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Further observations on mesenteric vasoconstriction, survival and the clotting defect after endotoxin administration

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Summary

1. The initial response after endotoxin administration (3 mg/kg) in cats involved pulmonary vasoconstriction. This was not seen when endotoxin was given by slow infusion and it could be prevented after a bolus injection of endotoxin by pretreatment of the cats with aspirin (10 mg/kg). Intense mesenteric vasoconstriction occurred in all the cats.

2. The mesenteric vasoconstriction was a specific response of the mesenteric blood vessels. At the time the mesenteric bed constricted, the renal bed dilated, the hepatic arterial bed remained unchanged and the smooth muscle of the intestinal wall relaxed.

3. Arterial blood from cats with a fully developed mesenteric vasoconstriction after endotoxin administration was perfused through a normal intestine. No immediate vasoconstriction developed but the perfused intestine constricted slowly over 60 minutes. This suggests that mesenteric constriction was not due to circulating vasoconstrictor factors or the intestinal innervation, but involved a slow local mechanism within the intestine. It could not be prevented or reversed by a variety of pharmacological agents.

4. These observations suggest that endotoxin caused a unique type of mesenteric vasoconstriction in cats by a local mechanism which took up to 60 min to develop, was sufficiently potent to reduce mesenteric flow to $<30\%$ control, and was maintained until death of the cats. Blood from these animals did not clot when placed in a glass tube.

5. The mesenteric constriction and the clotting defect could be prevented by repeated administration of aminophylline and dextran solution before and after a bolus intravenous injection of endotoxin. Arterial pressure and mesenteric flow were maintained for at least 10 h in these experiments. Inadequate treatment intensified rather than reduced the intestinal mucosal damage.

6. Cats were treated with aspirin, endotoxin and the optimal regimen for prevention of the mesenteric constriction and allowed to recover from the anaesthetic agent. In this series, 63% survived indefinitely compared to 25% after aspirin and endotoxin treatment and 0% after endotoxin alone.

7. The possible mechanisms of action of aspirin and aminophylline-dextran solution are discussed. Our failure to obtain 100% survival is probably due to pulmonary damage which develops 10–24 h after endotoxin administration. This delayed pulmonary action of endotoxin is not prevented by aspirin

treatment and it seems unlikely that aspirin will be of any value in the treatment of the pulmonary lesion in man.

Introduction

The current state of knowledge on the fundamental mechanisms of shock, including endotoxin shock, was extensively discussed at a recent symposium (Hinshaw & Cox, 1972). The problems involved are complex and it is difficult to dissociate primary and secondary responses. Marked species differences are also reported. In an attempt to avoid some of these difficulties, we have been examining a well-defined problem, namely, what are the pharmacological actions of Difco endotoxin No. 3105-25 (from *Salmonella enteritidis*) which result in death when 3 mg/kg are injected intravenously into cats anaesthetized with pentobarbitone.

Our initial studies confirmed earlier work (Kuida, Hinshaw, Gilbert & Visscher, 1958; Kuida, Gilbert, Hinshaw, Brunson & Visscher, 1961) which showed that an acute pulmonary vasoconstriction occurred. This resulted in pulmonary oedema and right ventricular failure and was immediately lethal in approximately half of the cats. This response could be prevented in some cats by alkaline hydrolysis of the endotoxin (Greenway, Lautt & Stark, 1969) and in all cats by pretreatment with aspirin (Greenway & Murthy. 1971). Initially we used a large dose of aspirin (100 mg/kg) but later we found that complete protection could be obtained with 10 mg/kg. In spite of prevention of the pulmonary response by aspirin, 75% of the cats still died after endotoxin administration. In these animals there was a slowly developing mesenteric vasoconstriction which was sufficiently intense and prolonged to be likely to cause death. Catecholamines, vasopressin and angiotensin played at most a minor role in the mechanism of this constriction and other unknown factors predominated (Greenway & Murthy, 1971).

The present study examines several questions which arose from this work, namely, (1) is the mesenteric vasoconstriction only seen when aspirin is present, (2) is the constriction occurring only in the intestine or is it part of a generalized contraction of smooth muscles, (3) is the constriction brought about by a circulating vasoconstrictor agent or is it due to a local mechanism within the intestine, and (4) will prevention of the mesenteric vasoconstriction result in survival after endotoxin administration to cats?

