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THROMBOXANE A_2 AND HEMODYNAMIC-BIOCHEMICAL PARAMETERS IN CANINE ENDOTOXIN SHOCK

BY

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ABSTRACT

Prostaglandins participate in the pathophysiology of endotoxin shock; however, their exact role has not yet been clear. In this study, we investigated the role of the proaggregatory vasoconstrictor, thoromboxane A_2 ($T\times A_2$), an arachidonic acid metabolite, during canine endotoxin shock. The central venous plasma levels of thromboxane B_2 ($T\times B_2$), the stable metabolite of $T\times A_2$, was measured by radioimmunoassay. We also investigated the therapeutic effect of reduced glutathione (GSH), a potential cell-stabilizing sulfhydryl compound, in canine endotoxin shock. Sixty minutes after the intraveous administration of E. coli endotoxin (1 mg/kg), the plasma $T\times B_2$ levels were significantly increased from 68.8±49.0 pg/m*l* to 318.3±117.2 pg/m*l* (N=5) in the control group and from 67.9±68,4 pg/m*l* to 222.6±133.2 pg/m*l* (N=5) in the GSH (300 mg/kg/hr) group. The levels in the GSH group were somewhat lower than in the control group for 60 to 180 minutes after the injection of endotoxin. Thromboxane A_2 value appear not to relate to early thrombocytopenia and pulmonary hypertension but to relate to the change of late coagulopathy and of pulmonary vascular resistance. The administration of GSH suppressed the lactic acidemia significantly, however there was a much more decrease in the mean arterial pressure in the GSH group than in the control group. In addition, there was a tendency to inhibit the increase of the serum β-glucronidase activity in the GSH group.

Key words: canine endotoxin shock, thromboxane A_2, thromboxane B_2, reduced glutathione

INTRODUCTION

Gram-negative septic shock is a serious clinical condition and despite the great advances in intensive care therapy, still carries a high mortality of greater than 50 per cent (Ledingham *et al.* [1]; Nakagawa *et al.* [2]). Live gram-negative organisms and bacterial endotoxin have similar pathophysiological effects (Guenter *et al.* [3]), and so the experimental endotoxin shock is a recognized model for the study of gram-negative septic shock.

It is generally accepted that platelets play an important role in the pathogenesis of endotoxin shock (Müller-Berghaus *et al.* [4]) because thrombocytopenia is a constant feature of this state and disseminated intravascular coagulation (DIC), which so frequently accompanies it (Beller [5]: Prager *et al.* [6]). The precise interaction between the endotoxin and the cellular elements of the blood has not yet been defined clearly. Over the past decade it has become increasingly apparent that prostaglandins are involved in the cellular function of the blood. Recently the arachidonate metabolite, thromboxane A_2 ($T\times A_2$), which is known as a potent proaggregator and vaso-

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constrictor, has been shown to be increased markedly in the rat (Cook *et al.* [7]) and baboon (Fletcher *et al.* [8]) during endotoxemia. So we investigated the possibility of the mechanism that $T\times A_2$ may mediate the endotoxin-induced alterations in hemodynamics as well as disseminated coagulopathies during canine endotoxin shock.

The administration of sulfhydryl compounds such as reduced glutathione (GSH) and cysteine has been demonstrated to diminish the susceptibility of the animals to the endotoxin (Szymanski *et al.* [9]) and traumatic shock (Yamada [10]). We also investigated some of the circulatory and biochemical actions of GSH in canine endototxin shock.

Materials and Methods

Ten mongrel dogs (7–15 kg) were anesthetized with 20 mg/kg of intravenous secobarbital sodium and intubated with succinylcholine chloride. Ventilation was kept with mixed air and oxygen to adjust the control values of Pao_2 and $Paco_2$ within the normal range by a volume-preset ventilator. Catheters were introduced into the right femoral artery, left femoral vein and right jugular vein. The position of the pulmonary artery-catheter (Swan-Ganz thermodilution) and central venous-catheter were monitored by pressure tracings. The latter catheter was utilized for blood sampling and for the administration of endotoxin and fluid during the experiment. Five m*l*/kg of Ringer's solution were infused until the end of the experiment. Cardiac output (C.O) was measured by the thermal dilution method and calculated on a computer program.

