Studies on Clinico-Biochemical and Pathological Changes Associated with Hypomagnesemia In Buffalo Calves

by
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(L-83-V-170-M)

THESIS

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1 Calves- Diseases

2 Magnesium deficiency diseases

Dedicated

To

My Parents

And

All Who Love Me

CERTIFICATE I

This is to certify that this thesis entitled "Studies on clinico-biochemical and pathological changes associated with hypomagnesemia in buffalo calves" submitted for the degree of M.V.Sc. in the subject of Veterinary Medicine (Minor : Veterinary Pathology) of the Punjab Agricultural University, is a bona fide research work carried out by Kirti Dua (L-83-V-170-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.

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(Dr D.C. Nauriyal)
Major Advisor

CERTIFICATE II

This is to certify that the thesis entitled "Studies on clinico-biochemical and pathological changes associated with hypomagnesemia in buffalo calves" submitted by Kirti Dua (L-83-V-170-M) to the Punjab Agricultural University in partial fulfilment of the requirements for the degree of M.V.Sc. in the subject of Veterinary Medicine (Minor: Veterinary Pathology) has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.

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C O N T E N T S

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INTRODUCTION

The disease panorama in cattle, ewes and poultry during the last decade shows considerable changes. The frequency of certain diseases has increased while that of others has decreased. In this concern, it has been observed that frequency of metabolic disorders has increased especially in high performance stock, such as dairy cows (Samson, 1973). The productivity of the dairy herd is primarily dependent upon metabolic and nutritional status of each dairy cow. Frequently there is a tendency for high output demands - milk and calves - to be associated with inadequate feed inputs resulting in imbalance of body metabolites which, if sustained, eventually becomes clinically apparent as 'production diseases', such as hypocalcemia and hypomagnesemia (Wilson, 1976).

The clinical disorder of cattle known as 'grass tetany', was linked with hypomagnesemia around 1930, and has since been cured by provision of extra-dietary magnesium. But still it is unknown which biochemical systems and pathways are altered, which enzymes suffer actually from magnesium deficiency and what metabolic disturbances give rise to well known clinical symptoms of hypomagnesemia.

Hypomagnesemic tetanies are less closely related to parturition, but lactation does play a role in their

precipitation as a clinical syndrome, since the mammary output of magnesium is in the order of 0.1 g/kg of milk (Todd, 1967). Grass tetany usually affects high-yielding dairycows inthe field during spring and autumn in relatively cold weather and when grass is still rich in nitrogen and potash (Samson, 1973; Wilcox and Hoff, 1974). Such conditions combined with poorly regulated magnesium homoeostasis facilitate the development of hypomagnesemia which is itself accompanied by hypocalcemia.

An effective wayof combating production diseases is necessary for ethical as well as for economic reasons. It is ethically not justifiable to buy increased productivity at the cost of increased morbidity or increased suffering on the part of the animal. It is also not justifiable that increased productivity and newer techniques discovered are counterbalanced by losses through animal diseases.

Effective combating of production diseases requires new methods to be used in veterinary medicine, which in turn require new knowledge.

The metabolic profile test is a diagnostic aid for production diseases. Individual herd test can be used either to elucidate the aetiology of outbreaks of metabolic disorders or to reveal theunsuspected abnormalities in normal herd. Useful indication can be gained from seasonal changes in the profile test. Magnesium can be low in winter as well as in summer indicating the need for

supplementation in winter rations.

In rapidly growing calves of 2-4 months age, there occurs a marked decrease in intestinal absorption of magnesium, while the need for its incorporation into the developing soft tissue is greater. In some of the calves suffering from scours, there is greater intestinal drainage of magnesium and as such severe hypomagnesemic tetany maydevelop irrespective of the diet on which they are maintained. The disease is invariably fatal in fast growing calves.

Sporadic occurrence of clinical cases of hypomagnesemia has been reported by Prasad et al. (1979) and Sinha (1980) in this country. Most of thecases are being diagnosed only on the basis of clinical recovery after therapy.

In view of the wide prevalence of the condition in high yielding cows and buffaloes in Punjab, it is necessary to understand how the environment impinges upon the milieu interieur. The present investigation was, therefore, undertaken with the following objectives in view :

1. To study the symptoms in the experimentally induced hypomagnesemia in buffalo calves.
2. To studythe biochemical changes in the various body secretions of the hypomagnesemic calves.
3. To study the physiological changes as a means of rapid diagnosis.
4. To find out pathological changes in the vital organs of the affected calves.

5. To find out suitable therapeutic measures based on biochemical changes in the blood and body fluids of buffalo calves.

REVIEW OF LITERATURE

Various workers using different parameters under varied conditions have multiple opinions about the induction, physiopathology and control of hypomagnesemia. Some of their important findings have been summarized as follows :

Induction of Hypomagnesemia

Todd and Horvath (1970) could produce hypomagnesemia in four cow calves reared on low magnesium milk. Two of them exhibited clinical symptoms in the form of tetanic convulsions. The electrolytes in the plasma remained unchanged.

Hvidston and Langebrekke (1972) concluded that combined reduction in the supply of energy and magnesium led to the reduction of serum magnesium to hypomagnesemic level.

Larvor and Rayssiguier (1972) found that hypomagnesemia could be induced in normal ewes within 90 minutes by giving intravenous infusion of theophylline @ 30 mg/kg. This is said to be due to increased urinary excretion of magnesium. Slight hypomagnesemia could also be developed in 90 minutes by infusing furosamide @ 7 mg/kg intravenously as a result of increased urinary excretion of magnesium.

Baker et al. (1976) induced hypomagnesemia in sheep by infusing milk low or inadequate in magnesium content by intra abomasal infusion.

Khalil (1977) reported that hypomagnesemia could be induced in sheep by feeding magnesium deficient or magnesium and sodium deficient diets.

Rayssiguier et al. (1977 a) observed hypomagnesemia in three calves by feeding them with magnesium deficient semi synthetic milk.

Rayssiguier et al. (1977 b) experimentally induced hypomagnesemia in ewes by intravenous infusion of adrenaline @ 40 µg/kg/hour. The effect was augmented by administration of phentolamine and inhibited by propranolol.

Scholz and Khalil (1978) observed low serum magnesium level (0.75-1.10 mg/dl) in 9 sheep (1-2 years) fed on a diet deficient in magnesium (0.113 g/kg) or sodium (0.083 g/kg) or both. Within 15 days, tetany was observed in magnesium deficient group only.

Yoshida (1978) reported hypomagnesemia (0.75-0.94 m mol/l) throughout the year in a herd of 20 cows maintained on magnesium deficient diet.

Baker et al. (1979) observed hypomagnesemia in adult sheep by changing their diet from chaff to a magnesium-deficient milk infused directly into abomasum.

Shiga and Shinozaki (1979 a) experimentally induced hypomagnesemia in four ewes by feeding a ration low in calcium and magnesium for 10 days. Serum magnesium level fell further when ration was supplemented with calcium

carbonate for eight days. Tetany was observed in one sheep.

Shiga and Shinozaki (1979 b) reported that old ewes developed tetany when fed on magnesium-deficient diet (2.9 mg/kg/day) as a result of increased Ca:Mg ratio in seven days.

Shiga et al. (1980) reported that plasma parathyroid hormone (PTH) levels fell sharply in four ewes kept on a diet low in calcium and magnesium for 10 days. In three of them, it remained low for four days after giving them extra calcium and then elevated to normal. In one old ewe, serum calcium rose but magnesium and PTH fell sharply on days two and clinical hypomagnesemia developed. Plasma PTH rose again from day five and death from tetay occurred on day seven.

Shiga et al. (1981) produced hypomagnesemia in four lactating ewes by feeding winter ration for 10 days and then spring grass containing more potassium but less magnesium, calcium, phosphorus and sodium for 9 days.

Sinha (1980) induced hypomagnesemia in 1-1½ months old buffalo calves by intra-ruminal administration of potassium chloride (1.5 g/kg) and citric acid (1.25 g/kg) for a week in addition to usual milk feeding.

El-Shrif and Mottelib (1983) experimentally produced hypomagnesemia in ten buffalo calves by feeding potassium

iodide (100 mg/kg body weight). Hypomagnesemic tetany occurred after 5-8 days.

Takashashi et al. (1983) induced hypomagnesemic experimentally by feeding three sheep with a magnesium-deficient diet and introducing into the rumen a VFA-triglyceride mixture (triacetin, tripropionin and tributyrin). Plasma magnesium level fell below 0.5 mg/dl within six days and one sheep died from severe hypomagnesemic tetany two weeks later.

Terashima et al.(1983) observed that plasma calcium and magnesium decreased to about 88% of initial values at 2-3 hours after intravenous infusion of norepinephrine @ 15 ug/kg body wt./min. in normal saline. The effect was more marked in fasting animals.

Pathophysiology of hypomagnesemia

Dobson et al. (1966) took saliva and blood samples from ewe lambs, both indoors and when grazing heavily fertilized pasture. Grazing produced rise in concentration of potassium (from 10 to 26 mEq/L) in saliva and fall in concentration of magnesium in blood from 1.8 mg/dl to about 1.1 mg/dl after two nights at pasture. Sodium concentration in the saliva fell when potassium concentration got elevated whereas the concentration of sodium in plasma was erratic.

Holtenius et al. (1970) analysed the blood samples of four groups of calves and two flocks of sheep affected

with tetany and observed that except in one group of calves with hypomagnesemia, the tetany in all cases was attributed to hypocalcemia. Investigation of the diets revealed inadequate vitamin D, calcium and in some cows inadequate magnesium in the diet.

Horvath et al. (1971) observed alterations in electrocardiogram in four experimentally induced hypomagnesemic bull calves. These included somatic nerve (TP deflection), increased heart rate and changed QRS complex when tetany was imminent. Inverting and peaking of T-wave were seen. Tissue calcification was seen in two calves examined on post-morterm but the heart was not involved.

House and Campen (1971) gave treatments in each of the four trials consisting of (1) the feeding one kg concentrate/day (control diet); (ii) feeding plus supplement of 60 g potassium chloride; (iii) feeding plus 30 g of citric acid; (iv) feeding plus both potassium and citric acid, to sheep. Plasma magnesium concentration, magnesium retention and exchangeable magnesium pool size were unaffected by the treatments. Increased intake of potassium chloride significantly depressed magnesium absorption, reduced urinary magnesium excretion and lowered endogenous faecal magnesium excretion.

Huber (1971) studied dehydration in glucose-induced acidosis in sheep. Increased haematocrit values coupled with reduced skin elasticity were indicative of severe

degree of haemoconcentration and dehydration in acidotic animals. A reduction in body water of approximately eight per cent of the body weight was observed. It was concluded that total body water loss was shared by plasma, interstitial and interacellular fluid compartments.

Mihai (1971) observed the symptoms associated with grass tetany included restlessness, bellowing, muscular contraction, opisthotonus, tetany and epileptic convulsions. Serum analysis for magnesium, calcium, sodium and potassium of 20 calves with tetanic symptoms and 10 calves without such symptoms revealed low values.

Hall and Reynolds (1972) analysed blood samples from twelve cows affected with hypomagnesemic tetany at the appearance of first clinical signs and 3-5 days later after tetany. Plasma magnesium concentration was found to be 0.66 $\pm$ 0.16 mEq/L at the time of tetany and 1.07 $\pm$ 0.41 mEq/L, 3-5 days later. Plasma calcium was 3.89 $\pm$ 0.59 mEq/L at the start of tetany and 5.69 $\pm$ 0.45 mEq/L, 3-5 days later.

Meyer and Scholz (1972) reported the mean values of magnesium in the cerebrospinal fluid and serum were 2.37 $\pm$ 0.14 mg/dl and 2.26 $\pm$ 0.16 mg/dl respectively. They found the relationship between the normal ratio of magnesium in the blood (x) to the magnesium in the cerebrospinal fluid (y) by deriving a formula :

$$Y = 2.405 - 0.261/(x)$$

They observed that in younger animals, cerebrospinal fluid magnesium decreased more slowly during magnesium deficiency and correlation of the clinical symptoms was greater with the cerebrospinal fluid magnesium level. Clinical signs could be anticipated at cerebrospinal fluid magnesium values below 1.6 mg/dl.

Murakami et al. (1972) made observations on five cows (3-5 years old) and suckling calves (4-6 months old) affected with grass tetany and reported symptoms which included excitement, hyperaesthesia, trembling, stiffness, salivation, breathing difficulty, diarrhoea, frequent micturition and ultimately the convulsions with tetany. Serum magnesium levels revealed hypomagnesemia, high calcium magnesium ratio and increased serum-glutamic-oxalo acetate transaminase activity. Serum magnesium levels generally ranged from less than 1-2 mg/dl in other 76 beef cattle. Hypomagesemia caused an increase in calcium magnesium ratio without affecting actual concentration of calcium and other serum ions.

Newton et al. (1972) conducted eight three-day balance trials on 12 crossbred lambs. Potassium bicarbonate @ 100 g was fed to the lambs and observed a decreased absorption of magnesium and increased absorption of sodium, potassium and calcium. Although blood serum, calcium, sodium and potassium showed no distinct trends, there was a trend toward lowered serum magnesium levels after 14 days of

potassium supplementation which disappeared after 27 days.

Osbaldiston et al. (1972) collected midstream urine samples from 48 healthy cows at various stages of lactation and found mean value of pH, sodium and potassium as 8.23, 34.3 mEq/L and 453.7 mEq/L respectively.

Rumsey and Putnam (1972) infused intraruminally a solution containing 500 g potassium chloride and 500 g citric acid in steers and found typical changes in the ECG patterns. There was increase in the P interval, decrease in the PR and QT intervals, a more negative QRS complex and an inverted T-wave. Concurrent with extreme toxic conditions, the P wave was lost and QRS and QT intervals broadened. Changes in the ECG pattern were associated with a serum potassium concentration of 9 mEq/L. Respiratory rate increased and salivary flow decreased after the infusion.

Scholz and Meyer (1972) observed that the range of normal calcium, phosphorus, potassium and sodium values in the cerebrospinal fluid of sheep were only a half of that in the blood plasma. The CSF values found were 5.23 ± 0.54 mg/dl for calcium, 1.27 ± 0.22 mg/dl for phosphorus, 12.3 ± 0.53 mg/dl for potassium and 349 ± 14.35 mEq/L for sodium. An experimental progressive hypomagnesemia induced a fall in blood calcium level and an increase in potassium content of both the blood and CSF. These changes were not considered significant for the development of hypomagnesemic tetany.

Bauda et al. (1973) collected cerebrospinal fluid samples from 23 cattle and found that pH of CSF was lower by 0.032 than that of blood. Sodium, potassium and inorganic phosphorus values of the CSF were also lower. However, chloride was higher in CSF than in blood.

Lotthammer and Alsewede (1973) observed that sodium content of the saliva of cattle remained fairly constant, whereas potassium content varied considerably according to the impurities present. On analysis of the saliva from parotid and sublingual glands of ten cattle, it was found that average sodium and potassium contents were 324.4 mg/dl and 58.6 mg/dl respectively.

Luthman et al. (1973) observed symptoms associated with hypomagnesemia in calves which included muscular spasms or inability to rise, besides observing serum magnesium values as low as 0.6 mg/dl. Also, serum magnesium levels in the range of 0.3-1.0 mg/dl were detected in tetanic hereford cows. They quoted the bovine renal threshold values for magnesium to be 1.7-1.8 mg/dl.

Ohshima et al. (1973) reported serum magnesium levels of 0.3 and 0.4 mg/dl respectively, in two clinical cases of hypomagnesemia in cattle. Post-mortem findings included focal haemorrhages in organs and tissues, cloudy swelling of skeletal muscles, degenerative changes in liver, kidneys, adrenals, lungs, spleen and heart. The primarily lesions of hypomagnesemia appeared to be tissue calcification and degeneration of blood vessels.

Garcia (1974) assayed rumen fluid, saliva and blood samples for magnesium, calcium, inorganic phosphorus, potassium and sodium during induced hypomagnesemia in sheep. It was observed that magnesium concentration in blood declined to as low as 0.5 mg/dl. The calcium content decreased to 7 mg/dl from a normal value of 9 mg/dl. No significant changes were observed in potassium, sodium or phosphorus in rumenfluid or saliva. Instead of a normal decrease of rumen fluid pH following a feed deficient in magnesium content, a post-prandial increase (upto pH 8.0) was detected.

Meyer et al. (1974) found that during hypomagnesemia, magnesium secretion from choroid plexes clearly diminished from 3.27 to 1.83 mg/dl. It was also found that typical tetanic seizures were induced in hypomagnesemic sheep by ventriculo-cisternal perfusion with magnesium free fluid, but the seizures did not occur in normomagnesemic sheep.

Pauli and Alsop (1974) estimated the concentration of magnesium in plasma and the cerebrospinal fluid in eleven cows with clinical grass tetany, four with hypomagnesemia but exhibiting no clinical signs and two calves with experimentally induced grass tetany. It appeared that level of magnesium in the cerebrospinal fluid was better index than the levels in plasma for indicating the onset of grass tetany. Hypocalcemia was not always present in tetanic cases; however, in acute tetany the plasma potassium got elevated to dangerous levels.

Beal et al. (1975) reported that salivary flow was depressed during hyperkalemia and decline in sodium : potassium ratio during potassium administration was diphasic. The decline in salivary flow during sodium depletion was associated with decreased salivary bicarbonate concentration and increasing salivary phosphate and pH, with the concentration of chloride showing no constant trend. During acute hyperkalemia the chloride and phosphate concentrations were negatively correlated with salivary flow while the pH was unaltered.

Furakawa et al.(1976) reported that intraruminal administration of citric acid @ 1.7 g/kg body weight produced no symptoms and had no effect on ECG. The administration of potassium chloride @ 1.7 g/kg body weight alone or in combination with citric acid produced a slight prolongation of P-wave and an elevation of the T-wave but no clinical symptoms. When both the substances were given and potassium chloride dose exceeded @ 1.75 g/kg body wt., there was highly abnormal ECG pattern and the high serum potassium concentration was responsible for cardiac failure in sheep.

Kariya et al.(1976) found increase in the hematocrit, serum proteins and potassium concentration after intra ruminal administration of potassium chloride @ 1.7 g/kg body weight but the levels of these ingredients returned to normal after 6 hours. Citric acid when given @ 1.7 g/kg body weight increased blood glucose, packed cell volume,

serum proteins and calcium concentration. Simultaneous administration of both substances in four sheep @ 1.7 -1.9 g/kg body weight of potassium chloride and 1.5-1.7 g/kg body weight of citric acid, produced a marked increase in blood glucose, lactic acid and potassium concentration. Three sheep of this group died within three hours. Administration of each substance produced a decrease in serum magnesium.

Meyer et al. (1976) investigated the incidence of clinical symptoms in hypomagnesemia in sheep and found that in the appearance of acute clinical symptoms neither a reduction in the calcium level in blood nor the uptake of high amounts of ammonia, phosphate or citric acid was involved. On the other hand, a strong correlation could be established between the magnesium content in CSF and clinical symptoms. A small reduction in the magnesium content in the intercellular fluid of the central nervous system might lead to functional reversible disturbances, probably by a lower glucose uptake of the nervous cells. During the magnesium deficiency the magnesium level in the blood decreased more rapidly than in CSF. The magnesium in CSF seemed to buffer the brain against large fluctuations of magnesium in the blood which were typical of this mineral. Tetanic seizures could, therefore, occur in different stages and/or after different times of hypomagnesemia.

Moreno et al. (1976) found an average blood magnesium level of 1.2-1.7 mg/dl in hypomagnesemic cows. However, blood calcium and phosphorus levels were within normal range in these animals.

Furakawa et al. (1977) made observations in eighteen young cattle after oral administration of potassium chloride and citric acid @ 2.0 g/kg body weight each. There had been an increase in blood potassium and a decrease in serum magnesium which did not lead to tetany within few hours of dosing.

Salama et al. (1977) found decrease in magnesium values in the rumen content from 11.7 to 2.6 mg/dl and that of calcium from 10.88 to 8.22 mg/dl in twelve Merino ewes of 4-6 years age which were given a magnesium deficient diet for 8 weeks.

Schafer & Neubert (1977) correlated the ECG changes with biochemical alterations in 155 cows. The R-wave amplitude value of cows with hypercalcemic (13.4 mg/dl), hypomagnesemic (1.4 mg/dl) and a combination of both conditions were 0.98, 1.09 and 1.22 mv respectively, while the corresponding values for T-wave were 0.33, 0.30 and 0.44 mv. The R-wave amplitude values for cows with normal blood calcium and magnesium levels (9 mg/dl and 2.7 mg/dl respectively) were 0.85 and 0.82 mv. The T-wave values for the cows with normal calcium and magnesium blood levels were 0.30 and 0.28 mv. Changes in blood ketone and inroganic phosphorus had no effect on ECG characteristics.

Smyth et al. (1977) revealed a tendency in calves to develop magnesium and copper deficiency at about 3-4 months of age when liver supplies had probably been exhausted. Symptoms including hyperaesthesia, incoordination and knuckling of fetlocks were noticed.

Baker et al. (1979) observed a striking positive correlation between calcium and magnesium in hypomagnesemic sheep. It was concluded that hypocalcemia was a general outcome of hypomagnesemia. Further, sheep which developed culvulsions also exhibited a rapid decline in plasma magnesium concentration associated with a decrease in plasma concentration of calcium as well.

Lal and Verma (1979) found that total proteins were maximum (28 $\pm$ 5.94 mg/dl) in the cerebrospinal fluid of 3-6 months old calves and minimum (17.5 $\pm$ 6.06 mg/dl) above 2 years of age. Calcium values were minimum (5.00 $\pm$ 0.5 mg/dl) in adult and maximum (8.00 $\pm$ 2.72 mg/dl) in young buffaloes in all the three age groups.

Nethery et al. (1979) made observations after infusing potassium chloride and sodium chloride in reticulo rumen of 6-months-old calves @ 0.29, 0.58 and 1.15, 1.73, 2.31 or 2.88 g of potassium/kg body weight or 1.35, 21.2 or 2.16 g of sodium/kg body weight in equal volumes of water. Potassium and total solids of plasma and PCV were increased at potassium doses greater than 0.29 g/kg body weight within an hour after dosing. At the higher doses

of potassium, sodium content of plasma increased about an hour after an increase in plasma potassium, Respiration generally increased and associated varibales of CO_2 pressure, pH and bicarbonate in blood were decreased. Clinical toxicity signs including excess salivation, muscular tremors of legs and excitability were observed with potassium doses greater than 0.58 g/kg body weight. Three of the five calves given 1.73 g/kg, 3 of the 4 values given 2.31 g/kg and one calf given 2.88 g/kg body weight of potassium chloride died.

