UNIVERSITY OF OKLAHOMA MEDICAL CENTER

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Technical Report No. 26
University of Oklahoma Medical Center THEMIS Contract

MEDICAL CENTER RESEARCH AND DEVELOPMENT OFFICE
OF THE UNIVERSITY OF OKLAHOMA FOUNDATION, INC.
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ABSTRACT

We tested the hypothesis that septic shock includes mesenteric vasoconstriction as an essential step in the pathogenesis of the disorder. This hypothesis has been based upon experiments using the canine model which does respond to endotoxin by developing mesenteric constriction and ischemia. We measured systemic arterial and portal venous pressures and mesenteric blood flow in 6 anesthetized rhesus monkeys and 6 anesthetized dogs during periods of control and for 4 hours after injection of lethal doses of E. coli endotoxin. Dogs responded as reported previously with abrupt but transient marked portal hypertension, early systemic arterial hypotension and a profound decline in mesenteric blood flow. Calculated vascular resistance steadily increased after endotoxin. In monkeys the circulatory responses were different: 1) arterial pressure fell gradually; 2) portal pressure increase was small; 3) mesenteric blood flow did not decrease; and 4) calculated mesenteric vascular resistance decreased steadily following injection of endotoxin. In contrast to previous findings in dogs, it appears that a key step in human septic shock may be mesenteric vasodilation, since the subhuman primate exhibits this hemodynamic response to endotoxin.

Most investigations of the hemodynamics of endotoxin shock have been performed in the dog. Results from these experiments showed evidence for a sympathomimetic effect of endotoxin resulting in vasospasm in small arteries and veins in splanchnic visceral organs, especially in the intestine (3-8, 15-19, 20 22). Based upon canine studies, a widely accepted hypothesis regarding the pathophysiology of human septic shock has been postulated. Its essential feature is the contention that the splanchnic vasculature responds to endotoxin with vasoconstriction.

The closer phylogenetic relationship between monkey and man suggests that results in the shocked dog should be corroborated in the monkey before extrapolating to man. There are features in the primate response to endotoxin which differ from those of the dog (2, 9, 10, 11, 14, 27, 28, 30), including the finding that the blood vessels of the perfused, denervated gut of the monkey failed to constrict with endotoxin (12).

Since the mesenteric vascular response to endotoxin seems to be a crucial point, the present study was undertaken to determine whether endotoxin evokes vasoconstriction in the intact intestinal circulation of the monkey.

METHODS

The experimental subjects consisted of 2 groups of adult animals: 9 male rhesus monkeys weighing 3.3 to 7.3 kg and 6 mongrel dogs of both sexes weighing 11 to 19 kg. The animals were anesthetized with intravenous sodium pentobarbital (30 mg/kg), and supplemental amounts were administered to maintain light surgical anesthesia. Escherichia coli endotoxin (Difco) from the same batch used in the present study was pretested for its lethality. In both dogs and monkeys a lethal dose (LD_{100}) of endotoxin was used: dogs, 1 mg/kg; monkeys, 4 mg/kg.

The surgical procedure for the 2 species was identical, except that we used an endotracheal tube and larger catheters and flow transducers in the dog. The monkeys were anesthetized and placed in a supine position. After a median incision, a cannula was introduced in the upper trachea. Every 15 minutes room air under position pressure was forced through this cannula with a piston respirator to inflate the lungs and prevent atelectasis. Otherwise, the animals breathed spontaneously. A midline abdominal laparotomy was performed, the superior mesenteric artery carefully exposed, a 2.5 mm size blood flow transducer (Micron Instruments) implanted on the primate vessel and connected to a gated sine-wave type electromagnetic blood flow amplifier (Biotronix Laboratory, Inc.). Distal to the transducer we implanted an hydraulic occluder (13) on the artery to permit occlusion for periodic measurement of zero flow. Absolute flow was determined by measuring the deflection from zero flow using pre-calibrated transducers (13). A PE 90 catheter was passed through a branch of the splenic vein into the portal vein to measure portal vein pressure. The abdomen was then closed with towel clamps. The left femoral artery was isolated and a PE 260 catheter was inserted into the abdominal aorta to measure systemic arterial pressure. Both catheters were connected to pressure transducers (Sanborn Co.). The left femoral vein was cannulated to inject additional anesthesia and endotoxin. A direct writing polygraph (Sanborn Co.) was used to record mean systemic arterial pressure, portal venous pressure, superior mesenteric artery blood flow and the ECG. Mesenteric vascular resistance was calculated as the pressure gradient (arterial-portal venous) divided by mesenteric blood flow and was expressed in mm Hg/ml/min.

