PREVALENCE AND PATHOLOGY OF POLIOENCEPHALOMALACIA IN GOATS

By

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THESIS

Submitted in partial fulfilment of the
requirement for the degree

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CENTRE OF EXCELLENCE IN PATHOLOGY
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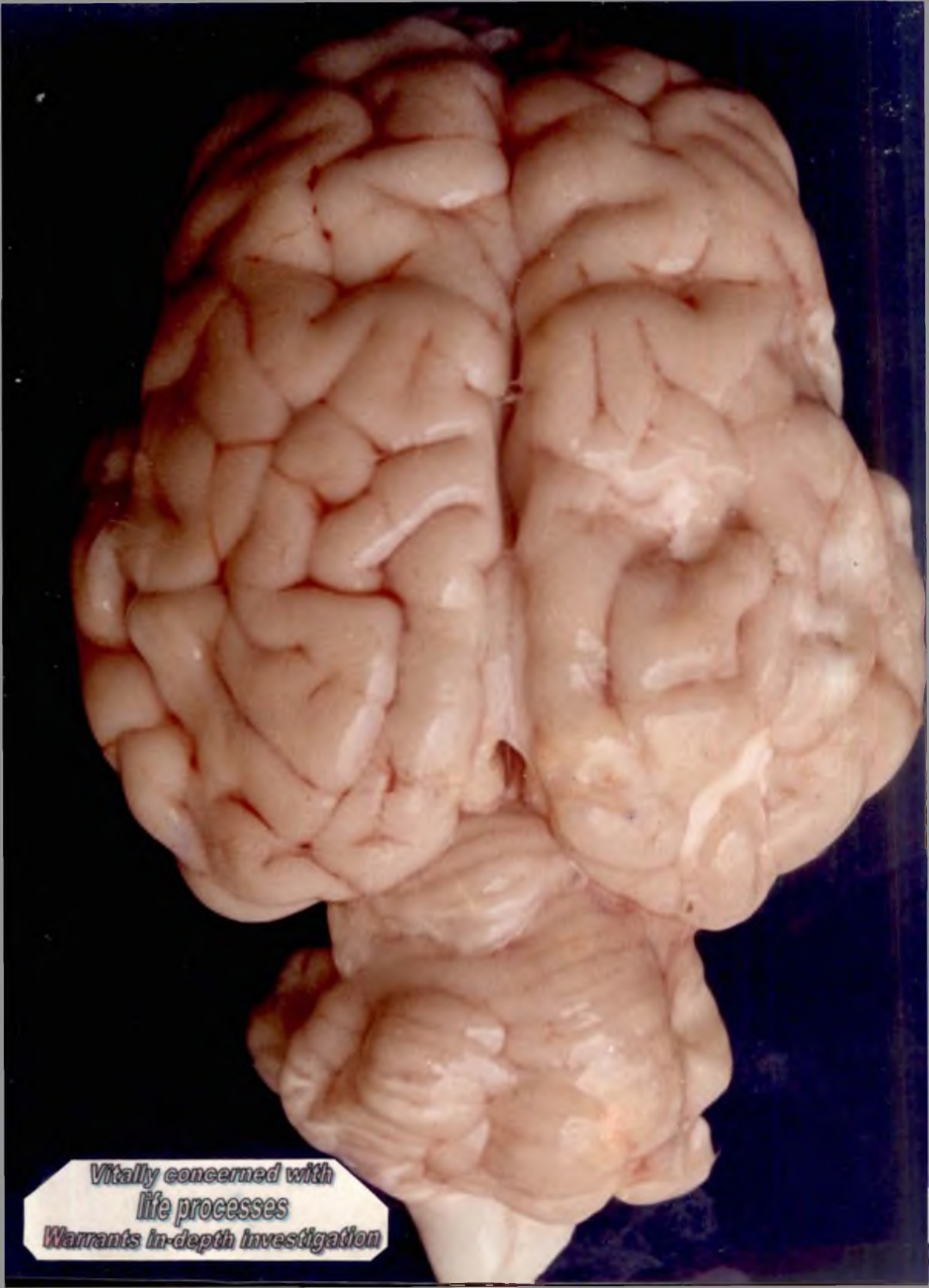
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life processes
Warrants in-depth investigation

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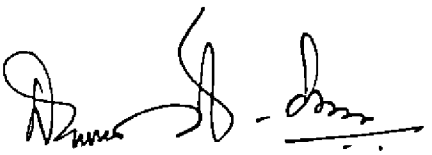

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Introduction

INTRODUCTION

Goats being poorman's cow remain in an exalted position among small ruminants. Yet the economic importance of goats often goes unnoticed. For house holds surviving on subsistent farming, small ruminants like goats act as an economic buffer. For a farmer, the goat serves as an important disposable asset and money provider to tide over times of financial stress. Therefore, the goat has an enviable position in the farmer's animal husbandry activities and play a significant role in the stability of the rural economy.

Against this background it is pertinent to point out that India is one among the first few countries with regard to goat population.. According to the 1994 estimates of the FAO the flock size of goats in India is 118.34 million representing 20 per cent of the world population and as per the existing growth rate India will have 149 million goats by 2000 AD. This phenomenal increase observed in all regions reflects the farmers perceptions and testifies to the useful role goat plays in different farming systems. Their contribution to the livelihood of a large percentage of small and marginal farmers and landless labourers is tremendous. The meat of goat has become a very precious

commodity and the farmers margin of profit on rearing is enviously high and goat farming has turned out to be the most rewarding proposition. The contribution of goats to the gross national product has been substantial and the annual contribution amounts to rupees 3500 million. Goats have made significant impact in the rural development by providing gainful employment and remunerative income to a majority of rural youths.

The trend for intensifying goat husbandry has already been set in Kerala and the farmers who had one or two goats conventionally have started rearing them commercially as the demand for goat meat is on the increase. Goat rearing has become an industry in certain parts of the state to cater to the needs of the population.

Intensification of goat production will certainly result in an increase in the number of high producing animals. These high quality animals when reared on high tech management will perforce be subjected to stress and this will precipitate disease problems resulting in high mortality and serious economic upsets.

Goats suffer from a variety of diseases and disorders. Because of the browsing habit they are exposed to a great variety of poisonous substances. The toxic plants constitute an important group and includes a variety of

natural vegetations. The industrial revolution has caused a bewildering array of man made noxious substances in the environment in the form of industrial effluents and fumes, heavy metals, automobile and machinery lubricants, disinfectants, herbicides, insecticides and rodenticides to name a few. Besides this the concentrate feeds on which the goats are to be fed, necessarily in the phase of shortage of fodder, have hidden poisonous substances in them like mycotoxins. It is also relevant to point out that the unconventional fodders which are fed or grazed by goats are also potential sources of various poisonous substances. In an intensive production system, the goats are, therefore, exposed to a variety of substances which are health hazards.

The conventional diseases of goats are well defined. However, there are a few emerging disease problems in goats which need special attention. In this category Polioencephalomalacia (PEM) is one of the most important problems leading to reduced feeding, delayed maturity and marketing. Though not an acute killer, it does account for total aggregate mortality and serious economic losses.

PEM is a non-infectious disease of cattle, sheep and goats characterized clinically by depression, blindness, inco-ordination and pathologically by laminar cerebral cortical necrosis and malacia. Severe cases may progress

rapidly to recumbency, convulsions and death. High incidence of this has been reported among feedlot cattle and nursing animals. It has a world wide distribution among pasture and farm stock and is of great concern to the farming community and the Veterinarians. It was first described in cattle and sheep in the USA.

In India relatively only little work has been done on goat diseases, probably due to lesser occurrence of diseases in them when they are grazing on pasture. However, the present day intensive system of management had looped around diseases of various aetiology.

Neurological disorders and their proper understanding has been a neglected field in small ruminants. Although PEM in goats was recognized and reported as early as 1956, its aetiology is poorly understood. Symptomatology, pathological features and therapeutic approach have not been well defined and documented. In the past, alteration of thiamine metabolism was attributed for nearly every outbreak of PEM. World wide, there is a large body of contradictory literature regarding the possible relationship between alteration of thiamine metabolism and PEM. A variety of other substances have also been suspected as incriminating agents for this disorder. These include poisonous plants,

chemicals, pesticides and an array of other toxic materials in addition to certain deficiencies.

In Kerala, considerable number of cases with clinical signs suggestive of PEM have been recorded. However, it has not been possible to pin point the cause and therefore the treatment was based on symptomatology and most of the treatments adopted have not met with success. Therefore, an investigation was undertaken on this problem to elucidate the aetiopathological features.

Review of Literature

REVIEW OF LITERATURE

2.1 Incidence

The disease polioencephalomalacia (PEM) was first described in cattle and sheep in the USA by Jensen *et al.* (1956). They reported that the estimated annual incidence of PEM in cattle for four areas in Colorado ranged from 4.5 to 100.5 cases per 10,000 cattle. In a series of 111 fatal cases 38 per cent were males and 62 per cent were females. The incidence by age in 110 cases showed the larger number in the 12-18 month age group. The incidence by breed in 107 cases revealed a higher incidence in Hereford cattle which predominated in pasture and feed lot. The monthly incidence in 103 cases in cattle from, feedlots and pastures showed a higher incidence during January and for feedlot during July. They also observed PEM in 70 out of 1,200 sheep in feedlot and seven out of 90 in pasture. The incidence among those in feedlot was more in January and in the other case it was in August.

Hartley and Kater (1959) and Terlecki and Markson (1961) reported the incidence of PEM in sheep to vary between one to 10 per cent. Fenwick (1967) reported PEM in 22 sheep. Out of these 21 had died and only one responded to treatment with thiamine. According to Little and

Sorensen (1969) the disease affects several ruminant species and has an almost world wide distribution. They reported that bovine PEM was sporadic in yearling feedlot cattle and the morbidity and mortality was in the range of 0.7 per cent to two per cent. In calves under six months of age, the morbidity and mortality could be over 50 per cent, but usually it was not more than 10 per cent.

Verdura and Zamora (1970) reported that 15 per cent of cattle fed a diet based upon molasses and urea in a large Cuban feedlot developed PEM during a six months period. In Colorado the estimated annual death loss in animals was 1.6 per cent of which 19 per cent were due to PEM (Pierson and Jensen, 1975). They observed that the disease has been reported in sheep, cattle, goats, white tailed deer, antelops and deer. They opined that it is an important problem of goats in New Zealand, Britain, France, Germany, USSR, America and India. Mella *et al.* (1976) reported many cases of PEM in cattle in Cuba co-incident with the use of a new molasses-urea based diet to fatten bulls. A mortality rate of 0.6 to one per cent in routine slaughter and in emergency slaughter from 0.4 per cent to 3.04 per cent was found to be due to the disease. Smith (1979) encountered six cases of PEM in goats of two to 2½ months age. Out of these, three responded to thiamine treatment and the other three died two to seven days after onset. He noticed the

cases during the winter or early spring. Dickie and Berrymann (1979) reported death of 14 cattle from PEM in a pasture where Kochia had been heavily grazed.

Tanwar (1987) was the first to report spontaneous cases of PEM in goats from the arid zone of Rajasthan and observed the disease in 50 female goats of different age groups. He reported the occurrence in late winter (January/February), mid summer (April), rainy seasons (August to October) and early winter (November). There was no correlation between the occurrence of PEM and temperature, humidity and rainfall. The incidence was more in January, February and March. However, there was no month in which PEM was not recorded.

Hamlen *et al.* (1993) observed that most feedlot cases developed during late autumn and winter or early spring, a time of greater concentrate feeding. They found the outbreaks in farm flocks following changes from over grazed to lush pasture. They noticed PEM in 11 out of 110 in a herd exposed to a source of water containing sodium sulphate.

Lonkar *et al.* (1993) in a retrospective study of 5593 Marwari goats brought for treatment at the Bikaner Veterinary College observed PEM in 158 cases and the percentage incidence was 2.6 per cent. They noticed the

disease throughout the year with the highest incidence in late winter followed by mid summer, rainy season and early winter. Domenech and Formenty (1994) recorded a nervous syndrome of sheep (Cerebral cortical necrosis - CCN) resembling PEM in the Cote d' Ivoire. They assessed the economic importance, conducted field trials and reported cost benefit analysis of prophylaxis programmes for CCN. The economic loss from cerebro-cortical necrosis was about 30-40 French francs/animal annually.

Domenech *et al.* (1994) reported that an ataxia and paralysis syndrome has been recognized since 1978 in West African Dwarf sheep in the humid south of the Cote d' Ivoire with 100 per cent mortality in untreated cases.

Moro *et al.* (1994) observed three cases of PEM in cattle aged 4-18 months from Minais Gerais in Brazil.

Jeffrey *et al.* (1994) observed a morbidity rate of 16-48 per cent and mortality of zero to eight per cent in animals affected with PEM. In the later part of 1991, they observed neurological diseases in several farms in England. Six calves from five such farms and two lambs aged from two to 44 weeks showed PEM. Bulgin *et al.* (1996) found PEM unresponsive to thiamine treatment in 40 out of 2000 ewes when they were moved to a field that had been sprayed 16 h earlier with elemental sulphur.

Low *et al.* (1996) observed an outbreak of PEM in 16 of 46 Swaledale lambs and five of 25 Scottish black face lambs 15 to 32 days after they were introduced to an *ad libitum* concentrate ration containing 0.43 per cent sulphur.

2.2 Etiology

2.2.1 Amprolium

Morgan *et al.* (1974) investigated encephalopathy and haemorrhagic syndrome in lambs and suggested amprolium poisoning as the cause. Spicer and Horton (1981) induced PEM in sheep using amprolium at the rate of 1 g/kg body weight. The animal showed clinical signs of PEM after three weeks of amprolium dosage and all affected sheep had succumbed after five weeks of treatment. Sobhanan (1981) attempted experimental induction of PEM in four male goats of two to three months of age. Amprolium at the rate of 280 mg/kg body weight was given as a drench for 60 days and the roughage ration was restricted. Only one animal showed mild symptoms and brain lesions suggestive of PEM. Fakhruddin *et al.* (1987) produced PEM in goats by feeding amprolium. All the animals receiving amprolium drench from 33rd to 52nd day developed symptoms of PEM. Animals were given amprolsol daily in three equally divided doses at the rate of 4.5 g as drench by stomach tube till the development of symptoms.

This daily dose was increased by 1.5 g in each subsequent week.

Itabisashi *et al.* (1990) produced neurological signs of PEM in sheep and cattle by giving amprolium at the rate of 600 mg/kg body weight daily intraruminally. Lonkar and Prasad (1992) induced PEM in goats which were drenched with amprolium three times a day at 225 mg/kg/day with periodic increase in the dose until the onset of clinical symptoms. Strain *et al.* (1992) produced the disease in sheep. Out of the 10 sheep dosed with amprolium at the rate of 1 g/kg/day, two only developed PEM which displayed clinical signs on the 41st and 43rd day. They observed a reduction in the mean transketolase levels from 58.9 to 32.7 ml/litre/minute at the onset of signs confirming PEM.

Chahar *et al.* (1993) induced PEM in sheep and noticed symptoms 40-77 days after the administration of amprolium. They observed a decrease in the level of thiamine in the serum and rumen contents. Lonkar and Prasad (1994) observed wide individual variation regarding the total quantity of amprolium required to induce PEM. The quantity required varied from 8.23 g to 23.8 g/kg and the time required also varied from 57-119 days. PEM was induced in six buffalo calves aged 6-12 months by drenching amprolium at the rate of 300 mg/kg body weight per day for 25-55 days (Tanwar and

Malik, 1995). They found biochemical changes characteristic of PEM after oral administration of amprolium for 4-6 weeks. They noticed a progressive decrease in erythrocyte transketolase activity and an increase in thiamine pyrophosphate (TPP) effect in amprolium fed animals until the onset of clinical signs. There was a significant increase in blood lactate and pyruvate concentrations and a decrease in lactate/pyruvate ratio at the onset of clinical signs. Serum sodium, potassium, calcium and magnesium showed no changes.

2.2.2 Sulphates

Feeding of rations containing high concentrations of gypsum ($CaSO_4$, $2H_2O$) or other sulphate salts such as sodium sulphate was noticed to be a common cause of several episodes of PEM (Raisbeck, 1982). Inclusion of 2% gypsum in the ration as an intake limiting additive for 1-2 weeks caused symptoms of PEM. He undertook a study to evaluate the possible relationship between PEM and high dietary sulphate intake. PEM developed in 18 of 21 herds on high sulphate rations. He opined that PEM was 43 times more likely to be diagnosed in herds fed with high sulphate ration. Environments known to have high sulphate water have been associated with PEM (Hibbs and Thilsted, 1983) since rumen microbes are capable of efficiently reducing sulphate

to sulphide which is toxic to the brain. Sadler *et al.* (1983) conducted a study to find out the relationship between sulphate and PEM in cattle. They observed the development of PEM in 20 out of 60 cattle when fed with 30 per cent roughage diet with an additional 0.72 per cent sulphate in the form of $MgKSO_4$. There was a weight reduction by 50 per cent in the group with a diet more in sulphate. The effect of high dietary sulphur supplementation on blood thiamine concentration was studied by Gooneratne *et al.* (1989). They found the development of nervous symptoms in the high sulphur supplemented groups. Those groups which were supplemented with thiamine at the rate of 230 mg/kg diet did not show clinical signs, but brain lesions suggestive of PEM were observed in all sheep. It was speculated that PEM may be caused by a direct toxic effect of sulphur, sulphur metabolites or B1 antimetabolites in the brain rather than by an *in vivo* B1 deficiency. Sulphur toxicity was implicated as the cause of PEM in Hereford heifers (Beke and Hironaka, 1991).

Rousseaux *et al.* (1991) induced PEM in sheep by high sulphur diet. A dose of 0.63 per cent sulphur in the diet produced PEM between the third and seventh week. They demonstrated an association between elevated sulphide concentration in the ruminal fluid of calves and the occurrence of PEM which supported the hypothesis that PEM

can result from excessive sulphide concentration in the rumen. They did not find any change in the thiamine status of the animals. Gould *et al.* (1991) suggested that PEM could result from sulphide toxicosis following excess production of sulphide in the rumen. Five of nine Holstein steers fed an experimental diet with added sodium sulphate developed PEM. The sulphide concentration in the ruminal fluid was high. PEM was observed in heifers within two months of exposure to saline well water (Beke and Hironaka, 1991). The water contained 3875 mg/litre of total dissolved salts, sulphates and sodium being the dominant ions. The calculated daily sulphur and sodium intakes were near the upper limit of their range for recommended maximum tolerance level of 0.4 per cent for sulphur. Olkowski *et al.* (1992) induced PEM in sheep with high sulphate diet. Sheep of about two months age when fed with a diet rich in sulphur (0.63%) developed clinical signs after three to eight weeks.

McAllister *et al.* (1992) induced PEM in four of ten lambs following the administration of a sulphide solution into the oesophagus at 20 min intervals for 40-120 minutes and they indicated that this disease can be caused by sulphide toxicosis independent of the metabolic status of the animal. Hereford cross-bred cows on pasture supplemented with a water source containing elevated levels of sodium sulphate (7200 ppm) developed PEM (Hamlen *et al.*

1993). They observed PEM in 11 of 110 mature Hereford cows. Jeffrey *et al.* (1994) observed PEM in calves and lambs when a proprietory concentrate ration containing ammonium sulphate was given rather than the normal urinary acidifier ammonium bicarbonate. Blowey and Packington (1994) discussed the use of ammonium salts as acidity regulators in mineral premixes and their association with PEM.

It was noted that the inclusion of sulphur as sulphates and flours of sulphur as copper antagonists in sheep feed may cause sulphur toxicity which had been identified as a possible cause of non-responsive case of PEM. Cummings *et al.* (1995) produced PEM in steers by feeding a diet with added sodium sulphate. The onset of signs of PEM was associated with increased sulphide concentration in the rumen fluid. An acute outbreak of PEM in lambs aged 15 to 32 days was observed by Low *et al.* (1996) after they were introduced to an *ad libitum* concentrate ration containing 0.43 per cent sulphur. Bulgin *et al.* (1996) observed PEM in ewes after they were moved to a field that had been sprayed 16 h earlier with elevated sulphur. PEM was induced in steers by adding sodium sulphate in the diet (Gould *et al.* 1997). High sulphate diet produced clinical signs which included episodic ataxia and absence of menace reaction. Ruminal hydrogen sulphide concentration was 40 to 60 times

higher in steers fed the diet with added sulphate compared with the one without sulphates.

2.2.3 Thiamine status and PEM

Though the lesions of PEM are not suggestive of a single etiology, it was believed for long that thiamine deficiency plays a major role in the pathogenesis of PEM and early stages of PEM were responsive to parenteral administration of thiamine. Considerable evidence was present to show that an abberation of thiamine metabolism plays a major role in the pathogenesis of PEM. High levels of thiaminase I activity and intraruminal degradation of thiamine and possibly production of thiamine antimetabolites were attributed to the factors responsible for PEM (Edwin and Jackman, 1970; Edwin and Lewis, 1971). Subsequently correlations were established between the occurrences of PEM and the presence of type I thiaminase in the rumen contents, faeces of affected animals, thiamine status as reflected by tissue thiamine content, urinary thiamine content and erythrocyte transketolase activity. Thiamine requirement varies with animal species, metabolic activity and feed intake. Ruminants may often be on the border line of deficiency. Thiamine mainly as thiamine pyrophosphate has an important role as a co-enzyme in carbohydrate metabolism both in the TCA cycle and the pentose pathway. Inhibition

of carbohydrate metabolism in thiamine deficiency results in elevated levels of blood lactate, pyruvate, oxoglutarate and lowered erythrocyte transketolase activity. This deficient state has general effects on metabolic activity that terminates in neuronal necrosis by energy dependent type (Edwin and Lewis, 1971; Edwin and Jackman, 1973). They found significantly lowered levels of thiamine in the brain and liver of animals affected with PEM. In cattle, sheep and goats, thiamine is supplied by the synthetic activity of ruminal microbes and a state of thiamine deficiency is believed to be due to thiaminase destroying enzymes within the gastro-intestinal tract. Thiaminase activity has been found in the bacteria, *Clostridium sporogenes* and *Bacillus thiaminolyticus* (Morgan and Lawson, 1974, Shreeve and Edwin, 1974). Increased thiamine degrading activity was demonstrated in the gastro-intestinal content of affected animals (Roberts and Boyd, 1974). The thiamine status, that is, thiamine concentration in the whole blood, plasma and erythrocytes of normal cattle consuming varying diets did not differ from that of cattle with PEM and lead poisoning (Loew *et al.* 1975). Dairy cattle had higher ruminal content of thiamine and lower thiamine destroying activity. The deficiency of thiamine caused by its excessive microbial destruction was not the only cause of PEM. Low thiamine concentration in the ruminal fluid and blood and high

thiamine degrading activity were documented in cases of ruminal indigestion and clinically normal sheep (Gupta *et al.* 1976 and Linklater and Dayson, 1977). Therefore, they reported that alterations in thiamine metabolism may sometimes be a consequence of the disease or an incidental finding rather than a cause of PEM. Boyd and Halton (1977) reported CCN in ruminants and found thiaminase as the cause for increased thiamine degradation. They identified the source of thiaminase and found that it could be derived from gut microflora.

Linklater and Dayson (1977) were unable to demonstrate low thiamine concentration in the tissue and rumen fluid of PEM affected animals and they reported that the activity of blood transketolase and gastro-intestinal thiaminase can be altered non specifically in conditions other than PEM.

World wide, there is no unanimity of view regarding the possible relationship between alteration of thiamine metabolism and PEM. Several investigators have found markedly low concentrations of thiamine in the body tissues, low activity of thiamine diphosphate dependent enzyme, transketolase in blood and high activity of thiamine destroying thiaminase in the gastro-intestinal tract of animals with PEM (Spicer and Horton, 1981; Jackman, 1985; Rammell and Hill, 1986; Fakhruddin *et al.* 1987 and Tanwar,

1987). The disease could be induced by prolonged feeding of thiamine antagonists and the animal often recovered when high doses of thiamine were administered. However, according to them these findings were not consistent as it was not possible to induce PEM utilizing various means including inducing a thiamine deficiency state.

2.2.4 Plants, pasture and mycotoxins

Evans *et al.* (1975) experimentally induced PEM by feeding diets containing 15 per cent to 25 per cent dried bracken fern rhizomes, a natural source of type-1 thiaminase. Basson *et al.* (1975) reported blindness and encephalopathy caused by *Helichrysum argyrophaerum* DC in sheep and cattle from Namibia. The toxicity was confirmed by feeding plant material to sheep. Bilaterally symmetrical status spongioses of the white matter of the brain and spinal cord were seen. The most severe and consistently affected areas included the cerebral white matter adjacent to the lateral ventricles, cerebellar peduncles and the brain stem particularly pons. The spongy changes were not accompanied by gliosis and inflammation. Grey matter involvement was indicated by the presence of perivascular and pericellular oedema. Cerebellar degeneration in cattle caused by the ingestion of *Solanum kwebense* was reported in South Africa (Pienaar *et al.* 1976). The disease is known by

the name Maldronksiekte in cattle and was experimentally reproduced by feeding plants to cattle. Temporary loss of balance and transient epileptiform seizures precipitated by a variety of stimuli, such as exercise handling and fright were the characteristic symptoms. When not disturbed, most of the affected animals appeared completely normal. The most conspicuous histopathological lesion was a neuropathy manifested by vacuolar degeneration and necrosis of neurons, particularly of the Purkinje cells in the cerebellum.

An isolate of *Fusarium moniliforme* from maize caused oedema of the brain, leukoencephalomalacia and hepatosis in horses (Marasas *et al.* 1976). The clinical signs of nervous disorder included ataxia, paresis, apathy, hypersensitivity, frenzy and other locomotory and psychic disturbances. Oedema and focal areas of liquefactive necrosis were present in the cerebral white matter. The subcortical white matter and cortical areas of the cerebrum showed malacic foci. Rarefication of the white matter, perivascular haemorrhage, oedema and cellular infiltration composed mainly of plasma cells and eosinophils were observed. Galitzer and Ochme (1978) observed Kochia poisoning in cattle with symptoms and lesions of PEM and they concluded that the plant thiaminase may be involved. They also reported that the consumption of hepatotoxin derived from *Kochia scoparia* might be a causative factor leading to impaired vitamin utilization and

PEM. Dickie *et al.* (1979) observed deaths in the cattle in the USA from PEM in a pasture where *Kochia scoparia* was heavily grazed. They observed photosensitization, toxic hepatitis, toxic nephrosis and PEM in cattle pasturing on this plant. PEM developed in 27 of 225 cattle grazing on 486 hectares of dry, short, grama grass pasture. Hereford, Angus and mixed breeds were affected. It was apparent that in the pasture where the problem occurred, probably a plant containing thiaminase or an unidentified toxin was causing the problem because when the cattle were removed from the pasture, the problem terminated. The plants present were *Kochia scoparia* (Medical fire weed) and *Salsola pestifer* (Russian thistle).

Newsholme *et al.* (1984) observed pushing disease as a result of ingestion of a plant *Matricaria negellifolia*. Five out of six steers developed this disease when fed with dried milled negellifolia after a latent period that varied from 16-44 days. The lowest dose of plant that proved toxic was 10 g/kg. Clinical signs included debility, clumsiness and pushing against objects.

Arguroudis *et al.* (1985) reported an outbreak of poisoning in a herd of goats browsing Amaranthus species with a high nitrate content. The symptoms observed were excitement, staggering, prostration, muscular tremors,

increased respiration and frothing at the mouth. Nitrites were demonstrated in the rumen contents. Kellerman *et al.* (1985) experimentally produced neurotoxicosis in ruminants by dosing with cultures of a common cob-rot fungus of maize namely *Diplodia maydis*. A subcortical laminar status spongiosis was observed on histopathological examination. The cultures of this fungus was found to be highly foetotoxic to ruminants. Still born also showed status spongiosis in the white matter. Divakaran Nair *et al.* (1985) produced *Ficus tsiela* Roxb (Chela) leaf toxicity in calves by feeding them with graded levels of the plant leaves. They observed various neurological disturbances and the characteristic histologic lesions were hyperaemia of the meninges, neuronal degeneration in the cortical grey matter and extensive vacuolation of the white matter neuropil. A moderate degree of gliosis was also observed. Neuronophagia, satellitosis and glial nodule formation were present occasionally. Rajan *et al.* (1986) described a nervous disorder in cattle caused by the ingestion of the leaves of the tree *Ficus tsiela* Roxb. The animals which ate this had a fixed staring look and within forty-eight hours showed uneasiness and bellowed frequently. Muscular twitching involving different parts of the body were seen more commonly involving muscles of the gluteal and shoulder region. After recumbency vague aimless movements of the

limbs were made and attempts to lift the head were not successful. The animals died during the course of 15-20 days.

The clinicopathological features of *Ficus tsiela* Roxb poisoning in calves in experimental toxicosis included transient hypoglycemia and increase in SGOT. There was no change in the blood calcium, haemoglobin and other parameters (Divakaran Nair *et al.* 1987). The calves were administered chela leaves at the rate of 550 g per day through the rumen fistula. The animals showed clinical symptoms on the 11th day after consuming 6.5 kg of chela leaf. A plant bracken fern (*Pterdium acquilinum*) was associated with CCN in Australia and USA. This plant was reported to cause bracken staggers, a nervous disorder of horses developing from a thiamine deficiency and a syndrome of cattle characterized by haemorrhagic tendencies and bone marrow suppression. These diseases were reported to be induced by a nonsesquiterpene glucoside present in the plant (Kellermann *et al.* 1988). Thiaminase type I was also present in the plant.

Marasas *et al.* (1988) found the development of oedema of the medulla oblongata in horses in Fumonisin B1 toxicosis. The principle lesions were severe oedema of the

brain and early, bilaterally symmetrical, focal necrosis in the medulla oblongata.

Gajendragad *et al.* (1992) observed death of several sheep in a herd of 150 after consuming the pods from a tree belonging to Acacia group. Signs were of nervous type which included dyspnoea, excessive salivation, recumbency and death. The histopathological lesions encountered in the cerebrum, hippocampus, pons, thalamus and medulla were classified into vascular, neuronal, glial, white matter changes, central chromatolysis, intracytoplasmic vacuolations and gliosis.

Hamlen *et al.* (1993) observed CCN in animals when they were shifted from over grazed to lush pasture. They suspected that some of the thiaminase mimicing substances present in the pasture might be causing destruction of thiamine and producing PEM associated with thiamine deficiency.

Van Der Lugt *et al.* (1996) studied the pathological effects of *Helichrysum argyrophaerum* poisoning in sheep and goat. They found lesions, mainly spongiform changes in the white matter of the brain in the sub-ependymal area adjacent to the lateral ventricles, cerebellar peduncles and brain stem. Bourke (1997) reported a new cerebellar degenerative disorder that developed in goat grazing pasture infested

with *Solanum cinereum*. All the goats affected showed an altered head posture and wide based stance. In the cerebellum, there was deficit of grey matter and Purkinje cells were absent in many folia. Some in the affected folia were undergoing degeneration and most of them were having foamy cytoplasm. Axonal swelling and marked reduction in the density of the granular layer and white laminae were observed.

2.2.5 Insecticides

Incidence of poisoning due to organophosphorus and organochlorine are on the increase in recent time due to their indiscriminate use. The herbivores like goats are more likely to suffer as a result of accidental ingestion of freshly sprayed crops. BHC enjoys a unique position in veterinary practice as it shows a wide spectrum of insecticidal activity, yet the incidence of accidental and malicious poisoning in livestock is not uncommon. The minimum toxic dose of BHC in baby calves was found to be 5 mg/kg and 25 mg/kg in older cattle and sheep (Radleff, 1970). Sheep appeared to be more susceptible. The average single oral lethal dose of BHC (containing 12% of the G-isomer) was reported to be about 1 g/kg. However, fatal poisoning was reported in dogs receiving only 20-40 g/kg. A variety of chlorinated hydrocarbon insecticides have been

used as sprays or dips to control ectoparasites on pigs, cattle, sheep and goats. They are CNS stimulants and in toxicity the characteristic clinical features observed were episodic seizures, salivation, champing of jaws, vomiting, ataxia and muscle tremors (Glastonbury *et al.* 1987).

Gilmour and Synge (1987) reported that neuro-pathological changes were usually lacking in most of the organochlorine compound poisoning. But sporadic focal anoxic and ischemic cerebro-cortical lesions were observed in dogs. The neuropathologic effects of a Bromethelin based rodenticide were studied by Dorman *et al.* (1992) in cats. The symptoms were characterized by ataxia, focal motor seizures, decerebrate posture, decrease in conscious proprioception, recumbency, depression and semicoma. Histologically, spongy change, oedema characterized by the formation of vacuoles in the extracellular spaces and myelin lamellae, hypertrophied fibrous astrocytes and oligodendrocytes were observed in the white matter of the cerebrum, cerebellum, brain stem, spinal cord and optic nerve. Spongy change often extended into the cerebellar Purkinge cell layer and cerebral cortical grey matter.