Choudhuri et al. (1980) induced rumen acidosis in four adult buffalo calves, by oral feeding of molasses @ 10 g/kg body weight. In the rumen liquor a decrease in pH was accompanied by a decrease in sodium and potassium and an increase in calcium, magnesium and inorganic phosphorus levels. Serum values of inorganic phosphorus and sodium increased while calcium, magnesium and potassium concentrations decreased. There was a decrease in the pH of urine which was associated with an increased urinary excretion of inorganic phosphorus.

Randhawa et al. (1980) induced rumen acidosis in four crossbred calves by oral feeding of molasses @ 25 g/kg body weight. With the progression of acidosis there was a decrease in the salivary pH, secretion rate and bicarbonate content and increase in sodium, inorganic phosphorus, lactic acid and protein contents of saliva. The osmolality

of the saliva and blood changed from hypertonic to hypotonic when compared with the rumen liquor.

Sinha (1980) reported that in induced hypomagnesemic calves blood, serum and rumen fluid pH, magnesium calcium and phosphorus (except rumen fluid phosphorus) decreased significantly; while the serum and rumen fluid potassium increased significantly from 4.833 to 13.26 and 36.083 to 330 ± 14 mEq/L respectively. Post-mortem examination revealed marked hyperaemia, congestion and mild haemorrhage in the mucosa of the rumen, abomasum, intestine, liver, lungs, kidney, spleen, meninges and brain in whole milk induced hypomagnesemia; while echymotic haemorrhages and petichae were observed in above mentioned organs in potassium chloride and citric acid induced diseases in calves.

Seoane (1981) measured the concentration of various electrolytes in the cerebrospinal fluid taken from the lateral ventricle of the brain in sheep. Concentrations in mEq/L of sodium, potassium, calcium and magnesium were found to be 156.6 ± 1.5, 2.94 ± 0.02, 2.37 ± 0.04 and 2.18 ± 0.08 respectively.

Shiga et al.(1981) found that in experimentally induced hypomagnesemia, ratio of absorption to intake of magnesium and phosphorus rose in three ewes but decreased in another, while that of calcium decreased and of sodium and potassium increased in all animals. Urinary secretion of potassium and phosphorus increased while magnesium and

sodium decreased with no change in calcium. Body retention of sodium and potassium increased whereas that of magnesium calcium and phosphorus decreased. Serum concentrations of magnesium and phosphorus decreased while calcium, sodium and potassium levels remained unchanged.

Vihan (1981) collected cerebrospinal fluid from ten healthy sheep and five with nervous system disorders. Physical attributes like turbidity and clotting were not seen in healthy sheep but the fluid was turbid in three clinical cases. Values for pH, total proteins, glucose, chloride and calcium averaged 8.2, 41.4 mg/dl and 43.5 and 20.02 mEq/L and 4.94 mg/dl respectively in healthy sheep.

West et al. (1981) observed the occurrence of hypomagnesemic tetany in a flock of 2500 mixed aged ewes in New Zealand. Twelve ewes died suddenly 2-3 weeks after lambing. One ewe was staggering before death and postmortem examination of two ewes exhibited blood tinged froth in the mouth and blood stained discharge from the anus.

El-Shrif & Mottelib (1983) observed that during hypomagnesemia the animals were sensitive to external stimuli and revealed opisthotonus, ears folded backwards, retraction of eyelids, muscle twitching, tremors, tonic and clonic spasms of limbs, clonic convulsions and tetanic contractions were observed. The blood chemistry revealed alkalosis, reduced calcium, magnesium, inorganic phosphorus, sodium and chloride and increased concentration of potassium.

Kariya et al. (1983) reported that increased levels of dietary potassium appears to further reduce serum levels of magnesium in steers fed diets with decreased magnesium content. Infusion of potassium chloride into the jugular vein did not have this effect which leads to the conclusion that the site of action where potassium induces reduction of magnesium has been in digestive tract.

Takahashi et al. (1983) induced hypomagnesemia in three sheep. Magnesium level declined below 0.5 mg/dl and one sheep died from severe hypomagnesemic tetany. Plasma levels of calcium also decreased. Plasma sodium level was unchanged, during the experimental period. The sheep which died during the experiment showed increase in potassium levels from 13.7 to 27.0 mg/dl during convulsions before death.

Treatment of Hypomagnesemia

Murakami et al. (1972) observed successful recovery of clinical cases of grass tetany (hypomagnesemia) in cows and suckling calves with subcutaneous injection of 100-200 ml of 25 per cent magnesium sulphate solution.

Ohshima et al. (1973) treated several clinical cases of grass tetany (hypomagnesemia) in cows successfully with magnesium sulphate solution.

Heen (1974) found that all cows suffering from puerperal paresis, receiving 15 g of magnesium -DL-aspartate alongwith 100 ml of calcium preparation, showed

only a slightly higher percentage of recovery (53%) than the control (48%) and this difference was not significant.

Hadlich and Kolb (1975) found that infusion of 2% calcium chloride and 10% magnesium chloride and 5% glucose one after other or in combination, produced severe functional changes in the cardiac activity and the respiration. The recommended concentration worked out by them for the treatment or prophylaxis of hypomagnesemia was 10 g magnesium chloride, 2 g magnesium adepate and 5 g calcium gluconate in 100 ml of distilled water (total dose = 500 ml). This was well tolerated and found to overcome the mineral deficiency in tissues.

Meyer and Busse (1975) infused magnesium chloride (20-30%) per rectally in cattle and sheep and observed 0.5 mg/dl increase in blood magnesium level. The maximum effect was seen only after one minute. Simultaneous infusion of magnesium and calcium showed that magnesium absorption in magnesium depleted sheep was less than when magnesium alone was infused and there was no change in the calcium level of the blood. Though magnesium is absorbed by the mucus membrane of the rectum, this method is not as certain as the use of intravenous subcutaneous injections as a part of the solution is sometimes ejected by the animal.

Meyer and Busse (1975) studied the effect of single intravenous injection of magnesium gluconate on the

magnesium content of cerebrospinal fluid in sheep with normal and low levels of magnesium in the cerebrospinal fluid. They found that in normally fed sheep, the administration of a single injection of magnesium gluconate resulted in a slight brief rise (about 0.25 mg/dl) in the magnesium levels of cerebrospinal fluid. The greatest effect (increase upto 0.4 mg/dl) lasting about 2 hours occured in magnesium deficient diet with a low level of magnesium in cerebrospinal fluid.

Alterskjaer and Mosdal (1976) treated cows with either hypocalcemia, parturient paresis, clinical paresis and hypomagnesemia. The recovery after intravenous injection of calcium and magnesium chloride solution was 63.7%, 76.7%, 86.9% and 94.4% respectively in the above-mentioned conditions. Supplementary treatment with subcutaneous injection was suggested inall cases.

May et al. (1976) administered calcium, magnesium, vitamin A and vitamin D in young hypomagnesemic calves and treated them successfully.

Mieth et al. (1976) studied the efficiency of various combinations of drugs in 224 cows with serum magnesium concentrations of less than 1.8 mg/dl. The treatment included intravenous injection of Parevert (calcium chloride and magnesium chloride solution). Solution A (magnesium adipate, calcium gluconate and boric acid) or 25% magnesium sulphate solution or various

combinations. Prolonged increase in serum magnesium concentration was obtained only by giving high doses (400-500 ml) of solution A. Low dose (100 ml) magnesium sulphate solution prevented a further drop in serum magnesium for a short period after the treatment.

Moreno et al. (1976) treated hypomagnesemic cows with 25% magnesium sulphate solution 100 ml intravenously. Some cases were also treated by them with calcium gluconate. In all cases recovery was followed in 2-3 hours. Later these cows were also given 100 ml of magnesium sulphate solution subcutaneously.

Schonherr et al. (1976) studied the efficacy of solution A (12 g magnesium adipate and 5 g calcium gluconate per 100 ml of distilled water) with that of solution B (12 g magnesium adipate and 12 g calcium gluconate per 100 ml) by intravenous infusion(500 ml)to 28 cattle suffering from grass tetany and hypocalcemia. After the treatment with solution B, there was a considerable increase of magnesium level in the serum. Efficacy of the treatment was found better when infusion was performed just after appearance of clinical signs. Grass tetany and paresis with tetany-like symptoms were recommended to be treated with solution B.

Vrzgula et al. (1976) administered a solution containing 10% calcium gluconate, 3% magnesium chloride and 0.5% trimicaine hydrochloride to 54 cattle, 79 sheep

and 30 pigs by intravenous, intramuscular or subcutaneous routes to see the persistence of the drug in the system for the treatment and control of hypomagnesemia. The intravenous and intramuscular routes produced rise in serum calcium and magnesium in one hour, which persisted upto 72 hours.

Flast and Meen (1978) treated a complicated case of hypocalcemic hypomagnesemia in cows with a solution containing calcium and magnesium salts with transitory improvement after a relapse (7 hours later). The cow was given thiamine chloride (3 g), calcium chloride (35 g) and magnesium chloride (15 g) intravenously. The animal improved rapidly within 20 minutes and regained its normal appetite after one and a half hour.

Haggard et al. (1978) treated clinical cases of tetany associated with magnesium deficiency in 4-months-old beef calves with intravenous magnesium salts which responded very well. Death losses ceased after a magnesium containing supplement was fed to the calves and cows.

Rutkowiak et al. (1978) treated an outbreak of grass tetany due to nitrogenous fertilizers application in the pasture in a herd of 200 cows with magnesium salts. Blood levels of magnesium enhanced from an average of 0.72 to 2.04 mg/dl, that of calcium from 7.72 to 8.17 mg/dl and the inorganic phosphorus from 4.08 to 5.19 mg/dl.

Yoshida (1978) conducted a therapeutic trial on two complicated ketotic cows with low serum magnesium

content by treating them with five daily injections of magnesium (1 g intravenous). This treatment decreased serum and urine acetone to normal after five injections. Another ketotic cow was injected daily with magnesium (1 g intravenous) and glucose (200 g intravenous), recovered within three days.

Sinha (1980) treated the whole milk induced hypomagnesemic calves with 10% solution of magnesium sulphate @ 1.5-2.0 ml/kg body weight intravenously daily for 2 days, followed by half the dose intravenously and remaining half dose subcutaneously daily for 2 days. Treatment of citric acid and potassium chloride induced hypomagnesemic calves was done with 50 ml of calcium borogluconate solution (calborol) in addition to 10% magnesium sulphate.

Teuffert et al.(1981) treated 14 cases of hypomagnesemia in cows by intravenous infusion of 500 ml solution of 60 g magnesium adipate and 60 g calcium gluconate. Concomitant hypocalcemia occured in one cow only. The disorder had no effect on the concentration of potassium and inorganic phosphorus of blood. There occurred a slight reduction in sodium concentration. Blood glucose was high in the animal with hypocalcemia in another that failed to respond to the treatment. The changes in magnesium and calcium concentration following infusion were similar to those reported in healthy cattle.

El-Shrif & Mottelib (1983) observed successful recovery in ten buffalo calves in which hypomagnesemia was induced,

when treated with xylazine (0.5 ml intramuscular), 150 ml of calphos intravenous or subcutaneous (containing various calcium and magnesium salts) and catorol 5 ml intramuscular (containing phosphoric acid and cyanocobalamin). Each animal was given one litre of electrolyte solution containing 8.5 g of sodium chloride, 0.4 g of calcium chloride, 0.2 g caffeine, sodium benzoate and distilled water for three consecutive days.

MATERIAL AND METHODS

The series of experiments were conducted on 22 clinically healthy male buffalo calves, 3-7 months old and weighing between 45-70 kg (mean ± SEM 63.2 ± 7.7). Deworming of the calves was carried out with Panacur* (@ 3 mg/kg body weight). The animals were randomly divided into four groups of 5,5,6 and 6 animals each. The animals of group I served as control, while hypomagnesemia was induced in group II, III and IV. All the animals were kept and maintained under similar managemental conditions.

Control (Group I)

The animals were allowed to eat green fodder and wheat chaff.

Induction of hypomagnesemia by intraruminal administration of potassium chloride and citric acid (Group II, III & IV)

The animals of these groups were kept on green fodder and wheat chaff. Potassium chloride @ 1.3 g/kg and citric acid 1.1 g/kg body weight, dissolved in water were administered slowly intraruminally daily. Five animals of group II were used for terminal studies and symptoms were observed. In the remaining animals of Group III and IV these chemicals were given intraruminally for four days daily till hypomagnesemia developed. Then they were subjected to

*Panacur (25% Fenbendazole), Hoechst-Pharmaceuticals Ltd.Bombay.

therapeutic trials using two different regimens.

COLLECTION OF SAMPLES

a. Blood and serum

Five ml of blood from jugular vein was collected in a sterile vial containing heparin (0.1 mg/ml blood). Another 15 ml of blood was collected into serum separating vials. Serum samples were separated by decanting within shortest possible time and kept in deep freeze. Blood samples were taken daily till death in group II animals. After 4 days treatment was started in animals of group III and IV and blood samples were collected for 3 days at 24-hour intervals.

b. Rumen fluid

Ten ml of rumen fluid was aspirated by using a sterile syringe fitted with 4-inch long 15 gauge needle, directly from the rumen of all the experimental animals. The rumen fluid was strained through gauze, collected and stored in deep freeze. Frequency of rumen fluid sampling was the same as for blood sampling.

c. Cerebrospinal fluid

Three to four ml of cerebrospinal fluid was aseptically aspirated on alternate days by puncturing into intervertebral space at lumbosacral joint or between 5th and 6th lumbar vertebrae, using a 4-inch long and 18 gauge needle. The samples were collected in capped vials and kept in deep freeze.

d. Saliva

Stenson's duct was fistulated on left side before the start of the experiment. Three-four ml of saliva was collected daily from fistulated tube. Fistulation was performed in a way that normal flow and supply of saliva was not hindered.

e. Urine

Five ml of urine was collected at natural urination every-day and stored in deep freeze for further analysis.

PARAMETERS STUDIED

1. Base apex electrocardiogram (Norr, 1913) was recorded through electrocardiograph[1], at a paper speed of 25 mv/sec and calibration setting at 1 MV/cm. The electrocardiogram (ECG) was analysed for duration and amplitude of P and T waves and mean QRS complex, PR interval and segment and QT interval and ST segment.

2. Heart rate was calculated from base apex ECG.

3. The packed cell volume was measured by micro-haematocrit method.

4. Haemoglobin (Hb) was measured by Sahli's method.

5. Temperature was recorded from rectum.

BIOCHEMICAL ANALYSIS

1. Calcium and magnesium in serum, saliva, rumen fluid and cerebrospinal fluid were determined by atomic absorption spectrophotometer[2].

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1. Cardiart, British Physical Laboratories, Hyderabad, India.
2. Atomic Absorption Spectrophotometer, Model AA6, Australia.

2. Phosphorus in serum, rumen fluid, cerebrospinal fluid and saliva was determined by microcalorimetric method (Tauasky and Shorr, 1953).

3. Sodium (Na^+) and potassium (K^+) in serum, saliva, rumen fluid, cerebrospinal fluid and urine were estimated by flame photometer[1].

4. pH of the rumen fluid, and urine was noted with the help of BDH pH paper strips[2].

5. Total protein in cerebrospinal fluid was estimated by Spectronic-20 (Meulemans; 1960).

6. Pandy's test was performed on cerebrospinal fluid to check turbidity.

THERAPEUTIC TRIALS

The treatment of the animals was done on the basis of electrolyte status of the body. The therapy commenced 96 hours after the start of potassium chloride-citric acid administration as by that time it was known that hypomagnesemia develops 3-4 days after the administration of the above-mentioned salts intraruminally. Two different therapeutic regimens were adopted in group III, and IV respectively.

PREPARATION OF SOLUTIONS

1. Magnesium sulphate (10% w/v)

This solution was prepared by dissolving 50 g of magnesium sulphate (BDH LR) in 500 ml of glass triple distilled water which has been autoclaved at 15 lb. pressure

1. Elico, Biomed Flame Photometer, Model CL Hyderabad, India
2. BDH, Paper, Glaxo

for 15 minutes. These bottles were stored subsequently in a refrigerator at 4°C for routine intravenous use in experimental calves.

2. Magnesium chloride (30% w/v)

This solution was prepared by dissolving 150 g magnesium chloride hexahydrate (BDH, Analar) in 500 ml of glass double distilled water.

TREATMENT OF HYPOMAGNESEMIA (Group III)

The treatment schedule consisted of magnesium sulphate 10% solution @ 1.5-2.0 ml/kg body weight. Half of the dose was given intravenously and remaining half subcutaneously daily for 3 days. In addition, calcium borogluconate[1] 50 ml was given slowly intravenously daily. Observations for recovery were made in all animals.

TREATMENT OF HYPOMAGNESEMIA (Group IV)

The treatment of calves of group IV comprised administration of magnesium chloride hexahydrate ($MgCl_2.6H_2O$) solution per rectally. For this a two-foot long plastic tubing was taken and after lubricating, it was pushed about one-foot deep into the length of rectum. Through it, 75 ml of solution was passed with the help of funnel at other end of the tube. The treatment started after 96 hours of daily infusion of potassium chloride and citric acid. Treatment continued for three consecutive days. Observations for recovery were made in all animals.

1. Calcium borogluconate injection B.P. (Vet.)- 450 ml containing : Caclium 8.03 g (i.e 1.79% w/v), proportion of boric acid to calcium 2 to 1, preservative cholorocresol, produced by BRIAH, Pune and marketed by NOCIL, Bombay-21

GROSS AND HISTOPATHOLOGICAL EXAMINATION

Post-mortem examination of the buffalo calves died of hypomagnesemia was conducted as early as possible. Various organs, viz. heart, kidneys, liver, lungs, skeletal muscles, brain, intestine and lymph nodes were collected and examined for gross lesions. Thereafter, they were preserved in 10% buffered formalin for histopathological examination.

The tissues were processed in a routine manner sectioned at 5 μ thickness and stained with hematoxylin and eosin (H & E) stain (Frankel et al., 1970).

STATISTICAL ANALYSIS FOR THE DATA

The data was analysed using one-way analysis of variance followed by a critical difference test. In all analysis probability level of P 0.05 was considered as statistically significant.

RESULTS

CONTROL GROUP (Group I)

The normal average values of magnesium, calcium phosphorus, sodium and potassium observed in serum, rumen fluid, CSF and saliva are presented in Tables 1-4. Table 5 depicts pH, sodium and potassium levels in urine. ECG recordings are presented in Table 6. The day-to-day observations did not reveal any significant change.

INDUCTION OF HYPOMAGNESEMIA (Group II, III &IV)

All the 5 animals of the untreated group (Group II) died in 4-6 days due to induced hypomagnesemia, while the animals belonging to group III recovered after receiving the treatment. But in animals of Group IV values of various electrolytes remained below normal even after treatment.

SYMPTOMS ASSOCIATED WITH HYPOMAGNESEMIA

Following intraruminal administration of potassium chloride and citric acid restlessness was observed. After two days a slight inappetence coupled with decrease in ruminal movements was noted. Associated symptoms included wildness of facial expression and exaggard limb movements. Polypnoea was observed. On the 3rd day animals were dull, depressed, lathargic, keeping head down while standing in apathetic condition with drowsy eyes. It was preceded by spasmodic urination, polyurea and frequent defecation. Shaking of the head, opisthotonus, ataxia, without circling

Table 1 : Biochemical analysis of serum, haematological changes and body temperature variation in buffalo calves of control Group I (Mean ± SEM)

Parameter	Time (hr)						
	0	24	48	72	96	120	144
Magnesium (mg/dl)	2.17 ±0.56	2.16 ±0.16	2.20 ±0.45	2.05 ±0.15	2.07 ±0.19	2.06 ±0.14	2.18 ±0.35
Calcium (mg/dl)	9.87 ±1.45	9.97 ±1.10	9.88 ±0.30	9.36 ±0.16	9.52 ±0.55	10.26 ±1.00	9.83 ±0.16
Phosphorus (mg/dl)	6.76 ±0.74	6.81 ±0.61	6.29 ±0.80	5.92 ±0.75	6.34 ±0.81	6.82 ±0.42	7.12 ±0.68
Sodium (mEq/L)	131.37 ±2.14	130.35 ±3.07	131.76 ±3.85	132.88 ±2.36	132.83 ±3.33	129.33 ±2.50	128.49 ±2.01
Potassium (mEq/L)	5.20 ±0.44	5.04 ±0.23	5.48 ±0.19	6.05 ±0.43	5.80 ±0.16	6.25 ±0.59	6.10 ±0.88
PCV (%)	26.00 ±2.31	27.50 ±0.58	28.25 ±0.50	24.50 ±1.33	25.54 ±0.88	27.26 ±1.78	26.85 ±0.35
Haemoglobin (g/dl)	10.20 ±0.42	10.33 ±0.17	11.00 ±1.04	10.50 ±0.46	10.67 ±0.17	10.83 ±0.33	11.17 ±0.17
Rectal Temp. (^{O}F)	100.60 ±0.35	100.53 ±0.34	101.00 ±0.20	101.20 ±0.58	100.50 ±0.58	100.53 ±0.24	100.00 ±0.55

Table 2 : Biochemical analysis of rumen fluid from buffalo calves of control Group I (Mean ± SEM)

Parameters	Time (hr)						
	0	24	48	72	96	120	144
pH	6.90 ±0.24	6.83 ±0.17	6.88 ±0.27	6.60 ±0.28	6.65 ±0.18	6.75 ±0.16	6.67 ±0.17
Magnesium (mg/dl)	7.65 ±0.78	7.06 ±0.53	7.25 ±0.60	7.60 ±0.85	7.44 ±0.78	7.25 ±1.09	7.22 ±1.23
Calcium (mg/dl)	13.65 ±1.41	13.13 ±0.97	13.74 ±2.78	13.65 ±2.14	12.90 ±1.78	12.94 ±0.97	13.40 ±1.34
Phosphorus (mg/dl)	14.23 ±0.76	14.55 ±0.80	14.15 ±0.76	13.60 ±0.76	13.15 ±0.75	13.96 ±0.75	14.10 ±0.91
Sodium (mEq/L)	130.62 ±5.58	128.42 ±0.66	126.29 ±5.52	127.68 ±5.71	123.25 ±5.16	126.99 ±2.36	123.26 ±4.26
Potassium (mEq/L)	38.75 ±1.75	37.59 ±2.58	38.20 ±3.21	37.67 ±2.76	37.18 ±1.84	35.83 ±2.26	37.17 ±2.51

Table 3 : Biochemical analysis of cerebrospinal fluid from buffalo calves of control Group I (Mean ± SEM)