Methods

Acute experiments

Eighty-two cats (1·4–4·4 kg body weight, mean 2·2 kg) were used; they were anaesthetized by intraperitoneal injection of sodium pentobarbitone (Abbott, 30 mg/kg). When reflex limb and swallowing movements returned, additional doses (8 mg) of sodium pentobarbitone were given through a cannula in a forelimb cutaneous vein. Mean arterial pressure (1 mmHg≡1·333 mbar) was recorded from a cannula in a femoral artery and mean right atrial pressure from a cannula inserted through the right external jugular vein. Mean portal pressure was recorded from a small cannula introduced into the portal vein through the small branch from the appendix. The following procedures were carried out in some of the experiments as described in the **Results.**

Superior mesenteric arterial flow or hepatic arterial flow was recorded with a non-cannulating flow probe connected to an electromagnetic flowmeter (Nycotron, Oslo) as previously described (Greenway, Lawson & Mellander, 1967 ; Greenway & Murthy, 1971). Renal flow was recorded by a long-circuit from the left renal vein to the left external jugular vein through an extracorporeal flow probe ; these cats were given heparin (10 mg/kg) intravenously before the long-circuit was set up. In all these experiments, calibration of the flowmeter was carried out *in situ* at the end of each experiment. Vascular conductance was calculated by dividing the flow by the arterial pressure minus right atrial pressure (renal and hepatic beds) or portal pressure (intestinal bed). Since arterial and venous pressures did not change markedly in these experiments, the graphs for flow and conductance were similar. Peristaltic movements of the intestine were recorded by inserting a small balloon into the intestinal lumen through an incision in the wall ; the balloon was positioned at least 10 cm from the site of the incision and connected to a pressure transducer (Statham type P23BC). All recordings of pressures and flows were made on a dynograph recorder (Beckman).

In experiments involving cross-circulation of an intestine, two cats were given heparin (10 mg/kg) intravenously. Cat A (the donor) was prepared for the recording of arterial and portal pressures and mesenteric arterial flow as described above. The intestine of cat B was isolated *in situ* by ligatures, its superior mesenteric artery was perfused through a long-circuit from the carotid artery of the same cat and the flow was recorded by an extra-corporeal flow probe ; blood was returned from the mesenteric vein to the external jugular veins. T-pieces in the long-circuits allowed the isolated intestine of cat B to be perfused from the carotid artery and jugular veins of cat A when required.

Survival experiments

For this series, all solutions and catheters were sterile. Twenty-eight cats were given procaine-penicillin G (200,000 u) and streptomycin (100 mg) by intramuscular

TABLE 1. *Protocol for the survival experiments*

Time	Procedure
−60 min	Sodium pentobarbitone by intraperitoneal injection (30 mg/kg)
−45 min	Insert intravenous catheter. Take blood sample
−30 min	Aspirin (10 mg/kg) intravenously
−15 min	Aminophylline (5 mg/kg)+dextran solution (5 ml/kg) intravenously
0	Endotoxin (3 mg/kg) intravenously
20 min	Repeat aminophylline-dextran solution
40 min	Repeat aminophylline-dextran solution
60 min	Repeat aminophylline-dextran solution
2 h	Repeat aminophylline-dextran solution
3 h	Repeat aminophylline-dextran solution
4 h	Dextran solution (5 ml/kg)
6 h	Dextran solution (5 ml/kg)
9 h	Dextran solution (5 ml/kg)
10 h	Take blood sample
12 h	Dextran solution (5 ml/kg)
15 h	Take blood sample
	Dextran solution (5 ml/kg)
30 h	Take blood sample
	Dextran solution (5 ml/kg)
	Remove intravenous catheter

Dextran solution was a mixture of equal parts Rheomacrodex (10% dextran in saline) and Ringer-Locke solution.

injection 16 h and 30 min before the experiments were begun. The cats were anaesthetized by intraperitoneal administration of sodium pentobarbitone (30 mg/kg) and a catheter (Steril-Peel 8 inches, Bard Inc.) was inserted through a cutaneous hindlimb vein into the lower part of the inferior vena cava. This catheter was used for administration of drugs and removal of blood samples for determinations of haematocrit and clotting time. In the survivors, the catheter was removed after 30 hours. No other surgery was carried out. Gastric contents were aspirated through a rubber tube (6 mm i.d.) introduced through the mouth.

The final protocol for these experiments was based on the results of the acute experiments (see Results) and is given in Table 1.

Drugs

Aspirin (10 mg/ml) was dissolved in ammonium acetate solution (1·15 g/100 ml water) and given intravenously in a dose of 10 mg/kg body weight. Endotoxin (Bactolipopolysaccharide B from *Salmonella enteritidis*, Difco Lab. No. 3105, 3 mg/ml) was suspended in 0·9% w/v NaCl solution (saline) and given intravenously in a dose of 3 mg/kg either as a bolus injection or as an infusion (0·1 ml/minute). Vasopressin (Pitressin, Parke-Davis), disodium cromoglycate (Fisons), streptokinase (Behringwerke AG), proteinase inhibitor (Trasylol K, Bayer), procaine hydrochloride (U.S.P.) and cortisol (Solu-cortef, Upjohn) were dissolved or diluted in saline and given intravenously in the doses stated in **Results**. Adrenaline hydrochloride (Sigma) and isoprenaline hydrochloride (B.D.H.) were dissolved in saline containing ascorbic acid (0·2 mg/ml). Indomethacin (Merck, Sharp & Dohme, 1 mg/ml) was dissolved in phosphate buffer pH 8·0. Ethylene glycol tetra-acetic acid (EGTA, Baker, 50 mM) was dissolved in water and titrated to pH 7·4 with 5 N NaOH solution. Aminophylline (sterile solution for injection, U.S.P. Glaxo-Allenbury) was diluted with sterile saline to give 5 mg/ml. Dextran solution was prepared by mixing equal volumes of Rheomacrodex (10% dextran, average molecular weight 40,000, in saline Pharmacia) and Ringer-Locke solution (NaCl 154, KCl 5·6, $CaCl_2$ 2·2, $NaHCO_3$ 4·7, glucose 5·1 mM). Rheomacrodex has been shown to be a suitable plasma substitute in cats (Eliasson & Samelius-Broberg, 1963) but in 3 experiments a solution of 5% bovine albumin in Ringer-Locke solution was used instead of dextran solution to exclude pharmacological actions of dextran.