Vashisht *et al.* (1996) observed depression, ataxia and increased secretions and excretions in buffalo. Calves inoculated with dichlorvos at the rate of 4 mg/kg for 21

days recorded cerebellar lesions characterized by meningo-encephalitis and necrosis of Purkinge cells. Cerebrum showed lymphocytic meningitis and perivascular cuffing along with demyelination. Foci of necrosis with haemorrhages and infiltration of glial cells, satellitosis and neuronophagia were observed. Cerebellum also showed necrotic areas infiltrated with microglial cells.

2.2.6 Acidosis and PEM

Polioencephalomalacia occurs because an enzyme, thiaminase develops in the rumen catalyzing the production of a thiamine antagonist. Lactic acidosis might set up ruminal conditions that encourage this chain of events (Brent, 1976). According to Elam (1976) acidosis in cattle was caused by excessive ingestion of feeds which were rich in readily available carbohydrates. Those factors which contribute to excessive ingestion of high energy diets also predisposed to acidosis. Episodes of PEM have been associated with specific nutritional factors including high levels of dietary carbohydrate, molasses and urea (Mella *et al.* 1976).

Dietary composition most commonly associated with the development of PEM was high levels of concentrates or low quantities of roughage (Mc Guirk, 1987).

Rice a carbohydrate rich diet forms the main component of concentrate ration for goats. Excess feeding of rice at times of availability often account for majority of ruminal dysfunctions and lowering of pH (Aleyas and Vijayan, 1981) and this possibly created an environment for increased thiaminase activity. This was attributed as the possible predisposing factor in the etiology of PEM in goats in Kerala by Aleyas and Vijayan (1981).

Tanwar and Mathur (1983) induced ruminal acidosis in goats by administering whole wheat grain at the rate of 80, 100, 120 g/kg through rumen canula. There was fall in rumen pH and increase in rumen lactic acid concentration.

Patra *et al.* (1995) produced experimental acidosis in sheep by oral feeding of wheat grains at the rate of 90 g/kg. Dullness, diarrhoea and anorexia developed. Signs were attributed to excessive production of lactic acid and its absorption into the circulation. Brain showed congestion of meningeal vessels. Histologically there was widening of the perivascular space, perivascular cuffing and mild form of neuronal degeneration with satellitosis.

2.3 Symptoms

Jensen *et al.* (1956) observed two degrees of severity of symptoms of PEM in cattle, in the more severe type, the

animals were often found prostrate and comatose. They showed severe muscular tremors, twitching of the ears, eyelids, flacid muscles and convulsions. Bilateral impairment of vision was consistent. Those affected with the sub-acute form, occasionally pushed against solid objects. They were bilaterally blind. Empty masticatory movements, excessive salivation and bulbar paralysis were observed.

Complete anorexia, marked depression, weakness of limbs, progressive ataxia of hind limbs, incoordination of movements, staggering gait, salivation, loss of vision, aimless walking, head pressing, opisthotonos, hyperaesthesia and paddling movements were observed in amprolium induced PEM (Fakhruddin *et al.* 1987).

Tanwar (1987) observed opisthotonos, tonoclonic convulsions, rigidity of forelimbs, clamping of the jaw, absence of menace response, nystagmus, dorsomedial strabismus and paddling movements in goats with PEM. Impaired vision, decreased response to external stimuli and abnormal gait were observed in calves with PEM (Sager *et al.* 1990). Ricardo *et al.* (1991) induced PEM in calves by feeding a semipurified, low roughage diet of variable copper and molybdenum composition. Clinical signs of the disease developed as early as 15 days and included impaired vision, decreased response to external stimuli and lethargy followed

by ataxia, compulsive walking, head pressing and standing in a corner. Ataxia, blindness, dysphagia and death were the symptoms observed by Olkowski *et al.* (1992). McAllister *et al.* (1992) observed stupor, visual impairment and seizures in lambs with PEM. Pugh (1993) noticed recumbency, depression, apparent blindness, head pressing and opisthotonos in a herd of Illamas in PEM. Depression, blindness, sternal recumbency with extended neck and lowered head, bilateral absence of menace response and dorsomedial strabismus were the symptoms observed by Sargison *et al.* (1994) in lambs with PEM. Moro *et al.* (1994) observed apathy, excessive mastication, opisthotonos, blindness and ataxia as the main symptoms. Tanwar and Malik (1995) observed the following symptoms in amprolium induced PEM in buffalo calves. Initial signs were lacrimation, depression, dullness, anorexia, standing motionless for several hours, muscle tremors and abnormal posture and gait. Posture and gait abnormalities were characterized by ataxia, kyphosis and abduction of limbs. In the later stages they observed muscular weakness, blindness, lateral recumbency and convulsions. Menace reflex was absent but pupillary light reflex was present. In a few, they found circling movement, aimless wandering and torticolis. Oedema of the optic disc was observed in six calves on examination with ophthalmoscope. Depression, central blindness and head

pressing, nystagmus, dorsiflexion of the neck or opisthotonos were the symptoms observed in sheep (Low *et al.* 1996).

2.4 Gross and histopathology

Dow *et al.* (1963) observed widespread vacuolation and laminar necoris of the cerebral cortex in sodium sulphate induced PEM in pigs. Christian and Tryphonas (1971) studied lead encephalopathy. They observed brain oedema, swelling and congestion of cerebral cortical tissues. Prominance of capillaries and endothelial swelling, petechial haemorrhages, laminar cortical neuronal necrosis and oedema of the white matter were characteristic. Astrocyte proliferation and microglial accumulation and infiltration of leptomeninges were also seen. The histological changes in all respects were similar to the lesions of PEM. The histological lesions in the cerebral cortex in natural cases of PEM and amprolium induced ones were similar (Morgan, 1974). However, he found that a haemorrhagic diathesis resulting from thrombocytopenia may occur in amprolium induced PEM. Spicer and Horton (1981) observed swelling and cerebral coning together with separation of the cortex from the white matter in amprolium induced PEM. Microscopically the cerebrocortical grey matter showed a range of changes varying in severity from acute neuronal necrosis to almost

complete destruction of the cerebral cortex. There was capillary endothelial hypertrophy and mild perivascular lymphoid cuffing. The white matter was oedematous. Moderate to severe encephalitis characterized predominantly by microgliosis, perivascular lymphocytic infiltration and reactive changes in the astrocytes were present in the white matter of the forebrain and midbrain in pushing disease in cattle (Newsholme *et al.* 1984). Yellowish discolouration of the dorsal cerebral cortex especially the occipital lobes was observed in PEM of goats (Tanwar, 1987). The histological lesion consisted of focal necrosis of the cerebral cortex with shrunken neurons, perineuronal vacuolation and dilation of perivascular space.

Moderate to severe diffuse enlargement of the brain, meningeal hyperaemia, widening of the gyri and moist glistening appearance of the convolutions of the brain were observed in cattle poisoned by *Ficus tsiela* Roxb (Rajan *et al.* 1986). There was moderate diffuse hyperaemia and oedema of the meninges. The Virchow-Robin space was very much distended. Many of the neurons showed degenerative changes characterized by swelling of the neurons and eccentric nucleus. Status spongioses characterized by multiple focal areas of vacuolation either in groups or in a scattered manner were seen in the neuropil. This was more extensive in the white matter of the cerebrum and cerebellum.

Enlarged, soft and spongy brain with yellowish discolouration particularly cerebral hemisphere was observed in PEM (Fakhruddin *et al.* 1987). Focal areas of microcavitation, loose matrix and oedematous brain tissue with congested vessels were the lesions. Enlargement of the perivascular space, congestion of capillaries and occasional prominant swollen vascular endothelial cells were noticed. Neurons were shrunken and in some places they were triangular in outline. A few lymphocytes and monocytes were seen at the perivascular spaces.

Sager *et al.* (1990) noticed laminar areas of cavitation in the cerebrocortical areas in calves. Cerebrocortical and subcortical necrosis with microvascular fibrinoid necrosis predominantly in the thalamic region were the characteristic lesions in sulphate induced PEM (Olkowski *et al.* 1992). Gajendragad *et al.* (1992) studied the pathology of the brain in acute hydrocyanic acid poisoning in sheep. Microscopically vascular congestion, distention of the virchow-Røbin space, cerebral haemorrhage and disruption of the endothelial lining were noticed by them in the cerebrum, hippocampus, pons, thalamus and medulla. Besides this, neuronal nucleus showed various stages of nuclear degeneration, Nissl degeneration, chromatolysis, intracytoplasmic vacuolation, satellitosis and focal glial cell accumulation. Congestion and Purkinje cell degeneration

were also observed in the cerebellum. Swarup and Dwivedi (1992) observed congestion of the blood vessels with occasional evidence of focal hemorrhage, reduction in the density of neurons and degenerative changes with loss of cell outline in lead toxicity in calves. Cerebellum revealed reduction in Purkinje cells. Congestion, haemorrhages in the meninges, swollen central gyri and yellowish discolouration of the cerebral cortex were the gross lesions observed in PEM (Tanwar *et al.* 1993). The histological lesions were limited to the grey matter of the cerebral and cerebellar cortex, caudal colliculi of mid brain and thalamus. In these locations shrinkage of neurons, perivascular and pericellular oedema, necrosis of the neurons, satellitosis, glial nodules and gliosis were observed. They observed thickening of the walls of the vessels, degeneration of the Purkinje cells and bilateral malacia of the caudal colliculi of the mid-brain. In the necrotic areas neuropil was fragmented, oedematous and hypercellular. Neocapillary formation was also prominent. Chahar *et al.* (1993) observed necrotic changes in the cerebral cortex in sheep. Sargison *et al.* (1994) reported widespread diffuse gliosis with occasional neuronal degeneration throughout the brain and spinal cord particularly in the cerebral cortex in PEM in sheep. Laminar cerebro-cortical necrosis and severe bilateral

necrosis of the thalamus and or striatum progressing to cavitation were recognized in calves and lambs with PEM (Jeffrey *et al.* 1994).

Domenech *et al.* (1994) found the following lesions in the cerebellum of African dwarf sheep with CCN. In the most severely affected areas of the culmen and declive of the cerebellar hemisphere folia had become thin because of the disappearance of Purkinje cells. The granular layer was reduced in thickness with a diminished granule cell population.

Cerebral lesions in PEM were characterized by congestion, oedema, microcavitation, laminar necrosis, increased perineuronal and perivascular spaces, neuronal degeneration with shrunken angular and triangular neurons, foci of malacia, extravasation of erythrocytes, gliosis, satellitois, perivascular cuffing and prominance of capillary epithelium confined to the cerebral cortex (Lonkar and Prasad, 1994).

2.5 Ultrastructural pathology

Morgan (1974) studied the detailed ultrastructural pathology of ovine PEM. He classified the lesions into early spongy change and severe malacia of all the cortical elements. The cortex was oedematous. Consistent features of

oedematous cortex were swollen watery astrocytes and satellite cells, many of which showed an apparent increase in nuclear volume. Organelles were dispersed in the electronlucent cytoplasm. Mitochondria were either small and dense or hypertrophied. Smooth endoplasmic reticulum and RER were preserved. Oligodendrocytes were well preserved in the moderately oedematous cortex. Clustered osmiophilic granules were largely seen in the swollen processes of the astrocytes. Myelinated axons were intermittently separated from their myelin sheaths by an electronlucent space. At the areas of severe oedema, many axons showed increased density of the axoplasm. Microtubules were seen clumped in the dendrites. Oedematous neuropil showed a marked increase in the number of glycogen granules. Neurons were compressed and showed variable electron density. Those most affected appeared as electron dense angular bodies. Mitochondria appeared hypertrophic. Many had ruptured to produce crescentic or irregular profiles.

Necrotic neurons showed distension of golgi saccules. Stacks of golgi saccules showed loss of compact arrangement and ultimately appeared as an array of bizzare membraneous fragment and vesicles. The endothelial cells were swollen and bulged into the lumen.

Akai *et al.* (1977) studied the cellular changes at the ultrastructural level in lithium neurotoxicity in non-human primates. The RER and SER showed various degree of dilatation in nerve cells and oligodendroglia, particularly in the cerebral cortex, temporal and frontal lobes, hypothalamus and medulla. The dilated cisterne of RER presented irregular distribution and loss of ribosomes. Some membrane profiles were disorganised and disrupted. The fine structure of the ribosomes appeared, at times well preserved, but at times showed various degree of disorganization with irregular pattern of distribution and variable degree of reduction. Golgi complex frequently showed unusual pattern of distribution. A large number of synaptic terminals displayed qualitative and quantitative variations of the dense core vesicles. Zachary and O'Brien (1985) observed spongy degeneration of the central nervous system in two canines. They found intramyelinic vacuoles with separation of lamellae at intraperiod lines and larger spaces formed by coalescence of ruptured vacuoles. Hypertrophy of the fibrous astrocytes was observed and such cells had abundant glial filaments, oedmatous cytosol, membrane bound crystalline inclusions, dilated cytocavitary systems and abnormal mitochondria.

Sasaki *et al.* (1986) observed spongiform lesions in the grey matter of the cerebrum, mid brain, pons and medulla

oblongata and the white matter of the cerebrum in three scrapic sheep aged 4 to 5 years. Vacuolation and swelling of the astrocytic and oligodendroglial perikarya with enlargement of the endoplasmic reticulum or disappearance of the organelles were observed. Focal clearing was seen in the neuronal perikarya. The clearing appeared to be caused by rarefication of the cytoplasmic matrix, deformation of the endoplasmic reticulum or Golgi apparatus and disappearance of the organelles.

The ultrastructural pathology of neurons and myelin sheaths in bovine spongiform encephalopathy and experimental scarpie in hamsters was studied by Liberski *et al.* (1992). They also compared the lesion with that found in a pan encephalopathic model of Creutzfeldt Jacob disease (CJD). Myelin ballooning, dystrophic axons, phagocytic astrocytes and macrophages were found in all three models but to different degree. Axons containing numerous cellular processes and concentric cisterns were observed in experimental scrapie and CJD. Dystrophic axons contained electron dense pleomorphic inclusions or masses of neurofilaments or both. Dorman *et al.* (1992) found cytosolic oedema of the astrocytes and oligodendroglial cells with disruption and vacuolization of associated myelin, formation of intramyelinic vacuoles and rupture and coalescence of intramyelinic vacuoles into larger

extracellular spaces in cats in Bromethalin rodenticide toxicity.

A novel spongiform myelinopathy of the CNS of eleven African dwarf goats was examined by light and electron microscopy (Obermaier *et al.* 1995).

Ultrastructurally, vacuoles were shown to be intramyelinic, resulting from splitting of the outer myelin or lamellae at the intraperiod line. A few oligodendrocytes showed vacuolar degeneration of cell bodies and processes.

Divakaran Nair *et al.* (1995) studied the ultrastructural changes in the brain of calves in *Ficus tsiela* (Roxb) poisoning. They observed a comparable pattern of ultrastructural changes in different parts of the brain. Structural changes were more pronounced in the neuropil than in the cell body of the neurons. The cytoplasm of neurons and glial cells had an expanded volume. Many neurons of the cerebral cortex and the Purkinje cells showed partial degranulation of ribosomes with the rough endoplasmic reticulum showing fragmentation. Mitochondria showed a variety of changes like swelling, deformed cristae and lysis. Most of the axons showed variable structural alterations. They had prominent and dilated stacks of flat saccules which were separated by electron dense material. The axonal mitochondria were pleomorphic. Many myelinated

axons showed focal lysis. Loosening and splitting of the myelin lamellae and separation from axons by wide spaces were the features observed. Most of these dendrites appeared swollen. The spine apparatus consisted of arrays of well formed smooth walled sacs separated by electron dense material. There was an increase in the swollen mitochondria and vesicular structures. The axodendritic and axosomatic synapses showed a reduction in the number of vesicles. One of the consistent findings was the loosening, separation and fragmentation of the surface membranes of the neuropil both in the cerebrum and cerebellum forming vacuoles and dilated intercellular spaces. The astrocytes and oligodendrocytes also showed varying ultrastructural changes. The cytoplasm of these cells were expanded in volume. The RER was degranulated. Mitochondria showing structural alterations and phagolysosomes containing mitochondrial remnants were seen.

2.6 Autofluorescence

Lee and Little (1980) reported that brain autofluorescence at the area of cortical necrosis could be utilized as a method to diagnose cases of PEM. They opined that the autofluorescence was apparently as a consequence of the degradation of the lipoidal material within the macrophages. Autofluorescence according to them was related

to a high molecular weight collagen like material. The areas of CCN were identified by autofluorescence under the ultraviolet light of fresh or formalin fixed tissue (Markson and Wells, 1982). Ricardo *et al.* (1991) found cerebro-cortical laminar cavitation and a creamy white autolfuorescence under the UV illumination of the brains of calves in induced PEM. Gould *et al.* (1991) observed blue green cortical ribbons of autofluorescene in calves with signs of PEM when illuminated with UV light. Autofluorescent lesions in the cerebro-cortical grey matter were present twenty-four hours after sulphide administration (Mc Allister *et al.* 1992). Formalin fixed brain slices of amprolium fed calves showed disseminated areas of greenish yellow autofluorescence in the cerebral cortex when viewed under UV light at 365 nm (Tanwar *et al.* 1993). Sager *et al.* (1996) observed spontaneous yellowish green fluorescence in the cortical areas in calves in induced PEM by feeding a semipurified diet deficient in thiamine.

2.7 CSF changes

Rebhun and deLahunta (1982) reported that examination of CSF was a valuable diagnostic aid for bovine listeriosis. They observed an increased number of white blood cells and increased protein values. Sorjonen (1987) opined that the albumin quota and total CSF albumin values could be used as

indicators of blood brain barrier disturbance. Sundar *et al.* (1992) observed a significant elevation in glucose, protein, pyruvate, lactate, total cell count and SGOT in PEM affected calves. Scott (1992) analysed CSF samples from normal sheep and from cases of certain neurological disorders. He observed a significant increase in the group mean CSF protein concentration in meningitis, listeriosis and spinal abscesses, but not in PEM. Swarup and Dwivedi (1992) studied the biochemical changes in blood and CSF in experimental lead toxicity. The changes in the CSF included hyperglycorrhacia and increased urea nitrogen and creatinine levels on day 42. Positive Pandy's test and higher leucocyte count were seen in CSF. They observed no changes in the physical characters.

Changes in the cell count and total protein concentration of CSF in animals with rabies were not diagnostic or characteristic (Green and Smith 1992). Braund *et al.* (1993) observed an increased protein concentration in the CSF sampled from the atlanto-occipital and lumbosacral spaces in lambs with congenital hypomyelination neuropathy. Scott (1993) opined that examination of cell counts and protein concentration in CSF was of little value in the diagnosis of slow viral encephalopathies of ruminants and infection by the agents causing bovine spongiform encephalopathy.

Sargison *et al.* (1994) observed normal CSF values in lambs with PEM. The electrolytes sodium, potassium, calcium, phosphorus and magnesium did not show any change in the CSF of calves in PEM induced by amprolium (Tanwar *et al.* 1994). A significant increase in lactate and pyruvate concentration was noticed.

Materials and Methods

MATERIALS AND METHODS

3.1. Prevalence

To study the prevalence of PEM, a proforma was prepared and sent to 200 randomly selected Veterinary Hospitals in the state covering all the fourteen districts and the responses were collected. The number of hospitals covered in each district were as follows. Trivandrum (14), Quilon (14), Alleppey (17), Pathanamthitta (8), Idukki (7), Kottayam (18), Ernakulam (19), Thrissur (16), Palakkad (22), Malappuram (13), Kozhikode (17), Wynad (12), Cannanore (1) and Kasaragod (17). The data procured were for a period of four years from 1991 to 1994. The Veterinary Surgeons were requested to fill up the proforma after going through their case register. They were also advised to send back the proforma with a 'nil' statement if they were not able to find any information from the relevant registers. The data obtained were analysed statistically and the extent of the disorder was assessed.

3.2 Experimental studies

The effect of amprolium, amprolium and rice gruel, rice gruel, Benzene hexa chloride (G isomer of BHC), sodium sulphate and *Ficus tsiela* Roxb (Chela leaves) on the brain

of goats were studied as per the schedule shown in the Table 1.

Forty-two goats were utilized for the experiment. The cross-bred male goats were procured from the University Goat Farm, Mannuthy and dewormed. The animals were in the age group of 3-4 months weighing on an average 8 to 9 kilogrammes. They were maintained on standard concentrate and roughage diet for a week. The animals were examined for the presence of any disease. After a week the animals were divided into seven groups containing six animals each. Six groups were subjected to treatment as shown in the table and one group served as the control.

Table 1. Details of the experimental schedule

	Chemical/ plant	Initial dose	No. of animals	Age	Period of treatment
I	Amprolsol (Amprolium soluble powder 20%)	350 mg/kg	6	3-4 months	45 days
II	Amprolsol + rice gruel	350 mg/kg rice gruel *ad lib*	"	"	"
III	Rice gruel	*Ad lib*	"	"	"
IV	BHC (isomer of BHC)	2.5 mg/kg	"	"	"
V	Sodium sulphate	150 mg/kg	"	"	"
VI	*Ficus tsiela* Roxb tender leaves (Chela leaves)	50 g/animal	"	"	"

3.2.1 Group A (Amprolium)

Amprolium soluble powder USP 20 per cent (Merind Limited, Bombay) was administered after mixing thoroughly in water as per the dose shown.

3.2.2 Group B (Amprolium and rice gruel)

Amprolium was orally administered to six animals as per the dose given in the table. These animals were fed with rice gruel *ad libitum*. The total amount of raw rice used for the preparation of rice gruel for six animals was 2.25 kg. The standard concentrate diet was restricted throughout the experiment. Grass and water were given *ad libitum*.

3.2.3 Group C (Rice gruel)

The rice gruel was prepared out of 2.25 kg of raw rice by boiling. *Ad libitum* rice gruel was fed to the animals every day till the end of the experimental period. The standard concentrate ration was restricted to 50 per cent of the daily ration.

3.2.4 Group D (BHC - isomer of BHC)

Fifty per cent emulsifiable powder procured from Keyes Agro Industries, Salem was dissolved in water and the dose was administered as per the schedule (Table 1). Six animals

were administered the solution with a syringe followed by 20 ml of water to ensure that the entire amount has gone into the rumen. The animals were observed for symptoms and the dose was increased to 5 mg/kg on the 10th day. Concentrate and water were given *ad libitum*.

3.2.5 Group E (Sodium sulphate)

The chemical was procured from the Nice Chemicals, Edappally, Ernakulam. The solution was prepared in water and drenched to six animals at the rate of 150 mg/kg body weight for the first nine days and on the 10th day the daily dose was increased to 500 mg/kg and the same dose was maintained up to the end of the experiment. Water, concentrate and green grass were given *ad libitum*.

3.2.6 Group F (*Ficus tsiela* Roxb (Chela)

Fresh tender leaves procured locally were given daily at the rate of 50 g/kg body weight. The animals were observed for any symptoms and since the animals relished it, on the 10th day onwards *ad libitum* quantity of leaves (6-7 kg of leaves for six animals) were provided and continued upto 45 days. Only 50 per cent of the concentrate was given and no green grass was provided to animals in this treatment group.

3.2.7 Group G (Control)

Six healthy animals in the same age group served as the control for the six sets of experiments. They were maintained on standard diet.

3.3 Group H (Natural cases)

Animals which were suffering from PEM and not responding to thiamine treatment, yet diagnosed as PEM by the veterinary surgeons were purchased, observed for symptoms, sacrificed at the extremis and materials were collected for histopathology and ultrastructural studies. The weight of the animals was recorded and CSF was collected before sacrifice. After autopsy the brain was weighed and detailed examination of the brain was made. The histopathological and ultrastructural changes of the brain were compared with the experimental cases.

3.4 Observation for the clinical sings

All the animals in each treatment group were thoroughly examined at each dosing and several times throughout each day for the appearance of clinical signs.

A subjective assessment of vision was made which included response to fingers flicked in front of each eye, head and eye reaction in response to movement of persons.

3.5 Weight of the animals

All the animals were weighed before the treatment and at fortnightly intervals. The group mean weight was analysed statistically and compared with the control.

3.6 CSF collection and evaluation

Prior to the start of the experiment and immediately before sacrifice CSF was collected from all the animals.

The site was the midline position midway between the last palpable lumbar dorsal spine and first palpable sacral dorsal spine. The animal was restrained in sternal recumbency with the hips flexed and the pelvic limbs held extended along the abdomen. The site was prepared and local anaesthetic, xylocaine injected subcutaneously. The interarcuate ligament presented considerable resistance to the passage of the needle followed by a 'pop' as the needle entered the epidural space. The CSF was collected by attaching a syringe to the needle hub. The CSF so collected was subjected to physical examination and the total protein concentration was evaluated by the modified Biuret method (Inchiosa, 1964). The data were analysed statistically (Snedecor and Cochran, 1967) and compared with the control.

3.7 Sacrifice and collection of samples

Animals were sacrificed by exsanguination in extremis and also at the termination of the experimental period. Post-mortem examination was performed and the brain was subjected to detailed examination. The brain and the cervical part of the spinal cord after separation from the cranial cavity were preserved immediately by immersion in 10 per cent buffered neutral formalin. After 24 h of fixation sections were sliced across all the brain tissue to ensure examination of a wide range of neuroanatomical structures. Several regions of the CNS including the anterior, middle and caudal parts of the cerebral hemispheres, the hippocampus, caudate nucleus, mid brain, thalamus, cerebellum, pons, medulla oblongata and the cervical region of the spinal cord were further fixed in neutral buffered formalin.

3.8 Histopathological studies

The sections were stained with routine Haematoxylin and Eosin (Sheehan and Hrapchack, 1980). Toluidine blue, phosphotungstic acid haematoxylin (PTAH), Kluver-Barrera method for myelin and nerve cells and Glees and Marsland's modification of Bielschowskey's were employed wherever necessary to representative sections for further elucidation

of neuropathological changes (Kluver and Barrera, 1953; Luna, 1960, Sheehan and Hrapchak, 1980).

3.9 Ultrastructural studies

Immediately after sacrifice, tissues from different parts of the brain were collected for electron microscopy. Tissues were collected from the cerebral grey and white matter, cerebellar grey and white matter, pons, medulla oblongata and spinal cord. Tissues were fixed immediately in 2.5 per cent phosphate buffered glutaraldehyde, rinsed in phosphate buffer and post fixed in one per cent Osmium tetroxide. The tissue blocks were processed through graded acetone series and embedded in spurr. Sections were cut with glass knives in a Leica ultracut. Thin sections were picked up on uncoated copper grids, stained with uranyl acetate and lead citrate and examined in a Hitachi 600 A electronmicroscope at an accelerating voltage of 50 KV.

3.10 Weight of the brain

Immediately after sacrifice the brain was carefully separated from the cranial cavity and weighed. The average brain: body weight ratio was subjected to analysis and compared with the controls.

3.11 Autofluorescence

Neutral buffered formalin fixed coronal slices of the brain from different regions of the cerebral hemisphere were examined for autofluorescence in a UV cabinet at 360 nm wavelength (Markson and Wells, 1982).

Results

RESULTS

4.1 Prevalence of PEM

A detailed proforma was prepared and sent to 200 randomly selected veterinary hospitals in the state covering all the fourteen districts. The data requested were for a period of four years from 1991 to 1994. The information requested included the number of cases recorded in these years and the month-wise distribution of the disease. Information was also sought on the etiology suspected, symptoms manifested, the age group affected, sex affected and other detailed information on various neurological disorders encountered with particular reference to PEM.

Out of the 200 Veterinary Hospitals, only 76 had data available on PEM and they had been properly recorded in the register. Not even a single case was recorded in 49 hospitals as they reported the incidence to be nil in the proforma. No information was received from 75 hospitals.

The number of cases reported from the 76 Veterinary Hospitals of the fourteen districts are set out in Table 2. A progressive increase in the number of cases could be observed from the year 1991 to 1994 (Fig.1). The total

number of cases recorded in 76 hospitals varied from 683 in the year 1991 to 1047 in 1994.

Since the number of hospitals was not uniform (Table 1), the cases recorded were calculated for one hospital and the percentage prevalence in each district for the year 91 to 94 was calculated and depicted in the diagram (Fig.2). The highest prevalence of PEM was recorded at Malappuram district followed by Ernakulam, Kottayam and Calicut districts.

The month-wise prevalence of the disease was analysed statistically and significant difference was found in the prevalence in certain months. The data are depicted in Fig.1a. The disease was recorded throughout the year and there was no month in which the disease was not recorded. From the diagram it is seen that the highest maximum incidence occurred in the first five months of which the peak incidence was noticed in the month of April. This was followed by January, February, March and May. The incidence was also significantly high in the month of August, September and October with the peak in the month of October. Comparatively low incidence was observed in the months of June, July, November and December.

The response of the veterinarians of the 76 Veterinary hospitals with regard to certain queries was as follows:

The cause of the condition was reported to be deficiency, particularly of thiamine. Forty-three out of the 76 had this opinion. The observation by them was based on the effectiveness of the treatment that thiamine administration in the initial phase of the problem cured the condition. Plant poisoning was suspected by 21 veterinarians. The plants suspected were *Anamritha cocculus*, *Ficus tsiela* Roxb B (Chela), Kavath and Bracken fern.

Eight suggested the cause to be chemical poisoning, but did not suggest any specific one. One attributed the cause to poisoning by organochlorine compounds which are being used indiscriminately for pest control.

Four of the veterinarians suggested the cause to be either chemical poisoning, plant poisoning, thiamine deficiency or a combination of these factors.

Twelve among the entire group of 76 again stated that unreliable feeding practices and administration of only rice gruel during the time of maximum availability caused the condition of PEM.

Sixty of the 76 Veterinary Surgeons who have recorded PEM reported the incidence to be more in the female. Thirteen found the incidence more in males and three said both sexes were equally susceptible.

The age of the affected animals, as per the opinion and observation of 47 veterinarians was [illegible] one year and above. Twenty-one observed it to be more between six months to one year and the other eight reported the maximum occurrence in the age group of 3-6 months.

Twenty-eight veterinarians reported that the administration of thiamine in the early stages cured the condition. Eighteen informed that thiamine treatment was not effective as it gave erratic results. Twenty of them had no opinion about a specific treatment. They tried vitamin B1, Dextrose, liver tonics and calcium and found little success.

As an answer to the question about the areas to be focussed for further studies, majority of them suggested detailed investigations on the etiology and associated pathological alterations in the brain and the treatment modalities to be followed.