The rectal temperature was continuously measured by a thermometer and the spontaneous decrease in temperature during anesthesia and endotoxin shock was

prevented by means of a warming blanket. CO_2 and pH determinations of arterial blood were made at hourly intervals throughout the experiment (Astrop Microtonometer, Radiometer).

After the preparatory surgery was completed and the animal was allowed to stabilize, control measurements were obtained every 15 minutes for 1 hour. Then a lethal dose of endotoxin was injected intravenously and the animal was observed for 4 hours, with measurements recorded every 15 minutes. After 4 hours the animals were sacrificed with a lethal dose of pentobarbital.

Statistical analysis was performed within each series of animals using the Sign Test (25). We also compared results of monkeys versus dogs for each parameter using the Mann-Whitney U Test (26).

RESULTS

1) Dogs, experimental group.

This group consisted of 6 dogs. Mean systemic arterial pressures dropped within 30 seconds after the injection of endotoxin and reached its lowest value at 3 minutes. There was a recovery with a maximum at 30 minutes, then a second gradual drop over the next 3 1/2 hours. All values recorded after endotoxin were significantly ($p < .05$) lower than pre-injection values, except the pressures at 30 minutes post-injection (Fig. 1). Portal venous pressures showed a sharp increase within 30 seconds after endotoxin with a maximum at 3 minutes; all portal pressure values were significantly ($p < .05$) greater than control for 30 minutes. By 45 minutes post-injection, portal pressures had returned to pre-injection values and were not significantly different from control for the subsequent 3 1/2 hours (Fig. 2). Superior mesenteric artery blood flow changes approximately mirrored those of systemic arterial pressure: sudden decrease with a nadir at 3 minutes, slight recovery with a maximum at 30 minutes, then a subsequent gradual decline

(Fig. 3). However, all values for mesenteric blood flow were significantly lower than control and the decrease in flow exceeded the fall in pressure. Mesenteric vascular resistance increased following injection of endotoxin, and by the end of our observation period was 75% above pre-injection values (Fig. 4). The increase in resistance was significant for all values from the 2nd to the 4th hour after endotoxin.

Significant ($p < .05$) changes in arterial pCO_2 occurred at 3 and 4 hours post-injection and in respiratory and heart rates at various times after endotoxin. These values appear in Table I.

2) Monkey, control group.

We used 3 monkeys for control measurements. No endotoxin was injected into these animals, but they were observed for 4 hours past the time the lipopolysaccharide would have been administered. There were no significant changes in either systemic arterial pressure, portal venous pressure or mesenteric blood flow during the observation period.

In these animals arterial pressure was 110 ± 5 (S.E.) mm Hg at the start of control and 108 ± 3 mm Hg 4 hours later. Comparable values for portal venous pressure were 4 ± 1 and 5 ± 2 mm Hg; for mesenteric blood flow these values were 75 ± 15 and 80 ± 8 ml/min.

3) Monkey, experimental group.

This group consisted of 6 animals. Following injection of endotoxin, mean systemic arterial pressure fell gradually, the lowest pressure coming at 90 minutes after the intravenous injection of endotoxin (Fig. 1). The hypotensive response occurred in all 6 animals and was significantly ($p < .05$) lower than control values for all times from 45 minutes to 4 hours after endotoxin. Portal pressure increased slightly and reached its maximum at 45 minutes after endotoxin application but the post-injection values were not statistically

