CHAPTER V

DISCUSSION
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There is little doubt that the most effective treatment of hemorrhagic shock is to restore the lost blood volume. With timely volume replacement, cardiac output is definitely increased. The sequence of events is represented in figure I. However, with the progression of hypovolemic shock, without any treatment, cardiac output can no longer be maintained and the system enters a high resistance-low output state due to stimulation of sympatho-adrenal system along with vasoconstrictor prostaglandins. The decrease in renal flow also activates renin-angiotensin-aldosterone system and further contributes to an increase in total peripheral resistance resulting in a total decrease in tissue perfusion causing metabolic acidosis and accumulation of myocardial depressant factor. The progression of hypovolemic shock with this new cycle of positive feedback interactions leading to myocardial failure is represented in figure-2.

Unfortunately, because of the complexity of the body, and poor understanding of the pathophysiology
of shock, no drug has been found clinically useful, although numerous ones have been tried and reported to be effective. Some of the broad categories of the pharmaceutical agents, that have been studied in an attempt to prevent irreversible phase of hemorrhagic shock include, catecholamines, adrenergic blockers, glucocorticoids, prostaglandins, angiotensin and its inhibitors. The role of opioids and opioid receptors in shock has just begun to unfold. Endogenous opioids are released in response to stressful conditions, both as hormones and as central and peripheral neurotransmitters (Guillemin et al, 1977 and Rossier et al, 1977). This led Faden and Holaday (1979) to consider the possibility that beta-endorphin—an endogenous opioid released during hemorrhagic stress might contribute to the hypotension seen in shock. This hypothesis was tested by administering, the opiate receptor antagonist—naloxone and it did indeed reverse the hypotension produced in hemorrhagic shock. Naloxone has been proved to be effective and safe during its years of use as a narcotic antagonist in narcotic coma and shock. Though its effectiveness in hemorrhagic shock is still under investigation, naloxone has been
found to improve the cardiovascular status, such as blood pressure, LV dP/dt, cardiac output, peripheral resistance and coronary flow, which are depressed in experimental animals (Faden and Holaday, 1979; Vargish et al, 1980; Lechener et al, 1985).

Kidney has a very important contribution to make in regulating the homeostatic mechanism in hemorrhagic shock. Hence any derangement in the renal function will also affect this homeostatic mechanism and further deteriorate the already depressed cardiovascular function in hemorrhagic shock. This acts as a vicious cycle, fall in blood pressure - affecting kidney function - leading to further fall in blood pressure, which in turn will further deteriorate the kidney function and so on and so forth. Hence it is extremely important that this type of deterioration in the cardiovascular status may not be allowed. Naloxone by improving the cardiovascular status as referred to above should improve the kidney function and improve cardiovascular status by its homeostatic mechanism.

The present work was undertaken to study the role of naloxone on renal function and indirectly
demonstrating its role on the cardiovascular status as well as homeostatic mechanism. The positive role of naloxone was compared with that of catecholamines, since there are many reports in the literature (Catchpole et al, 1955; Simeone et al, 1958 and Chien, 1967), where, these drugs have been administered in the treatment of hemorrhagic shock. Their ultimate usefulness is however controversial. Animal's survival was taken as the ultimate parameter in testing, the efficacy of the administered drugs.

The results of the present study will be discussed under the following headings:

1) **Effect of hemorrhage on cardiovascular function, kidney function and metabolic status** (control study).

2) **Effect of naloxone on cardiovascular status, renal function and metabolism in hemorrhagic shock.**

3) **Effect of administration of catecholamines** (noradrenaline and dopamine) on the above parameters in hemorrhagic shock.
4) Comparison of the effects of naloxone with that of catecholamines, including angiohistopathological study of the kidney and survival in hemorrhagic shock.

I) Effect of hemorrhage on Cardiovascular Status, renal function and metabolism in hemorrhagic shock (control study):

a) Effect of hemorrhage on cardiovascular status:

The mean arterial pressure measured in this group of animals before producing hemorrhage was 116±1.5 mmHg in the present study. This pressure was reduced to 45 mmHg in about 15 minutes time by bleeding the animals from the femoral artery and maintained at this pressure (44.8 ± 0.5 mmHg) for one hour by adjusting the height of the reservoir in which the shed blood was collected. This procedure prevents the effectiveness of the compensatory responses of the animals since a vasopressor response promotes additional loss of blood to the reservoir. The maximal bleed out volume in this group of animals was 43.0±1.6 ml/kg (Table 23).
and was not significantly different from other groups of animals in the present study. Gurll et al (1981) also removed 66-75 per cent of the blood volume to maintain 45 mmHg pressure for one hour in a similar type of study. Even though heart rate increased during hemorrhage, no statistically significant difference was seen between basal level of heart rate (168.6±6.5) and heart rate at t60 minutes (183.0±8.3) (Figure-6). The increase in heart rate is due to increased sympathetic activity (Chien, 1967).