Table 2. District-wise distribution of PEM cases (data from 76 Veterinary Hospitals) - Number of cases/number of Hospitals

District	1991	1992	1993	1994
Trivandrum	31/5	38	39	43
Quilon	27/4	29	20	19
Pathanamthitta	9/2	17	21	21
Idukki	2/1	4	5	8
Kottayam	96/6	103	82	27
Alleppey	8/5	18	46	47
Ernakulam	48/5	59	149	136
Thrissur	69/7	72	93	96
Palakkad	34/8	53	77	111
Malappuram	113/6	110	163	132
Calicut	129/10	143	151	162
Wynad	37/6	40	39	111
Cannanore	68/8	87	106	121
Kasaragod	12/3	10	16	23
	683	784	1007	1047

Fig.1: Prevalence of PEM during the period 1991-1994 (Data from 76 Veterinary Hospitals)

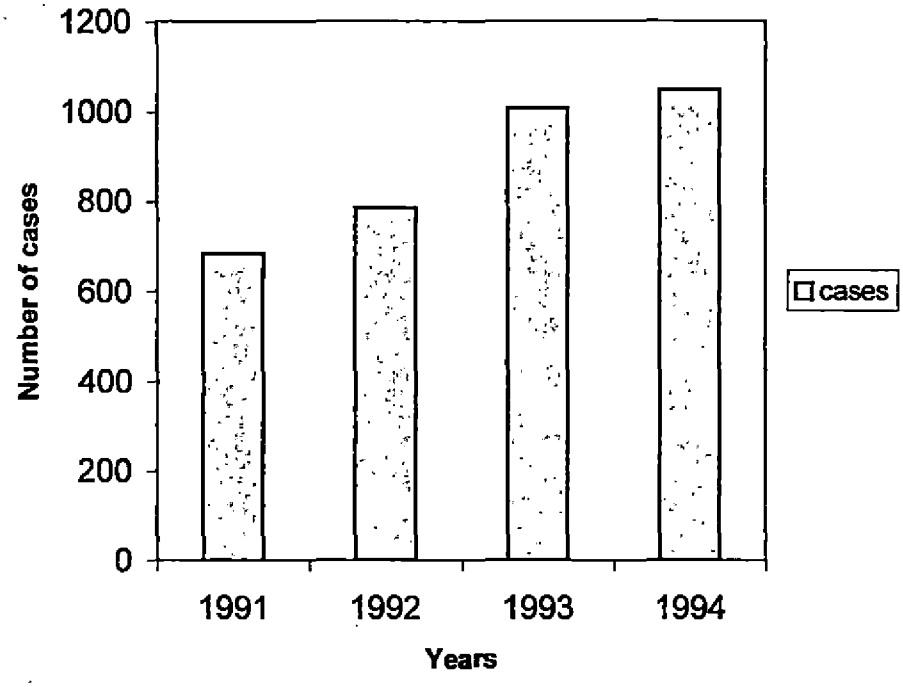


Fig.1(a) : PEM during 1991-94 (Average monthly prevalence at 76 Veterinary Hospitals)

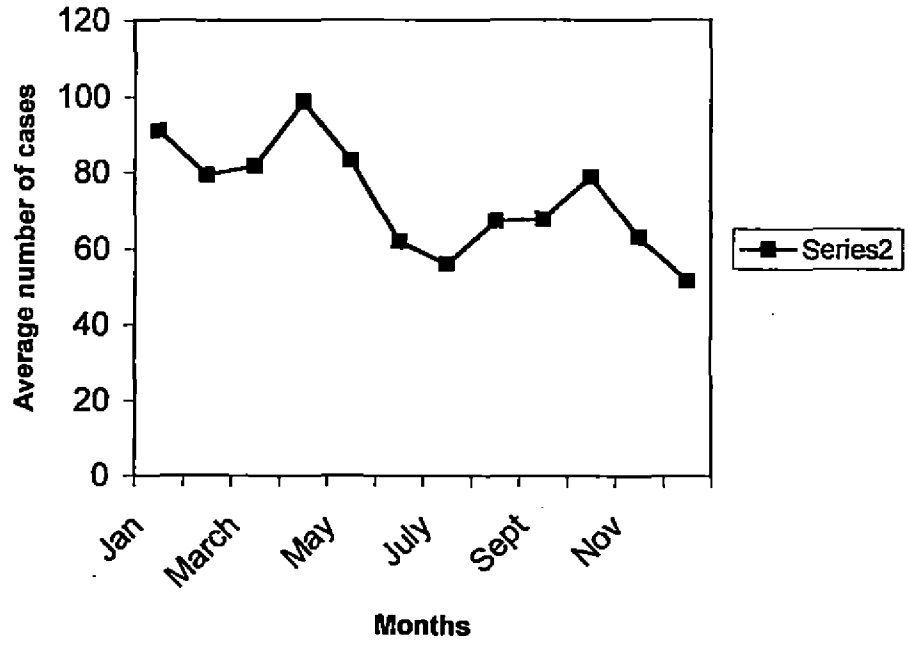
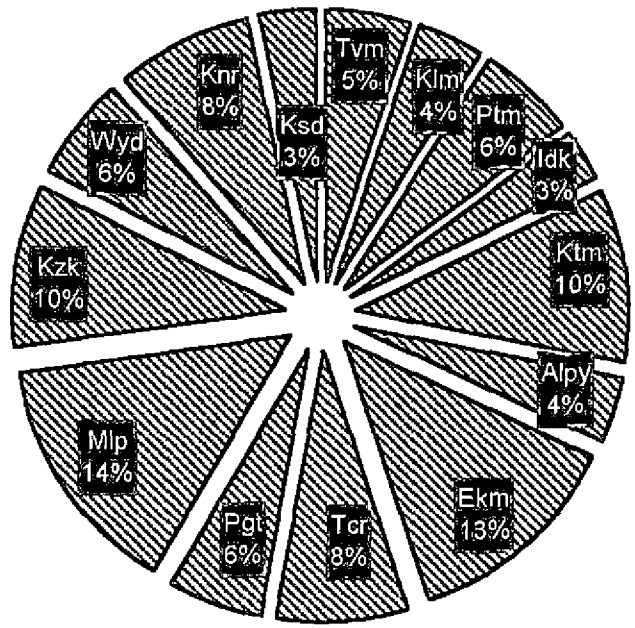


Fig.2: District-wise prevalence of PEM during the period from 1991-1994



Tvm = Trivandrum
Klm = Kollam
Ptm=Pathanamthitta
Idk = Idukki
Tcr= Trichur
Pgt= Palghat
Mlp= Malappuram
Kzk= Kozhikode
Ktm= Kottayam
Alpy = Alleppey
Ekm= Ernakulam
Wyd= Wayanad
Knr= Kannur
Ksd= Kasaragode

Group A (Amprolium)

4.2 Clinical signs

There were six animals in the group. None of the animals developed any symptoms of nervous disorder. All the animals were found to be active till the termination of the experiment.

4.2.1 Weight of the animals

This is depicted in Fig.3 and also shown in the Table 3. The weight was significantly different throughout the experimental period which remained low as compared to the controls. There was no significant difference in the pre and post treatment weights.

4.2.2 Cerebrospinal fluid

The fluid was clear in all the cases. The flow was slow. The total protein concentration remained low and both the pre and post exposure values were significantly different from that of the controls (Table 4 and Fig.4).

4.2.3 Weight of the brain

The average brain body weight ratio was compared with that of the control. The values as compared to the control did not show any significant difference. The data are shown in Table 5.

4.2.4 Brain autofluorescence

No autofluorescent foci could be detected in any part of the cerebral cortex.

4.2.5 Gross lesions

Examination of the brain did not show any abnormalities. All the internal organs appeared normal.

4.2.6 Histopathology

Though the cerebral cortical neurons in all the segments remained normal, occasionally in certain segments there was neuronal necrosis and neuropil vacuolation. The external granular and external pyramidal neurons, and the internal pyramidal neurons were involved. There was perineuronal and perivascular oedema and gliosis (Fig.5). Generalised congestion was also seen in the cerebellum, pons, medulla oblongata and spinal cord. The white matter of the cerebrum in all the animals remained intact.

Occasionally in one or two animals there was diffuse vacuolation (Fig.6).

4.2.7 Ultrastructural pathology

Most of the neurons of the cerebral cortical laminae remained normal. But occasional neurons had structural changes in the cytoplasmic organelles and nuclei indicative of necrobiotic processes. Clumping and margination of chromatin appeared in such neurons. There was disaggregation of polyribosomes and they appeared as irregular clumps in an expanded cytoplasmic matrix. A few distended cisternal profiles were seen and mitochondria appeared in between as homogenised electrondense structures.

Glial cells often appeared swollen and had an expanded cytoplasm wherein the organelles were not discernible. In most of the areas the astrocytes remained normal within the cortex and also in the white matter. Oligodendrocytes appeared as compact structures along the course of the fibers as well as in the cerebrocortical grey matter. Glial cells mostly microglia with swollen nuclei were seen in the cerebrocortical neuropil and also in the white matter (Fig.7).

Structural changes were not observed in the cerebellar neurons. The Purkinje cells remained intact with regular nuclear membrane in foldings. The granule layer was compact.

Both the myelinated and non-myelinated axons of the white matter and cortical neuropil were moderately affected. Axons showed splitting of myelin sheath, swelling and loss of axoplasm (Fig.7 and 8). In some other areas of the white matter the axons were of normal size and contained a usual component of microtubules, neurofilaments and mitochondria. The myelin sheath appeared thin.

Table 3. Group mean body weight (kg) at fortnightly intervals

Group	Days			
	0	15	30	45
Amprolium	8.417± 0.52	8.467± 0.52 *	8.517± 0.54 *	8.500± 0.54 *
Amprolium + rice Gruel	8.867± 0.51	9.033± 0.87 *	9.217± 0.86 *	9.330± 0.99 *
Rice Gruel alone	10.017± 0.21	10.750± 0.31 *	10.883± 0.33	11.033± 0.33
BHC	8.375± 0.71	8.917± 0.67 *	8.433± 0.85 *	8.167± 0.86 *
Sodium sulphate	7.808± 0.89	8.017± 0.76 *	7.958± 0.86 *	8.567± 0.91 *
Chela	8.658± 1.05	8.233± 0.94 *	9.467± 1.15 *	10.383± 1.09 *
Control	11.733± 0.57	12.967± 0.88	12.933± 0.92	13.050± 0.98

* Significantly different at 5% level ($P<0.05$)

Table 4. Average pre and post exposure CSF protein concentration (mg/dl)

Amprolium	Pre :	6.575 ± 0.44*
	Post :	6.715 ± 0.30*
Amprolium + rice gruel	Pre :	7.793 ± 0.90
	Post :	7.955 ± 1.15
Rice gruel alone	Pre :	9.290 ± 0.57
	Post :	8.400 ± 0.74
BHC	Pre :	8.533 ± 0.76
	Post :	8.667 ± 0.70
Sodium sulphate	Pre :	9.798 ± 0.90
	Post :	10.440 ± 0.82
Chela	Pre :	8.700 ± 0.84
	Post :	9.338 ± 0.72
Control	Pre :	10.323 ± 0.66
	Post :	10.430 ± 0.68

* Significantly different at 5% level ($P<0.05$)

Table 5. Mean brain: body weight ratio (g/g)

Amprolium (Group A)	0.00880 ± 0.00082
Rice gruel + Amprolium (Group B)	0.00876 ± 0.00082
Rice gruel (Group C)	0.00705 ± 0.00016
BHC (Group D)	0.00952 ± 0.00122
Sodium sulphate (Group E)	0.00935 ± 0.00082
Chela (Group F)	0.00761 ± 0.00041
Control (Group G)	0.00696 ± 0.00041

The mean brain : body weight ratio was estimated in grams and the same was compared with the control. On analysis the mean brain:body weight ratio of the treatment and control showed no significant difference.

Fig. 3 : Average weight at fortnightly intervals

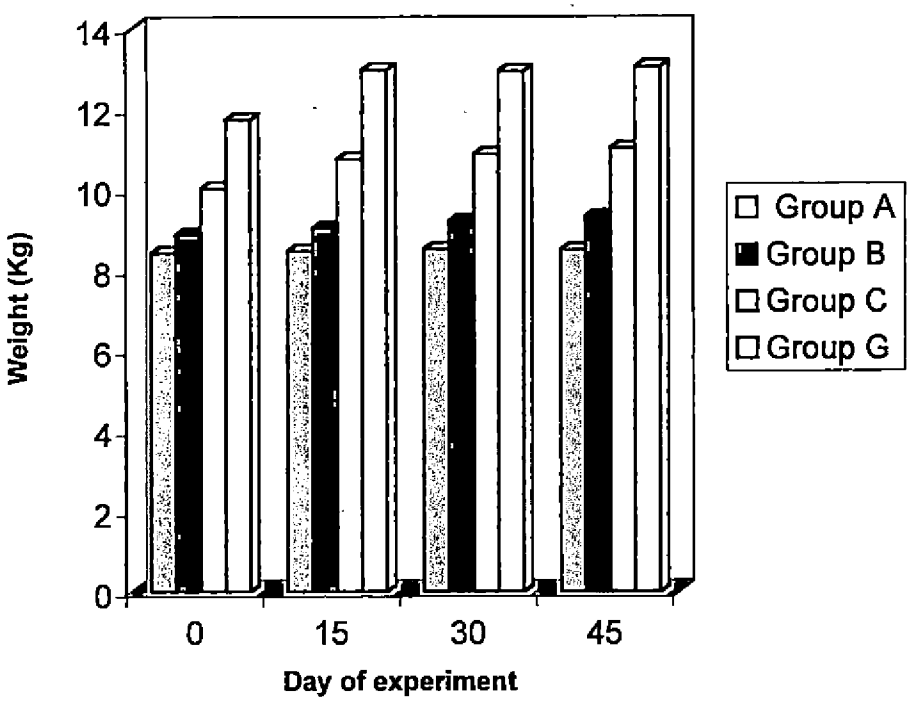


Fig.4 : Average CSF protein concentration (mg/dl)

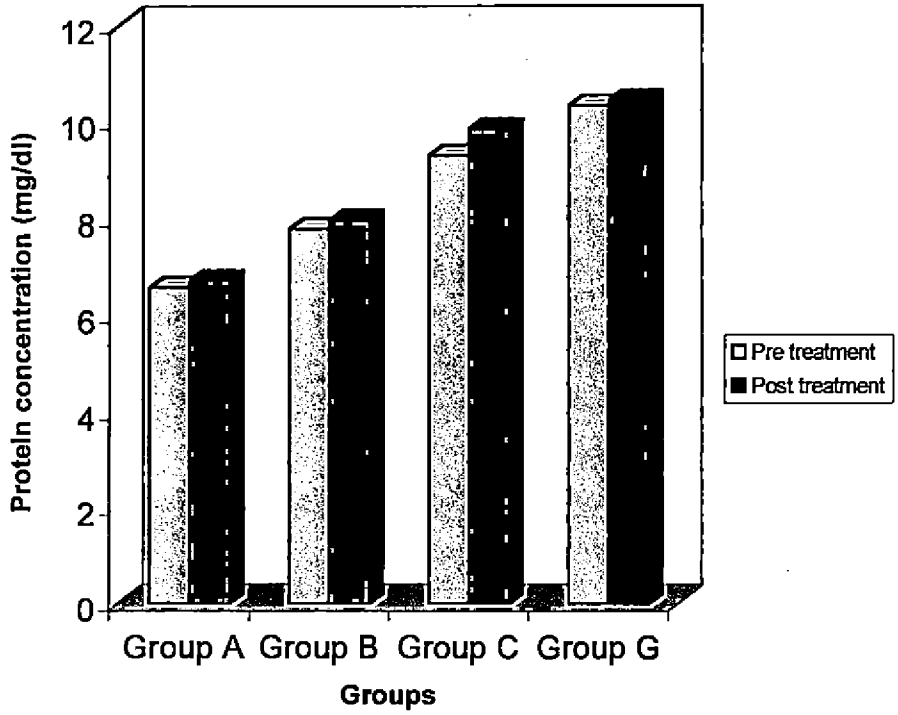
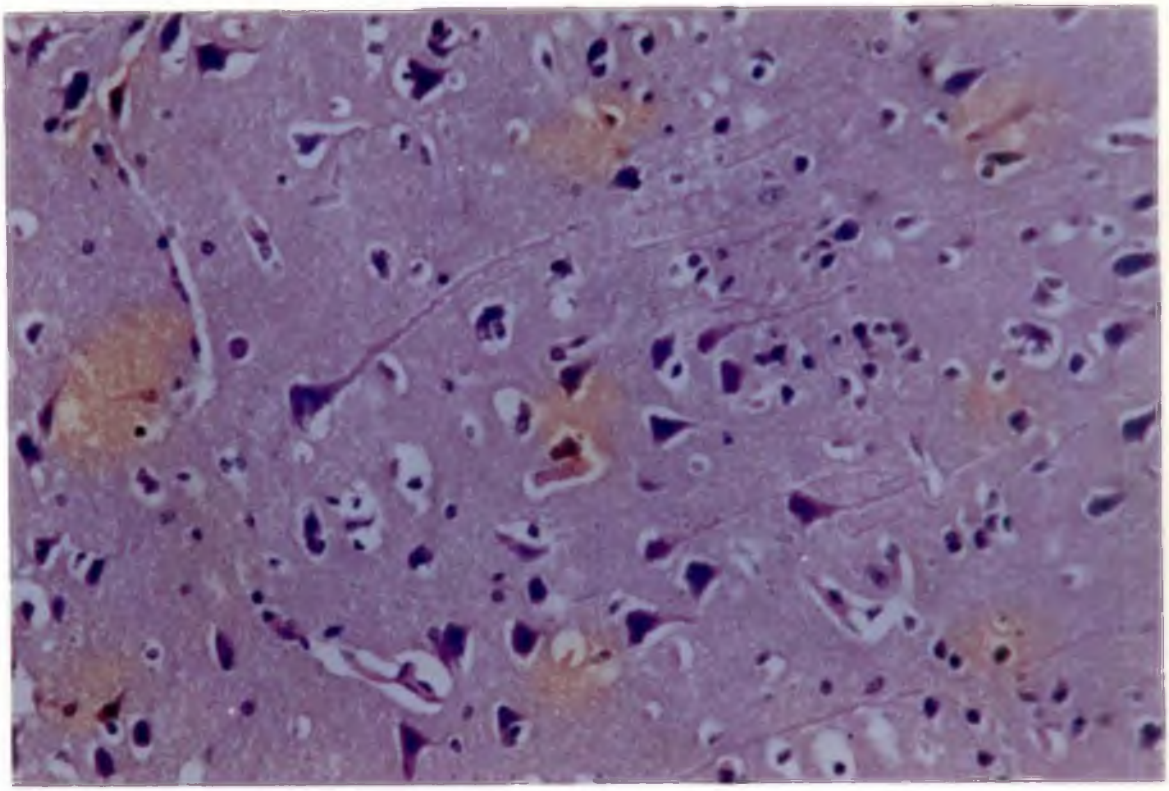


Fig.5. Group A. Cerebral cortex: deeper cortical lamina-internal pyramidal layer-perineuronal and perivascular oedema and gliosis. H&E x 400

Fig.6. Cerebrum : White matter-diffuse vacuolation. H&E x 250



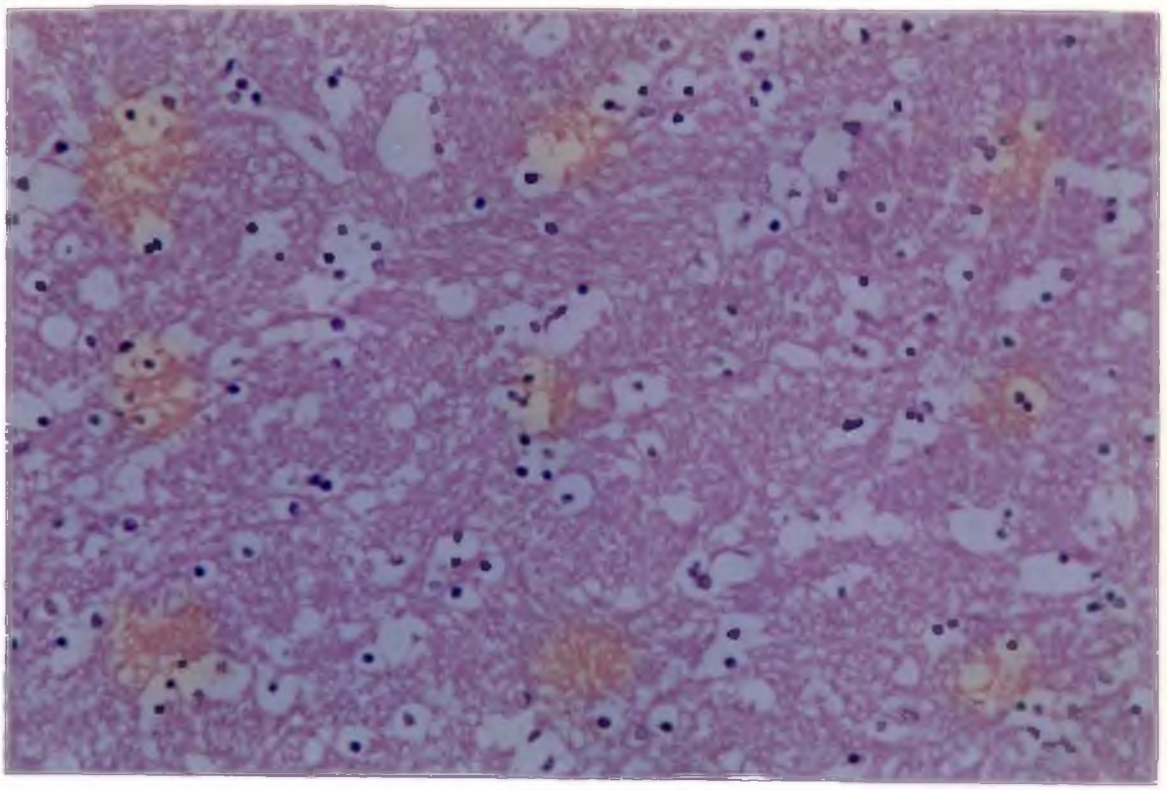
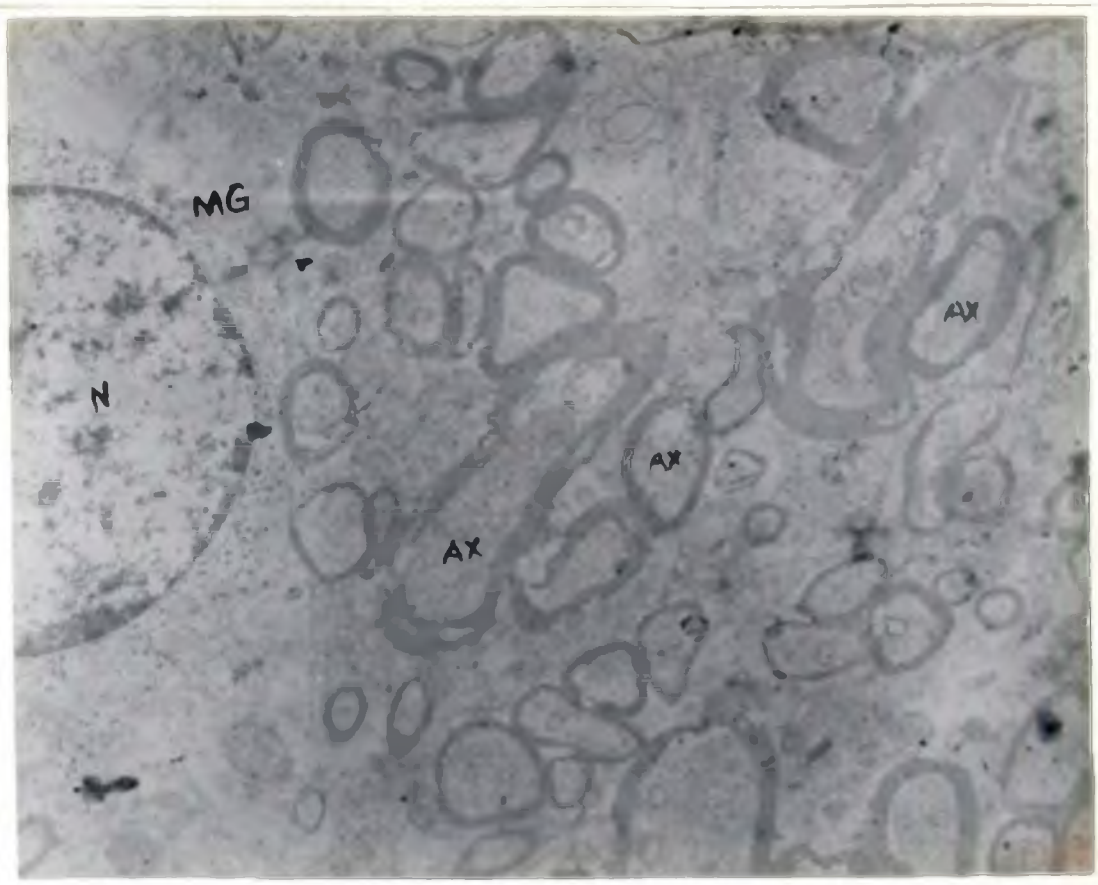
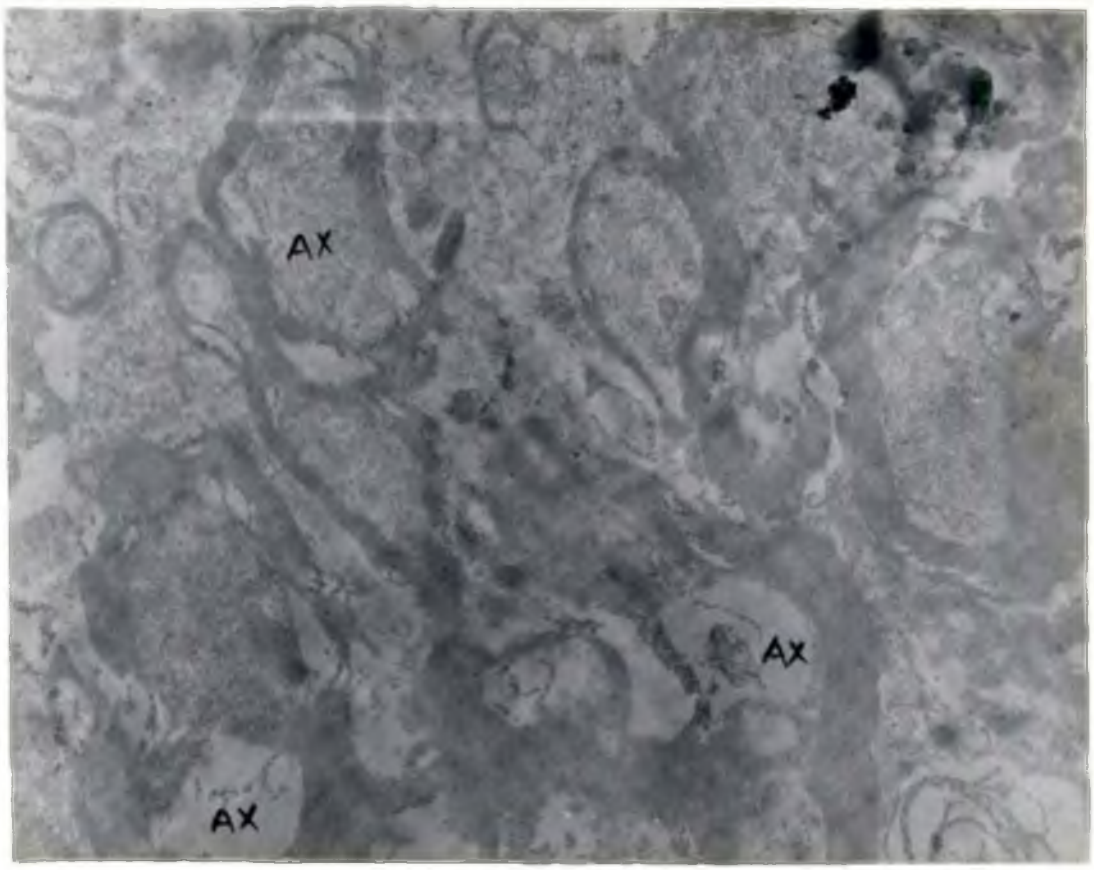


Fig.7. E/m: White matter showing a microglia (MG) with swollen nucleus(N). Axons show splitting of myelin sheath (MS) and swelling and loss of axoplasm x 10,000

Fig.8. E/m white matter showing severally damaged axons (AX) x 16000


MG
N
AX
AX
AX


AX
AX
AX

Group B (Amprolium and rice gruel)

4.3 Clinical signs

Out of the six animals, two developed moderate neurological disturbances. The disorders were not progressive. Both of these animals manifested the symptoms abruptly on day 39. They had intermittent diarrhoea. Abdomen was very much distended. Frequently they lied down and got up. Nystagmus and intermittent convulsions were observed. The animals could not bear weight on their limbs and preferred to lie down at the height of the disorder. The neck was stretched forward and the animals had dyspnoea. These symptoms remained for few hours and afterwards the animals became alert and started drinking water. On the subsequent days the animals appeared dull and depressed. There was reduction in the feed consumption. Occasional tremors were seen on the subsequent days. The symptoms never aggravated. The eye preservation reflex was normal. The animals remained dull until they were sacrificed on the 45th day. The other four animals too were inactive, but were feeding normally. They remained alert and did not show any discomfort.

4.3.1 Body weight

The body weight is depicted in the graph (Fig.3) and the mean group average is given in the Table 3. On statistical analysis of the groups means, the body weight appeared significantly less as compared to the control on the 15th, 30th and 45th day.

4.3.2 Cerebrospinal fluid

The CSF was collected before the start of the experiment and also on the day of sacrifice. The fluid was clear in all the animals on the pre-exposure period. The fluid was clear in two cases and all the other four cases the fluid was mixed with blood when collected on the day of sacrifice. On centrifugation the fluid appeared homogeneously lightly pink staining. No coagulation was observed. The mean pre and post-exposure protein concentration did not show any significant variation from the controls. The data are presented in Table 4 and depicted in the Fig.4.

4.3.3 Weight of the brain

The group mean brain weight per gram of body weight was 0.00876 ± 0.00082 and this was not significantly different from the control (Table 5).

4.3.4 Autofluorescence

Samples of cerebral segments fixed in buffered formalin were examined under UV from both the cases. Autofluorescent lesions were not evident in any of them.

4.3.5 Gross lesions

The meningeal covering of the brain of the two affected animals showed generalised congestion. Focal areas of gelatinous changes in the cortex were observed in one of them. Loss of glistening appearance, and moderate swelling of the few gyri at the caudal cerebral hemisphere on both the sides were observed.

Flabbiness of the heart, pulmonary congestion, greyish white diffuse spots of necrosis of the liver, petechiae in the kidney and distention of the rumen were the other lesions noticed. There were no gross lesions in the other four animals.

4.3.6 Histopathology

The neuropathological changes in the affected ones consisted of generalised congestion of different parts of the central nervous system, diffuse neuronal degeneration in different cerebral cortical regions, vacuolation and astrocytic reaction.

Meningeal congestion was observed in both the affected animals (Fig.9). The molecular layer remained intact, but the cellularity was little more. Capillary structures were prominent. Different regions of the cerebral cortex showed lesions of varying type starting from the involvement of a few neurons to a large number of neurons both at the crown of the gyrus and sulcal depression. Lesions in the cerebral cortex were more or less diffuse. A number of neurons in the superficial and mid laminae of the cortex appeared swollen. The nucleolus was absent in many and the whole structure appeared as circumscribed round vesicular bodies where in the chromatin material was seen diluted or had disappeared. Luxol fast blue stained structures showed swollen neurons with loss of nucleolus and narrow rim of violet staining homogenous perikaryon (Fig.10). Shrunken neurons with eosinophilic cytoplasm and basophilic, pyknotic eccentrically placed nucleus and astrogliosis were also seen. There was often grey matter neuropil vacuolation, and they were seen mostly at the deeper cortex and cortico-medullary areas. Astrocytic reaction was more in a few of the cerebral gyri and a few of the neurons were seen fading away into the

substance of the neuropil (Fig.11). Dark neurons were also seen surrounded by widened perineuronal space. Some of the astrocytes were swollen, others condensed leaving space around it indicating a cytoplasmic dissolution. Diffuse microglial reaction, congestion of vessels, wide dilatation of the perivascular space, oedema and cyst like cavitation were observed in the grey matter neuropil. Many of the neurons surrounding the vessels had degenerated. Moderate astrogliosis was also noticed. There was perineuronal vacuolation and spongy change in the brain of one of them. The neurons involved were of the pyramidal type. The cell body was shrunken and eosinophilic (Fig.12). Nucleus of the neurons appeared condensed and appeared dark blue with phosphotugstic acid-haematoxylin stain (PTAH). Linear vacuolation was seen extending along the dendrites (Fig.13). The astrocytes in the neuropil had pericellular vacuolation.

The cortico-medullary neurons of the cerebral hemispheres appeared as highly condensed hyperchromatic structures. The cortical white matter immediately following this layer showed congestion of vessels.

The cerebral white matter showed occasional vacuolations involving focal areas and group of fibers. Most of the small vacuoles were coalesced forming honeycomb like cavitations. This contained a few microglial cells with elongated nuclei and vesicular oligodendroglia. A few gitter cells also were seen (Fig.14). The white matter deeper to the ependymal lining of the corpus callosum of the ventricles revealed congestion of vessels and pronounced haemorrhage (Fig.15). The neuropil in these areas surrounding the congested vessels appeared sieve like.

The choroid plexus in one case revealed vacuolar degeneration of the lining cells and congestion of vessels. The caudate nucleus showed degeneration of groups of neurons in the amorphous layer. Most of them were condensed. Neuronal degeneration, satellitosis and neuronophagia were seen in the sub-nuclear neuropil. Some of the neurons appeared homogenous and pale staining indicating chromatolysis.

Congestion, oedema and haemorrhages were seen in the molecular layer of the cerebellar folium. Many Purkinje cells were shrunken. Widespread loss of Purkinje cells and

spongy degeneration of the white matter of the cerebellum were also observed.

There was dilatation of the central canal of the spinal cord and the ependymal cells were swollen. The canal was filled with pale staining CSF. Congestion was noticed. Endothelial cells of the vessels were damaged. Gliosis of the grey column was also seen. Congestion was also observed in the medulla oblongata and some of the larger neurons in it showed chromatolysis.

Occasional degeneration of the brain stem nuclei, congestion of vessels and focal areas of haemorrhage in the thalamic area were noticed. Diffuse haemorrhages were present in all the CNS components.

There was pronounced malacic changes in the neuropil of the pons in one of the affected animals (Fig.16) Congestion was also observed. Spinal cord grey column revealed extensive gliosis, congestion of vessels and diffuse haemorrhage.

The hippocampus of the animals which showed non-progressive neuronal signs revealed loss of cells from the fusiform layer, indicating a loose texture and an increased glial cell reaction and invasion in those areas. Sub

hippocampal neurons showed degeneration, necrosis and satellitosis. A few of the neurons were well preserved.

Diffuse neuronal degeneration was also noticed in few areas of the brain in those animals which were unaffected with the treatment. Diffuse collections of dark staining neurons were seen amidst normal neurons in few of the gyri in those cases. The lesions were confined to the cerebral cortex and the mid laminar granule cell layer showed more pronounced changes. The upper and deeper laminar neurons remained intact. The white matter in a few cases showed vacuolations. The lesions in the hippocampus mentioned previously were more or less constantly present in all the six cases, although mild in nature.

Hepatocyte degeneration, vacuolar changes in the lining cells of the kidney tubules, congestion of the kidney, congestion and focal haemorrhage in the lungs were the other extra neuronal lesions observed.