different from pre-injection values until 2 hours and 45 minutes after endotoxin (Fig. 2). Blood flow in the superior mesenteric artery showed no significant change at any time after injection of endotoxin (Fig. 3). Calculated mesenteric vascular resistance decreased after endotoxin, reflecting the fall in arterial pressure with unchanged flow (Fig. 4). All resistance values from 45 minutes to 4 hours after endotoxin were significantly ($p < .05$) less than control. There were significant ($p < .05$) increases in respiratory and heart rates and a decline ($p < .05$) in arterial pCO_2 after endotoxin (Table I). Comparison of canine versus primate hemodynamic responses after injection of endotoxin revealed significant ($p < .05$) differences as follows: 1) arterial pressure decreased more in dogs at 15 minutes and more in monkeys at 45, 60 and 75 minutes; 2) portal pressure elevation was greater in dogs at 3, 15 and 30 minutes and greater in monkeys at 2 1/2 to 3 1/2 hours; 3) dogs had a larger decrease in mesenteric blood flow at all times after endotoxin; 4) mesenteric vascular resistance decreased in monkeys and increased in dogs with the difference being significant from 30 minutes on.

DISCUSSION

Our findings indicate that the circulations of dogs and monkeys do not respond in the same way to the injection of lethal doses of endotoxin. The early circulatory responses of the dog to endotoxin have been reported often (1, 10, 15-19). The dog reacts to endotoxin with hepatic venoconstriction, portal hypertension, and sequestration of blood in the splanchnic viscera. Venous return falls and there follows an abrupt decline in cardiac output, arterial pressure and mesenteric artery blood flow. These changes show some mitigation by 15 minutes and portal pressure is restored to normal by 30 minutes after injection of endotoxin. The recovery in arterial pressure exceeds that of

mesenteric blood flow, so calculated mesenteric vascular resistance progressively increases after endotoxin. Similar changes occur with endotoxin in the rat (29). Our findings fully confirm previous reports of canine mesenteric vascular responses to endotoxin.

The monkey, however, does not exhibit such hemodynamic responses to endotoxin. There is no massive sequestration of blood in the abdominal viscera and no striking portal hypertension (15-19). Arterial hypotension is a more gradual development after endotoxin. The most significant difference between the canine and the primate circulatory changes in endotoxin shock is the stable mesenteric blood flow in the monkey. This indicates that in the primate the mesenteric vasculature has dilated, whereas in the dog mesenteric vasoconstriction is the characteristic shock response.

The hypothesis developed by Fine (3-8), Lillehei (15-19) and Nickerson (20-22) concerning human septic shock is predicated on the existence of splanchnic vasoconstriction in the animal model. If human septic shock resembles experimental endotoxin shock in dogs or rats, mesenteric ischemia should be a characteristic of the human disorder. If, on the other hand, the disease in man resembles the shock model in another primate, the monkey, then mesenteric vasoconstriction is not an early essential step in the pathogenesis of septic shock. It is apparent also that had the proponents of the aforementioned hypothesis used monkeys instead of dogs as their experimental animal, they would have evolved a different hypothesis.

In support of our finding that the monkey exhibits mesenteric vasodilation in endotoxin shock is the report of a similar response in the denervated, pump-perfused primate gut (12). Furthermore, total peripheral resistance declines in the monkey in endotoxin shock (9, 11, 23, 30). Since the mesenteric circulation may either constrict or dilate in lethal shock states in various animal models, it would appear hazardous to speculate that the circulation of the gut is a preferential target in human septic shock.

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	DOGS				MONKEYS			
Time (hrs.)	pH	pCO_2	Resp. Rate	Heart Rate	pH	pCO_2	Resp. Rate	Heart Rate
0	7.24	43	8	198	7.38	40	33	194
1	7.28	41	9	196	7.38	38	35	200
2	7.22	34	17	*181	7.40	*31	*49	*226
3	7.20	31	*16	197	7.35	*30	*54	*222
4	7.22	*31	15	191	7.32	*31	*52	217
5	7.27	*28	*19	185	7.32	*32	48	208

TABLE I. Effects of intravenous injection of a LD_{100} of endotoxin in a series of dogs and monkeys in which the following measurements were made: arterial pH, arterial pCO_2 (mm Hg) respiration rate (breaths/min), and heart rate (beats/min). Endotoxin was injected 1 hour after starting the experiment. An asterisk indicates significant differences ($p < .05$) from pre-injection values (mean of 6 dogs and 6 monkeys). Significance was determined with the Sign Test (25).