Parameters	Time (hr)			
	0	48	96	144
Magnesium (mg/dl)	2.03 ±0.06	2.05 ±0.09	1.97 ±0.07	2.05 ±0.08
Calcium (mg/dl)	5.08 ±0.57	5.00 ±0.14	4.92 ±0.23	5.03 ±0.18
Phosphorus (mg/dl)	1.67 ±0.38	1.66 ±0.17	1.73 ±0.10	1.63 ±0.07
Sodium (mEq/L)	140.30 ±1.34	141.86 ±1.00	138.40 ±2.00	139.94 ±1.05
Potassium (mEq/L)	3.43 ±0.58	3.23 ±0.05	3.11 ±0.37	3.10 ±0.23
Total protein (mg/dl)	40.66 ±1.70	41.33 ±3.63	39.60 ±3.71	42.50 ±5.21
Pandy's test	(-)	(-)	(-)	(-)

Table 4 : Biochemical analysis of saliva from buffalo calves of control Group I (Mean ± SEM)

Parameter	Time (hr)						
	0	24	48	72	96	120	144
Magnesium (mg/dl)	1.52 ±0.06	1.53 ±0.07	1.53 ±0.03	1.55 ±0.03	1.54 ±0.06	1.51 ±0.08	1.50 ±0.09
Calcium (mg/dl)	2.55 ±0.58	2.50 ±0.32	2.61 ±0.16	2.75 ±0.50	2.70 ±0.45	2.88 ±0.73	2.62 ±0.57
Phosphorus (mg/dl)	30.57 ±0.60	31.83 ±0.70	33.60 ±3.36	30.67 ±0.32	30.02 ±1.02	32.88 ±1.66	30.38 ±0.84
Sodium (mEq/L)	140.13 ±6.24	137.25 ±2.88	136.34 ±3.28	137.82 ±2.51	140.17 ±4.93	142.53 ±3.21	144.82 ±4.93
Potassium (mEq/L)	7.41 ±0.85	7.18 ±0.58	6.92 ±1.00	7.35 ±0.86	7.50 ±1.02	8.00 ±1.40	7.80 ±0.84

Table 5 : Biochemical analysis of urine from buffalo calves of control Group I (Mean ± SEM)

Parameter	Time (hr)						
	0	24	48	72	96	120	144
pH	8.80 ±0.15	8.83 ±0.17	8.67 ±0.17	8.63 ±0.24	8.80 ±0.29	8.85 ±0.17	8.70 ±0.29
Sodium (mEq/L)	43.42 ±3.71	42.89 ±9.24	45.03 ±5.90	48.67 ±3.71	48.03 ±5.90	44.23 ±3.68	38.11 ±5.19
Potassium (mEq/L)	440.75 ±40.74	436.11 ±38.01	420.00 ±30.55	455.51 ±38.62	450.66 ±40.48	435.22 ±53.82	428.67 ±30.79

Table 6 : Various components of ECG from buffalo calves of control Group I (Mean ± SEM)

Parameters	Time (hr)						
	0	24	48	72	96	120	144
P Wave							
D	0.040 ±0.006	0.045 ±0.005	0.047 ±0.007	0.045 ±0.006	0.040 ±0.007	0.050 ±0.006	0.045 ±0.002
A	0.093 ±0.007	0.093 ±0.007	0.087 ±0.014	0.090 ±0.009	0.096 ±0.013	0.100 ±0.015	0.095 ±0.007
Mean QRS							
D	0.060 ±0.008	0.060 ±0.005	0.060 ±0.005	0.057 ±0.003	0.057 ±0.003	0.063 ±0.003	0.060 ±0.008
A	-0.825 ±0.370	-0.817 ±0.235	-0.867 ±0.017	-0.830 ±0.130	-0.820 ±0.132	-0.763 ±0.206	-0.763 ±0.177
T Wave							
D	0.067 ±0.013	0.067 ±0.013	0.070 ±0.013	0.073 ±0.018	0.072 ±0.020	0.070 ±0.030	0.068 ±0.007
A	0.173 ±0.013	0.173 ±0.018	0.160 ±0.014	0.170 ±0.013	0.172 ±0.018	0.160 ±0.012	0.165 ±0.014
PR Int. (Sec)	0.173 ±0.013	0.173 ±0.018	0.160 ±0.014	0.160 ±0.012	0.160 ±0.050	0.172 ±0.013	0.172 ±0.018
QT Int. (sec)	0.366 ±0.059	0.370 ±0.010	0.320 ±0.020	0.310 ±0.030	0.315 ±0.033	0.293 ±0.015	0.318 ±0.013
PR seg. (sec)	0.147 ±0.007	0.147 ±0.013	0.140 ±0.012	0.133 ±0.024	0.132 ±0.016	0.138 ±0.008	0.142 ±0.012
ST seg (sec)	0.170 ±0.030	0.173 ±0.024	0.170 ±0.020	0.165 ±0.018	0.173 ±0.008	0.175 ±0.015	0.174 ±0.011
HR (per min)	60.800 ±2.750	66.730 ±4.140	64.250 ±2.400	66.570 ±6.140	65.400 ±3.220	62.450 ±2.350	61.240 ±1.250

D = Duration in seconds; A = Amplitude in mv; Int. = Interval ; seg. = segment

and droopy backward carriage of the ears were constant features. On the 4th day, the animals were weak, unable to get up. Muscle tremors of the hind legs and tail were observed. Salivation, stiffness and apparent opthistonus were found as characteristic symptoms. Death was preceded by darkening of blood, intense muscular tremors, protruding eyes and lost ability to stand. Considerable strugling was noted after the ability to stand was lost. Tetanic symptoms of hypomagnesemia were not observed in the present study.

PATHOPHYSIOLOGICAL GROUP (Group II)

Haematological studies

Both haemoglobin and packed cell volume revealed a steady progressive rise in their respective levels, the same remained statistically significant ($P < 0.05$) throughout the study (Table 7; Fig. 7 and 8).

Biochemical studies

a. Serum

Potassium exhibited a statistically significant ($P < 0.05$) upward trend throughout the experiment (Table 7; Fig. 5). On the other hand, regularly decreasing trend in the levels of magnesium (Table 7; Fig. 1) which became significant ($P < 0.05$) from 48 hours onwards, persisted till the end of study. Both calcium (Table 7, Fig. 2) and sodium (Table 7, Fig. 4) remained almost unaltered while phosphorus, which increased significantly ($P < 0.05$) at 24 hours of hypomagnesemia, but decreased progressively and significantly ($P < 0.05$) from 72 hours onwards upto 144 hours of study (Table 7, Fig. 3).

b. Rumen fluid

pH of the rumen fluid (Table 8) decreased continuously and significantly ($P < 0.05$) begining from 24 hours of experiment. Potassium (Table 8, Fig. 5) and magnesium (Table 8, Fig. 1) revealed exactly the opposite trend; the former revealed a progressive rise while the later showed a regular fall; all the changes being statistically significant ($P < 0.05$) The fall in the levels of calcium (Table 8;Fig. 2) and sodium (Table 8, Fig. 4) again remained statistically non-significant. The significant ($P < 0.05$) fall in phosphorus level was observed at and beyond 24 hours till the end of the study period (Table 8, Fig. 3).

c. Cerebrospinal fluid

Potassium sustained a regularly upward and statistically significant ($P < 0.05$) trend throughout the study (Table 9; Fig. 5). Magnesium levels decreased significantly ($P < 0.05$) beginning from 48 hours onwards (Table 9; Fig. 1) while calcium also revealed a similar behaviour; however, the decrease was significant at 96 hours (Table 9, Fig. 2). Once again an initial significant ($P < 0.05$) upsurge in phosphorus values recorded at 48 hours was succeeded by statistically significant ($P < 0.05$) fall at 96 and 144 hours of study (Table 9; Fig. 3). Sodium was the only ion which remained unchanged during the study (Table 9, Fig. 4). Total proteins concentration showed a regularly upward and statistically significant ($P < 0.05$) trend throughout the study. Pandy's test which was slight positive (++) after 48 hours

of hypomagnesemia became intense positive (++++) after 96 and 144 hours of study (Table 9).

d. Saliva

Changes in saliva were nearly the same as in other body fluids. The potassium level increased regularly and significantly only at 24 hours of induction and thereafter decreased steadily, the change becoming significant ($P < 0.05$) from 72 hours onwards (Table 10, Fig. 5). Magnesium decreased significantly ($P < 0.05$) 48 hours after administration of potassium chloride with citric acid and kept decreasing significantly ($P < 0.05$) till the end of the study (Table 10; Fig. 1). The appreciable increase in calcium levels remained statistically insignificant (Table 10; Fig. 2) while sodium remained unchanged throughout the study (Table 10; Fig. 4). The significant ($P < 0.05$) rise in phosphorus observed at 24 hours of hypomagnesemia was followed by statistically significant fall ($P < 0.05$) from 72 hours onwards till the end of study.

e. Urine

Urine pH revealed a straight and statistically significant fall throughout the study (Table 11). Potassium levels increased drastically throughout the experimental phase, all the changes being statistically significant ($P < 0.05$) (Table 11; Fig. 6). End study values were nearly 4 times greater than base values. Sodium showed a decreasing trend which became significant from 48 hours onwards (Table 11; Fig. 4).

Table 7 : Biochemical analysis of serum, haematological changes and body temperature of hypomagnesemia induced untreated buffalo calves of Group II (Mean ± SEM)

Parameters	Pathophysiological study (Time in hr)						
	0	24	48	72	96	120	144
Magnesium (mg/dl)	2.26 ±0.11	2.06 ±0.09	1.80* ±0.12	1.39* ±0.13	1.07* ±0.13	0.95* ±0.15	0.90* ±0.10,
Calcium (mg/dl)	11.29 ±0.62	11.74 ±0.97	10.26 ±1.00	9.97 ±1.10	9.87 ±1.45	9.08 ±0.97	9.40 ±0.65
Phosphorus (mg/dl)	6.53 ±0.26	8.49* ±0.19	6.89 ±0.40	5.31* ±0.31	4.58* ±0.34	4.68* ±0.34	4.10* ±0.07
Sodium (mEq/L)	132.83 ±3.33	135.75 ±2.35	136.39 ±2.22	132.88 ±2.36	136.47 ±2.15	141.89 ±5.50	146.15 ±8.15
Potassium (mEq/L)	5.04 ±0.23	6.46* ±0.33	6.96* ±0.33	8.16* ±0.32	9.16* ±0.32	9.63* ±0.08	9.65* ±0.32
PCV (%)	23.75 ±0.85	28.25* ±0.48	32.50* ±1.32	35.50* ±1.32	38.25* ±1.32	39.50* ±1.50	42.00* ±1.00
Haemoglobin (g/dl)	10.50 ±0.46	12.18* ±0.32	12.85* ±0.40	13.55* ±0.49	14.40* ±0.87	16.20* ±1.70	17.00* ±1.00
Rectal Temp. (°F)	100.60 ±0.34	99.15* ±0.38	99.35* ±0.43	98.68* ±0.20	97.25* ±0.43	97.16* ±0.44	96.75* ±0.25

* Significantly different from base value

Table 8 : Biochemical analysis of rumen fluid of hypomagnesemia-induced untreated buffalo calves of Group II (Mean ± SEM)

Parameters	Pathophysiological study (Time in hr)						
	0	24	48	72	96	120	144
pH	6.80 ±0.12	6.30* ±0.12	6.20* ±0.12	5.60* ±0.10	4.90* ±0.19	4.83* ±0.17	4.50* ±0.00
Magnesium (mg/dl)	7.06 ±0.53	5.82* ±0.38	4.92* ±0.32	4.40* ±0.29	3.92* ±0.41	4.10* ±0.90	3.90* ±0.63
Calcium (mg/dl)	12.70 ±0.20	11.66 ±1.09	10.36 ±0.29	10.18 ±0.46	10.22 ±1.21	9.98 ±0.49	9.75 ±0.50
Phosphorus (mg/dl)	14.18 ±0.58	12.55* ±0.48	9.84* ±0.70	8.33* ±0.30	7.51* ±0.50	6.65* ±0.11	5.70* ±0.14
Sodium (mEa/L)	119.23 ±4.77	109.24 ±2.36	116.07 ±6.36	109.94 ±6.37	116.52 ±8.09	107.46 ±7.73	109.30 ±5.60
Potassium (mEq/L)	33.89 ±1.11	121.78* ±4.85	184.40* ±6.71	254.30* ±8.54	320.50* ±8.56	335.35* ±20.1	357.97* ±7.29

* Significant different from base value

Table 9 : Biochemical analysis of cerebrospinal fluid of hypomagnesemia-induced untreated buffalo calves of Group II (Mean ± SEM)

Parameters	Pathophysiological study (Time in hr)			
	0	48	96	144
Magnesium (mg/dl)	2.05 ±0.09	1.89* ±0.09	1.69* ±0.04	1.62* ±0.01
Calcium (mg/dl)	4.15 ±0.35	3 .75 ±0.24	3.16* ±0.07	3.03* ±0.08
Phosphorus (mg/dl)	1.73 ±0.10	2.39* ±0.15	1.14* ±0.55	0.85* ±0.04
Sodium (mEq/L)	140.61 ±1.34	139.94 ±1.00	138.59 ±1.28	136.25 ±1.77
Potassium (mEq/L)	3.52 ±0.14	5.54* ±0.19	5.95* ±0.17	7.50* ±0.10
Total proteins (mg/dl)	39.60 ±3.71	87.60* ±5.21	180.83* ±3.63	345.00* ±22.91
Pandy's test	(-)	(++)	(++++)	(++++)

* Significantly different from base value

Table 10 : Biochemical analysis of saliva of hypomagnesemia-induced untreated buffalo calves of group II (Mean ± SEM)

Parameters	Pathophysiological study (Time in hr)						
	0	24	48	72	96	120	144
Magnesium (mg/dl)	1.53 ±0.03	1.43 ±0.06	1.34* ±0.05	1.24* ±0.06	1.19* ±0.05	1.20* ±0.04	1.16* ±0.03
Calcium (mg/dl)	2.89 ±0.17	4.51 ±0.22	3.90 ±0.82	3.97 ±0.16	4.44 ±0.61	3.97 ±0.35	3.90 ±0.25
Phosphorus (mg/dl)	31.83 ±0.70	15.69* ±0.94	30.38 ±0.84	11.11* ±0.51	9.40* ±0.22	8.25* ±0.15	9.35* ±1.35
Sodium (mEq/L)	134.21 ±3.23	135.00 ±2.29	131.17 ±5.04	132.45 ±5.44	131.93 ±6.75	133.00 ±5.50	130.50 ±0.00
Potassium (mEq/L)	7.18 ±0.58	21.53* ±2.99	29.25* ±1.94	36.11* ±4.65	39.85* ±1.81	45.31* ±1.62	47.35* ±1.70

* Significantly different from base value

Table 11 : Biochemical analysis of urine of hypomagnesemia-induced untreated buffalo calves of Group II (Mean ± SEM)

Parameters	Pathophysiological study (Time in hr)						
	0	24	48	72	96	120	144
pH	8.63 ±0.24	7.88* ±0.24	7.75* ±0.14	7.38* ±0.13	7.25* ±0.14	7.00* ±0.20	6.75* ±0.25
Sodium (mEq/L)	43.90 ±5.86	35.82 ±3.17	29.11* ±4.80	26.70* ±3.65	18.87* ±1.51	16.35* ±2.15	12.68* ±1.66
Potassium (mEq/L)	454.43 ±35.05	853.65* ±45.68	958.99* ±55.77	1141.53* ±59.92	1308.37* ±40.20	1368.93* ±39.71	1417.99* ±46.71

* Significantly different from base value

Table 12 : Various components of ECG of hypomagnesemia-induced untreated buffalo calves of Group II (Mean ± SEM)

Parameters	Time (hr)						
	0	24	48	72	96	120	144
P Wave							
D	0.045 ±0.007	0.060 ±0.012	0.053 ±0.007	0.060 ±0.012	0.067 ±0.007	0.060 ±0.020	0.090 ±0.030
A	0.100 ±0.029	0.093 ±0.007	0.107 ±0.023	0.063 ±0.019	0.075 ±0.019	0.075 ±0.025	0.070 ±0.030
Mean QRS							
D	0.067 ±0.003	0.057 ±0.003	0.063 ±0.003	0.057 ±0.003	0.053 ±0.003	0.060 ±0.020	0.050 ±0.010
A	1.270 ±0.350	0.830 ±0.070	0.970 ±0.270	0.820 ±0.130	0.570 ±0.150	1.030 ±0.080	0.680 ±0.100
T Wave							
D	0.060 ±0.012	0.060 ±0.012	0.057 ±0.012	0.067 ±0.013	0.067 ±0.007	0.050 ±0.010	0.070 ±0.010
A	-0.033 ±0.120	0.017 ±0.073	0.167 ±0.033	0.116 ±0.072	0.133 ±0.088	0.125 ±0.025	0.200 ±0.100
PR Int. (sec)	0.173 ±0.013	0.173 ±0.018	0.160 ±0.023	0.153 ±0.035	0.173 ±0.018	0.220 ±0.060	0.190 ±0.010
QT Int. (sec)	0.390 ±0.010	0.300* ±0.012	0.293* ±0.013	0.280* ±0.023	0.280* ±0.120	0.320* ±0.020	0.310* ±0.030
PR seg. (sec)	0.140 ±0.020	0.127 ±0.007	0.120 ±0.012	0.093 ±0.013	0.127 ±0.033	0.160 ±0.040	0.100 ±0.020
ST seg. (sec)	0.263 ±0.023	0.183 ±0.012	0.173 ±0.024	0.157 ±0.023	0.153 ±0.019	0.210 ±0.010	0.190 ±0.010
HR (per min.)	60.800 ±2.730	78.180* ±3.450	80.980* ±2.970	82.610* ±3.360	85.360* ±4.910	76.130* ±6.660	82.830* ±10.04

D = Duration in seconds; A = Amplitude in mv; Int = Interval; Seg. = segment
* Significantly different from base value

Fig. 1 : Periodic alterations in the levels of Mg^{++} in serum, rumen fluid, CSF and saliva of untreated buffalo calves studied till death.

Control value : Zero hour observation

GROUP II

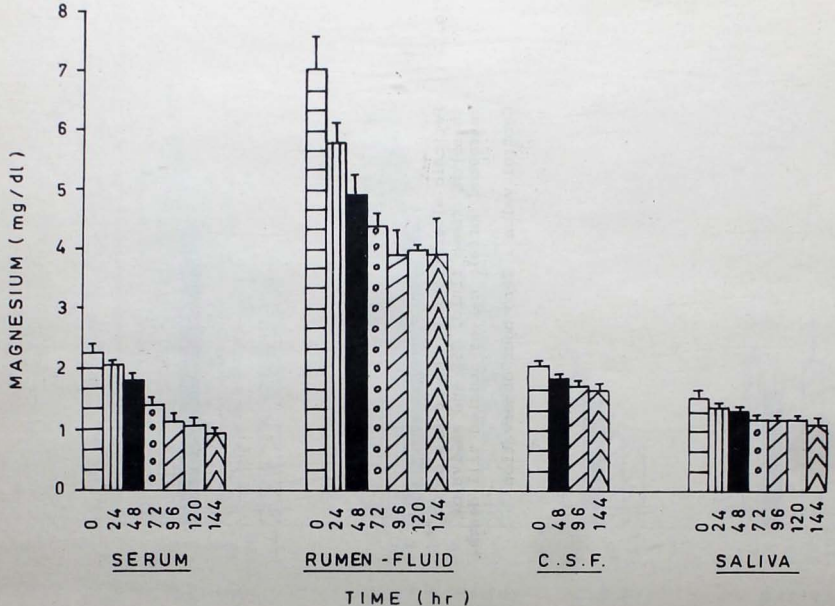

MAGNESIUM (mg / dl)
8
7
6
5
4
3
2
1
0
0 24 48 72 96 120 144
SERUM
0 24 48 72 96 120 144
RUMEN - FLUID
0 48 96 144
C.S.F.
0 24 48 72 96 120 144
SALIVA
TIME (hr)

Fig. 2 : Periodic alterations in the levels of Ca^{++} in serum, rumen fluid, CSF and saliva of untreated buffalo calves studied till death.

Control value : Zero hour observation

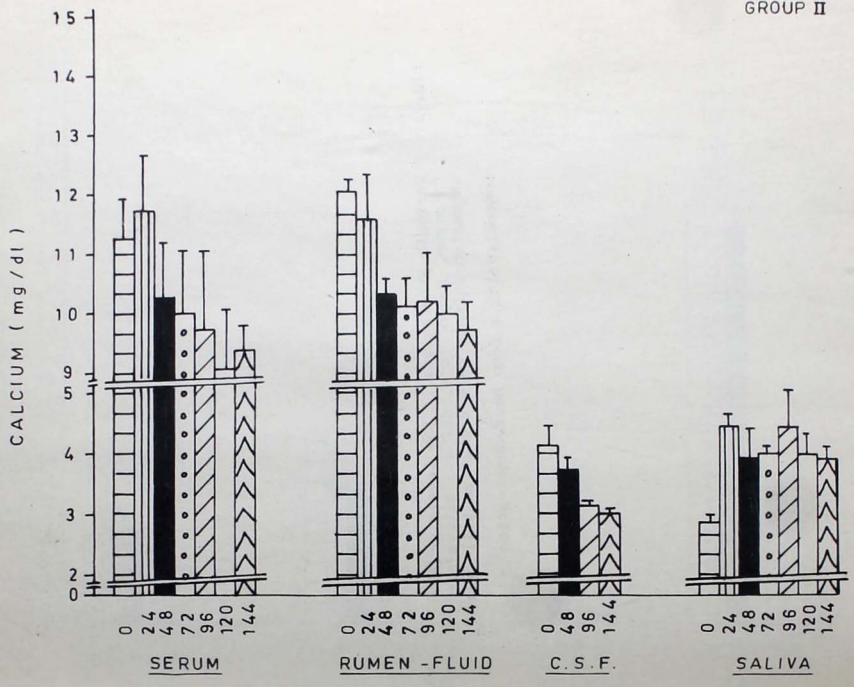

GROUP II
CALCIUM (mg / dl)
15
14
13
12
11
10
9
5
4
3
2
0
0 24 48 72 96 120 144
SERUM
0 24 48 72 96 120 144
RUMEN - FLUID
0 48 96 144
C.S.F.
0 24 48 72 96 120 144
SALIVA
TIME (hr)

Fig. 3 : Periodic alterations in the levels of pH in serum, rumen fluid, CSF and saliva of untreated buffalo calves studied till death.