Results

Slow infusions of endotoxin

In 5 cats, endotoxin was infused intravenously (0·1 mg/min) until a total dose of 3 mg/kg body weight had been given ; this required a mean time of 86 minutes. In 4 control cats, saline was infused at the same rate (0·1 ml/minute). Aspirin was not given to these 9 cats. Before the infusions were begun, mean arterial pressure was 141 ± 8 mmHg (mean $\pm$ S.E.), superior mesenteric arterial flow was 25 ± 3 (ml/min)/kg body weight and right atrial pressure was $1{\cdot}0 \pm 0{\cdot}4$ mmHg.

The results in these experiments are shown in Figure 1. Within 10–20 min after beginning the endotoxin infusion, arterial pressure decreased gradually and after the infusion ended it recovered slowly towards the pre-infusion level. Superior mesenteric flow decreased steadily to 33% of the control flow 2 h after beginning

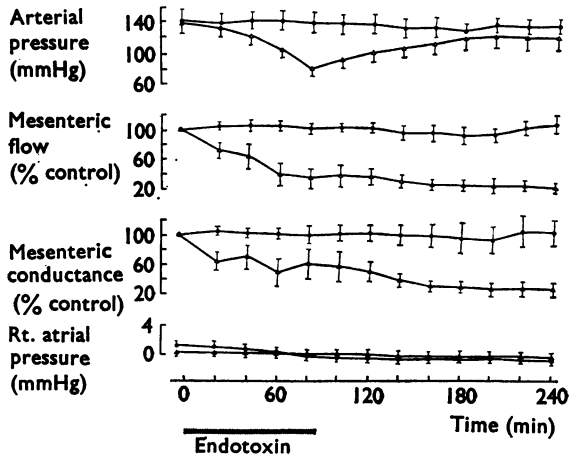

FIG. 1. The responses (means ± S.E.) to intravenous infusions of endotoxin (0·1 (mg/min)/kg to a total dose of 3 mg/kg ; 5 cats ; open triangles) or saline (0·1 ml/min ; 4 cats ; closed circles).

the endotoxin infusion. Right atrial pressure did not change and after termination of the experiments, the lungs showed no congestion or haemorrhagic areas. In 3 of these 5 cats, a bolus injection of endotoxin (3 mg/kg) was given before the experiment was terminated. This did not produce any change in arterial pressure or right atrial pressure. In the control experiments where saline was infused, the measured variables did not change over the 2 h period.

Thus, slow intravenous infusion of endotoxin does not produce the severe initial pulmonary vasoconstriction and right ventricular failure which occur after a bolus injection of the same dose (Greenway *et al.*, 1969) and it prevents these responses to a bolus injection 2 h later. The mesenteric vasoconstriction was similar to that seen in cats given a bolus injection of endotoxin after aspirin pretreatment and it is concluded that this aspirin pretreatment does not cause or modify the mesenteric vasoconstriction produced by endotoxin administration.

Does vasoconstriction occur in other abdominal organs?

The effects of intravenous administration of endotoxin (3 mg/kg) on mesenteric, hepatic arterial and renal blood flows were compared in three series of cats pretreated with aspirin. In 8 cats, control mean arterial pressure was 122 ± 3 mmHg and mean superior mesenteric arterial flow was 37 ± 6 (ml/min)/kg body weight. In 3 cats, control mean arterial pressure was 145 ± 29 mmHg and mean hepatic arterial flow was 99 ± 3 (ml/min)/100 g liver. In 4 cats, control mean arterial pressure was 131 ± 6 mmHg and left renal flow was 194 ± 54 (ml/min)/100 g kidney.

The changes in mesenteric, renal and hepatic arterial conductances after administration of endotoxin in these 3 series of experiments are shown in Figure 2. A marked mesenteric vasoconstriction occurred but hepatic arterial conductance did not change markedly and renal conductance increased. It is concluded that the mesenteric vasoconstriction was not part of a generalized vasoconstrictor response but was a specific response of the intestinal bed.

In 2 cats, peristaltic activity and tone of the intestinal wall were recorded from a balloon in the gut lumen. Within 15 min after administration of endotoxin, peristaltic activity ceased and the mean pressure within the balloon decreased.