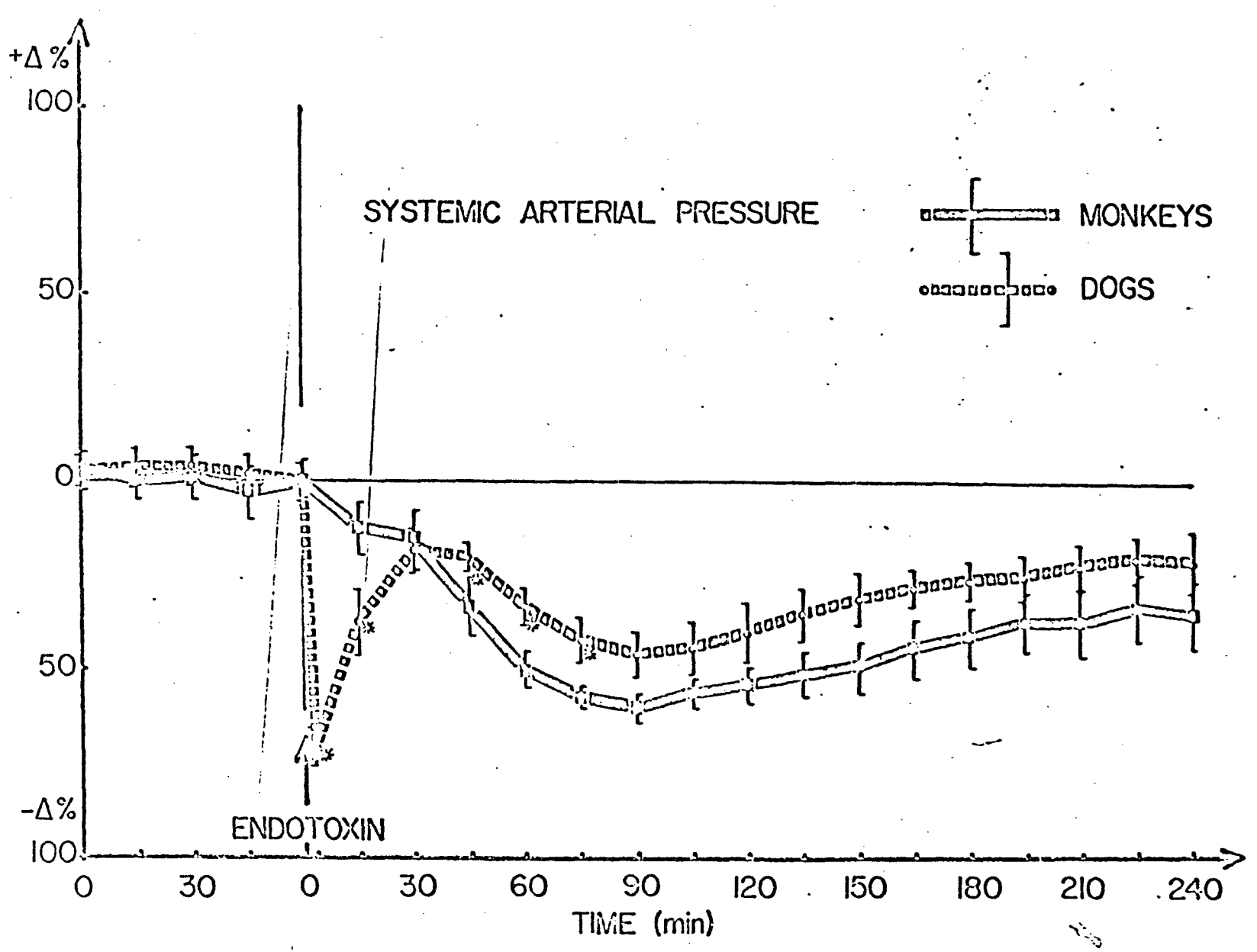
LEGENDS

Figure 1. Comparison of the effect of endotoxin on mean systemic arterial pressure in dogs and monkeys. Values are expressed in % change (± S.E.M.) from control (pre-injection values). An asterisk indicates significant difference in values between the two series (dog versus monkey).