At t60 minutes, the reservoir was clamped to prevent to and fro movement of blood and at the same time, saline was infused at the rate of 0.5 ml/min. All the dogs died within one hour of clamping the reservoir. Gurll et al (1981) and Vargish et al (1980) also have reported similar observations.

In the pre-terminal stage of this study, the mean arterial pressure of dogs decreased significantly (p<0.05) from 45.0 mmHg±1.0 at t60 to 30.0 mmHg±3.0 at t90 minutes (Table11). The heart rate per minute also decreased (p<0.05) from 183.0±8.3 (at t60) to 160.0±5.3 (at t90) indicating cardiovascular deterioration. Which portion of the cardiovascular system fails
first, if the hypovolemic shock is left untreated is still a subject of controversy. Crowell and Guyton (1962) supported the evidences of the theory of myocardial failure in contributing to irreversible shock. Myocardial depressant factor which is produced from the splanchnic bed due to ischemia enters the circulation and causes a direct negative inotropic effect (Lefer, 1978 and Lefer and Martin, 1970). Myocardial failure can also be a direct consequence of insufficient coronary flow (Sarnoff et al, 1954; Granata et al, 1969 and Bethea et al, 1972) due to fall in blood pressure.

A defect in the peripheral vascular capacitance system also has been established by many investigators (Rothe et al, 1963; Bond et al, 1967 and Rothe, 1970). Rothe et al (1963) observed an initial increase in total peripheral resistance followed by a decrease in TPR and heart rate in the later stages of hypovolemic shock and suggested that this may be due to partial loss of neurogenic control (Rathe et al, 1970). The mechanism of this vascular decompensation has shown to be independent of reflex vasodilation (Bond et al, 1980) and may be due to the production of
prostaglandin E$_1$ (Bond et al, 1979).

Beta-endorphin, an endogenous opioid peptide released from pituitary during hemorrhagic stress might also contribute to hypotension and decreased heart rate in shock state (Faden and Høladay, 1979). Beta-endorphin level in the blood increased in experimental animals subjected to hemorrhagic shock (Machuganska and Zaharieva, 1985). Either intra-cerebroventricular or intravenous injection of beta-endorphin can produce a shock state indicating central and peripheral opioid receptors.

b) Effect of hemorrhage on renal function:

In the present study, when the MAP of hemorrhaged dogs was kept at 45 mmHg for one hour, it was observed that the parameters of renal function like inulin clearance and PAH clearance decreased significantly (p < 0.001) in all the animals. Inulin clearance decreased from a basal level value of 39.4 ml/min + 1.1 to 4.9 ml/min + 0.6 and PAH clearance from a basal value of 152.6 ml/min + 10.3 to 18.0 ml/min + 2.4
during the period of hemorrhagic hypotension (Table 22). Marked impairment in renal circulation during shock has been observed previously by many investigators using clearance techniques (Corcoran and Page, 1943; Lauson et al, 1944; Phillips et al, 1945 and Selkurt, 1945). Vasoconstriction seen in kidney is considered as a part of homeostatic mechanism during shock. The compensatory mechanism in hemorrhagic shock involves the redistribution of the remaining circulating blood volume, apparently in an attempt to maintain adequate blood flow to brain and heart. Penner and Bernheim (1940) concluded from autopsy findings that ischemia of the kidney and intestine occurred as a part of the compensatory mechanism during shock. The cause of low inulin clearance might be due to the powerful vasoconstriction in the kidney that results from the continued activity of the sympathetic nervous system or through the renin-angiotensin axis (Conger and Schrier, 1980).

Urine flow and excretory rate of sodium also decreased tremendously ($p/0.001$) in this study during the period of hypotension. Urine flow in ml per hour decreased from a basal value of 5.9±0.68
Fig. 2A: Histological section of the cortical region of hemorrhaged dog's kidney infused only with saline, taken just before the death of the animal at t₀₀ minutes (majority of the glomeruli filled with ink particles).
to $0.16 \pm 0.02$ after hemorrhage and excretory rate of $\text{Na}^+$
from a value of $3.8 \mu\text{eq/min} \pm 0.4$ to $0.32 \mu\text{eq/min} \pm 0.1$
during hypovolemic period (Table 22). Shires et al (1960) also found little urine and marked increase in
the sodium and urea retention during hemorrhagic shock.
Sodium and water are conserved by the kidney under the
influence of aldosterone and ADH produced in excess
during hemorrhage to maintain the effective homeostasis

There was complete anuria in all the
animals till death, after clamping the reservoir while
infusing only saline ($0.5 \text{ ml/min}$) in this study. The
anuria seen in the current study may be due to further
reduced perfusion pressure in the kidney. The presence
of ink particles in the majority of glomeruli (Figure 24)
of the kidneys removed just before death of the
animals indicate reduced renal resistance (Schadet
al, 1984) which was seen reflexly increased to maintain
the homeostatic mechanism, in the early phase of
hemorrhagic hypotension due to sympathoadrenal
stimulation. Although the death of saline treated
animals within one hour of saline treatment cannot
be attributed to renal failure, the status of the renal
function is a reflection of the general perfusion of vital tissues.