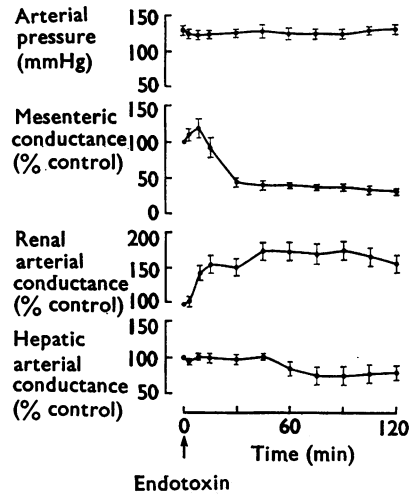

FIG. 2. The changes (means ± S.E.) in mesenteric (8 cats), renal (4 cats) and hepatic arterial (3 cats) conductances after a bolus intravenous injection of endotoxin (3 mg/kg) to cats pretreated with aspirin. The arterial pressures are the means for all 15 cats.

These observations suggest that the mesenteric constriction was not accompanied or caused by a contraction of the smooth muscle of the gut wall.

Is the mesenteric vasoconstriction due to circulating vasoconstrictor agents?

In 5 cross-perfusion experiments, cat A was pretreated with aspirin and given endotoxin (3 mg/kg). After the mesenteric vasoconstriction was allowed to develop for 2 h, the isolated intestine from cat B was perfused from the carotid artery and jugular veins of cat A. The results are shown in Figure 3. The mean arterial pressure of cat B before cross-perfusion was 137 ± 12 mmHg and this was slightly

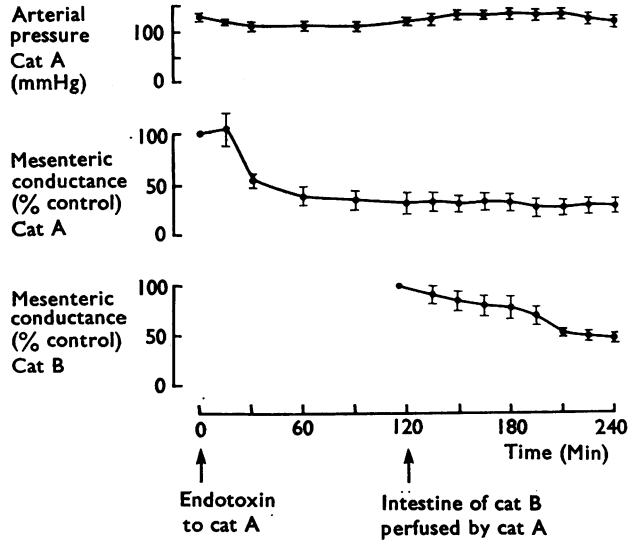

FIG. 3. Mean responses (± S.E.) in 5 experiments where the isolated intestine of cat B was cross-perfused with arterial blood from cat A which had been given endotoxin (3 mg/kg) 2 h earlier.

higher but not significantly different from the arterial pressure of cat A (Fig. 3) at the time of cross-perfusion ($P>0{\cdot}05$, paired t-test). When blood from cat A perfused the intestine from cat B, the mesenteric conductance of this intestine decreased about 10% in the first 15 min of cross-circulation. It is concluded that the arterial blood of cat A contained no significant intestinal vasoconstrictor agents. However, the mesenteric conductance of the intestine from cat B continued to decrease over 2 hours. This suggests that the arterial blood of cat A contained endotoxin and that the slow development of the vasoconstriction after endotoxin administration was occurring in the intestine itself since it did not develop faster when the intestine was perfused from an animal which had received endotoxin 2 h previously.

The following control experiments were carried out to validate the results and conclusions just described. In 3 experiments, cat B was set up with its intestine perfused from its own carotid artery and jugular veins ; aspirin and endotoxin were administered. The mesenteric vasoconstriction which developed was not significantly different from that in cats without isolation of the intestine (mesenteric conductance in Fig. 2 ; $P>0{\cdot}1$, unpaired t-test). Thus isolation *in situ* and perfusion through long-circuits does not modify the mesenteric vasoconstriction after endotoxin. In 3 experiments, cross-circulation was carried out as described but no endotoxin was given to either cat. Mesenteric conductance did not change significantly ($P>0{\cdot}1$, unpaired t-test) in either cat A or the isolated perfused intestine of cat B. On 3 occasions in each of these experiments, vasopressin (10–46 mu/min) was infused intravenously into cat A while it perfused the intestine from cat B. The decreases in mesenteric conductances in the two intestines were measured and were not significantly different ($P>0{\cdot}1$, paired t-test). These control experiments show that the procedure of cross-circulation did not change mesenteric conductance and that both intestines were equally responsive to a circulating vasoconstrictor agent.