Control value : Zero hour observation

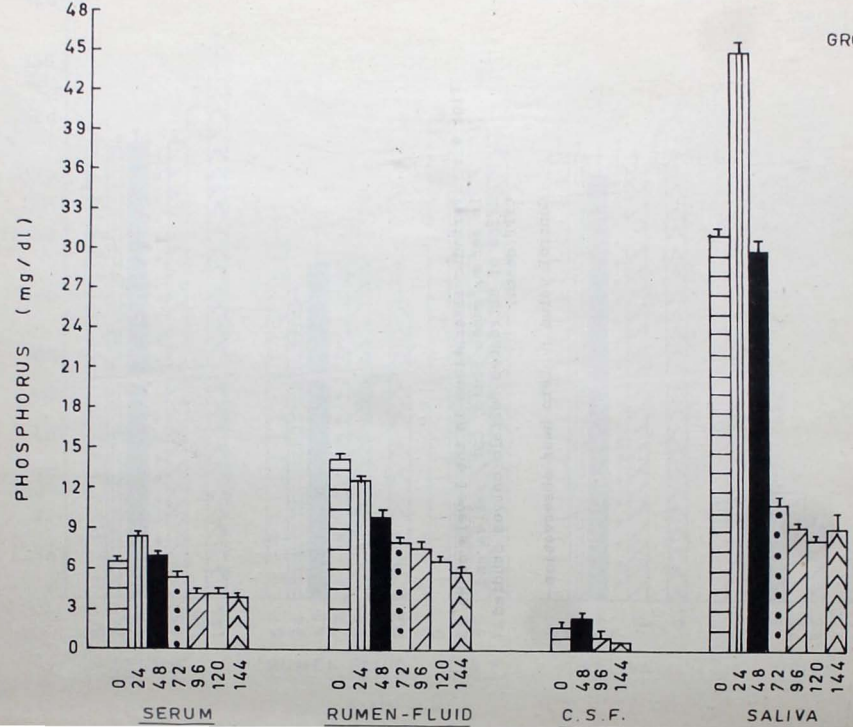

GROUP II
PHOSPHORUS (mg/dl)
48
45
42
39
36
33
30
27
24
21
18
15
12
9
6
3
0
0 24 48 72 96 120 144
SERUM
0 24 48 72 96 120 144
RUMEN-FLUID
0 48 96 144
C.S.F.
0 24 48 72 96 120 144
SALIVA

Fig. 4 : Periodic alterations in the levels of Na^+ in serum, rumen fluid, CSF, saliva and urine of untreated buffalo calves studied till death.

Control value : Zero hour observation

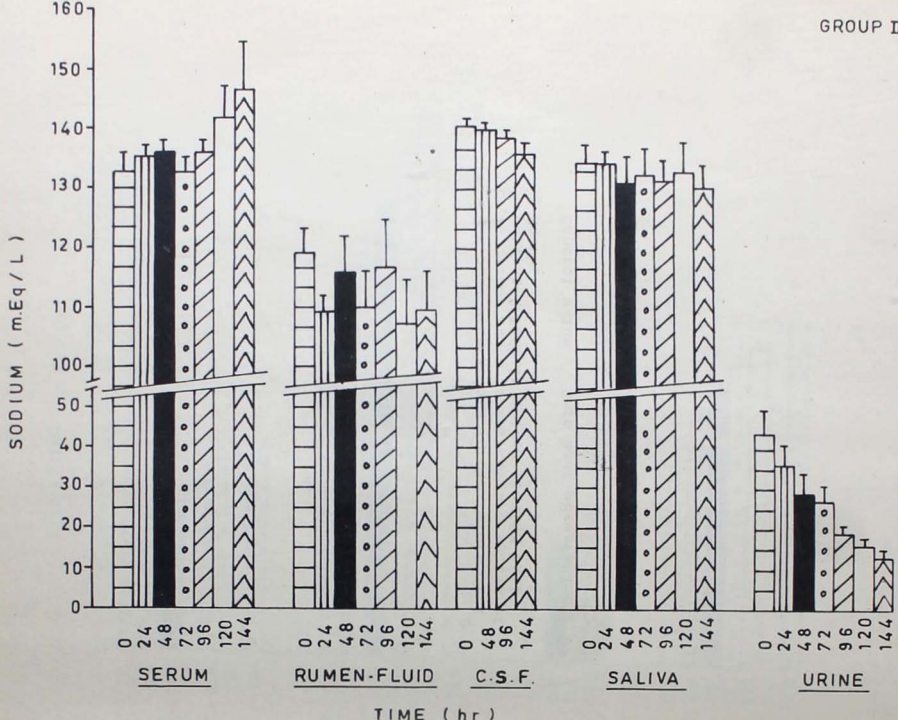
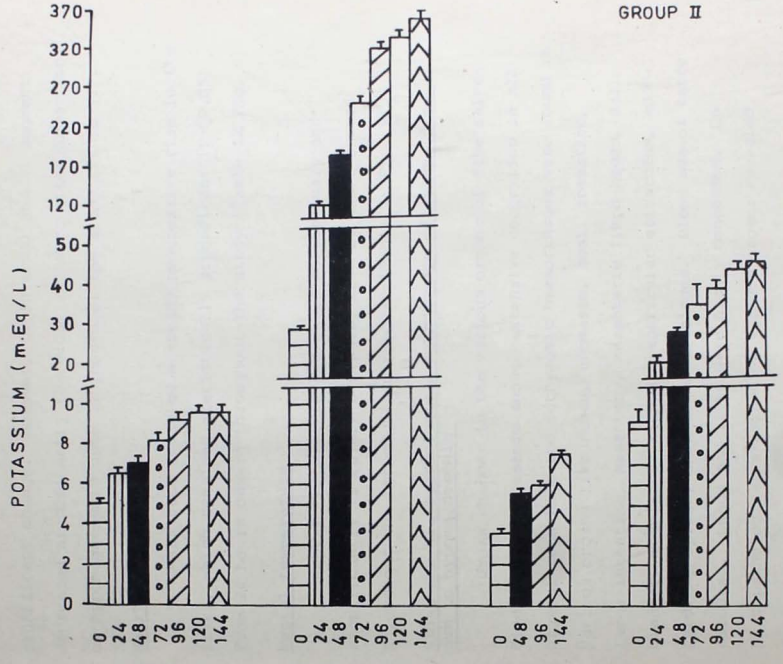

GROUP II
SODIUM (m.Eq / L)
160
150
140
130
120
110
100
50
40
30
20
10
0
0 24 48 72 96 120 144
SERUM
0 24 48 72 96 120 144
RUMEN-FLUID
0 48 96 144
C.S.F.
0 24 48 72 96 120 144
SALIVA
0 24 48 72 96 120 144
URINE
TIME (hr)

Fig. 5 : Periodic alterations in the levels of K^+ in serum, rumen fluid, CSF and saliva of untreated buffalo calves studied till death.

Control value : Zero hour observation


GROUP II
POTASSIUM (m.Eq/L)
370
320
270
220
170
120
50
40
30
20
10
8
6
4
2
0
0 24 48 72 96 120 144
SERUM
0 24 48 72 96 120 144
RUMEN-FLUID
0 48 96 144
C.S.F.
0 24 48 72 96 120 144
SALIVA

Electrocardiographic changes

Various components of the ECG did not show any significant change. However, QT interval and ST segment decreased significantly ($P < 0.05$) 24 hours after induction of hypomagnesemia upto 120 hours of the study (Table 12).

Heart rate

Heart rate revealed a steady progressive rise in its level, which remained statistically significant ($P < 0.05$) from 24 hours onward throughout the study (Table 12, Fig. 6).

Rectal temperature

Rectal temperature decreased continuously and significantly ($P < 0.05$) beginning from 24 hours onward throughout the hypomagnesemic phase (Table 7; Fig. 9).

Gross and histopathological examination of calves died due to hypomagnesemia

Gross changes in the various organs of five calves died of hypomagnesemia showed extensive congestion in all organs, petechiae and ecchymotic haemorrhages were found in various organs like rumen, abomasum, small intestine, large intestine, mesentery, mesenteric lymph nodes, gall bladder, medulla of kidney, ventricular epicardium, endocardium, spleen, omentum and meninges. Blood was of tarry coloured. Lungs and liver were highly congested. On histopathological examination various organs revealed following changes :

Heart : revealed degeneration of Purkinje fibre (Figs. 22 and 23) and cloudy swelling, fatty changes (Fig. 24) and

foci of necrosis (Fig. 25) in cardiac muscle cells. In one animal fibroelastosis of endocardium leading to its thickening and non purulent epicarditis with infiltration of lymphocytes, plasma cells and macrophages (Fig. 26) was also seen. Endocardial haemorrhage was seen in one animal.

Skeletal muscles : Hyaline degeneration (Fig. 27) was the prominent feature in all the animals.

Stomach : revealed mild necrotic gastritis.

Intestine : There was mild to severe acute necrotic entertis (Fig. 28) in all the animals.

Liver : showed degenerative changes in the form of cloudy swelling (Fig. 29). In one case mild fatty change and small eosinophilic intracytoplasmic inclusion bodies were seen in hepatocytes (Fig. 30). Diffuse coagulative necrosis (Fig. 31) was seen in one animal.

Kidney : Nephrosis was seen in all the animals and nephrotic changes varied from cloudy swelling (Fig. 32) vacuolar degeneration (Fig. 33) and diffuse coagulative necrosis (Fig. 34) of the tubular epithelial cells.

Lungs : showed severe congestion (Fig. 35) and alveolar emphysema (Fig. 36).

Spleen : There was depletion of red pulp and marked proliferation of macrophages and plasma cells (Fig. 37).

Lymph nodes : showed severe congestion (Fig. 38) infiltration of macrophages and plasma cells and perivascular oedema.

Brain : There was severe congestion in the meninges (Fig. 39) and brain substance. Encephalomalacia with myelin degenera-

tion led to spongiois of brain substance (Fig. 40). There was a marked infiltration of macrophages and microglia cells in the brain (Fig. 41). The microglia cells became hypertrophied and their cytoplasm was granular and vacuolated (gitter cells) because of phagocytosis of the necrotic brain tissue. Other changes in the brain were chromatolysis, satellitosis and neuronophagia (Fig. 42). Other organs did not show any significant pathological changes.

TREATMENT GROUP (Group III & IV)

Hematological changes

Following administration of potassium chloride and citric acid, a significant rise in packed cell volume (PCV) was noticed throughout the phase of hypomagnesemia in Group IV animals. However, in Group III animals this rise became significant ($P < 0.05$) from 48 hours onward. Following treatment in Group III, all the observations revealed a significant rise ($P < 0.05$) at 48 and 72 hours of treatment (Table 13 & 19; Fig. 7).

In both the groups haemoglobin revealed a significant ($P < 0.05$) rise at 72 and 96 hours of hypomagnesemia. The treatment resulted in a progressive and significant fall ($P < 0.05$) in haemoglobin level upto 72 hours in animals of both the groups (Table 13 & 19; Fig. 8).

Biochemical changes

a. Serum

There was regularly increasing trend in the levels of

Fig. 6 : Periodic alterations in the levels of K^+ as observed in urine of buffalo calves.

Control value : Zero hour observation
Untreated Animals : Group II
Treated Animals : Group III & IV
Pathophysiological study : 24-96 hr.
Treatment study : 120-168 hr

POTASSIUM (m·Eq/L) IN URINE
1600
1400
1200
1000
800
600
400
0 24 48 72 96 120 144
0 24 48 72 96 120 144 168
0 24 48 72 96 120 144 168
GROUP: II III IV
TIME (hr)

Fig. 7 : Periodic alterations in the values of Packed Cell Volume in the blood of buffalo calves :

Control value : Zero hour observation
Untreated Animals : Group II
Treated Animals : Group III & IV
Pathophysiological study : 24-96 hr
Treatment study : 120-168 hr

PACKED CELL VOLUME (%)
50
40
30
20
10
0
24
48
72
96
120
144
168
GROUP:
II
III
IV
TIME (hr)

Fig. 8 : Periodic alterations in the values of haemoglobin in the blood of buffalo calves.

Control value : Zero hour observation
Untreated Animals : Group III
Treated Animals : Group IV
Pathophysiological study : 24-96 hr
Treatment study : 120-168 hr

HAEMOGLOBIN (g/dl)
18
17
16
15
14
13
12
11
10
9
8
7
6
0
24
48
72
96
120
144
168
GROUP:
II
III
IV
TIME (hr)

Fig. 9 : Periodic alterations in the values of rectal temperature in buffalo calves.

Control value : Zero hour observation
Untreated Animals : Group II
Treated Animals : Group III, IV
Pathophysiological study : 24-96 hr
Treatment study : 120-168 hr

101
100
99
98
97
96
95
0
TEMPERATURE (°F)
0 24 48 72 96 120 144
0 24 48 72 96 120 144 168
0 24 48 72 96 120 144 168
GROUP:
II
III
IV
TIME (hr)

Fig. 10 : Periodic alterations in the values of heart rate in buffalo calves.

Control value : Zero hour observation
Untreated Animals : Group II
Treated Animals : Group III & IV
Pathophysiological study : 24-96 hr
Treatment study : 120-168 hr

HEART RATE (per minute)
100
90
80
70
60
50
0
24
48
72
96
120
144
168
GROUP:
II
III
IV
TIME (hr)

potassium (Table 19 and 13) which became significant ($P < 0.05$) at 24 (Fig. 20) and 48 (Fig. 15) hours in Group IV and III respectively. The treatment resulted in significant ($P < 0.05$) fall in the potassium level in both the groups. Although these values were appreciably above the zero hour observation after 72 hour of treatment. A significant ($P < 0.05$) fall in serum magnesium level was evident throughout in the group III (Table 13) animals following potassium chloride citric acid administration while in Group IV animals this decrease was recorded at 48, 72 and 96 hours respectively (Table 19). The post-treatment values in both the groups revealed a significant ($P < 0.05$) rise. However, in Group III after 72 hours these values were as good as the control value (Fig. 11) whereas in Group IV they were appreciably below the zero hour observation (Fig. 16).

Calcium (Table 13 & 19; Fig. 12 and 17) and sodium (Table 13 & 19; Fig. 14 and 19) fluctuated insignificantly before as well as after the treatment in both the groups.

In Group III and also in Group IV the significant ($P < 0.05$) increase in the phosphorus at 24 hours was followed by a significant ($P < 0.05$) decrease throughout the rest of the hypomagnesemic phase. Following treatment in Group III, all the values exhibited a significant ($P < 0.05$) rise (Table 13, Fig. 13) while in Group IV this rise in phosphorus did not become significant ($P < 0.05$) before 72 hours (Table 19; Fig. 18).

b. Rumen fluid

pH of the rumen fluid progressively decreased significantly ($P < 0.05$) after potassium chloride and citric acid administration in both the groups. Again the post-treatment phase revealed a continuous significant ($P < 0.05$) rise in the pH in both the Groups (Table 14 and 20).

The rise in potassium level was uniformly progressive and significant ($P < 0.05$) as compared to control values upto 96 hours of experiment in both the groups (Table 14 and 20). The treatment resulted in a significant fall upto 72 hours in both the groups. However, the last values of study were still significantly high as compared to control value (Fig. 15 and 20).

The gradual fall in magnesium content of rumen fluid became statistically significant ($P < 0.05$) during 48, 72 and 96 hours of study (Table 14 and 20). However, the post-treatment rise was not significant in Group IV (Fig. 16) while in Group III, it did become significant ($P < 0.05$) at 48 hours after treatment (Fig. 14)

Alteration in the calcium (Fig. 12 and 17) and sodium (Fig. 14 and 19) remained insignificant in both the groups (Table 14 and 20) throughout the study.

The decrease in phosphorus value became significant ($P < 0.05$) at 24 hours in Group III (Table 14) and 72 hours in Group IV (Table 20) animals, following potassium chloride and citric acid administration. The trend was sustained upto 96 hours of pathophysiological study in both the groups. Following treatment, there was insignificant increase of

phosphorus in Group IV (Fig. 18) while in Group III there was a statistical significant ($P < 0.05$) rise at 24 hours and 72 hours of treatment (Fig. 13).

c. Cerebrospinal fluid

The significant ($P < 0.05$) rise in the potassium level was recorded at 48 and 96 hours after commencing the experiment in both the groups (Table 15 and 21). They were followed by significant ($P < 0.05$) fall after 48 hours in the post treatment phase in both the groups (Fig. 15 and 20).

A significant decrease ($P < 0.05$) in the levels of magnesium was recorded at 48 and 96 hours of induction phase of both the groups (Table 15 and 20) were followed by a rise in the post-treatment phase. This rise became significant ($P < 0.05$) at 72 hours in Group IV (Fig. 16) while in Group III both 48 and 72 hours values after reversal were statistically significant ($P < 0.05$) (Fig. 11).

No significant change occured in the level of calcium (Fig. 12 and 17) and sodium (Fig. 14 and 19) throughout the observation period (Table 15 and 21).

Phosphorus values which fluctuated insignificantly in Group III (Table 15) animals (Fig. 13) but revealed a significant ($P < 0.05$) increase in Group IV animals (Table 21) at 48 hours post induction. In the same group the post treatment value revealed a statistically significant ($P < 0.05$) rise at 48 and 72 hours (Fig. 18).

Total proteins showed a trend exactly similar to potassium in the respective groups (Tables 15 and 21).

d. Saliva

A progressive and statistically significant ($P < 0.05$) rise in the potassium value were observed throughout the pathophysiological study in both the groups (Tables 16 and 22). The trend of fall in potassium level following the treatment became significant ($P < 0.05$) only after 72 hours in both the groups (Fig. 15 and 20).

In the GroupIII animals (Table 16) the significant ($P < 0.05$) fall in the magnesium levels observed at 72 and 96 hours of induction phase was followed by a uniformally significant ($P < 0.05$) rise in the treatment phase (Fig. 11). On the other hand in Group IV (Table 22) animals the significant ($P < 0.05$) fall of the magnesium observed at 48, 72 and 96 hours after potassium chloride and citric acid administration was not followed by any significant rise in the treatment phase (Fig. 16).

The alteration in the calcium (Fig. 12 and 17) and sodium (Fig. 14 and 19) remained without significant change in both the groups (Tables 16and 21).

In both the groups (Table 16 and 22) there was an initial significant ($P < 0.05$) rise in phosphorus level at 24 hours post induction of hypomagnesemia. The trend was however, reversed in subsequent observations as significantly ($P < 0.05$) subnormal values were recorded in both the groups at 72 and 96 hours before treatment. All the three post treatment observations revealed a progressive and significant ($P < 0.05$) rise in phosphorus (Fig. 15 and 20).

e. Urine

A significant ($P<0.05$) fall in the pH of the urine was recorded in all the animals of both the groups (Tables 17 and 23) at all the intervals before treatment. Conversely the treatment phase revealed a significant rise of pH during all the observation hours.

Potassium level increased progressively and significantly ($P<0.05$) in both the groups (Tables 17 and 23) as the hypomagnesemic state progressed. The trend was exactly reversed following treatment in Group III, that all the subsequent values showed a significant ($P<0.05$) fall while in Group IV this decrease become significant ($P<0.05$) only at 72 hours post treatment (Fig. 6).

The alterations in sodium levels which remained insignificant in Group III (Table 17; Fig. 14), however, revealed a significant fall ($P<0.05$) in Group IV animals at 48, 72 and 96 hours intervals before treatment (Table 23). The treatment failed to raise the sodium level significantly ($P<0.05$) in this group (Fig. 19).

Electrocardiographic changes

Various components of ECG did not show any significant change in both the groups (Table 18 and 20). The amplitude of the P wave in Group III increased significantly ($P<0.05$) at 72 hours and decreased at 96 hours of hypomagnesemic phase. It decreased significantly ($P<0.05$) at 72 hours of post treatment phase.

Heart rate

Heart rate which showed increasing trend in both the groups (Tables 18 and 20) became significant ($P < 0.05$) after 72 and 96 hours of hypomagnesemic phase in Group III animals. Following treatment there was a significant ($P < 0.05$) decrease in the heart rate after 72 hours in the same group (Fig. 10). In Group IV animals the increase in the heart rate remained statistically insignificant throughout the course of study (Fig. 10).

Rectal temperature

The regularly decreasing trend in the rectal temperature which became significant ($P < 0.05$) after 24 hours and persisted till the hypomagnesemic phase in Group III animals (Table 11) was noted. Following treatment, a significant ($P < 0.05$) rise in the rectal temperature was recorded (Fig. 9). However, the decrease in the rectal temperature in Group IV (Table 19) animals remained statistically insignificant throughout the course of study (Fig. 9).

