Endotoxin shock (7 mgr/kg). Biphasic adrenergic blockade. n = 3. Blood flow in ml/min.

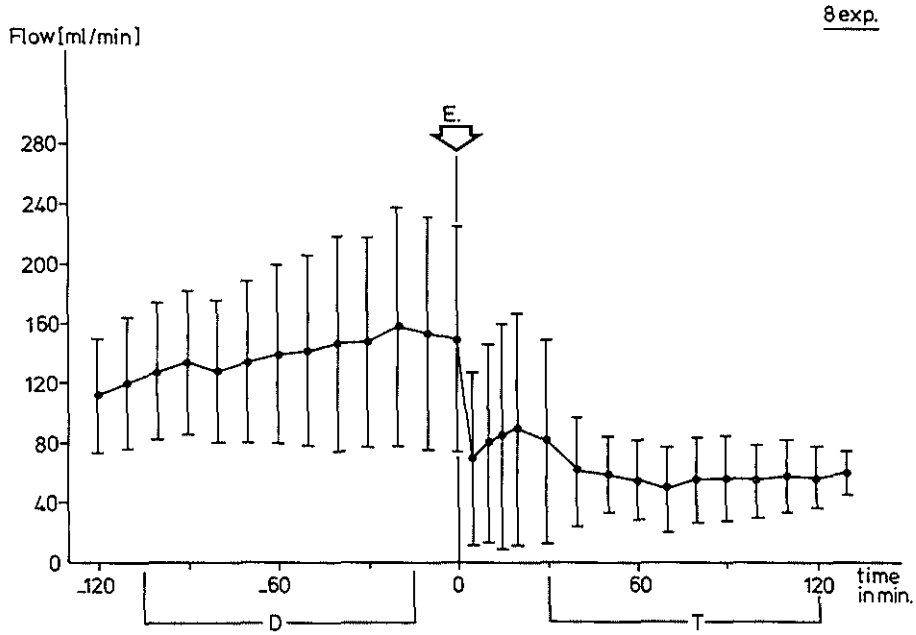

Fig. 27. Endotoxin shock (6→7 mgr/kg). Biphasic adrenergic blockade. Blood flow ± S.D.

	PRE-ENDOTOXIN Time in min.	Blood flow	S.D.		POSTENDOTOXIN Time in min.	Blood flow	S.D.
Start dibenzyline →	- 120	112,01	38,31	Endotoxin injection →	0	150,96	75,03
	- 110	120,57	43,61		5	71,52	58,40
	- 100	128,42	45,88		10	81,38	66,30
	- 90	134,62	48,19		15	85,43	75,57
	- 80	128,56	47,96		20	89,61	77,58
	- 70	143,43	54,74	Start trasicor →	30	81,65	68,67
	- 60	139,11	59,79		40	61,41	36,91
	- 50	142,78	63,77		50	59,55	24,96
	- 40	146,90	72,66		60	55,38	26,01
	- 30	147,35	71,82		70	49,84	27,93
End dibenzyline →	- 20	157,22	79,52		80	55,32	27,21
	- 10	154,72	77,90		90	57,04	28,05
Endotoxin injection →	0	150,96	75,03		100	56,69	24,99
					110	58,98	24,15
				End trasicor →	120	57,17	20,44
					130	60,58	13,72

Table 27. Endotoxin shock (6→7 mgr/kg). Biphasic adrenergic blockade. n = 8. Blood flow in ml/min.

Discussion:

In the 6 and 7 mgr/kg endotoxin experiments the significant improvement of survival with biphasic adrenergic blockade was confirmed.
The increased doses of 6 and 7 mgr/kg endotoxin did still not represent a L.D. 95%, but a L.D. 77%.
The biphasic pattern of blood pressure in endotoxin shock is confirmed also in these experiments with a higher dose of endotoxin.
Biphasically treated dogs with 6 or 7 mgr/kg endotoxin had a significantly higher blood flow in comparison with untreated 5 mgr/kg endotoxin cases, only in the range of T = 0 → T = 30 min. after endotoxin injection (first phase of shock) and T = 110 → T = 130 min. (well into the second phase), but not in between.
With 5 mgr/kg endotoxin (C. 3-7-1), the flow in the treated cases was at all moments after endotoxin injection significantly higher.
The heart rates in the treated cases were not essentially different from those in experiments with 5 mgr/kg endotoxin.

Conclusion:

It can be concluded that the increased dosages of endotoxin had a stronger impact on the blood flow, but they still did not represent a L.D. 95%.

C. 3-7-3 Combined biphasic blockade (compiled data)
5 → 7 mgr/kg endotoxin

Objectives: To analyse the compiled data on survival, blood pressure, -flow and heart rates in the experiments with all three doses of endotoxin.
To evaluate the blood gas measurements in these experiments and to make a systematic analysis of the autopsy findings.

Materials and methods: Survival: The blood pressure increases or decreases in the T = 5 → 10 min. interval have been investigated with regard to its prognostic value for survival. This time interval was chosen, because it had proved to be a characteristic range in the shock parameters.
A similar analysis was not made for the blood flow, because here the subgroups of survivors and non-survivors with reliable flow curves were not large enough.

Blood pressure: Trends in pressure changes for the whole group of 31 experiments with biphasic blockade and 5 → 7 mgr/kg endotoxin were analyzed and compared with those trends in the untreated controlgroup, injected with 5 mgr/kg endotoxin, applying the Wilcoxon matched-pairs signed rank test, the test against trends in related samples and the Mann-Whitney-U-test with alpha=0,05. Because the turning points and the nature of the trends (increasing or decreasing) were similar in the biphasic blockade experiments with 5,6 or 7 mgr/kg endotoxin, a dose differentiation was not necessary in studying these trends.

Blood flow and peripheral resistance: In 17 experiments of the whole group of biphasic blockade with 5 → 7 mgr/kg endotoxin, the blood flow and P.R. were calculated together and compared with those parameters in the untreated controlgroup, injected with 5 mgr/kg endotoxin (C. 3-4). Peripheral resistances were also calculated in these experiments. The trends of the flow changes were investigated for the same groups of experiments in the range of 0 → 130 min. after endotoxin injection (Wilcoxon-test, test against trends in related samples and Mann-Whitney-U-test). The tests were two sidedly performed

with alpha= 0,05. Here also, because of the similarity of the turning points and the nature of the blood flow trends after the three different doses of endotoxin, a dose differentiation could be omitted in studying these trends.

Heart rates: The trend of the heart rates was analyzed with the test against trend in related samples, two sidedly performed at a significance level of alpha= 0,05. As with the blood pressure and -flow trends, it was not necessary to distinguish the differences in endotoxin dosages here.

Blood gas analysis: Differences between treated and untreated dogs and survivors and non-survivors in pH- and HCO_3^- values at T = 130 after endotoxin at the end of the experiment, were analyzed with the Mann-Whitney-U test (two sidedly performed) with alpha= 0,05 (standard normal distribution test statistic is Z.). Because no significant difference was found for various doses endotoxin, a dosage differentiation could be omitted.

Autopsy findings: Macroscopic and microscopic autopsy findings of all non-surviving animals were rated, according to the criteria given in chapter C. 3-2 and subjected to a statistical test, based on an equidistant score attribution from 0 → 4, in which 0 = normal (Yates Cochran test, two sidedly performed, alpha = 0,05). The data made it acceptable not to differentiate in endotoxin dosage.

Results:

1. Survival:

	Number of dogs	Dibenzyline mgr/kg bw	Endotoxin mgr/kg bw	Trasicor mgr/kg bw	Survival/ death
Biphasic blockade	31	15	5 → 7	1,5	23 survived >14 days 8 died
Control-group	29	-	5 → 7	-	7 survived >14 days 22 died

The percentage of surviving treated animals in the combined group is significantly higher than the percentage of untreated survivors with a p-value <0,001 (Binomial test).

Survival prediction: At alpha = 0,05, no significant differences in blood pressure increase or decrease were found in the T = 5 → 10 min. interval between the survivors and non-survivors, both in the treated and untreated group.

2. Blood pressure: The average values of the systolic blood pressure in this combined group with biphasic blockade decreased from 99 mm Hg at 0 min. → 80 mm Hg at 5 min after endotoxin injection. It increased to 89 mm Hg at 10 min., remained at this level up till 30 min. after endotoxin injection and decreased again to 68 mm Hg at 120 min. after endotoxin injection.

Trends of the blood pressure changes: from 0 → 5 min. after endotoxin injection: S.D.T.; 5 → 10 min.: S.I.T.; 10 → 30 min.: N.S.T., and 30 → 120 min. after endotoxin injection: S.D.T. Fig. 28 gives the graphic illustration and comparison with the untreated group, described in chapter C. 3-4.

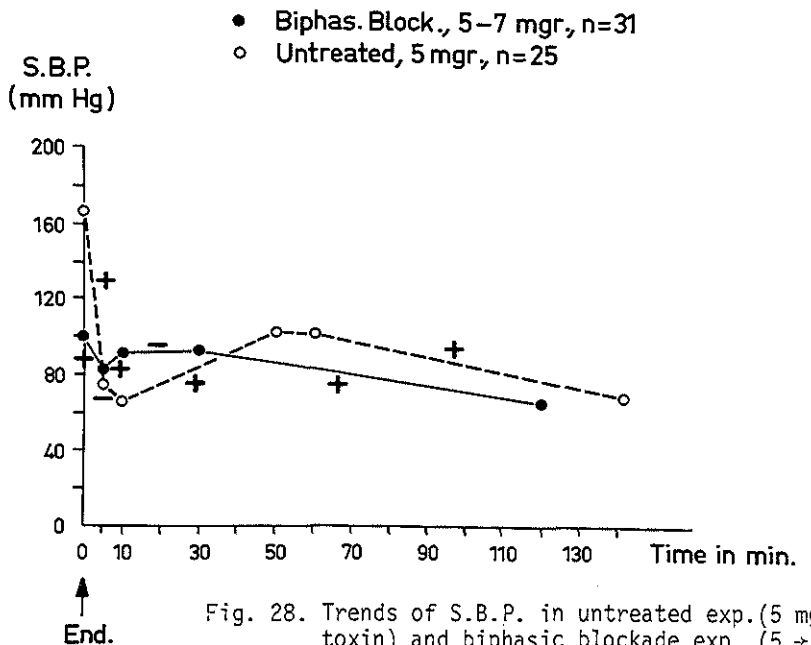

Fig. 28. Trends of S.B.P. in untreated exp.(5 mgr/kg endotoxin) and biphasic blockade exp. (5 → 7 mgr/kg endotoxin).