4.3.7 Ultrastructural pathology

The neurons of the cerebral cortex showed almost uniform pattern of changes. Neurons of all the cortical laminae were found affected including the pyramidal type to the innermost mixed type neurons of the fusiform layer. Ultrastructural changes leading to necrosis were evident in

many of these cells. The neuropil and glial cells also showed definite retrograde changes.

4.3.7.1 Neurons

Many of the neurons of the cerebral cortex were highly swollen (Fig.17). Nuclear membrane though remained intact in many showed disruption occasionally. The outer membrane was not discernible in many. In some of the cortical neurons the nucleoli were not seen. While in a few others they appeared bigger with clumping of chromatin. Dissociation of the granular and filamentous components could be seen in a few of the internal pyramidal neurons. The nucleoli in such cases were not compact and had focal electronlucent areas. Perichromatin granules were prominent in few of the neurons. Clumps of euchromatin appeared in an electronlucent and expanded nucleoplasm. Heterochromatin appeared as narrow rim along the inner membrane. In few of the neurons the nucleoplasm was finely granular with a few condensations of chromatin attached to the inner nuclear membrane.

The cytoplasm of many of the neurons appeared highly vesicular indicating an expansion of its volume. Normal stacks of RER were not discernible. Occasional fragments of RER were seen. Dilated segments of the RER were in

continuation with the outer membrane. Partial degranulation was observed. Fragments of the RER with attached ribosomes were present in a completely disintegrated cytoplasmic matrix in few of the neurons. No other visible stainable structures could be seen in those affected neurons. Ribosomes were seen scattered in the cytoplasm and were not numerous. SER was present in lesser quantities and appeared as circular profiles scattered within the cytoplasm. Depletion of the glycogen granules occurred in many neurons. A few of the neuronal cytoplasm contained small vesicular profiles which appeared to be detached from Golgi stalks. Mitochondria appeared pleomorphic. Most of them were either electrondense or dilated with complete dissolution of cristae.

Some of the neurons of the middle cerebral cortex especially the globular ones had nucleus with a wooly appearance. In some of these nucleoli were not seen and there was irregular clumping of the euchromatin around vacant spaces. The outer membrane appeared discontinuous in many of the mid cortical neurons. Cytoplasmic Nissl substance was visible in some, but the RER was not discerned. A few segments of the fragmented RER with ribosomes could be seen close to the outer membrane. Ribosome aggregates in the form of polysomes could be

observed. Disorganised array of RER was seen in few of the inner pyramidal neurons. Damaged microtubules, bundles of fragmented neurofilaments were scattered in the cytoplasm. Some of the necrotic neurons had only cytocavitary cisterns of RER and there was complete absence of nuclear membranous structures. Such necrosed cells were seen surrounded by microglia having elongated nucleus. Many capillaries in the cerebrocortical neuropil had swollen endothelium and electrondense homogenous basement membranes (Fig.18 and 19).

4.3.7.2 Glial cells

There was dissolution of most of the glial cells in the cortex. Astrocytes appeared with compact nucleus and the cytoplasm around it appeared vesicular containing few mitochondria and segments of dilated RER. Some of the astrocytes appeared electrondense having free ribosomes and few lysosomal bodies. Mitochondria appeared damaged (Fig.20). The processes of astrocytes appeared swollen in which mitochondria were visible as electrondense masses having a compact cell membrane. The mitochondrial membrane in some were not discernible. Some mitochondria were pleomorphic in appearance with round and elongated profiles. The neurotubules of the processes appeared

clumped. The neuropil immediately surrounding the astrocytes appeared loose. Some of the astrocytes in the deep cortical grey matter of the cerebrum were swollen. The cell bodies and foot plates of these were swollen and had an electronlucent cytoplasm. The cytoplasmic boundary was imperceptible in many as the neuropil around such astrocytes appeared highly loosened. The dilated fragments of RER appeared naked in some and in few others were dotted with ribosomes.

Microglial reaction was not prominent. A few of them present had invaginated nuclear membrane with highly condensed chromatin. The cytoplasmic structures were hardly visible as they were highly distorted and diluted. Few of the oligodendrocytes of the grey matter appeared swollen with swollen nucleus (Fig.18). Cytoplasmic contents were hardly visible.

The granule cells of the cerebellum also showed varying grades of structural changes. The nucleus of such neurons showed condensation of chromatin and the organelles were disorganised. Astrocytic fibers were running irregularly between the damaged granule cells. Purkinje cells, though limited in number appeared normal with

compact nuclear structures and the cytoplasm with mild organellar disorganisation.

4.3.7.3 The neuropil and white matter

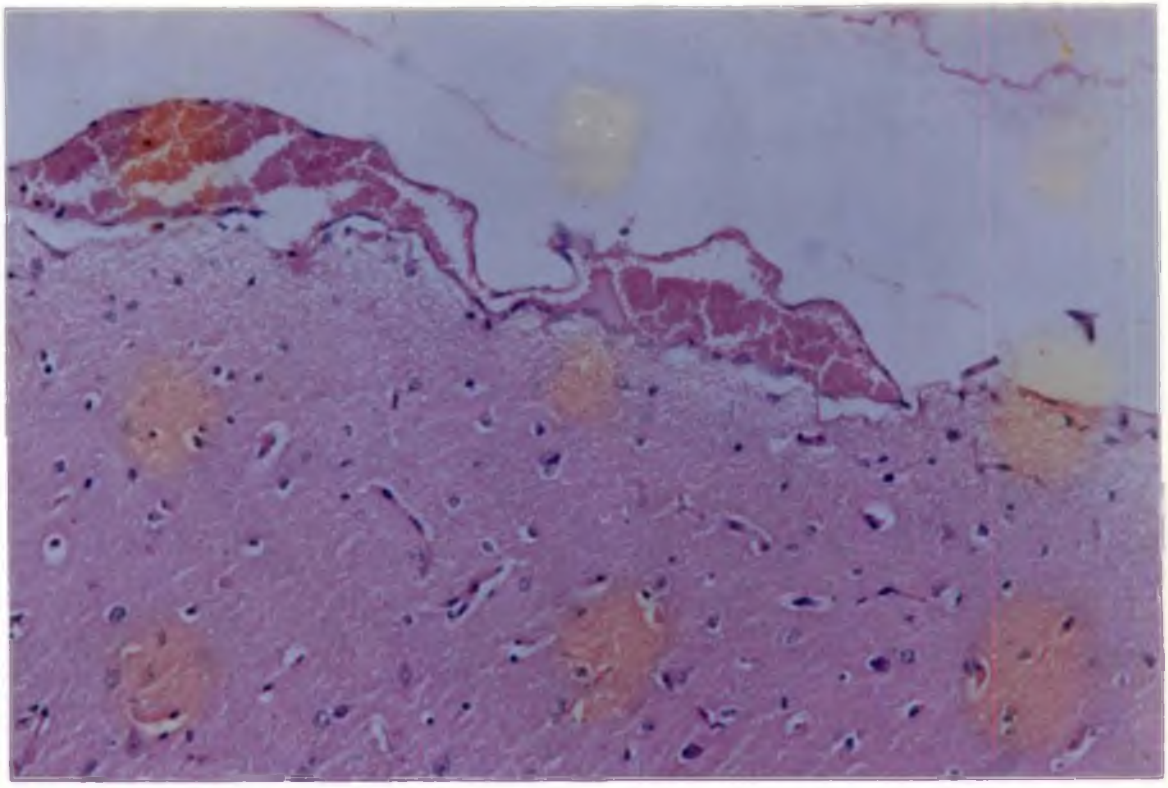
Neuropil surrounding the neurons of the cerebral cortex had a loose texture (Fig.17). The white matter also had a very loose texture at certain areas. Myelinated fibers showed vacuolation of the myelin. Polycavitation was formed by coalescence of ruptured discrete vacuoles in the myelin lamellae. The myelin surrounding most of the axons was thin. The oligodendroglia appeared preserved in the interfascicular areas of the undamaged white matter. A few such cells had irregular nuclear membrane. Cytoplasm had few segments of RER and the mitochondria appeared preserved. Osmiophilic homogenous electrondense structures could be seen in the neuropil close to oligodendroglia which appeared to be completely damaged oligodendroglial structures. The myelin appeared homogenous with loss of laminations. In other locations there was complete destruction and they assumed a whorled appearance. Cleft like structures were also seen separating the myelin laminae (Fig.18).

Occasional swelling of the axons was evident. Numerous profiles of the axons contained clumped and disorganised microfilaments and tubules and there was axonolysis in some as only a thin layer of myelin lamellae surrounded such spaces. A majority of axon profiles showed a number of damaged mitochondria and neurovesicles. Synapses were dilated containing a few compact vesicles.

Fig.9. Group B : Cerebrum : molecular layer - meningeal congestion. Molecular layer intact H&E x 250

Fig.10. Cerebral cortex : Superficial layer - swelling of neurons, loss of nucleoli, narrow rim of violet staining homogeneous perikaryon and gliosis

Kluver Barrera Luxol fast blue stain x 400



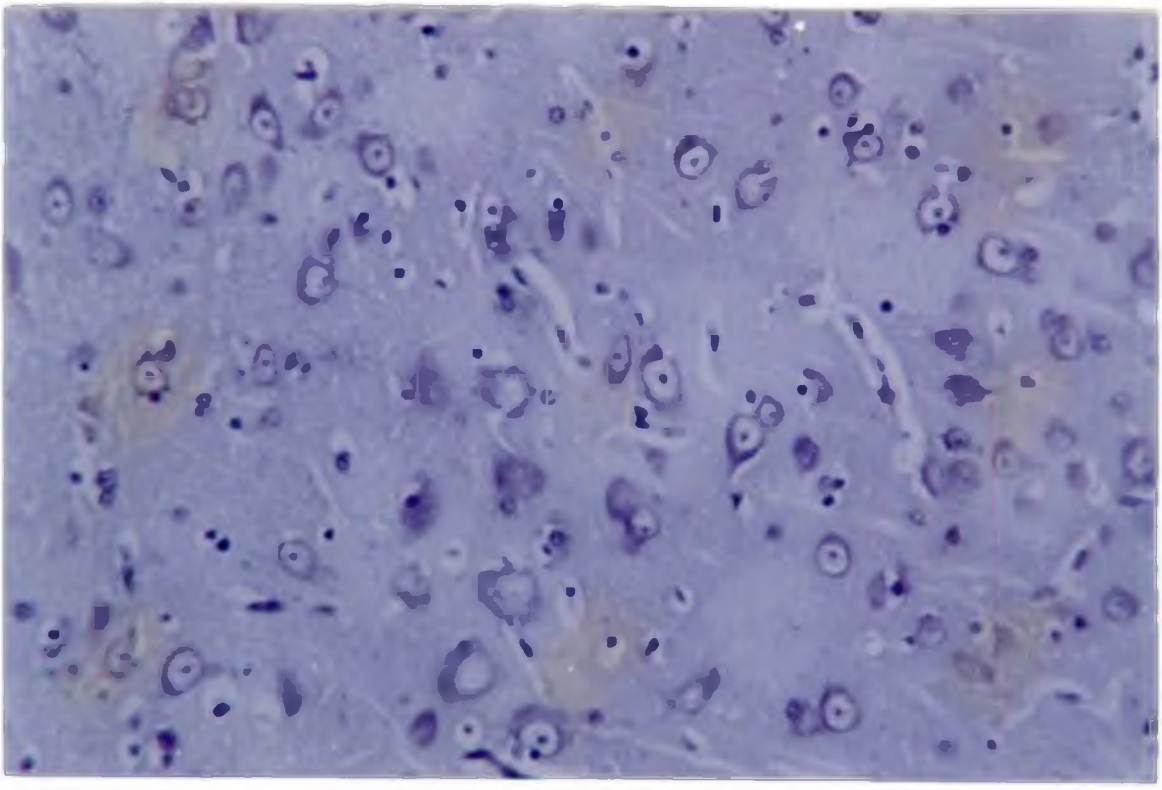
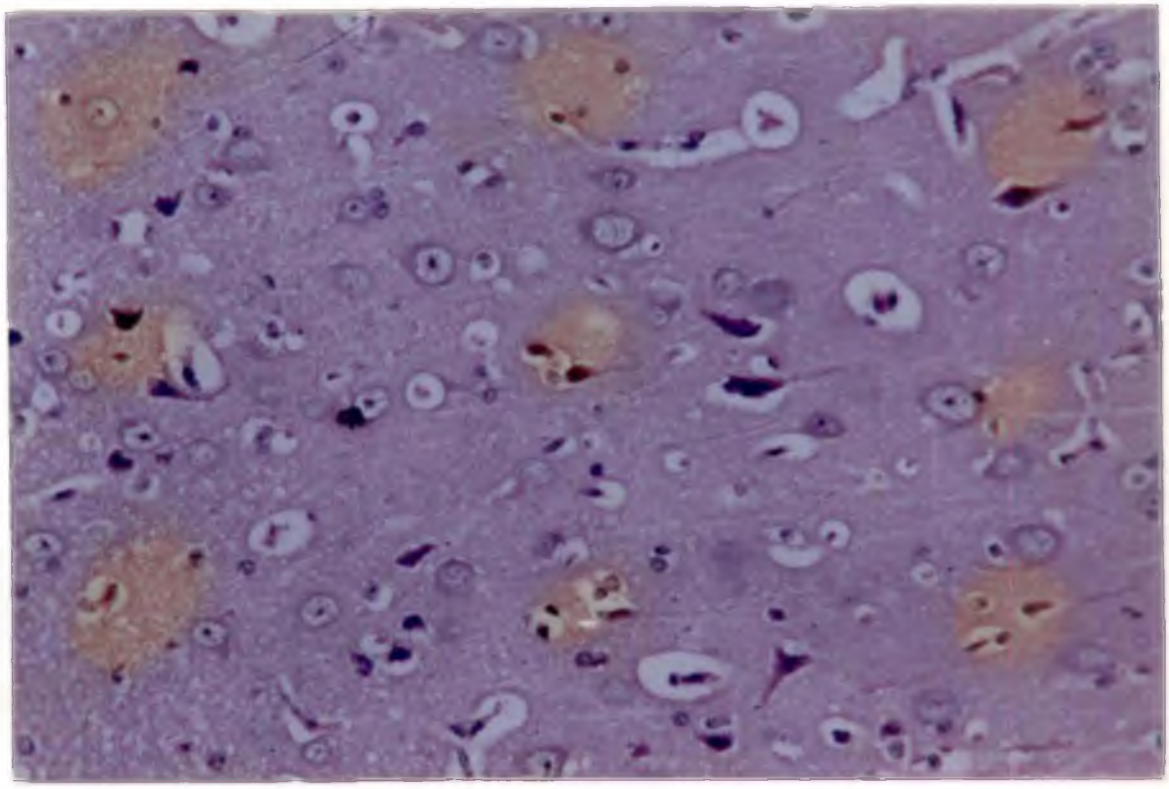


Fig.11. Cerebrum : deeper cortex - swollen and fading neurons, dark staining neurons, astrogliosis and neuropil vacuolation. H&E x 400

Fig.12. Cerebral cortex : middle lamina -shrunken and eosinophilic neurons surrounded by widened perineuronal space and neuropil spongiosis. H&E x 400



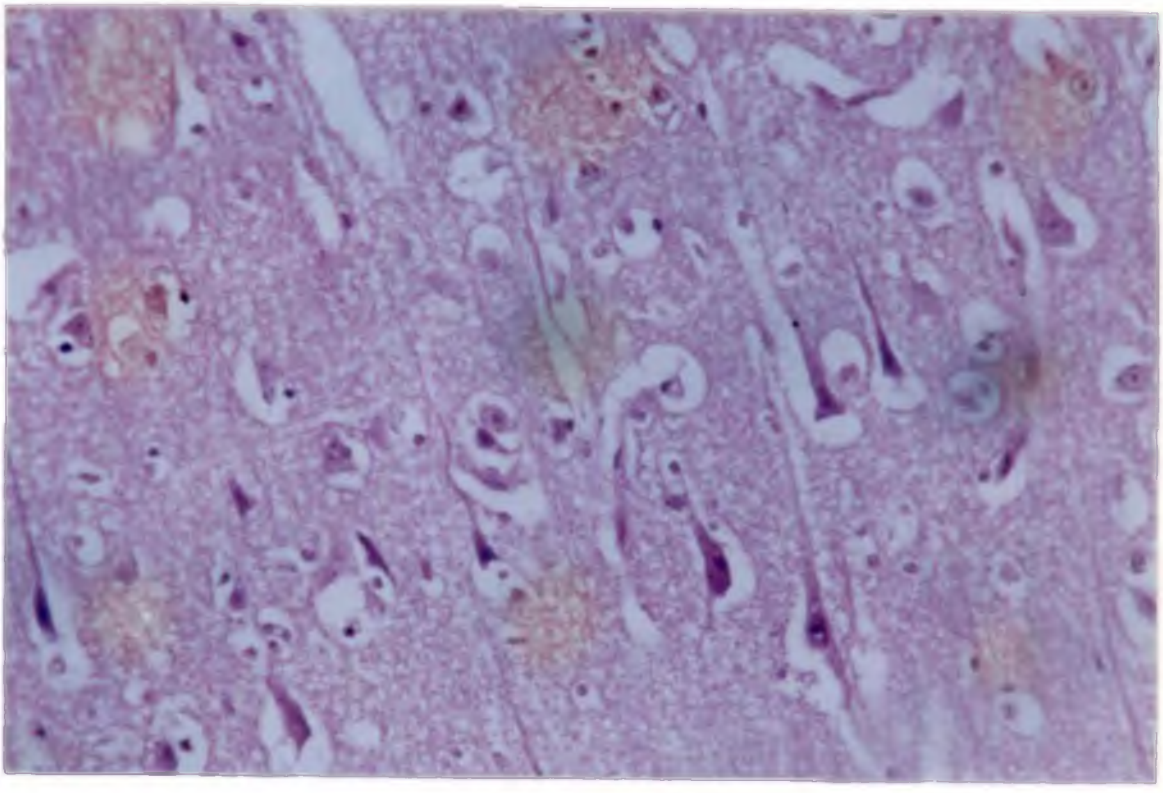
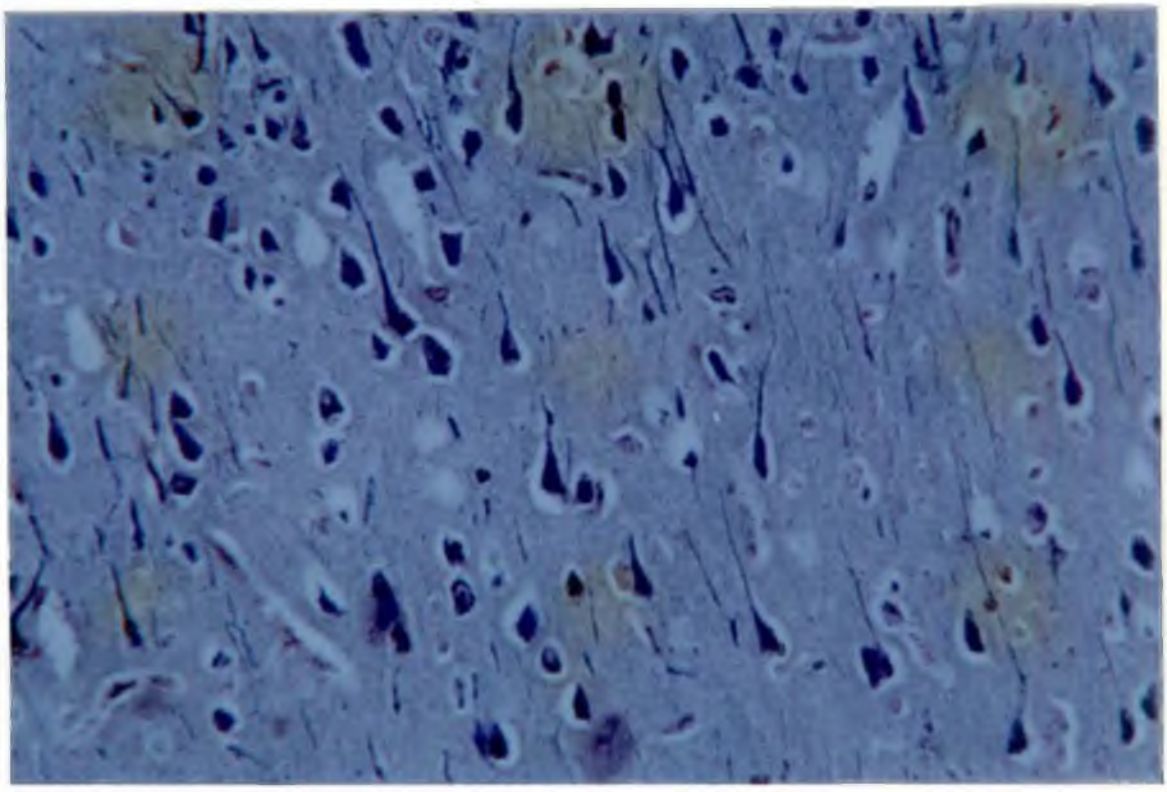


Fig.13. Cerebral cortex - condensed neurons and vacuolation extending along the dendrites. PTAH stain x 400

Fig.14. Cerebrum : White matter - inter and intrafascicular vacuolation, crowding of oligodendroglia, moderate microglial reaction and few gitter cells. H&E x 400



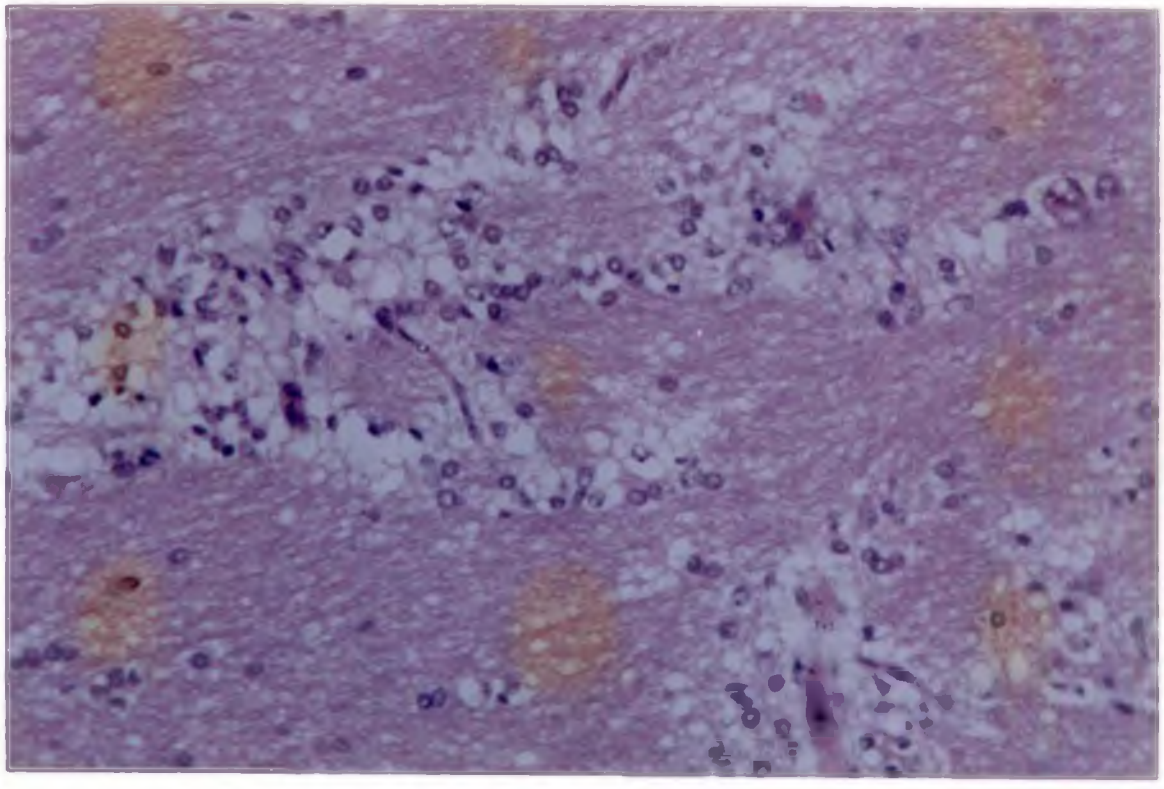
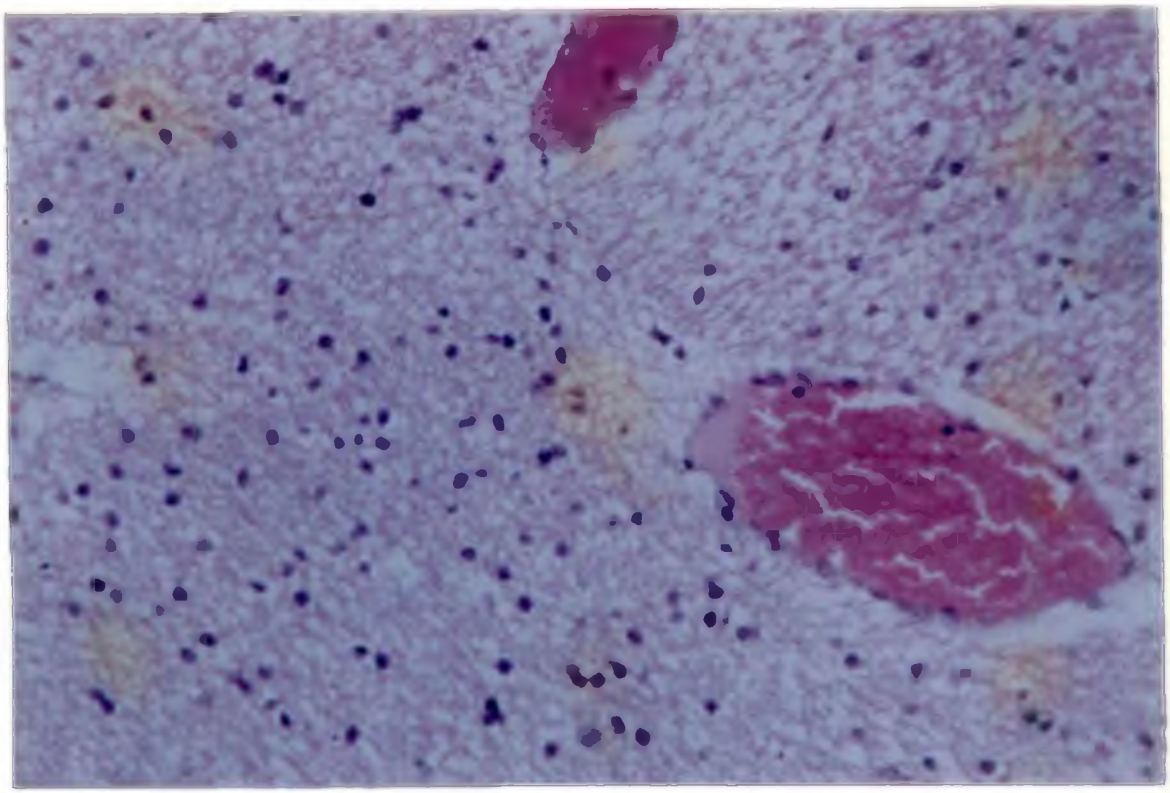


Fig.15. Cerebrum : white matter - deeper ependymal area of lateral ventricle - congestion and spongy change around the vessel. H&E x 250

Fig.16. Pons - malacic changes in the neuropil. H&E x 400



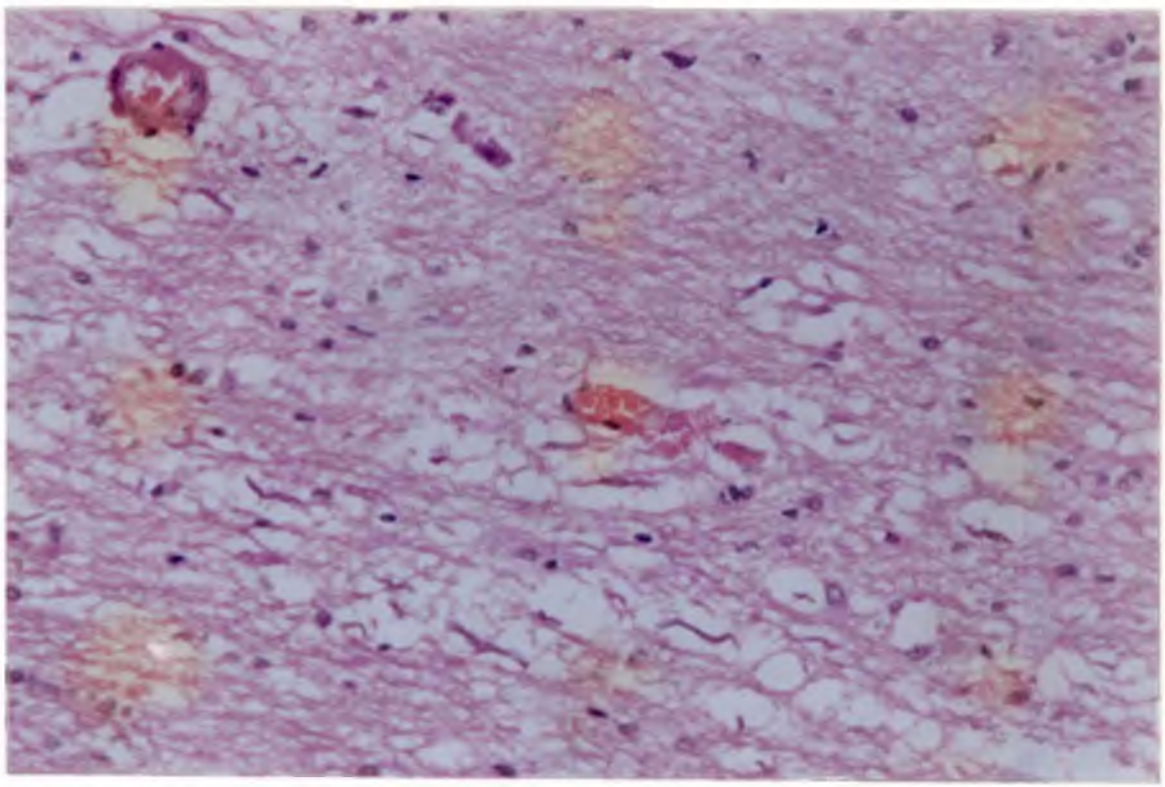
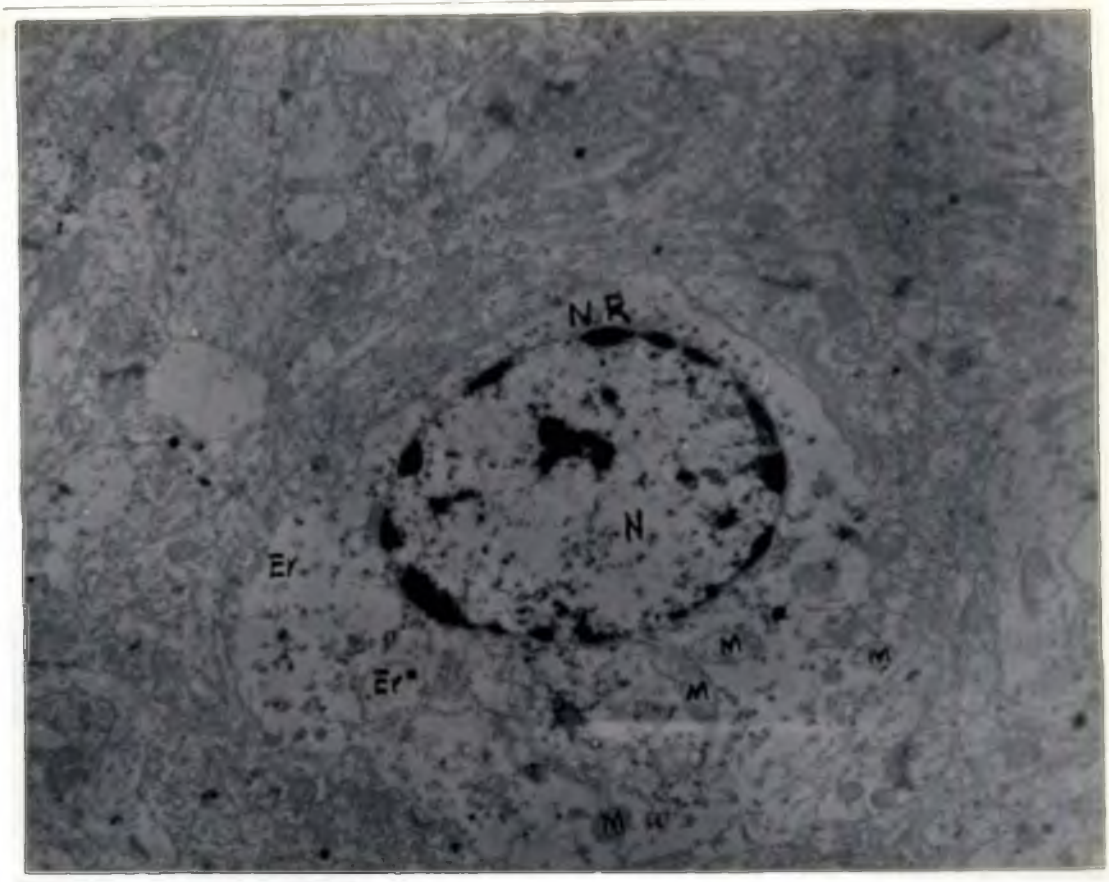