Table 13 : Biochemical analysis of serum, haematological changes and body temperature of hypomagnesemia-induced (Group III) and treated buffalo calves (Mean ± SEM)

Parameters	Pathophysiological study (time in hr)					Treatment study (time in hr)		
	0	24	48	72	96	24	48	72
Magnesium (mg/dl)	2.07 ±0.09	1.79* ±0.10	1.48* ±0.09	1.17* ±0.09	0.95* ±0.03	2.08** ±0.02	2.27** ±0.06	1.94** ±0.07
Calcium (mg/dl)	9.88 ±0.30	9.36 ±0.16	9.52 ±0.55	9.36 ±0.32	8.57 ±0.73	8.73 ±0.69	9.36 ±0.16	9.83 ±0.16
Phosphorus (mg/dl)	6.64 ±0.15	8.25* ±0.27	5.69* ±0.22	4.78* ±0.32	4.35* ±0.28	5.32** ±0.12	6.65** ±0.54	6.65** ±0.19
Sodium (mEq/L)	126.38 ±2.43	127.68 ±2.12	129.33 ±2.50	130.35 ±3.07	131.76 ±3.85	127.86 ±2.30	130.67 ±2.74	130.74 ±3.68
Potassium (mEq/L)	5.48 ±0.19	6.74 ±0.69	7.09* ±0.54	7.27* ±0.93	9.41* ±0.46	7.01** ±0.32	6.03** ±0.47	7.63** ±1.69
PCV (%)	24.00 ±1.15	27.67 ±0.88	31.00* ±1.53	33.67* ±1.86	34.33* ±0.58	27.30** ±2.40	26.33** ±3.84	25.67** ±2.19
Haemoglobin (g/dl)	10.20 ±0.16	10.63 ±0.45	11.33 ±0.60	11.90* ±0.67	13.80* ±1.09	12.36** ±0.82	11.30** ±0.62	10.60** ±0.44
Rectal Temp. (°F)	101.00 ±0.20	98.01* ±0.58	98.20* ±0.59	98.70* ±0.94	98.80* ±0.77	100.50** ±0.58	101.20** ±0.58	100.53** ±0.24

* Significantly different from base value ** Significant different from the value just before the treatment

Table 14 : Biochemical analysis of rumen fluid of hypomagnesemia-induced (GroupIII) and treated buffalo calves (Mean ± SEM)

Parameters	Pathophysiological study (Time in hr)					Treatment study (time in hr)		
	0	24	48	72	96	24	48	72
pH	6.83 ±0.17	6.23* ±0.17	6.33* ±0.17	5.67* ±0.17	5.00* ±0.29	6.16** ±0.17	6.67** ±0.17	6.70** ±0.20
Magnesium (mg/dl)	6.93 ±0.45	6.13 ±0.50	5.43* ±0.49	4.93* ±0.19	4.47* ±0.22	4.93 ±0.15	5.87** ±0.64	5.23** ±0.64
Calcium (mg/dl)	13.65 ±2.14	13.74 ±2.70	11.27 ±1.50	9.98 ±2.30	9.73 ±1.21	12.73 ±1.78	12.14 ±3.03	13.65 ±1.41
Phosphorus (mg/dl)	14.57 ±0.40	11.99* ±0.26	10.71* ±0.20	8.81* ±0.21	8.33* ±0.10	10.70** ±1.28	9.62 ±0.44	10.42** ±0.54
Sodium (mEq/L)	135.37 ±2.16	123.25 ±5.16	126.29 ±5.52	123.26 ±4.26	123.99 ±5.86	133.22 ±4.58	127.68 ±5.71	130.62 ±5.58
Potassium (mEq/L)	38.20 ±3.21	103.73* ±4.00	172.24* ±4.55	237.08* ±7.24	265.84* ±13.30	220.01** ±20.37	198.57** ±28.57	163.99** ±11.81

* Significantly different from base value; ** Significantly different from the value just before the treatment

Table 15 : Biochemical analysis of cerebrospinal fluid of hypomagnesemia-induced and treated buffalo calves of Group III (Mean ± SEM)

Parameters	Pathophysiological study (time in hr)			Treatment study (time in hr)	
	0	48	96	48	72
Magnesium (mg/dl)	1.97 ±0.07	1.69* ±0.05	1.57* ±0.02	1.84** ±0.14	1.95** ±0.05
Calcium (mg/dl)	5.08 ±0.57	5.15 ±0.29	4.20 ±0.70	4.29 ±0.48	5.66 ±0.70
Phosphorus (mg/dl)	1.66 ±0.17	2.47* ±0.24	1.33* ±0.35	2.36** ±1.02	2.86** ±0.32
Sodium (mEq/L)	140.19 ±0.06	140.13 ±1.00	138.40 ±2.00	139.55 ±0.58	138.73 ±1.05
Potassium (mEq/L)	3.23 ±0.05	5.41* ±0.63	5.59* ±1.20	3.10** ±0.23	3.11** ±0.37
Total proteins (mg/dl)	47.00 ±4.04	88.60* ±6.12	163.83* ±17.79	45.60** ±13.37	69.33** ±20.18
Pandy's test	(-)	(++)	(++++)	(-)	(+)

* Significantly different from base value; ** Significantly different from the value just before the treatment

Table 16 : Biochemical analysis of saliva of hypomagnesemia-induced (Group III) and treated buffalo calves (Mean ± SEM)

Parameters	Pathophysiological study (Time in hr)					Treatment study (Time in hr)		
	0	24	48	72	96	24	48	72
Magnesium (mg/dl)	1.55 ±0.03	1.46 ±0.06	1.38 ±0.08	1.24* ±0.13	1.07* ±0.15	1.30** ±0.08	1.34** ±0.03	1.39** ±0.04
Calcium (mg/dl)	3.31 ±0.45	1.96 ±0.49	3.97 ±0.88	4.44 ±0.64	4.78 ±0.73	4.28 ±0.82	4.44 ±0.57	4.12 ±0.63
Phosphorus (mg/dl)	30.67 ±0.32	43.27* ±1.64	30.57 ±0.60	9.90* ±0.95	9.62* ±0.73	30.02** ±1.02	31.50** ±1.50	37.24** ±0.78
Sodium (mEq/L)	144.17 ±4.93	144.53 ±3.21	136.09 ±2.31	137.82 ±2.51	140.13 ±6.24	130.82 ±11.92	131.07 ±11.20	130.92 ±9.21
Potassium (mEq/L)	7.89 ±0.85	14.28* ±1.00	33.61* ±3.98	31.10* ±2.15	35.72* ±3.85	32.98 ±0.71	28.92 ±3.60	23.46** ±2.20

* Significantly different from base value; ** Significantly different from the value just before the treatment

Table 17 : Biochemical analysis of urine of hypomagnesemia-induced (Group III) and treated buffalo calves (Mean ± SEM)

Parameters	Pathophysiological study (Time in hr)					Treatment study (time in hr)		
	0	24	48	72	96	24	48	72
pH	9.17 ±0.73	8.50* ±0.58	8.33* ±0.17	7.50* ±0.00	7.33* ±0.17	8.50** ±0.29	8.67** ±0.17	8.67** ±0.17
Sodium (mEq/L)	48.67 ±2.99	43.42 ±3.71	35.43 ±4.96	28.29 ±5.18	24.34 ±4.31	77.27 ±25.90	83.04 ±33.11	42.89 ±9.24
Potassium (mEq/L)	436.11 ±49.74	923.42* ±67.61	1007.21* ±19.21	1253.32* ±107.77	1190.45* ±38.01	880.33** ±40.48	776.57** ±53.82	658.26** ±38.62

* Significantly different from the base value; ** Significantly different from the value just before the treatment

Table 18 : Various components of ECG of hypomagnesemia-induced and treated buffalo calves of Group III (Mean ± SEM)

Parameters	Pathophysiological study (Time in hr)					Treatment study (Time in hr)		
	0	24	48	72	96	24	48	72
P Wave								
D	0.047 ±0.007	0.073 ±0.007	0.053 ±0.007	0.087 ±0.018	0.060 ±0.012	0.047 ±0.007	0.040 ±0.000	0.053 ±0.013
A	0.093 ±0.007	0.100 ±0.000	0.080 ±0.017	0.130* ±0.017	0.060* ±0.021	0.093 ±0.007	0.093 ±0.007	0.050** ±0.000
Mean QRS								
D	0.060 ±0.005	0.060 ±0.005	0.060 ±0.005	0.056 ±0.005	0.053 ±0.003	0.050 ±0.005	0.053 ±0.003	0.050 ±0.005
A	-0.827 ±0.373	-1.150 ±0.161	-0.900 ±0.225	-1.100 ±0.058	-0.817 ±0.235	-0.750 ±0.202	-0.583 ±0.148	-0.867 ±0.017
T Wave								
D	0.067 ±0.013	0.067 ±0.013	0.047 ±0.007	0.067 ±0.013	0.087 ±0.018	0.080 ±0.000	0.073 ±0.018	0.053 ±0.013
A	-0.100 ±0.100	0.067 ±0.088	0.020 ±0.040	0.267 ±0.088	0.183 ±0.188	0.133 ±0.067	0.133 ±0.067	-0.067 ±0.033
PR Int. (sec)	0.190 ±0.007	0.187 ±0.035	0.193 ±0.007	0.173 ±0.018	0.127 ±0.064	0.187 ±0.007	0.173 ±0.018	0.173 ±0.013
QT Int. (sec)	0.300 ±0.010	0.366 ±0.059	0.293 ±0.024	0.273 ±0.007	0.287 ±0.024	0.280 ±0.000	0.293 ±0.027	0.313 ±0.024
PR seg (sec)	0.147 ±0.007	0.113 ±0.029	0.140 ±0.012	0.087 ±0.018	0.133 ±0.060	0.014 ±0.012	0.133 ±0.048	0.126 ±0.007
ST seg (sec)	0.170 ±0.020	0.240 ±0.050	0.190 ±0.020	0.150 ±0.010	0.147 ±0.045	0.150 ±0.050	0.190 ±0.030	0.200 ±0.020
HR (per min)	66.730 ±1.630	71.100 ±6.680	72.770 ±2.330	80.370* ±1.370	81.100* ±2.070	77.700 ±2.700	81.230 ±6.230	61.100** ±5.610

D = Duration in seconds; A = Amplitude in mv; Int. = Interval; Seg. = segment
* Significantly different from base value ; ** Significantly different from the value just before the treatment

Table 19 : Biochemical analysis of serum, haematological changes and body temperature of hypomagnesemia-induced buffalo calves (Group IV) treated with alternative method (Mean ± SEM)

Parameters	Pathophysiological study (Time in hr)					Treatment study (Time in hr)		
	0	24	48	72	96	24	48	72
Magnesium (mg/dl)	2.06 ±0.04	1.95 ±0.10	1.60* ±0.09	1.25* ±0.04	0.99* ±0.05	1.31** ±0.04	1.34** ±0.09	1.43** ±0.10
Calcium (mg/dl)	8.89 ± 0.85	7.93 ±0.79	8.16 ±0.44	9.84 ±1.27	8.05 ±0.76	7.31 ±1.77	9.84 ±1.14	9.76 ±1.19
Phosphorus (mg/dl)	6.62 ±0.27	8.70* ±0.35	5.62* ±0.26	4.81* ±0.33	4.23* ±0.39	4.69 ±0.30	4.79 ±0.47	6.00** ±0.62
Sodium (mEq/L)	132.19 ±5.55	131.37 ±2.14	126.45 ±1.71	127.70 ±2.20	130.83 ±5.21	124.56 ±6.19	130.25 ±5.20	128.49 ±2.01
Potassium (mEq/L)	5.20 ±0.44	7.48* ±0.88	7.31* ±0.43	8.17* ±0.56	9.01* ±0.16	7.12** ±0.59	7.07** ±0.87	6.50** ±0.90
PCV (%)	26.00 ±2.31	31.67* ±2.73	32.67* ±1.76	33.33* ±1.33	35.67* ±0.88	32.00 ±0.57	30.33** ±0.88	2[illegible].00** ±0.57
Haemoglobin (g/dl)	10.67 ±0.17	10.83 ±0.33	11.17 ±0.17	11.83* ±0.17	12.83* ±0.17	10.20** ±0.42	11.00** ±1.04	10.33** ±0.17
Rectal Temp. (°F)	100.53 ±0.35	97.93 ±0.93	98.06 ±0.93	97.90 ±0.86	97.90 ±0.86	98.90 ±0.48	99.20 ±1.66	99.40 ±0.81

* Significantly different from the base value; ** Significantly different from the value just before treatment

Table 20 : Biochemical analysis of rumen fluid of hypomagnesemia-induced buffalo calves (Group IV) treated with alternative method (Mean ± SEM)

Parameters	Pathophysiological study (Time in hr.)					Treatment study (Time in hr)		
	0	24	48	72	96	24	48	72
pH	6.75 ±0.15	6.25* ±0.15	6.00* ±0.20	5.63* ±0.13	5.00* ±0.20	5.63** ±0.13	5.86** ±0.13	6.25** ±0.15
Magnesium (mg/dl)	7.83 ±0.81	6.93 ±0.71	6.05* ±0.64	5.35* ±0.58	4.53* ±0.48	5.30 ±0.58	5.00 ±0.82	5.53 ±1.09
Calcium (mg/dl)	14.03 ±1.20	12.30 ±2.23	14.38 ±1.68	11.52 ±1.65	10.48 ±1.18	12.55 ±2.11	11.27 ±1.38	10.22 ±1.84
Phosphorus (mg/dl)	14.37 ±0.58	12.22 ±0.44	10.75 ±0.23	8.73* ±0.21	8.05* ±0.13	9.00 ±0.90	9.07 ±0.55	10.07 ±0.35
Sodium (mEq/L)	128.42 ±0.68	112.18 ±5.20	110.68 ±6.75	107.08 ±6.86	104.08 ±8.03	113.51 ±8.52	117.09 ±6.66	111.58 ±5.28
Potassium (mEq/L)	36.70 ±3.14	128.07* ±3.08	173.69* ±7.17	242.16* ±4.06	280.29* ±5.02	264.67* ±2.36	253.45** ±3.63	202.44** ±4.33

* Significantly different from the base valu ; ** Significantly different from the value just before the treatment

Table 21 : Biochemical analysis of cerebrospinal fluid of hypomagnesemia-induced buffalo calves (Group IV) treated with alternative method (Mean ± SEM)

Parameters	Pathophysiological study (time in hr)			Treatment study (Time in hr)	
	0	48	72	48	72
Magnesium (mg/dl)	2.12 ±0.04	1.83* ±0.06	1.61* ±0.02	1.70 ±0.02	1.80** ±0.05
Calcium (mg/dl)	5.21 ±0.50	4.83 ±0.44	4.28 ±0.27	5.00 ±0.14	4.28 ±0.83
Phosphorus (mg/dl)	1.63 ±0.07	2.33* ±0.17	1.12* ±0.12	1.67** ±0.38	2.27** ±0.07
Sodium (mEq/L)	133.47 ±2.78	133.70 ±2.78	126.03 ±4.32	129.52 ±4.80	130.39 ±4.71
Potassium (mEq/L)	3.87 ±0.22	6.03* ±0.36	7.56* ±0.72	3.43** ±0.58	5.12** ±0.34
Total proteins (mg/dl)	40.66 ±4.70	106.33* ±14.62	180.83* ±3.63	63.50** ±18.50	94.50** ±10.50
Pandy's test	(-)	(+++)	(++++)	(+)	(++)

* Significantly different from the base value; ** Significantly different from the value just befor the treatment

Table 22 : Biochemical analysis of saliva of hypomagnesemia-induced buffaloes calves treated with alternative method of Group IV (Mean ± SEM)

Parameters	Pathophysiological study (Time inhr)					Treatment study (Time in hr)		
	0	24	48	72	96	24	48	72
Magnesium (mg/dl)	1.52 ±0.06	1.45 ±0.08	1.37* ±0.06	1.29* ±0.06	1.22* ±0.04	1.26 ±0.04	1.28 ±0.01	1.30 ±0.04
Calcium (mg/dl)	2.50 ±0.58	4.60 ±0.16	4.70 ±0.92	4.92 ±0.88	4.32 ±0.51	4.37 ±0.53	4.20 ±0.50	3.67 ±0.32
Phosphorus (mg/dl)	31.47 ±0.65	45.11* ±0.93	28.79 ±1.30	10.42* ±0.42	9.68* ±0.56	18.48** ±1.44	22.03** ±1.97	31.20** ±1.50
Sodium (mEq/L)	137.25 ±2.88	136.34 ±3.28	135.34 ±3.95	132.46 ±3.18	131.51 ±2.71	129.40 ±4.93	127.85 ±5.46	127.44 ±4.53
Potassium (mEq/L)	7.41 ±0.86	23.09* ±1.80	32.70* ±1.90	35.26* ±4.62	38.00* ±3.88	32.17 ±3.20	31.35 ±3.13	30.32** ±2.90

* Significantly different from the base value; ** Significantly different from the value just before the treatment

Table 23 : Biochemical analysis of urine of hypomagnesemia-induced buffalo calves treated with alternative method of Group IV (Mean ± SEM)

Parameters	Pathophysiological study (Time in hr)					Treatment study (Time in hr)		
	0	24	48	72	96	24	48	72
pH	8.83 ±0.17	8.00* ±0.29	8.00* ±0.29	7.50* ±0.29	7.16* ±0.17	8.00** ±0.29	8.33** ±0.17	8.53** ±0.17
Sodium (mEq/L)	48.03 ±5.90	38.11 ±5.19	30.59* ±5.74	28.37* ±4.17	22.23* ±3.68	30.08 ±5.43	24.11 ±7.35	26.12 ±9.66
Potassium (mEq/L)	420.00 ±30.55	1013.49* ±30.79	1064.19* ±92.54	1126.62* ±154.15	1143.51* ±138.75	1066.66 ±40.74	942.22 ±77.49	757.67** ±43.19

* Significantly different from the base value; ** Significantly different from value just before the treatment

Table 24 : Various components of ECG of hypomagnesemia-induced buffalo calves (Group IV) treated with alternative method

Parameters	Pathophysiological study (Time in hr)					Treatment study (Time in hr)		
	0	24	48	72	96	24	48	72
P Wave								
D	0.040 ±0.000	0.060 ±0.008	0.060 ±0.012	0.047 ±0.007	0.045 ±0.005	0.050 ±0.006	0.047 ±0.007	0.050 ±0.006
A	0.115 ±0.015	0.100 ±0.020	0.087 ±0.032	0.100 ±0.000	0.125 ±0.025	0.125 ±0.014	0.117 ±0.017	0.125 ±0.014
Mean QRS								
D	0.060 ±0.008	0.055 ±0.006	0.067 ±0.007	0.053 ±0.003	0.045 ±0.015	0.053 ±0.010	0.053 ±0.009	0.053 ±0.008
A	-0.663 ±0.206	-0.763 ±0.177	-0.838 ±0.175	-0.800 ±0.225	-0.725 ±0.149	-0.838 ±0.160	-0.988 ±0.171	-0.963 ±0.129
T Wave								
D	0.080 ±0.008	0.070 ±0.013	0.080 ±0.000	0.060 ±0.012	0.045 ±0.005	0.060 ±0.008	0.053 ±0.013	0.055 ±0.010
A	0.175 ±0.063	0.063 ±0.165	0.267 ±0.069	0.133 ±0.169	0.000 ±0.196	0.238 ±0.055	0.150 ±0.104	0.088 ±0.097
PR Int. (sec)	0.160 ±0.005	0.160 ±0.014	0.160 ±0.000	0.147 ±0.013	0.160 ±0.000	0.160 ±0.000	0.160 ±0.012	0.140 ±0.012
QT Int. (sec.)	0.315 ±0.033	0.330 ±0.044	0.293 ±0.035	0.267 ±0.013	0.250 ±0.025	0.260 ±0.012	0.280 ±0.023	0.260 ±0.012
PR seg (sec)	0.125 ±0.017	0.100 ±0.013	0.127 ±0.017	0.107 ±0.007	0.125 ±0.013	0.125 ±0.013	0.133 ±0.024	0.105 ±0.019
ST seg. (sec)	0.170 ±0.030	0.199 ±0.056	0.127 ±0.037	0.123 ±0.022	0.143 ±0.028	0.169 ±0.043	0.157 ±0.043	0.135 ±0.012
HR (per min)	66.570 ±6.140	77.500 ±12.27	76.870 ±6.700	88.750 ±6.960	86.240 ±3.980	84.700 ±3.720	82.170 ±3.850	86.920 ±1.210

D = Duration in seconds; A = Amplitude in mv; Int. = Interval; Seg. = segment

Fig. 11 : Periodic alterations in the levels of Mg^{++} in serum, rumen fluid, CSF and saliva of buffalo calves treated with 10% $MgSO_4$ and calborol intravenously.

Control value : Zero hour observation
Pathophysiological study : 24-96 hr
Treatment study : 120-168 hr

GROUP III

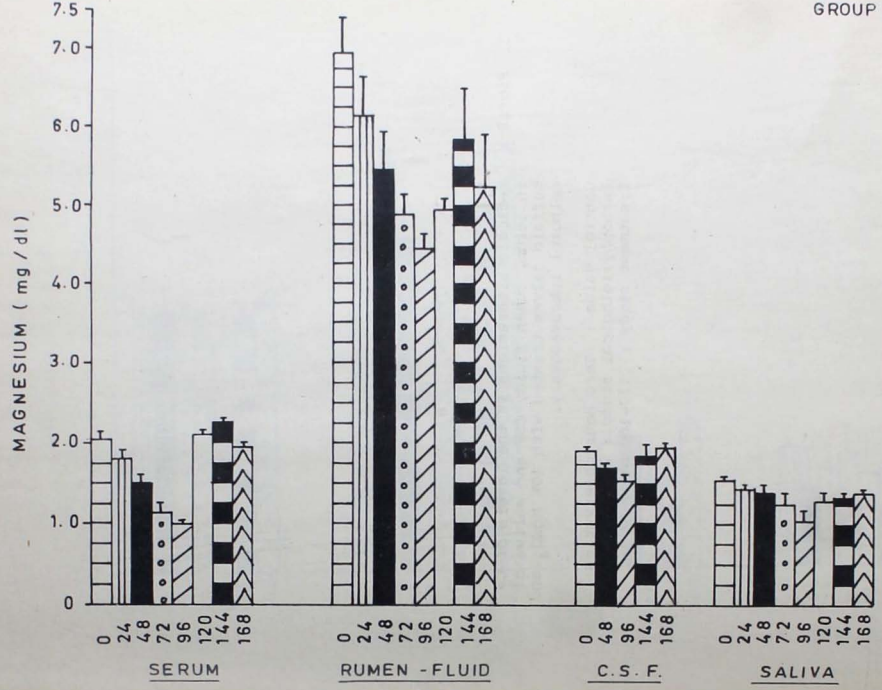

MAGNESIUM (mg / dl)
7.5
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
0 24 48 72 96 120 144 168
SERUM
0 24 48 72 96 120 144 168
RUMEN - FLUID
0 48 96 144 168
C.S.F.
0 24 48 72 96 120 144 168
SALIVA
TIME (hr)

Fig 12 : Periodic alterations in the levels of Ca^{++} in serum, rumen fluid, CSF and saliva of buffalo calves treated with 10% $MgSO_4$ and calborol intravenously.

Control value : Zero hour observation
Pathophysiological study : 24-96 hr
Treatment study : 120-168 hr

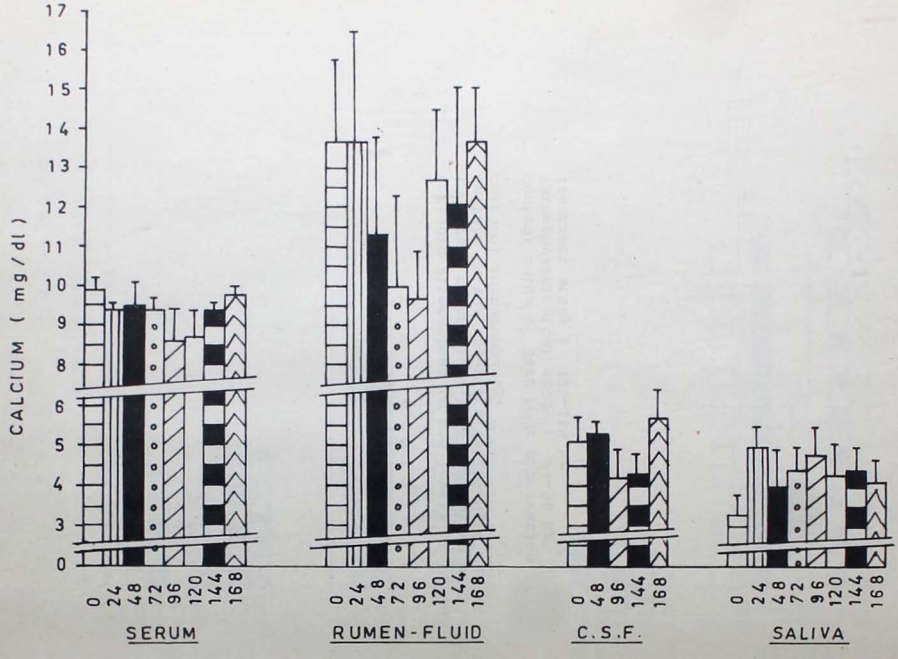

CALCIUM (mg / dl)
17
16
15
14
13
12
11
10
9
8
6
5
4
3
0
0 24 48 72 96 120 144 168
SERUM
0 24 48 72 96 120 144 168
RUMEN-FLUID
0 48 96 144 168
C.S.F.
0 24 48 72 96 120 144 168
SALIVA
TIME (hr)

Fig 13 : Periodic alterations in the levels of Pi in serum, rumen fluid, CSF and saliva of buffalo calves treated with 10% $MgSO_4$ and calborol intravenously.