c) **Effect of hemorrhage on metabolism**

In the present study metabolic acidosis was evident in all dogs following hemorrhage as observed by increased arterial lactic acid concentration, low pH with a concomitant fall in bicarbonate at t₆₀ minutes (Table 22). Lactic acid level in the blood increased from a basal level of 12.9 mg%+1.2 to 35.1 mg%+1.1 at t₆₀ minutes (p<0.001). On the other hand bicarbonate concentration, pH and pCO₂ fell from the basal levels of 22.8 MML+1.3; 7.35+0.01 and 42.3 MMHg+2.7 to 13.9 MML+1.0; 7.20+0.03 and 36.4 mmHg+3.4 respectively, one hour after hemorrhage. The acidosis is attributed to the tissue hypoxia resulting from the anaerobic production and reduced metabolism of lactate. Whether increased production, impaired lactate utilization or both are responsible for the lactic acidosis to occur is not clear (Arieff et al, 1980). Excess lactic acid is produced during hypovolemia from the peripheral tissues, especially
from the muscles, skin and the splanchnic bed due to ischemia (Wiener and Spitzer, 1974 and Yudkin and Cohen, 1975). In addition lactate metabolising organs like liver and kidney may not be able to utilize this excess lactic acid due to their impaired blood flow during hemorrhagic hypotension (Tashkin et al, 1972 and Yudkin and Cohen, 1975). There are many other clinical evidences also indicating increased lactic acid concentration in hemorrhagic shock (Beaty, 1945; Broder and Weil, 1964 and Daniel et al, 1976). The respiratory adjustment following hemorrhage lower the arterial PCO$_2$ (Lassen, 1959) which was also observed in this study. There were no significant differences in P0$_2$(90.9 mmHg±9.1 and 93.5 mmHg±10.8), serum sodium (153 meq/l±5.8 and 149.2 meq/l±4.0) and serum potassium (4.4 meq/l±0.5 and 4.4 meq/l±0.5) before and one hour after hemorrhage.

Metabolic acidosis was aggravated further from t$_{60}$ to t$_{90}$ minutes after clamping the reservoir and during saline infusion in this study. Lactic acid concentration in the arterial blood increased further (44.0±0.8 mg%) while bicarbonate level
(9.5±1.1 MML) and PH (7.0±0.03) fell at t90 minutes (Table 21). The metabolic acidosis is attributed to the tissue hypoxia due to prolonged hypovolemia and hypoperfusion, resulting in anaerobic production and reduced metabolism of lactate (Arief et al, 1980). The excess hydrogen ions titrate the body buffer system causing a metabolic acidosis and impaired utilization of lactate causes a serious loss of potential energy to the body (Cohen and Simpson, 1975). Excess level of lactic acid in the blood corresponds to the severity of circulatory failure and prognosticates a fatal outcome in hemorrhagic shock in human beings (Broder and Weil, 1964).

Carbon dioxide tension which increased tremendously (51.4±4.5 mmHg) at t90 minutes (Table 21), indicates respiratory acidosis. The decrease in venous return can lead to ventilation perfusion defects in the pulmonary bed (Hess et al, 1983). No significant difference in arterial oxygen tension and serum sodium level were seen at t60 and t90 minutes (Table 21) as observed by other investigators (Lechner et al, 1985 and Sayeed, 1987). The high serum potassium concentration (6.0±1.1 meq/L) seen in these hypovolemic dogs,
can be attributed to the shift of potassium from the intracellular to the extracellular compartment (Haloamae, 1970). Significant depression of the skeletal muscle transmembrane potentials and abnormal levels of high energy phosphates (Albert et al, 1982) reflect the inability of the cells to maintain intracellular potassium.

The above observations (hypotension, decreased heart rate, metabolic acidosis, respiratory acidosis, renal failure and increased serum potassium) which are noted at the preterminal stages of hypovolemic dogs treated only with saline, support the view of multiple organ failure during the irreversible phase of hemorrhagic shock due to total body ischemia. These factors do not act entirely independently but they try to potentiate their individual action by acting on each other. Even though the interplay of various mechanisms in the pathogenesis of hemorrhagic shock leading to death has been documented by various authors, the irreversible phase of hemorrhagic shock is still an enigma to the investigators and there are still more questions than answers.
II) Effect of naloxone on cardiovascular function and metabolic status in hemorrhagic shock

Effect of naloxone on cardiovascular status:

In the present study, haloxone, the opiate receptor antagonist improved the mean arterial pressure (MAP) in dogs subjected to hemorrhagic shock (this shock was produced by bleeding the animals to bring the MAP to 45 mmHg(t₀) and maintained at this level for one hour (t₀–t₆₀) and then clamped the reservoir at t₆₀ minutes in which the shed blood was collected). With 1 mg/kg i.v. bolus administration of naloxone the MAP increased, from a post hemorrhagic value of 45.0±1.8 mmHg at t₆₀ to 81.9±3.0 mmHg at t₁₂₀. The improvement in MAP was not dose dependent as there was not any significant difference between the action of 1 mg/kg or 2 mg/kg administration of naloxone (Figure 18). Faden and Holaday (1979); Varghish et al (1980); Gurll et al (1980) also have found increase in MAP after administration of naloxone in their experimental studies which is in line with the results of the current study. The increase in MAP seen in this study may be due to an increase in cardiac output (CO).
or due to an increase in total peripheral resistance or both. Vargish et al (1980) observed in a canine shock model, that naloxone improved the MAP primarily by increasing CO, secondary to an increase of left ventricular contractility (dp/dt max). In another hemorrhagic shock models Saleno et al (1981) and Sahadt et al (1984) demonstrated that naloxone could improve the MAP by increasing the total peripheral resistance, whereas Toth et al (1982) and Lechner et al (1985) observed an increase in CO without any change in total peripheral resistance.

No significant difference in the heart rate was seen after 1 mg/kg i.v. bolus administration of naloxone in the present study. The heart rate before treatment at t_{60} minutes was 164.0±7.3 and one hour after treatment at t_{120} minutes it was 180.0±8.7 per minute. After 2 mg/kg i.v. bolus administration of naloxone, the heart rate decreased significantly (p<0.05) from 165.0±13 at t_{60} to 143.0±14 at t_{120}. These observations are in agreement with some of the previous findings as well (Gurll et al, 1980; Schadt and York, 1982; Horton et al, 1980 and Lechner et al, 1985).

From the available literature, the mechanism
of action of naloxone in improving cardiovascular status in hemorrhagic shock does not seem to be clear. Normal animals treated with naloxone demonstrated no significant difference in MAP when compared with control group of animals perfused only with ringer solution (Alberts et al, 1982 and Reynolds et al, 1979). According to Faden and Holaday (1979) beta endorphin, an endogenous opioid peptide released from the pituitary during hemorrhagic shock might also contribute to hypotension and bradycardia seen in shock state, and naloxone the opiate receptor antagonist did indeed reverse this effect. Beta endorphin level in the blood increased in experimental animals subjected to hemorrhagic shock (Lange et al, 1982 and McIntosh et al, 1985). Lechner et al (1985) in their various studies have demonstrated that naloxone appears to improve the cardiovascular status by potentiating the effect of released catecholamines but does not increase the sympatho-adrenal discharge. They found that the increase in MAP, CO and LV (dp/dt max) due to naloxone in hemorrhagic shock are unaffected by cardiac denervation but are attenuated by alpha or beta adrenergic blockade. Prior infusion
of alpha and beta adrenergic agonists completely restores the cardiovascular responses to naloxone which were abolished by adrenal denervation and ganglionic blockade. They have also shown, that sustained sympathetic and parasympathetic responses appeared to result from the action of naloxone at the myocardial site. The net effect of sympathetic and parasympathetic activity results in either increase or decrease in the heart rate after naloxone administration in hemorrhagic shock.

As to the site of action of naloxone, causing improvement in the cardiovascular status in hemorrhagic shock, there are controversial views. Some workers have demonstrated in animal studies that the action of naloxone is centrally mediated via pituitary (Faden and Holaday, 1979; Machuganska and Zaharieva, 1985). They have suggested that the beneficial effect of naloxone in hemorrhagic shock is mediated through central opioid receptors, as it is seen only in animals with intact pituitary. Machuganska and Zaharieva (1985) have also found that neither intracerebroventricular nor intravenous administration of naloxone in hypophysectomised rats
showed any improvement in the cardiovascular function after hemorrhagic shock. But Gurll et al (1937) argue that opiate receptor mediated cardiovascular depression, seen in shock, is mediated centrally only in endotoxic shock and is peripheral in hemorrhagic. The small dose of 0.1 mg/kg of naloxone was effective when given directly into the coronary circulation during hemorrhagic shock and was not effective when given into the ventriculo-cisternal system of the brain. This supports the peripheral site (cardiac) of action for naloxone in canine hemorrhagic shock.

b) Effect of naloxone on renal function:

All the parameters of renal function which were seen depressed during hypotension, improved significantly after naloxone (1 mg/kg) administration in all the dogs subjected to hemorrhagic shock in this study. Inulin and PAH clearances increased from the pretreatment values of 5.6±0.4 ml/min to 13.3±1.4 ml/min and 18.2±1.5 ml/min to 38.6±4.4 ml/min, respectively. Urine flow per hour and excretory rate of sodium also improved significantly from
0.19±0.02 to 1.4±0.25 ml/h and 0.32±0.1 to 2.58±0.57 μeq/min respectively (Figures 7, 8, 9, 10). There was no significant difference between 1 mg/kg and 2 mg/kg administration of naloxone in any of the parameters of renal function (Figure 18).