Fig.17. E/m: Cortical white matter showing a neuron (NR) with cytoplasmic oedema. Organelles loosely arranged. Swelling of the endoplasmic reticulum. (Er) and free ribosomes seen. Nucleus (N) swollen with small clumps of heterochromatin along inner nuclear membrane. Neuropil shows loose texture and cavitary pattern. Most of the mitochondria (M) are with out structural details. x 12000

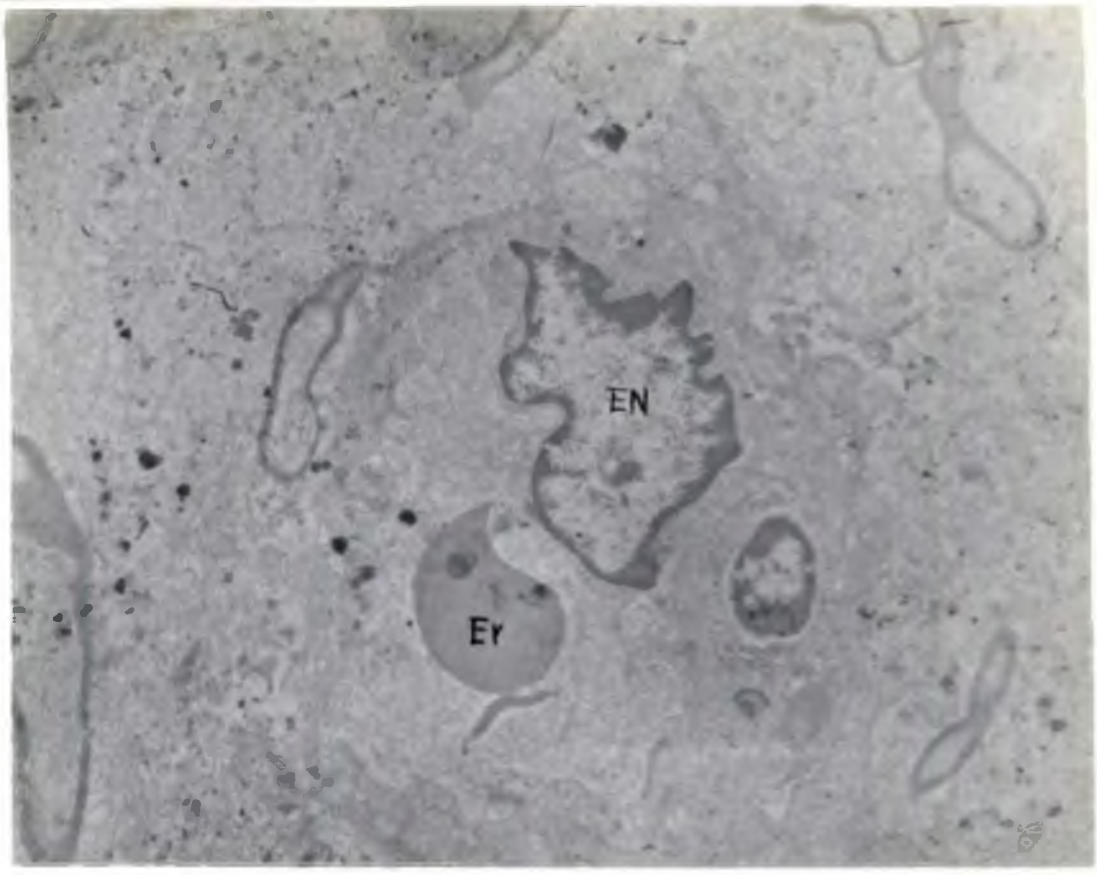
Fig.18. E/m: Cerebral cortex. Notice the neuropil with degenerated axons. (AX) having homogenous, vesicular or whorled myelin. Portion of an Oligodendroglia (OG) with swollen nucleus and a section of a capillary with dense basement membrane are seen. There is loss of internal structure in the mitochondria (M). x 8000

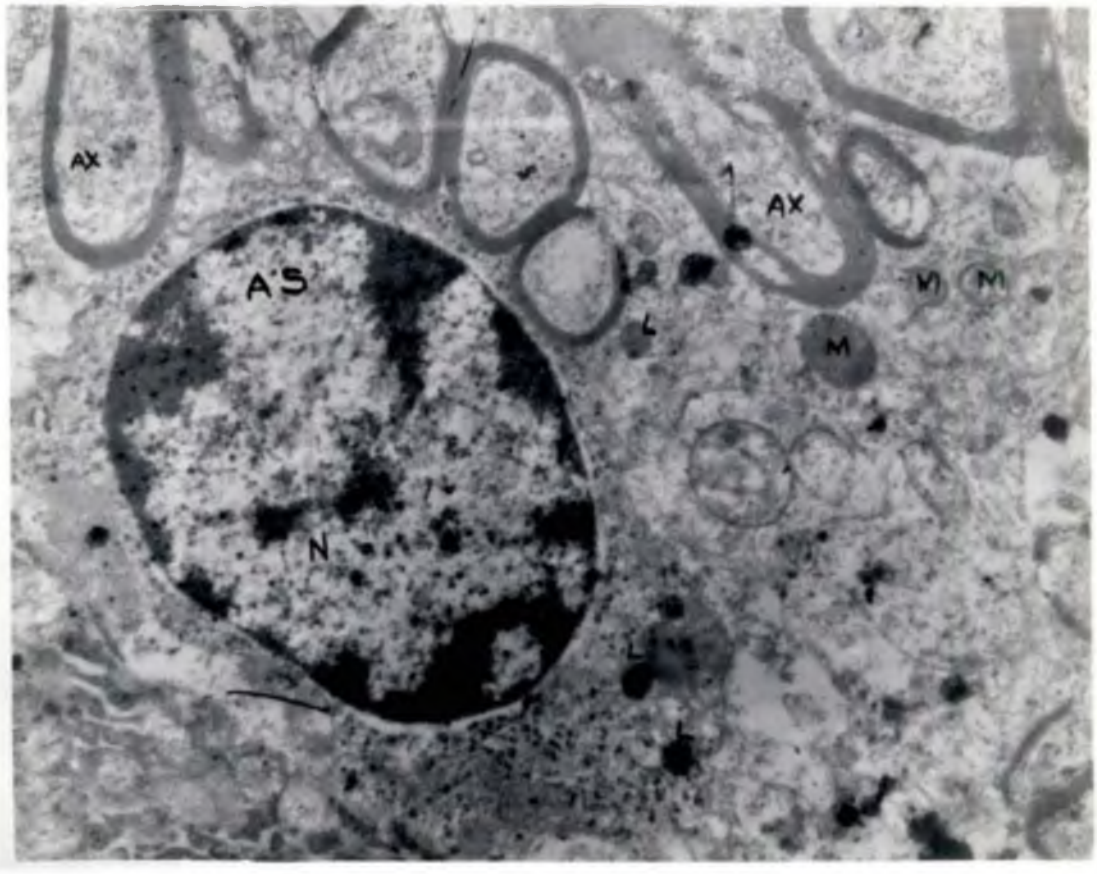

N.R
N
Er
Er
M
M
M
M


M
G
N
AX
AX
AX
Er

Fig.19. E/m: A capillary in a necrotic area in the cerebral cortex. Notice swollen endothelium (EN). Er. Erythrocyte. x 10000

Fig.20. E/m: The granular layer of corticomedullary junction. An astrocyte (AS) shows electrondense granular cytoplasm with free ribosomes, lysosomes (L) and damaged mitochondria (M). Nucleus with condensed chromatin Neuropil with axons having fragmented and vacuolated myelin and swollen and lytic axoplasm. Mitochondria (M) swollen with loss of cristae. x 16000


EN
Er


AX
AX
AS
M
M
M
L
N
L

Group C (Rice gruel)

4.4. Clinical signs

One of the animals out of the six died suddenly on the 42nd day. The animal did not show any neurological discomfort earlier. The animal was dull and the abdomen appeared very much distended. The conjunctival mucous membrane was very much congested.

The other animals did not show any discomfort except for remaining dull after ingestion of rice for some hours and all the animals became active on the next day. Moderate diarrhoea was present in all the cases for a few days. The animals were sacrificed on the 43rd day.

4.4.1 Body weight

The weight of the animals was significantly low on the 0 to 15th day (Table 3 and Fig.3) and there after on the 30th and 45th day, no significant variation was noticed from that of the control. There was a weight gain from the 30th day onwards.

4.4.2 Cerebrospinal fluid

CSF protein concentration did not vary significantly from that of the controls (Table 4 and Fig.4).

4.4.3 Weight of the brain

The weight of the brain compared with the body weight in grams did not show any variation when compared to the control (Table 5).

4.4.4 Autofluorescence

None of the brains examined showed any autofluorescent foci in the cerebral cortex.

4.4.5 Gross lesions

On autopsy it was found that the whole compartments of the stomach were packed with rice which was partially digested. The stomach mucosa was also congested. There was distension of the urinary bladder. No lesions could be seen in any of the other internal organs except for mild congestion. Brain in all the cases remained normal.

4.4.6 Histopathology

The animal which died all of a sudden had some lesions in the brain. The cerebral cortex, close to the molecular layer, that is the external granular and pyramidal layer showed neuronal degeneration (Fig.21). Most of them appeared shrunken surrounded by widened perineuronal space. Most of the damaged neurons were dark staining. Diffuse

gliosis was also observed. The cerebral grey matter close to the white matter (corticomedullay region) had extensive vacuolation and the neurons within appeared shrunken and eosinophilic. No cellular reaction was seen around these lesions. Pulverization of the nucleus and complete loss of neurons were observed in focal areas in this case.

The hippocampal neurons showed occasional necrosis and those necrotic neurons were dark staining. Inter fascicular vacuolation of the white matter of the cerebrum was noticed (Fig.22). In the cerebellum the granule layer was less cellular and many dark staining granule cells could be seen amidst homogenous pink staining structures. Purkinje cell layer was intact. In the spinal cord a few cystic dilatations in the neuropil of the grey column were seen. There was moderate gliosis.

Except for few occasional dark staining neurons in the cortex of the cerebral hemisphere, no other lesions could be noticed in the CNS in all the other cases.

4.4.7 Ultrastructural pathology

A few of the neurons of the cerebral cortex showed necrotic changes. The nuclear membrane was intact. There was condensation of the chromatin. Nucleolus was prominent. Neuronal cytoplasm had cisterns of RER which at times

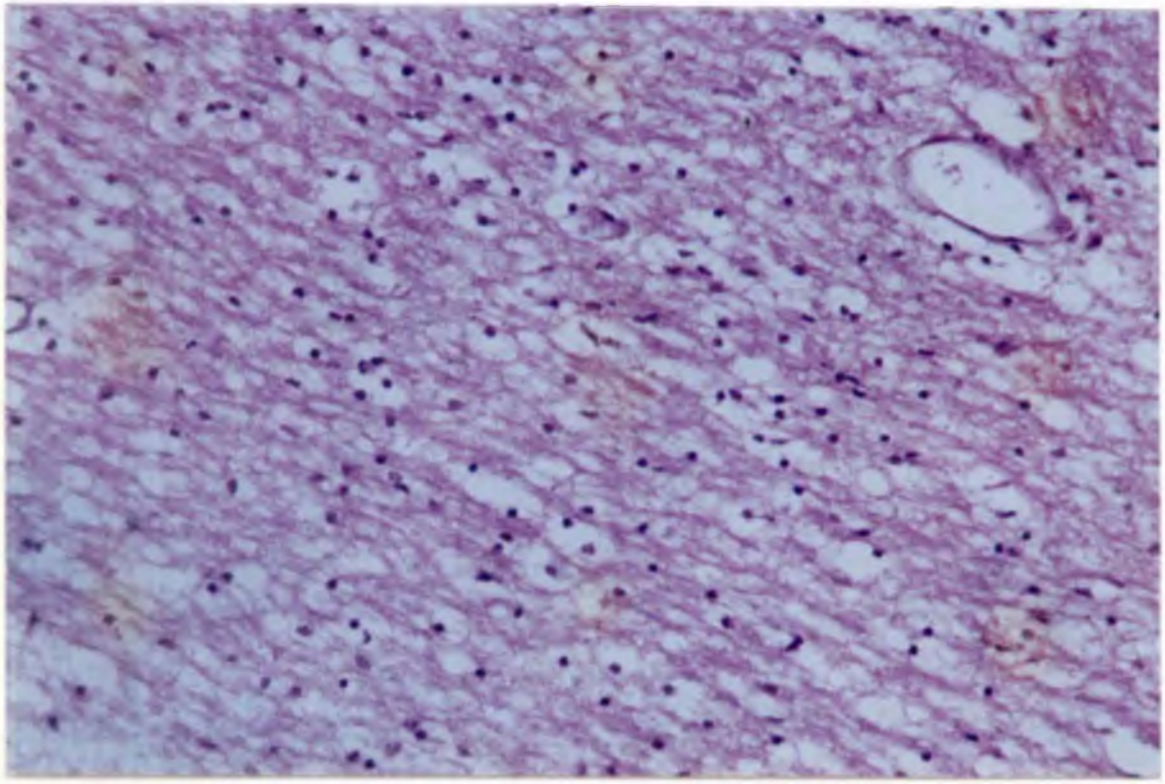
showed partial degranulation of ribosomes. Mitochondrial structures were well preserved in some neurons while in few others partial cristolysis was observed. Also there was homogeneity and electrondensity of the matrix. Sparse accumulation of glycogen appeared in the cytoplasm.

Glial cells appeared normal. Cerebellar granule cells occasionally showed margination of chromatin and many of the nuclei appeared swollen. The perineuronal spaces were distended (Fig.23).

White matter remained normal except for the presence of small vacuolations in the myelin. A few axons had myelin with small vacuoles. Occasionally there was separation of axon and focal lysis of axoplasm (Fig.24). Synaptic structures remained compact with normally appearing vesicles. Organelles in the cortical neuropil were well preserved.

Fig.21. Group.C. cerebral cortex : external granular and pyramidal layer - neuronal degeneration and neuropil vacuolation. H&E x 250

Fig.22. Cerebral white matter - interfascicular linear vacuolation. H&E X 250



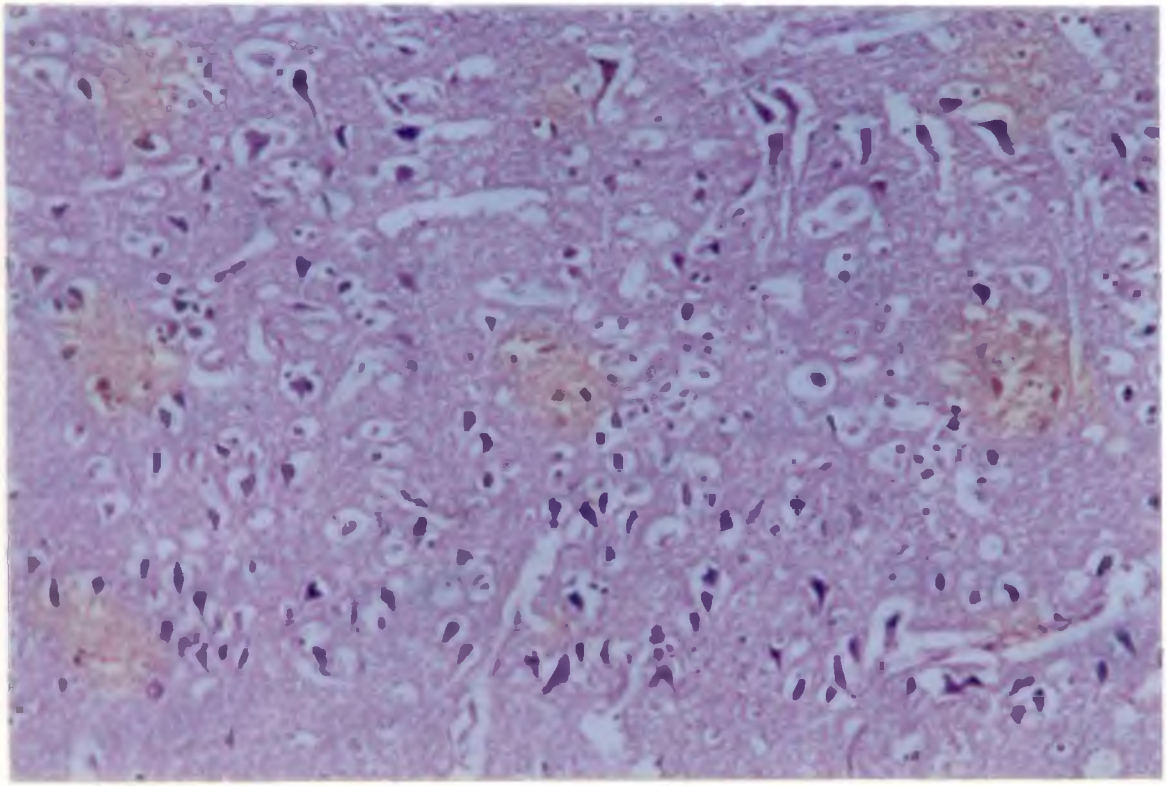
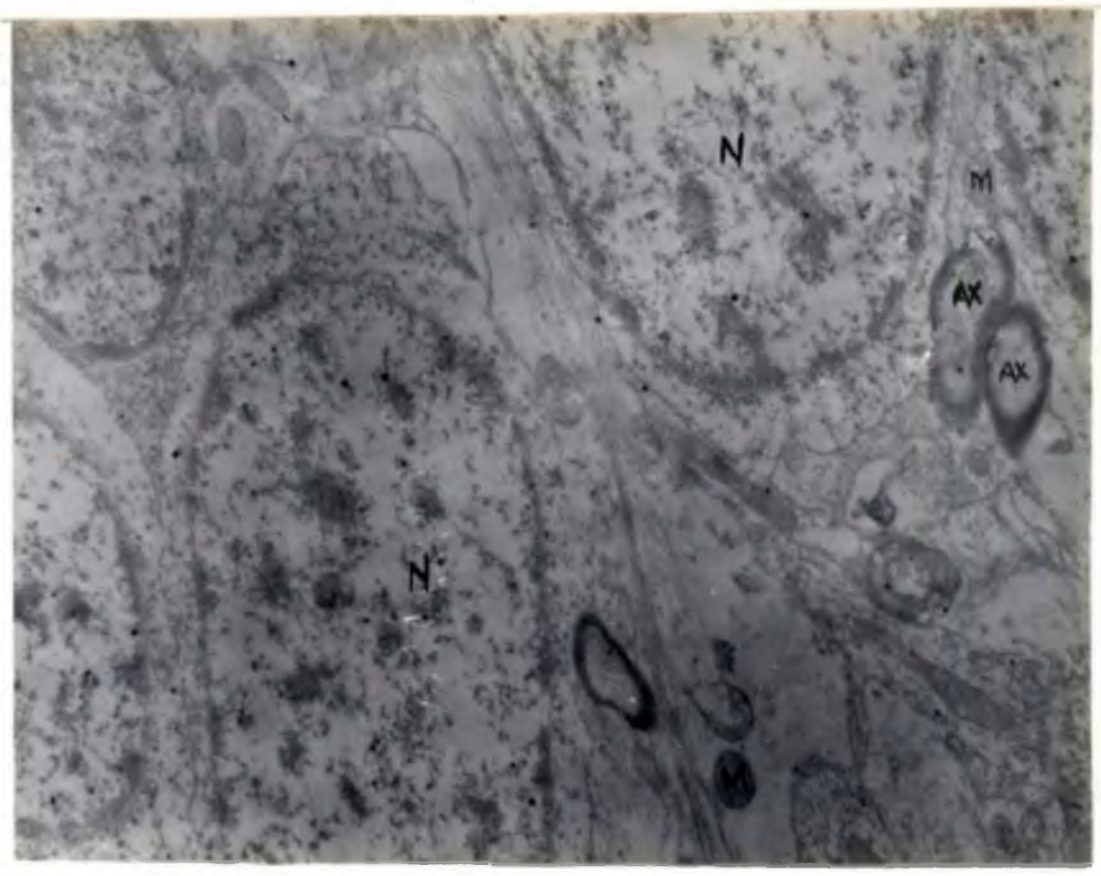
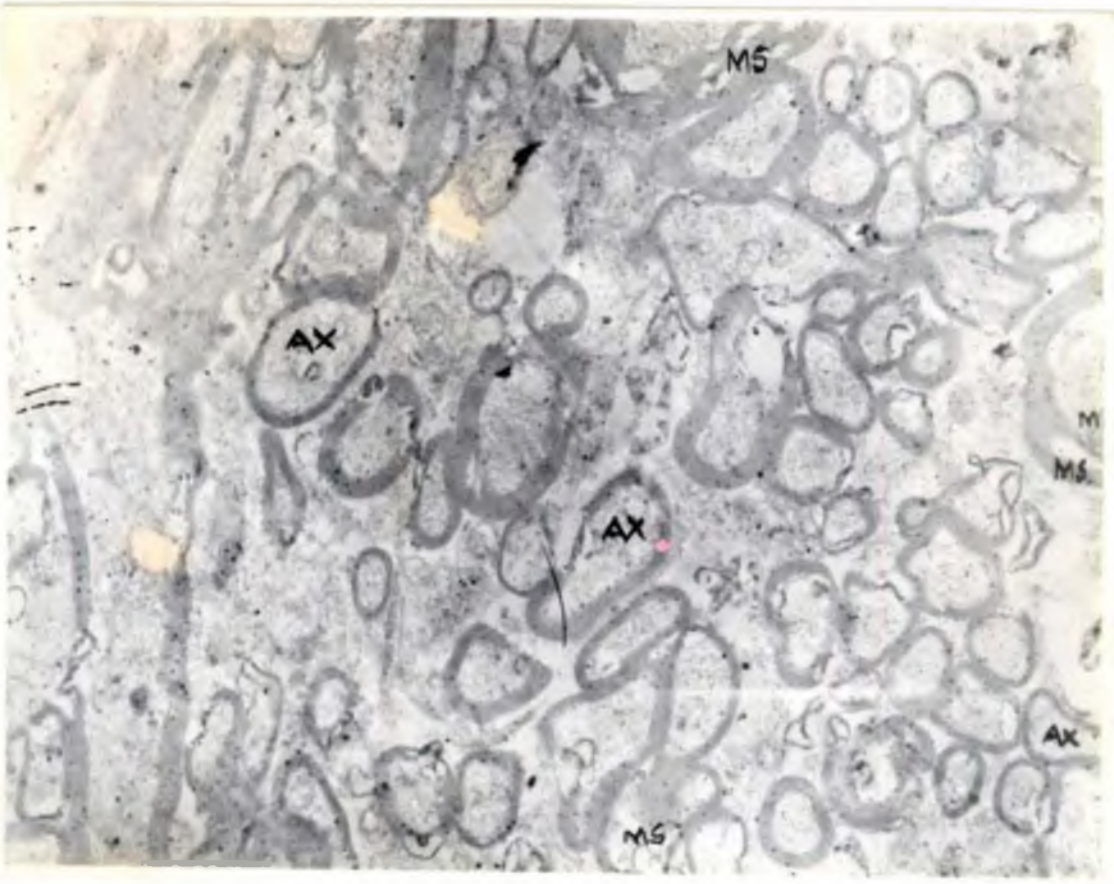


Fig.23. E/m Cells of the granular layer of cerebellum with swollen nucleus (N). The perineuronal spaces are distended. x 12000

Fig.24. E/m: Cerebrum - white matter. There are sections of axons with swelling and focal lysis of axoplasam and partial degeneration of myelin sheath x 12000


N
M
AX
AX
N


MS
AX
AX
M
MS
AX
MS

Group D (BHC)

4.5 Clinical signs

Out of the six animals, four of them developed nervous signs at different latent periods. Neurological signs were noticed in one as early as the 17th day post exposure. The symptoms were not readily progressive, but had intermittent remissions. The nervous symptoms, though mild to start with remained for two to three days and the animals recovered after each spurt of disturbances of the nervous system. The animals were sacrificed on the 36th, 38th and 41st day and one died in extremis of the symptoms on the 43rd day.

The symptoms were not consistent. Varying manifestations were observed. These were not specific. Lethargy, sleepiness, facial twitching, frequent tremors, salivation and wobbling gait were observed. The animals showed nystagmus at the time of development of symptoms. Frequent bleating and lachrimation were also observed. At times the animals remained anxious. The animals fell down and got up occasionally. This type of symptoms persisted for one to one and a half hours as it occurred intermittently and they spontaneously recovered. Rigidity, spasms, grinding of teeth, frequent diarrhoea, retraction

of the skin of the lower jaw were also observed. The animal could manage to eat and drink, but remained dull when recovered from the symptoms. One animal, in extremis could not straighten the forelimbs and they remained folded. When forced to stand, the animal stood with abducted forelimbs and with the head held down. Facial oedema was also observed (Fig.25). At the later stages before sacrifice, they appeared dull, depressed and could not stand erect. Animals were emaciated.

4.5.1 Body weight

There was progressive reduction in the body weight (Table 3 and Fig.26) throughout the period of observation and the weight reduction was significant as compared to the control.

4.5.2 Cerebrospinal fluid

CSF collected just before sacrifice appeared roseate in three animals. The flow was very poor in two of the animals which were very weak and all the others the flow was good. The CSF did not coagulate. The total protein concentration of CSF, both in the pre-exposure state and post exposure period did not show any significant variation from the control (Table 4 and Fig.27).

4.5.3 Weight of the brain

The weight of the brain showed no significant difference when compared to the control (Table 5). The group mean brain weight in grams per gram of average body weight was 0.00952 ± 0.00122.

4.5.4 Brain autofluorescence

None of the animals brain showed any autofluorescence in any part of the cerebral cortex when the fomalin fixed slices were examined under UV at 365 nm.

4.5.5 Gross lesions

There was no detectable gross lesions in the brain except for generalised congestion and scattered petechiae in different segments of the brain.

In two of the affected animals liver was moderately enlarged and necrotic. Diffuse and mild foci of necrosis could also be observed in all the other animals. Kidney showed petechiae, foci of necrosis and a few infarcts. The other lesions observed included pulmonary congestion and oedema, cardiac hypertrophy and mild congestion of all other internal organs.

4.5.6 Histopathology

Although significant microscopic lesions were seen in the brains of the affected goats, the distribution, pattern, extent, severeity and type of lesion in any one brain varied with the duration of clinical illness with minor differences.

Moderate engorgement of the capillaries, dilation of the perivascular space and several small areas of spongy transformation were randomly distributed within the molecular layer (Fig.25a).

Spongy transformation was also seen in the cerebral cortex in which most of the pyramidal and globular neurons appeared condensed with pyknotic dark nucleus. The cytoplasm appeared eosinophilic. Astrogliosis with vacuolation of glial cells especially along the vicinity of the vessels were constantly seen in different segments of the cerebrum including the rostral, middle and caudal hemispheres bilaterally. Infiltration of microglia was observed. Congestion of capillaries, degeneration of neurons, perineuronal vacuolation, astrogliosis and many randomly distributed circumscribed granular eosniophilic bodies which were interpreted to be degenerating axons were a feature in the cerebral cortex in two of the animals

(Fig.28). Spongy changes in the neuropil containing uniformly distributed and regular sized vacuoles with scanty degenerating neurons were also observed in the middle part (Fig.29).

Astrogliosis, swelling of the neurons, peripheral displacement and condensation of nucleolus, presence of one or two very small to large circumscribed intra nuclear vacuoles and perivascular cavitation were seen in the middle of the cortical lamina in all the cases. Most of the astrocytes were also vacuolated. There was complete chromatolysis in some of the neurons and some other neurons were seen getting faded into the substance of the brain. Dark staining neurons with eosinophilic cytoplasm and condensed nucleus surrounded by widened perineuronal space, central and peripheral chromatolysis were seen amidst normally appearing neurons in one case. Astrogliosis was predominant (Fig.30). Most of the spherical neurons were seen getting faded and some of them appeared swollen. In some areas many of the astrocytes were seen shruken leaving circumscribed spaces around. Neuronal nuclei were marginated in many at the depth of the sulci and crown of the gyri.

Vacuolation was pronounced in the deeper cortical layer close to the white matter. Vacuoles were spherical to elliptical and coalesced larger ones were also observed. The capillaries were highly engorged (Fig.31) and the endothelial cells were prominant. The neuropil around such capillaries were found retracted. The neurons within the vacuoles appeared shrunken and marginalised. Many of the spaces were seen infiltrated by oligodendroglial cells. Most of the vacuoles contained a pink staining hazy material indicating oedema.

The white matter of the cerebrum showed spongiform changes characterized by multiple diffuse circumscribed vacuoles of varying sizes both in the inter and intrafascicular regions (Fig.32). Many of these contained very dark staining oligodendroglial cells.

Vacuolation was also seen along the course of the fibers in the form of regular chambers separating the fiber tracts. Such cavitations were traversed by thin fiber strands and contained numerous oligodendrocytes (Fig.33). Vascular damage, perivascular oedema and extensive haemorrhages were seen in the white matter (Fig.34). The spongy appearance of the white matter was attributed to numerous, round or oval empty spaces of varying size

occurring independently in rows particularly evident along the long axis of the fibers. Demyelination and oedema occurred in highly vacuolated areas indicated by relative loss of staining intensity of myelin with luxol fast blue stain (Fig.35). The spongiform changes occurred throughout the central nervous system in severely affected animals whereas in midly affected cases there was prediliction for the grey and white matter particularly of the cerebrum.

Subependymal and perivascular oedema was prominent in all the four cases. Stratified subependymal infiltrates were present below the ependyma of the lateral ventricle. Subependymal neuropil showed malacic changes.

Hippocampus showed mild astrogliosis in three cases. Pyramidal neurons of the hippocampus had shrunken cell bodies and dark staining nucleus. The pericellular space was dilated. There was loss of cells, sub-hippocampal spongy changes, astrogliosis and large neuronal necrosis.

Cerebellum revealed multiple areas of haemorrhage and congestion in the molecular layer, interfolial areas and in the granule cell layer. Purkinje cell layer was dicontinuous.

Multiple haemorrhages and malacic foci were consistently present in the midbrain, in the medulla

oblongata (Fig.36) and pons. The neuronal nucleus of the medulla oblongata was well preserved. The spinal cord also showed congestion, haemorrhage and very big vacuolations in one case. The left ventro-lateral grey column neurons were highly shrunken, appeared angular with pyknotic and laterally placed nucleus (Fig.37). Moderate degeneration of the ependymal cells could also be seen.

The animals which did not show any symptoms had mild neuronal degeneration in different segments of the brain. Diffuse dark staining neurons could be observed amidst normal neurons.

Fatty change, necrosis and focal infiltration were observed in the liver. Tubular degeneration, congestion and necrosis were the lesions observed in the affected kidney.

4.5.7 Ultrastructural pathology

The neurons of the cerebral cortex showed varying degree of changes. But the white matter changes were predominant in all the affected goats.

4.5.7.1 Neurons

Many neurons showed cytoplasmic and nuclear changes indicative of necrosis. In most of the neurons of the

cerebrocortical laminae, the neuronal nuclei were swollen. Occasional lysis of the nuclear membrane was observed in some. In some other areas the nucleus appeared compact in a cytoplasm which was over distended (Fig.38). Along with this neurons were also seen having electrondense cytoplasm with complete loss of organellar structures (Fig.38). The nucleoplasm appeared highly electronlucent leaving only small aggregations of chromatin. Euchromatin was sparse. Heterochromatin appeared as electrondense clumps on the inner nuclear margin. In few of the neurons there was separation of the outer and inner nuclear membrane. The granular and fibrillar components of the nucleoli were not easily distinguishable giving a homogenous appearance. In a few there was segregation of the pars granulosa and pars amorpha (Fig.39). An increase in the perichromatin granules was observed in few of the neurons. Some of the necrotic neurons of the outer pyramidal and inner fusiform layer appeared elongated with irregular nuclear membrane and clumping of chromatin. Nucleoli in such cells appeared condensed and intensely electrondense. Enlargement of a nucleoli appeared in a few neurons. The cytoplasm of many neurons was characterized by absence of stacks of linear RER. But fragments of RER were present. Dilatation of RER was noticed occasionally and the lumen contained an

electronlucent material. Crowding of ribosomes were observed in the dilated intercisternal areas. Strands of RER with collapsed lumen could be observed at focal areas. Fragmented bits of RER with ribosomes attached to it were seen diffusely in the cytoplasm. Occasional degranulation of the ribosomes was seen in few of the neurons. Very great dilatation of the cisterns was noticed in such neurons. An increase in the SER was a feature and extensive arborization was seen in many of the neurons. They appeared as branching tubular structures and tubular profiles were abundant in the cytoplasm of certain neurons. Glycogen was seen dispersed between such tubular arborizations. In many neurons the cytoplasm contained mitochondria of varying shape and size. Some appeared round and others elongated. Swollen mitochondria were seen in many of the external granular layer neurons of the cerebral cortex. Focal separation of the outer membrane was seen in a few mitochondria. The cristae appeared lysed in many and in some others there was only partial loss. The mitochondrial matrix in most of them were electrondense. Mitochondria with well preserved cristae could be seen amidst some of the damaged ones. Matrix condensation and wooly densities appeared in few of the mitochondria. A few membrane bound

lysosomal structures could be discerned in many neuronal cytoplasm.