Control value : Zero hour observation
Pathophysiological study : 24-96 hr
Treatment study : 120-168 hr

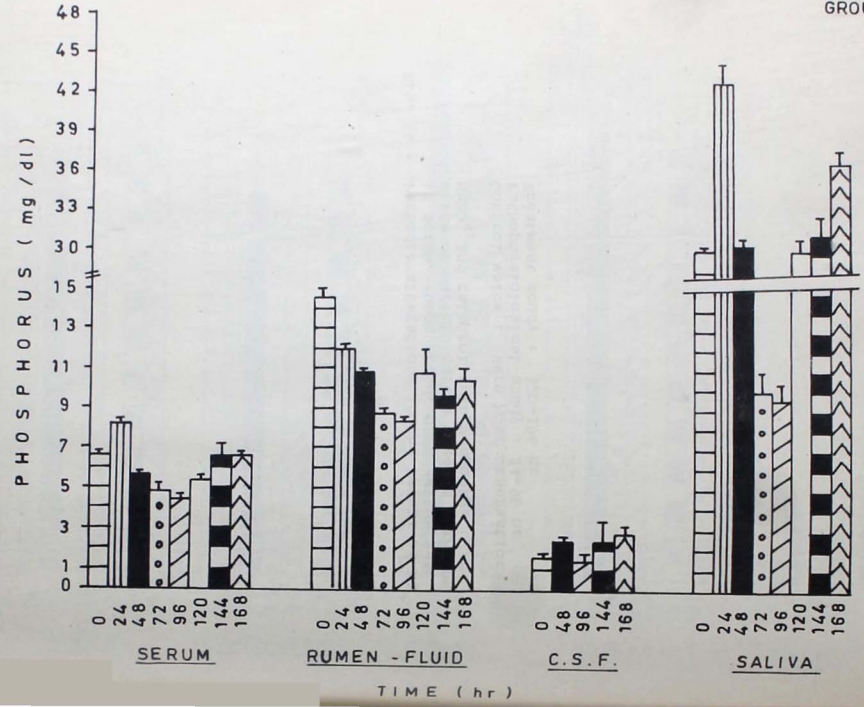

GROUP III
PHOSPHORUS (mg / dl)
48
45
42
39
36
33
30
15
13
11
9
7
5
3
1
0
0 24 48 72 96 120 144 168
SERUM
0 24 48 72 96 120 144 168
RUMEN - FLUID
0 48 96 144 168
C.S.F.
0 24 48 72 96 120 144 168
SALIVA
TIME (hr)

Fig. 14 : Periodic alterations in the levels of Na^+ in serum, rumen fluid, CSF, saliva and urine of buffalo calves treated with 10% $MgSO_4$ and calborol intravenously.

Control value : Zero hour observation.
Pathophysiological study : 24-96 hr
Treatment study : 120-196 hr

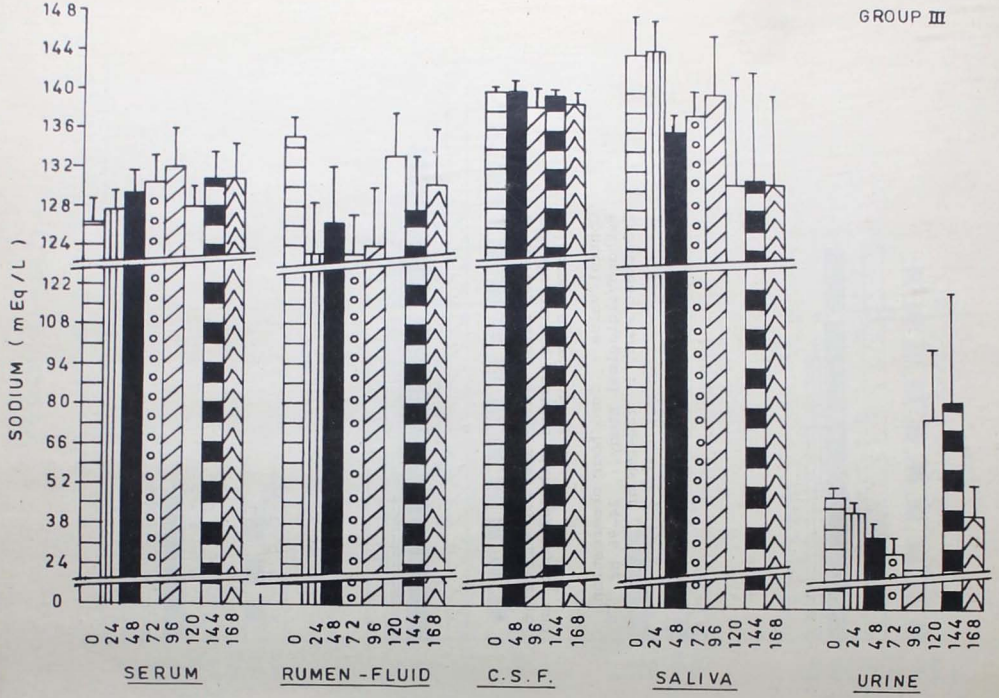

GROUP III
SODIUM (mEq/L)
148
144
140
136
132
128
124
122
108
94
80
66
52
38
24
0
0 24 48 72 96 120 144 168
SERUM
0 24 48 72 96 120 144 168
RUMEN-FLUID
0 48 96 144 168
C.S.F.
0 24 48 72 96 120 144 168
SALIVA
0 24 48 72 96 120 144 168
URINE
TIME (hr)

Fig. 15 : Periodic alterations in the levels of K^+ in serum, rumen fluid, CSF and saliva of buffalo calves treated with 10% $MgSO_4$ and calborol intravenously.

Control value : Zero hour observation
Pathophysiological study : 24-96 hr
Treatment study : 120-168 hr

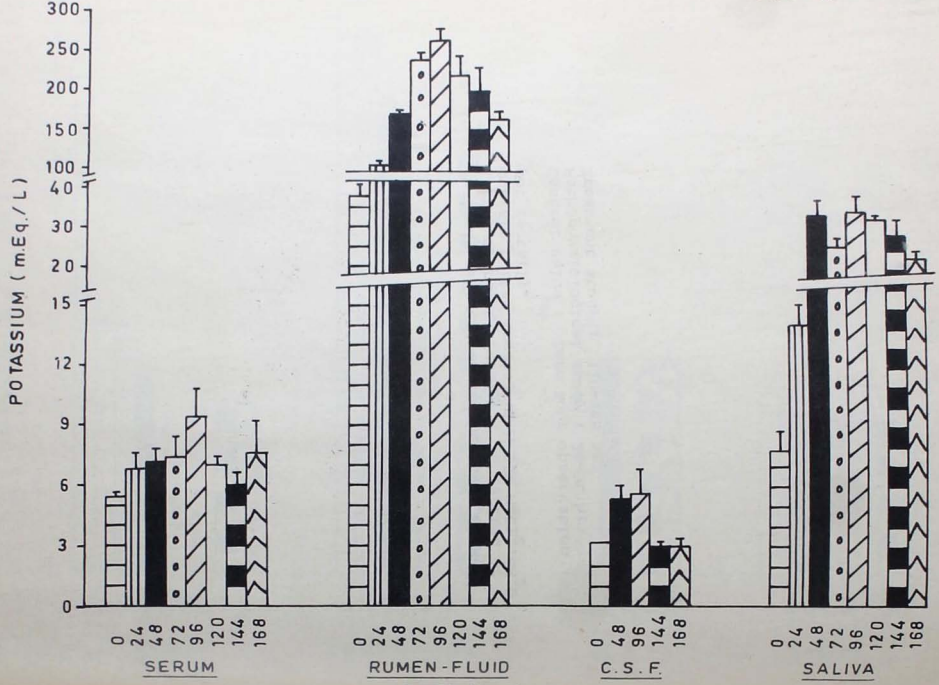

GROUP III
POTASSIUM (m.Eq./L)
300
250
200
150
100
40
30
20
15
12
9
6
3
0
0 24 48 72 96 120 144 168
SERUM
0 24 48 72 96 120 144 168
RUMEN-FLUID
0 48 96 144 168
C.S.F.
0 24 48 72 96 120 144 168
SALIVA
TIME (hr)

Fig. 16 : Periodic alterations in the level of Mg^{++} in serum, rumen fluid, CSF and saliva of buffalo calves treated with 30% $MgCl_2.6H_2O$ per rectally.

Control value : Zero hour observation
Pathophysiological study : 24-96 hr
Treatment study : 120-168 hr

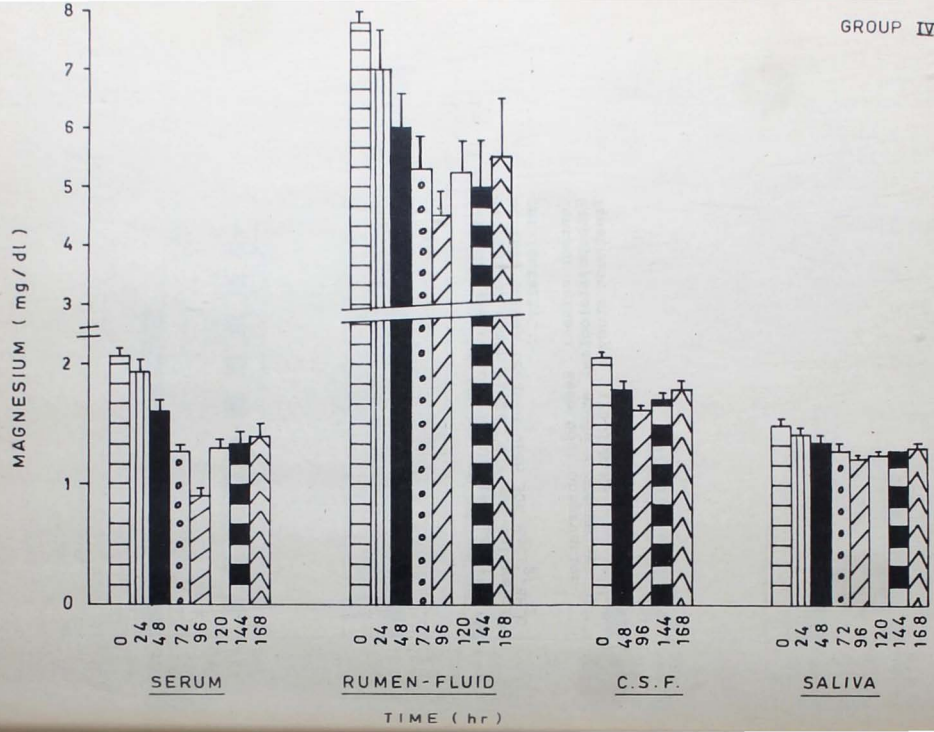

GROUP IV
MAGNESIUM (mg / dl)
8
7
6
5
4
3
2
1
0
0 24 48 72 96 120 144 168
SERUM
0 24 48 72 96 120 144 168
RUMEN-FLUID
0 48 96 144 168
C.S.F.
0 24 48 72 96 120 144 168
SALIVA
TIME (hr)

Fig. 17 : Periodic alterations in the levels of Ca^{++} in plasma, rumen fluid, CSF and saliva of buffalo calves treated with 30% $MgCl_2 \cdot 6H_2O$ per rectally.

Control value : Zero hour observation
Pathophysiological study : 24-96 hr
Treatment study : 120-168 hr

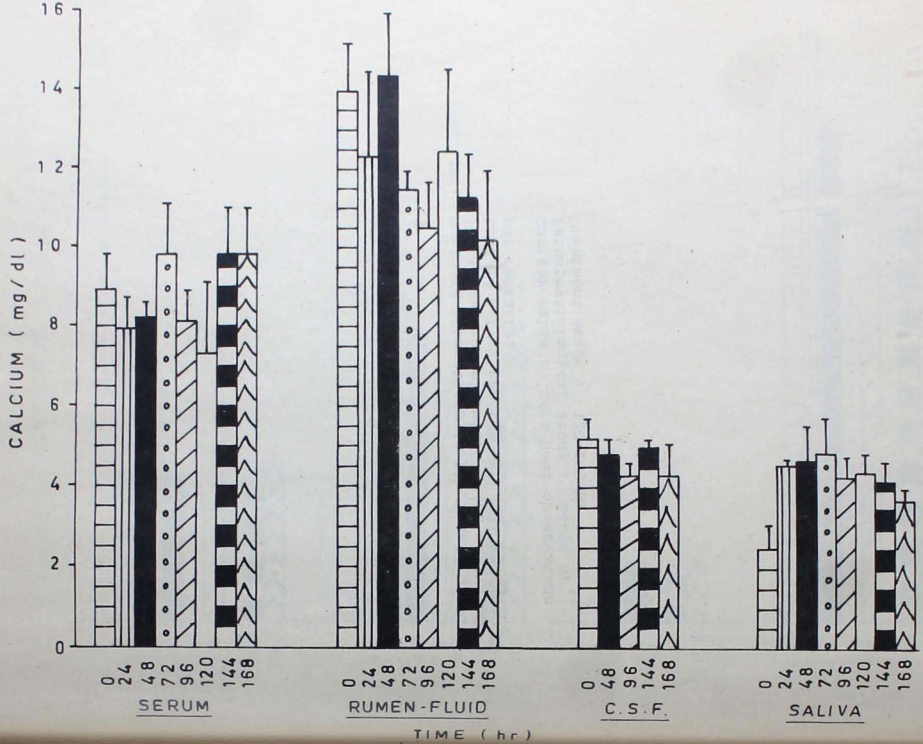

CALCIUM (mg/dl)
16
14
12
10
8
6
4
2
0
0 24 48 72 96 120 144 168
SERUM
0 24 48 72 96 120 144 168
RUMEN-FLUID
0 48 96 144 168
C.S.F.
0 24 48 72 96 120 144 168
SALIVA
TIME (hr)

Fig. 18 : Periodic alterations in the levels of Pi in plasma, rumen fluid, CSF and saliva of buffalo calves treated with 30% $MgCl_2.6H_2O$ per rectally.

Control value : Zero hour observation
Pathophysiological study : 24-96 hr
Treatment study : 120-168 hr

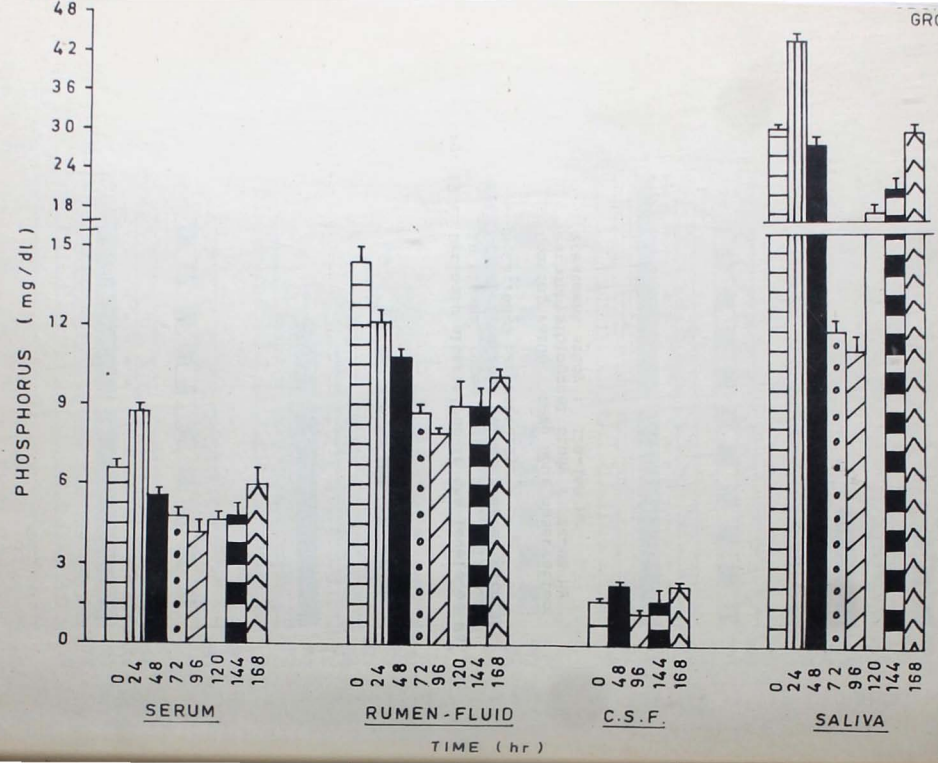

PHOSPHORUS (mg/dl)
48
42
36
30
24
18
15
12
9
6
3
0
0 24 48 72 96 120 144 168
SERUM
0 24 48 72 96 120 144 168
RUMEN-FLUID
0 48 96 144 168
C.S.F.
0 24 48 72 96 120 144 168
SALIVA
TIME (hr)

Fig. 19 : Periodic alterations in the levels of Na^+ in plasma, rumen fluid, CSF, saliva and urine of buffalo calves treated with 30% $MgCl_2.6H_2O$ per rectally.

Control value : Zero hour observation
Pathophysiological study : 24-96 hr
Treatment study : 120-168 hr

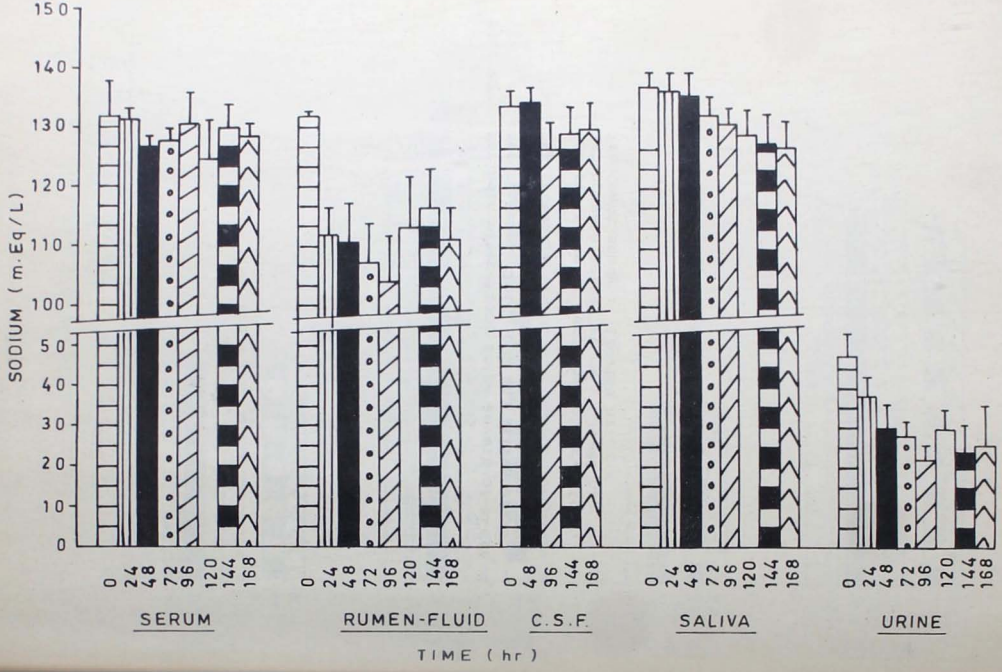

SODIUM (m.Eq/L)
150
140
130
120
110
100
50
40
30
20
10
0
0 24 48 72 96 120 144 168
SERUM
0 24 48 72 96 120 144 168
RUMEN-FLUID
0 48 96 144 168
C.S.F
0 24 48 72 96 120 144 168
SALIVA
0 24 48 72 96 120 144 168
URINE
TIME (hr)

Fig. 20 : Periodic alterations inthe levels of K^+ in serum, rumen fluid, CSF and saliva of buffalo calves treated with 30% $MgCl_2.6H_2O$ per rectally.

Control value : Zero hour observation
Pathophysiological study : 24-96 hr
Treatment study : 120-168 hr

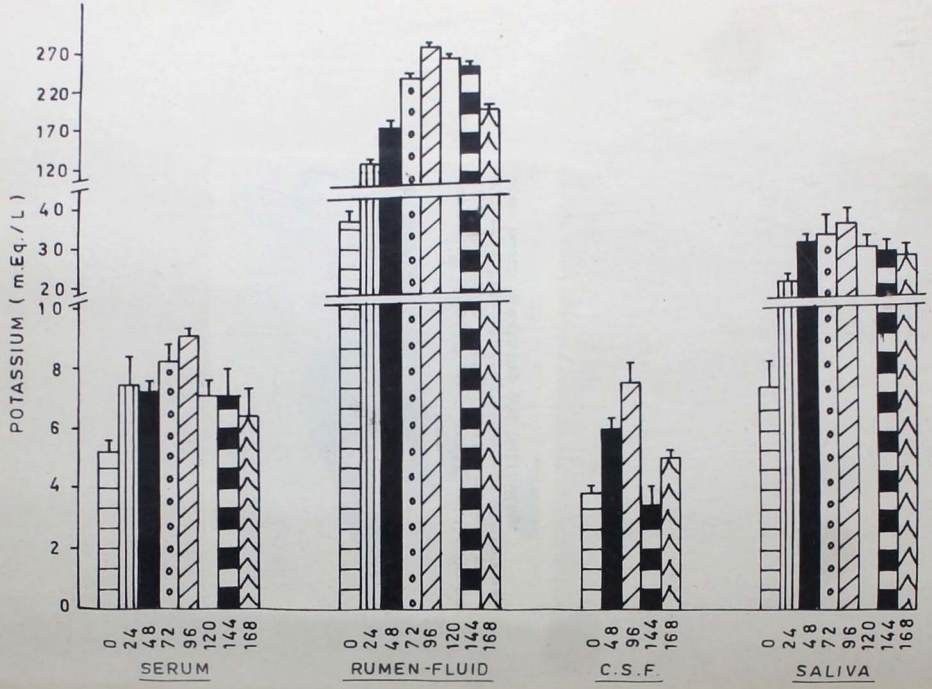

POTASSIUM (m.Eq./L)
270
220
170
120
40
30
20
10
8
6
4
2
0
0 24 48 72 96 120 144 168
SERUM
0 24 48 72 96 120 144 168
RUMEN-FLUID
0 48 96 144 168
C.S.F.
0 24 48 72 96 120 144 168
SALIVA
TIME (hr)

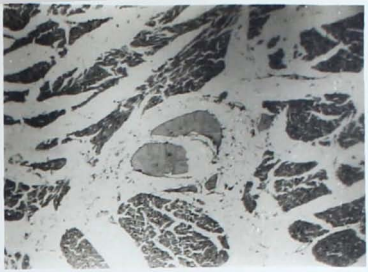
Fig. 21 : Potassium chloride and citric acid induced hypomagnesemic buffalo calf manifesting symptoms like staring eyes, opisthotonous, open mouth breathing and bellowing.