Systemic arterial and central venous blood samples for blood gas and coagulation and biochemical parameters were obtained during the control period and at 5 minutes and at hourly intervals for three hours after the administration of endotoxin. Hemodynamic parameters were measured just before giving the endotoxin and at 5 and 30 minutes and at hourly intervals for three hours after the administration of endotoxin.

After the measurements of the baselines for all variables, endotoxin shock was established by an intravenous bolus injection of 1 mg/kg of Difco E. coli endotoxin. All endotoxins used were prepared from the same lot and were resolved every time, using a 0.9% saline solution. Following the administration of endotoxin, the dogs were divided into two groups, 5 into the control and other 5 into the GSH group. In the GSH group, 300 mg (5 m*l*)/kg/hr of GSH were injected instead of Ringer's solution for three hours immediately after the administration of endotoxin.

The hemodynamic parameters measured were the heart rate (H.R), arterial blood pressure (ABP), pulmonary artery pressure (PAP), pulmonary capillary wedged pressure (PCWP), central venous pressure (CVP) and cardiac output (C.O). The blood samples were used to perform the following determinations: Blood gas, plasma $T\times B_2$, plasma lactic acid, serum β-glucronidase, platelet count, fibrinogen, prothrombin time (P.T) and fibrinogen/fibrin degradation products (FDP).

The blood for $T\times B_2$ assay was collected in the heparinized syringes containing 30 μl of 10^{-2} M indomethasin dissolved in pH 8.0, 0.1M sodium phosphate buffer. The blood was centrifuged at 4°C at 3,000 rpm for 10 minutes and kept frozen (−70°C) until assayed. $T\times B_2$ was determined using the previously described radioimmunoassay method (Koh *et al.* [11]). The plasma lactic acid concentration was determined by the lactic

acid analyzer (Abbot-ABA 50). The concentration of serum β-glucronidase was measured by the modified Fishman's method (Shimazu UV 150–02) and FDP was determined by the FDPL test (Teikoku Zoki).

Statistical analysis was performed by the paired, two-tailed student t test for the values of the experiment subtracted by that of the baseline in the same animals and also performed by the unpaired, two-tailed t test for the difference between two groups. A P value of 0.05 or less was considered significant. All date are expressed as the mean±standard deviation of the mean.

Results

I. *Hemodynamic evaluation*

Figure 1 shows the typical tracings of the endotoxin shock in the early time course. The control tracings of ABP, PAP, CVP, standard Lead III of the electrocardiogram and C.O preceded the

