EFFECT OF ENDOTOXIC SHOCK ON CARDIOVARCULAR SYSTEM IN CALVES

Thesis

Submitted to the Punjab Agricultural University
in partial fulfilment of the requirements
for the degree of

MASTER OF VETERINARY SCIENCE

in

VETERINARY PHYSIOLOGY

(Minor Subject : Animal Nutrition)

by

Jasdeep Singh Grewal

(L-92-V-290-M)

Department of Veterinary Physiology

College of Veterinary Science

PUNJAB AGRICULTURAL UNIVERSITY

LUDHIANA-141 004 (India)

1995

C E R T I F I C A T E - I

This is to certify that this thesis entitled, "Effect of endotoxic shock on cardiovascular system in calves" submitted for the degree of M.V.Sc. in the subject of Veterinary Physiology (Minor subject : Animal Nutrition) of the Punjab Agricultural University is a bonafide research carried out by Jasdeep Singh Grewal (L-92-V-290-M) under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

S P Singh

Dr. S.P.S. Sodhi
Professor
Veterinary Physiology
Deptt. of Veterinory Physiology
Punjab Agricultural University
Ludhiana 141001, India.

Physiology, PAU Ludhiana
Dept of Vety
Dean/Head
25-9-95

C E R T I F I C A T E - II

This is to certify that the thesis entitled, "Effect of endotoxic shock on cardiovascular system in calves" submitted by Jasdeep Singh Grewal (L-92-V-290-M) to the Punjab Agricultural University in partial fulfilment of requirement of the degree of M.V.Sc. in the subject of veterinary physiology (Minor subject : Animal Nutrition) has been approved by the student's advisory committee, after an oral examination of the same in collaboration with an external examiner.

27/7/95

Dr. S.P.S. Sodhi
Major Advisor

27.7.95

External Examiner
(DR. O. P. NANGIA)
DEAN, COVS
H.A.U. HISSAR

27/7/95

Head of the Department
(DR. P.J.S. RATTAN)

25/8/95

Dean Post Graduate Studies
(DR. K. D. MANNAN)

A C K N O W L E D G E M E N T

I wish to express my sincere gratitude and appreciation to my guide Dr. S.P.S. Sodhi, Professor, veterinary physiology for his able guidance, constructive criticism and cooperative attitude.

My sincere thanks are due to members of my advisory committee : Dr. P.J.S. Rattan, Professor, Reproductive physiology, Dr. M.S. Setia, Professor, Veterinary physiology, Dr. B.K. Gupta, Senior Animal Nutritionist and Dr. A.K. Srivastava, Associate Professor, Veterinary Pharmacology and toxicology for their valuable suggestions and encouragement during the course of study and prepration of manuscript.

My warm thanks are also due to other faculty members of the Department, Dr. Rajvir Singh, Dr. S.P.S. Sangha, Dr. R.S. Kahlon, Dr. D.V. Singh and Dr. Shashi Nayyar for their constant encouragement and helpful attitude. Help rendered by Dr. V.K. Sobti, Professor, Veterinary clinics and Dr. Sandhu, Asstt. Professor, Veterinary Pharmacology and Toxicology is acknowledged.

I shall ever remain thankful to my friends - Amar Iqbal, Bhupinder, Rama Krishna, Navdeep, Ravinder, Hirdesh, Raman, Charanbeer, Manohardeep, Opinder, Gurpreet, Sarvpreet, Ajitpal and Jaspreet for their ever willing help and encouragement.

The constant, untiring, ever encouraging and selfless sacrifice of my family will always remain as indelible in my heart.

I acknowledge the help rendered by laboratory staff S/Sh. Raj Singh, Sita Ram and Animal attendants Shishpal and ANil. The office staff is also thanked for their cooperation during the period of study.

LUDHIANA

DATED 23rd JUNE, 1995

Jasdeep Grewal

(JASDEEP SINGH GREWAL)

TITLE OF THESIS	:	Effect of endotoxic shock on cardiovascular system in calves.
NAME OF STUDENT	:	JASDEEP SINGH GREWAL
ADMISSION NO.	:	L-92-V-290-M
MAJOR SUBJECT	:	Veterinary Physiology
MINOR SUBJECT	:	Animal Nutrition
MAJOR ADVISOR	:	Dr. S.P.S. Sodhi Professor Veterinary Physiology
TOTAL NO. OF PAGES	:	50
TOTAL NO. OF PAGES OF BIBLIOGRAPHY	:	xv
YEAR OF AWARD	:	1995
DEGREE AWARDED	:	M.V.Sc.
NAME OF AWARDING UNIVERSITY	:	PUNJAB AGRICULTURAL UNIVERSITY, LUDHIANA.

A B S T R A C T

Eight male buffalo calves between 8-10 months of age were given E-coli endotoxin @ 0.6 mg/kg Body weight IV into jugular vein after catheterization. Effect of endotoxin on ECG, Heart rate, Blood Pressure, Biochemical Profile, Histamine, Prothrombin time and minerals was evaluated at 0, 5, 15, 30, 60 and 120 minutes. Prolongation of P-R interval at 5 and 15 minutes of shock followed by shortening in late stages was observed. Prolongation of S-T segment was observed in late stages of shock. Significant decline in MAP and CVP was observed. Acid phosphatase and GOT enzymes were elevated whereas Alkaline phosphatase was depressed. There was significant increase in the level of histamine in blood. Prothrombin time was prolonged significantly. Plasma zinc and calcium levels were decreased during endotoxic shock.

Jasdeep Singh Grewal

I N D E X

CHAPTER – I

INTRODUCTION

Shock frequently eluding clinical diagnosis is often associated with high mortality. In general shock is characterized by hypovolumea, decreased cardiac output, hypotension, fall in mean arterial pressure, central venous pressure and hypoxia of tissues. In fact endotoxic shock is a peripheral circulatory failure leading to death of cells due to inadequate tissue perfusion secondary to an infectious process. Modern concept based on pathophysiological processes inducing shock is as per endotoxin administration to animals.

In living beings gastro-intestinal tract is a storehouse of Gram negative bacteria and a potential pool of endotoxin in animal body. The access of endotoxins to the body may presumably be ending up into many metabolic disorders and even lethal effects on the body by its absorption from the intestine. Normally the entry of enteric endotoxin to the cirulation is prevented by intraluminal bile acids, intact mucosal membrane and functional hepatic clearance mechanisms. But as and when these barriers are disrupted endotoxic shock ensues. These organisms are responsible for many diseases in ruminants viz neonatal coliform septicaemia, coliform mastitis, pasteurellosis, pneumonias, brucelosis, metritis, compylobacteriosis, infections of cornea and sclera and thromboembolic

meningoencephalitis. Most of clinical symptoms and morbid changes developed from reaction of Gram negative organisms specifically **Escherichia coli** and Salmonella species.

Current treatments and managemental practices remain only moderately successful in reducing the morbidity and mortality following Gram negative bacteremia. Therefore a better understanding of pathophysiologic changes of this syndrome is of importance. So the present investigation was planned with the objectives :-

1. To study the lethal levels of intrcirculation and intracardiac endotoxin administration.
2. To study the general clinical behaviour of animals during and after endotoxic shock.
3. To study the changes in cardiac, nervous and respiratory systems.
4. To study the changes in haemodynamics and biochemical profile in blood.
5. To study changes in hemogram of animal during endotoxic shock.

CHAPTER - II

REVIEW OF LITERATURE

ELECTROCARDIOGRAPHIC STUDIES (ECG)

Hartman and Kronbeger (1976) reported that during coli dysentry in 100 calves heart rate increased, ventricular systole QRS complex prolonged and TP and ST intervals shortened.

Singh **et al** (1982) recorded ECG alteretions during septic shock induced in seven healthy buffalo calves, between the age of 6 months to one year, by strangulating a segment of bowel. A peaked T wave alongwith progressive increase in its amplitude, ventericular tachycardia and evaluation of ST segments were consistent changes observed in all calves. Prolongation of QRS Complex was observed in terminal stages only.

Singh (1990) also reported marked increase in potential of QRS complex decrease in T wave potential. QRS complex increased to 0.3, 0.5 and 0.8 mv at 10, 15 and 30 minutes and T wave decreased from 0.16 mv to 0.12 mv at 30 minutes of shock. There was prolongation of QRS complex and decrease in Q-T. interval and S-T segment. Heart rate decreased from 68.1 to 30 beats/min at 30 minutes after endotoxin shock induction.

Datta and Pathak (1992) studied ECG changes at different times of haemorrhagic shock and observed changes in the

configration of T wave, shortening of P wave, P-R and Q-T intervals, S-T segment and increase in the amplitude of QRS complex.

BLOOD PRESSURE

Hinshaw **et al** (1961) Lethal doses of **E.coli** endotoxin to anaesthetized dogs induced hypotension

Tikoff and Kuida (1965) studied the haemodynamic effect of **E.coli** endotoxin @ 0.25 - 0.5 mg / kg and reported an increase in pulmonary arterial pressure on an average by 43 mm Hg over control often for longer duration while cardiac output either remained constant or decreased. Left ventricular pressure scarcely changed while pulmonary resistance increased. The decrease in systemic arterial pressure was for a variable duration.

Gilder **et al** (1970) Intravenous infusion of **E.coli** endotoxin @ 4.4 mg/kg body weight in dogs over a course of 1 minute caused a fall in blood pressure to 20 mm of Hg within a minute which subsequently gradually rose to 60-80 mm of Hg.

Wyler **et al** (1970) studied the effect of endotoxin on haemodynamics and reported fall in cardiac output.

Osborne and Meredith (1971) studied the influence of **E.coli** endotoxin (O111:B4) on cardiac action, cardiac output and haemodynamics in anesthetized piglets. There were cardiac arhythmia, variable fall in cardiac output, fluctuating

aortic flow pattern, arterial hypotension and pulsatile venous hypotension.

Lewis and Eyre (1972) examined cardio respiratory responses to histamine in 18 anaesthetized calves and reported systemic hypotension. After initial hypotension endotoxic shock induced elevation of pulmonary arterial and venous blood pressure was followed by terminal hypotension and death.

Brungardt **et al** (1972) recorded arterial and portal pressure of dogs given **E.coli** endotoxin @ 0.125 mg/kg. There was significant and sustained fall in mesenteric blood flow. Portal pressure was increased transiently. The arterial pressure exhibited an early fall, a recovery and a second decline to a sustained level of hypotension.

Reece and Wahlstrom (1973) studied effect of **E. coli** endotoxin in cow calves and reported hypotension and tachy cardia. Blood pressure decreased early and was low even after 24 hours of effect of endotoxin. Heart rate decreased during first 50 minute and thereafter it increased and persisted till 24 hours.

Pingleton **et al** (1974) reported a fall of more than 50% in systemic pressure as well as cardiac output following injection of **E.coli** endotoxin in rhesus monkeys.

Daniel **et al** (1976) induced endotoxic shock in dogs by

infusing **E.coli** endotoxin @ 1 mg / kg B. wt. which led to an immediate fall in arterial pressure .

Singh (1979) reported that in buffalo calves **E.coli** endotoxin induced a significant increase in PCV, atrial fibrillalion, significant hypotension and steep fall in CVP.

Shrauwen **et al** (1985) induced endotoxin @ 0.5 mg / kg which resulted in progressively evolving deterioration of cardio vascular performance i.e. hypotension, decrease in cardiac output, an increase in pulmonary vascular resistance and death of all animals in 3.5 hours. Carotid artery pressure fell from 105 $\pm$ 4 mm Hg to 36 $\pm$ 4 mm Hg and cardiac output from 2.51 $\pm$ 0.26 litres/min. to 1.51 $\pm$ 0.19 litres/min at 120 minutes after injection as compared to normal pre-injection levels.

Olson and Talmage (1985) studied the effects of **E.coli** endotoxemia on haemodynamics of concious calves 4-6 weeks old. Continuous slow infusion at dose level of 4 Mg / Kg for 5 hours led to an increase in MAP and pulmonary vascular resistance while cardiac index, central plasma volume and mean systemic arterial pressure decreased.

Olson **et al** (1986) studied haemodynamics in endotoxemic pigs given **E.coli** endotoxin continuously by slow intravenous infusion @ 5 mg/kg the first hour followed by 2 mg/kg/hour. After 1 hour cardiac index deceased by 0.38 ml/s/kg and mean pulmonary arterial pressure and pulmonary vascular

resistance increased by 9.4 mm Hg and 7.5 mm Hg/ml/s/kg respectively. After pigs were endotoxmic for 4.5 hours cardiac index decreased by 1.03 ml/s/kg while mean pulmonary arterial pressure increased by 16.9 mm Hg and pulmonary ventricular pressure by 27.5 mm Hg/ml/s/kg respectively.

Clark **et al** (1991) studied the effects of 1/V infusion of endotoxin for 60 minutes at cumulative dose of 0.03 mg/kg B.Wt. in concious horses. Heart rate was increased significantly although systolic and diastolic arterial pressure, cardiac output, total peripheral resistance and right arterial pressures did not change significantly.

Average pulmonary arterial pressure was increased significantly. It was suggested that low dosage of endotoxin produce pulmonary hypertension causing hypotensive hypodynamic shock.

TRANSMINASES AND PHOSPHATASES

Paul and Vadlamudi (1973) studied the normal levels of enzymes in buffalo calves and found that the Asparate transaminase and alanine transaminase activity was 55.31 $\pm$ 1.68 and 42.56 $\pm$ 1.81 micro moles of pyruvate liberated per minute per litre. Serum alkaline phosphatase and serum acid phosphatase activity in Murrah buffalo calves was 2.54 $\pm$ 0.51 and 1.85 $\pm$ 0.16 units per liter respectively.

Nagaraja **et al** (1979) reported that intravenous administration of rumen bacterial endotoxin in calves

induced signs and pathophysiologic changes typical of endotoxin shock. There was elevation of serum GOT (AST), LDH, acid phosphatase and B glucuronidase.

Kumar (1989) reported the normal values of ALT and AST as 23.17 ± 0.92 and 56.67 ± 0.96 IU per litre. Acid phosphatase value was 2.02 ± 0.82 IU and Alkaline phosphatase values ranged between 10.36 ± 0.36 and 47.48 ± 16.55 KAU/100 ml.