3. Blood flow: Table 28 gives the calculations of the mean blood flow in this combined group of animals treated with biphasic blockades. Fig. 29 (p. 109) is the graphic illustration. Fig. 30 (p. 110) represents the mean blood flow with S.D. of the biphasic blockade group of 5 → 7 mgr/kg and the untreated 5 mgr/kg group together (C. 3-4), in order to visualize the difference of this parameter in endotoxin shock with biphasic treatment and endotoxin shock without treatment. Finally, Fig. 31 (p. 110) gives the graphic illustration of the compiled data on mean blood flow after endotoxin in various groups studied (C. 3-4 up till C. 3-7-3),to be able to compare the effects of various dosages and treatments on mean blood flow. The statistical comparisons of these data are described in the various chapters.

Trends of the blood flow changes: from 0 → 5 min. after endotoxin: S.D.T.; 5 → 10 min.: S.I.T.; 10 → 30 min.: N.S.T.; 30 → 70 min.: S.D.T. and 70 → 130 min. after endotoxin injection: S.I.T. These results are illustrated in Fig. 32 (p.111).

Statistical comparison of these data with the untreated control-group led to the following conclusions:

1. After endotoxin injection the flow drops immediately in the treated as well as in the untreated dogs.
   In all cases the flow decrease in the 0 → 5 min. period is larger in the untreated dogs.
2. In most cases the recovery (≈ flow increase) in the biphasically treated dogs already sets in after 5 min.
   In untreated dogs a recovery only takes place in the period of 10 → 50 min.
3. The recovery of blood flow in the 5 → 10 min. period after endotoxin is significantly greater in the group of treated dogs than the flow increase during this interval in the group of untreated dogs.
4. In the 70 → 130 min. period after endotoxin, blood flow in untreated 5 mgr/kg experiments had a significantly decreasing trend. In the same period biphasically treated animals

with 5 → 7 mgr/kg endotoxin had a significantly increasing trend in blood flow.

4. Peripheral resistance: The calculations of the peripheral resistances in this combined group are given in Table 29. Fig. 34 (p. 112) is the graphic illustration of these data. Fig. 33 (p. 111) represents the P.R. of the biphasic blockade group (5 → 7 mgr/kg) and the untreated 5 mgr/kg (C. 3-4) together, in order to visualize the difference of this parameter in endotoxin shock with biphasic treatment and without treatment.

5. Heart rates: With biphasic blockade, in the range of T = -120 → T = 0 min.,a significantly increasing trend in heart rate was found; in the range of T = 0 → T = 120 min. the trend was significantly decreasing.In untreated experiments the trend from T = 0 → T = 120 was significantly increasing.

6. Blood gas analysis (Astrup method): In blood samples of 29 animals without treatment and of 31 animals with biphasic adrenergic blockade,pH- and HCO_3^- values were measured at various moments during the experiments.

   The data concern 22 non-survivors and 7 survivors in the untreated group and 8 non-survivors and 23 survivors in the treated group:

   a. Untreated dogs, 5 → 7 mgr/kg endotoxin.

      Both pH- and HCO_3^- values in the group of 22 non-survivors are significantly lower than in the group of 7 survivors (pH: Z = - 3,43; HCO_3^-: Z = - 3,31).

   b. Biphasically treated dogs, 5 → 7 mgr/kg endotoxin.

      The pH-value in the group of 8 non-survivors is significantly lower than in the group of 23 survivors.
      For the variable HCO_3^-, no significant difference was found here
      (pH: Z = - 2,47; HCO_3^-: Z = - 0,64).

In view of these results, survivors and non-survivors have been separated in the following groups:

a. Non-surviving dogs, 5 → 7 mgr/kg endotoxin.

No significant difference was found for both pH- and HCO_3^- values between the group of 22 untreated dogs and the group of 8 biphasically treated dogs.
(pH: Z = - 1,02; HCO_3^-: Z = 1,38).

b. Surviving dogs, 5 → 7 mgr/kg endotoxin.

No significant difference was found here either for both pH- and HCO_3^- values between the groups of 7 untreated dogs and 23 biphasically treated dogs.
(pH: Z = - 0,13; HCO_3^- : Z = - 0,73).

7. Autopsy findings: Macroscopic and microscopic findings were rated according to the criteria, described in chapter C. 3-2.
Macroscopically,the characteristic haemorrhagic necrosis of the bowel mucosa was severe in untreated dogs and mostly absent in biphasically treated dogs.
Intestines and mesenterial bloodvessels usually were contracted and pale in untreated cases.
The mucosal haemorrhagic necrosis was mostly severe in duodenum and small intestines and less severe in large bowel and rectum.
Pulmonary oedema was found in a number of treated dogs.
Microscopically, it proved impossible to differentiate the lesions of the intestinal mucosa in shock effects and autolysis.

Statistical analysis:

a. M a c r o s c o p i c findings:

The amount of untreated animals in which the lesions of the intestinal mucosa were rated as severe, was significantly higher than in the biphasically treated animals, the amount of biphasically treated animals in which the degree of pulmonary oedema was rated as severe, was significantly higher than in the untreated animals.

b. M i c r o s c o p i c findings:
The difference in microscopic changes in lungs, small bowel, colon and kidneys between treated and untreated dogs were not statistically significant.

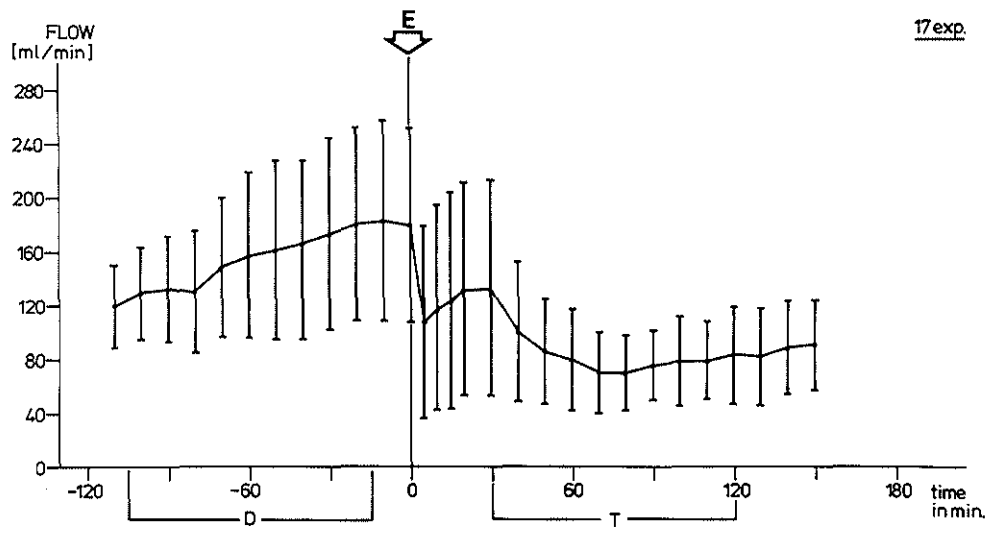

Fig. 29. Endotoxin shock (5→7 mgr/kg). Biphasic adrenergic blockade. Blood flow ± S.D.

	PRE-ENDOTOXIN Time in min.	Blood flow	S.D.		POSTENDOTOXIN Time in min.	Blood flow	S.D.
Start dibenzyline →	- 110	117,22	30,18	Endotoxin injection →	0	117,85	72,94
	- 100	124,88	34,31		5	105,81	70,63
	- 90	130,69	40,87		10	116,37	75,83
	- 80	129,91	45,02		15	122,18	79,39
	- 70	146,71	51,92		20	130,68	78,94
	- 60	155,07	61,63	Start trasicor →	30	130,94	79,14
	- 50	159,04	66,21		40	98,67	52,72
	- 40	165,04	70,84		50	84,76	40,57
	- 30	172,52	71,97		60	79,85	37,45
End dibenzyline →	- 20	179,00	72,69		70	69,09	30,95
	- 10	181,10	74,00		80	69,24	28,52
Endotoxin injection →	0	177,85	72,94		90	73,78	26,40
					100	76,45	33,06
					110	77,95	28,59
				End trasicor →	120	81,86	35,63
					130	81,70	35,96
					140	87,00	33,05
					150	89,40	34,72

Table. 28. Endotoxin shock (5→7 mgr/kg). Biphasic adrenergic blockade. n = 17. Blood flow in ml/min.

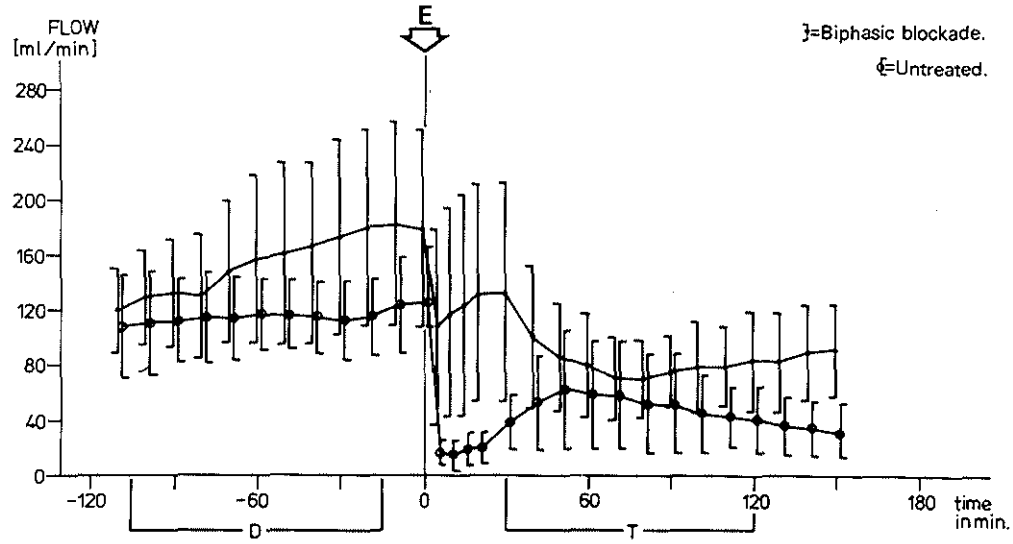

Fig. 30. Mean blood flow differences between biphasic blockade exp. (5 → 7 mgr/kg endotoxin) and untreated exp. (5 mgr/kg endotoxin).