Figure 2. Comparison of the effect of endotoxin on portal venous pressure in dog and monkey. Values are expressed in % change (± S.E.M.) from control. An asterisk indicates significant differences between the two series.

Figure 3. Comparison of the effect of endotoxin on superior mesenteric artery blood flow in dog and monkey. Values expressed in % change (± S.E.M.) from control. An asterisk indicates significant differences between the two series.

Figure 4. Comparison of the effect of endotoxin on mesenteric vascular resistance in dog and monkey. Values are expressed in % change from control. An asterisk indicates significant differences between the two series.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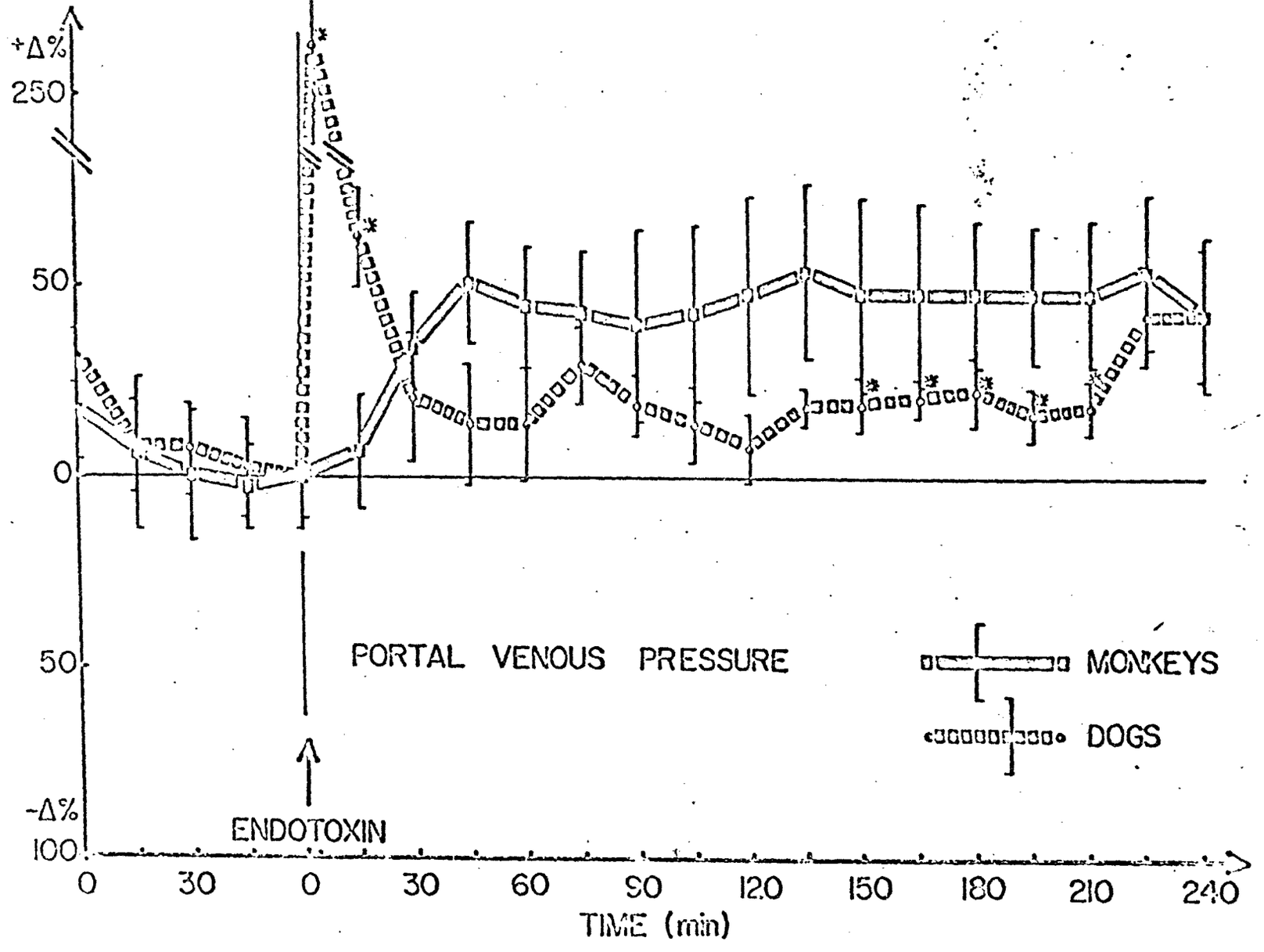
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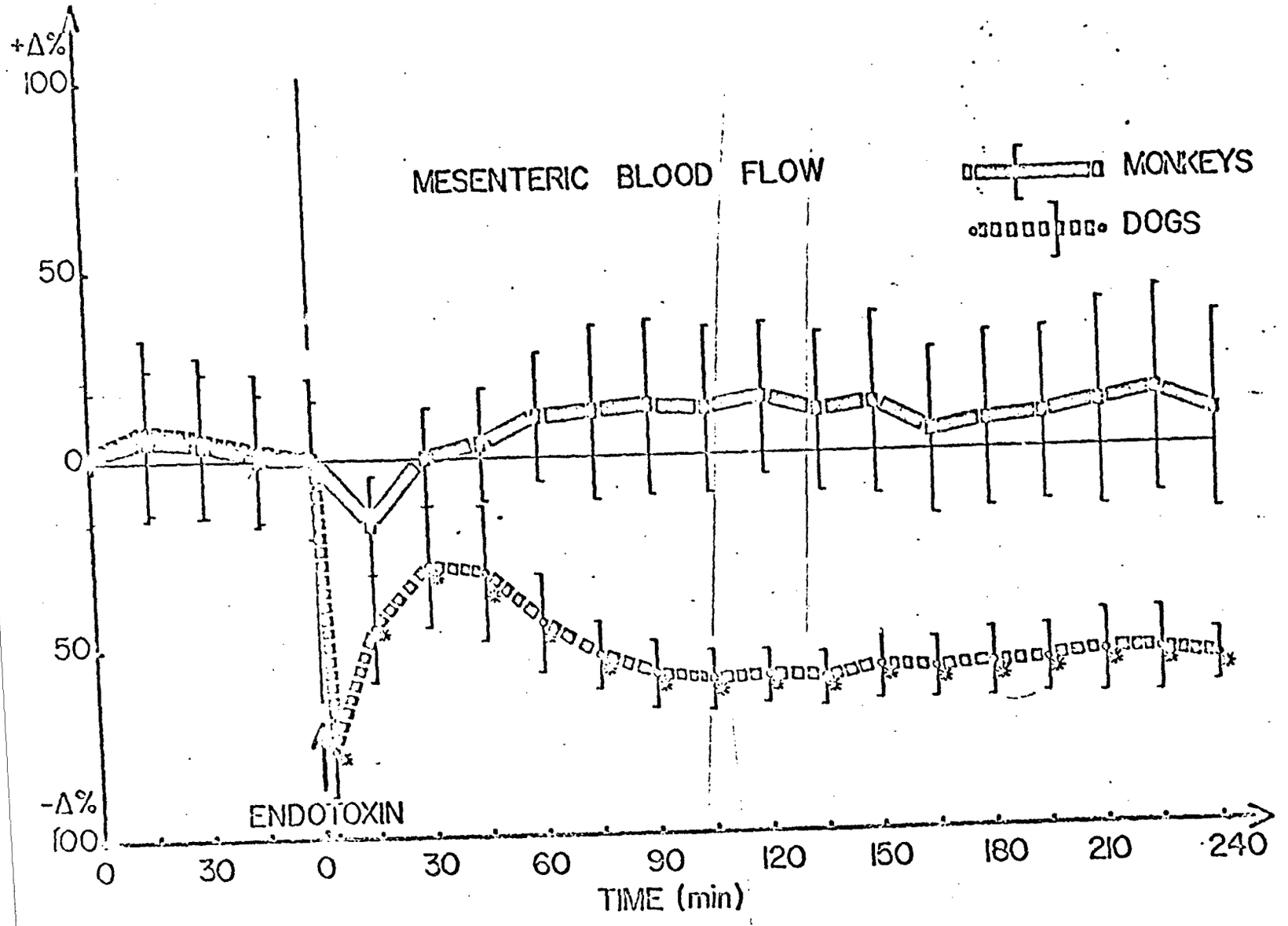
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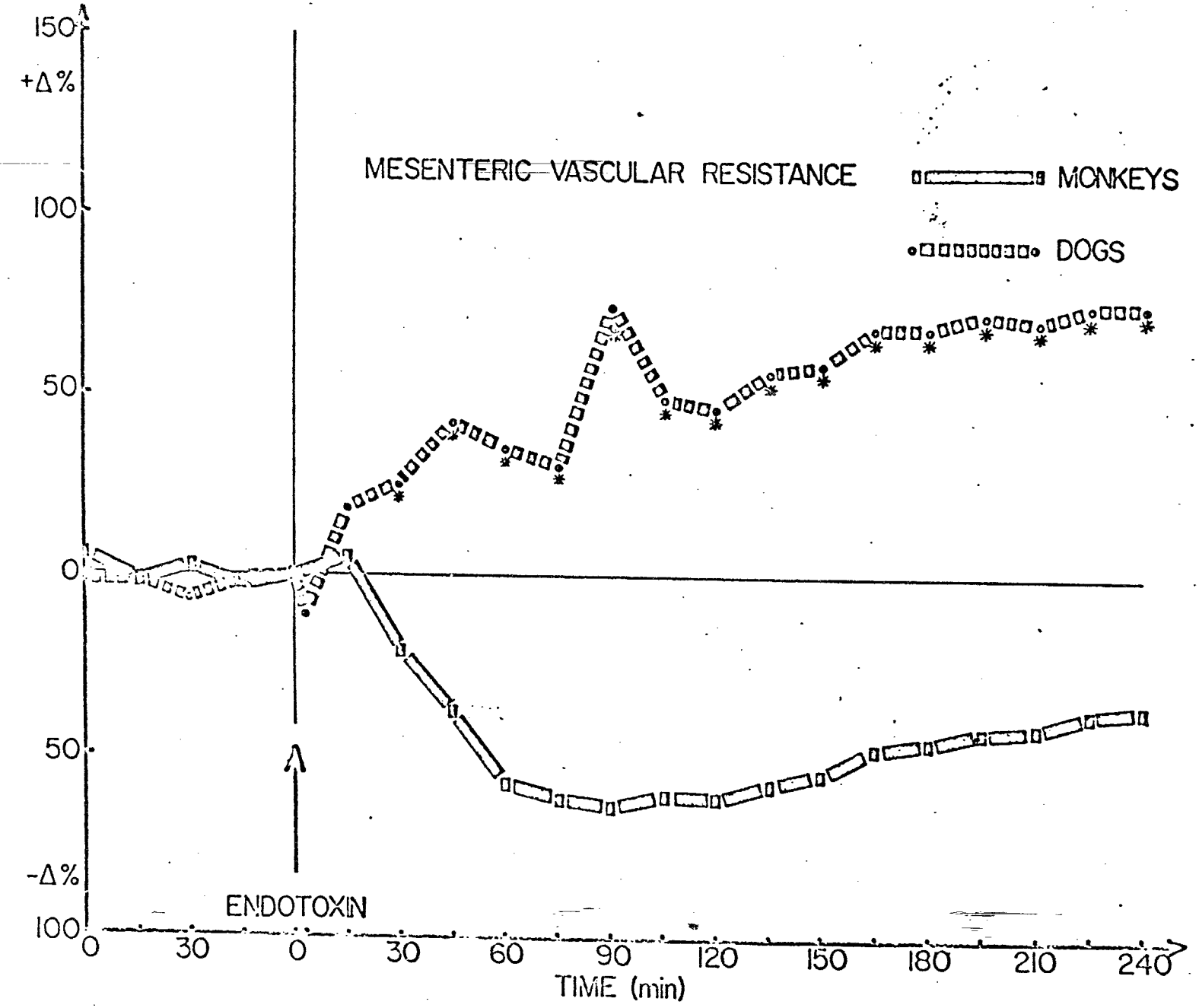
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