Singh (1990) induced endotoxic shock in buffalo calves @ 0.6 mg / kg body weight and reported a significant decline in ALT from 21.47 ± 7.52 to 16.50 ± 7.50 IU within 10 minutes of shock and an elevation of AST from 63.59 ± 8.59 to 81.93 ± 2.07 IU at 30 minutes of shock. Alkaline phosphatase activity increased sharply from 7.32 ± 1.24 to 28.18 ± 10.20 KAU/100 ml at 30 minutes of shock and acid phosphatase activity increased marginally from 4.36 ± 0.88 to 5.64 ± 1.20 KAU/100 ml at 15 minutes of shock.

Sudhan **et al** (1993) induced endotoxin shock in 6 Black Bengal goats by intravenous administration of endotoxin @ 100 micrograms per kg. They reported significant increases in the levels of serum aspartate aminotransfeease, lactate dehydrogenase and alkaline phosphatase.

HISTAMINE

Spink and Vick (1961) reported that **E.coli** endotoxin causes peripheral vascular collapse due to histamine

liberation following activation of proteolytic system by endotoxin. Hinshaw **et al** (1961). The **E.coli** endotoxin administration in lethal doses to the anaesthetized dogs induced hypotension, increase in blood histamine and accelarated rate of conversion of histidine to histamine.

Wrenn **et al** (1963) showed that a small intravenous dose of living **E.coli** increased level of histamine in blood by 0.061 mg/ml, whereas a lethal dose caused a slight decrease.

Vick (1964) reported that trigger mechanism of endotoxic shock involved plasma, platelets and endotoxin. The product of this combination is blocked by anti-histaminics and has similar effect on vessel contraction. Further the platelets and plasma factor also are a rich source of histamine.

Stewart and Anderson (1971) reported that endothelial cells from endotoxin treated animals revealed extensive degeneration of subcellular organelles except vescicles and caveolae. Endotoxin caused swelling and protrusion of endothelial cells into lumen, detatchment of these cells from internal elastic laminae and marked thickening of the internal elsatic lamina. Vascular endothelium reacts to histamine, serotonnin, adrenaline and noradrenaline in a similar manner, suggesting that the effects observed after administration of endotoxin may be due to the release of such chemical mediators.

Aitken and Vanford (1972) studied the effects of histamine

and 5-HT on calves. The effects produced after histamine and 5-HT administration of the calves are similar to anaphylaxis. In anaesthetized calves histamine, 5-HT and brady-kinin produced respiratory and circulatory changes which also occur during anaphylactic shock.

Lewis and Eyre (1972) examined the cardio-respiratory responses to histamine, 5-HT and compound 48/80 in 18 anaesthetized calves. After administration of histamine and 5-HT, systemic BP decreased, pulmonary arterial BP increased and venous BP increased after an initial fall. During infusion of compound 48/80 whole blood histamine and plasma 5-HT rose in biphasic manner although changes in mast cells were minimal and there was no change in tissue histamine concentration. An initial rise in femoral arterial BP was followed by terminal hypotension and death. Both pulmonary arterial and venous BP rose and there was dyspnoeic respiration. Antihistaminics, mepyramine and methylsergide inhibited the toxicity of compound 48/80 which suggests that histamine and 5-HT play a major role in toxic response of calf to compound 48/80.

Verma and Gangapathy (1973) reported that normal blood histamine levels in healthy cows ranged between 0.10 to 0.20 mcg/ml (mean 0.16 mcg/ml).

Shrauwen **et al** (1985) showed that in anaesthetized piglets intravenous administration of **E.coli** endotoxin @ 0.5 mg/kg

was lethal and resulted in progressive deterioration of cardiovascular performance, an increase in pulmonary vascular resistance and death in all animals within 3.5 hours. Pretreatment with 5-HT$_2$ receptor antagonists increased pulmonary vascular resistance and survival time of animal suggesting serotonin to be involved in typical pulmonary vascular changes during endotoxic shock.

Anderson **et al** (1987) incubated leucocytes from Holstein Freisian calves with fleurescein bovine albumin (FBA) or FBA-histamine receptors. They identified 21% of the leucocyte population possessing histamine receptors. Inokuma **et al** (1994) demonstrated bovine peripheral lymphocytes to have both H_1 and H_2 receptors on their surface.

PROTHROMBIN TIME

Thomson **et al** (1974) reported that intravenous administration of sublethal dose of piromen, a pseudomonas species endotoxin to four calves resulted in disseminated intravascular coaggulation, Significant increases in prothrombin time from +4 to +40 hours was demonstrated. The peak increase of prothrombin time at +8 to +10 hours was 180%.

Nagaraja **et al** (1979) demonstrated that rumen bacterial endotoxin induced signs and pathophysiological changes typical of endotoxic shock. The signs and pathophysiological

changes were similar to those observed in calves that received Escherichia coli endotoxin. **E.coli** endotoxin injected at dose rate of 200 mg/kg body weight resulted in prothrombin time values of 15.5 seconds prior to endotoxin administration and 14.6, 15.3, 15.1, 17.3, 19.7, 20.7 and 19.5 seconds at 5, 15, 30, 60, 180, 360 and 720 minutes after endotoxin administration.

Patrick (1982) administered **E.coli** endotoxin @ 40 mg/kg Body weight to horses intravenously. They reported a consumptive coaggulopathy developing with gradually increasing activated clotting, prothrombin and activated partial thromboplastin times.

Yagi and Nakajima (1983) injected **E.coli** 0111 endotoxin in two 8 month old calves and reported prolongation of prothrombin time.

Nakam (1984) infused **E.coli** endotoxin 1/V in dogs for 10 hours and reported prolonged prothrombin and partial thromboplastin times.

Ewert **et al** (1985) injected 18 anaesthetized ponies **E.coli** endotoxin 055:B and reported no statistically significant differences for any of the clotting values except platelet counts. prothrombin time remained normal until 6 hours at wlhich time there was slight prolongation.

Naylor and Kronfield (1986) reported prolongation of prothrombin time in preguant sheep given **E.coli** endotoxin.

Welles **et al** (1993) reported significant increase in prothombin time in cows with experimentally induced endotoxemia and mastitis after intramammary infusion of 1 mg **E.coli** derived endotoxin. Prothrombin time in-creased from 17.2 ± 0.4 seconds prior to endotoxemia to 18.0 ± 0.4 and 19.0 ± 0.5 seconds at 12 and 24 hours after challange.

Semrad and Dubielzig (1993) studied effect of progressively increasing doses of endotoxin administration (0.1 to 15 mg/kg) on homeostasis in neonatal calves. Prolongation of prothrombin time, compared to base line values, was observed only at the 96 hours sample collection time. Prothrombin time prior to endotoxin administration was 15.3 ± 0.9 seconds which increased to 16.4 ± 1.0, and 17.0 ± 1.0 and 17.5 ± 1.1 at 24, 48 and 72 hours after endotoxin administration.

GENERAL CLINICAL BEHAVIOUR

Johannsen **et al** (1972) Three pigs induced endotoxemia died from shock like cardiovascular collapse, with edema of stomach wall and colonic mesentry. Those that survived showed sign of intoxication viz prostration and apathy (14), vomiting (9), muscular tremor (10), convulsions (2), paralysis of limbs (4), skin pallor (15) sometimes occompanied by ear cyanosis, hypothermia (7) and hyperthermia (1).

Johannsen **et al** (1972) infused endotoxemia by continuous intravenous drip and reported severe illness in every case. The illness lasted for 27-490 minutes (average 195 - minutes). Hyperthermia, either as progressive febrile rise in body temperature until death (14) or as a febrile rise in body temperature followed by a fall to normal or subnormal level (17) Rate and depth of respiration increased.

Wray and Thomlinson (1972) reported high rectal temperature, congested mucous membranes, collapse to sternal recombancy and death after **E.coli** endotoxemia.

Reece and Wahlstrom (1973) opined that the responses after endotoxin administration are similar to the clinical signs of naturally acquired neonatal colibacillosis, namely, dyspnoea and hyperpnoea, metabolic acidosis, blood-gas value changes and later hypotension.

Tennant **et al** (1973) induced endotoxic shock by 1/v administration of **E.coli** endotoxin in male calves @ 0.1 to 0.2 mg/kg body weight and observed hyperpnoea, salivation and restlessness within 5 minutes of administration. Within 15-25 minutes the calves become recombent. Liquid faces were passed once or twice but profuse watery diarrhoea was not observed. Eight calves died after an average time of 6.2 hours.

Miert **et al** (1978) administered 0.01 mg **E.coli** LPS into third ventricle of concious goats to induce shivering rise

in body temperature and inhibition of extrinsic ruminal contractions. A similar dose of pyrogen given 1/v was less effective.

Nagaraja **et al** (1979) reported an immediate hyperpnoea gradually leading to dyspnoea, profuse salivation, diarrhoea and collapse into laterel recombancy after intravenous injection of calves with rumen bacterial endotoxin.

Patrick **et al** (1982) reported that clinical signs in 16 horses given **E.coli** @ 40 micro gram/kg 1/v were divided in 3 phases. The first phase was characterised by cyanotic mucous membranes, sweating and lethargy developing into ataxia followed by prostration. Five ponies became comatose and died during this phase. The 11 remaining horses progressed to second phase and were more alert, drank water and urinated, had normal mucous colour but intermittent diarrhoea and quadrapedal laminitis. The third phase was slow progressive development of circulatory insufficiency with cyanosis, dyspnoea and prostration in ponies 26 to 36 hours after infusion.

Torano **et al** (1984) induced **E.coli** endotoxemia in 14 calves aged 1-3 weeks and estimated LD 50 at 4.4 $\pm$ 0.3 mg/kg body weight. A dose of 6 mg/kg was 100% lethal while 3.1 mg/kg caused diarrhoea in 3 of 5 calves with death of one. There was transient hypertension followed by severe hyertension.

Cullor (1992) reported that general effects of endotoxins include lethargy, respiratory distress, transient hyperthermia followed by hypothermia, diarrhoea and alterations in blood coaggulation systems.

Semrad and Dubielzig (1993) reported that calves receiving endotoxin became lethargic, with transient recombancy, tachypnea and diarrhoea. After 72-80 hours of administration there was piloerection, more prolonged recombancy and increased respiratory effort. The clinical status of calves improved within 2-3 hours after each endotoxin injection. Endotoxin in this study was injected every 8 hours. Four calves developed acute respiratory distress i.e. dyspnea, respiratory grunting, open-mouth breathing necessitating euthanasia from 97 to 104 hours.

CHAPTER - III

MATERIALS AND METHODS

Eight normal apparently healthy male buffalo calves between 8-10 months of age procured from the local market were used for the study. The calves were kept under same managemental conditions as are practised at PAU dairy farm. The samples were collected and normal values were evaluated after perfecting various techniques to be used. The same animals were given endotoxic shock by intravenous infusion of E.coli endotoxin @ 0.6 mg/kg body weight. Before administration of endotoxin[1] total dose of endotoxin was dissolved in 50 ml of normal saline (0.9% Nocl). The endotoxin was later infused into jugular vein over a period of 5 minutes.

CATHETERIZATION

Prior to catheterization the calves were kept off feed and water for 12 hours. The animals were placed under lateral recombancy without restraint. An area of 2-3 square inches of the skin over the left jugular furrow was cleaned and shaved. The area was anaesthetised by 20-30 ml of local anaesthesia 2% Lignocaine.

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1. Lypholized (Phenol extracted) E.coli endotoxin - SIGMA Chem. USA 0111 : B (Lipopolysaccharide).

An incision 4-5 cms long was made parallal to jugular furrow and the carotid sheath was isolated. The vagus nerve was separated from the carotid artery and ligated with silk thread anteriorly around the isolated artery. Another knot was applied by using silk thread posteriorly on the artery and pulled. A stab incision was made in the carotid artery and then saline heparinized polyethylene catheter[2] already connected to mercury manometer was slowly inserted and pushed towards the direction of heart. The catheter was inserted ensuring sufficient pressure to check coagulation of blood and to prevent rush of blood back along the tip of the carotid catheter. The catheter was held in the lumen of artery by tying the knot of the posteriorly placed silk thread in the carotid artery. The polyethyne tubing was flushed with heparinized saline solution to prevent blood coagulation.

Similarly the jugular vein was catheterized by inserting the free end of saline manometer[3] for the measurement of central venous pressure and for intravenous infusion of dye and endotoxin.

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2. PE 2000, 1/D of 0.14 cms, length 90 cms.

3. CVP manometer set, Vilfor Laboratories, Bombay.

The following studies were undertaken during normal conditions and after the effect of endotoxic shock in buffalo calves.

BLOOD PRESSURE

MEAN ARTERIAL PRESSURE (MAP)

The mean arterial pressure was evaluated from the tracings of blood pressure record of mercury manometer and kymographic assembly. For recording of blood pressure the carotid artery was exteriorized and the artery was catherized. The artery after catheterization was attached to the mercury manometer through a 3 way cannula for flushing and infusing heparin saline into the pressure transmission system to avoid coagulation in the catheter. The mean arterial pressure was calculated by using the equation :

$$\text{Mean arterial pressure (MAP)} = \text{Diastolic pressure} + \frac{1}{3}\text{ pulse pressure}$$

$$\text{Pulse pressure} = \text{Systolic pressure} - \text{Diastolic pressure}$$

CENTRAL VENOUS PRESSURE (CVP)

It was recorded by connecting the jugular vein on catheterization to the CVP saline manometer.

ELECTROCARDIOGRAPHY (ECG)

It was recorded by using cardiart ECG machine[3]. The recordings were made in horizontal plane by standard lead system. The electrodes were implanted on left foreleg, right foreleg, left hind leg and right hindleg. Before applying the electrodes the area was shaved and rubbed with jelly to ensure proper contact. The recordings were made under lead II of standard lead system.

BIOCHEMICAL AND ENZYME STUDIES

Normal blood samples for biochemical and enzyme studies were collected before infusion of endotoxin. The endotoxin was then infused over a period of 5 minute. Immediately after infusion zero minutes sample was collected thereafter samples were collected at 5 minutes, 15 minutes, 30 minutes, 60 minutes, 90 minutes and 120 minutes after infusion of total dose of endotoxin. The blood samples were collected for the study of enzymes and histamine using EDTA as anticoaggulant and sodium citrate for evalating prothrombin time. Histamine was estimated by taking duplicate samples of 1 ml each for regrigeration. The rest of blood samples were centrifuged and plasma separated out for study of enzymes.

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3. Cardiart ECG machine.

ASPARTATE TRANSAMINASE (AST) OR GLUTAMIC-OXALOACETIC TRANSAMINASE (GOT)

It was estimated by Wootton (1964). AST causes the conversion of aspartate to oxaloacetate which decarboxylate spontaneously to pyruvate. On reaction with DNPH it gives brown colored hydrazine which is measured at 546 nm wavelength.