4.5.7.2 Glial cells

Necrosis and lysis of certain oligodendroglia could be observed in the molecular layer of cerebral cortex and also in the white matter. Lysed glial cells appeared granular with complete dissolution of the nuclear membrane. Most of the astrocytes were swollen. The nuclear membrane appeared thin without the separation into outer and inner ones. The nucleoplasm appeared lucent. Euchromatin appeared dispersed within the lucent matrix and extensive clumping of heterochromatin along the rim of nuclear membrane. Cytoplasmic organelles were seen dispersed in a loose cytoplasmic matrix. The cell boundary was not discernible. The processes of the astrocytes extended into the neuropil. A few segments of RER and round mitochondria were seen. The mitochondrial cristae were not prominant and the matrix was electrondense. Few clumps of filaments could be observed in many of the swollen astrolytes. Astrocytes with condensed nucleus were also seen in some of the segments. Microglia with crenated nuclear membrane and electrondense nucleoplasm were seen occasionally. Many microglial cells with well formed nuclei with vacuoles and

lysosomes were noticed (Fig.40). Free ribosomes and liposomes were also seen in the cytoplasm.

Oligodendroglial structures in the cortex were observed in a few. Some of the cells were necrotic and had cytoplasm with a homogenised appearance. In some the plasmalemma appeared detached from the cytosol (Fig.41).

Many neurons of the cerebellum showed degranulation of ribosomes with the rough endoplasmic reticulum showing fragmentation. The granule cells of the cerebellum had electrondense nucleoplasm in many. The nuclear membrane appeared irregular and crenated. Abnormal notching was present in a few of the granule cells. The nucleoplasm in many appeared diluted and electronlucent. Rupture of nuclear membrane at focal areas was noticed. Chromatin was sparse in some and in some others chromatin clumping was observed. Mitochondria occasionally showed electrondensity. There was partial cristolysis in few of the cells. The neuropil between the cells appeared loosely textured and expanded and only less stainable material was evident.

4.5.7.3 Neuropil and white matter

Very extensive vacuolation of the neuropil was seen. This presented a cavitary pattern (Fig.41). The mitochondria were pleomorphic in nature. Some of them were

very elongated while others assumed rounded or saucer shaped appearance. There was varying grades of loss of internal structure (Fig.41).

The vacuolar changes were predominant in the cerebral as well as cerebellar white matter. Most of the axons were highly distended and lined by a thin layer of myelin. Marked changes were observed in myelinated axons. There was segmental loss of myelin in some. Distortion, separation, rupture and homogenisation of myelin (Fig.40) lamellae were evident. Lysis of axolemma and clumping of matrix material was observed. Mitochondria and other structures were not discernible in many axons. There was rupture of surface membranes of axons and those appeared connected with adjacent membranes forming cavitary areas containing electronlucent material (Fig.42). There was splitting of myelin at the intraperiod lines in focal areas. Interfascicular oligodendroglia appeared condensed in certain areas whereas in other areas appeared elongated. The cytoplasm of such glial cells contained few dilated vesicular profiles of RER. Dispersed ribosomes were evident in the cytoplasm. The nuclear membrane in many of the glial cells was preserved, but the perinuclear space was widened at regular intervals. The loosely textured white matter contained many axons which were undergoing

lysis. Electrondense globular aggregates could be observed irregularly in the white matter. Most of the axodendritic and dendrodendritic synapses appeared with few vesicular profiles. Spinous processes were not discernible. Few of the dendritic processes were found lysed. Mitochondria of dendritic processes were swollen.

Fig.25. Group D. BHC treated animal showing facial oedema and emaciation

Fig.25a. Cerebrum molecular layer and superficial cortex - spongy transformation, congestion of capillaries and perineuronal oedema. H&E x 250

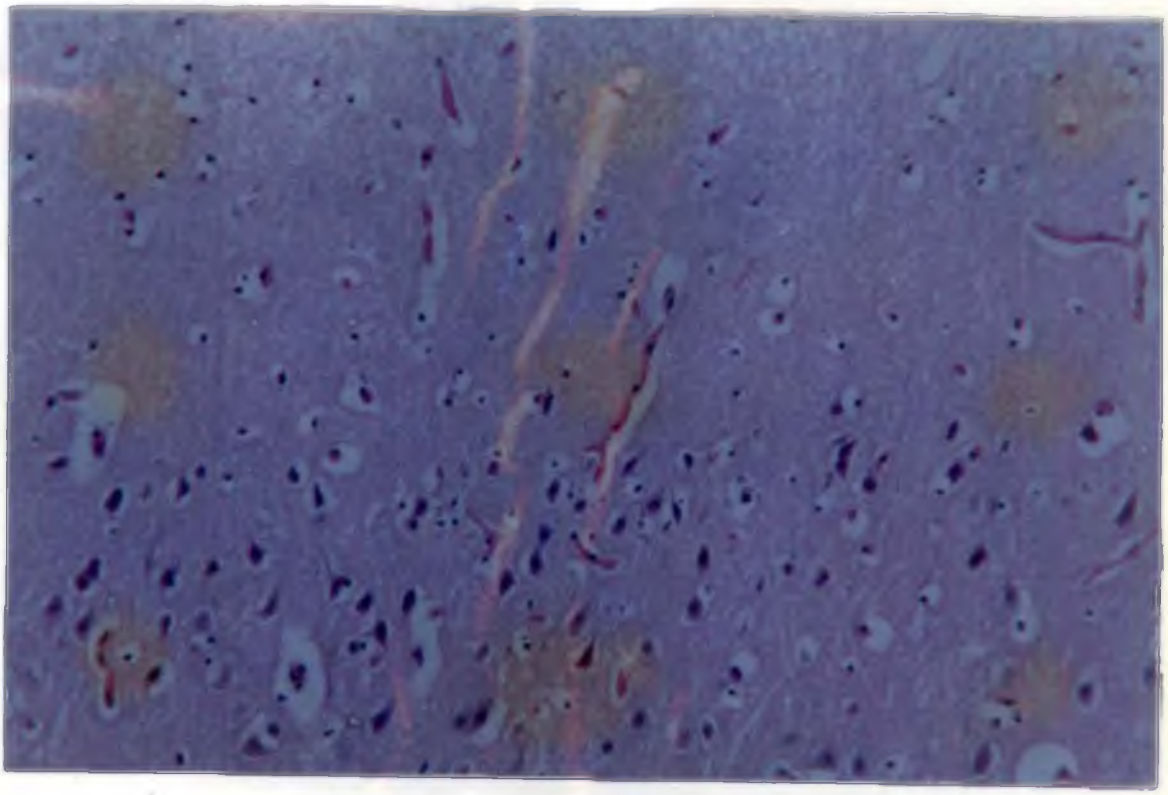


Fig.28. Cerebral cortex : moderate astrogliosis, neuronal degeneration, perineuronal oedema and degenerating axons. H&E x 400

Fig.29. Cerebrum - middle part spongy neuropil, absence of neurons and degeneration of neurons. H&E x 400

Fig. 26 : Average body weight at fortnightly intervals

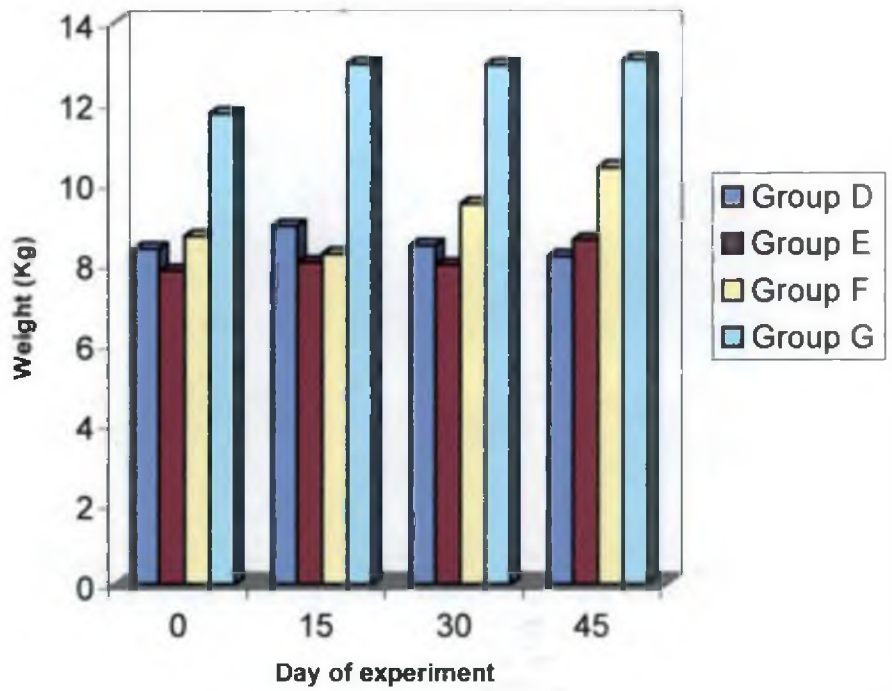
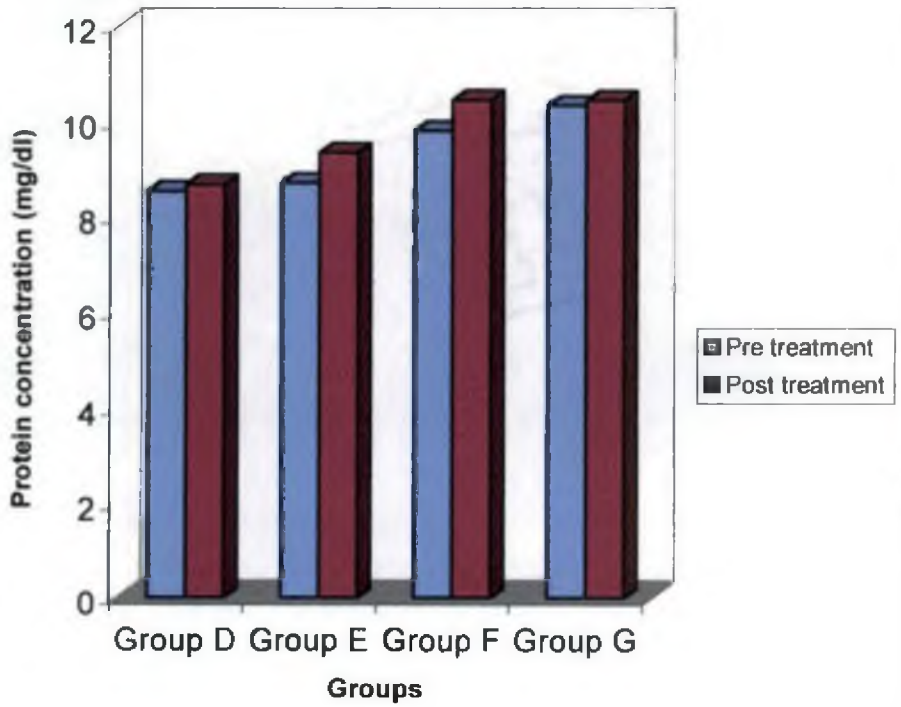
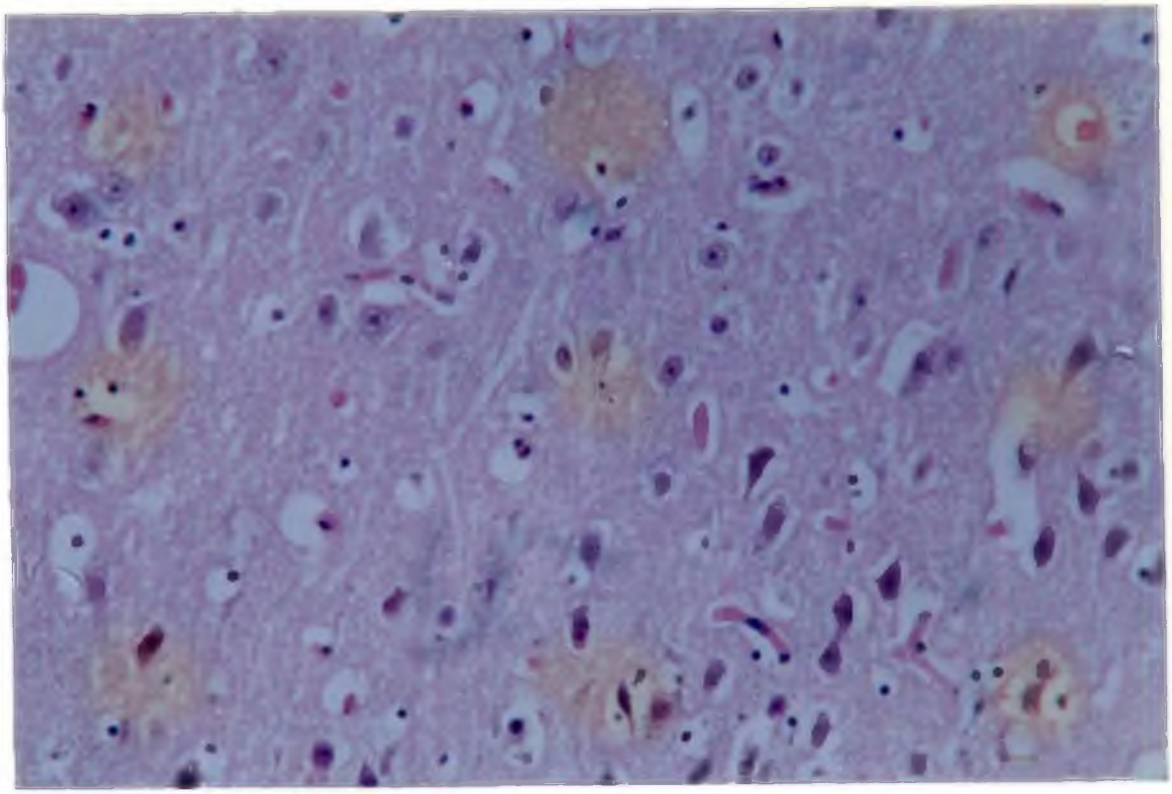


Fig. 27 : Average CSF protein concentration (mg/dl)





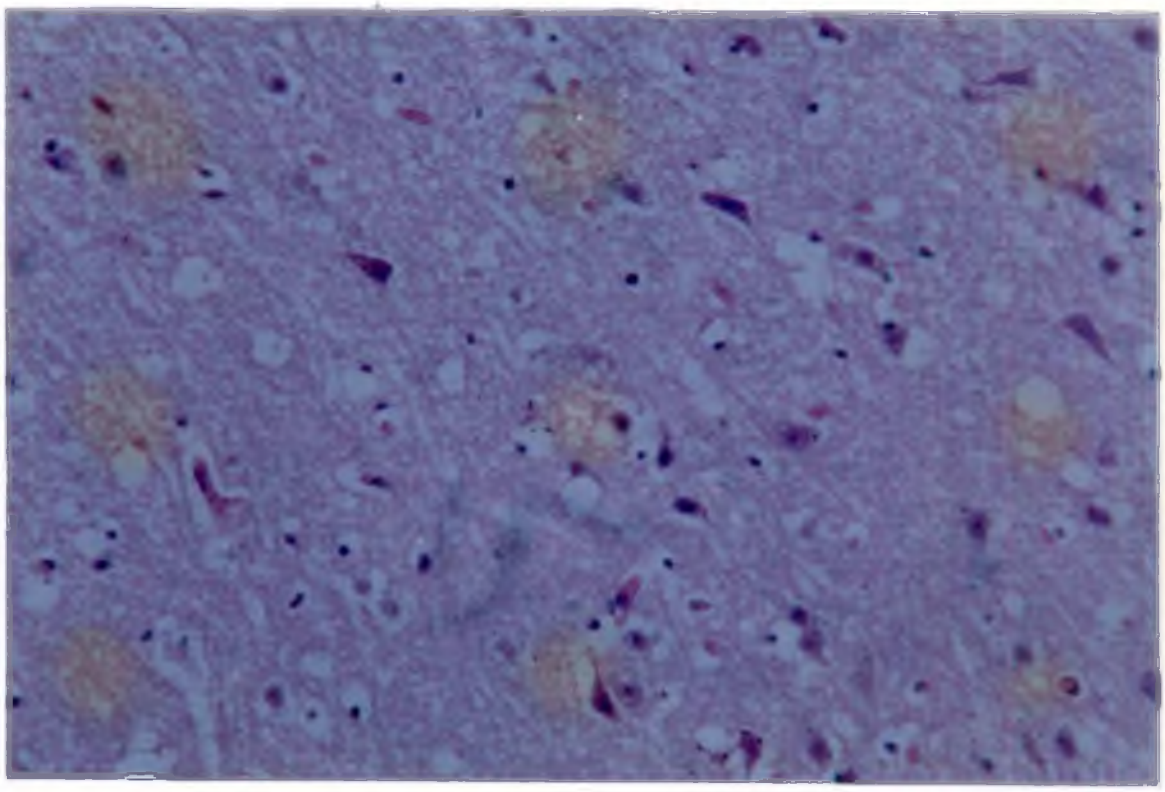
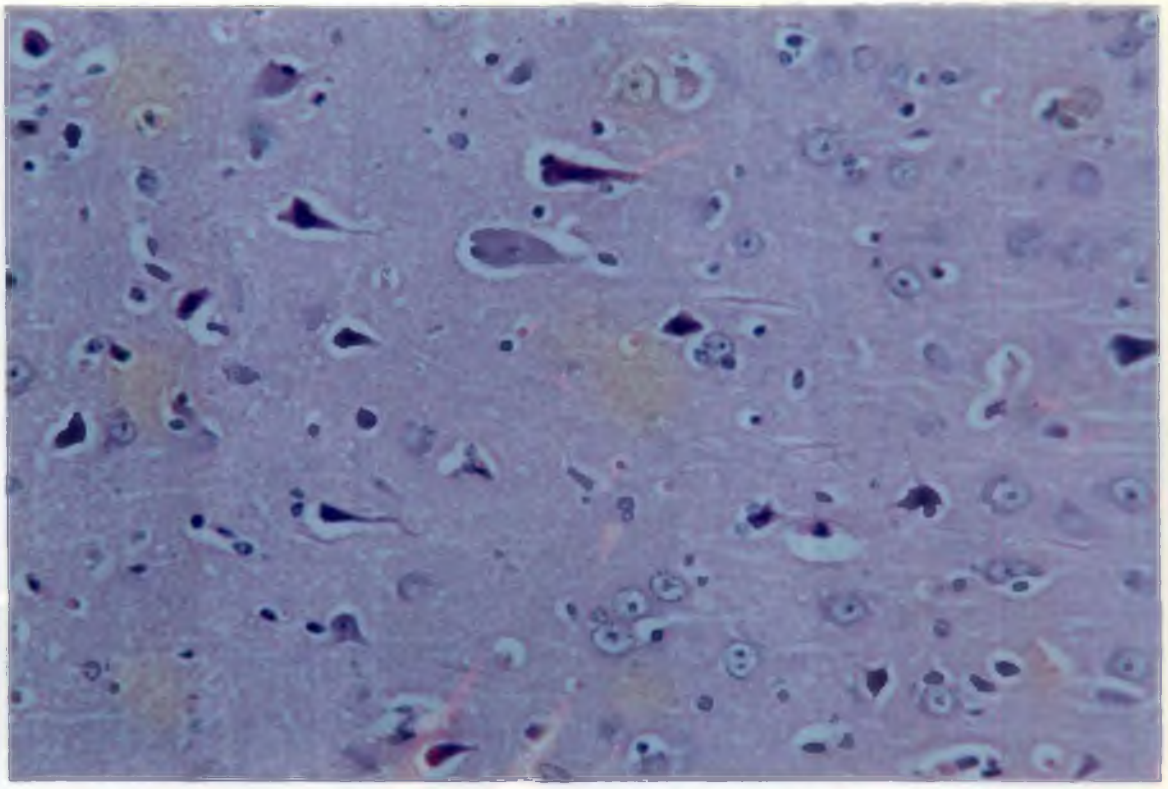


Fig.30. Cerebrum : middle part- dark staining neurons with condensed cell body, eosinophilic cyctoplasm amidst normally looking neurons and astrogliosis. H&E x 400

Fig.31. Cerebrum : deeper cortical area : pronounced vacuolation, neuronal degeneration and capillary congestion. H&E x 400



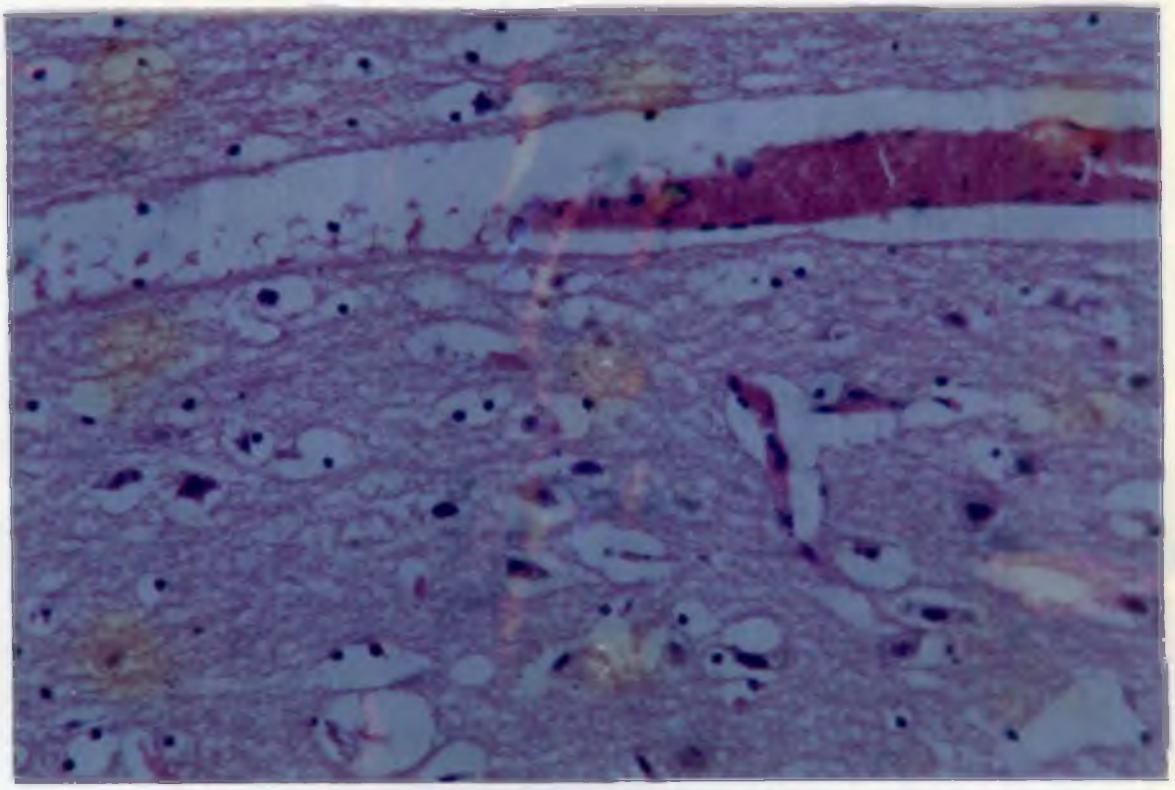
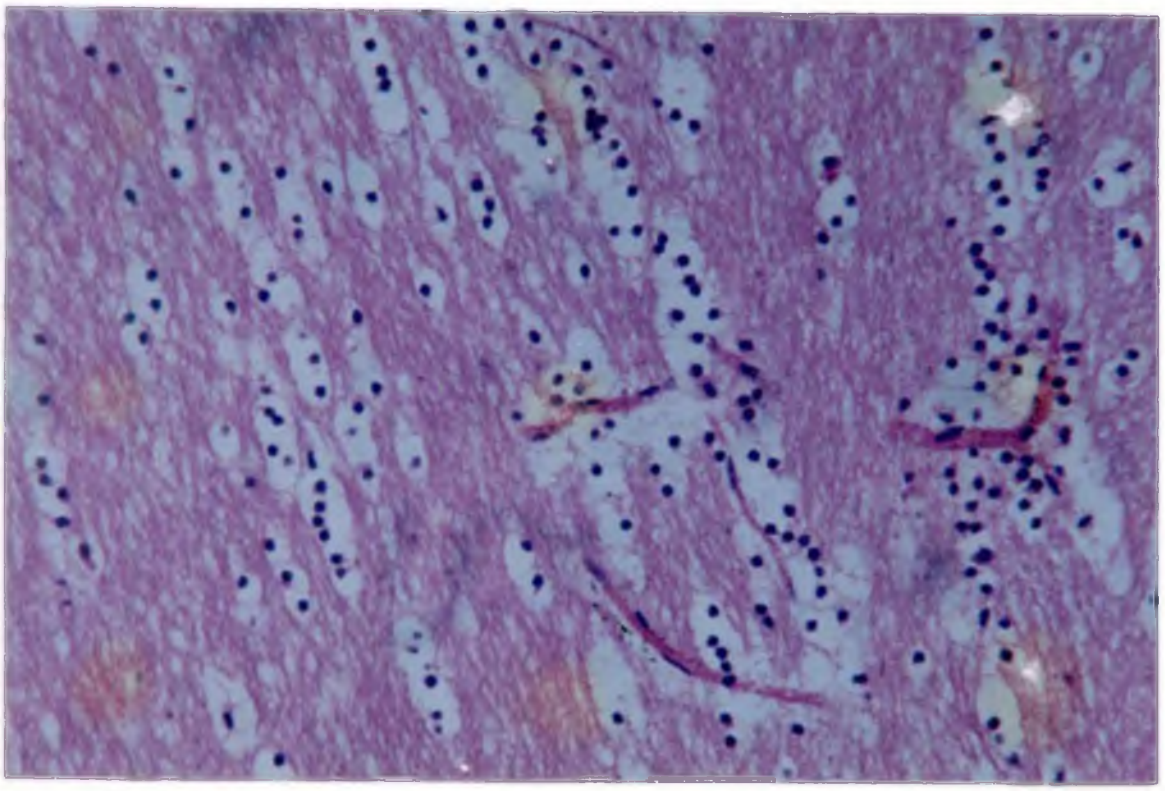


Fig.32. Cerebral white matter - spongiform change. H&E x 250

Fig.33. Deep cerebral white matter spongiform changes along the fiber tract. H&E x 400



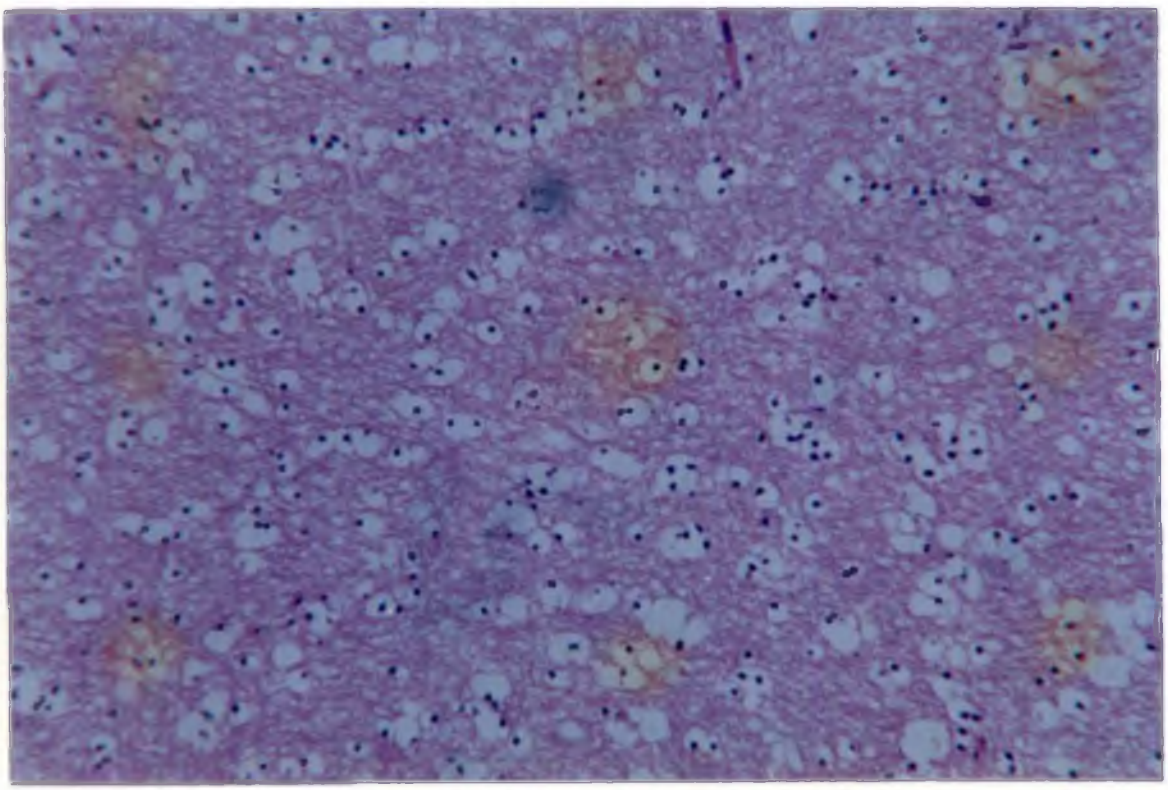
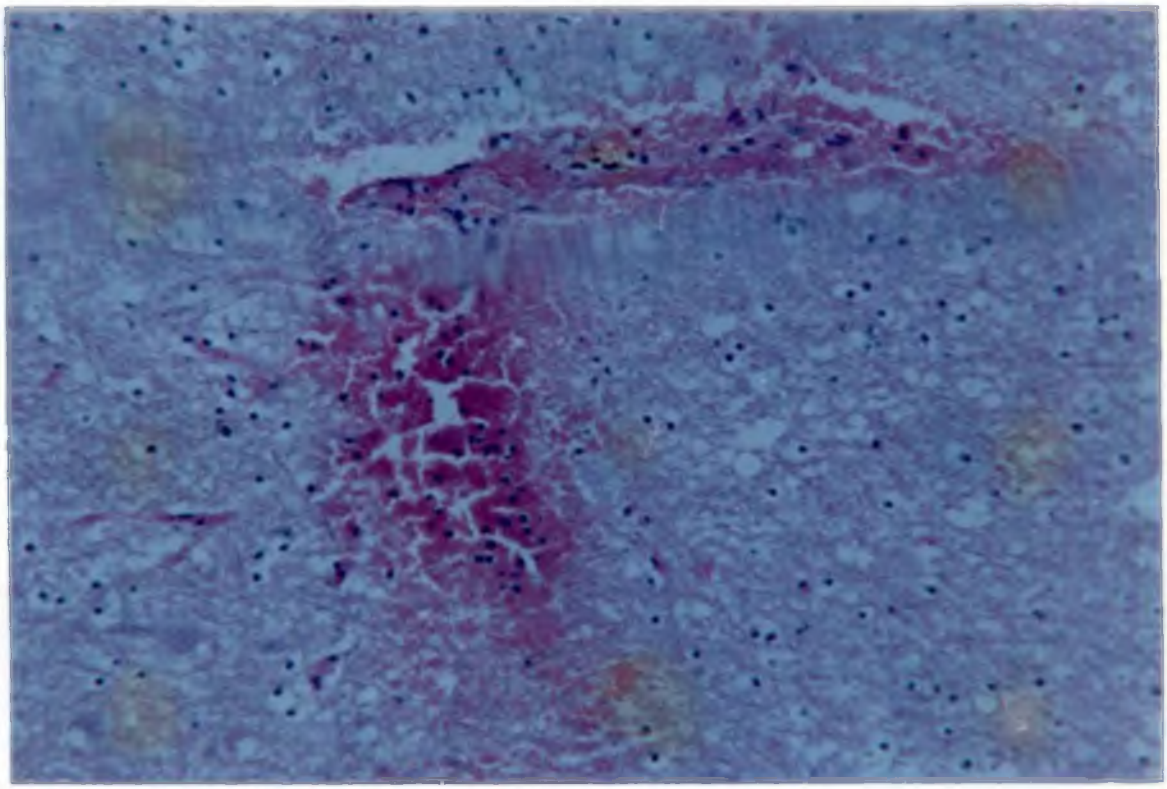


Fig.34. White matter: spongiform change, focal area of haemorrhage and vascular damage. H&E x 250

Fig.35. Cerebral white matter : oedema of myelin and loss of staining intensity.