Unsuccessful attempts to prevent mesenteric vasoconstriction

A variety of agents were tested for ability to prevent or reverse the mesenteric vasoconstriction after endotoxin administration to cats pretreated with aspirin. Since these attempts were unsuccessful, the results are presented only briefly. The mean arterial pressure in these cats was 130 ± 9 mmHg and the superior mesenteric arterial flow was $32 \pm 5{\cdot}1$ (ml/min)/kg before the endotoxin was administered. The following agents, given intravenously before the endotoxin, did not prevent the development of a severe mesenteric vasoconstriction: indomethacin (2 mg/kg ; 2 cats) ; phenoxybenzamine (5 mg/kg ; 4 cats) ; disodium cromoglycate (10 mg/kg ; 2 cats) ; proteinase inhibitor (Trasylol K, 10,000 i.u./kg ; 2 cats) ; cortisol (50 mg/kg ; 3 cats). Intravenous infusions of streptokinase in doses sufficient to prolong coagulation time to >20 min (50,000 i.u. over 2 h ; 2 cats) did not prevent development of the constriction and infusions of procaine hydrocaine hydrochloride (2 μg–2 mg/min ; 2 cats) into the superior mesenteric artery did not increase flow before or after the vasoconstriction had developed. Infusions (2–20 μg/min) or injections (0·5–20 μg) of isoprenaline into the superior mesenteric artery (4 cats) and infusions (2–20 μg/min) of adrenaline intravenously (3 cats) produced an increase in mesenteric arterial flow when given before or after development of the vasoconstriction. However, after the administration of endotoxin, we were unable to hold the mesenteric flow at the control level for more than 15–20 min by infusions of

these drugs and repeated bolus injections of isoprenaline produced progressively smaller increases in flow. A similar progressive decrease occurred in the reactive hyperaemia response to 20 s occlusions of the superior mesenteric artery (flowmeter zero checks ; all cats).

At this point it was clear that the mesenteric vasoconstriction was very resistant to the vasodilator agents tested and the possibility of some type of physical block of the vessels had to be considered (see **Discussion**). In 3 cats with an established mesenteric vasoconstriction, EGTA (20 ml, 50 mM) was placed in the peritoneal cavity. Mesenteric flow increased to above the pre-endotoxin level. Irrigation of the peritoneal cavity with saline containing $CaCl_2$ (20 mM) resulted in a rapid redevelopment of the vasoconstriction. EGTA could not be used to maintain mesenteric flow at the pre-endotoxin level for more than a few minutes due to the development of hypocalcaemia.

Successful attempt to prevent mesenteric vasoconstriction

It was found possible to prevent the mesenteric constriction by repeated administration of aminophylline and dextran solution before and for 3 h after administration of endotoxin. Administration of these agents after the constriction developed had no effect (2 cats). The optimal regimen to prevent the constriction was analysed in the following way.

In 3 cats pretreated with aspirin, superior mesenteric arterial flow was recorded and endotoxin (3 mg/kg) was given. As soon as mesenteric flow decreased to 80% of control, aminophylline (5 mg/kg) and dextran solution (5 ml/kg) were administered. These were repeated as often as was necessary to prevent the flow falling below 80% of control. In a further 5 cats, the procedure was similar except that one dose of aminophylline and dextran solution was given before the endotoxin. The results in the 8 experiments are shown in Figure 4. Before administration of endotoxin or aminophylline-dextran solution, mean arterial pressure was

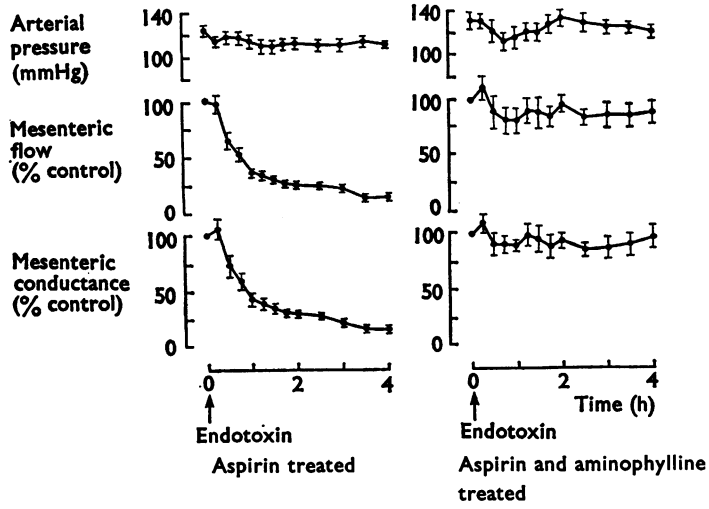

FIG. 4. The mesenteric vascular responses (means ± S.E.) to endotoxin administration in 20 cats pretreated with aspirin and in 8 cats pretreated with aspirin and given aminophylline (5 mg/kg) and dextran solution (5 ml/kg) whenever mesenteric flow decreased below 80% of the control level.

$129 \pm 6{\cdot}3$ mmHg and mean superior mesenteric flow was $23 \pm 1{\cdot}7$ (ml/min)/kg body weight. The mesenteric flows and conductances at all times after 45 min were significantly greater in those 8 cats treated with aminophylline-dextran solution than in the 20 control experiments where this treatment was not given (Fig. 4 ; $P<0{\cdot}001$, unpaired *t*-test).