ALANINE TRANSAMINASE (ALT) OR GLUTAMIK PYRUVIC TRANSAMINASE (GPT)

It was estimated by Wootton (1964) ALT catalyses the transamination of alanine to pyruvate which reacts with DNPH to give brown colored hydrazine that is measured spectronically at 510 nm wavelength.

ACID PHOSPHATASE AND ALKALINE PHOSPHATASE

These were estimated by Bergmeyer (1974). Phosphatases cause the hydrolysis of phenyl phosphate at a specific PH and the phenol released is estimated colorimetrically at 405 nm wavelength.

HISTAMINE

It was estimated by using the method of Klimkina and Plitman (1989). Whole blood sample is precipitated with 10% TCA solution (in a blood to acid ratio of 1:9) for histamine extraction. Before use the blood sample is kept in a refrigerator for 24 hours.

Principle - The method is based on reaction between histamine and diazotized paranitroaniline yielding red - orange products.

Procedure - Filter the trichloroacetic acid extract from blood through filter paper. Measure out 2ml of filtrate into a test tube (SAMPLE). In another tube put 0.2 ml of standard histamine solution (200 micro mol/L) and 1.8 ml of distilled water. (STANDARD). Add 1 ml of 4% sodium nitrite solution to the two tube. Mix the contents with vigorous shaking and place the tubes in a boiling water bath. Wait for 2 minutes and then cool under tap water. Add 1 ml of fresh diazotized paranitroaniline solution to both test tubes.Diazotized para nitroaniline solution is prepared prior to use from a 0.1% P-nitroaniline solution in 0.1 M Hcl; to this effect, 1 ml of 4% aqeous sodium nitrite solution is added to 10 ml of ice cooled P-nitroaniline solution. Mix thoroughly the contents and adjust their PH to 10.0 by adding 20% sodium carbonate solution. Mix the contents by shaking. Cool under tap water and add 2-3 drops of 5 M sodium hydroxide solution to allow coloration to develop. Measure the absorbance of sample and standard solutions at 520 - 540 nm wavelength on spectronic against control solution. Control solution is prepared by mixing 10 ml of distilled water, 2 ml of 4% sodium nitrite solution, 2 ml of diazotized P - nitroaniline solution, 4 ml of 20% sodium carbonate solution and 0.6 ml of 5 M sodium hydroxide solution.

Calculations :

Concentration of histamine in whole blood (micro mol/Litre)

$$= \frac{\text{E sample}}{\text{E standard}} \times \text{concentration of standard (200)}$$

E sample - Absorbance of sample.

E standard - Absorbance of standard.

CHOLINESTERASE

It was estimated by using the method of Voss and Sachsse (1970).

PROTHROMBIN TIME

Its was estimated by using LIQUIPLASTIN, a calcium thromboplastin reagent derieved from rabbit brain. (Tulip Diagnostics (P) Ltd., Goa).

PLASMA MINERALS

They were estimated by Duncan (1976) using atomic absorption spectroscopy.

STATISTICAL ANALYSIS

Statistical analysis of the data was done by Snedecor and Cochran (1976).

CHAPTER IV

RESULTS AND DISCUSSION

ELECTROCARDIOGRAPHIC STUDIES (ECG)

The electrocardiographic tracings which depict the functional state of heart appeared in the form of P wave, QRS complex and T wave were recorded under normal state and after endotoxic shock. The heart underwent changes in position, pressure and structure. As a result significant changes were recorded during shock which are shown in table-

13. CARDIAC POTENTIALS

The overall mean potentials of P wave, QRS complex and T wave during normal state and under the effect of endotoxic shock in buffalo calves are presented in table-1.

The overall potential of P wave in different buffalo calves ranged between 0.05 and 0.15 mv with a mean of 0.11 $\pm$ 0.01 mv. The differences between the different buffalo calves was found non significant.

Upadhayay *et al* (1976) reports that in 1 to 6 month old Jersey cattle the average potential of P wave was 0.10 $\pm$ 0.01 and in Red Dane cattle as 0.05 mv. They further report that the potentials in 3-6 years old animals were lower than smaller age groups. Sobti (1979) reported that overall mean P wave potential developing in buffalo calves ranged between 0.10 to 0.20 mv with a mean value of 0.13 mv.

TABLE -1 ELECTROCARDIOGRAPHIC CHANGES DURING ENDOTOXIC SHOCK DURING ENDOTOXIC SHOCK IN BUFFALO CALVES

PARAMETERS	NORMAL	0	5	15	30	60	90	120
1. Potentials (mv)								
Pwave	0.11	0.22**	0.22**	0.25**	0.07**	0.11	0.11	0.11
	±0.01	±0.01	±0.02	±0.02	±0.01	±0.01	±0.01	±0.01
QRS Complex	0.70	0.65*	0.77*	0.72	0.47**	0.65	0.52**	0.70
	±0.02	±0.02	±0.02	±0.01	±0.1	±0.01	±0.00	±0.01
Twave	0.08	0.10	0.06	0.10	0.16**	0.25**	0.11	0.15*
	±0.02	±0.03	±0.04	±0.04	±0.01	±0.01	±0.02	±0.02
2. Times (seconds)								
Pwave	0.05	0.06	0.46	0.6	0.05	0.05	0.06	0.04
	±0.01	±0.01	±0.01	±0.02	±0.02	±0.02	±0.01	±0.01
QRS Complex	0.07	0.10	0.10	0.10	0.08	0.08	0.08	0.06
	±0.02	±0.01	±0.02	±0.02	±0.01	±0.01	±0.2	±0.02
Twave	0.06	0.05	0.04	0.06	0.06	0.06	0.06	0.07
	±0.01	±0.00	±0.02	±0.01	±0.00	±0.00	±0.00	±0.00
3. Intervals (seconds)								
P-R interval	0.24	0.30	0.32*	0.32*	0.32	0.28	0.20	0.18*
	±0.02	±0.07	±0.02	±0.02	±0.07	±0.07	±0.07	±0.02
Q-T Interval	0.40	0.41	0.42	0.41	0.41	0.042	0.44	0.44
	±0.03	±0.02	±0.04	±0.07	±0.07	±0.02	±0.01	±0.01
S-T Intervals	0.34	0.32	0.30	0.36	0.36	0.36	0.37	±0.38
	±0.01	±0.04	±0.02	±0.07	±0.07	±0.07	±0.07	±0.04
S-T Segment	0.27	0.26	0.26	0.28	0.28	0.30*	0.30*	0.31*
	± 0.04	±0.04	±0.02	±0.02	±0.02	±0.04	±0.07	±0.02

* P (< 0.05)
** P (< 0.01)

Under the effect of endotoxic shock the mean potential of P wave increased from 0.11 to 0.22 mv immediately after infusion of total dose of endotoxin. Thereafter the effect of endotoxin on cardiac potential was inconsistent. After 30 minutes of endotoxic shock the P wave potential decreased to 0.07 mv which again returned almost to normal level after 60 minutes of shock. The potential thereafter stablized at the normal preinjection level till the end of study period of 120 minutes.

Similar observation under the effect of endotoxic shock in buffalo calves were recorded by Singh (1990). He reported that the potential of P wave did not show any marked variation. The alternations in the amplitude of P wave at different intervals of shock was non significant in buffalo calves (Sobti et al, 1982). However, Singh et al (1982) in their studies on septic shock by strangulating a segment of jejunum reported an increase in potential of P wave from pre-shock normal of 0.13 mv to 0.18 mv during septic shock.

The amplitude of QRS complex under normal state and endotoxic shock are given in table-1. The overall mean potential of QRS complex of lead II in different buffalo calves ranged between 0.60 and 0.80 mv with an average of 0.70 $\pm$ 0.02 mv. The QS and QR forms of QRS complex occured more frequently than QRS as reported by Upadhayay et al (1976) in Jessey cattle. Sobti (1979) reported that very

young calves exhibit more QRS potential than the grown up buffalo calves. Similarly Upadhayay et al (1976) also reported a higher potential in younger stock than older ones.

During the effect of endotoxic shock the normal mean ventricular potential (QRS Complex) increased significantly ($P < 0.05$) from 0.70 to 0.77 mv within 5 minutes of endotoxin induction. However, the ventricular potential remained low thereafter during the remaining period of study.

Singh et al (1982) reported that preshock QRS potential of 0.70 mv increased to 1.05 mv following septic shock in buffalo calves. Earlier Singh (1990) following infusing endotoxin and Sobti et al (1982) after haemorrhagic shock also reported an increased QRS complex amplitude.

The overall ventricular repolarization potentials i.e. T wave in different buffalo calves ranged between 0.05 and 0.10 mv with a mean of 0.08 ± 0.02 mv. The T wave was mostly of V shaped configration. The incidence of biphasic and notched T wave was infrequent in lead II. These observations well similar to those made by Sodhi (1979) in buffalo calves and contrary to the one made by Upadhayay et al (1976) and Alfredson and Sykes (1942).

The potential of T wave recorded an increase in general under the effect of endotoxin in buffalo calves. T wave potential increased significantly ($P < 0.01$) after 30

to 60 minutes endotoxic shock from normal preinjection level os 0.08 mv to 0.25 mv.

Septic shock following strangulating a piece of jejunum revealed a peaked T wave in buffalo calves (Singh et al 1982). They reported a significant increase in the potential of T wave from preshock level of 0.37 mv to 0.67 mv during shock. Sobti et al (1982) also reported an increase in potential of T wave from normal level of 0.19 mv to 0.27 and 0.34 mv after 1 hour following mild and severe haemorrhagic shock respectively. However Singh (1990) observed a decrease in potential of T wave from normal 0.16 mv to 0.12 mv following endotoxic shock in buffalo calves.

The increase in ventricular potential and changes in configuration of T wave following haemorrhagic shock were indicative of myocardial ischaemia (Sobti et al, 1982). The prolongation of QRS complex and peaked T wave might be due to hyperkalemia as recorded by Singh and Flear (1969) and Drucker (1971).

CARDIAC TIMINGS

The normal mean timings of P wave, QRS comples and T wave were 0.05, 0.07 and 0.06 seconds respectively. These normal timings are comparable to 0.06, 0.08 and 0.10 seconds (Sobti et al, 1982); 0.11, 0.10 and 0.17 seconds (Singh et al, 1982) of P wave, QRS complex and T wave respectively in buffalo calves.

The data presented in table - 1 revealed that endotoxin infusion increased the timings of QRS complex from normal 0.07 seconds to 0.10 seconds within 5 minutes and it remained prolonged thereafter. The prolongation of QRS complex was also recorded by Sobti et al (1982), Singh (1990) and Singh et al (1982). This prolongation of QRS Complex could be due to hyperkalemia (Sobti et al, 1982).

No significant variation was recorded in the timings of P wave and T wave following endotoxic shock. Similar observations were made by Sobti et al (1982). However Singh et al (1982) observed a decrease in duration of T wave followed by an increase in the terminal stages of septic shock.

CARDIAC INTERVALS

The various cardiac intervals i.e. auriculo-ventricular conduction time (P-R interval), intra-ventricular conduction time (Q-T interval), isoelectric phase of ventricle (S T interval) and S-T segments during normal state and under the effect of endotoxic shock in buffalo calves are presented in table - 1.

P-R INTERVAL

The normal mean auriculo-ventricular conduction time (P-R interval) was 0.24 $\pm$ 0.02 seconds. These normal values were slightly higher than the normal mean PR interval of 0.16 $\pm$ 0.07 (Singh et al, 1982), 0.17 $\pm$ 0.00 (Sobti et

al, 1982) and 0.20 seconds (Singh, 1990) in buffalo calves.

After the induction of endotoxic shock the P-R interval was prolonged significantly ($P < 0.05$) at 5 and 15 minutes of E.coli endotoxin injection. However, P-R interval decreased significantly ($P < 0.05$) from normal level of 0.24 $\pm$ 0.02 seconds to 0.18 $\pm$ 0.02 seconds during late stages of shock viz 90 and 120 minutes.

The shortening of P-R interval during late stages of shock was also reported by Singh et al (1982) following septic shock and Sobti et al (1982) after haemorrhagic shock in buffalo calves. However, no clinical significant could be attached to decrease in P-R and Q-T intervals in septic shock (Singh et al, 1982).

Q-T INTERVAL

The mean normal intraventricular conduction time (Q-T interval) was 0.40 $\pm$ 0.03 seconds. This was comparable to the mean normal value of 0.43 $\pm$ (Singh et al, 1982), 0.48 $\pm$ 0.00 (Singh, 1990) and 0.51 $\pm$ 0.03 seconds (Sobti et al, 1982) in buffalo calves.

Following endotoxic shock Q-T interval registered a non significant prolongation in buffalo calves (table-1). Singh (1990) and Singh et al (1982) reported a significant shortening of Q-T interval following septic shock in buffalo calves. However, Sobti et al (1982) recorded a prolongation followed by shortening of Q-T interval after haemorrhagic shock in buffalo calves.

S-T INTERVAL

The mean values recorded for isoelectric phase of ventricle (S-T interval) and S-T segments before infusion of endotoxin were 0.34 ± 0.01 and 0.27 ± 0.01 seconds respectively. These values were comparable to 0.40 and 0.24 seconds recorded by Singh (1990) for S-T interval and S-T segment respectively. Singh et al (1982) recorded a normal S-T segment of 0.14 ± 0.03 seconds in buffalo calves.

Following the induction of endotoxic shock S-T interval and S-T segment were shortened during initial 5 minutes of shock. However in late stages of endotoxic shock viz 60, 90 and 120 minutes S-T segment recorded a significant increase ($P < 0.05$) from normal 0.27 seconds to 0.30, 0.30 and 0.31 seconds respectively.

The increase in S-T segment following septic shock was also observed by Singh et al (1982) and Celly and Prasad (1989) in buffalo calves. The elevation of S-T segment indicating myocardial hypoxia may develope due to improper ventilation which has been reported to occur in bovine septic shock (Celly and Parsad, 1987).

The decrease in mean arterial pressure, central venous pressure, pulse pressure, cardiac output after endotoxin injection lead to low flow pressure in the coronary vessels which produced myocardial ischaemia and thus producing a typical S-T segment prolongation.