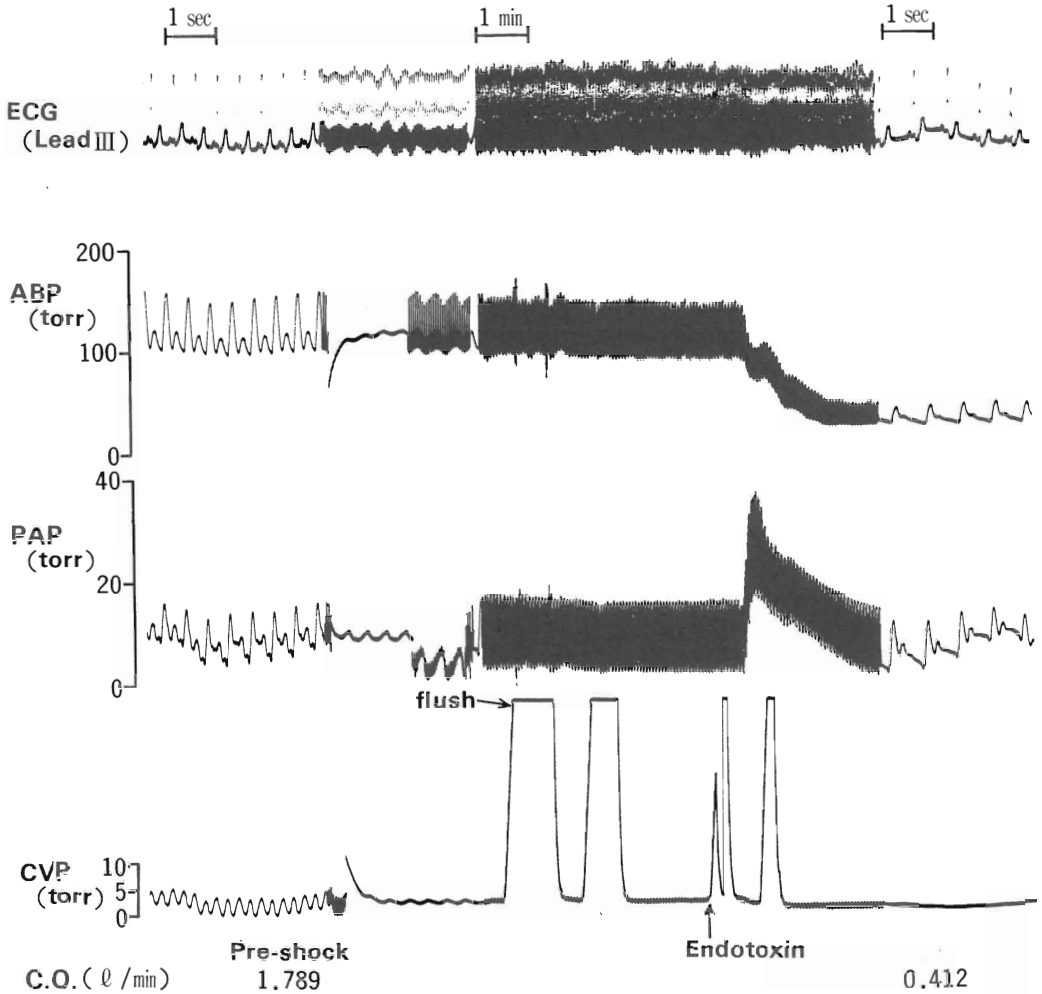

Fig. 1. Direct Hemodynamic Measurements Demonstrating the Early Time Course in the Endotoxin-treated Dog.
ECG: Standard lead III of the scalar electrocardiogram; ABP: Arterial blood pressure; PAP: Pulmonary artery pressure; CVP: Central venous pressure; C.O: Cardiac output.

infusion of endotoxin. Immediately after the injection of endotoxin, an abrupt fall was observed in the ABP, CVP and C.O and an abrupt rise in PAP. However, PAP returned to the control level within 5 minutes. Hemodynamic changes in the two groups are shown in Table 1. Following the injection of endotoxin, the MAP in both groups immediately fell down significantly to approximately 60% of the control value, followed by a slow progressive rise. The MAP of the GSH group was somewhat lower than in the control group throughout the experimet (Fig. 2). The TPR in both groups increased transiently at 5 minutes and decreased for 60 minutes after the endotoxin injection and then gradually increased. Although there were no significant differences between the TPR in the two groups, the TPR in the GSH group showed a tendency to become lower than that in the control gruop 30 to 180 minutes after the injection of endotoxin (Fig. 3). The C.O decreased immediately after the injection of endotoxin in both groups followed by a slow progressive increase, and two hours later both

Table 1. Hemodynamic Changes during Endotoxin Shock, MAP: Mean arterial blood pressure; MPA: Mean pulmonary artery pressure; PCWP: Pulmonary capillary wedge pressure; CVP: Central venous pressure; C.O: Cardiac output; H.R: Heart rate; TPR: Total peripheral resistance; PVR: Pulmonary vascular resistance; Values=mean±SD
*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$