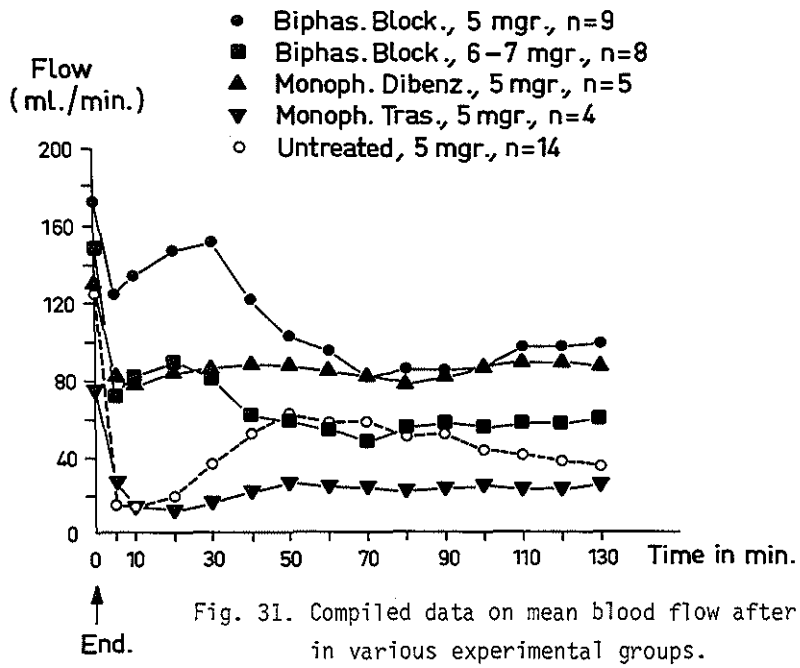

Fig. 31. Compiled data on mean blood flow after endotoxin in various experimental groups.

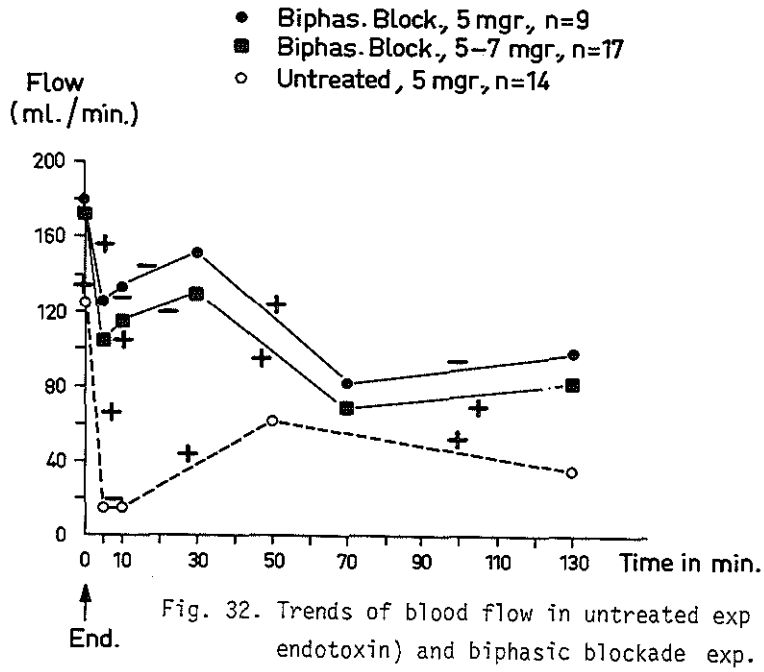

Fig. 32. Trends of blood flow in untreated exp (5 mgr/kg endotoxin) and biphasic blockade exp. (5 and 5 → 7 mgr/kg endotoxin).

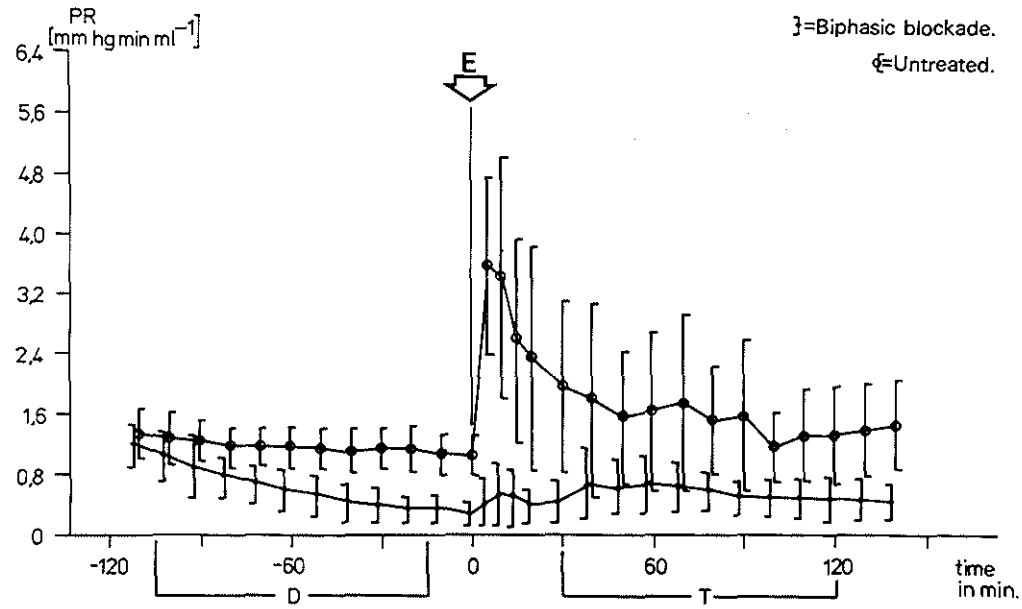

Fig. 33. Peripheral resistance differences between biphasic blockade exp. (5 → 7 mgr/kg endotoxin) and untreated exp. (5 mgr/kg endotoxin) ± S.D.

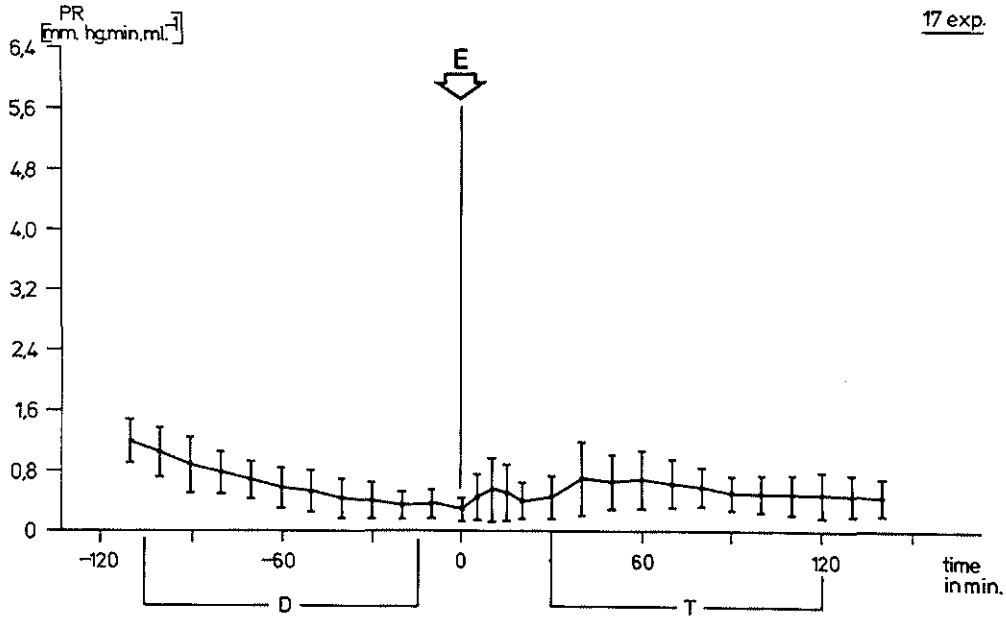

Fig. 34.Endotoxin shock (5→7 mgr/kg).Biphasic adrenergic blockade. Peripheral resistance ± S.D.

	PRE-ENDOTOXIN Time in min.	P.R.	S.D.		POSTENDOTOXIN Time in min.	P.R.	S.D.
Start dibenzyline →	- 110	1.15	0,28	Endotoxin injection →	0	0,29	0,16
	- 100	1,02	0,32		5	0,42	0,30
	- 90	0,86	0,27		10	0,52	0,41
	- 80	0,74	0,28		20	0,46	0,37
	- 70	0,65	0,24	Start trasicor →	30	0,39	0,24
	- 60	0,56	0,26		40	0,42	0,26
	- 50	0,51	0,28		50	0,69	0,47
	- 40	0,43	0,26		60	0,65	0,36
	- 30	0,37	0,24		70	0,65	0,38
End dibenzyline →	- 20	0,33	0,18		80	0,62	0,33
Endotoxin injection →	- 10	0,33	0,17		90	0,55	0,26
	0	0,29	0,16		100	0,48	0,21
					110	0,47	0,25
				End trasicor →	120	0,45	0,25
					130	0,46	0,31
					140	0,43	0,27
					150	0,42	0,24

Table 29.Endotoxin shock (5→7 mgr/kg).Biphasic adrenergic blockade. n = 17. P.R. in mm Hg/min./ml.