Renal insufficiency seen in hemorrhagic shock can be due to reflex renal circulatory changes, hormonal changes or various combination of both (Passmore, 1983). Conger and Schrier (1980) attributed the cause of low clearance values to the powerful vasoconstriction in the kidney resulting from the continued sympathetic activity or through renin-angiotensin mechanism. It is also possible, that, a proximal tubular failure to reabsorb sodium causes an intense secretion of renin via the tubuloglomerular feedback system (Loen and Meng, 1976) which in turn can decrease renal flow and glomerular filtration rate.

The beneficial effect of naloxone seen in this study on renal function, could be due to the local effect of naloxone on renal blood vessels, decreasing the reflex renal vasoconstriction, or by
diffusing renin-angiotensin mechanism or as a consequence of generalized improvement in the mean arterial pressure. The direct effect of naloxone on renal blood vessels may be ruled out as Schadt et al (1984) found no significant difference in the renal resistance after administration of naloxone in experimental rabbits, where hemorrhage was produced, as compared to control animals infused with only saline. Moreover, even though opioid peptides have high affinity for opiate receptors in the brain, they have negligible effect on opiate bindings in guinea pig and rabbit kidney (Simantov et al, 1978).

It is known that hemorrhagic hypotension is a potent stimulus for the release of renin. Some reports indicate that angiotensin II is the primary factor involved in the increased renal resistance (Rocchini and Barger, 1979) and may be a factor responsible for the outer cortical ischemia after hemorrhage (Beregovich et al, 1974; Lachance et al, 1974 and Hock et al, 1982). The amount of Na\(^+\) delivered to the macula densa alters the release of renin by the juxta glomerular apparatus and thus the level of angiotensin II. This mechanism is considered as a
regulator of glomerular filtration rate. As naloxone attenuates the release of renin from the kidney (Szilayi, et al, 1986) explains partially the improvement of renal function seen in this study after naloxone administration.

Water and sodium are conserved by the kidney under the influence of ADH and aldosterone produced in excess during hemorrhagic shock (Skillman et al, 1967; and Haljamae, 1978). The increase in ADH may be mediated through the beta-endorphin released during hemorrhage (Lightman et al, 1980). ADH is known to cause redistribution of blood flow from the renal cortex to renal medulla (Fisher et al, 1970 and Johnson et al, 1977) leading to renal cortical ischemia. Therefore naloxone may help in the improvement of renal function by decreasing the ADH release as well.

C-Effect of naloxone on metabolism and electrolyte status:

The metabolic status of the animals also improved significantly in the present study after naloxone treatment. The lactic acid concentration which rose significantly (35.4±1.0 mg%) at t₅₀ minutes,
decreased significantly (p<0.05) to 32.8±1.2 mEq/L at 20 minutes (Figure 11) after naloxone (1 mg/kg IV bolus) injection. It is also known that naloxone increases the MAP without increasing the total peripheral resistance in hemorrhagic shock (Lechner et al, 1985). Decreased arterial lactate concentration after naloxone might be due to increased perfusion of the lactate metabolising organs and/or due to fall in anaerobic production of lactate. Lechner et al (1985) in another study observed a significant increase in the myocardial, splanchnic and hepatic blood flow in hypovolemic dogs after naloxone treatment. The arterial lactate uptake is proportional to the hepatic and renal blood flow (Muller and Smith, 1963).

A substantial recovery of bicarbonate (14.0±0.7 to 17.7±0.8 MML) concentration in the blood of naloxone treated dogs, in hypovolemia also indicates effective tissue perfusion, aerobic metabolism and rapid utilization of lactic acid in the present study. Recently in the rabbit, the tissue metabolic capacity particularly of liver and lung has been shown to improve after naloxone administration (Matheyse et al, 1986). PCO₂ of the arterial blood is
restored after naloxone treatment (36.4±3.4 to 45.6±5 mmHg) indicating again improved metabolic status of the animals. There was no significant difference in PO₂, serum sodium, and serum potassium levels at t<sub>60</sub> and t<sub>120</sub> minutes after administration of naloxone. These observations are in line with other research workers. The maintenance of sodium potassium level even after prolonged, fall in blood flow indicates the integrity of the cell membrane and adequate functioning of the sodium potassium pump. Alberts et al (1982) have found that naloxone enables the cell to maintain normal transmembrane potential even at a low flow state.

The present results introduce naloxone as a new effective therapeutic agent against the deleterious consequences of hemorrhagic shock on renal function in dogs. However, the mechanism of action of naloxone needs further investigations. The present findings also assert the ability of naloxone to vanquish the pernicious effects of hemorrhage on cardiovascular function and metabolism.
III. Effects of Catecholamines in Hemorrhagic Shock:

a) Effects of Noradrenaline in Hemorrhagic Shock:

In the present study noradrenaline infusion (2 µg/kg/min) for one hour in hemorrhaged dogs produced a significant improvement in the MAP from a post-hemorrhagic value of 45.5 ± 1.6 mmHg to 77.2 ± 6.6 mmHg (Fig. 5).