Parameter	Group	Control reading	Time course after administration of endotoxin (min)				
			5	30	60	120	180
MAP (%)	control	100	61.0± 33.9*	79.6 ± 20.0	68.9 ± 17.0**	69.5 ± 13.4***	80.7± 10.7**
	GSH	100	54.0± 22.1**	60.8 ± 15.1***	62.3 ± 15.1***	65.9 ± 27.8*	71.7± 29.7
MPA (%)	control	100	149.8± 50.4	120.1 ± 25.7	119.7 ± 27.1	120.9 ± 31.4	131.3± 34.5
	GSH	100	79.0± 12.9**	98.1 ± 20.2	109.4 ± 18.2	111.7 ± 26.8	112.4± 23.2
PCWP (torr)	control	4.4 ± 2.3	2.9± 3.5	3.2± 2.8	3.3± 3.5	2.6± 2.7	2.6± 3.4
	GSH	3.8 ± 1.5	1.8± 1.3	2.9± 1.1	1.8± 0.8*	1.6± 1.3*	1.6± 1.3*
CVP (torr)	control	4.0 ± 1.9	2.6± 1.9	3.2± 1.6	2.8± 1.8	2.8± 1.9	2.4± 2.6
	GSH	2.6 ± 1.5	1.3± 1.0	1.7± 1.0	1.8± 0.8	1.4± 0.9	1.6± 0.9
C.O. (%)	control	100	66.1± 31.6*	75.0 ± 18.2*	80.8 ± 12.3**	72.8 ± 15.0***	62.8± 24.5**
	GSH	100	34.8± 14.3***	69.1 ± 32.0	77.7 ± 28.8	68.0 ± 21.2**	62.4± 20.6**
H.R. (%)	control	100	102.4± 13.2	96.8 ± 12.3	105.3 ± 30.6	102.6 ± 30.5	99.9± 27.5
	GSH	100	94.4± 17.4	88.8 ± 14.9	109.1 ± 13.4	111.1 ± 9.7	113.2± 9.7
TPR (%)	control	100	123.6± 42.9	119.5 ± 14.5*	106.9 ± 30.1	131.2 ± 40.8	155.9± 59.6
	GSH	100	157.6± 45.8*	95.4 ± 28.2	81.1 ± 16.6*	95.1 ± 16.6	112.1± 22.8
PVR (%)	control	100	313.4 ± 298.8	130.5 ± 24.2*	131.5 ± 7.5***	181.4 ± 40.5**	200.5± 86.0*
	GSH	100	303.8± 95.6**	172.0 ± 28.6**	204.9 ± 89.2*	238.0 ± 97.0*	278.7 ± 159.7*

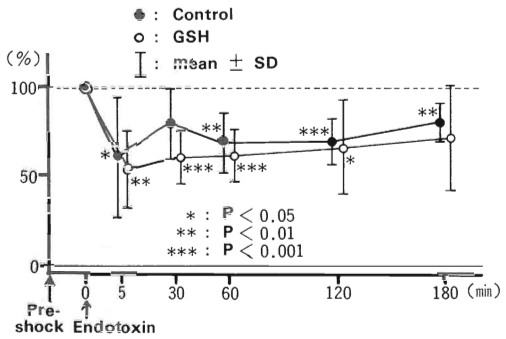

Fig. 2. Percent Changes in Mean Arterial Blood Pressure.

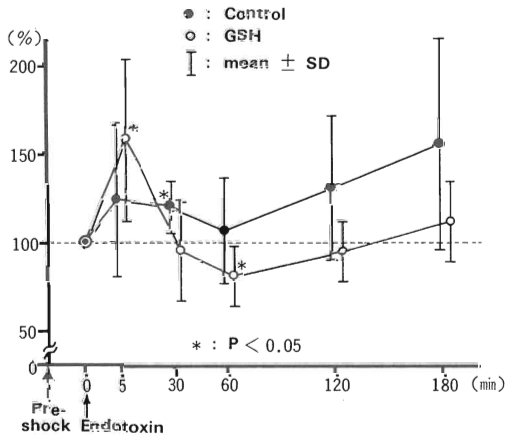

Fig. 3. Percent Changes in Total Peripheral Resistance.