Discussion:

In the experiments with biphasic blockade, the turning points and the nature of the blood pressure and flow trends (increasing or decreasing) are similar with those of the experiments with injection of different endotoxin dosages.
It seemed therefore reasonable not to distinguish between the different dosages of endotoxin in studying these trends.
Fig. 30 (p. 110) suggests that the trend of the blood flow in the second phase is upwards in the treated cases and downwards in the untreated cases for this compiled group.
These trends are confirmed in the statistical analysis (Fig. 32, p. 111). The values of blood flow and peripheral resistance are inversely related.
Changes of heart rates in the combined group gave no new information. The values of pH and HCO_3^- at 120 min. after endotoxin did not correlate with the treatment but with survival: pH-values were low in non-surviving animals whether or not the animals had received an adrenergic blockade and significantly higher in surviving animals, also in treated and untreated cases alike.
A dose differentiation could be omitted in this analysis also, because no significant difference was found for various doses of endotoxin. The same applies for the statistical analysis of the autopsy findings; the data made it acceptable not to differentiate in endotoxin dosages.

Conclusions:

The compiled data on survival, blood pressure, -flow and peripheral resistance led to the same conclusions as in the preceding chapters.
The survival increase with biphasic blockade in this combined group was significant with a p-value <0,001. The severity of acidosis in late shock proved significantly linked with survival prognosis.
Blood pressure changes shortly after endotoxin did not correlate with survival prognosis.Biphasic blockade prevented mostly the haemorrhagic intestinal lesions, characteristic for endotoxin shock.

C. 3-8 Potency of endotoxin

Objective: To analyse the survival times of untreated animals which received endotoxin in a dose of 5 → 7 mgr/kg bodyweight in relation to the batches of endotoxin used.

Introduction: In the main study in which Beagles were used as experimental animal, a number of untreated control animals survived the endotoxin shock >14 days.
In the pilot study, in which mongrel dogs were used, none of the experimental animals survived the shock procedure.
This difference could have been due to the different dog model used in the main study, to differences in potency of the several endotoxin batches used, or to a decline of potency during storage time of the different batches.
Interpretation of the eventual efficacy of therapy in treated animals was therefore hampered.
It was thus necessary to investigate the lethality of the various batches and dosages of endotoxin used.

Materials and methods: In total 9 batches were used. The survival data of 48 Beagles,which reveived no anti-adrenergic treatment, were compared.

Results:

In Table 30 the survival data are given for the animals receiving 5, 6 and 7 mgr/kg endotoxin.

Batchnumbers:	Total	Number of dogs Surviving	Non-surviving
	5 Mgr/kg bodyweight endotoxin		
260253	4	2	2
581374-1	6	0	6
581374-2	6	6	0
582495-1	5	2	3
582495-2	1	0	1
573093-2	5	2	3
572818	8	2	6
	6 Mgr/kg bodyweight endotoxin		
568487	7	2	5
581374-2	1	0	1
	7 Mgr/kg bodyweight endotoxin		
573093-1	4	1	3
581374-2	1	0	1

Table 30. Survival data in the groups with different dosages endotoxin.

Differences of potency of different batches are clear. In only one case, batchnumber 5813742, 6 out of 6 dogs survived 5 mgr/kg bodyweight endotoxin injection.
When other batches were used, only a few animals survived the endotoxin shock procedure.
On summation, 5 mgr/kg endotoxin appears to be a L.D. 60% and the 5,6 and 7 mgr/kg endotoxin doses together represented a L.D. 64%.

Discussion:

Information in literature (18,106) indicated 5 mgr/kg Endotoxin coli, codenumber 0127:B8, Difco Laboratories, Detroit, as a L.D. 95%. These data were not confirmed in our studies.
The non-lethal outcome of these control experiments could be explained by a resistance in the experimental animal, by an unintended change in the methodology or by a diminished lethality of the endotoxin batch used.
Most likely, the batches of endotoxin were variable in lethality. As a logical consequence, higher dose levels were used, specifically 6 and 7 mgr/kg. Later experiments were done on a paired basis: treated and untreated animals at the same time, using the same batch of endotoxin,in an effort to eliminate batch differences.
Our results implicate that the generally accepted canine shock model, in which 5 mgr/kg endotoxin is considered to be a L.D. 95%, should be looked at with considerable scepsis.
It appeared that even higher doses of endotoxin (6 and 7 mgr/kg bodyweight) did not result in death of all the untreated animals.

Conclusions:

5 Mgr/kg Endotoxin coli 0127:B8 from different batches appeared not to be a L.D. 95%, but a L.D. 60% in our experimental set up.

C. 3-9 Extended registrations

Objective: To investigate the trends of blood pressure, -flow and peripheral resistance later on in the second phase.

Introduction: The experimental set up in which haemodynamic parameters were registrated up till 2½ hrs. after the injection of endotoxin was based on considerations as described in chapter C. 3-2.
Two hours after endotoxin administration the shock pattern was usually in a steady state.
Swan (103) proved that haemodynamic parameters in late shock only changed in the last 15 min. before death of the experimental animal. The small number of experiments described in this chapter were performed to check whether in our experimental model any changes in the trend of blood pressure and -flow occurred later on in the second phase of shock.

Materials and methods: Two paired experiments were done with 6 mgr/kg and 7 mgr/kg endotoxin resp.
The treated animals received biphasic blockade with 15 mgr/kg dibenzyline and 1,5 mgr/kg trasicor.
Registration of shock parameters was continued for 5½ hrs. after endotoxin.

Results:

Fig. 35,36,37,38,39,40,41 and 42 give the data (p. 120 up till 123).

1. Experiments with 6 mgr/kg endotoxin: the S.B.P. in the treated animal (Fig. 35, p. 120) shows an upward course later on in the second phase. The same parameter in the untreated animal shows a steady decline in the second phase of shock Fig. 36, p. 120). A comparison of the blood flow in these experiments (Fig. 37, p. 121) shows a steady course in the later part of the second phase in the experiment with adrenergic blockade: the blood flow 5 hrs. after endotoxin (43 ml/min.) is roughly 2/3 of the flow at the start of the experiment (69 ml/min). The untreated animal has a low flow state in the second phase with some further decline: the blood flow values 5 hrs. after

endotoxin (4,4 ml/min) is here approximately 1/20 of the flow at start (85 ml/min).
The peripheral resistances (Fig. 38, p. 121) showed a steady state at 5 hrs. after endotoxin.

2. Experiments with 7 mgr/kg endotoxin: The S.B.P. in the treated and untreated experiments alike does not change essentially in the later phase of shock (Fig. 39,p.122 and Fig. 40,p. 122).
The same applies for the flow in these experiments (Fig. 41, p. 123):the flow 5 hrs. after endotoxin in the experiment with biphasic blockade (39 ml/min) is between 1/3 and 1/2 of the flow at the start of the experiment (90 ml/min).
In the untreated animals the value 5 hrs. after endotoxin (5 ml/min) is approximately 1/14 of the flow at start (71 ml/min).
The peripheral resistances(Fig. 42,p. 123) do not change anymore in the later phase of endotoxin shock.

Discussion and conclusions:

Trends of blood pressure, -flow and peripheral resistance values did not change significantly later on in the second phase of shock up till 5½ hrs. after endotoxin injection.
Blood flow in the treated cases remained higher than in the untreated cases.

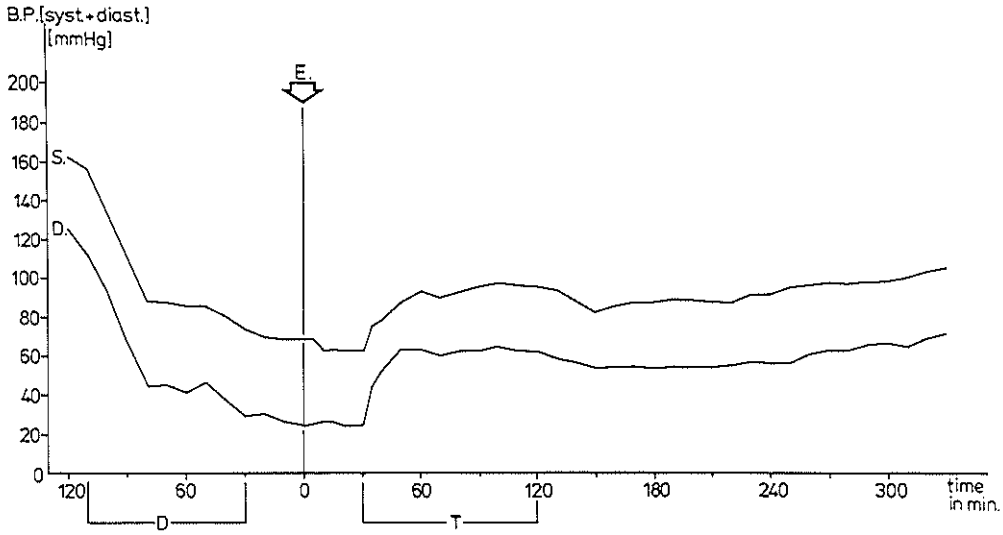

Fig. 35. Endotoxin 6 mgr/kg. Biphasic adrenergic blockade.
Systolic and diastolic blood pressure.

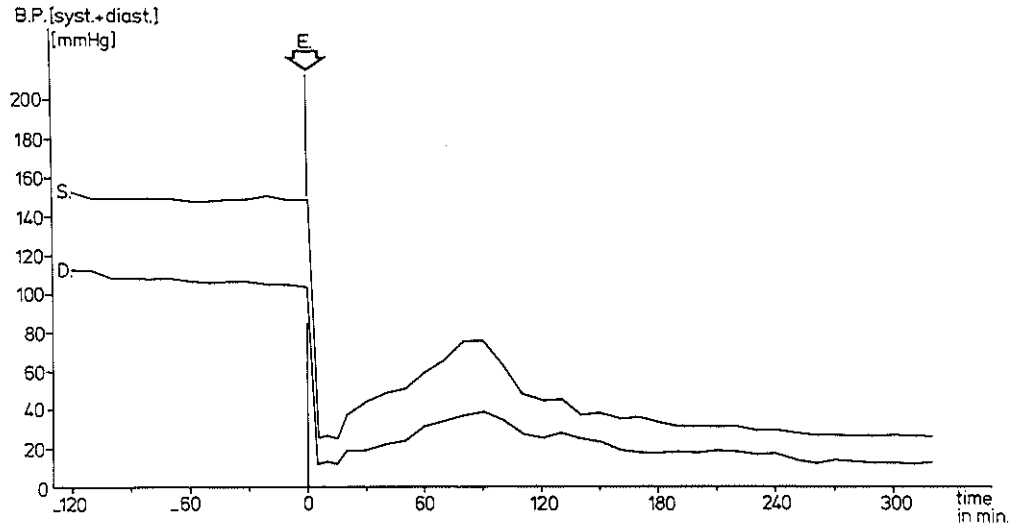

Fig. 36. Endotoxin 6 mgr/kg. No adrenergic blockade.
Systolic and diastolic blood pressure.