Kluver Barrera Luxol fast blue stain x 400



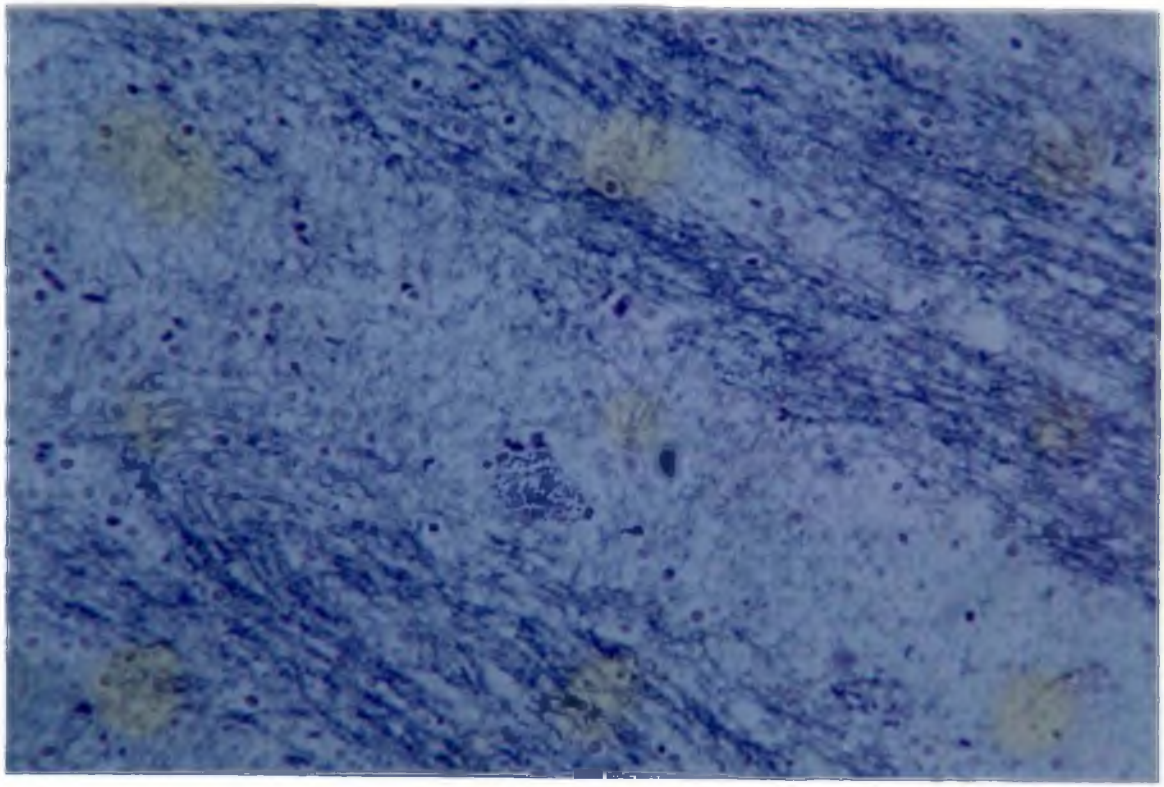
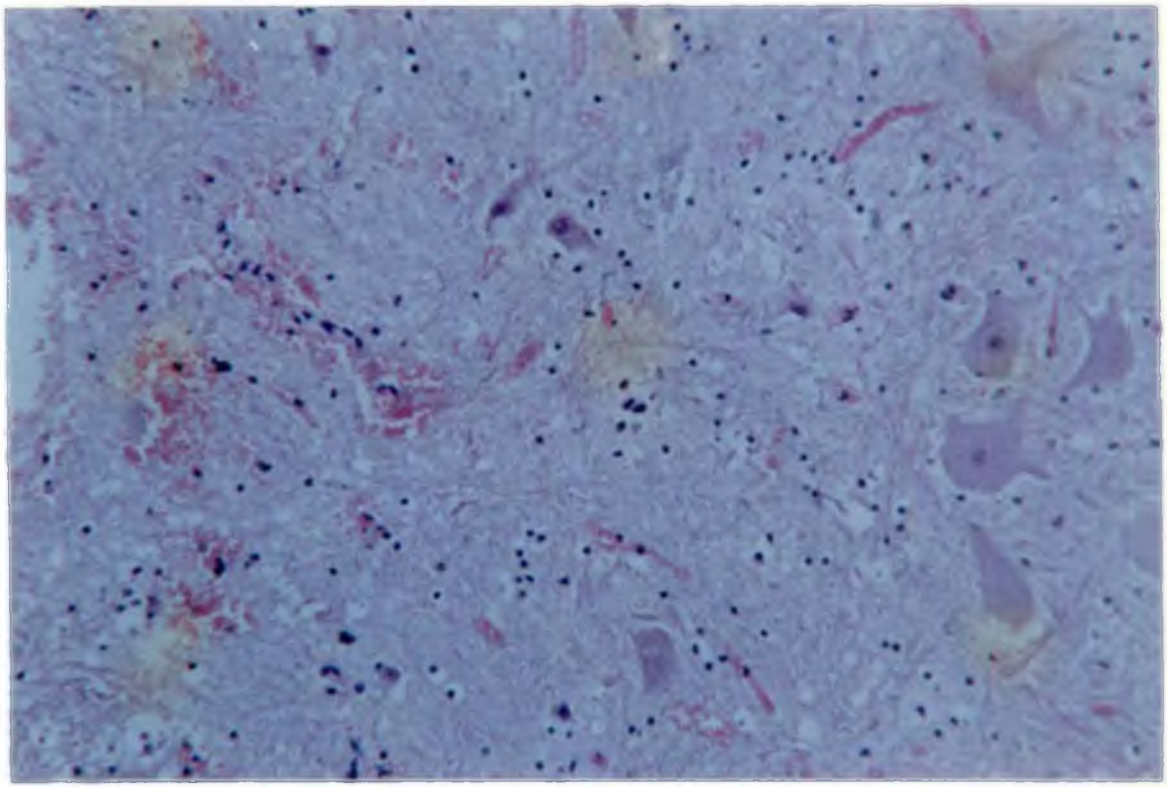


Fig.36. Medulla oblongata : congestion and multiple haemorrhage. H&E x 250

Fig.37. Spinal cord (SPC) : Left ventrolateral grey column-spongy change, perineuronal vacuolation and degeneration of neurons. H&E x 250



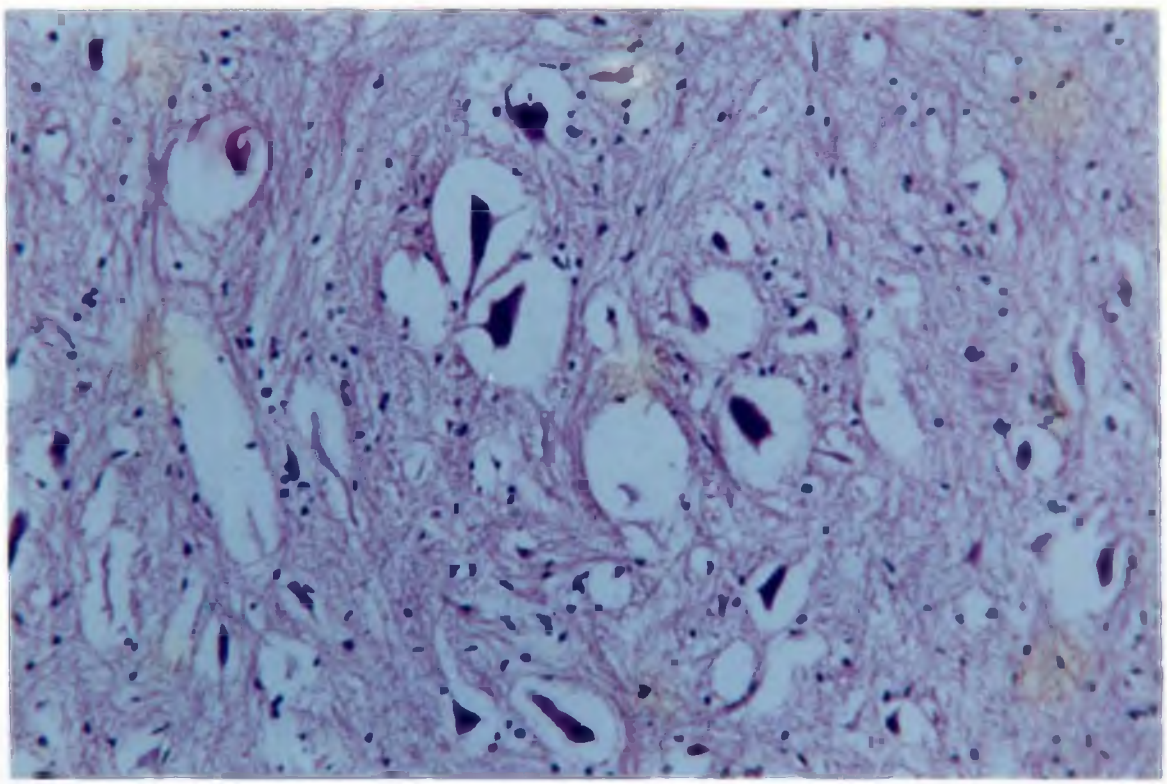
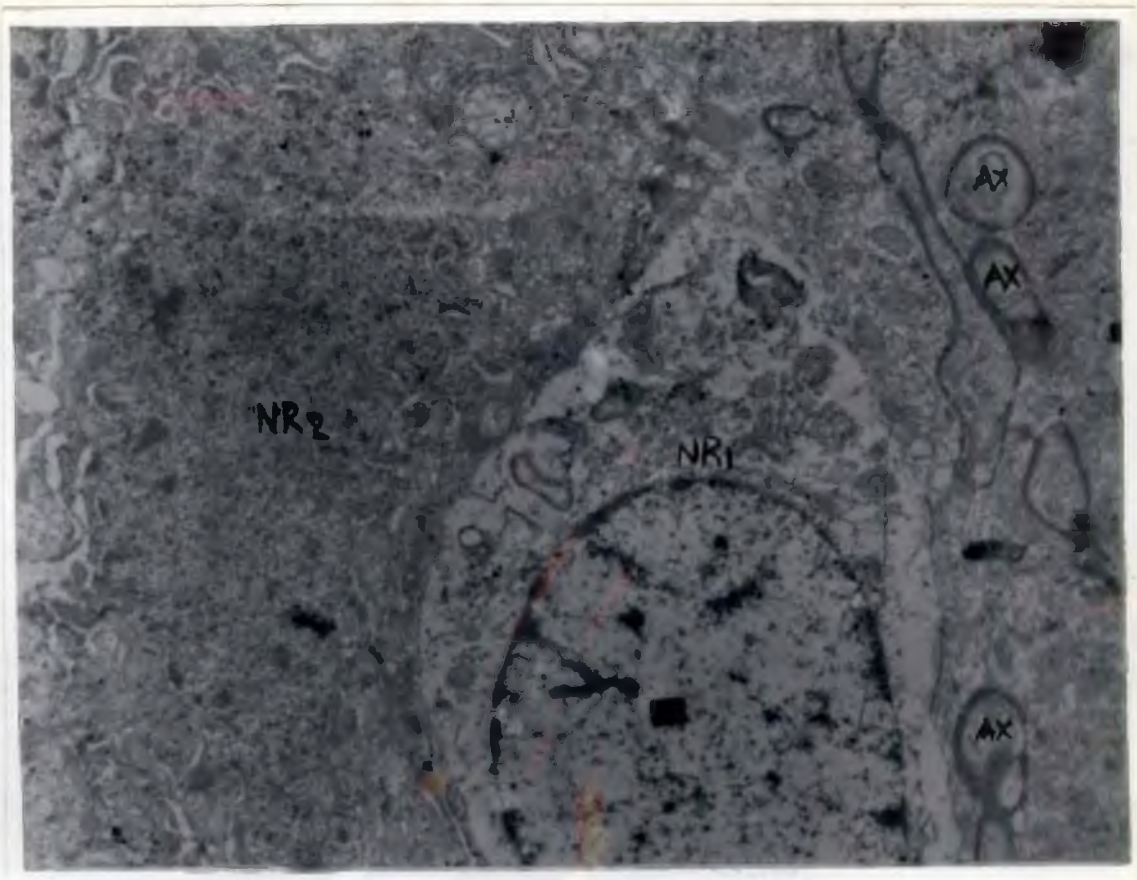


Fig.38. E/m: Portion of two neurons. One of which (NR-1) shows distension and loosening of cytoarchitecture and sparse organelles. The other neuron (NR-2) has electrondense cytoplasm with complete loss of organelle structure. Few profiles of myelinated axons (AX) showing degeneration of myelin sheath and swelling of axoplasm. x 10,000

Fig.39. E/m: Nucleus (N) of a neuron having predominantly euchromatin and with a well developed nucleolus (NL) showing segregation of pars amorpha and pars granulosa. Few lysosomes (L) and free ribosomes are seen in the cytoplasm. x 20000


AX
AX
NR2
NR1
AX

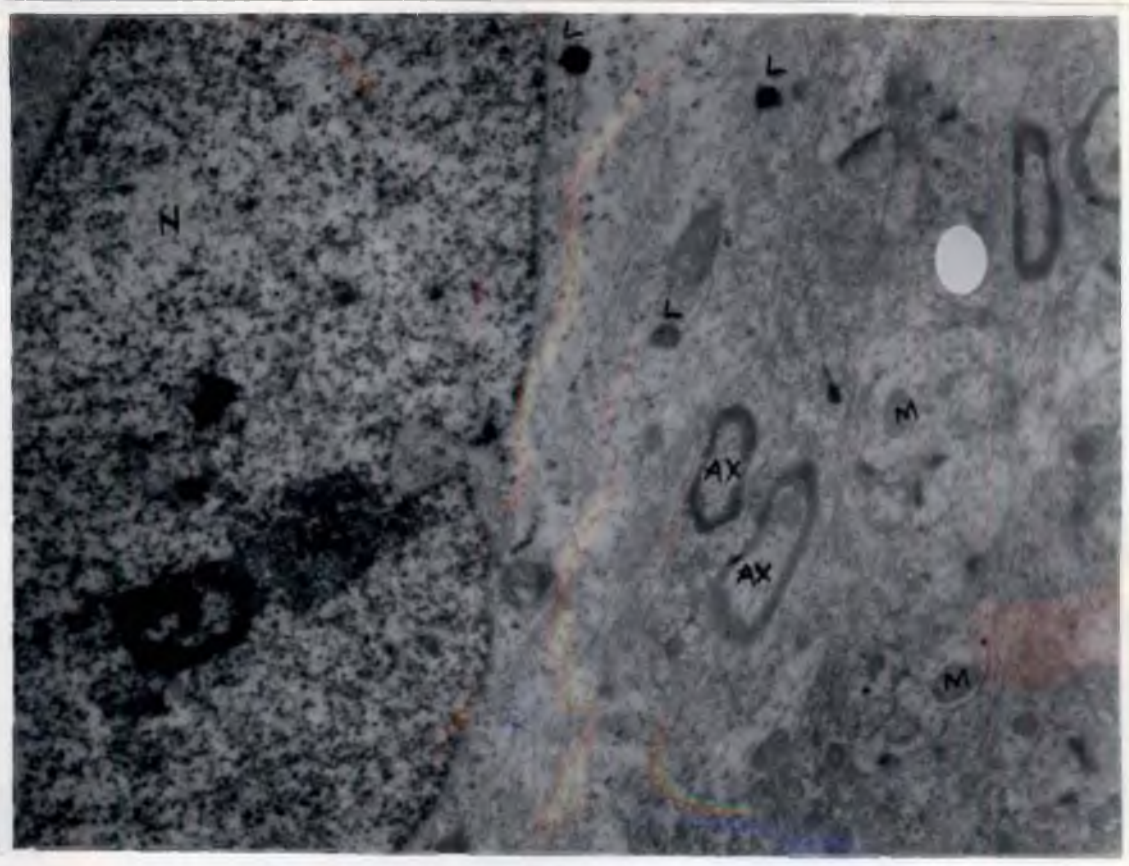
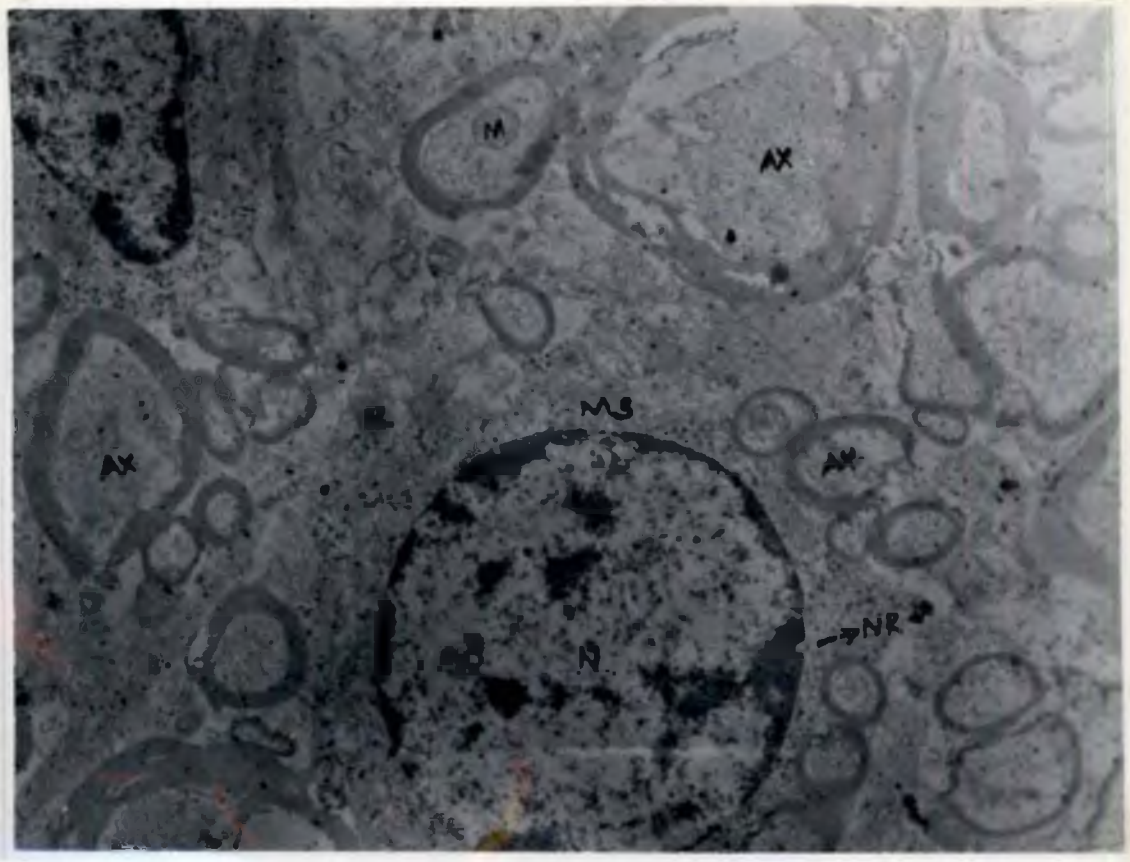

L
L
N
L
M
AX
AX
M

Fig.40. E/m: A microglial cell (MS) with well formed nucleus (N) having a rim of hetero chromatin along the inner nuclear membrane. Notice vacuoles (V), free ribosomes and liposome (L) in the cytoplasm. Numerous necrotic axons (AX) NP - nuclear pore x 12000

Fig.41. E/m An area of neuropil with moderate changes in the myelin sheath and axoplasm. Separation and fragmentation of fibres with cavitary pattern seen. Mitochondria (M) are pleomorphic. Also seen is part of an Oligodendroglia (OG) with cytoplasmic homogenisation and loosening of plasmalemma (PL) x 8000


M
AX
MS
AX
AX
NR
N

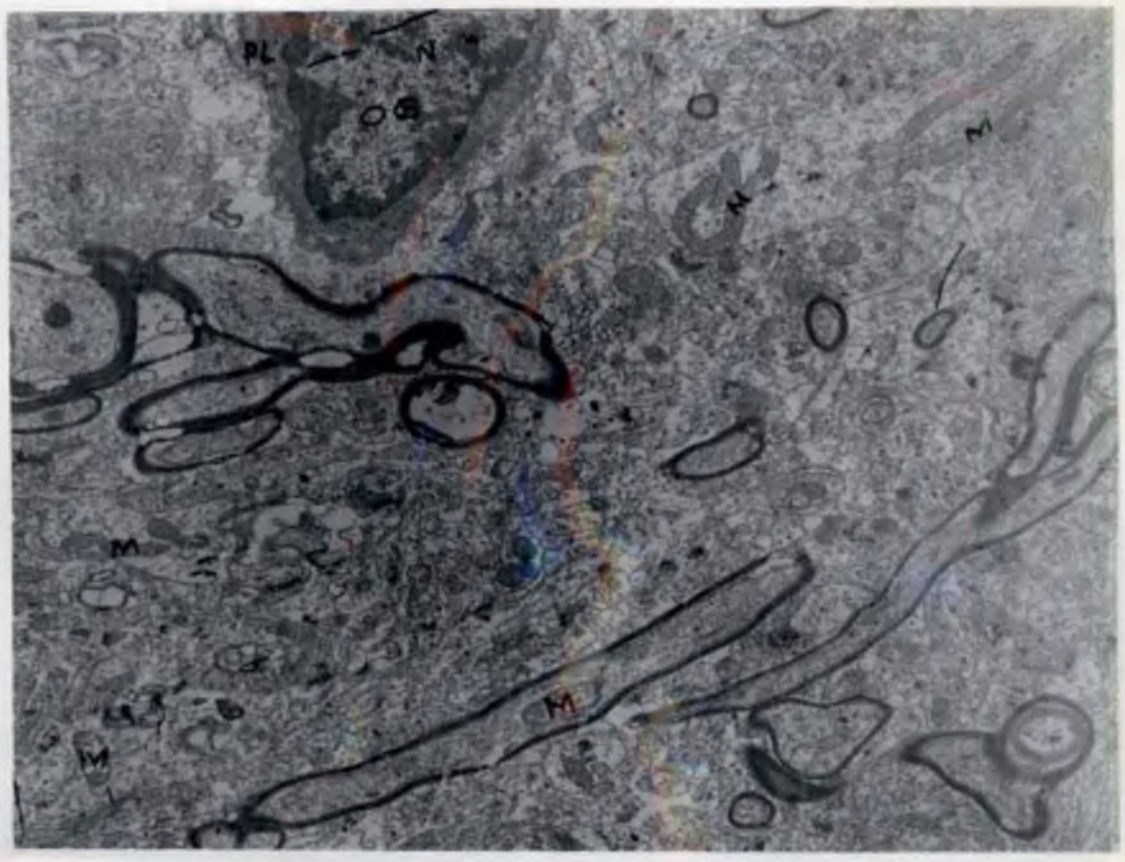
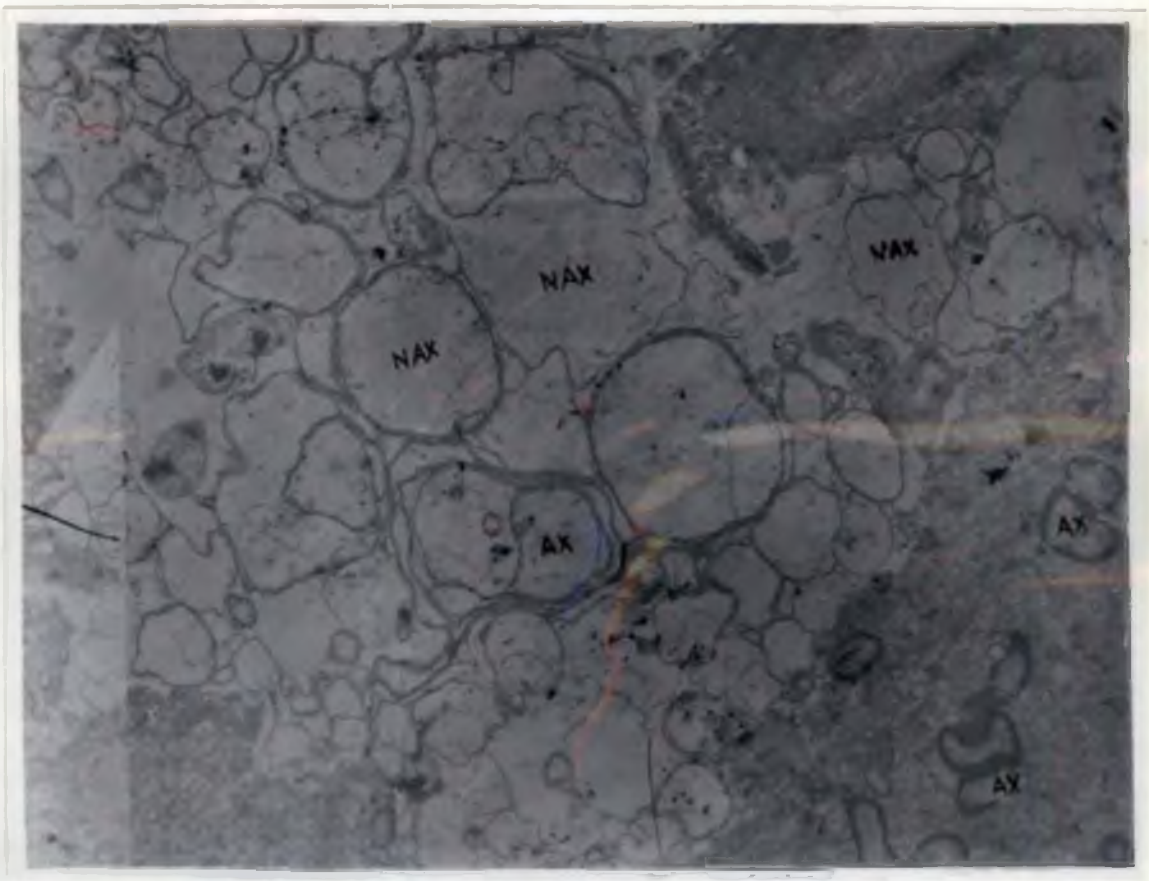

PL
N
M
M
M
M
M

Fig.42. E/m Necrotic myelinated axons (AX) and non myelinated axons (NAX) most of which are organelle free. Fusion of the axonal structures are seen forming cavitary areas. x 7000


NAX
NAX
VAX
AX
AX
AX

Group E (Sodium sulphate)

4.6 Clinical signs

Out of the six animals only three manifested nervous symptoms. It varied from lethargy, depression and anorexia to facial twitching, knuckling at the fetlock, spasticity of limbs, grinding of teeth, frequent urination, falling down and getting up frequently to recumbency. The animals preferred to lie down on one side. When forced to move, the animal moved to a distance and stood motionless. Another one showed pressing of the head against objects, salivation, lameness and occasional tremors of the body. Animal staggered when forced to move. Frequent bleating was observed in one of the animals. All on a sudden, the animal shivered and started moving backward aimlessly and fell down. At this position the animal developed spasms of the legs, opisthotonous (Fig.43) and respiratory distress. Eye preservation reflex in two cases was absent. One animal developed symptoms on the 34th day, another on the 39th day and the other on the 42nd day. The other three animals, although did not show any nervous symptoms were dull and preferred to lie down. All the animals had

intermittant diarrhoea. All the animals were sacrificed on the 45th and 47th day.

4.6.1 Body weight

Significant differences were observed in the weight of the experimental animals as compared to the control as shown in Table 3 and Fig.26.

4.6.2 Cerebrospinal fluid

The pre and post exposure total protein concentration of CSF is shown in Table 3 and Fig.27. The group mean values on statistical analysis did not show any variation in both the periods when compared to the control.

4.6.3 Weight of the brain

The group mean weight of the brain in grams per gram of body weight was 0.00935 ± 0.00082 and this was not significantly different from the controls (Table 5).

4.6.4 Brain autofluorescence

The cerebrocortical regions of two of the affected animals showed focal as well as diffuse blue green fluorescence at the superficial laminae, mostly at the gyral convolutions. All the others were negative.

Autofluorescence was also observed in focal areas in the white matter of one animal.

4.6.5 Gross lesions

The animals which showed nervous symptoms had more or less similar lesions in the brain. The meninges were highly congested. Moderate oedema of the brain was evident by the presence of moderately flattened gyri and the shallow sulci. In fact a few of the cerebral gyri at the rostral pole and caudal pole of the hemisphere revealed increased bulging and flattening (Fig.44). The vessels were highly engorged. In one of the animals there was haemorrhage into the spinal dura. The gyri had a glistening appearance. Yellowish discolouration of the cerebral cortical white matter was observed and it was diffuse. The mid brain region contained areas of petechiae and malacia (Fig.45). The malacic foci were seen consistently in the caudal colliculi of the mid brain. Petechiae and slightly dark brown spots of necrosis were seen restricted to the grey matter and in one animal dark pin head sized areas of softening were seen in the white matter. In two of the animals the cortex appeared slightly yellowish. There were dark brown or dark grey pin head sized lesions in the cortex and white matter. Such lesions

were situated mostly in the lateral and dorsal regions of the tempero-parietal and mid frontal lobes and occipital lobes. Lesions were more common in sulcal than in gyral grey matter. Vessels in the medulla oblongata, pons and spinal cord were engorged. The animals which did not show symptoms had no lesions in the brain.

Pulmonary congestion, cardiac dilatation, pale liver, petechiae in the kidney, congestion of intestinal mucosa were the other gross lesions observed.

4.6.6 Histopathology

Predominant histopathological lesions were present in those which showed nervous symptoms. Extensive vascular damage characterized by multiple haemorrhages were present in different segments of the brain. Cortical, subcortical and deeper cortical laminae of the grey matter showed varying degree of degeneration and necrosis.

Molecular layer cavitation or sponginess and vascular changes characterized by congestion, perivascular oedema, endothelial damage and neovascularisation were prominant (Fig.46). Diffuse gliosis was also observed.

Cerebral cortical lesions varied from segment to segment and also in different regions. In two of the cases

both in the superficial and mid-cortical laminae, individual or groups of neurons were found to be shrunken with intensely acidophilic cytoplasm and pyknotic basophilic nucleus. In some, the nucleus was seen condensed towards the periphery. Most of the shrunken neurons were found within or surrounded by empty spaces in the neuropil both at the cell body and dendritic processes. The perivascular space was also widened (Fig.47). Most of the neurons were seen associated with prominant astrocytes (Alzheimertype 2). Oligodendroglial satellites were seen surrounding a few neurons and also found occupying many of the vacuoles. A few of the astrocytes were vacuolated. There was condensation of chromatin as dots along the nuclear membrane. The cytoplasm was imperceptible. Few of the globular neurons present were pale staining and nucleoli were absent. The cytoplasmic condensation left a space around the cell. The neuropil was hypereosinophilic in one case. Endothelial swelling and diffuse glial cell reaction were noticed in them. Shrunken eosinophilic neurons and vacuolated parenchyma were seen in the full thickness of the cortex in one case. The dendrites in many of the neurons had a spiral appearance. The cytoplasm of a few neurons had small vacuoles. In one animal the superficial cortex of the cerebrum remained dense with

compact neuropil and the deeper areas appeared pale and rarefied. Disfigured dark pink staining neurons with potential perineuronal space were present in many of the cerebral cortical segments and mostly they were confined to the base of the sulci. Occasionally it was seen also amidst other neurons in the different laminae of the cerebrum. In one animal less cellularity was evident in the middle granule layer of the cerebral cortex. Few of the neurons present were pale staining and astrocytes were found associated with them.

Laminar cortical necrosis involving the entire length was observed in a few of the cerebral gyri. Increase in the cellularity of the molecular layer, cortical neuronal damage and mid laminar hypocellularity and pale areas were observed. Microglial nodules were seen in the superficial laminae in two of the cases (Fig.48). Diffuse and focal microglial accumulation were also observed in many of the cortical gyral areas. Damaged and eosinophilic neuronal remnants were seen amidst those cells. A few lymphocytes also could be seen along with those collections.

Vascular changes were predominant in the subcortical regions of animals which showed severe symptoms. Neovascularisation, congestion represented by prominent

capillary structures, perivascular neuropil vacuolation, endothelial damage and gliosis were seen. Extensive cavitation of the neuropil especially surrounding the vessels and neurons were observed in the deeper cortical areas close to the white matter. Vacuoles were spherical to ovoid or elliptical, sometimes multilocular and coalescing (Fig.49). The neurons were highly shrunken and most of them were seen pushed to the side of the vacuole. There was scant oligodendroglial reaction in these areas. There was severe congestion of the vessels in few segments of the cerebral cortex, midbrain, cerebral crus and lateral ventricular areas. Swelling of the endothelium of the vessels was evident.

Congestion, haemorrhage, vascular endothelial swelling and discontinuity and extensive accumulation of lymphocytes, monocytes and glial cells (Kolmer cells) were also seen in the subependymal areas of the lateral ventricle (Fig.50). The ependymal cells lining the ventricles were separated from its surroundings by oedema.

Cerebro-cortical white matter in all the affected animals showed varying degree of vacuolation and congestion of capillaries. Small circumscribed to elliptical and large vacuoles were seen (Fig.51). The white matter at

focal sites had a sieve like appearance having uniform small vacuolation forming irregular pattern of arrangement between regular and thick fibers, which were in turn separated by regular big vacuoles. Demyelination and intrafascicular oedema were evident as the myelin which was blue to green stained, appeared discontinuous and pale staining with luxol fast blue stain (Fig.52). The intact myelin stained blue. The myelinated fibers also appeared broken and the demyelinated area appeared less impregnated with silver (Fig.53). Gliosis was also evident.