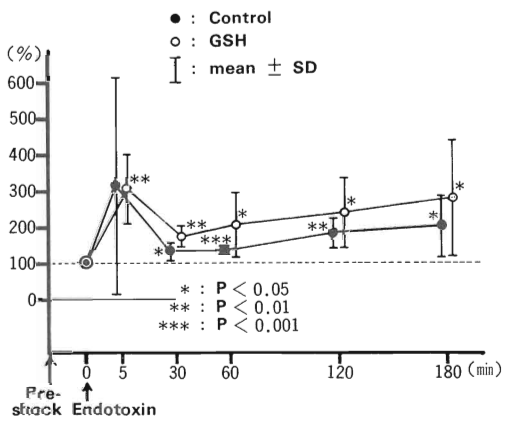

Fig. 4. Percent Changes in Pulmonary Vascular Resistance.

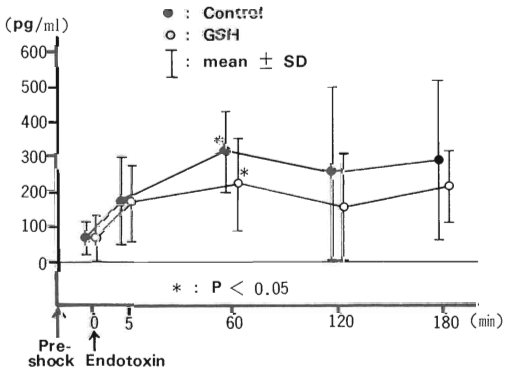

Fig. 5. Plasma Thromboxane B_2 levels during Endotoxin Shock.

groups exhibited a second decrease of the same degree. A few minutes after the injection of endotoxin, the MPA rose abruptly in both groups (Fig. 1). However, within 30 minutes after the injection of endotoxin, the MPA in both groups returned to near the baseline values (Table 1). The PVR in both groups increased progressively from 30 minutes to the end of the experiment after the injection (Fig. 4). In the GSH group, the PCWP fell significantly 60 to 180 minutes after the injection of endotoxin.

II. *Plasma T×B₂ formation*

Plasma samples were obtained from the inferior vena cava. The time course of the changes in the plasma T×B_2 levels following the injection of endotoxin in both groups was evaluated (Fig. 5, Table 2). Sixty minutes after the injection of endotoxin, a significant ($p<0.05$) increase in the plasma T×B_2 levels was observed from 68.8±49.0 pg/m*l* to 318.3±117.2 pg/m*l* (N=5) in the control group and from 67.9±68.4 pg/m*l* to 222.6±133.2 pg/m*l* (N=5) in the GSH group. Although there was no significant difference between the baseline and the experimental values at 120 and 180 minutes after the injection of endotoxin, the plasma T×B_2 levels in the both groups showed a tendency to be still

Table 2. Serum β-Glucronidase, Plasma Thromboxane B_2, Lactic Acid and Coagulation Parameter Levels During Entotoxin Shock, FDP: Fibrinogen/fibrin degradation products, *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ★: Significant difference between the two groups at $p<0.05$; ★★: $p<0.01$