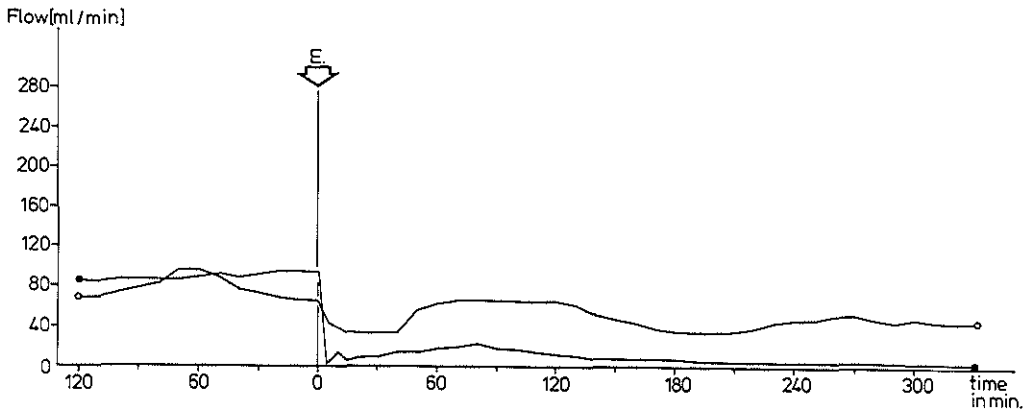

Fig. 37. o = Biphasic adrenergic blockade. Endotoxin 6 mgr/kg.
● = No adrenergic blockade. Endotoxin 5 mgr/kg.
Mean blood flow.

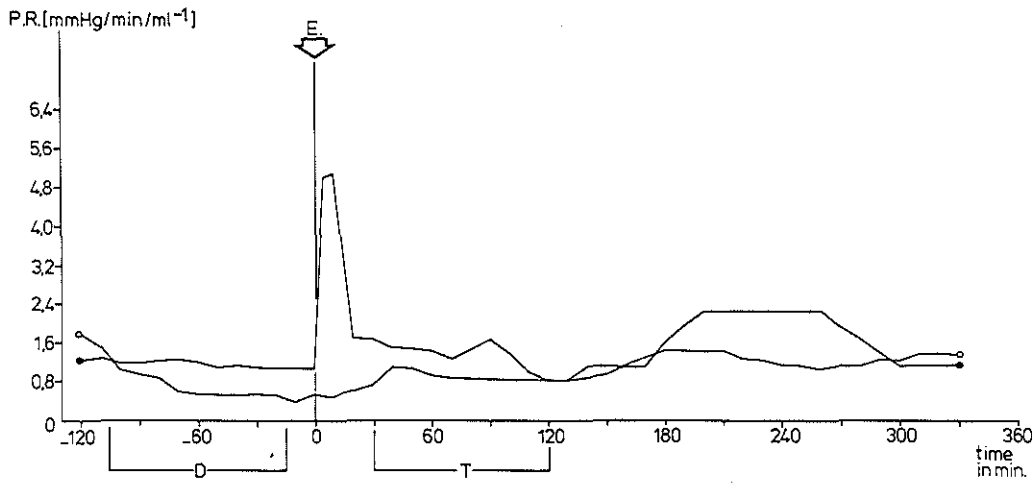

Fig. 38. o = Biphasic adrenergic blockade.Endotoxin 6 mgr/kg.
● = No adrenergic blockade. Endotoxin 6 mgr/kg.
Peripheral resistance.

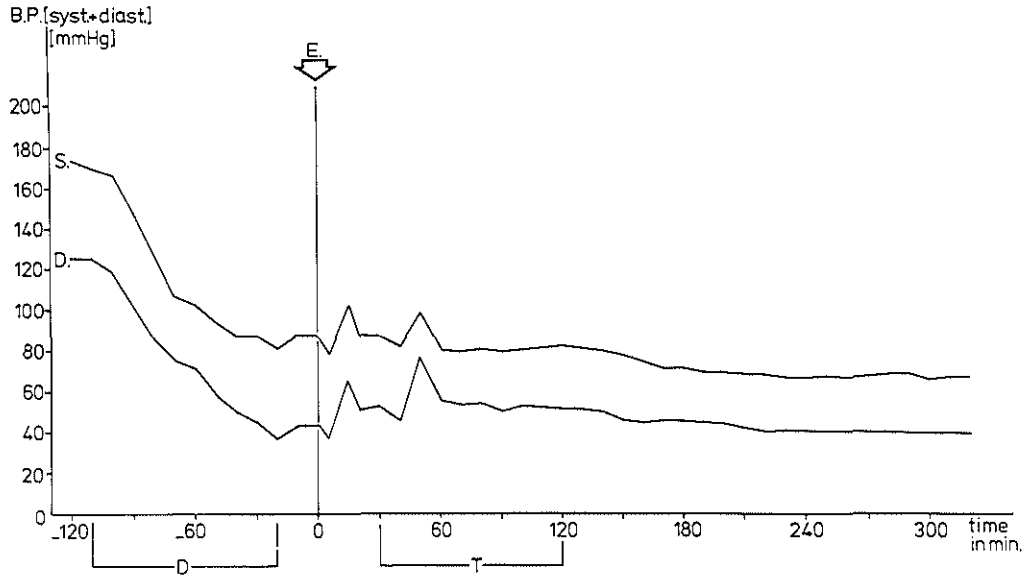

Fig. 39. Endotoxin 7 mgr/kg. Biphasic adrenergic blockade.
Systolic and diastolic blood pressure.

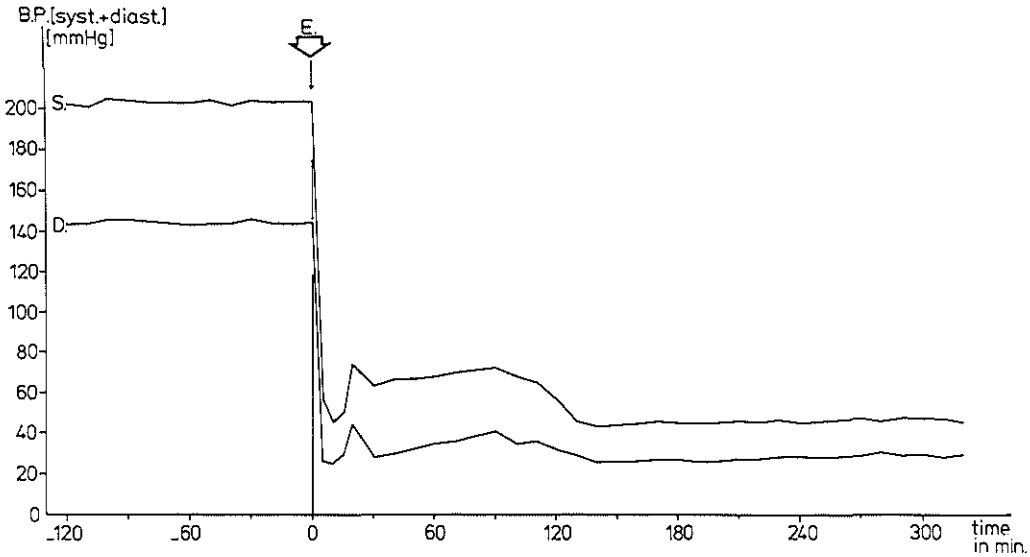

Fig. 40. Endotoxin 7 mgr/kg. No adrenergic blockade.
Systolic and diastolic blood pressure.

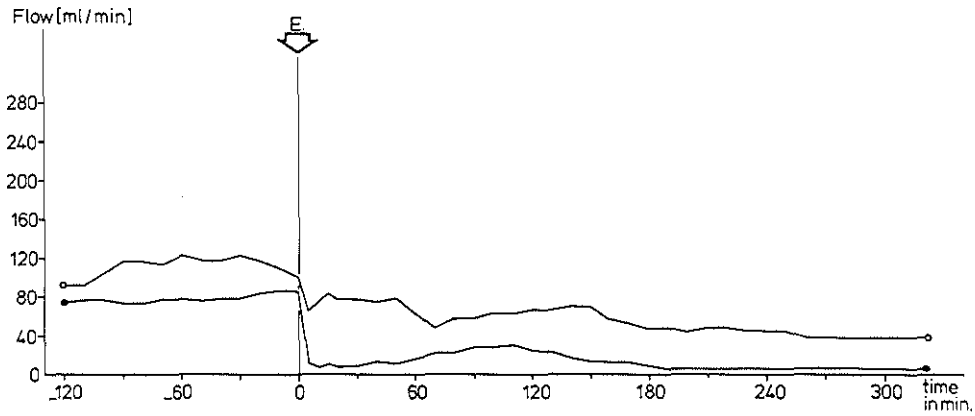

Fig. 41. o = Biphasic adrenergic blockade. Endotoxin 7 mgr/kg.
● = No adrenergic blockade. Endotoxin 7 mgr/kg.
Mean blood flow.

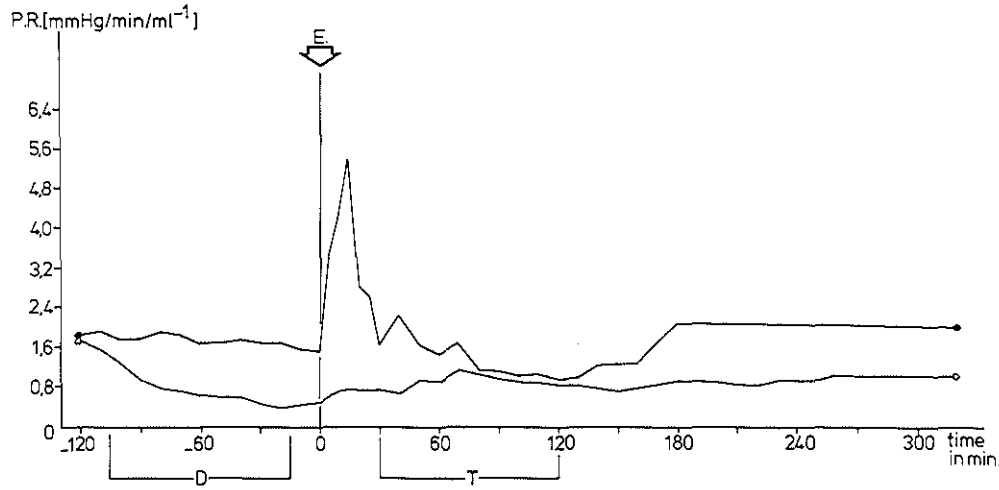

Fig. 42. o = Biphasic adrenergic blockade. Endotoxin 7 mgr/kg.
● = No adrenergic blockade. Endotoxin 7 mgr/kg
Peripheral resistance.