The normal heart has a tremendous amount of reserve pumping ability. Decreasing the cornary arterial pressure to as low as 50 mm hg ordinarily will not decrease the cardial output. Decreasing the cornoary artery pressure to 80 mm Hg decreases 10% pumping effectiveness of heart. The pumping ability of heart is highly dependednt on the coronary arterial pressure. A fall in coronary arterial pressure below 40 mm Hg was sufficient to cause cardiac deterioration and death (Donald, 1968).

HEART RATE (HR)

The mean heart rate during normal state and under the effect of endotoxic shock in buffalo calves are given in table - 2.

The normal heart rate ranged between 53.57 and 71.42 (65.83 ± 2.70) beats per minute in buffalo calves (table - 2). These values are within the range of 45.02 and 68.18 beats/minute (Singh and Sodhi, 1992), 54.20 ± 3.97 beats/minute (Sobti, 1979) and 65.0 ± 11.9 beats/minute (Singh, 1979) in buffalo calves.

The endotoxin infusion did not show any tachycardia or bradycardia like effects in buffalo calves. Although there was fluctuating frend (figure-1), the heart rate was slightly increased after 5 minutes of endotoxic shock. But it decreased thereafter and remained low till 2 hours after endotoxic shock. The results were never consistent.

TABLE - 2 HEART RATE AFTER ENDOTOXIC SHOCK IN BUFFALO CALVES (beats/min)

→ Calf No. / ↓ Minutes after endotoxic shock	1	2	3	4	5	6	Mean ± SE
NORMAL	69.18	65.21	71.42	71.42	53.57	65.21	65.833±2.70
0	50.00	78.95	75.00	71.42	45.45	55.55	62.723±5.77
5	83.30	81.08	62.50	65.21	62.50	50.00	67.433±5.15
15	62.50	90.90	66.60	51.72	65.71	57.69	65.853±5.49
30	60.00	75.00	48.38	46.87	75.00	65.21	61.743±5.05
60	68.18	75.00	57.69	46.87	60.00	68.18	61.103±4.68
90	62.50	68.18	60.00	45.45	53.57	65.21	59.153±3.41
120	69.76	-	62.50	46.87	65.21	62.50	61.373±3.86

* P (<0.05)
** P (< 0.01)

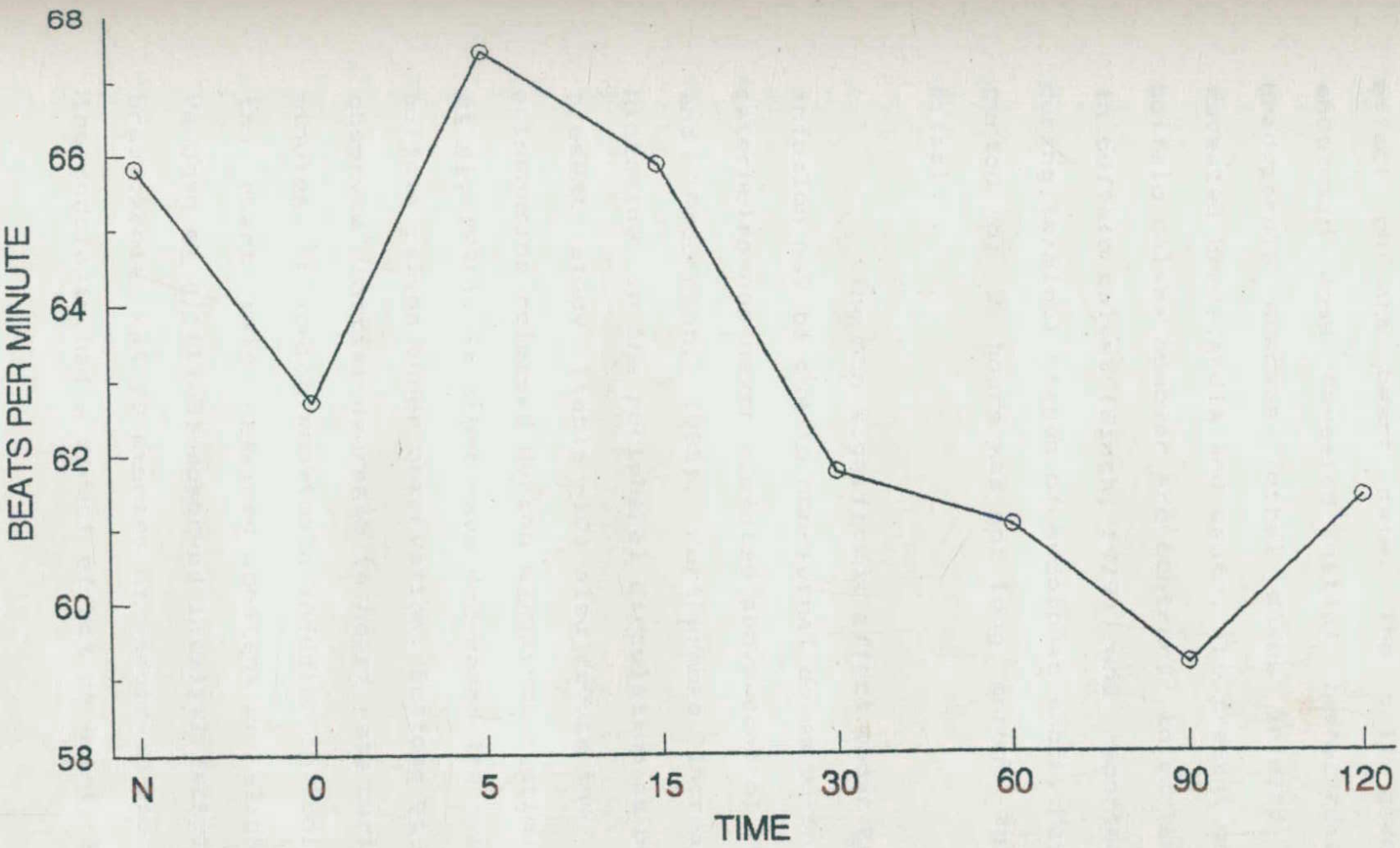

Fig. 1. HEART RATE DURING ENDOTOXIC SHOCK IN BUFFALO CALVES (BEATS PER MINUTE)

Singh and Sodhi (1992) while studying the effect of endotoxic shock in buffalo calves reported a variable effect on the heart rate. The calf given non lethal endotoxin dose revealed initial tachycardia followed by bradycardia whereas lethal doses infused intracardially revealed bradycardia and death. The present observations in buffalo calves however are contrary to earlier observations in buffalo calves (Singh, 1979) who reported tachycardia during terminal stages of endotoxic shock. May be the study period of 2 hours was not long enough to observe this effect.

The non significant effect under E.coli endotoxin infusion may be due to non-lethal doses during which plasma catecholamines never elevated above control levels (Dusapin and Mcdonough, 1985). Furthermore increased level of histamine in the peripheral circulation as observed in the present study (table -10) also reduce the amount of nor epinephrine released during sympathetic stimulation (Robert et al, 1991). It might have decreased the heart rate in some buffale calves under observation. Bottoms et al (1981) also observed initial decrease in heart rate during the first 5 minutes following endotoxin infusion in ponies. Thereafter the heart rate remained constant or slightly increased. Vaughen et al (1968) observed intitial tachycardia and late bradycardia at 30 minutes of endotoxic shock stating that the endotoxin had a direct effect on heart. They stated that

the release of catecholamines during induction of endotoxic shock may have induced tachcardia during early stages of shock.

The sympathetic system and catecholamines have synergestic effects with endotoxin in the production of ischaemia and death. The progressive slowing of heart beat with widening of QRS complex and rising of central venous pressure as observed in buffalo calf that died following infusion of endotoxin, was more pronounced when the arterial pressure was at the irreversible shock level i.e. just before death.

Those buffalo calves that survived the endotoxic shock rarely had an abnormality of the heart beat and conduction was usually normal.

MEAN ARTERIAL PRESSURE (MAP)

The mean arterial pressures during normal state and under the effect of endotoxic shock in buffalo calves are presented in table-3. The normal values of mean arterial pressures in buffalo calves ranged between 128.6 and 163.3 (150.6 $\pm$ 4.4) mm Hg. These values were close to the values of 131.2 and 166.3 mm Hg in calves (Singh, 1990). However, Sobti and Singh (1979) reported slightly lower normal MAP of 127 $\pm$ 4.5 mm Hg in buffalo calves.

The buffalo calves given E.coli endotoxin revealed significant haemodynamic alterations (table-3). Within

TABLE -3 MEAN ARTERIAL PRESSURE DURING ENDOTOXIC SHOCK IN BUFFALO CALVES (mm of Hg)

→ Calf No. Minutes after ↓ endotoxic shock	1	2	3	4	5	6	7	Mean ± SE
NORMAL	152.0	150.0	162.0	163.3	144.6	128.6	154.0	150.64±4.43
0	129.3	150.0	66.0	111.3	128.6	78.6	120.0	111.97±11.24
5	110.6	122.0	71.3	112.6	107.3	68.6	100.0	98.91±7.88*
15	118.6	92.6	77.3	120.0	82.0	45.3	96.6	90.34±9.74**
30	109.3	94.6	84.0	109.3	70.6	48.6	92.0	86.91±8.20**
60	116.0	88.0	88.6	113.3	65.3	37.3	98.0	86.64±10.46**
90	74.6	81.3	82.0	110.6	60.6	30.6	96.6	76.61±9.74
120	79.3	81.3	82.0	111.3	81.3	34.6	103.3	79.24±9.16

* P (<0.05)
** P (< 0.01)

minutes of endotoxin injection there was a decrease in the mean arterial pressure. It was also observed that the infusion of endotoxin in any concentration lead to an immediate fall in MAP. Thereafter MAP remained at same low level after 5, 15, 30, 60 and 90 minutes of endotoxxic shock (figure-2). A slight non significant recovery in MAP was recorded after 120 minutes of shock. However, MAP never returned to normal preinjection level in buffalo calves even after 3 to 4 hours of endotoxic shock although modest recovery was observed. The arterial pulse pressure decreased along with the decrease in MAP following endotoxin injection.

Singh (1990) in an earlier study also reported a fall in mean arterial pressure in buffalo calves when infused E.coli endotoxin @ 0.8 mg/kg body weight. Miller et al (1987) reported a decrease in MAP from base line (115 ± 5 mm Hg) to 55 ± 2, 54 ± 5 & 53 ± 3 mm Hg at 60, 90 and 120 minutes after endotoxin administration, respectively. Hypotension following endotoxin infusion was also observed by Bottoms et al (1981), Burrows (1970) and Bond et al (1991).

Burrows (1970) reported decrease in venous return, cardiac output and systemic arterial pressure during slow intravenous administration of E.coli endotoxin in anaesthetized ponies. He thought that during endotoxic shock there is stagnant anoxia due to relative lack of peripheral blood flow. Foklow (1962) suggested that locally released

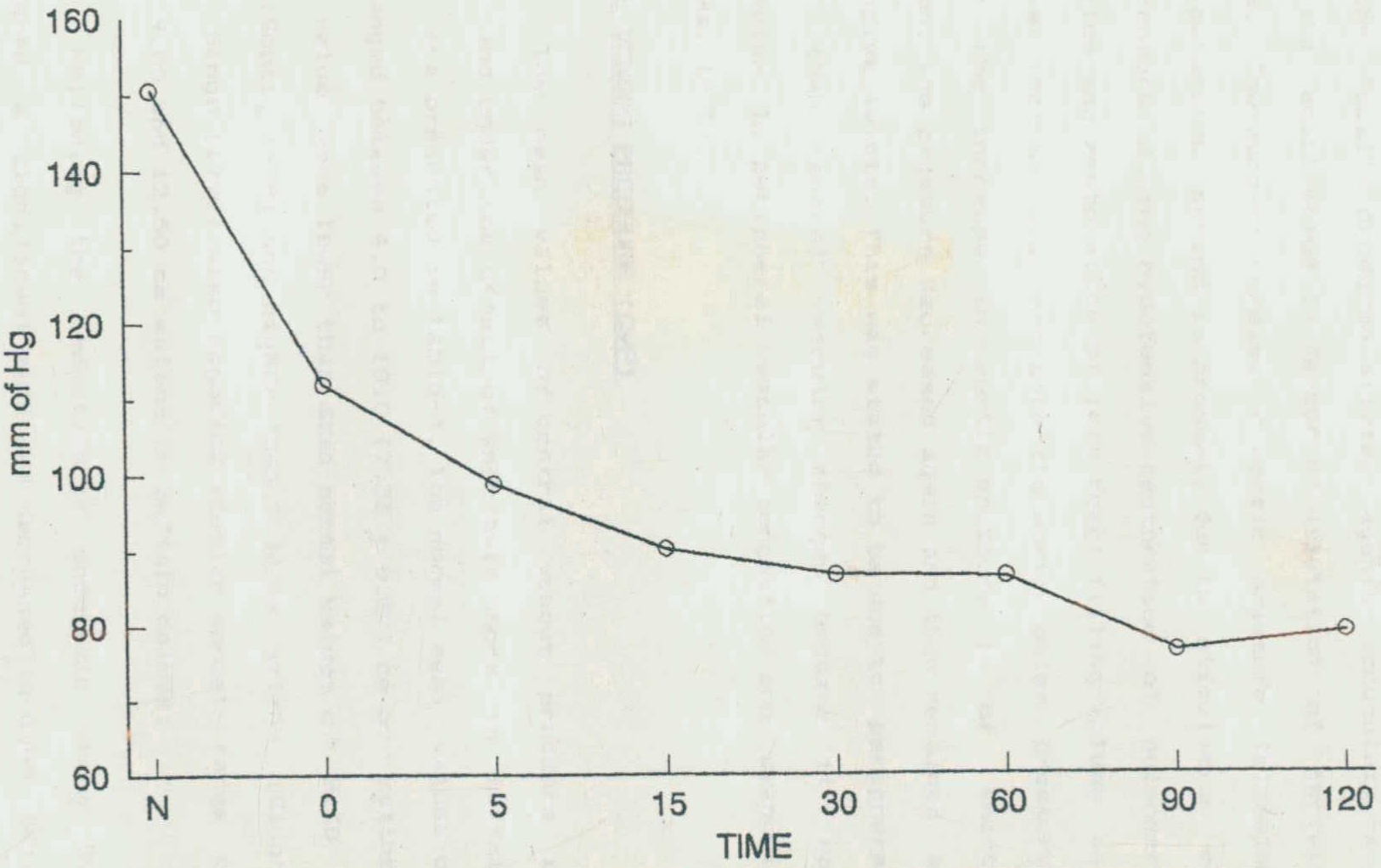

Fig. 2. MEAN ARTERIAL PRESSURE DURING ENDOTOXIC SHOCK IN BUFFALO CALVES (mm oF Hg)

constricter fibre transmitter and locally produced dilater agents are in equilibrim. Under conditions of reduced blood flow the locally produced dilater agents accumulate and shift the equilibrium in favour of dilatation of certain vessels. The sudden increase in aortic pressure following the hypotensive period is probably due to stimulation of baroreceptors during hypotensive restoration of pulmonary blood flow and restoration of left heart filling volume and pressure. That may also increase the aortic pulse pressure. However the increase in aortic pressure is of short duration. The pressure decreased again and then remained at hypotensive levels. This was stated to be due to peripheral rather than central vascular changes becuase it was accompanied by peripheral vascular congestion and cyanosis (Burrows, 1970).