Parameter	Group	Control reading	Time course after administration of endotoxin (min)			
			5	60	120	180
β-glucronidase (μg/dl)	control	4538 ± 1702	7646 ± 1534 *	15646 ± 4826 **	19675 ± 4570 ***	19076 ± 5120 ***
	GSH	5491 ± 2473	5784 ± 3637	11040 ± 5370	13189 ± 5713 *	15058 ± 5771 **
Thromboxane B_2 (pg/ml)	control	68.8 ± 49.0	175.3 ± 126.6	318.3 ± 117.2 **	258.0 ± 248.6	295.0 ± 231.3
	GSH	67.9 ± 68.4	169.4 ± 110.4	222.6 ± 133.2 *	159.4 ± 178.2	218.1 ± 103.6
Lactic acid (mg/dl)	control	18.8 ± 8.0	37.3 ± 11.6 *	43.6 ± 9.7 **	47.0 ± 7.9 ***	52.7 ± 8.9 ***
	GSH	22.0 ± 5.8	33.9 ± 6.1 *	38.3 ± 16.3	32.1 ± 11.6 ★	30.0 ± 9.0 ★★
Platelet count ($\times 10^4/mm^3$)	control	12.2 ± 4.6	2.9 ± 3.1 **	10.8 ± 4.2	11.0 ± 2.8	12.2 ± 3.9
	GSH	11.8 ± 3.6	1.6 ± 0.3 ***	7.8 ± 2.1	9.1 ± 2.0	10.3 ± 1.5
Fibrinogen (mg/dl)	control	335.6 ± 111.4	289.0 ± 107.2	266.2 ± 93.5	188.4 ± 76.8 *	185.4 ± 83.8 *
	GSH	325.0 ± 34.5	318.6 ± 55.9	262.4 ± 38.6 *	191.2 ± 26.5 **	190.6 ± 36.7 **
Prothrombin time (sec)	control	7.9 ± 0.3	8.2 ± 0.5	8.9 ± 1.0	9.8 ± 1.6 *	10.4 ± 1.7 *
	GSH	7.7 ± 0.4	8.1 ± 0.6	8.9 ± 1.3	10.4 ± 1.9 *	11.2 ± 2.3 *
FDP (μg/ml)	control	5.0 ± 5.0	13.0 ± 17.2	41.0 ± 38.8	62.0 ± 41.0 *	214.0 ± 119.1 *
	GSH	6.0 ± 5.5	18.0 ± 14.8	42.0 ± 31.0	64.0 ± 42.0 *	196.0 ± 127.0 *

elevated compared to the baseline values. The level in the GSH group was somewhat lower than in the control group from 60 to 180 minutes after the injection of endotoxin.

III. *Metabolic and lysosomal integrity*

A marked disruption of the tissue metabolism was evident during the shock (Fig. 6, Table 2). In the control group, the plasma lactic acid level was progressively elevated from 18.8±8.0 mg/d*l* to 52.7±8.9 mg/d*l* 180 minutes after the injection of endotoxin, coincident with the progression of metabolic acidosis in the arterial blood gas. In contrast to the fulminant lactic acidemia of the control group, minimal alterations of the tissue metabolism were apparent in the GSH group. In the GSH group, the level was also elevated significantly from 22.0±5.8 mg/d*l* to 33.9±6.1 mg/d*l* immediately after the injection of endotoxin and began to decline 120 to 180 minutes after the injection of endotoxin, and there were significant differences in the levels between the two groups.

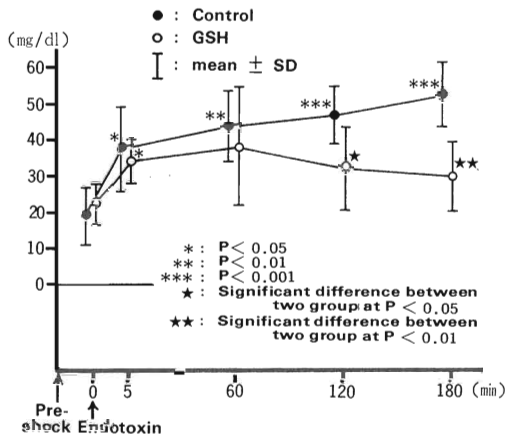

Fig. 6. Plasma Lactic acid levels during Endotoxin Shock.