It has long been recognized that excessive vasoconstriction due to sympathetic activity in hemorrhagic shock is aggravated further after infusion of noradrenaline (Frank et al., 1956 and Corday and Williams, 1960). Noradrenaline infusion increases the cardiac output also (Gillmore et al., 1954; Levy and Brind, 1957; and Fowler and Franch, 1957). Increased heart rate seen in this study (160.0±10 to 201.0±11.8 per min) after noradrenaline infusion for one hour can be detrimental to the heart. There are plenty of evidences (Sarnoff and Mitchell, 1962; Berne, 1964; and Rushmer, 1965), that a rapid heart rate causes a decrease in cardiac filling time, myocardial efficiency, cardiac output and coronary flow. Thus although noradrenaline is a potent stimulator of myocardial beta-adrenergic receptor, the clinical
use of it has been limited by its reflex chronotropic action on heart and potent effect on vascular alpha-adrenergic receptors which causes vasoconstriction (Goldberg and Baij, 1985).

Inulin and PAH clearances decreased significantly \( (p/0.05) \) in this study after infusion of noradrenaline from \( t_{60} \) to \( t_{120} \) minutes. The inulin and PAH clearances before infusion of noradrenaline were \( 4.7 \pm 1.0 \text{ ml/min} \) and \( 17.6 \pm 1.6 \text{ ml/min} \). After one hour treatment further decreased to \( 3.0 \pm 1.0 \text{ ml/min} \) and \( 7.7 \pm 2.0 \text{ ml/min} \) respectively (Figure 7 and 8). As oligemic hypotension progresses, the renal blood flow usually decreases further and renal resistance increases to higher levels (Kovach et al, 1958; Corday and Williams, 1960; Selkurt, 1964). Administration of noradrenaline in hemorrhagic shock further intensifies such vasoconstriction in kidney (Corday and Williams, 1960; Hinshaw et al, 1961 and Nelson et al, 1961) and other viscera (Nelson et al, 1961; Lillehel et al, 1964). In fact, infusion of noradrenaline in hemorrhaged dogs has been associated with increased hemorrhagic lesions in viscera and kidney (Frank et al, 1956 and Rao, 1968).
No statistically significant change in either urine flow or excretory rate of Na$^+$ was observed after noradrenaline infusion in this study. Urine flow before and after treatment were 0.14±0.05 ml/h and 1.1±0.69 ml/h and excretory rate of sodium was 0.46±0.1 and 2.46±1.1 p eq/min respectively. An involvement of catecholamines in vasopressin secretion is clearly indicated by the heavy catecholaminergic innervation of the paraventricular and supraoptic nuclei (Sladek et al, 1930). Both stimulatory and inhibitory action on vasopressin release have been ascribed to noradrenaline (Kimura et al, 1980; Armstrong et al, 1982 and Sklar and Schrier, 1983). These observations might explain why there was increased urine flow and excretory rate of Na$^+$ in some dogs and why there was decreased water and electrolyte excretion in some dogs after infusion of noradrenaline in hemorrhaged animals. Norepinephrine can reduce vasopressin secretion, only when it is under the influence of angiotensin II (Armstrong et al, 1982) and renin-angiotensin system is activated during hemorrhage.

Metabolic acidosis was further increased by way of lactic acidosis (43.2±1.6 mg%) low PH(7.13±0.06) and decreased bicarbonate concentration(11.8±1.5
in the arterial blood at $t_{120}$ minutes after infusion of noradrenaline for one hour in this part of the study (Figure 11, 12 and 13). The aggravation of metabolic acidosis and elevation of circulatory catecholamines may contribute a vicious cycle, since detecholamines intensify metabolic acidosis, and accumulation of lactate can provoke a further release of endogenous catecholamines (Nahas et al, 1960; Woods et al, 1956; and Darby and Watts, 1964). The exaggeration of metabolic acidosis as a result of norepinephrine induced vasoconstriction can exert many deleterious effects on cardiovascular system. There was no significant changes in $\text{PCO}_2$, $\text{PO}_2$, serum sodium and potassium levels after administration of noradrenaline from the pretreatment values. These observations indicate the absence of respiratory acidosis, integrity of cellular membrane and maintenance of sodium, potassium pump.

Even though mean arterial pressure increased in this study after noradrenaline treatment, there was decrease in the renal function. The aggravation of metabolic acidosis indicates a profound vasoconstriction. Temporary treatment with noradrenaline may be a life
saving mechanism, but with the exaggeration of metabolic acidosis and elevation of circulating catecholamines may constitute a vicious cycle and can lead to many deleterious effects.

b) Effects of Dopamine in Hemorrhagic Shock:

Intravenous infusion of dopamine (10 μg/kg/min) for one hour increased the mean arterial pressure and heart rate significantly in this study. MAP increased from a post hemorrhagic value of 44.6±3.5 mmHg to 65.2±4.7 mmHg at 120 minutes (Figure 5). Heart rate per minute increased significantly from a value of 180.0±18.6 (at 60) to 218.0±17.4 (at 120) (Figure 6). Dopamine is known to have both inotropic and chronotropic effects on heart (Carvalho et al, 1969 and Goldberg, 1974). At mid range doses of dopamine it stimulates alpha adrenergic receptors, resulting in increased systemic arterial blood pressure (Beregovich et al, 1974 and Leier et al, 1973). This vasoconstrictor action is frequently undesirable for a weak heart with increased heart rate (Goldberg and Rajfer, 1985).
No urine was formed during the period of dopamine treatment in this study. Many workers have found that only at lower doses (below 6 µg/kg/min) dopamine stimulates renal dopaminergic receptors resulting in increased renal blood flow and urine flow without any improvement in cardiovascular function (Goldberg et al, 1961; Gifford et al, 1968; Goldberg, 1974). But Bergovich et al (1974) observed that at doses of 6-10 µg/kg/min dopamine caused an increase in the MAP and a decrease in renal blood flow. The lack of improvement in the renal function after dopamine treatment in this study may be partly due to extremely high plasma renin activity induced by hemorrhage per se as well as dopamine infusion (Neiberger and Levin, 1980).

Even though arterial lactic acid concentration increased from 35.0±1.0 mg/dl (at t60) to 38.8 mg/dl±0.6 after dopamine treatment at t120 minutes, there was no further deterioration from the posthemorrhagic levels of PH, HCO3-, PCO2 and PO2 in this study (Figures 11,12,13,14,15). No further deterioration of metabolic acidosis after dopamine infusion is probably because of lesser effect of
dopamine on total peripheral resistance (Carvalho, 1969 and Goldberg, 1974). There was no alteration in serum sodium and potassium also after dopamine treatment (Figures 16 and 17).

IV) **Comparison of the effects of Naloxone with that of Catecholamines, including angiohistopathological studies of the kidney and survival in dogs in hemorrhagic shock.**

Five dogs selected at random from each group were reinfused with the shed blood at 120 minutes and kept for noting the survival rate for seven days. Administration of naloxone (1 mg/kg iv bolus) improved the survival rate of hemorrhaged dogs to 100 per cent as against 100 per cent mortality in the case of saline treated ones. In noradrenaline (2 µg/kg/min) treated dogs the survival rate was 40 per cent in dopamine (10 µg/kg/min) group of animals it was only 20 per cent.

All the five dogs which were treated with naloxone survived for seven days and they were looking quite well on the 7th day and would have survived for a longer period. Gurll et al (1982) also
noted 100 per cent survival rate for 72 hours after treating with naloxone in hemorrhagic shock. In another study Gutt et al (1982) observed prolonged survival and enhanced cardiovascular performance even without the reinfusion of the shed blood. Naloxone is a specific opiate receptor antagonist with minimal effect on cardiovascular function in normal animals (Reynolds et al, 1980). Therefore it has to be assumed that the effect of naloxone is seen only in the presence of pathological conditions. Hemorrhagic shock is a pathological state and is associated with the production of an endogenous opioid peptide, beta-endorphin which depresses the cardiovascular function seen in shock. The salutory effects of naloxone is due to the blockade of opiate receptors either centrally or peripherally (Faden and Holaday, 1979 and Machuganska and Zaharieva, 1985) and Lechner et al, 1987). Naloxone is also known to prevent the formation of myocardial depressant factor by stabilising the lysosomal membrane in hemorrhagic shock (Curtis and Lefer, 1980).

Improvement of renal function, in addition to the improvement in cardiovascular function in the
Fig. 20: Histological section of the cortical region of normal dog's kidney, after injecting India ink through the renal artery (glomeruli densely filled with ink particles uniformly).
Fig. 21: Histological section of the cortical region of hemorrhaged dog's kidney, one hour after treatment with naloxone (majority of the glomeruli partially fitted with ink particles).
Fig. 22: Histological section of the cortical region of hemorrhaged dog's kidney, one hour after treatment with nor-adrenaline (only very few glomeruli partially filled with ink particles).
Fig. 23: Histological section of the cortical region of hemorrhaged dog's kidney, one hour after treatment with dopamine (few glomeruli partially filled with ink particles).
present study reflect, the improvements of other visceral perfusion. The improvement in inulin clearance and PAH clearance seen in this study after administration of naloxone is due to the generalized improvement in the mean arterial pressure. Lechner et al (1985) observed that in hypovolemia naloxone produced a significant improvement in myocardial intestinal, hepatic and adrenal blood flow. In this present work, kidneys, perfused with India ink at t=20 minutes after naloxone injection in hemorrhaged dogs showed about 75 per cent of the glomerular capillaries partially filled with ink particles at the cortical region (Figure 21) as against 100 per cent glomerula capillaries densely filled with ink particles (Figure 20) in the case of normal dogs. This shows the partial patency of the majority of the arterioles in the cortical region after naloxone treatment, even at the low flow state.

Metabolic acidosis produced during hemorrhage reversed after naloxone administration. This substantial recovery in metabolic acidosis also indicates effective tissue perfusion, aerobic metabolism and rapid utilization of arterial lactate. Naloxone is known to improve the cardiac output and mean arterial pressure without increasing the total
peripheral resistance (Vargish et al, 1980 and Lechner et al, 1985). Adequate peripheral perfusion and prompt restoration of PH are essential for the reversibility of shock and naloxone might have met these requirements and improved the survival rate.