Fig. 22 : Section of heart showing degeneration of Purkinje fibres, HE x 70

Fig. 23 : Higher magnification of Fig. 22, showing Purkinje fibres without nuclei, HE x 300

Fig. 24 : Section of heart, showing fatty changes, HE x 300

22



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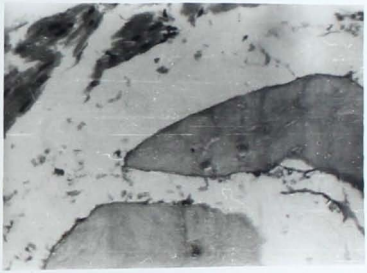

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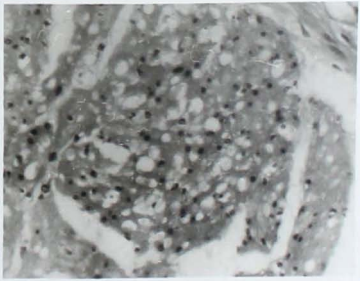
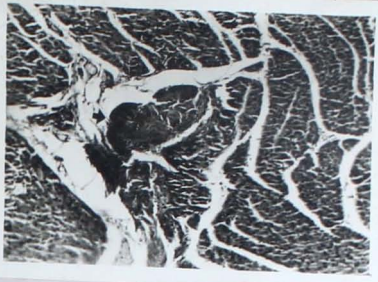


Fig. 25 : Section of heart of a hypomagnesemic calf showing focal areas of necrosis (arrow) in the cardiac muscle, HE x 70

Fig. 26 : Section of heart showing non-purulent epicarditis with infiltration of serous fluid, lymphocytes, plasma cells and macrophages.

Fig. 27 : Section of skeletal muscle showing hyaline degeneration, HE x 300

25



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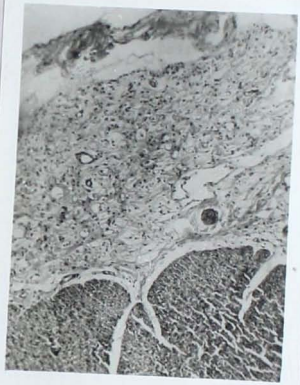

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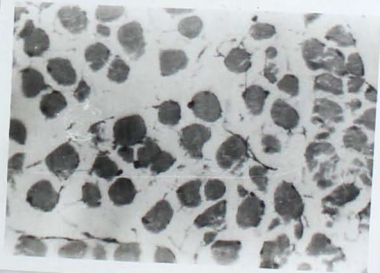


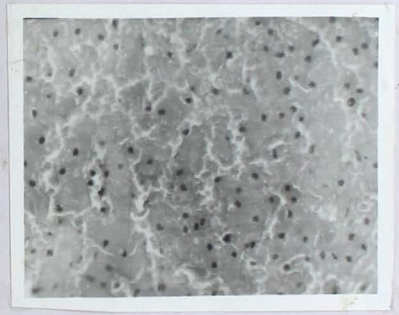
Fig. 28 : Section of intestine showing acute enteritis, HE x 70

Fig. 29 : Section of liver showing cloudy swelling, HE x 300

Fig. 30 : Section of liver showing mild fatty changes and intracytoplasmic inclusion bodies in the hepatocytes, HE x oil

28

29



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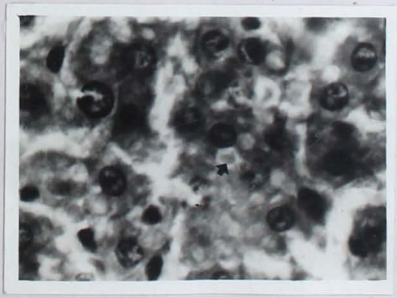
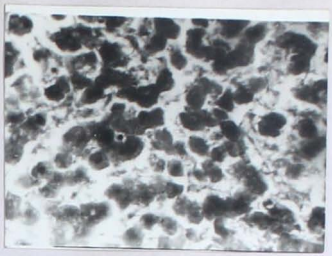


Fig. 31 : Section of liver showing diffuse coagulative necrosis, HE x 300

Fig. 32 : Section of kidney showing cloudy swelling in the tubular epithelium, HE x 300

Fig. 33 : Section of kidney showing vascular degeneration in the tubular epithelial cells; HE x 300

31



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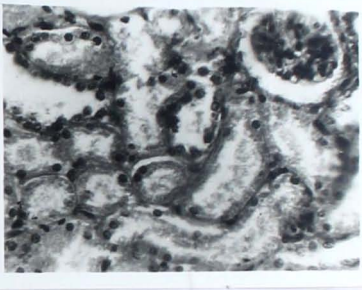

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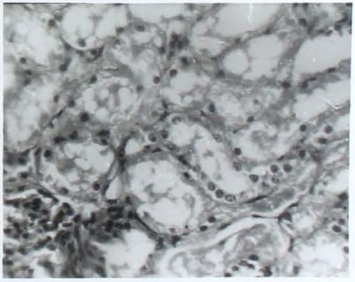

Fig. 34 : Section of kidney showing diffuse coagulative necrosis of tubular epithelial cells. HE x 300

Fig. 35 : Section of lung showing severe congestion in the small blood vessels and the capillaries in interalveolar septa, HE x 300

Fig. 36 : Section of lung showing alveolar emphysema, HE x 70

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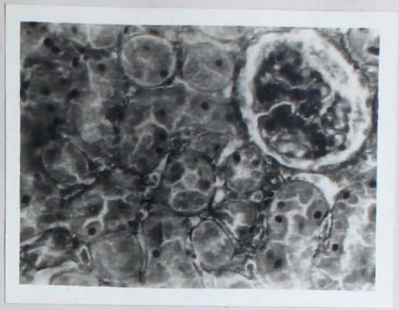

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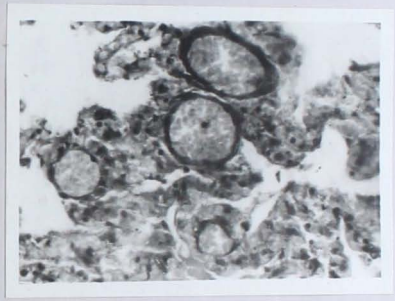

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Fig. 37 : Infiltration of plasma cells in spleen, HE x 300

Fig. 38 : Section of lymph node showing intense congestion, HE x

Fig. 39 : Section of brain showing severe congestion in the meningeal blood vessels, HE x 70

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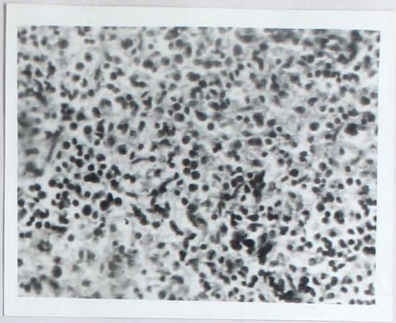

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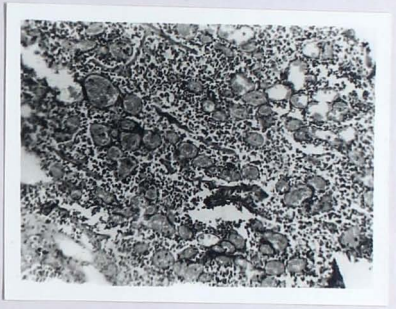

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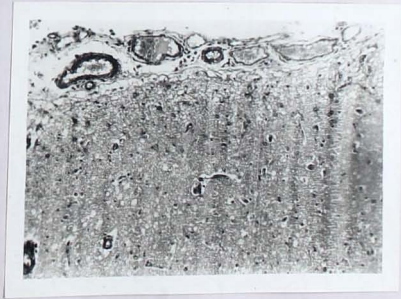
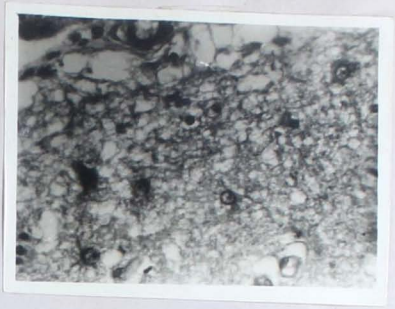


Fig. 40 : Section of brain showing myelin degeneration in brain substance leading to spongiosis, HE x 300

Fig. 41 : Section of brain showing infiltration of hypertrophied microglia cells, HE x 300

Fig. 42 : Section of brain showing satellitosis and neuronophagia, HE x 300

40



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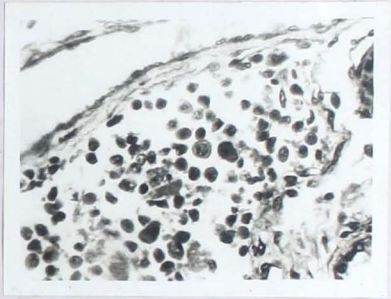

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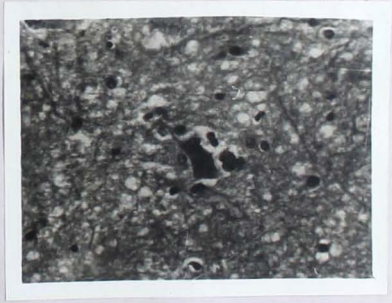

DISCUSSION

Hypomagnesemia, with or without clinical signs of tetany, has been recognised in ruminants for over fifty years but, in spite of the fact that some measures of control has been achieved, the syndrome is not fully understood as yet. Although the conditions under which hypomagnesemia can develop are fairly well known, the immediate cause is still not certain, and even less certain is the relation between the low concentration of plasma magnesium and the presence or absence of clinical signs.

CONTROL GROUP (Group I)

The concentration of various electrolytes which were recorded in the serum in the present study and served as control are in accord with those of Choudhuri et al. (1980), carried out in 2 years old healthy buffalo calves. Studies on PCV of healthy buffalo calves are in agreement with the earlier reports (Nauriyal, 1975 and Sethuraman (1976) in buffalo calves.

A wide variability in the pH (5.7 to 7.6) of rumen fluid has been reported in cattle and buffaloes maintained on different feed and fodder (Mishra and Singh, 1974; Nauriyal, 1975 and Sethuraman, 1976). Normal pH value observed in the present study was 6.8. The values of pH of rumen fluid, magnesium, calcium, phosphorus, sodium and potassium observed in the present study are in

accordance with those reported by Choudhari et al. (1980) in buffalo calves, Randhawa et al. (1980) in crossbred calves and Sinha (1980) in buffalo calves.

In CSF, the concentration of sodium, potassium and magnesium as observed in the present study are similar to those reported by Seoane (1981) observed in sheep, the lone exception being the level of calcium which was more in buffalo calves, the subjects of present study than in sheep. The values for total proteins and calcium are similar to those reported by Scholz (1972) in sheep Lal and Verma (1979) in buffalo calves and Vihan (1981) in sheep. It has been reported that the normal concentration of calcium in CSF is much lower than that in plasma. The principal reason for this has been attributed to the greater degree of protein binding of calcium that occurs in plasma dialyzate and CSF. This discrepancy, however, is not very large, but at the same time the concentration of ionized calcium in plasma has been reported to be greater than that of CSF (Kaneko, 1980). The potassium concentration in the CSF is considerably less than that of plasma dialyzate (Kaneko, 1980). Sodium concentrations which is slightly greater in CSF than in plasma suggests the presence of an active secretory mechanism for the substance in brain (Kaneko, 1980). The comparative concentrations of these electrolytes observed in the present study confirm the observations reported by Bauda et al. (1973).

The electrolyte concentrations of sodium and potassium in saliva are comparable to those reported by Lothamer (1973) and Randhawa et al. (1980) in crossbred calves while electrolyte concentrations of magnesium and phosphorus are in agreement with those reported by Randhawa et al. (1980) in crossbred cows. Calcium level which was found slightly higher in buffalo calves and may be due to the species variations.

Values of pH, sodium and potassium as reported by Osbaldiston (1969) in urine of cattle are similar to those found in the present study.

Electrocardiographic changes recorded in the present study show variability, but still the various components of ECG are in normal range. R-wave amplitude recorded in the present study is similar but T-wave amplitude is less than that reported by Schafer & Neubert (1977) in cows.

Heart rate and rectal temperature recorded were in normal range.

Induction of hypomagnesemia

Of the various animal models evolved to induce hypomagnesemia (Kariya et al., 1976; Furukawa et al. 1972; Sinha, 1980; Shiga et al., 1981; El-Shrif & Mottelib, 1983 Takahashi et al., 1983; and Terashima et al., 1983), oral administration of potassium chloride and citric acid appears to be more natural and appropriate from clinical point of view. It allows sufficient time for hypomagnesemia

to develop, apparently simulating the clinical situations. In the present investigation, all the animals of untreated Group II died owing to hypomagnesemia as detected by biochemical analysis.

Symptoms and haematological changes associated with hypomagnesemia

Clinical symptoms include inappetence, rumen stasis, diarrohea, dehydration, systemic acidosis, cardiovascular and respiratory failure. The clinical symptoms observed after the experimental induction of hypomagnesemia in buffalo calves, were similar to those described by Murakami et al. (1972) in cows and Luthman et al (1973) in calves.

Daily administration of potassium chloride and citric acid into the rumen, decreased the ruminal fluid pH significantly after 24 hours in all the animals and the low pH continued throughout the course of study in untreated group. It resulted in decreased tone and frequency of rumen movements. Similar findings have been reported by Sinha, (1980) in buffalo calves. Complete ruminal stasis was observed clinically and finally this was accompanied by inappetance. The general condition was also aggravated by the derangement of the normal rumen metabolism. The stasis was followed by diarrhoea, which might be due to the reduction in the net absorption of water from the colon (Randhawa et al., 1980). The normal rumen fluid remains hypotonic to blood and saliva, but with the onset

of acidosis, as in the case of lactic acidosis, the rumen content became hypertonic as compared with blood and saliva. This increase in the osmolality of rumen fluid has been considered responsible for withdrawl of fluid from vascular compartments in the rumen, leading to haemo concentration (Huber, 1971).

Symptoms like sunken eyeballs, reduced skin elasticity, subnormal temperature and comatose conditions were indicative of severe degree of haemoconcentration and dehydration, which may be due to pheripheral circulatory failure.

In the absence of measurement of total plasma proteins, it was not possible to infer whether the haemoconcentration was a result of spleenic concentrations or dehydration. This was marked by significant increase in PCV as reported by Kariya et al. (1976) in sheep and Nethery et al.(1979) in calves and haemoglobin in all the animals.

Polyponea was observed following induction of hypomagnesemia and it may be attributed to the metabolic acid base alterations. Ruminal acidosis is usually followed by metabolic acidosis. Nethery et al. (1979) reported that in severe cases of acidosis, the reserves of plasma bicarbonate were reduced in an attempt to buffer the pH change in the blood and this resulted in steady decline of blood pH. The clinically observable effects of this were related mainly to the respiratory

systems. The increased CO_2 tension of blood and depletion of bicarbonate caused an increase in the depth and rate of respiration (Kaussaul breathing). Respiratory compensation was usually evident, when bicarbonate levels were diminished to 50 per cent of the normal.

Immediately after induction of hypomagnesemia, normal saliva which was clear, colourless, watery and translucent, became viscous and later on thin in consistancy. The increase in the viscosity of saliva might be due to excessive secretion of electrolytes, particularly inorganic phosphorus and increase in the protein content (Randhawa et al., 1980). During hypomagnesemia, the salivary flow rate also decreased, possibly as a result of marked increase in the osmolality of rumen fluid, and partially due to a slight increase in osmolar concentration of plasma (Warner, 1977; Beal et al., 1975). The decrease in the flow rate can also be either due to the reduction in rumen motility which affects the receptors for salivation in mouth or due to the direct inhibition of parasympathetic stimulation (Obara et al., 1972).

A fall in the urine pH was observed. This may be due to increased excretion of acids in the acidotic state. This decrease in the pH of urine has a direct correlation with rumen pH (Choudhuri et al., 1980). The excess hydrogen ions were excreted as such by tubular cells for want of a suitable substrate like bicarbonate or ammonia ions, and resulted in the decreased pH of the

urine (Kaneko, 1980). The increased urinary excretion of acids causes polyurea, also observed by Murakami et al., (1972) in cows, which is an adaptive consequence of increased solute load per nephron or polyurea of renal failure, which became evident in the late stage of hypomagnesemia and was due to a lack of maintenance of medullary osmotic gradient. In the present study histopathological findings suggest degenerative changes in the renal medulla. This polyurea may be sufficiently severe to cause dehydration or accentuate the concomitant dehydration.

Polydipsia was observed in the hypomagnesemic animals. The water deficiency in the body resulting in the diminished effective circulatory volume and altered sympathetic tone was corrected in part by increased water resorption in the kidney and polydipsia.

Reluctance to stand and lameness, as observed in the present study have also been reported by Luthman et al. (1973) and Smyth et al. (1977) in calves and West et al. (1981) in sheep. These symptoms were indicative of laminitis caused by histamine release from damaged tissue (Dirkson, 1970). Symptoms like hanging of head and opisthotonus, as suggested by Mihai (1971) in calves were observed. These can be attributed to the entry of toxic products across the blood brain barrier, which may result in the lower glucose uptake of nervous cells (Meyer et al., 1976). Pauli and Alsop (1974) and

Furukawa et al.(1977) did not observe tetanic symptoms in hypomagnesemic cows. Similar results have also been obtained in the present investigation.

BIOCHEMICAL CHANGES

Potassium

Potassium levels of rumen fluid increased significantly 24 hours after administration of potassium chloride and citric acid intraruminally. Similar trend was observed by Sinha (1980) in buffalo calves. In normal animals the potassium concentration of the fluid fraction throughout the GIT is consistently several times higher than plasma levels. On the other hand, the contents of the tract are generally electro-negative. Therefore, the concentration gradient must be sufficiently great to overcome an adverse electrical gradient for absorption into circulation following potassium supplementation (House and Campen 1971). Potassium is readily absorbed from the rumen (Parthasarthy and Phillipson, 1953) and from omasum (Oyaert and Boucqué, 1961) as indicated by high percentage of intake in excreted urine (Ward, 1969).

The present finding that intraruminal administration of potassium chloride and citric acid result in hyperkalemia after 24 hours is in line with the observations made by earlier workers (Kariya et al., 1976 in ewes; Furukawa et al. 1977 in cattle; Nethery et al., 1979 in calves; Sinha, 1980 in buffalo calves).

Hyperkalemia was further aggravated owing to metabolic acidosis which resulted in increased extracellular concentration of hydrogen ions. As the body cells tend to take up hydrogen ions and release potassium ions inthe extra-cellular fluid. Thus, there may occur a marked elevation of serum potassium (Kaneko, 1980).

The variations of potassium in the CSF followed more or less the same trend as that of plasma which has also been observed by Scholz and Meyer (1972) in sheep. It has been suggested (Cornelius, 1963) that potassium alterations in the CSF are likely to occur when whole plasma or blood escapes into CSF.

A highly significant rise of potassium was observed in the urine after 24 hours. A similar observation was made by Shiga et al. (1981) in ewes. Ward (1969) reported that 80% of the potassium was eliminated through urine in non-lactating animals. Potassium is excreted mainly through kidney by glomerular filtration and tubular secretion. However, when aldosterone causes increased resorption of sodium, it does so by promoting the exchange of sodium in tubular fluid for potassium in therenal tubular cells. Thus aldosterone promotes excretion of potassium, when sodium is scarce in the tubular fluid and potassium excretion by this mechanism is enhanced.

Hyperkalemia was accompanied by significant rise in salivary potassium concentration. The trends of potassium variation in the saliva vis-a-vis plasma has been demonstrated in induced hyperkalemia in calves (Sobti et al. 1982). The trends of changes in potassium excretion in saliva was similar to those reported by Dobson (1966).

Magnesium

There was a significant decrease in the magnesium level of serum, rumen fluid, CSF and saliva following the administration of potassium chloride and citric acid. Similar observations have been made by Sinha (1980) in buffalo calves and Kariya et al. (1983) in sheep. However, House and Campen (1971) did not find depression in the blood magnesium and opined that potassium intake did not affect the magnesium retention and exchangeable magnesium pool size. Administration of potassium had a negative influence on magnesium absorption from the alimentary tract, and this supports the previous conclusions by Care (1967) and Care et al., (1967). The high potassium intake resulted in an average decrease in apparent magnesium absorption by 46 per cent (Newton et al., 1972).

The exact mechanism of reducing magnesium uptake is not known. Depression of magnesium absorption may have resulted from an increased potential gradient in the alimentary tract following potassium administration (House and Campen, 1971). But since magnesium uptake

apparently involves a carrier system rather simple diffusion (Care, 1965), potassium may have reduced the available magnesium absorption sites or by inhibiting transport mechanism.

A significant decrease in magnesium levels was seen following daily administration of potassium chloride and citric acid. This was in accord with the results obtained by Kariya et al. (1976) in sheep; Furukawa et al. (1977) in cattle; Sinha, (1980) in buffalo calves and Kariya et al. (1983) in ewes.

House and Campen (1971) attributed the decline in plasma magnesium partially to decreased magnesium absorption but mainly to the direct depressing effect of potassium on circulating magnesium. It has been postulated that cells take up and retain more magnesium as levels of potassium in the cells increase. Citric acid ameliorates the effect of potassium on both distribution and intercompartmental flux of magnesium; the reason for this is not known exactly.

Citric acid chelates with magnesium and these complexes may contribute to tetany syndrome by affecting the normal flux of magnesium in the animal. Burt and Thomas (1961) suggested that feeding of citric acid to heifers lowered serum magnesium concentration but Kennedy (1968) reported that magnesium level in plasma and urine of sheep were not substantially modified by feeding either of these acids.

In this work, magnesium level in CSF showed the same trend as in plasma. Meyer and Scholz (1972) in sheep investigated whether the clinical symptoms during magnesium deficiency were of central origin and related to changes of magnesium content in CSF. They observed that tetanies occurred only after the magnesium in CSF declined to values lower than 1.6 mg/dl, indepedent of blood magnesium level. Further even when the magnesium in the blood came down to 0.3 mg/dl or less, clinical symptoms were absent as long as magnesium in CSF was normal or only slightly reduced. Similar observations have been made in the present studies. This shows that clinical symptoms are more related to magnesium level in CSF than in blood. This relationship has also been confirmed in field cases (Pauli and Alsop, 1974).