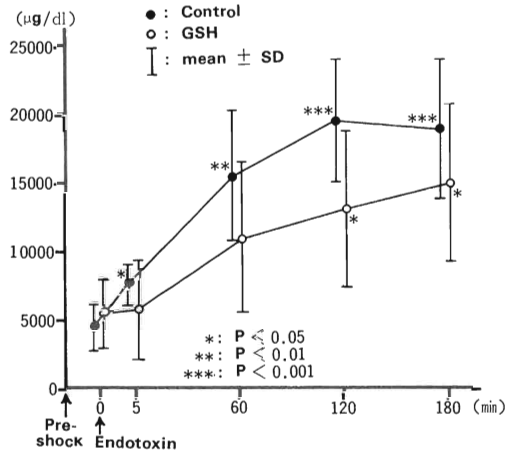

Fig. 7. Serum β-Glucronidase Activity during Endotoxin Shock.

Extensive alterations of the lysosomal integrity were also apparent within 180 minutes after the injection of endotoxin (Fig. 7). At 180 minutes, the serum β-glucronidase activity of the control group was elevated approximately fourfold ($p<0.001$) over the baseline values. The serum β-glucronidase activity of the GSH group, however, was elevated threefold ($p<0.05$) over the baseline values.

IV. *Platelet counts, fibrinogen, prothrombin time (P.T) and FDP*

Endotoxin administration produced a significant coagulopathy as denoted by the thrombocytopenia, decreased plasma levels of fibrinogen, elongated P.T and increased serum levels of FDP in both groups (Table 2). The changes in these parameters were almost of the same degree in both groups.

Discussion

I. *Plasma thromboxane formation*

In recent years considerable attention has been focused on the prostaglandin production during endotoxin shock and also elevated levels both of PGE and $PGF_{2\alpha}$ have been observed (Anderson *et al.* [12]; Fletcher *et al.* [13]). The production of these prostaglandins can be inhibited by the non-steroidal anti-inflammatory drugs such as aspirin and indomethasin. It has been shown by several workers that both the prophylactic and therapeutic administration of these drugs improved the hemodynamic function and increased the survival in experimental endotoxin shock (Paratt *et al.* [14]; Fletcher *et al.* [15]).

Radioimmunoassay is now available which enables us to measure the other prostanoid, thromboxane. As $T\times A_2$ is a potent constrictor of the vascular smooth muscle and initiator of platelet aggregation (Sammuelsson *et al.* [16]), its behaviour is of particular interest in endotoxin shock.

This is the first study that demonstrated that the administration of endotoxin to dogs is associated with a rise in $T\times B_2$. $T\times B_2$ of the central venous plasma increased within 60 minutes after the intravenous administration of endotoxin, and maximal thrombocytopenia and pulmonary artery hypertension were observed within 5 minutes after the injection of endotoxin. The levels of $T\times B_2$ at 60 minutes were higher than at 5 minutes after the injection of endotoxin.

Although there were no significant differences between the baseline and experimental values 120 and 180 minutes after the injection of endotoxin, the plasma $T \times B_2$ levels in both groups showed a tendency to be still elevated compared to the baseline values. These data suggest that the rise in the levels of $T \times B_2$ appears not to relate to early thrombocytopenia and pulmonary artery hypertension but to relate to late coagulopathy and the pulmonary vascular resistance changes. Cook *et al.* [7] have demonstrated that the plasma $T \times B_2$ increases significantly within 30 minutes after the intravenous administration of endotoxin (20 mg/kg) in the rats and that the pretreatment with imidazole, which inhibits the thromboxane synthetase, abolished the elevation and improved the survival. In the rat in shock (Cook *et al.* [7]), the $T \times B_2$ levels were higher in the vena cava than in the aorta, suggesting that the splanchnic circulation as well as the platelets may be the source of $T \times A_2$ production. Fletcher *et al.* [8] have reported that the plasma $T \times B_2$ increases significantly within 15 minutes after the intravenous injection of endotoxin (6 mg/kg) in the baboons and the $T \times B_2$ levels correlate with the changes in the pulmonary artery pressure. The present work coupled with these observations suggests that the $T \times A_2$ may be a significant factor in endotoxin shock, perhaps mediating such pathologic sequelae as pulmonary and/or splanchnic ischemia (Pennington *et al.* [17]; Kuida *et al.* [18]; Brobmann *et al.* [19]) or disseminated coagulopathies (Müller-Berghaus [4]). Additional studies need to be done in the septic animal models and humans in order to better understand the roles of $T \times A_2$ in pathophysiology of endotoxin or septic shock.