D. DISCUSSION AND CONCLUSIONS

In chapter A. the outline of this study is given: the complexity of the syndrome of clinical gram-negative shock and the confusing and contradictory information found in literature on cause and mechanisms of this type of circulatory failure, led to further investigation of the subject.
Evidence indicates that the bacterial endotoxins lead to shock via release of vasoactive mediators (chapter A.2).
The catecholamines may play the primary role as vasoactive mediators, although histamine, serotonine, kinin and bradykinin also may have some vasoactive function in endotoxin shock(chapter B.3).
Adrenergic effects are distinguished in alpha-receptor functions and beta-receptor functions , which can both be blocked pharmacologically (chapter B. 4).
As discussed in chapter A. 2, this study on combined adrenergic blockade in canine endotoxin shock was started when no information on such a complete blockade could be found in literature.
The dog was chosen as experimental animal, although it was realized that the canine model differs in certain haemodynamic aspects from the subhuman primate model and therefore most likely also from man (chapter A. 3).
The endotoxin shock model was preferred over the viable bacteria shock model, because it are the endotoxins from the bacterial cell wall which cause shock, and it seemed that the endotoxin model would have more precise haemodynamic parameters (chapter A. 4).
5 Mgr/kg bodyweight Endotoxin coli (0127:B8, Difco) was chosen for our experiments, because several authors consider this dose to represent a L.D. 95% (subchapter B. 1-1-1).
Endotoxin shock in dogs follows a biphasic pattern with hepatosplanchnic pooling of blood in the first phase of shock and probably diffuse vasodilatation in the second phase (subchapter B. 1-2). More evidence is given in chapter B. 4, which seems to endorse the idea of blocking both adrenergic effects : Swan (104, 105) proved that norepinenephrine and epinephrine have both alpha- and beta-receptor functions.

If a conclusion can be made on the data given in literature, it would be that most likely alpha-blockade influences the first phase of canine endotoxin shock, and beta-blockade the second phase.
Such a hypothesis and consequently a biphasic alpha-beta-adrenergic blockade has not been described in literature.
Therefore, such a working hypothesis formed the basis of our investigation (chapter C. 1).
An intriguing aspect of any treatment that would improve endotoxin shock is the fact that such a treatment might also be beneficial in the endstages of haemorrhagic and cardiogenic shock.
This can be concluded from the so called "final common pathway theory", described in subchapter B. 2-1, in which is stated that shock of any kind becomes ultimately endotoxic in nature, because the intestine after prolonged ischaemia loses its barrier function against passage of bacteria and endotoxins into the bloodstream.
Evidence as outlined by Fine (28, 29) and Lillehei (65, 67) seems conclusive on this point.
In chapter C. 2, the results of the pilot study are described, in which the influence on canine endotoxin shock of alpha-blockade with dibenzyline and beta-blockade with inderal or trasicor was investigated.
Biphasic blockade with dibenzyline and trasicor in high doses led to a significant improvement of survival (subchapter C. 2-3).
It was demonstrated that even 15 mgr/kg dibenzyline was not the maximum dose with regard to its vasodilatory effects (subchapter C. 2-4).
Another argument in favour of these high dosages was found in the observation that endotoxin circulated for a prolonged period of time after injection (subchapter C. 2-5).
The increased levels of plasma catecholamines, found after endotoxin injection, seemed to support the validity of the catecholamine hypothesis in our model (subchapter C. 2-6).
In the main study, the results of further analysis of this biphasic blockade is described (chapter C. 3).
The alpha-blockade with dibenzyline was given as premedication

before the injection of endotoxin, and directed to the first phase of shock, immediately after endotoxin injection.
The beta-blockade with trasicor was given as medication after endotoxin injection and directed to the second phase of shock (subchapter C. 3-2).
Alternative methods of adrenergic blockade were also more extensively investigated. At first, firm conclusions were impossible, because the supposedly lethal dose 95% (L.D. 95%) of 5 mgr/kg endotoxin appeared to have a lower lethality in control experiments with endotoxin shock without treatment (subchapter C. 3-4). Whereas in the pilot study, no animals, treated or untreated, survived the procedure, in the main study not only treated, but also some untreated animals survived.
This difference could have been due to the different dog model, used in the main study, to differences of potency of the several endotoxin batches used, or to a decline of potency during storage time of the endotoxin. This problem is discussed in subchapter C. 3-8.
The solution was then sought and found in extending the amount of experiments and comparing a larger group of treated animals with untreated counterparts.
Experiments with higher doses of endotoxin (6 and 7 mgr/kg) were performed as well in an effort to reach a L.D. 95% shock model (subchapter C. 3-7-2).
When even 7 mgr/kg endotoxin appeared not to be a L.D. 95%, experiments were paired for comparison of treated and untreated animals, injected with the same batch of endotoxin (subchapter C. 3-7).
On the completed experimental data, statistical analysis was possible. The biphasic pattern of endotoxin shock in dogs was confirmed (subchapter C. 3-4).
The conclusion was reached that biphasic blockade improved survival significantly and resulted in a significantly higher blood flow after endotoxin injection (subchapter C. 3-7).
Contrary to some information in literature (subchapter B. 4-3), dibenzyline was found to give a significant improvement of shock parameters in the first phase of canine endotoxin shock, which seems to endorse the hypothesis that this phase is largely an

alpha-adrenergic phenomenon.
When solely dibenzyline premedication was given without trasicor in the second phase, the blood flow also remained high in the second phase after endotoxin, but at the expense of a significantly increased heart rate (subchapter C. 3-5-1).
It can be concluded that the beneficial effect of beta-blockade in the second phase is mainly that the higher blood flow can be maintained with a normal heart rate (subchapter C. 3-7-1).
In this respect also,the working hypothesis seemed to be supported by the results of our investigation.
Beta-blockade after endotoxin injection without premedicative alpha-blockade (subchapter C. 3-5-2) and combined simultaneous blockade as premedication (subchapter C. 3-6) were of no benefit.
Adrenergic blockade prevented the sharp rise in peripheral resistance, that is characteristic for endotoxin shock (subchapters C. 3-4, C. 3-5-1 and C. 3-7).
This prevention of general vasoconstriction is apparently one of the most important effects of adrenergic blockade in endotoxin shock.
Evidently, in a clinical condition one cannot give a premedication with dibenzyline as in the experimental model.
The eventual "translation" to a clinical situation should then probably be found in a very early start of such a therapy, when the first symptoms of septic shock are suspected.
Nonetheless, application of this method in human septic shock seems premature and should be preceded by investigation of biphasic adrenergic blockade in subhuman primates.

E. SUMMARY

In the introduction the motives to undertake this investigation are outlined: although septic shock in man still has a high mortality, basic research in this field did not give a satisfactory explanation of the syndrome.
In the experimental model,it was found that septic or gram-negative bacterial shock is caused by the bacterial endotoxins. The endotoxins lead to shock via vasoactive mediators.
Among these possible mediators, the catecholamines may be the most important ones. In this case, adrenergic blockade could be of benefit in experimental endotoxin shock and probably also in man.
Documentation of a combined adrenergic-receptor blockade in experimental endotoxin shock could not be found in literature. Therefore, such a combined blockade was investigated in the animal experiment.
As the experimental model, endotoxin shock in dogs was chosen.
In a literature survey this model and the possible role of the catecholamines in endotoxin shock was studied.
Reports of various authors on separate adrenergic blockades in canine endotoxin shock are given.
The own investigation started with a pilot study, in which the influence of several dosages and combinations of adrenergics on survival in canine endotoxin shock was studied.
The effect of increasing dosages of dibenzyline (alpha-blockade) and additional trasicor (beta blockade) on vasodilatation, endotoxin circulation and plasma catecholamine levels were investigated.
Biphasic blockade with 15 mgr/kg bodyweight dibenzyline and 1,5 mgr/kg bodyweight trasicor in canine endotoxin shock was the main topic of interest.
Alternative methods of adrenergic blockade were also investigated in this model.
Results led to the conclusion that the biphasic blockade improved survival significantly, as compared with control experiments with endotoxin shock without treatment.

The blood flow was significantly higher in the treated group as compared to untreated animals.
The higher blood flow was reached with a much lower heart rate, when beta-blockade was applied in the second phase of shock.

SAMENVATTING

In de inleiding wordt vermeld waarom dit onderzoek werd verricht: hoewel de mortaliteit van septische shock bij mensen nog steeds hoog is, heeft het elementaire onderzoek op dit gebied nog geen afdoende verklaring voor het syndroom opgeleverd.
In het experimentele proefmodel is aangetoond, dat septische of gram-negatieve bacteriële shock veroorzaakt wordt door bacteriële endotoxinen. De endotoxinen veroorzaken shock door middel van stoffen die een invloed op de vaattonus hebben.
Van deze mogelijke tussenstoffen zouden de catecholaminen de meest belangrijke kunnen zijn. Wanneer dit het geval is, zou een adrenergische blokkade van nut kunnen zijn in experimentele endotoxische shock en mogelijk ook bij de mens.
In de literatuur kon geen beschrijving worden gevonden van een gecombineerde adrenergische receptor blokkade in experimentele endotoxische shock.
Om deze reden werd een dergelijke gecombineerde blokkade onderzocht in het diermodel.
Endotoxische shock bij honden werd gekozen als experimenteel model.
In een literatuuroverzicht werd dit model en de mogelijke rol van de catecholaminen in endotoxische shock bestudeerd.
Mededelingen van verschillende auteurs over aparte adrenergische blokkades in endotoxische shock bij de hond worden vermeld.
Het eigen onderzoek begon met enkele voorlopige experimenten waarin de invloed van verscheidene doseringen en combinaties van anti-adrenergica op overleving in endotoxische shock bij de hond werden bestudeerd.
Het effect van toenemende doseringen dibenzyline (alpha-blokkade) en toegevoegde trasicor (beta-blokkade) op vaatverwijding, endotoxine-circulatie en plasma catecholamine-gehalten, werd onderzocht.
Biphasische blokkade met 15 mgr/kg lichaamsgewicht dibenzyline en 1,5 mgr/kg lichaamsgewicht trasicor in endotoxische shock bij de hond was het belangrijkste doel van het onderzoek.
Alternatieve methoden van adrenergische blokkade werden ook onderzocht in dit model.