CENTRAL VENOUS PRESSURE (CVP)

The mean values of central venous pressure in normal and under the effect of endotoxic shock in buffalo calves are presented in table-4. The normal mean values of CVP ranged between 4.0 to 10.0 (7.33 $\pm$ 0.80) cm of saline. These value were lower than mean normal values of 8.30 $\pm$ 1.67 (Sobti, 1979) and higher than 5.00 cm saline (Singh, 1979). Singh (1990) also reported similar normal range of CVP of 9.00 and 12.50 cm saline in buffalo calves.

Following the induction of endotoxic shock CVP registered a significant fall and decreased to even below

TABLE -4 CENTRAL VENOUS PRESSURE DURING ENDOTOXIC SHOCK IN BUFFALO CALVES (cm of Saline)

→ Calf No. ↓ Minutes after endotoxic shock	1	2	3	4	5	6	Mean ± SE
NORMAL	8.0	7.5	4.0	8.0	6.0	7.5	7.3 ± 0.8
0	6.0	5.5	3.0	6.0	4.0	6.0	5.3 ± 1.6
5	4.5	4.0	1.5	4.0	3.5	4.0	3.5* ± 0.6
15	2.5	2.5	0	1.0	3.0	2.5	2.1** ± 0.7
30	3.0	2.5	-1.0	0	3.0	2.5	2.2** ± 0.5
60	3.0	2.5	0	1.0	3.0	2.5	2.8** ± 0.7
90	3.0	2.0	1.0	1.5	2.5	2.5	2.7**± 0.7
120	3.5	2.5	2.0	2.5	3.5	4.0	3.0**± 0.9

* P (<0.05)
** P (< 0.01)

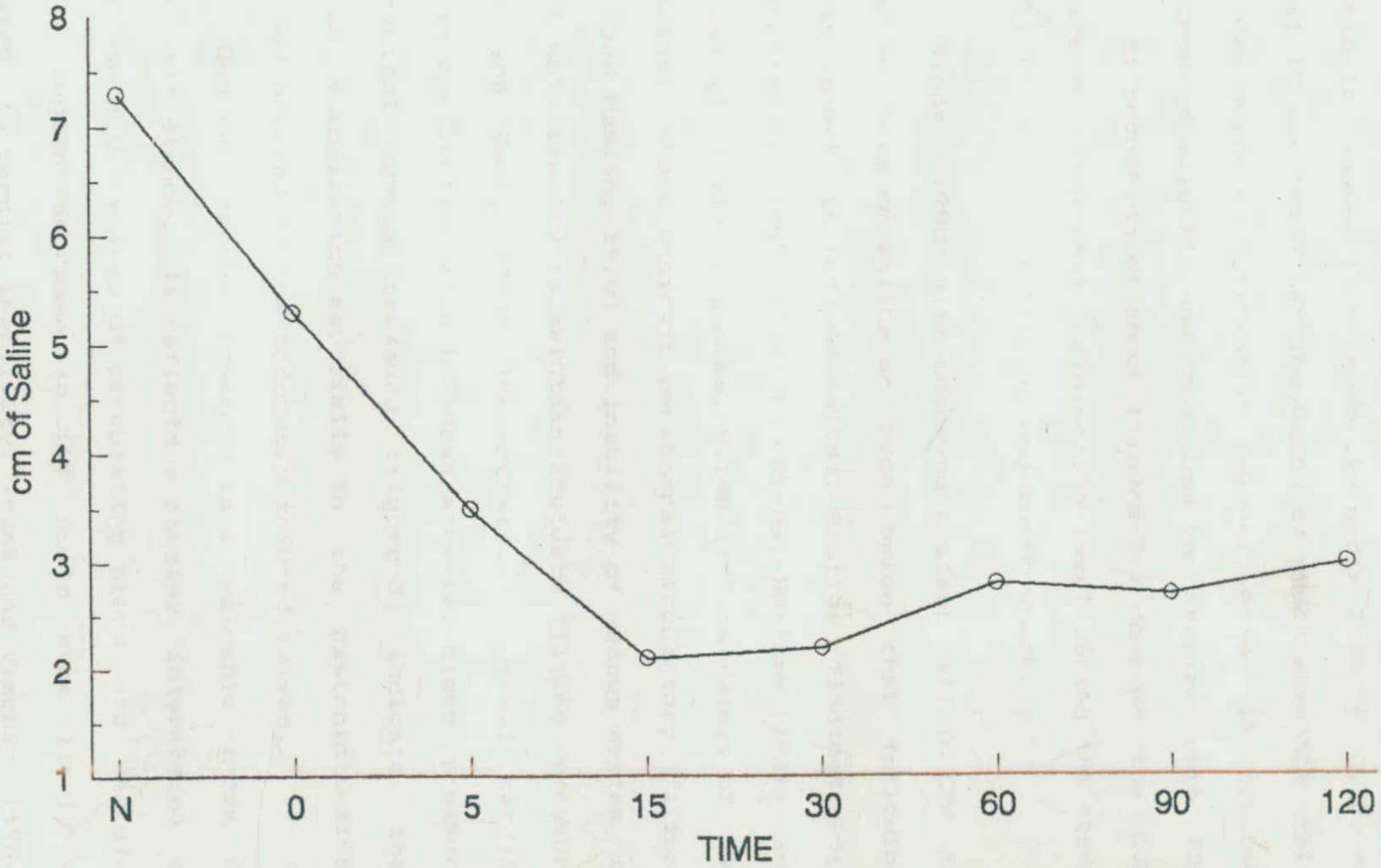

Fig. 3. CENTRAL VENOUS PRESSURE DURING ENDOTOXIC SHOCK IN BUFFALO CALVES (cm of saline)

zero cm saline in some animals. The mean level of CVP remained below the normal level throughout the study period of endotoxic shock. It reached its nadir 2.10 ± 0.78 cm saline at 15 minutes after induction of shock when the fall in CVP was quite significant ($P < 0.01$). After 15 minutes CVP recovered slightly and continued to recover upto 120 minutes of induction of shock (figure-3). However the CVP never reached its normal preinjection level during the study period of 120 minutes following endotoxic shock.

Singh (1990) also observed a steep fall in CVP to as low as zero cm saline or even below that following endotoxic shock in buffalo calves. Similar findings were also reported by Singh (1979) in calves, Bottoms (1981) and Robert et al (1991) in ponies. Extremely low values of CVP in endotoxic shock indicate peripheral circulatory failure (Smith and Hamlin, 1970) and inability of venous system to contract sufficiently to maintain adequate filling pressure (Nelson and Swan, 1974). The relatively normal cardiac output in conjunction with low mean arterial blood pressure and central venous pressure (figure-3) indicate that extensive vasodilation especially in the gastrointentinal region had occured in the endotoxin infused calves.

Central venous pressure is a valuable index of cardiac efficiency. It reflects a complex interaction of cardiac output, volume of circulating blood and vascular tone. A sudden decrease in CVP would more likely be correlated to cardiac inactivity. Trank and Vischer (1962)

postulated that the hemoregulatory barostatic mechanism is reset at a lower level during period of prolonged hypotension. The reset mechanism then does not intitiate a compensatory response until a very low blood pressure are attained. In calves, failure of capacitance changes due to lack of venous contraction may be one of the important factor leading to death following endotoxin administration (Singh, 1979). A reduction in blood flow to the CNS during shock is believed to result in irrepairable damage and may be contributing to mortality in clinical cases.

A sudden marked rise in central venous pressure as observed in the buffalo calf that died following intravenous endotoxin administration suggests myocardial failure with overtransfusion as a possible cause. Pericardial temponade is also a possibility (Moore, 1968).

PLASMA BIOCHEMICAL PROFILE

The mean values of different constituents during normal state and endotoxic shock are presented in tables 5 to 10.

ACID PHOSPHATASE

The mean concentration of acid phosphatase under normal and under the effect of endotoxic shock is given in table - 5. The mean normal acid phosphatase values in buffalo calves ranged between 1.40 and 2.22 units per litre (1.62 $\pm$ 0.11 units/litre). These values were lower than the normal values of 2.02 $\pm$ 0.82 IU (Kumar, 1989) and higher

TABLE - 5 ACID PHOSPHATASE CONCENTRATION DURING ENDOTOXIC SHOCK IN BUFFALO CALVES(Units/litre)

→ Calf No. / ↓ Minutes after endotoxic shock	1	2	3	4	5	6	7	Mean ± SE
NORMAL	1.4	1.4	1.6	1.6	1.6	1.4	2.2	1.6 ± 0.1
0	2.1	1.8	1.1	1.0	1.4	-	1.6	1.5 ± 0.1
5	4.6	1.1	0.4	1.1	-	1.1	1.6	1.7 ± 0.6
15	-	2.8	1.2	1.4	1.9	1.5	1.9	1.8 ± 0.2
30	3.9	2.4	1.1	2.4	2.2	1.4	2.5	2.3** ± 0.1
60	6.6	6.0	-	3.0	3.8	2.2	2.1	4.2** ± 0.7
90	7.6	7.3	4.4	3.5	3.5	2.2	3.8	4.6** ± 0.7
120	-	5.7	4.4	3.5	3.8	2.7	3.8	4.0** ± 0.4

* P (<0.05)
** P (< 0.01)

than 1.20 ± 0.29 IU (Mirakhur, 1979). Singh and Sodhi (1992) reported that normal acid phosphatase in buffalo calves ranged between 0.57 ± 0.02 and 6.26 ± 0.11 KAU/100 ml (3.41 ± 2.28 KAU/100 ml).

The biochemical changes in response to E.coli endotoxin injection elevated plasma concentration of acid phosphatase. Within 15 minutes following injection there was an increase in the concentration of acid phosphatase. The increase in concentration at 60 minutes after injection of endotoxin was significant ($P < 0.05$). The acid phosphatase level increased siginificantly from normal values of 1.62 ± 0.11 IU/L to 2.31 ± 0.13, 4.29 ± 0.70, 4.66 ± 0.70 and 4.03 ± 0.40 IU/L at 30, 60, 90 and 120 minutes of endotoxic shock (table - 5; Fig. 4).

Singh and Sodhi (1992) also recorded an increase in acid phosphatase activity in plasma in cow and buffalo calves following shock. Similar observations were also recorded by Mirakhur (1979) and Ewert et al (1985) in buffalo calves. Nagaraja at al (1979) in their study on endotoxic shock in calves observed a definite increase in serum acid phosphatase at 6 hours after injection of endotoxin. They reported that elevated serum enzyme particularly hydrolytic enzymes (acid phosphatase) indicate endotoxin induced tissue damage (Sleeman et al, 1971).

Intravenous administration of endotoxin produces characteristic increase in acid phosphatase due to prostrate

fraction. The hypoxia subsequent to ischaemia of prostrate and disintegration of cell wall elevated the acid phosphatase level (Wood word, 1959). The elevated serum acid phosphatase reflected the lysosomal disruption secondary to local cellular anoxia (Clermount et al, 1972). During shock there is insufficient production of ATP. The hydrolytic enzymes contained in the lysosomal membrane are released due to regression of the membrane. The enzyme enters the blood and thus elevates acid phosphatase concentration.

ALKALINE PHOSPHATASE

The mean concentration of alkaline phosphatase in normal state and under the effect of endotoxic shock are given in table - 6.

The normal mean values of alkaline phosphatase ranged between 4.00 and 8.25 IU per litre (5.75 ± 0.55 IU/L) in buffalo calves (Table-6). These normal values of alkaline phosphatase in buffalo calves are within the range of 2.42 to 5.41 IU recorded by Singh (1995) in neonatal buffalo calves. Kumar (1989) reported a normal range of 10.36 ± 0.37 to 47.48 ± 16.55 KAU/100ml and Singh and Sodhi (1992) reported a normal range of 4.89 ± 0.84 to 7.32 ± 1.24 KAU/100 ml (5.72 ± 1.23 KAU/100 ml).