In these experiments, 4–8 (mean 5) doses of aminophylline-dextran solution were required to prevent mesenteric flow decreasing below 80% of control. The mean times of administration were 18 min before and 27, 50, 85 and 104 min after endotoxin administration. After this period, arterial pressure and mesenteric flow could be maintained by administration of dextran solution (5 ml/kg) only, at 2–3 h intervals for up to 10 h (the longest period tested). Post-mortem examination of these animals revealed no remarkable features. The lungs and intestines appeared normal although there was some fluid in the stomach and small intestine. Histological studies showed no pathological lesions of the intestinal mucosa in one cat treated with aminophylline-dextran solution after endotoxin, while a control cat given only aspirin and endotoxin showed acute necrosis of the intestinal villi.

In 3 experiments, the procedure was similar except that a solution of 5% bovine albumin in Ringer-Locke solution was used instead of dextran solution. In all 3 experiments, the mesenteric vasoconstriction was prevented and 5–7 (mean 6) doses of aminophylline-protein solution were required to prevent mesenteric conductance decreasing below 80% of control.

Survival experiments

From the above observations, a protocol was worked out for the treatment of cats in which the abdomen was not opened and recovery from the anaesthetic agent was allowed (see **Methods** and Table 1). Since preliminary experiments showed that inadequate treatment with aminophylline-dextran solution resulted in gross haemorrhagic lesions of the intestinal mucosa after 24 h, this protocol was based on the largest doses required to prevent the vasoconstriction in the acute experiments. Nineteen cats ($2{\cdot}2 \pm 0{\cdot}2$ kg) were treated according to this protocol. Of these, 12 (63%) recovered and survived indefinitely. The remaining 7 died in $24 \pm 3{\cdot}6$ h (mean $\pm$ S.E.). At the time of death, the heart continued to beat strongly for 1–2 min after respiration stopped. Post-mortem examination showed marked haemorrhagic and congested areas in the lungs of 6 of the cats. The lumen of the intestine was filled with a white gel in 4 cats but a similar gel was aspirated from the stomach of some of the cats which survived. In one cat, the lungs and intestine appeared normal but there was marked haemolysis ; the death of this atypical cat may have been unrelated to our experiment.

It seemed possible that the delayed pulmonary lesions were developing because the cats were surviving long enough for the protective effect of the aspirin pretreatment to disappear. A further 5 cats were therefore treated according to the protocol except that aspirin (20 mg/kg) was given before and at 4 h intervals after the endotoxin administration. Two of these cats died at 16 and 22 h after endotoxin administration with gross haemorrhagic lesions of the lungs. The remaining 3 survived for 3 days and were then killed. Haemorrhagic lesions were visible in the lungs but were much less marked than in the cats which died. In all 5 cats, the intestinal mucosa was macroscopically normal and no white gel was present in the intestinal lumen.

The survival rates in the different groups of experiments are compared in Table 2.

TABLE 2. *% Survival in the various groups of experiments*

Treatment	Number of cats	% Survival
Endotoxin alone	5	0
Aspirin pretreatment + endotoxin	8	25
Aspirin pretreatment, no endotoxin	4	100
Aspirin pretreatment + endotoxin + aminophylline-dextran (Table 1)	19	63
Aspirin pretreatment + aminophylline-dextran (Table 1), no endotoxin	4	100
Aspirin repeated at 4 h intervals + endotoxin + aminophylline-dextran (Table 1)	5	60

Haematocrits and clotting times

Preliminary experiments had shown that the dextran solution had to be given with the aminophylline to prevent hypotension and haemoconcentration. Haematocrits were measured in 6 acute experiments where mesenteric flow was recorded and in 12 survival experiments. These cats were all treated according to the protocol in Table 1. The haematocrits were $32 \pm 1{\cdot}4\%$ (mean ± S.E.) before, $32 \pm 1{\cdot}2\%$ at 2–4 h, $35 \pm 2{\cdot}0\%$ at 5–8 h, $35 \pm 1{\cdot}7\%$ at 10–15 h and $33 \pm 2{\cdot}2\%$ at 15–24 h after endotoxin administration. These haematocrits were not significantly different from the pre-endotoxin values when analysed by the paired t-test ($P > 0{\cdot}1$).

In our previous studies (Greenway & Murthy, 1971), it was found that shortly before death of cats given endotoxin after pretreatment with aspirin, a sample of blood taken into a glass tube remained fluid for more than 30 minutes. Clotting times were therefore measured in cats treated according to the protocol in Table 1; data were obtained in 3 acute experiments where mesenteric flow was recorded and 10 survival experiments. The clotting times were $7{\cdot}3 \pm 0{\cdot}7$ min (mean ± S.E.) before, $6{\cdot}5 \pm 0{\cdot}9$ min at 4–5 h, $8{\cdot}5 \pm 2{\cdot}1$ min at 10 h and $6{\cdot}2 \pm 0{\cdot}3$ min at 15–24 h after endotoxin administration. These clotting times were not significantly different from the pre-endotoxin values when analysed by the paired t-test ($P > 0{\cdot}1$).