II. *Effects of GSH*

In recent years, sulfhydryl compounds such as reduced glutathione (GSH) (Szymanski [9]; Horeisi [20]; Yamada [10]; Kosugi *et al.* [21]) as well as cysteine (Galvin *et al.* [22]) has been demonstrated to have salutary effects on various forms of shock. In addition, the tissue sulfhydryl concentrations, particulary in the liver, are lower following many types of shock stimuli such as hemorrhage (Beck *et al.* [23]), drum trauma (Yamada [10]) and endotoxemia (Jeffries [24]). These findings suggest that the sulfhydryl compounds are involved in the shock sequelae, although the exact nature of their role in shock is not clear. Several actions of these compounds include maintaining the enzymes in their active form (Christopherson [25]), participating in the protein synthesis (Zehavi-Willner [26]), maintaining the glucocorticoid receptors in the membranes (Granberg *et al.* [27]), inhibiting the cathepsic activity and stabilizing the lysosomes (Chaneghem [28]), and inhibiting the endotoxin-induced platelet agglutination, histamine and serotonin release (Jokay *et al.* [29]). Because of these diverse actions of the sulfhydryl compounds, the lowered tissue levels of these compounds in shock may impair the biochemical and functional integrity of a variety of systemic cells.

These findings in our study can be summarized as follows: 1) MAP and TPR are somewhat lower in the GSH group than in the control group, 2) administration GSH improves the progression of lactic acidemia and disruption of lysosomal integrity during endotoxin shock and 3) the $T \times B_2$ levels are somewhat lower in the GSH group than in the control group during endotoxin shock.

Takenaka *et al.* [29] have demonstrated that intravenous infusion of GSH to the anesthetized dogs produces a sig-

nificant reduction of MAP with a concomitant increase in the mesenteric and renal blood flow. In the canine endotoxin shock, the decrease in the fractional distribution of the cardiac output to the splanchnic lesion has been oberved (Okada *et al.* [30]). In this aspect, the peripheral vasodilatory action of GSH may have a beneficial effect in canine endotoxin shock.

The significant suppression of plasma lactic acidemia in the GSH group as observed in this study is in agreement with Kosugi *et al.* [21]. One of the mechanisms of this beneficial tissue oxygenation is probably due to the effect of GSH decreasing the oxygen affinity of hemoglobin during shock with severe metabolic acidosis (Kosugi *et al.* [31]).

Although there were no significant differences in the serum β-glucronidase levels between the two groups, the serum lysosomal enzyme release during endotoxin shock was attenuated by GSH, an effect previously shown to be of benefit in shock (Yamada [11]; Kosugi *et al.* [21]; Galvin *et al.* [22]). The beneficial effects of GSH may be partially due to lysosomal stabilization.

Disseminated coagulopathy as observed in this study was almost of the same degree in both groups. There are a few reports concerning the effect of GSH on the platelet function; Jokay *et al.* [28] reported that sulfhydryl compounds inhibit in vitro the endotoxin-induced platelet agglutination and the release of histamine and serotonin; Saito *et al.* [32] demonstrated that the intravenous infusion of GSH (300 mg/kg) to dogs prior to endotoxin injection decreases the screen filtration pressure (SFP), suggesting that GSH inhibits the formation of microaggregates during endotoxin shock. Although there were no significant differences in the $T\times B_2$ levels between the two groups, the $T\times B_2$ levels were somewhat lower in the GSH group than in the control group. Further studies need to be done concerning the effect of GSH on the platelet function including thromboxane formation.

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