Singh and Sodhi (1992) reported an increase in alkaline phosphatase activity during the effect of endotoxic shock. However, their values were contrary to the

TABLE - 6 ALKALINE PHOSPHATASE CONCENTRATION DURING ENDOTOXIC SHOCK IN BUFFALO CALVES (units/litre)

→ Calf No. / ↓ Minutes after endotoxic shock	1	2	3	4	5	6	7	Mean ± SE
NORMAL	5.0	8.2	5.0	5.0	6.0	4.0	7.0	5.7 ± 0.5
0	4.0	6.0	1.2	1.2	2.5	-	3.5	3.0** ± 0.7
5	6.5	3.0	2.0	2.0	-	2.0	2.3	2.9** ± 0.7
15	-	3.5	2.0	2.0	1.2	4.0	3.5	2.7** ± 0.4
30	6.0	3.0	3.0	2.5	1.2	2.0	2.3	2.8** ± 0.5
60	6.5	3.5	-	2.5	2.0	2.0	2.3	3.4** ± 0.6
90	11.5	6.0	7.0	4.5	5.5	2.0	5.5	6.0 ± 1.0
120	-	6.0	9.0	4.5	6.0	3.8	6.5	5.9 ± 0.7

* P (<0.05)
** P (< 0.01)

By definition, 1 unit of AKP is the amount of enzyme which liberates 0.05 micromoles p-nitrophenol under the described conditons (0.1 ml serum/plasma, 11.1 ml assay volume, 30 minutes)

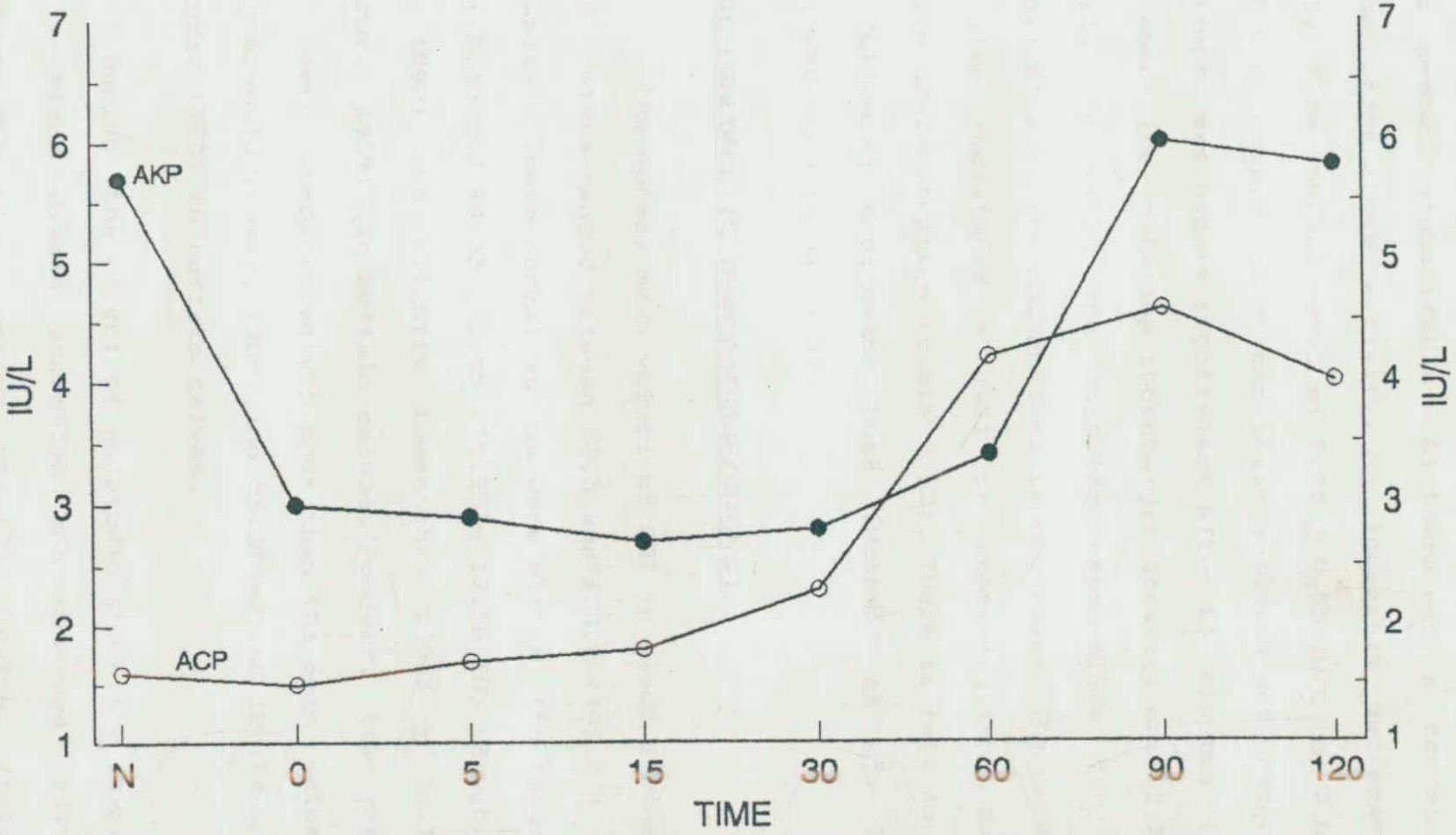

Fig. 4. ACID AND ALKALINE PHOSPHATE CONCENTRATION DURING ENDOTOXIC SHOCK IN BUFFALO CALVES (UNITS/LITRE)

present observations under the effect of E.coli endotoxin. In the present study (table - 6) there was a decrease in alkaline phosphatase activity. An immediate decrease in activity from normal level of 5.75 ± 0.55 IU/L to 3.08 ± 0.74 IU/L on completion of endotoxin infusion was recorded. The effect was highly significant after 15 minutes ($P < 0.01$) when the alkaline phosphatase activity was 2.70 ± 0.45 IU/L possibly the cofactor responsible for the synthesis of alkaline phosphatase is decreased. The cofactor zinc also registered a fall in concentration during endotoxin administration (table - 12). There is fall in zinc level following endotoxic shock (Withman et al, 1992; kindall and Airumlamai, 1991).

GLUTAMIC-OXALOACETIC TRANSAMINASE/GOT/AST

The normal mean values of GOT in blood plasma of buffalo calves ranged between 66.5 and 133.0 (101.3 ± 10.3 IU) Table-7. These normal values were within the range of 61.48 ± 2.68 and 94.35 ± 1.55 (75.95 ± 17.56 IU) (Singh and Sodhi, 1992) and slightly lower than 150.5 ± 22.1 IU (Mirakhur, 1979) in buffalo calves. However, the present values were comparitively higher than the mean value of 56.67 ± 0.96 IU (Kumar, 1989) and 55.31 ± 1.68 IU (Paul and Vadlamudi, 1973) in buffalo calves.

During the effect of endotoxic shock the level of GOT in blood plasma increased and remained elevated throughout the study period of 120 minutes following

TABLE -7 GLUTAMIC-OXALOACETIC TRANSAMINASE DURING ENDOTOXIC SHOCK IN BUFFALO CALVES (IU)

→ Calf No. / ↓ Minutes after endotoxic shock	1	2	3	4	5	6	7	Mean ± SE
NORMAL	99.7	110.8	133.0	66.5	66.5	99.7	133.0	101.3±10.3 *
0	166.2	177.3	199.5	116.3	83.1	-	221.6	160.7±21.2 **
5	219.4	221.6	199.5	83.1	-	166.2	177.3	177.8±20.9
15	-	221.6	199.5	99.7	66.5	99.7	177.3	144.0±25.9
30	186.2	177.3	199.5	66.5	66.5	133.0	133.0	137.4±20.6
60	172.0	221.6	-	99.7	66.5	166.2	133.0	143.3±22.6
90	106.4	133.0	199.5	83.1	83.1	133.0	221.6	137.1±20.6
120	-	110.8	-	83.1	83.1	199.5	117.3	130.7±24.3

* P (<0.05)
** P (< 0.0

infusion of endotoxin. The increase in GOT from 101.3 ± 10.3 to 160.7 ± 21.2 IU immediately following endotoxin infusion was highly significant ($P < 0.01$). Thereafter i.e. 15 minutes of shock, although the GOT level decreased but the plasma concentration was still higher than mean normal level (Table - 7; **Fig.5**).

Earlier Mirakhur (1979) and Singh and Sodhi (1992) while studying the effect of endotoxic shock also reported an increase in plasma GOT level in buffalo calves and Musa *et al* (1971) and Nagaraja *et al* (1979) in cow calves.

Mirakhur (1979) reported that the increased concentration of GOT in blood plasma during endotoxic shock was due to loss of hepatic cellular integrity. In general, during endotoxic shock there is ischaemia of body cells that injures the cell wall and on disintegration of cell wall the contents of the cells are released into peripheral circulation, thus increasing the concentration of GOT as observed in the present study.

GLUTAMIC PYRUVIC TRANSAMINASE/GPT/ALT

The normal mean value of GPT in blood plasma of buffalo calves ranged between 22.0 and 33.0 IU (27.0 ± 1.5 IU) Table - 8. The present values were comparable to normal values of 12.73 ± 0.16 to 24.76 ± 0.49 (19.65 ± 7.33 IU) (Singh and Sodhi, 1992) and 23.17 ± 0.92 IU (Kumar, 1989) in buffalo calves.

TABLE - 8 GLUTAMIC PYRUVIC TRANSAMINASE DURING ENDOTOXIC SHOCK IN BUFFALO CALVES (IU/L)

→ Calf No. / ↓ Minutes after endotoxic shock	1	2	3	4	5	6	7	Mean ± SE
NORMAL	25.0	25.0	30.0	33.0	22.0	24.0	30.0	27.0±1.5
0	20.0	30.0	24.0	32.5	23.0	-	24.0	25.5±1.9
5	25.0	14.0	24.0	35.0	-	32.0	32.0	27.0±3.1
15	-	18.0	22.0	31.0	27.0	30.0	27.0	25.8±2.0
30	20.0	14.0	27.0	32.5	25.0	35.0	32.0	26.5±2.8
60	23.0	12.0	-	38.0	25.0	24.0	30.0	25.3±2.0
90	24.0	14.0	27.0	31.0	24.0	27.0	27.0	24.8±2.0
120	-	16.0	28.0	30.0	28.0	32.0	30.0	27.3±2.3

* P (<0.05)
** P (< 0.01)

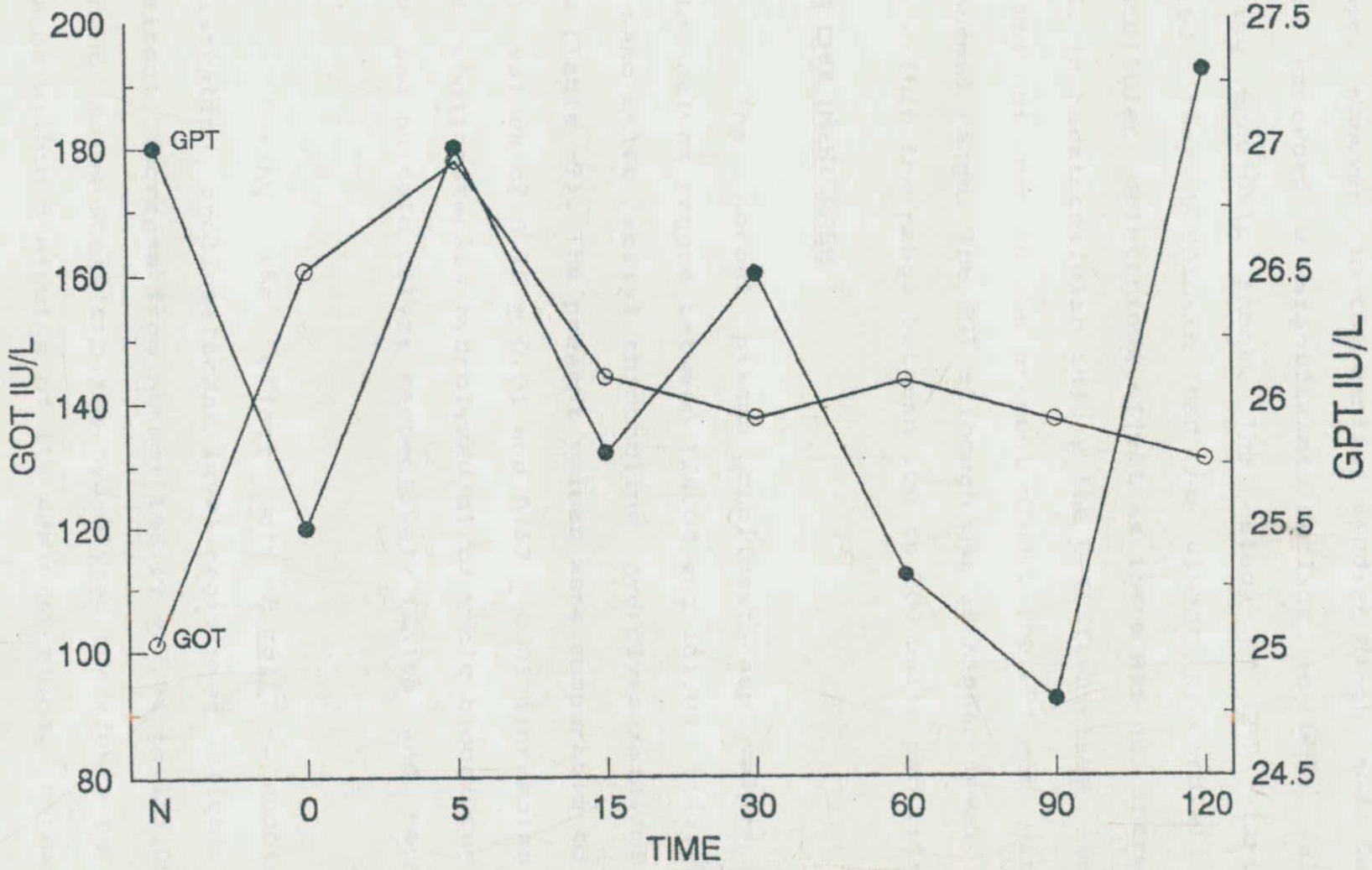

Fig. 5. TRANSAMINASES DURING ENDOTOXIC SHOCK IN BUFFALO CALVES (IU/L)

In the present study, during the effect of endotoxic shock the GPT level revealed a non-significant decrease. However in the earlier studies Singh and Sodhi (1992) recorded a significant decline in GPT values following endotoxic shock. The study of GPT further revealed that endotoxin had no direct myocardial or hepatocellular deleterious effect as there was no increase in GPT. In hepatocellular injury the GPT often rises sooner which was not seen in the present study. The GPT was within the normal range. The GOT although was at higher level but always within the range between 100 to 200 units per litre.

PLASMA CHOLINESTERASE

The normal plasma cholinesterase values in buffalo calves ranged between 124.04 and 161.04 (143.19 ± 7.94) nano moles acetyl thiocholine hydrolysed/min./ml of plasma (Table -9). The present values were comparable to the normal values of 0.72 ± 0.01 and 0.67 ± 0.01 micromoles of acetyl cholinesterase hydrolysed/ml of whole blood/hour in calves and buffalo calves respectively (Salem and Nounow, 1978).