Discussion

In these experiments, it was confirmed that the acute pulmonary vasoconstriction after endotoxin administration to cats could be prevented in all animals by pretreatment with a small dose of aspirin (10 mg/kg). This acute response was also absent if the endotoxin was given by slow infusion and this infusion protected the animal against a subsequent bolus injection of endotoxin. This confirms the earlier suggestion that the acute response is of an anaphylactoid nature (Greenway *et al.* 1969; Weil & Spink, 1957). The possible mechanisms for the protective effect of aspirin have been discussed previously (Greenway & Murthy, 1971; Murthy & Greenway, 1972). The recurrence of severe pulmonary damage in cats which died 24 h after treatment with the protocol (Table 1) is discussed below.

Endotoxin administration caused mesenteric vasoconstriction in cats anaesthetized with pentobarbitone and pretreated with aspirin. This was a consistent and intense response and it has occurred in every one of more than 100 cats studied up to this time. The constriction occurred only in the intestinal vascular bed, and the smooth muscle of the intestinal wall was not contracted. It occurred in the absence of aspirin when endotoxin was administered slowly to avoid acute death due to pul-

monary vasoconstriction. The constriction did not involve the intestinal innervation since it occurred in the isolated donor-perfused intestine and it was not due to circulating vasoconstrictor agents. This confirms our previous conclusions (Greenway & Murthy, 1971).

In view of the intensity of this constriction and the complete inability of a variety of agents to prevent or reverse it, the possibility of some type of physical block of the vessels has to be considered. Disputed possibilities include obstruction by fibrin threads produced during disseminated intravascular coagulation (Attar, Hanashiro, Mansberger, McLaughlin, Firminger & Cowley, 1970; Beller, 1969; Beller, Graeff & Gorstein, 1969; Bergentz & Leandoer, 1971; Hardaway, 1969; Lucas & Kitzmiller, 1972; McKay, Linder & Cruse, 1971; Muller-Berghaus, 1969), platelet aggregates (Swank & Edwards, 1968) or obstruction to capillaries secondary to endothelial damage (McGrath & Stewart, 1969). Although these seemed unlikely since the constriction was confined to the intestinal bed and was unaltered after administration of heparin or streptokinase in doses sufficient to prevent coagulation, we examined the effects of intraperitoneal administration of EGTA. The constriction was rapidly abolished and it returned within a minute or so after replacement of Ca^{++} ions. Although not conclusive proof, these observations suggest that the decrease in mesenteric flow was due to a true constriction of the vascular smooth muscle and not to a physical block of the vessels.

The mechanism producing the mesenteric constriction was a local one within the intestine and no significant circulating vasoconstrictor agents were present in arterial blood. The constriction took at least 30 min to develop and this delay was due to some mechanism within the intestine since it was not shortened when an isolated intestine was perfused with blood from an animal previously exposed to endotoxin for 2 hours. Our experiments do not show if the stimulus in the arterial blood of the donor cat was endotoxin itself, some metabolite of endotoxin or some other substance released by the animal and further studies are necessary to examine these possibilities. Studies on isolated organ preparations have not revealed any direct effects of endotoxin on smooth muscle (Filkins, 1969; Hinshaw, Kuida, Gilbert & Visscher, 1957; Kobold, Lovell, Katz & Thal, 1964; Kutner & Cohen, 1966; Vargas & Beck, 1957; Vick, 1964; Greenway, C. V. unpublished observations) but mesenteric arteriolar smooth muscle has not been examined. We have not so far been able to produce an isolated intestinal preparation which is perfused by an artificial medium and which responds to vasoactive drugs for 2 hours. It therefore remains possible that endotoxin reacts with some component of the blood within the intestine to release a vasoconstrictor agent or that it causes local release from some intestinal tissue. It is also possible that endotoxin is metabolized by intestinal tissue to an active vasoconstrictor agent.

In the experiments where the mesenteric constriction was prevented by treatment with aminophylline-dextran, several but not all of the cats had a thick white gel in the intestinal lumen and a similar gel was aspirated from the stomach of some of the cats which survived. This may be a mucosal secretion since *Escherichia coli* enterotoxin has been shown to stimulate jejunal secretion (Pierce & Wallace, 1972). It was not seen in the cats given repeated doses of aspirin at 4 h intervals and this suggests that the endotoxin-induced secretion is blocked by aspirin. A similar antagonism by aspirin of intestinal secretion induced by cholera enterotoxin has been described by Jacoby & Marshall (1972).

A primary aim of this study was to determine the lethal effects of endotoxin in cats (see **Introduction**). As we have discussed previously, a marked reduction in mesenteric blood flow is known to be lethal in cats (Greenway & Murthy, 1971) and Hagland & Lundgren (1972) have recently shown that mechanical reduction of superior mesenteric arterial flow caused hypotension and progressive deterioration of the whole cardiovascular system. Mesenteric obstruction can also produce many of the characteristic features seen after endotoxin (Fine, Palmerio & Rutenburg, 1968 ; Hershey, Baez & Rovenstine, 1961). Thus mesenteric vasoconstriction of the degree seen in our experiments is probably lethal but until it could be prevented, it was impossible to state whether there were additional lethal effects of endotoxin. Many agents were tested in attempts to prevent or reverse this vasoconstriction. Most were used to test a variety of possible theories on the mechanism of the constriction but since they were ineffective, these theories are not worth elaboration. Only brief details of these experiments were included to emphasize the intensity and apparent irreversibility of the constriction.