De resultaten leidden tot de conclusie dat de biphasische blokkade de overleving,in vergelijking met controle experimenten met endotoxische shock zonder behandeling, significant verbeterde.
De bloedstroom was ook significant hoger in de behandelde groep in vergelijking met niet behandelde dieren.
Met beta-blokkade in de tweede fase van shock werd deze hoge bloedstroom bereikt met een veel lagere hartfrequentie.

F. LITERATURE INDEX

1) ABRAMS, J.S. and R.W. Hopkins, Effect of phenoxybenzamine on endotoxic shock in dogs. I. Hemodynamic changes.
J. Trauma 9; 603 (1969).

2) AHLQUIST, R.P., A study of the adrenotropic receptors.
Am. J. Phys. 153; 586 (1948).

3) ANTON, A.H. and D.F. Sayre, The distribution of dopamine and dopa in various animals and a method for their determination in diverse biological material.
J. Pharm. Exp. Ther. 145; 326 (1964).

4) ARCHER, L.T. and M.R. Black, Myocardial failure with altered response to adrenaline in endotoxin shock.
Br. J. Pharm. 54; 145 (1975).

5) ARCHIE, J.P. Jr., Anatomic arterialvenous shunting in endotoxic and septic shock in dogs.
Ann. Surg. 186; 171 (1977).

6) ARCHIE, J.P., Systemic and regional arteriovenous shunting in endotoxic and septic shock in dogs.
Surg. Forum 27; 55 (1976).

7) ASPEREN de BOER, F.R.S. van , Combined α- and β-adrenergic blockade in endotoxin shock. (Abstract)
Eur. Surg. Res. 4; 358 (1972).

8) BAUE, A.E., Recent developments in the study and treatment of shock.
Surg. Gynec. & Obstet. 127; 849 (1968).

9) BERK, J.L. and J.F. Hagen, The effect of epinephrine on arteriovenous shunts in the pathogenesis of shock.
Surg. Gynec. & Obstet. 124; 317 (1967).

10) BERK, J.L. and J.F. Hagen, The treatment of hemorrhagic shock by Beta adrenergic receptor blockade.
Surg. Gynec. & Obstet. 125; 311 (1967).

11) BERK, J.L. and J.F. Hagen, The treatment of endotoxin shock by Beta adrenergic blockade.
Ann. Surg. 169; 74 (1968).

12) BLAIR, E., Hypocapnia and gram-negative bacteremic shock.
Am. J. Surg. 119; 433 (1970).

13) BLATTBERG, B. and M.N. Levy, Extrahepatic factors in the early hypotensive response to bacterial endotoxin.
Can. J. Phys. Pharm. 48; 583 (1970).

14) BLATTBERG, B. and M.N. Levy, Sites of extrahepatic pooling in early response to endotoxin.
Am. J. Phys. 220; 1267 (1971).

15) BOIVIN, A. and I. Mesrobeanu, Extraction d'un complexe toxique et antigénique à partir du bacille d'aertrycke.
C. R. Soc. Biol. 114; 307 (1933).

16) BOIVIN, A. and I. Mesrobeanu, Technique pour la préparation des polysaccharides microbiens.
C. R. Soc. Biol. 113; 490 (1933).

17) BORDEN, C.W. and W.H. Hall, Fatal transfusion reactions from massive bacterial contamination of blood.
N. Engl. J. Med. 245; 760 (1951).

18) BROCKMAN, S.K. and C.S. Thomas, Jr., The effect of escherichia coli endotoxin on the circulation.
Surg. Gynec. & Obstet. 125; 763 (1967).

19) CAVANAGH, D. and P.S. Rao, Endotoxin shock in the subhuman primate.
Arch. Surg. 99; 107 (1969).

20) CAVANAGH, D. and P.S. Rao, Pathophysiology of endotoxin shock in the primate.
Am. J. Obstet. Gynec. 108; 705 (1970).

21) CARRETERO, O.A. and A. Najsletti, Kinins and kininogen in endotoxin shock.
Experientia 26; 63 (1970).

22) CHRISTY, J.H., Treatment of gram-negative shock.
Am. J. Med. 50; 77 (1971).

23) COHN, J.D. and M. Greenspan, Arteriovenous shunting in high cardiac output shock syndromes.
Surg. Gynec. & Obstet. 127; 282 (1968).

24) CRONENWETT , J.L. and S.M. Lindenauer, Arteriovenous shunting in the canine hind limb with sepsis.
Surg. Forum 27; 24 (1976).

25) DIETZMAN, R.H. and J. Feemster, Peripheral resistance changes during shock in man.
Angiologie 19; 268 (1968).

26) ELIN, R.J. and R.A. Robinson, Lack of clinical usefulness of the test in the diagnosis of endotoxemia.
N. Engl. J. Med. 293; 521 (1975).

27) FORSCHER, B.K. and R.C. Lillehei, Shock in low and high-flow states. Proceedings of a symposium, (held at) Brook Lodge, Augusta,Michigan, June 1-3, 1971.
Excerpta Medica, Amsterdam 1972.

28) FINE, J., Current status of the problem of traumatic shock.
Surg. Gynec. & Obstet. 120; 537 (1965).

29) FINE, J., Septic shock.
JAMA 188; 127 (1964).

30) FINE, J. and S. Rutenburg, Therapeutic effect of an extract of spleen in shock caused by liver ischemia:an experimental study.
Ann. Surg. 170; 947 (1969).

31) FREEMAN, N.E., The production of shock by the prolonged continuous injection of epinephrine in dogs.
Am. J. Phys. 131; 545 (1941).

32) FRONEK, K. and B.W. Zweifach, Changes of splanchnic haemodynamics in hemorrhagic hypotension and endotoxemia.
J. Surg. Res. 11; 232 (1971)

33) GILBERT, R.P., Mechanisms of hemodynamic effects of endotoxin.
Phys. Rev. 40; 245 (1960).

34) GRANDJEAN, T. and J.L. Rivier, Cardio-circulatory effects of beta-adrenergic blockade in organic heart disease. Comparison between propranolol and CIBA 39,089-BA.
Br. Heart J. 30; 50 (1968).

35) GRIFFITHS, J. and A.C. Groves, The relationship of plasma catecholamine to serum triglycerides in canine gram-negative bacteremia.
Surg. Gynec. & Obstet. 134; 795 (1972).

36) GUNTHEROTH, W.G. and A.J. Felsenfield, Splenic constriction with endotoxin shock in the dog.
J. Appl. Phys. 30; 517 (1971).

37) GUYTON, A.C., Textbook of physiology. 3rd ed. Saunders, Philadelphia 1968.
p. 249: Basic principles of the circulation:haemodynamics.

38) HALMAGYI, D.F.J. and M. Kennedy,Combined adrenergic receptor blockade and circulating catecholamines in hemorrhagic shock.
Eur. Surg.3; 378 (1971).

39) HARDAWAY, R.M., Clinical management of shock. Charles C. Thomas, New York 1968.

40) HARDAWAY,III, R.M., Gram-negative shock.
Antibioth. Chemother. 21; 208 (1976).

41) HERMRECK, A.S. and A.P. Thal, Mechanisms for the high circulatory requirements in sepsis and septic shock.
Ann. Surg. 170; 677 (1969).

42) HINSHAW, L.B. and L.T. Archer, Effects of endotoxin on myocardial hemodynamics, performance and metabolism.
Am. J. Phys. 221; 504 (1971).

43) HINSHAW, L.B. and L.T.Archer,Effects of glucose or insulin on myocardial performance in endotoxin shock.
Proc. Soc. Biol. Med. 152; 529 (1976).

44) HINSHAW, L.B. and D.L. Nelson, Venous response of intestine to endotoxin.
Am. J. Phys. 203; 870 (1962).

45) HINSHAW, L.B. and S.E. Owen, Correlation of pooling and resistance changes in the canine forelimb in septic shock.
J. Appl. Phys. 30; 331 (1971).

46) HINSHAW, L.B. and M.D. Peyton, Prevention of death in endotoxin shock by glucose administration.
Surg. Gynec. & Obstet. 139; 851 (1974).

47) HINSHAW, L.B. and L.A. Solomon, Comparison of canine responses to escherichia coli organisms and endotoxin.
Surg. Gynec. & Obstet. 127; 981 (1968).

48) IAMPIETRO, P.F. and L.B. Hinshaw, Effect of an adrenergic blocking agent on vascular alterations associated with endotoxin shock.
Am. J. Phys. 204; 611 (1963).

49) INTERNATIONAL symposium on metabolic changes in shock.Held in Freiburg/Breisgau (West Germany), Oct 30th - Nov. 1 st, 1969.

50) ISAKSON, P.C. and F. Schofer, Prostaglandins and the renin-angiotensin system in canine endotoxemia.
J. Pharm. Exp. Ther. 200; 614 (1977).

51) JACOBSON, E.D., Considerations of the pathophysiology and treatment of endotoxin shock.
Arq. Gastroent. 2; 107 (1965).

52) JACOBSON, E.D. and W.E. Farrar, Jr., Influence of route of administration on hemodynamic effects of endotoxin.
Am. J. Phys. 205; 799 (1963).

53) JACOBSON, E.D. and J.P. Kals, Is histamine the "trigger mechanism" of endotoxin shock?
Am. Heart J. 68; 716 (1964).

54) JACOBSON, E.D. and B. Mehlman, Vasoactive mediators as the "trigger mechanism" of endotoxin shock.
J. Clin. Invest. 43; 1000 (1964).