During the effect of E.coli endotoxin administration cholinesterase level registered slight non significant increase from normal 143.19 ± 7.94 to 151.02 ± 7.68 nano moles acetylcholine hydrolysed per minute per ml. of plasma within 5 minutes of its administration. Thereafter

TABLE - 9 PLASMA CHOLINESTERASE CONCENTRATION DURING ENDOTOXIC SHOCK IN BUFFALO CALVES

(Nanomoles Acetyl Thiocholine Hydrolysed/Min./Ml. Plasma)

→ Calf No. / ↓ Minutes after endotoxic shock	1	2	3	4	5	6	Mean ± SE
NORMAL	154.5	124.0	-	161.0	124.0	152.3	143.1±7.9
0	147.9	167.5	117.5	169.7	-	141.4	148.8±9.5
5	143.6	171.9	126.2	158.8	-	154.5	151.0±7.6
15	117.5	180.6	117.5	171.9	121.8	163.2	145.4±12.0
30	134/9	-	117.5	182.8	106.6	158.8	140.1±13.8
60	139.2	191.5	124.0	171.9	115.3	147.9	148.3±11.8
90	-	-	124.0	178.4	108.8	108.8	130.0±16.5
120	130.5	-	108.8	156.6	104.4	126.2	125.3±9.2

* P (<0.05)
** P (< 0.01)

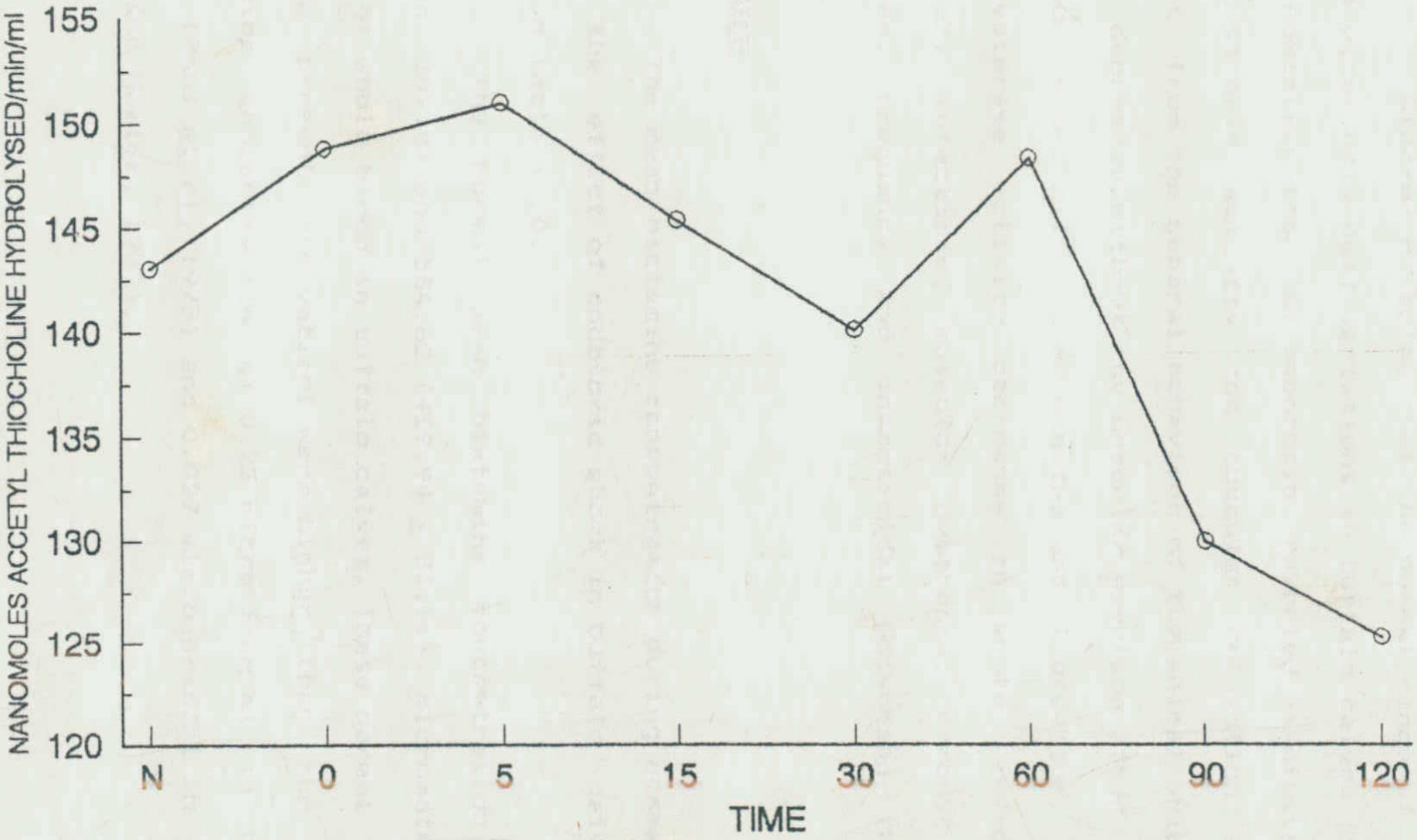

Fig. 6. PLASMA CHOLINESTERASE CONCENTRATION DURING ENDOTOXIC SHOCK IN BUFFALO CALVES (NANOMOLES ACETYL THIOCHOLINE HYDROLYSED/MIN/ML)

plasma cholineterase level declined till 120 minutes and recorded the lowest level of 125.35 ± 9.27 nano moles acetylthiocholine/min/ml plasma **(Fig.6).**

Studies revealed that the normal range of values showed wide individual variations in buffalo calves (table - 9). Administration of endotoxin revealed inhibition of cholinestrease activity and muscular relaxation as was evident from the general behaviour of the animal which was dull, depressed without any muscular exertion after E.coli infusion especially at 60 minutes and thereafter. Plasma cholinesterase activity decreases in acute infections, pulmonary infections, muscular dystrophy, chronic renal diseases, pregnancy and insecticidal poisoning (Kramer, 1989).

HISTAMINE

The mean histamine concentration during normal and under the effect of endotoxic shock in buffalo calves is given in table : 10.

The normal mean histamine concentration ranged between 309.09 and 554.62 (419.94 ± 34.11) micromoles per litre of whole blood in buffalo calves. These normal values in the present observations were higher than the normal histamine concentration of 0.22 micro gram/ml in buffalo blood (Paul et al, 1973) and 0.027 micro gram/ml in claves (Code and Hester, 1939).

TABLE -10 HISTAMINE CONCENTRATION IN WHOLE BLOOD (μ mol /Litre) DURING ENDOTOXIC SHOCK IN BUFFALO CALVES

→ Calf No. ↓ Minutes after endotoxic shock	1	2	3	4	5	6	7	8	Mean ± SE
NORMAL	551.26	554.62	436.97	453.78	346.15	323.07	309.09	384.61	419.9±34.11
0	798.31	756.30	655.46	613.45	384.61	476.92	-	461.53	592.36* ±59.22
5	974.78	806.72	773.10	756.30	-	-	400.00	523.07	705.66**±84.95
15	-	823.52	705.88	705.88	692.30	-	618.18	584.61	688.39**±33.95
30	1142.85	924.36	672.26	672.26	461.53	-	763.64	569.23	758.10**±85.48
60	907.05	873.94	722.69	722.69	575.00	-	781.81	553.84	735.80**±60.5
90	1008.40	756.30	689.07	689.07	753.84	-	581.81	553.84	726.10**±56.36
120	-	672.26	739.50	739.50	576.92	-	690.90	569.23	659.12**±28.61

* P (< 0.05)
** P (< 0.01)

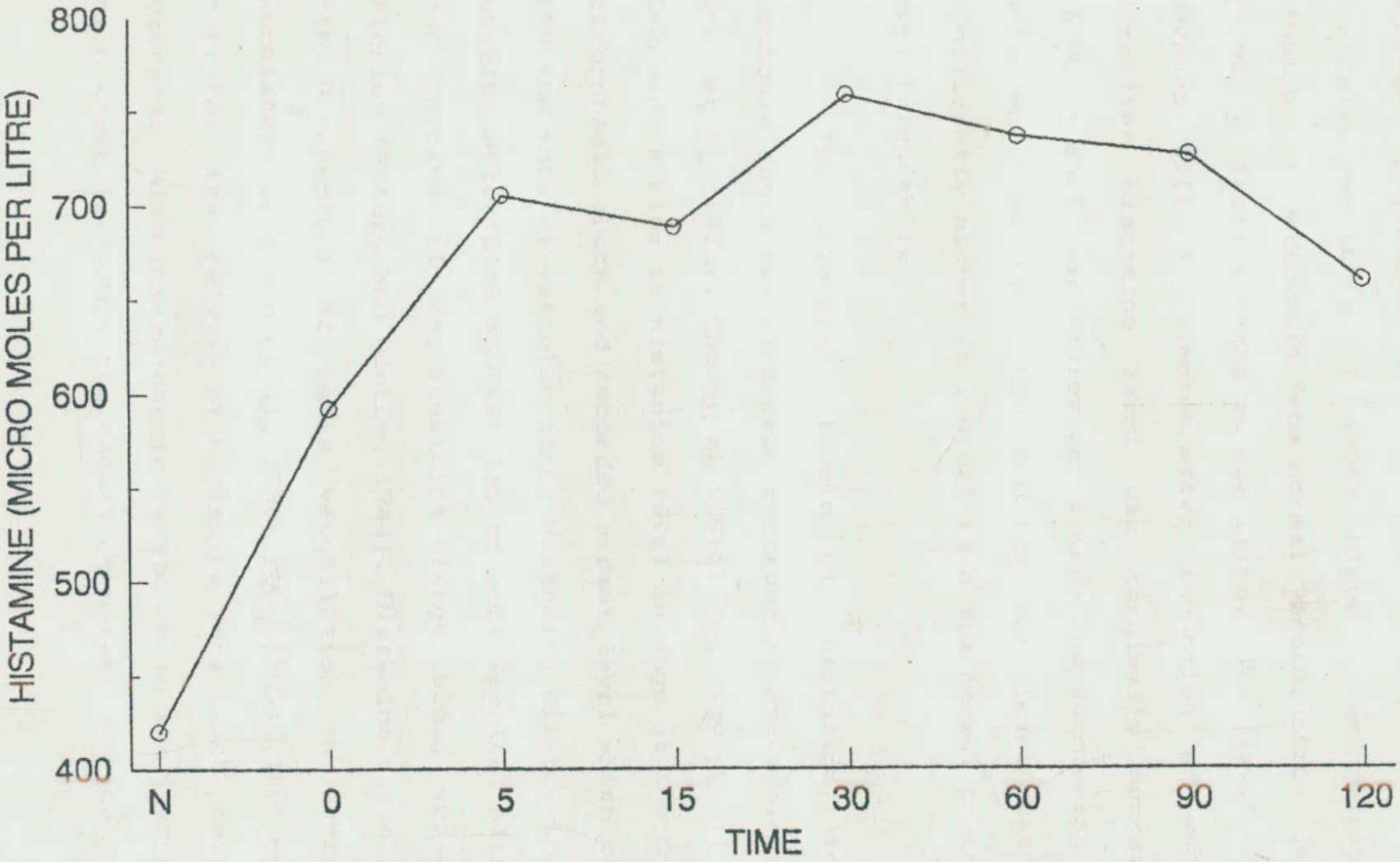

Fig. 7 HISTAMINE concentration during endotoxic shock in buffalo calves (MICRO MOLES PER LITRE)

The results revealed that the level of histamine was increased under the effect of endotoxic shock. The level increased significantly ($p < 0.05$) to 592.55 $\pm$ 59.22 micromoles per litre of whole blood immediately after infusion of endotoxin from normal preinjection level of 419.94 $\pm$ 34.11 micromoles per litre. The level remained elevated till 30 minutes after induction of endotoxin. Thereafter histamine level was marginally decreased to 735.80 $\pm$ 60.50 micromoles per litre. The decreasing pattern continued even upto 120 minutes but level was still significantly higher ($p < 0.01$) than the normal preinjection level (figure-7).

The elevated levels of histamine following endotoxic shock has also been recorded by Emerson (1985) and Vick *et al* (1971). Cho *et al* (1965) and Nagy *et al* (1986) observed a rise in histamine level in dogs at the onset of haemorrhagic shock and recorded a peak level which coincided with the loss of vascular tone. Histamine dilates the minute vessels, arterioles and venules of most specics, although in some species it may constrict large blood vessels and arteries (Peters and Horton, 1944). Histamine could interact with H_2 receptors to cause vasodilation in peripheral vasculature (Charbon *et al*, 1980; KOO, 1983). The endotoxin initiates the release of histamine from mast cells that interacts with histaminergic receptors in the vasculature, which serve to reduce the amount of norepinephrine released

during sympathetic stimulation, causing a loss of smooth muscle contracting ability. A significant loss of peripheral vascular tone accompanies the systemic hypotension associated with endotoxemia as is observed in the present observations in buffalo calves (table-3).

PROTHROMBIN TIME

The normal mean prothrombin time in buffalo calves ranged between 16.0 and 18.0 seconds (16.8 ± 0.4 seconds). This is comparable to mean normal prothrombin time to 17.2 ± 0.4 seconds (Welles et al, 1993) but higher than 15.5 seconds (Nagaraja et al, 1979) and 15.2 ± 0.8 seconds (Semrad and Dubielzig, 1993) recorded in cow calves.

After administration of endotoxin there was a significant increase in prothrombin time ($P < 0.01$). The normal mean value of 16.8 ± 0.4 increased to 22.0 ± 0.6 seconds within 5 minutes of endotoxic shock. Thereafter the values of prothrombin time almost stablized at a higher level till 60 minutes of shock. After 90 minutes of endotoxic shock the prothrombin time was further increased (Table-11; **Fig.8**).

The prolongation of prothrombin time following administration of endotoxin was also recroded by Semrad and Dubielzig (1993), Welles et al (1994), Yagi and Nakajima (1983), Patrick (1982) and Nagaraja et al (1979) in calves.