In the case of noradrenaline infused dogs, two out of five dogs only could survive for seven days in this study. It seems likely that noradrenaline possesses both beneficial and detrimental effects in hemorrhagic shock. There are no consistent results on survival rate in hemorrhagic shock after treating with noradrenaline, inspite of large number of reports (Simeone et al, 1958; Lillehei et al, 1964 and Chien, 1967). Individual variation in their response to hemorrhage and treatment, make it difficult to get a statistically significant result. Simeone et al (1958) are having the opinion that if noradrenaline is given very early after hemorrhage, increases the survival rate. Administration of noradrenaline after marked sympathetic activity decreased the survival rate (Lillehei et al, 1964). According to some workers the survival rate after treatment with noradrenaline in hemorrhagic shock depends on the dose
and time of administration (Lausing et al, 1957; Griffin et al, 1958 and Szakacs and Mchlman, 1960).

Eventhough mean arterial pressure, increased significantly, the parameters of renal function like inulin clearance and PAH clearance decreased significantly in this study during noradrenaline infusion. Only very few glomeruli (25 per cent) and its surrounding area showed ink particles (Figure 22) in the cortical region of kidney perfused with India ink at t=120 minutes after treatment with noradrenaline. These observations indicate severe vasoconstriction in the cortical region and support the reported observations that sympathetic nerve fibres are found mainly in the renal cortex and intrarenal perfusion of noradrenaline produces a pattern of progressive cortical ischemia (Mitchel, 1951 and Carrier et al, 1966). In hemorrhagic shock vasoconstriction in the kidney seems to play a prominent role in causing decompensation and administration of noradrenaline aggravates further these decompensatory changes (Howard, 1962; Howard, 1965 and Moore, 1965).

Severe metabolic acidosis also was seen in the present study after noradrenaline infusion
indicated by the presence of lactic acidosis, decreased bicarbonate level and PH of the arterial blood. The fate of animals in hemorrhagic shock is related to the degree of overall anaerobiosis. The exaggeration of metabolic acidosis as a result of noradrenaline infusion seen in this study would be detrimental to survival.

Only one dog out of five survived for seven days, in the case of dopamine treated hypovolemic animals. Daugher et al (1970) also found no beneficial effect on 48 hours survival in dogs subjected to severe hemorrhagic shock treated with dopamine and suggested that the beneficial effect of dopamine is overcome by alpha adrenergic vasoconstriction resulting from hypovolemia. There is no protective effect of dopamine with a hypovolemic circulation, despite reinfusion of the blood (Carvalho et al, 1969).

There was complete anuria during the period of dopamine infusion (10 µg/kg/min) inspite of the significant improvement in the mean arterial pressure in hemorrhaged dogs in this study. Only about 45 per cent of the glomeruli and its surrounding area
showed a slight tinge of ink particles in the cortical region (Figure 23). Partial patency of only 45 per cent of the glomerular capillaries after dopamine infusion shows increased alpha adrenergic activity (Daugher et al, 1970). Anuria seen in the present study reflects decreased renal and splanchnic perfusion. At this dose (10 μg/kg/min) dopamine stimulates alpha adrenergic receptors, resulting in increased arterial pressure and decreased renal blood flow (Beregovich et al, 1974 and Leier et al, 1973).

Increased lactic acid level in the arterial blood after dopamine infusion seen in this study indicates anaerobic metabolism and metabolic acidosis. Acidosis and accumulating lactate can provoke a further release of endogenous catecholamines (Woods et al, 1956; Nahas et al, 1960 and Darbin and Watts, 1964). No further deterioration of PH and bicarbonate levels in the arterial blood after dopamine treatment from the post hemorrhagic levels indicate lesser effect of dopamine on total peripheral resistance (Carvalho, 1969 and Goldberg, 1974) as compared to noradrenaline.

The dose of naloxone (1 mg/kg) selected in this study, proved to be effective in rendering
optimum survival rate in hemorrhaged dogs, whereas clinically used drugs like noradrenaline (2 µg/kg/min) and dopamine (10 µg/kg/min) could improve the survival rate only partially. Naloxone improved the depressed renal function in hypovolemia and improved the metabolic status of the animals in addition to the improvement in cardiovascular function. On the other hand noradrenaline and dopamine treatment deteriorated further the renal function and metabolic acidosis, even though there was significant improvement in the mean arterial pressure. According to Baue (1975) renal insufficiency is one of the contributory factors in limiting the survival of war wounded patients successfully resuscitated after hypovolemic shock. Adequate peripheral perfusion and prompt restoration of pH are also essential for the reversibility of shock. Naloxone meets these requirements as compared to noradrenaline and dopamine by improving the renal function and metabolic status. The clinical relevance of these findings seems evident and naloxone may be a better adjunct to fluid resuscitation than noradrenaline or dopamine, which have been used so far.