In view of these failures, it was surprising that it was possible to prevent the constriction with aminophylline-dextran solution. Large doses were required (Table 1) and inadequate treatment resulted in a marked haemorrhagic necrosis of the intestine which was not seen when no treatment was given. Thus inadequate treatment intensified rather than reduced the macroscopically obvious intestinal mucosal damage, possibly because it partially dilated the arterioles after ischaemic mucosal damage had occurred. The relative roles of the aminophylline and the dextran solution are not yet clear. Aminophylline alone causes hypotension and haemoconcentration while dextran solution alone had some protective effect but was much less effective than when combined with aminophylline (Cohen, M. M., unpublished observations). Since aminophylline is a diuretic and the cats to which it was given produced large volumes of urine, administration of dextran solution alone is not a satisfactory way to assess the role of fluid in this combination. A 5% solution of bovine albumin was successfully substituted for the dextran in 3 experiments and this appears to exclude any specific pharmacological action of dextran, for example, on platelets (Weiss, 1967). Aminophylline is a vasodilator agent and is most likely to be acting as a physiological antagonist to some constrictor agent produced after endotoxin administration. However, it also has effects on platelets (Ardlie, Glew, Schultz & Schwartz, 1967 ; Cole, Robison & Hartmann, 1971). Aspirin will not prevent the mesenteric constriction after endotoxin but until more is known about the effects of aspirin and aminophylline on platelets, the involvement of platelets in the constriction cannot be excluded. Aminophylline-dextran solution are not yet clear. Aminophylline alone causes hypotension and administration. This may be an independent effect but it is more likely that the clotting defect is a secondary consequence of the mesenteric vasoconstriction. Aminophylline has been shown to shorten coagulation time, probably by increasing the plasma level of factor V, and its smooth muscle relaxant effect may involve increased levels of cyclic 3′,5′-AMP due to inhibition of phosphodiesterase (Ritchie, 1970). However, isoprenaline infusions would not prevent the mesenteric vasoconstriction. Thus there are many intriguing possibilities which can be considered in relation to the mechanism of prevention of both the clotting defect and the mesenteric constriction by aminophylline-dextran solution but further studies are required before a reasonable hypothesis can be suggested.

The protocol used in the survival studies resulted in survival of 63% of the cats.

This is an improvement but is still clearly unsatisfactory. The cats which died showed marked pulmonary lesions. The development of these lesions was not prevented either by prolonged treatment with aspirin or by careful attempts to prevent aspiration of gastric contents during vomiting. Pulmonary lesions were present, but were less severe, in 3 survivors which were killed after 3 days. Thus prevention of the acute pulmonary vasoconstriction and of the intestinal vasoconstriction reveals a delayed pulmonary damage which is not prevented by aspirin. This appears to be sufficiently severe to account for our failure to obtain 100% survival after endotoxin administration.

The relationship of this work to endotoxin shock in other species including man remains obscure. In dogs, although the acute response to endotoxin administration involves outflow block of the liver which is not seen in cats (Greenway & Stark, 1971), a severe maintained vasoconstriction of the intestine develops over 60 min (Brungardt, Reynolds & Swan, 1972; Hinshaw, 1968; Kux, Holmes, Hinshaw & Massion, 1971; Lillehei, Dietzman & Movsas, 1967; Rayner, McLean & Grim, 1960). Mesenteric vasoconstriction does not appear to occur in sub-human primates (Brobmann, Ulano, Hinshaw & Jacobson, 1970; Wyler, Forsyth, Nies, Neutze & Melmon, 1969) but there have been some reports of haemorrhagic necrosis of the small intestine in man (Horton, Murthy & Seal, 1968). However, the clotting defect is prominent in both primates and man (Beller, 1969; Cavanagh, Rao, Sutton, Bhagat & Bachmann, 1970; Hardaway, 1969; Muller-Berghaus, 1969). The appearance of delayed pulmonary damage in the cats treated with aspirin and aminophylline-dextran solution suggests that the pulmonary lesions in man will not respond to aspirin as we hopefully suggested previously (Murthy & Greenway, 1972).

As is usual in studies on endotoxin, this work raises more problems than it solves. Nevertheless it suggests many further studies which can be done and even if the results are not applicable to man, we suggest that an understanding of the actions of endotoxin in cats may be of some value in elucidating the actions in other species. In addition, the signs and clinical course of feline panleucopenia (distemper) bear a remarkable resemblance to those following endotoxin administration. Although this is a viral disease of cats, infection of germ-free cats with the virus produces few clinical signs and no mortality (Rohovsky & Fowler, 1971) suggesting that secondary bacterial infection plays an important role. If an effective therapy against injected endotoxin can be found in the cat, it may prove useful in the treatment of panleucopenia which currently has a very high mortality.

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