TABLE -11 PROTHROMBIN TIME DURING ENDOTOXIC SHOCK (SECONDS) IN BUFFALO CALVES

→ Calf No. ↓ Minutes after endotoxic shock	1	2	3	4	5	6	Mean ± SE
NORMAL	16.0	16.0	16.0	18.0	17.0	18.0	16.8±0.4
0	22.0	22.0	20.0	24.0	-	22.0	22.0**±0.6
5	30.0	30.0	22.0	22.0	24.0	26.0	25.6**±1.5
15	29.0	30.0	29.0	21.0	24.0	23.0	26.0**±1.5
30	24.0	25.0	23.0	27.0	30.0	23.0	25.3**±1.1
60	24.0	23.0	25,0	24.0	26.0	26.0	24.6**±0.4
90	32.0	34.0	34.0	28.0	30.0	32.0	31.6**±0.9
120	33.0	36.0	36.0	28.0	31.0	33.0	32.8**±1.2

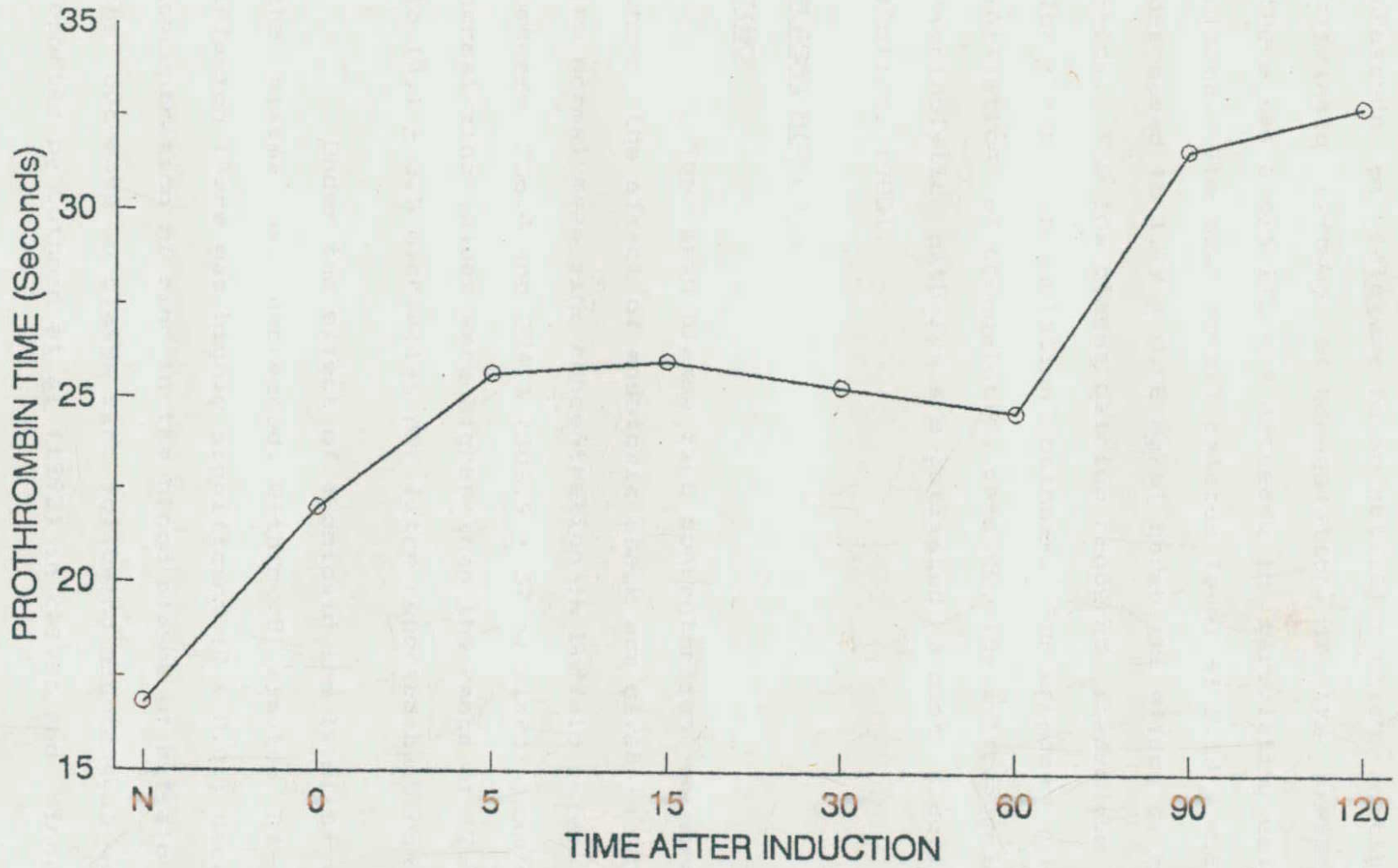

Fig. 8. PROTHROMBIN TIME (Seconds) DURING ENDOTOXIC SHOCK IN BUFFALO CALVES

Prothrombin time is a measure of extrinsic and common coaggulation pathways. Tissue thromboplastin, in the presence of calcium, is an activator which initiates the extrinsic pathway of coaggulation. In the present study there was a significant decrease in circulating calcium in plasma. The mean normal calcium level of 2.18 ± 0.14 mg/ml decreased to 1.77 ± 0.08 mg/ml under the effect of endotoxic shock. The low plasma calcium rendered inadequate calcium for the coaggulation pathway. Furthermore with the initiation of coaggulation cascade, the anticoaggulant and fibrinolytic pathways are activated almost simultaneously (Collen, 1980).

PLASMA MINERALS

ZINC

The mean plasma zinc concentration in normal and under the effect of endotoxic shock are given in table-12. The normal mean zinc concentration in buffalo calves ranged between 256.4 and 384.3 (306.9 ± 37.5) micro gram/dl. The normal zinc values were higher than the range of 12.9 ± 0.4 to 14.1 ± 0.5 micromoles per litre reported by Singh (1995).

Under the effect of endotoxin the level of zinc in the plasma was decreased. Within 5 minutes of E.coli infusion there was highly significant ($P < 0.01$) decrease in concentration of zinc in the blood plasma of buffalo calves. (Tab.12) The decrease in plasma zinc following endotoxemia was also reported by Luthman et al (1992) in calves and Kindahl and

TABLE -12 PLASMA ZINC CONCENTRATION DURING ENDOTOXIC SHOCK IN BUFFALO CALVES(µg/100 ml)

→

Calf No. → Minutes after endotoxic shock	1	2	3	4	Mean±SE
NORMAL	397.0	213.2	308.8	308.8	306.9±37.5
0	279.4	240.3	-	183.8	234.5±27.7
5	191.1	132.3	161.7	147.0	158.0**±12.5
15	220.5	102.9	-	110.2	144.6* ±38.0
30	323.5	117.6	279.4	220.5	235.2±44.5
60	205.8	147.0	264.7	191.1	202.1±24.2
90	213.2	308.8	205.8	191.1	229.7±26.7
120	176.4	264.7	235.2	264.2	235.1±20.7

* P (<0.05)
** P (< 0.01)

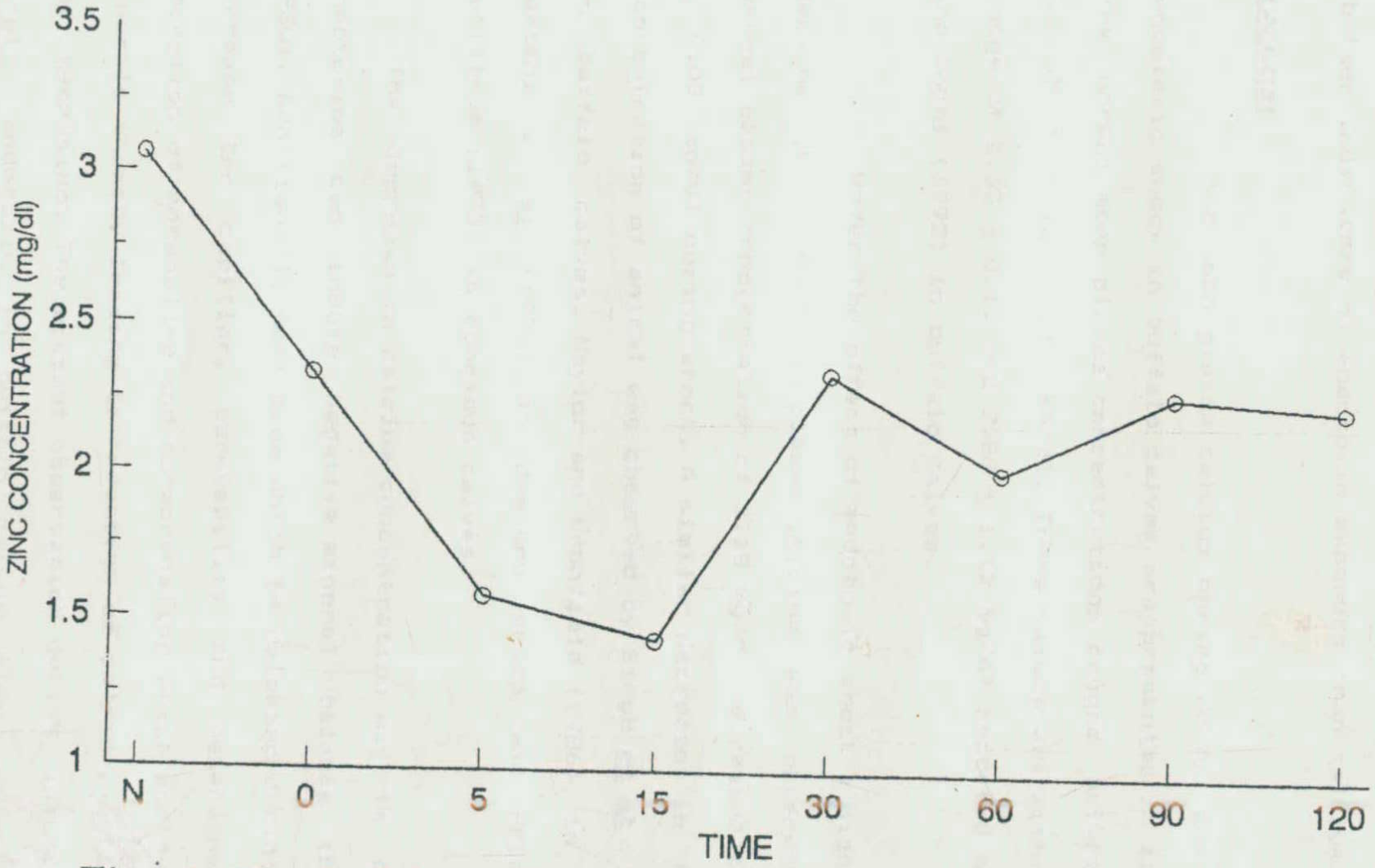

Fig. 9. ZINC CONCENTRATION DURING ENDOTOXIC SHOCK IN BUFFALO CALVES (mg/dl)

Aiumlamai (1992) in sheep. Kindahl and Aiumlamai (1992) suggest that levels of zinc and iron in blood plasma are better indicators of endotoxin exposure than calcium.

CALCIUM

The mean plasma calcium during normal and during endotoxic shock in buffalo calves are presented in table-13. The normal mean plasma concentrations ranged between 2.05 and 2.45 (2.18 ± 0.14) mg/dl. There values are within the range of 2.33 ± 0.11 and 2.88 ± 1.12 mg/dl reported by Singh and Sodhi (1992) in buffalo calves.

Under the effect of endotoxic shock a significant decline ($P < 0.05$) in plasma calcium was observed. The normal plasma concentration of 2.18 mg/dl decreased to 1.77 ± 0.08 mg/dl during shock. A similar decrease in calcium concentration of animal was observed by Singh **et al** (1992) in buffalo calves, Naylor and Kronfield (1986) in sheep, Bertoni et al (1989) in cows and sheep and Reece and Wahlstrom (1973) in concious calves.

The decrease in calcium concentration may be because endotoxins can induce negative mineral balance (Powada, 1980). Additionally histamine which is released during shock increase the capillary permeability and determine the secretion of adrenaline and noradrenaline which modify many minerals thereby leading to reduction of calcium, pottassium and magnesium. The present observation during infusion of E.coli endotoxin in buffalo calves also revealed a

TABLE -13 PLASMA MINERALS CONCENTRATION DURING ENDOTOXIC SHOCK IN BUFFALO CALVES (µg/ml)

	1	2	3	4	Mean SE
CALCIUM					
NORMAL	237.7	23.7	22.5	17.5	21.8 ± 1.4
ENDOTOXIC SHOCK	19.0	19.0	17.7	15.2	17.7* ± 0.8
MAGNESIUM					
NORMAL	55.0	41.2	47.5	43.7	46.8 ± 2.9
ENDOTOXIC SHOCK	47.5	40.0	38.7	32.5	39.6 ± 3.0

* P (< 0.05)
** P (< 0.01)

significant increase in histamine concentration in blood (table - 10).

PHYSICAL SIGNS

One of the eight buffalo calves injected E.coli endotoxin died. All the surviving calves exhibited signs of shock. Within a few minutes of intravenous administration the calves became hyperpnoeic and later dyspnoeic with respiratory movements markedly abdominal. The period of respiratory distress lasted for the entire period of study. Salivation was profuse. Diarrhoea often with mucous was observed. The animal usually collapsed into lateral recombancy within 15 to 30 minutes after injection. The calves adopted a typical dyspnoeac posture. The mucous membranes became cyanotic. An increase in rectal temperature by 1-2 degree C was usually observed.

The calf given E.coli dose at a faster rate collapsed suddenly into lateral recombency and exhibited shock like symptoms. Respiratory rate was rapid and markedly abdominal. Mucous membranes were cyanotic. Salivation was profuse followed by frothy fluid regurgitation. The calf died within a few minutes of endotoxin administration.

CHAPTER – V

SUMMARY AND CONCLUSIONS

Eight normal apparently healthy male buffalo calves (8-10 months of age) were given *E.coli* endotoxin @ 0.6 mg/kg body weight. The recordings and sampling was done at zero (just after endotoxin administration), 5, 15, 30, 60, 90 and 120 minutes. Significant cardiohaemodynamic and biochemical changes were observed. Histamine level in blood increased significantly during endotoxic shock. This could be attributed to endotoxin induced release of histamine from mast cells. The prothrombin time prolonged which could be due to activation of fibrinolytic pathways by endotoxin. Also calcium which is needed for coagulation is decreased. Mean arterial pressure and central venous pressures decreased. This drop could be ascribed to vasodilation, extravasation and peripheral pooling of blood due to endotoxin induced activation of kinin system. Immediate drop seen in MAP upon endotoxin administration is CNS mediated. Elevation of Acid phosphatase and Glutamic oxalotransaminase could be due to lysosomal diruption secondary to hypoxia of tissues. Inadequate tissue perfusion leads to damage to hepatic lysosomal membrane and seepage of contents into systemic circulation. However there was a decrease in level of alkaline phosphatase which could be attributed to corresponding decline in the level of its cofactor zinc. No significant alteration in the levels of plasma

cholinesterase or glutamic pyruvic transminase could be seen following endotoxin administration.

There was shortening of P-R interval and prolongation of S-T segment following endotoxic shock. The prolongation of S-T segment could be due to myocardial hypoxia which is known to occur in bovine endotoxic shock. No definite effect could be recorded on heart rate. The calf given E.coli endotoxin dose at a faster rate collapsed suddenly and died within a few minutes of endotoxin administration. The calves became hyperpnoeic and later dyspnoeic with markedly abdominal respiratory movements as endotoxic shock ensued. There was profuse salivation, diarrhoea along with fever followed by hypothermia in late stages of endotoxic shock.

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