From the results it can bededuced that during magnesium deficiency, the magnesium level in CSF does not fall as rapidlyas in the blood. This is in accordance with the findings of Meyer et al. (1974) in sheep. Magnesium in CSF seems to work like buffer for protecting brain against fluctuations inthe blood magnesium level. In general, it has been concluded that the ability of animal to defend the magnesium stock in CSF, depends upon the extent of magnesium deficiency and capacity to mobilize magnesium, which is greater in the case of calves than in adult lactating cows (Meyer et al., 1976).

The relationship between blood and CSF magnesium levels explains why animals with low blood magnesium level do not react clinically in each case or do so after quite different times.

A low concentration of magnesium, or more particularly a low magnesium/calcium ratio, potentiates the release of acetylcholine. It, therefore, appears that low concentration of magnesium in the extracellular fluid surrounding the muscle and plates leads to tetany through above-mentioned mechanism (Blaxter et al., 1954). It is however, known that cholinestrase activity is not affected during magnesium deficiency (Todd and Rankin, 1959).

Magnesium is excreted mainly through GIT and kidney and to some extent through saliva in cattle. In the present study a decrease in the salivary as well as plasma magnesium was noted which could be due to the tendency of the body to maintain blood magnesium levels by decreasing the excretion of magnesium through saliva.

Calcium

Calcium levels in the rumen fluid, serum, CSF and saliva showed a decreasing trend but exhibited no significant change during the progression of hypomagnesemia. Sinha (1980), however, observed a significant decrease of calcium in the blood and rumen fluid.

It has been reported that calcium is absorbed primarily from the anterior portion of small intestine by a passive or facilitated diffusion and active transport.

Increased potassium intake was responsible through mass action for reducing the absorptionof calcium and magnesium from the gut. Thus if theanimal is temporarily unable to mobilize sufficient amount of these elements from the body stores, then hypocalcemia accompanied by hypomagnesemia would result (Ward, 1969).

Most of the evidence indicates that increased acidity of theGIT favours calcium absorption (Loamba et al., 1978) as the decrease in the intestinal pH increases thesolubility of calcium. This effect is, however, countered by increased potassium concentrations in the rumen fluid. Thus, overall balance results in decreased calcium in the rumen fluid, which is in line with the observations of Salama (1977) in ewes and Sinha (1980) in buffalo calves. This was further attributed to loss of appetite and reduction in oral intake.

In the present study, there was a progressive decrease in the calcium level in the serum but decrease was not statistically significant which is in line of observations made by Murakami et al. (1972) in cows; Pauli and Alsop (1974) in cows and Shiga et al.,(1981) in sheep. However, Hall et al. (1972) in cows; Baker (1979) in sheep; Sinha (1980) in buffalo calves; El-Shrif and Mottelib, (1983) in cows andTakahashi et al. (1981) in sheep observed severe hypocalcemia in hypomagnesemic animals. But this bears no clear relationship to the onset of

acute stage of symptoms. Some animals with severe cramps had normal blood calcium level, while hypomagnesemic ones with a severe hypocalcemia did not always reacted in a typical manner. However, concomitant hypocalcemia may modify the clinical symptoms.

Meyer (1975) has clearly demonstrated in calves that there is an inverse sigmoid relationship between PTH secretion and plasma calcium concentration. He also showed that effect of magnesium concentration though similar was less effective than equimolar concentration of PTH. At very low plasma magnesium, there was not only PTH-target organ resistance but also impaired PTH secretion; both presumably the result of magnesium requirement for adenyl cyclase activity, which was involved in PTH release and subsequent action. The phenomenon of PTH-target organ resistance probably plays an important role in the development of hypocalcemia, which often accompanies hypomagnesemia and contributes to the clinical signs in grass tetany (Rude et al., 1972).

Calcium level in the CSF followed more or less the same pattern as observed in plasma.

Mineral acids fed to the cows increase calcium excretion (Stacy and Wilson, 1970). The same is true in non-ionized complexes such as EDTA and citrate. They are not subjected to tubular reabsorption in the kidney and therefore, renal calcium excretion is enhanced. In

the ruminants this may contribute to decrease in calcium levels in blood. Renal failure may additionally cause impaired calcium absorption from intestine because of the acquired defect invitamin D metabolism, which ultimately leads to decreased serum calcium (Kaneko, 1980).

Some calcium is excreted through saliva. As the hypomagnesemia progressed there was a non-significant increase excretion of calcium through the saliva was observed. Similar findings have so far not been available in the literature. This may be attributed to thedecrease inthe pH of the saliva which result in increase excretion of calcium.

Phosphorus

A significant increase in the blood inorganic phosphorus was observed after 24 hours of induction of hypomagnesemia and thereafter the phosphorus level decreased significantly. The increase in the phosphorus level during the first 24 hours is being reported for the first time though the decrease has been reported by Sinha (1980) in buffalo calves; Shiga et al. (1981) in sheep and El-Sherif & Mottelib, (1983) in calves. The initial increase in inorganic phosphorus levels might be due to the increased buffering action to maintain theacid base balance of blood.

As mentioned-above significant decrease in the phosphorus level occured later on. Lentz et al. (1976)

reported that hyperkalemia was responsible for increased level of insulin in blood which increased metabolism of glucose involving inorganic phosphorus (Doxy, 1971). This may be the reason for phosphorus depeltion in the blood.

Similarly in CSF the phosphorus decreased after 96 hours following the initial rise up to 48 hours. This indicates that phosphorus level followed the same pattern in CSF as in blood, but slowly due to buffering action in CSF.

It has been reported that in ruminants the main route of phosphate excretion is via saliva into the gut (Smith et al., 1956). Saliva which was clear otherwise became viscous which might be due to excessive secretion of electrolytes, particularly inorganic phosphorus and also due to increase in the salivary protein content. The salivary phosphorus increased significantly in the initial stages. This increase can be explained by the fact that in acidosis, there is depletion of plasma bicarbonate with a consequent decrease in the bicarbonate content of saliva. At this stage phosphate concentration of saliva and plasma increased, probably in an attempt to restore the buffering capacity as observed in the present study, and earlier by Turner and Hoggelts (1954). Later on, a significant decrease in phosphorus of saliva might be in response to decrease in the circulation.

A decrease in the concentration of phosphorus in the rumen liquor observed in the present study seems to be the result of dilution of rumen contents due to withdrawl of fluid from intravascular and extra-cellular compartments (Choudhuri et al., 1980)

Shiga et al. (1981) in ewes reported increased phosphorus excretion in urine during hypomagnesemia. When excess acid enters the ECF, a mechanism is necessary for excretion of hydrogen ions. This is accomplished in the kidney by excretion of dihydrogen phosphate and ammonia ions. Through the excretion of these ions in the urine, hydrogen ions are removed from the body and this may be the reason for decreased level of phosphorus in circulation later on.

Sodium

The present study has confirmed the earlier reports by Newton, (1972) in lambs; Shiga et al. (1981) in sheep; El-Shrif & Mottet (1983) in calves; Takahashi et al. (1983) in sheep, that plasma sodium does not exhibit any marked change during hypomagnesemia.

Sodium concentration in CSF followed the same trend as that of blood.

Salivary sodium did not show any marked change but rumen fluid sodium content showed a decreasing trend which may be due to the fluid withdrawl into the rumen. Another possible reason is that increase in the potassium

intake resulted in an increase in apparent sodium absorption (Newton et al., 1972). The principal regulation of body sodium is via kidney. During progression of hypomagnesemia, urine excretion of sodium significantly decreased and continued to remain at low profile, throughout the hypomagnesemic phase, thus conforming to the observations of Shiga et al. (1981).

In kidney, the regulation of body sodium is mediated through the effect of aldosterone on renal tubules, which increases resorption of sodium and promotes excretion of potassium (Kaneko, 1980).

Total proteins in cerebrospinal fluid

Normal calves have a clear CSF, whereas calves affected with hypomagnesemia have a turbid CSF with a clot formation property. Turbidity might be due to increased cellular elements and find shreds of fibrin due to inflammatory condition of the meninges resulting in an escape of fibrinogen into CSF. In the present studies, total proteins in CSF increased significantly after induction of hypomagnesemia. Alterations in CSF were generally present as neuropathological examination showed inflammation of CNS and meninges. Severe degenerative changes were also observed histopathologically. A parallalism has been reported to exist between the degree of CSF alterations and severity of histopathological picture. Marked increase in the cell count and

total proteins levels are rather significant which indicate extensive lesions in meninges (Frankhauser, 1962). A sginficant increase in the level of total proteins in CSF, might have been due to inflammation of CNS and meninges caused by the passage of toxic materials like acids and histamine across blood brain barrier (Brent, 1976).

Electrocariographic changes

Rumsey and Putnam (1972) observed decrease in QT interval, ST segment and P-wave amplitude in steers. Similar observations were made in the present study which may be attributed to hyperkalemia. Tachycardia may be another reason for decreasing in QT interval. Progressive increase in the extracellular potassium concentration produces a progressive decrease in the resting membrane potential (RMP). Hypokalemia also results in decreased P wave amplitude and increased duration on account of slower conduction of atria.

Heart rate

An increase in the heart rate was observed throughout the hypomagnesemic phase. This is in line with the observations of Horvath et al. (1971) in bull calves. Rumsey and Putnam (1972) in steers. Tachycardia can be attributed to activation of sympathetic nervous system stimulation (Slinker et al., 1982) due to acidosis causing increased cardiac contractility and resulting in increased cardiac output. Another possible reason of increased

heart rate can be due to necrotic foci on myocardium and respiratory distress observed during hypomagnesemia.

Rectal temperature

Rectal temperature decreased significantly throughout the present pathophysiological investigation. The decrease in temperature can be attributed to some defect in depression of the thermoregulatory centre, as degenerative changes were detected histopathologically during the progression of hypomagnesemia. Another possible reason for hypothermia can be the decrease in the basal metabolic rate at the cellular level.

Gross and histopathological findings

The post-morten findings in experimental buffalo calves that died owing to induction of hypomagnesemia include marked venous congestion of alimentary tract with haemorrhage in the mucosa of small and large intestine with ulcerative lesions. Enalrgement of liver with necrotic patches and marked cogestion and haemorrhage of lungs were also observed. This was similar to the gross lesions described by Ohshima et al. (1973) and Sinha (1980). Marked cogestion of meningeal blood vessels along with the swelling of brain was also observed inthe present study. This is in consonance with the findings of Haggard et al. (1970), and May et al. (1976). The lesions might have resulted from general passive hyperaemia and theentry of toxic metabolites such as acid and histamine in the brain (Brent, 1976).

All the parendymatous organs, viz. kidney, liver and myocardium, showed degerative changes due to hypoxia. The metabolically active sodium, potassium pump mechanism is located in the cell memberane. It consumes energy from ATP, which is split by magnesium, dependent sodium-potassium activated ATPase (Jones et al., 1977). During hypomagnesemia due to magnesium deficiency ATP enzyme decreased. The condition further accentuated because of hypoxia and hypophosphataemia which resulted in mitochondrial degeneration and a fall in ATP and a high energy phosphate concentration. Overall result was net deficiency of ATPase enzyme, which was responsible for injury to the sodium pump. Another contributing factor may be the rupture of lysosomal cell membrane due to the deficiency of ATP, resulted in the release of hydrolytic enzyme , responsible for further damage to the cell wall. Consequently there was net entry of sodium and chloride together with water into the cell, causing it to swell; when overhydration became severe, plasma membrane may rupture, producing lysis of the cell (Crocker et al., 1970). The ensuing interference of flow in kidneys and liver accentuated the pre-existing conditions.

The histopathological changes in lungs, like severe congestion and alveolar emphysema, were most probably due to increased ventilatory work. Both hypo-

xemia and acidosis led to vasoconstriction of pulmonary vascular resistance.

The pulmonary vascular resistance may be elevated further by the increased blood viscosity due to secondary polycythemia or by obliteration of pulmonary capillaries. As the alveolar walls expanded, it encroached on surrounding alveoli and caused its collapse. The high pulmonary vascular resistance leads to pulmonary hypertension, with subsequent right ventricular hypertrophy. The presence of pulmonary cogestion would increase the work of breathing further and also aggravate the respiratory insufficiency. With respiratory distress, there may be an element of left ventricular failure, perhaps because of hypoxic depression of myocardial function, the added load of a high ciruclating blood volume.

Foci of necrosis were observed in the cardiac muscles, which is in line with the observation of Cheville (1976), that magnesium depletion is characterized by small spoty foci degeneration and necrosis of heart. During the progression of hypomagnesemia, the dystrophic changes in the heart have been considered responsible for the development of cardiovascular insufficiency which further added to the pre-existing general passive hyperemia.

The renal tubules revealed coagulative necrosis and degenerative changes in the lining epithelial cells. Such type of lesions have been ascribed to peripheral

circulatory failure, leading to ischaemia of renal tubules. Haggard et al. (1978) have reported inflammatory changes of kidneys in clinical cases of beef calves which have not been observed in the present study as well as that reported by Sinha (1980).

Liver sections in the present investigations showed degenerative changes of hepatocytes especially cloudy swelling. Large intracytoplasmic inclusion bodies in the hepatocytes were observed which were presumably the megamitochondria, formed by progressive deposition of proteins in the matrix, and these are known to occur in several liver injuries (Gupta, 1981).

Spleen in most of the cases showed depletion of red pulp. It might have occurred due to the elevated level of circulating catecholamines to which spleen is very sensitive.

Marked venous congestion of alimentary tract with haemorrhages in the mucosa of small and large intestine and necrotic lesions were observed in the present study. These observations are in agreement with those made by Bohman et al. (1969) in young cattle. Lesions might have resulted from general passive hyperaemia coupled with local corrosive action of acid.

During hypomagnesemia, hyaline degeneration of skeletal muscles was observed as also reported by

Ohshima et al. (1973). This degnerative change might have occurred owing to increased depletion of ATP in muscle cells which is also responsible for skeletal muscle rigidity. Lymph nodes revealed severe congestion and perivascular edema, and this can be attributed to general passive hyperaemia. The presence of a large number of plasma cells and macrophages in the spleen and lymph node is indicative of increased antibody production, presumably due to the decreased resistance of the animals as a result of continuous stress.

The changes in the brain included severe congestion, perivascular lymphocytic cuffing, chromatolysis of neurons, setellitosis and neuronphagia. The presence of a large number of fat granular or gitter cells, which are modified microglia cells, indicates the phagocytosis of necrotic brain substance by the microglia cells. Encephalomalacia with myalin degeneration leading to spongiosis of the brain substance might have occurred due to hypoxia. This hypoxia is responsible for causing damage to blood brain barrier,

which later on showed inability to check the entry of undesirable substances to brain tissue. Moreover, blood brain barrier in bovine appears to be rather lenient, (Kanwar, 1982). Another possible reason for brain damage can be attributed to the deficiency of thiamine (Randhawa, 1979).

Other organs did not show any significant pathological changes.

TREATMENT

The treatment was aimed at restoring electrolytes of various body fluids which had undergone different changes. The various drugs and chemicals selected for the treatment of hypomagnesemia, in experimental buffalo calves were on the basis of the recommendations by earlier workers (Ohshima et al., 1973; Meyer et al., 1975; Mieth et al., 1976; Ruthkowick et al., 1978; and Teuffert et. al., 1981) Hypomagnesemic changes in untreated group provided the guidlines for adopting the treatment measures in subsequent study (Group III and IV).

The dosage varied depending upon the body weight of individual animals. The time selected for the treatment was based upon the biochemical analysis of the pathophysiological group, as indicated by alarming

symptoms which usually occured 96 hours after induction of hypomagnesemia.

The adaptation of treatment after four days of hypomagnesemic phase and repeated treatments to check relapses have been recommended for better recovery in experimental as well as in clinical cases of hypomagnesemia in calves (Mihai, 1971; Murakami et al., 1972; Morino et al., 1976; Haggard, et al., 1978 and Sinha, 1980).

Therapeutic measures were adopted by using two different regimens. Animals belonging to Group III and IV had a very low level of magnesium, calcium and phosphorus in serum, rumen fluid and CSF. The Group III animals were treated with 10% magnesium sulphate, half of which was given intravenous and half subcutaneous @ 1.5 ml/kg alongwith 50 ml of calcium borogluconate given intravenously. A remarkable improvement in the electrolyte concentration of serum, rumen fluid and CSF was observed. The treatment was repeated for three consecutive days. No relapses were seen in recovered animals within a week. Sinha (1980) has also made similar observations.

In this group, the initial phase of hypomagnesemia was covered up by intravenous administration of magnesium sulphate solution, whereas half of the magnesium sulphate was given subcutaneously and this seems to behelpful in slow release of the drug, for maintaining blood levels for longer period and avoiding relapse, because of the

presence of significantly high levels of potassium in the body fluids which hinders the gut absorption, as well as the maintenance of normal blood magnesium (Fontenot et al., 1973). Magnesium remained at subnormal level in the rumen fluid in which some ions from the blood were required to be added through various secretions for bringing it to the normal range. In the urine of Group III animals, there was a sharp rise of sodium level in the post-treatment phase. This might have been due to the fact that while the serum magnesium level was rising there was an increased urinary loss of sodium and chloride. Similar findings have been reported by Chesley and Tepper (1958). In addition, excretion of magnesium paralleled that of sodium as the excretion of sodium was increased or decreased the excretion of magnesium followed suit (Hills et al., 1959 and Ross, 1961). From this we can deduce that treatment with magnesium sulphate and calcarol was effective in animals of Group III. These buffalo calves were found clinically cured after being treated with both subcutaneous and intravenous injections, except that they had high potassium content in the rumen fluid, serum, saliva and urine as compared with the normal control values. It seems probable that fixed tissue potassium was being released very slowly to be excreted out to bring its blood levels to normal within the treatment period. Excess potassium retention in the tissues was evident from

biochemical studies which present a serious concern in counteracting potassium toxicity.

In Group IV animals, 75 ml of the 30% magnesium chloride hexahydrate solution was infused per ructally. Although there was a significant increase in the magnesium level in theserum, yet it remained much lower than the zero hour values. Same trend was observed in other body secretions viz. CSF, saliva and rumen fluid. Similarly values of calcium and phosphorus were much less than the normal values in the above-mentioned body secretions. This low level of magnesium could be due to three factors: (i) slow absorption per rectally as compared with intravenous administration; (ii) quick ejection of the drug by animals and (iii) lack of maintenance supply.

In the urine of Group IV animals; the rise of sodium level was negligible indicating only slight elevation of magnesium level in the blood. Potassium content remained high even after 72 hours of post-treatment phase in serum, rumen fluid, CSF, saliva and urine. So it can be concluded from theabove studies that treatment remained practically ineffective in restoring the electrolyte status of various body fluids and secretion to their control values.

Total proteins in the cerebrospinal fluid

A decreasing pattern was noticed in the concentration of total proteins in CSF, in both the groups, after

the initiation of therapeutic measures. Therapy, thus, might be effective to some extent in checking the damage of CNS as indicating by decrease in total protein content in CSF after treatment of neutralizing the effect of devitalizing factors present in the CNS.

Haemoglobin and Packed Cell Volume

Both the parameters showed a decreasing trend in the post-treatment phase in both the groups and their values came to near normal after 72 hours.

Electrocardiographic changes

Although electrocardiographs did not show any significant changes after the treatment, yet in animals of Group III, PR intervals, QT segment and PR segment, showed a decreasing trend and came to normal which had been decreased due to tachycardia. However, in animals of Group IV, they remained low even after the treatment. The amplitude of mean QRS complex which showed an increasing trend in the hypomagnesemic phase, came to normal after the treatment in Group III animals but maintained its increasing trend in Group IV animals. Similar findings have been reported by Horvath et al. (1971) in cow calves. The only exception was the significant decrease in the P wave amplitude after 72 hours in Group III animals. This may be due to persistent elevation of potassium ions in the body.

Heart rate

It remained higher even after 72 hours in Group IV animals, but came to normal after 72 hours of treatment in Group III animals. It appears that heart takes longer time to return to normal dynamics.

Rectal temperature

Temperature returned to near normal, after instituting the treatment in both the groups. This might be due to the fact that during the post-treatment phase, thermo-regulatory centre regained control as the nerve damage decreased and the basal metabolic rate of the body increased.

CONCLUSIONS

The results obtained in the present study point to the following conclusions :

1. Potassium chloride and citric acid administered intraruminally are effective in inducing hypomagnesemia in buffalo calves.

2. Hypocalcemia is insignificant in potassium chloride and citric acid induced hypomagnesemia.

3. Apperance of tetanic symptoms depends upon the concentration of magnesium level in CSF rather than its concentration in blood.

4. The kidney and salivary glands tend to relieve the hyperkalemic state.

5. Magnesium sulphate and calborol therapy is more effective than magnesium chloride treatment for hypomagnesemia.

SUMMARY

Experiments were performed on 22 healthy male buffalo calves, 3-7 months old and weighing between 45 and 70 kg (Mean ± SEM 63.2 ± 7.7 kg). The animals were randomly divided into four groups of 5, 5, 6 and 6 animals. The animals of Group I served as control, while hypomagnesemia was induced in Group II, III and IV, by daily administration of potassium chloride @ 1.3 g/kg and citric acid @ 1.1 g/kg body weight intraruminally. Animals of Group II were used for terminal studies while two therapeutic regimens were tried in rest of the animals, after 4 days of inducing hypomagnesemia.

Various parameters, such as calcium, magnesium, phosphorus, sodium, potassium, pH, haemoglobin, PCV and rectal temperature were studied to know electrolytes, biochemical and haematological changes in serum, rumen fluid, CSF, saliva and urine. Daily observations of ECG in all the groups were made. Gross and histopathological changes in the calves that died owing to hypomagnesemia (Group II) were studied.

Hypothermia, persisting tachycardia and haemo-concentration due to dehydration as indicated by elevation of haemoglobin and PCV were noticed during the progression of hypomagnesemia.

Magnesium and phosphorus decreased significantly, while calcium and sodium fluctuated non-significantly except that the latter showed a significant decrease in urine. Potassium showed marked elevation in serum and various body secretions during the course of hypomagnesemia.

Total proteins in CSF increased 4-5 times of the normal value indicating severe damage to the brain substance.

ECG did not reveal marked changes except the decreasing of P wave amplitude, QT interval and ST segment.

Tetanic symptoms were not observed during the present study, possibly because the levels of CSF did not fall below 1.6 mg/dl. Magnesium in CSF seems to act as buffer against the fluctuating levels of magnesium in serum.

Degenerative changes observed in various organs of the calves died owing to hypomagnesemia were suggestive of electrolyte imbalance, dehydration and anoxia.

Electrolyte concentration in various body fluids was restored with the therapy comprising 10% magnesium sulphate, half of which was given intravenously and the other half subcutaneously along with 50 ml of calcium borogluconate. Treatment with 30% magnesium chloride given per rectum was ineffective in restoring the electrolyte concentration.

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