55) JONGE, H.J. de, Inleiding tot de medische statistiek. Dl. 1. 2e dr. Wolters-Noordhof, Groningen 1963.p. 334:A test against trend in related samples.

56) JONGE, H.J. de, Inleiding tot de medische statistiek. Dl. 1. 2e dr. Wolters-Noordhof, Groningen 1963. p. 279: The Wilcoxon matched-pairs signed-ranks test.

57) KALAS, J.P. and E.D. Jacobson, The effect of vasoactive antagonists in endotoxin shock.
Am. Heart J. 67; 764 (1964).

58) KONDO, Y. and R.D. Culpepper, Heart transplantation in endotoxin shock.
Surg. Gynec. & Obstet. 140; 252 (1975).

59) LAVERTY, R. and K. Taylor,The fluorometric assay of catecholamines and related compounds.
Anal. Bioch. 22; 269 (1968).

60) LEES, N.W., The diagnosis and treatment of endotoxin shock.
Anaesthesia 31; 897 (1976).

61) LEVIN, J., Endotoxin and endotoxemia.
N. Eng. J. Med. 288; 1297 (1973).

62) LEVIN, J. and T.E. Poore, Detection of endotoxin in the blood of patients with sepsis due to gram-negative bacteria.
N. Eng. J. Med. 283; 1313 (1970).

63) LEWIS, A.A.G. and R.K. Rondel, Oxprenolol.
Postgrad. Med. J. Suppl.Nov., 1970.

64) LICHTENSTEIN, L.M., Sequential analysis of the allergic response: cyclic AMP, calcium and histamine.
Int. Arch. Allergy & appl. Immun. 49; 143 (1975).

65) LILLEHEI, R.C., The intestinal factor in irreversible hemorrhagic shock.
Surgery 42; 1043 (1957).

66) LILLEHEI, R.C. and J.K. Longerbeam, The modern treatment of shock based on physiologic principles.
Clin. Pharm.Ther. 5 ; 63 (1963).

67) LILLEHEI, R.C. and J.K. Longerbeam, The nature in irreversible shock: Experimental and clinical observations.
Ann. Surg. 160; 682 (1964).

68) LILLEHEI, R.C. and L.D. MacLean, The intestinal factor in irreversible endotoxin shock.
Ann. Surg. 148; 513 (1958).

69) LILLEHEI, R.C. and L.D. MacLean, Physiological approach to successful treatment of endotoxin shock in the experimental animal.
Arch. Surg. 78; 116 (1959).

70) MacLEAN, L.D. and A.P.H. MacLean, Hemodynamic and metabolic abnormalities.
Postgrad. Med. 48; 114 (1970).

71) MacLEAN, L.D. and M.H. Weil, Hypotension (shock) in dogs produced by escherichia coli endotoxin.
Circ. Res. IV; 546 (1956).

72) MANNY, J. and N. Rabinovici, Insulin response to continuous glucose load in endotoxin shock in the dog.
Surg. Gynec. & Obstet. 145; 198 (1977).

73) MEE, W.M., Some pathogenic mechanisms - their significance. Endotoxin shock - distinct entity or universal accompaniment?
Postgrad. Med. J. 45; 514 (1969).

74) MELA, L. and L.B. Hinshaw, Correlation of cardiac performance, ultrastructural morphology, and mitochondrial function in endotoxemia in the dog.
Circ. shock 1; 265 (1974).

75) MILLER, R.L. and K.L. Melmon, The related roles of histamine, serotonin and bradykinin in the pathogenesis of inflammation.
Series Haematol. III; 5 (1970).

76) MOORE, F.D., Shock and sepsis: some historical perspectives.
Surg. Clin. N. Am. 49; 481 (1969).

77) MORRIS, J.A. and R.W. Smith, Hemodynamic action of vasopressor agents in endotoxin shock.
Am. J. Obstet. Gynec. 91; 491 (1965).

78) MOTSAY, G.J., Gram negative septic shock.
Int. Surg. 54; 81 (1970).

79) MOTSAY, G.J. and R.H. Dietzman, Treatment of endotoxin shock.
Rev. Surg. 26; 381 (1969).

80) NAKANO, J. and T. Kusakari, Effect of beta-adrenergic blockade on the cardiovascular dynamics.
Am. J. Phys. 210; 833 (1966).

81) PALMERIO, C. and J. Fine, The nature of resistance to shock.
Arch. Surg. 98; 679 (1969).

82) PARK, M.K. and D. Baum, Lack of pooling in portal vein in endotoxin shock in the dog.
J. Appl. Phys. 28; 156 (1970).

83) PARRAT, J.R. and J. Grayson, Myocardial vascular reactivity after beta-adrenergic blockade.
Lancet I; 338 (1966).

84)PASSMORE , J.C. and R.E. Nelberger, Measurement of intrarenal anatomic distribution of krypton-85 in endotoxic shock in dogs.
Am. J. Phys. 232; 54 (1977).

85) PEYTON, M.D. and L.B. Hinshaw, The effects of coronary vasodilatation on cardiac performance during endotoxin shock.
Surg. Gynec. & Obstet. 143; 533 (1976).

86) PEYTON, M.D. and L.B. Hinshaw, Hypoglycemic effects of endotoxin in eviscerated dogs.
Surg. Gynec. & Obstet. 141; 727 (1975).

87) PIERONI, R.E. and E.J. Broderick, A simple method for the dequantification of submicrogram amounts of bacterial endotoxin.
Proc. Soc. Exp. Biol. Med. 133; 790 (1970).

88) RAI, D.K. and L.P. Gupta, A study of microcirculation in endotoxin shock.
Surg. Gynec. & Obstet. 139; 11 (1974).

89) RAO, P.S. and D. Cavanagh, Endotoxin shock in the subhuman primate.
Arch. Surg. 102; 486 (1971).

90) REICHGOTT, M.J. and K.L. Melmon, Cardiovascular and metabolic effects of whole or fractionated gram-negative bacterial endotoxin in the unanesthetized rhesus monkey.
Circ. Res. XXXIII; 346 (1973).

91) REINHOLD, R.B. and D.T. Caridis, Diagnosis of clinical endotoxemia.
J. Reprod. Med. 8; 335 (1972).

92) RETY, N.A. and C.M. Couves, Effects of slow infusion of endotoxin in the dog.
Can. J. Surg. 12; 493 (1969).

93) REYNOLDS, D.G. and J.M. Brungardt, Effects of endotoxin on the vascular architecture of intestinal mucosa.
In: The fundamental mechanisms of shock. Ed. by L.B.Hinshaw and B.G. Cox. Plenum Publ. Corp., New York 1972.(Repr.)p.113.

94) ROBERTS, J.M. and R.K. Laros, Hemorrhagic and endotoxic shock: a pathophysiologic approach to diagnosis and management.
Am. J. Obstet. Gynec. 110; 1041 (1971).

95) ROGERS,D.E. and M.G. Koenig, The problem of gram-negative bacteremia and its management.
South. Med. J. 58; 1391 (1965).

96) SHAH, J.P. and U.S. Shah, Studies on the release of bradykinin by the splanchnic circulation during endotoxic shock.
J. Trauma 10; 255 (1970).

97) SHOEMAKER, W.C., Cardiorespiratory patterns in complicated and uncomplicated septic shock: physiologic alterations and the therapeutic implications.
Ann. Surg. 174; 119 (1971).

98) SHUBIN, H. and M.H. Weil, Bacterial shock.
JAMA 235; 421 (1976).

99) SIEGEL, J.H. and M. Greenspan, Abnormal vascular tone, defective oxygen transport and myocardial failure in human septic shock.
Ann. Surg. 165; 504 (1967).

100) SIEGEL, S., Non-parametric statistics for the behavioral sciences. McGraw-Hill, New York 1956. p. 107: The X^2-test for 2 x 2 contingency tables.

101) SIEGEL, S., Non-parametric statistics for the behavioral sciences. McGraw-Hill, New York 1956. p. 116: The Mann-Whitney-U-Test.

102) SWAN, K.G. and R.W. Barton, Splanchnic bloodflow in experimental shock. In: The fundamental mechanisms of shock. Ed. by L.B. Hinshaw and B.G. Cox. Plenum Publ. Corp., New York 1972.(Repr.). p. 87.

103) SWAN, K.G. and E.D. Jacobson, Hemodynamics of endotoxin shock in the conscious animal.
Surg. Gynec. & Obstet. 125; 1 (1967).

104) SWAN, K.G. and D.G. Reynolds, Adrenergic mechanisms in canine mesenteric circulation.
Am. J. Phys. 220; 1779 (1971).

105) SWAN, K.G. and D.G. Reynolds, Effects of intra-arterial catecholamine infusions on blood flow in the canine gut.
Gastroent. 61; 863 (1971).

106) THOMAS, C.S. Jr. and S.K. Brockman, The role of adrenal corticosteroid therapy in escherichia coli endotoxin shock. Surg. Gynec. & Obstet. 127; 61 (1968).

107) THOMAS, C.S., Jr. and M. A. Melly, The hemodynamic effects of viable gram-negative organisms. Surg. Gynec. & Obstet. 128; 753 (1969).

108) VICK, J.A. and B. Mehlman,Early histamine release and death due to endotoxin. Proc. Soc. Biol. Med. 137; 902 (1971).

109) WEIL, M.H. and H. Shubin, Shock caused by gram-negative micro-organisms.Analysis of 169 cases. Ann. Int. Med. 60; 384 (1964).

110) WEIL, M.H. and H. Shubin, Gram-negative shock: definition, diagnosis and mechanisms. Antibioth. Chemother. 21; 178 (1976).

111) WEIL, M.H. and W.W. Spink, A comparison of shock due to endotoxin with anaphylactic shock. J. Lab. Clin. Med. 50; 501 (1957).

112) WRIGHT, C.J. Septic shock. Br. Med. J. I, 430 (1970).

113) WRIGHT, C.J. and J.H. Duff, Regional capillary blood flow and oxygen uptake in severe sepsis. Surg,. Gynec. & Obstet. 132; 637 (1971).

114) YARD, A.C., Shock produced in dogs by infusions of norepinephrine. Fed. Proc. 15; 502 (1956).

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