The cerebellar folial lesion included congestion, reduced cellularity, degeneration of granule cell layer and degeneration of a few Purkinje cells. Loss of glomerular pattern of arrangement of the cells was evident. Swollen and pyknotic Purkinje cells appeared within the loose granule layer matrix. In certain cerebellar folia, the fibers of the basket cell predominated. The cerebellar white matter appeared intact with regularly arranged intrafascicular oligodendrocytes.

Extensive subdural haemorrhage was seen in the spinal cord in one animal (Fig.54). Congestion, haemorrhage and diffuse vacuolation and gliosis of the grey column were evident in another case.

In the pons, certain neurons were swollen and there was diffuse neuropil vacuolation.

The corpus striatum was affected in all the affected goats with cortical lesions. The neuropil of the caudate nucleus was vacuolated and this change was accompanied by an astrocytic reaction and dark staining neurons.

The hippocampus showed mild astrocytosis, with some dark neurons in the large amorphous cell layer. Neuronophagia was occasionally present in para hippocampal areas.

Animals which did not show symptoms also had slight neuronal changes in the cerebral cortex. In some, the cerebro-cortical grey matter was more cellular.

4.6.7 Ultrastructural pathology

Cerebrocortical oedema was prominent in all the cases. The neuronal changes appeared varying in such an oedematous neuropil with large number showing lytic or pyknotic nucleus. In areas of oedema, glial fibrils, occasional mitochondria, fragments of RER and glycogen granules were seen.

4.6.7.1 Neuron

The cerebral cortical neurons showed changes such as swelling, shrinkage and cytocavitation of varying degrees. Many of the neuronal nuclei appeared swollen and there was an expanded volume of the karyoplasm (Fig.55). Dilatation of the perineuronal space was observed in a few of the neuronal nuclei. Nuclear membrane bleb formation was evident in few cases. There was irregular notching and crenation of the nuclear membrane in certain neurons especially the pyramidal ones of the inner laminae of the cerebral cortex. Some of the swollen nucleus had focal disruption of nuclear membrane and had an irregular outline. The nuclei were abnormal in their configuration, less dense and showed micro segregation of nucleoplasm.

Nuclear pores in some of the neurons appeared prominent. In the swollen neurons the chromatin was sparse and found dispersed in an electronlucent matrix. Irregular clumps of heterochromatin were observed amidst the electronlucent nuclear matrix. Heterochromatin was much less and when found was seen as electrondense linear condensations on to the nuclear membrane. In a few of the neurons perichromatin granules could be seen. The nucleolus appeared condensed and most of the cases they

appeared round and seen close to the nuclear membrane. There was no separation into nucleolonema and pars amorpha.

The cytoplasm of the neurons had an expanded volume and many of the organelles were not discernible. Most of them had a granular appearance (Fig.56). There was complete loss of stainable material in the cytoplasm of many neurons. Distension of parallel arrays of RER with detachment of ribosomes and fragmentation of RER could be seen in many. The distended RER appeared in the form of circular or elongated vesicular profiles. Cisternae of RER were less uniform in size and shape and had in some an electrondense contents. SER was sparse. The cytoplasm in few other neurons in many locations was devoid of RER. At some locations small strands of RER with a narrow slit like cisternae were present. The mitochondria seen in between the RER profiles were either swollen or condensed and electrondense (Fig.56). Round, elongated and bizzarre mitochondria were observed. Cristae were visible in some and in most of them there was partial to complete cristolysis. Few of the mitochondria were very small and round with a homogenous electrondense matrix. In some of the neurons partially damaged mitochondria with remnants of cristae were seen.

The cytoplasm contained free ribosomes and polysomes. Neurons showing lytic appearance of the cytoplasm and nucleus with focal condensation of the chromatin were evident. Glycogen rosettes were seen in a few neurons. Golgi apparatus were not apparent. Microtubules and few fragments of microfilaments were scattered in the cytosol in few of the neurons.

4.6.7.2 Glial cells

The astrocytic reaction was prominent. They, in the cerebral cortex, either appeared hypertrophic or condensed. There was invagination of the nuclear membrane in few of the astrocytes. The chromatin was found to be attached to such invaginated membranes. Nucleoli appeared less compact leaving spaces containing the lucent chromatin granules. Heterochromatin was sparse. Euchromatin appeared filling the nucleoplasm. Cytoplasm of such astrocytes did not reveal any cisternal sacs except for a few fragmented cisternae with ribosomes attached to it. There was partial degranulation of ribosomes in focal areas. Mitochondria appeared pleomorphic. Very small round mitochondria to larger ones with various electrondensity could be observed. Cristae in many were not discernible. The neuropil

surrounding such astrocytes contained both myelinated and non myelinated axons.

In some of the hypertrophic astrocytes cytocavitary profiles were very much predominant around a condensed electrondense perikaryon around the nucleus. Many of them contained abundant glial filaments which accompanied the degenerating neurons.

Satellite oligodendrites had disrupted nuclear membranes. The cytoplasm was scanty and found condensed around the nucleus. Organelles were not discernible (Fig.57). The axoplasm of the axons near the cell appeared swollen and necrotic. An increase in the number of microglia which contained individual or fused lipid droplets were seen in the

cerebral cortex. Membrane bound dense bodies appeared in few of the cells.

Purkinje cells often appeared regular with nuclear invaginations. Variable amount of degeneration of organelles was evident in few of the partially damaged Purkinje cells. Granule cell layer was less dense and the neurons showed varying changes. Some with crenated nuclear membrane, condensed chromatin, organellar changes and complete cytoplasmic dissolution were evident. Myelinated

tract of the neuropil showed intramyelinic vacuoles and there was microglial reaction.

There was marked perivascular oedema and these areas had only remnants of small round or ovoid structures with an electronlucent or dense contents. Endothelium of the vessels was swollen (Fig.58) with an enlarged cytoplasm. Cytoplasm was homogenous and moderately electrondense with sparse organelles. The tight junctions displayed mild separation indicative of a compromised blood brain barrier. The basement membrane showed focal areas of loosening (Fig.58) and blebbing. Irregular focal expansions on the basement membranes indicating the expanded foot pads of astrocytes were seen.

4.6.7.3 Neuropil and white matter

The cortical neuropil had an electronlucent appearance with separation and lysis of structures and many pleomorphic mitochondria showing different degrees of destruction were seen (Fig.58) in the neuropil element. Neuropil was loosely textured with many axons showing partial lysis. The oligodendrocytes amidst such axons had irregular nuclear membrane and condensed chromatin. The cells appeared clumped. The nuclear membrane appeared lysed in some.

The white matter appeared loosely textured. There was wide separation of white matter elements. The organelles especially the mitochondria in them showed complete lysis of internal structures with only the outer membranes remaining intact. The intercellular spaces in the neuropil were very much distended.

Complete lysis of axonal membrane, disruption and lysis of myelin and clearing of the matrix of such axons were seen in some. The mitochondria in them appeared electron dense without discernible cristae. Some of the distended axons contained electronlucent tubular profiles and condensed mitochondria. Many axons had prominant dilated stacks of flat saccules which were separated by electron dense material. The myelin vacuolation was extensive in the white matter. Multiple splitting of the myelin was observed occasionally. The several layered myelin surrounding the axons showed occasional lysis and such myelin appeared granular amidst the myelin lamella (Fig.57). Segmental loss of myelin was observed in few of the axons. Large masses of homogenised myelin sheath material were often seen in the white matter and also surrounding the damaged oligodendroglial cells. Some of the intramyelinic vacuoles infrequently contained myelin debris. In many the axolemma was often separated from the

myelin sheaths. Reactive astrocytes were often encountered. Some revealed disintegration and fragmentation of their processes. The cell bodies and mitochondria were swollen.

Dendritic processes were swollen and contained distorted mitochondria. The dendritic spine with spine apparatus was seen (Fig.59). The apparatus appeared as flattened or dilated sacs separated by an electrondense material. The mitochondria within the apparatus appeared swollen and there was cristolysis.

Synapses were seen at times. Synaptic vesicles were less and some appeared clumped. Most of the vesicles displayed variations in size and shape as well as dilatations and evacuation of their contents as they appeared translucid. In many axosomatic or axoaxonic synapses, the cleft was moderately widened and appeared irregular in shape with variable densities of osmiophilic materials.

Fig.43. Group E. (sodium sulphate treatment)-Animal dull, sleepy and showing opisthotons.

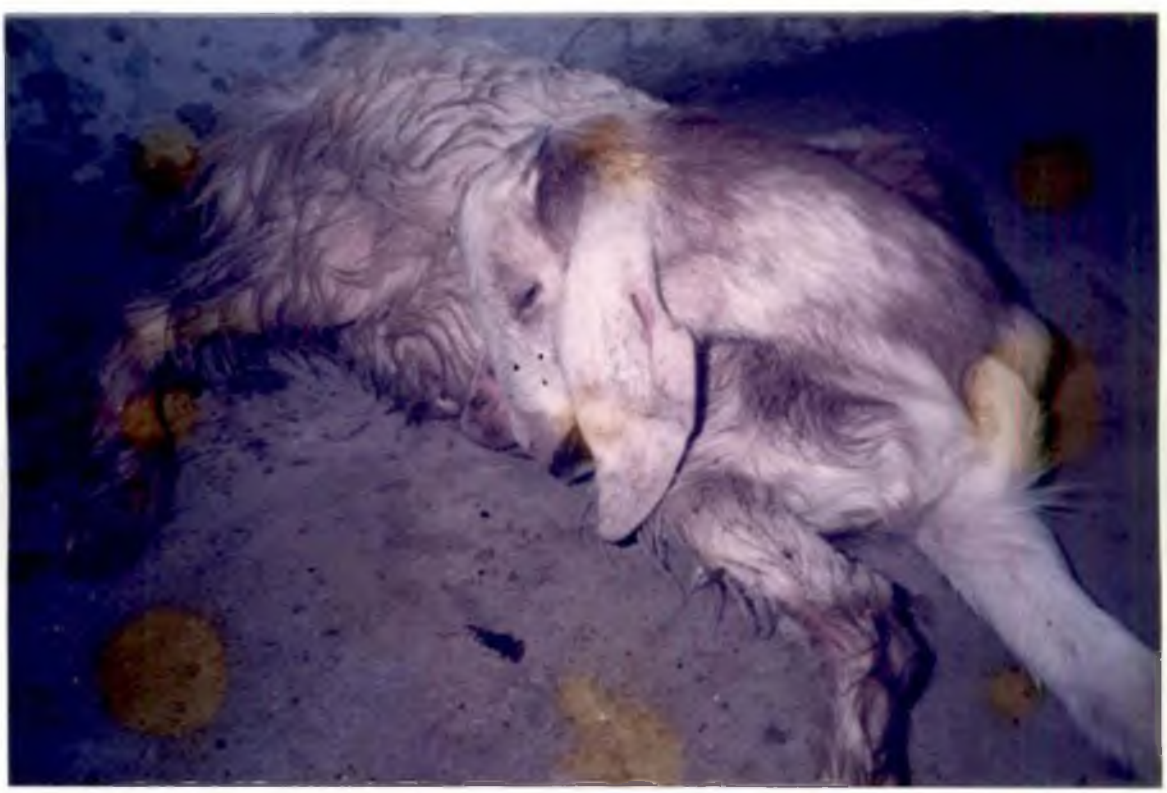


Fig.44. Group E. (Sodium sulphate) congestion, flattening of the gyri at the rostral and caudal pole of cerebral hemisphere

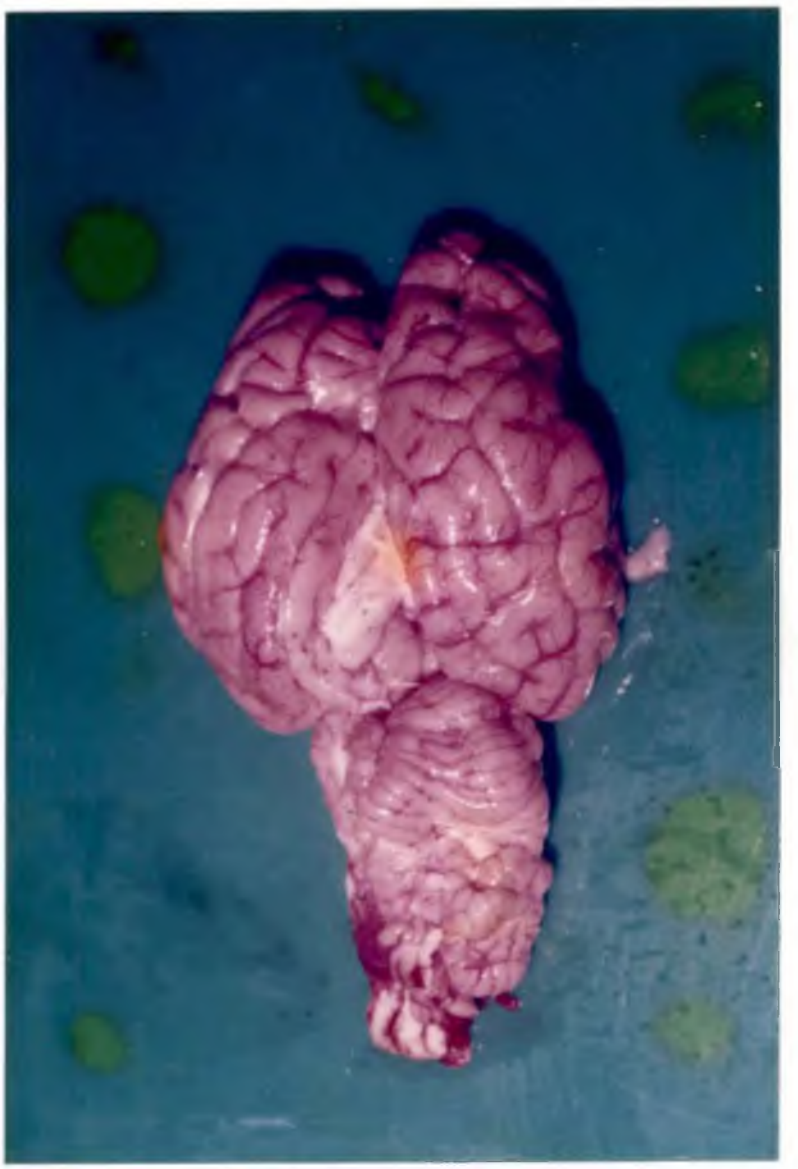


Fig.45. Cut surface of the brain showing scattered petechiae and focal malacia

Fig.46. Cerebrum : molecular layer - spongy, change, congestion and oedema. H&E x 250

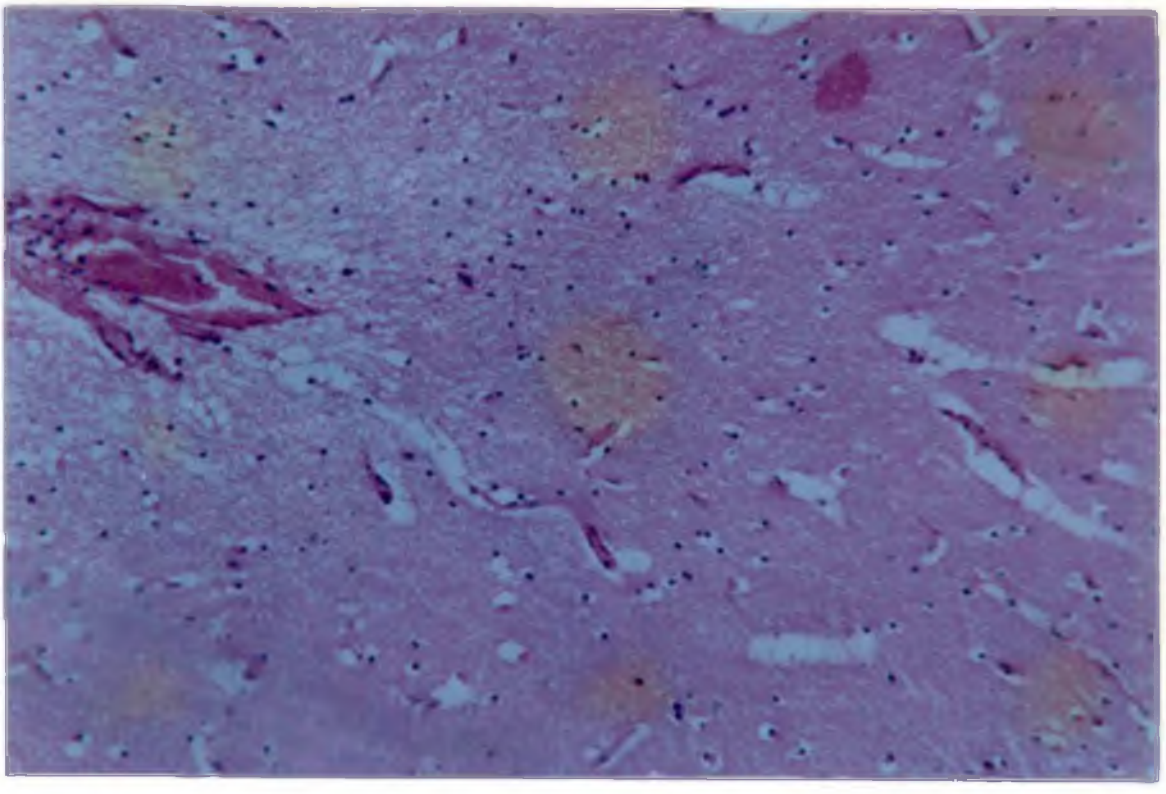
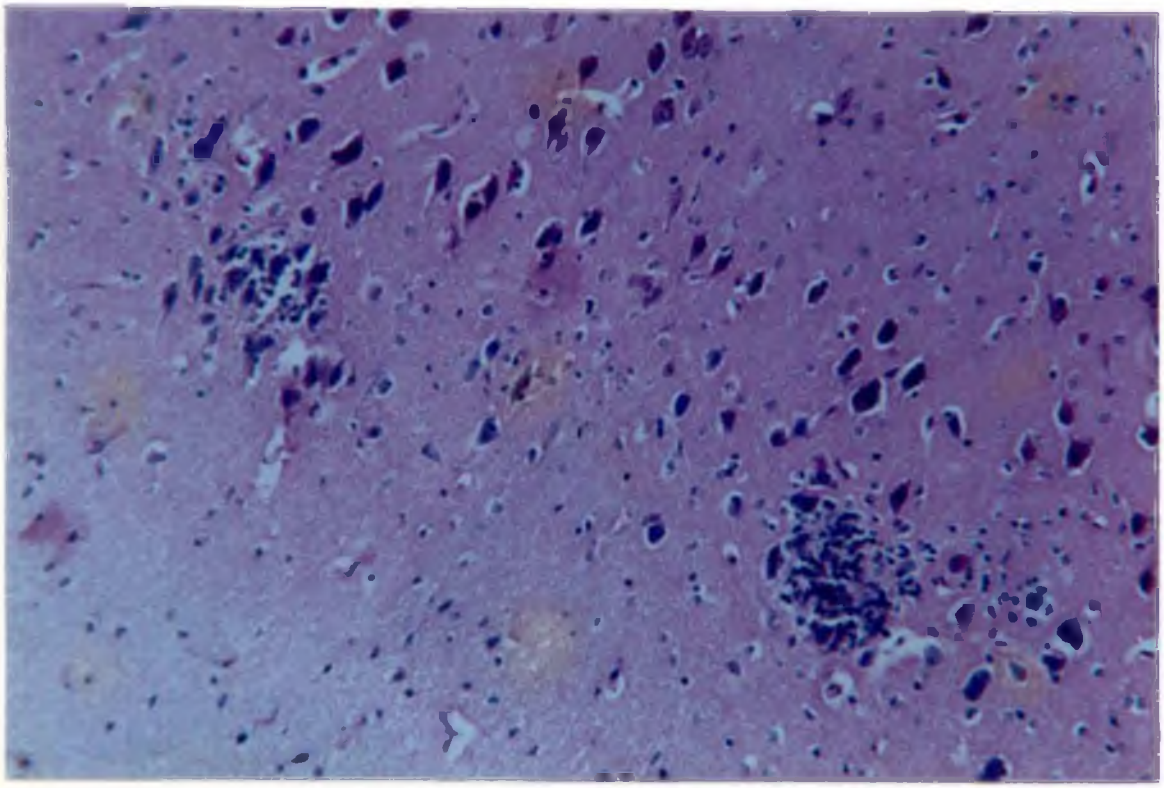


Fig.47. Cerebrum - middle lamina-shrunken neurons, pyknotic nucleus, perivascular and perineuronal oedema. H&E x 400

Fig.48. Cerebrum : Superficial laminae-microglial nodules. H&E x 250



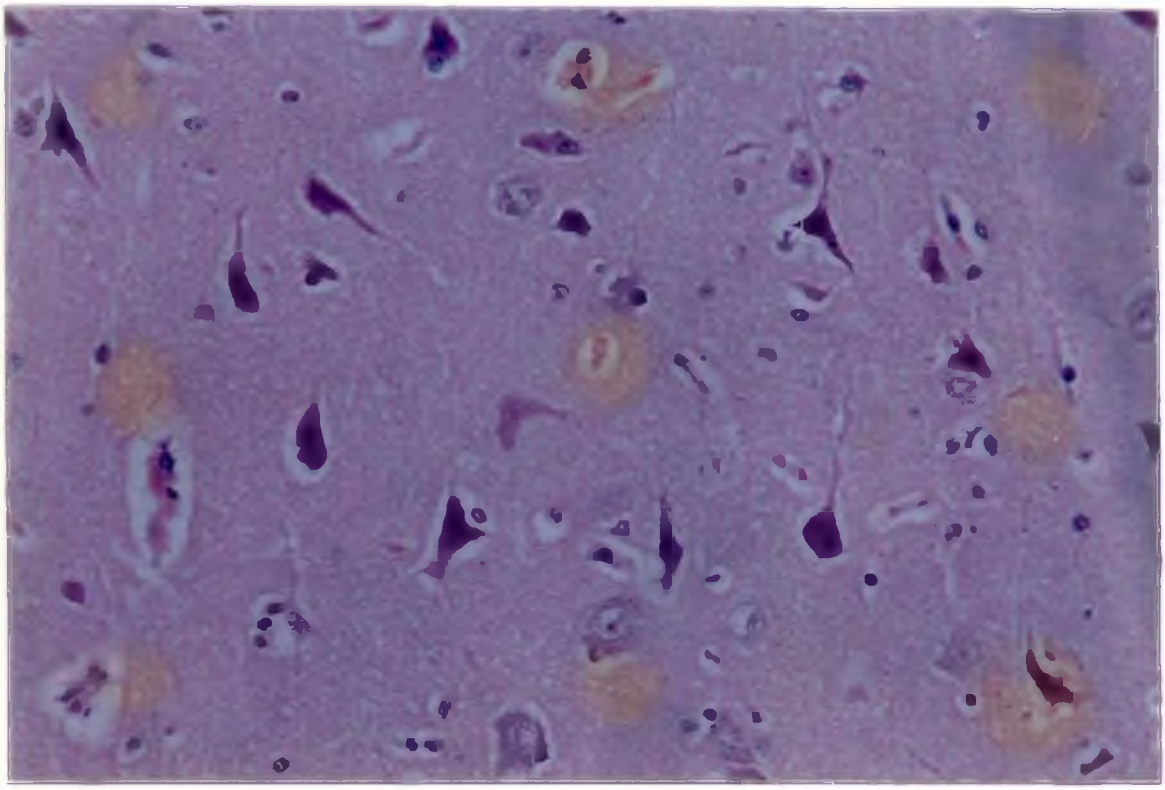
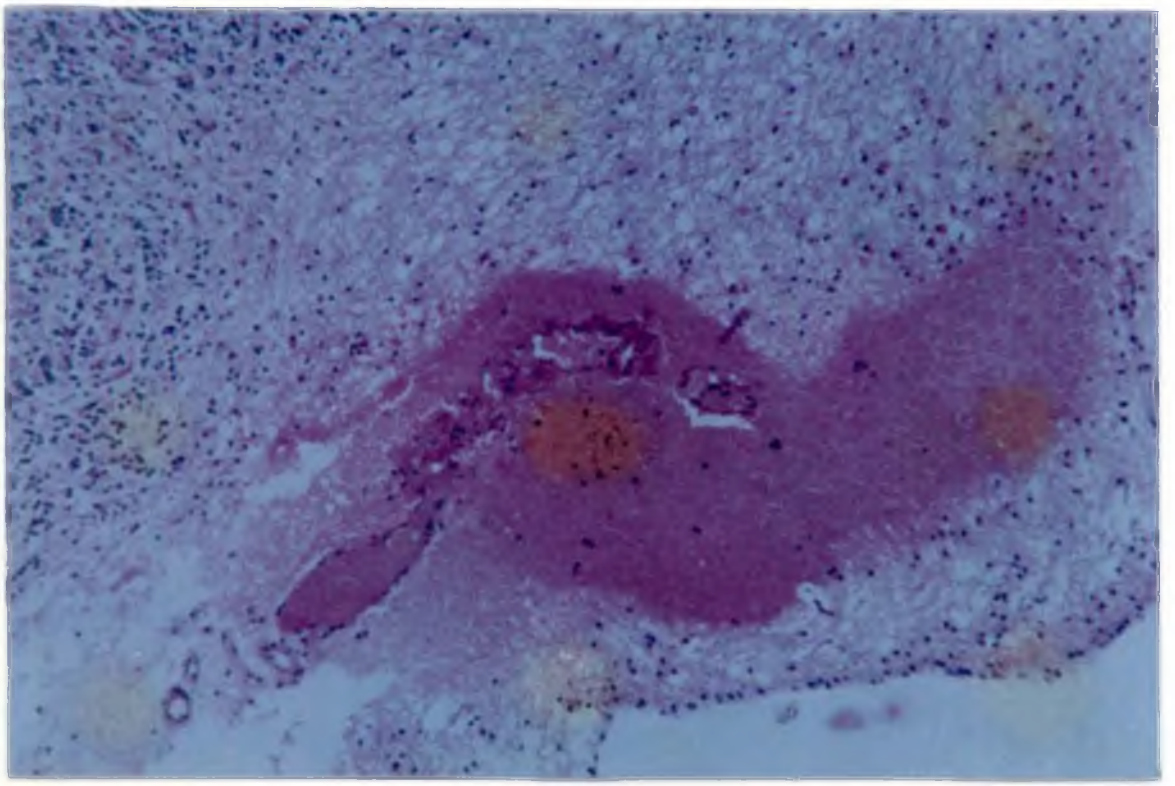


Fig.49. Cerebrum : deeper cortical laminae - vacuolation and neuronal condensation. H&E x 400

Fig.50. Lateral ventricle - sub-ependymal haemorrhage, vascular damage and Kolmer cell accumulation. H&E x 250



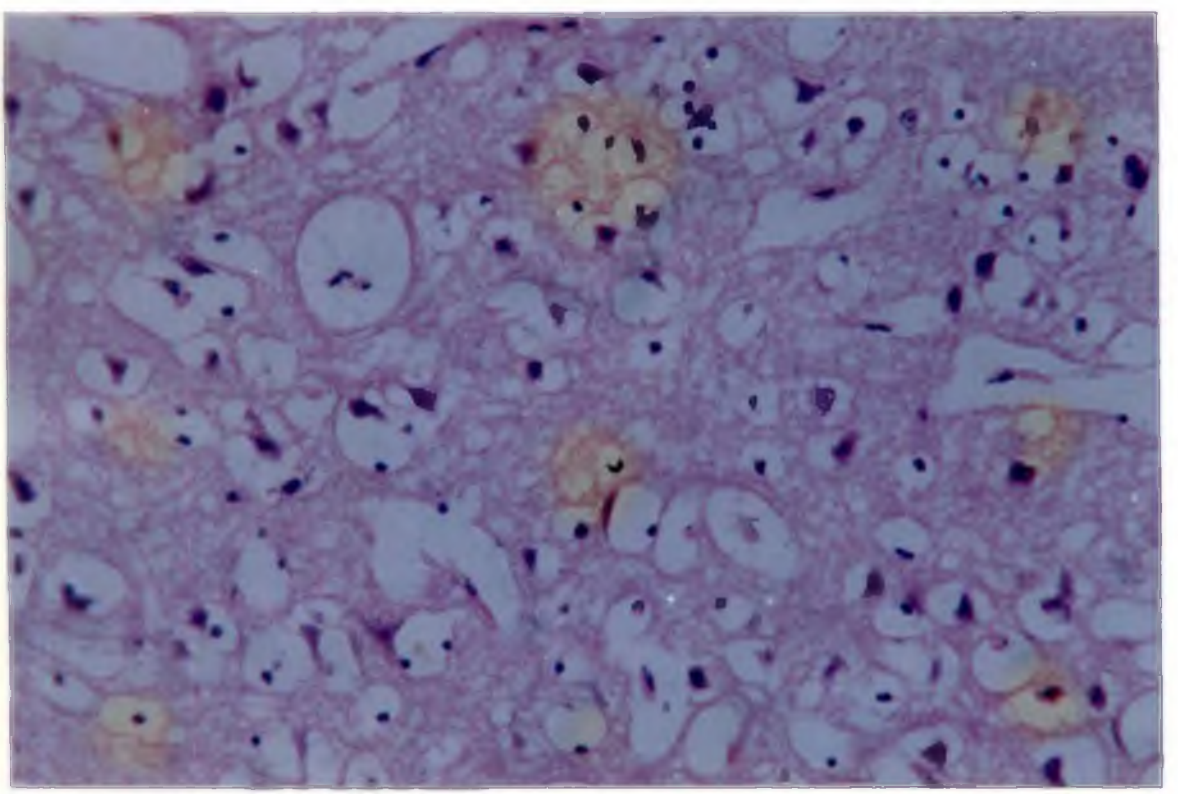
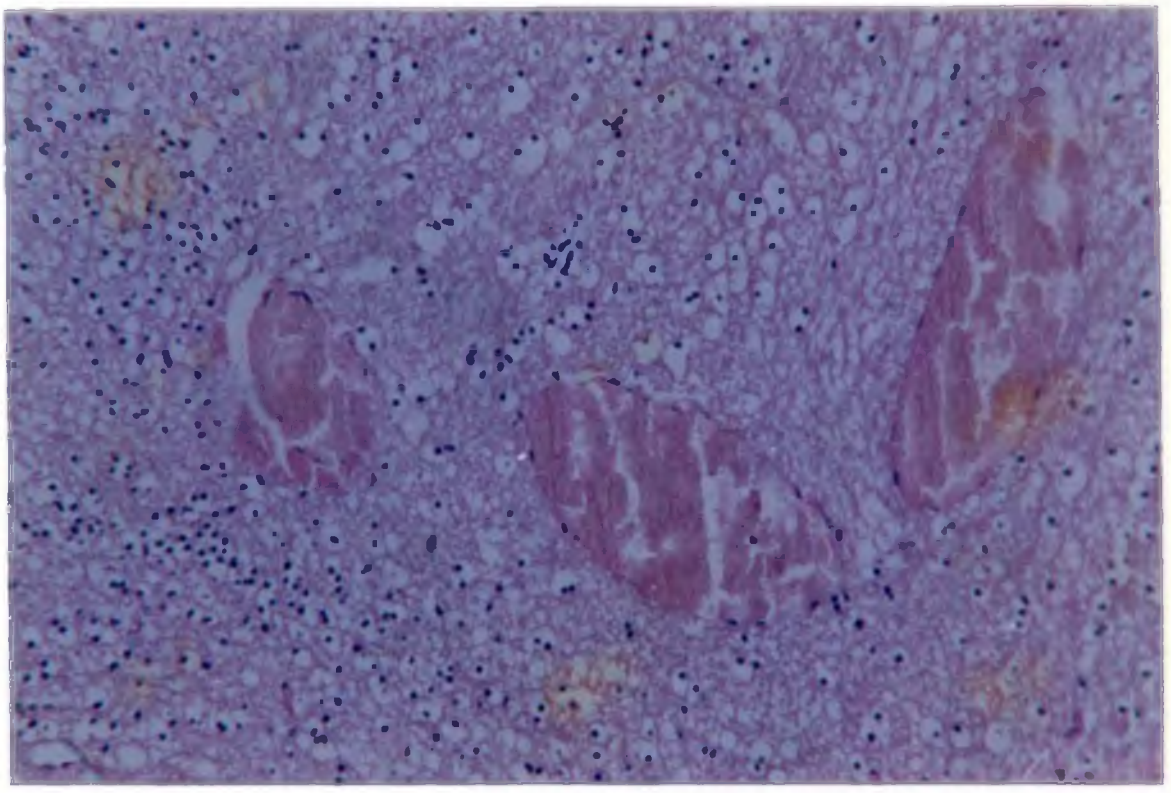


Fig.51. Cerebral white matter - vacuolation and congestion. H&E x 250

Fig.52. Cerebral white matter demyelination and interfascicular oedema. Reduced stainability of myelin. Kluver Barrera Luxol fast blue stain x 250



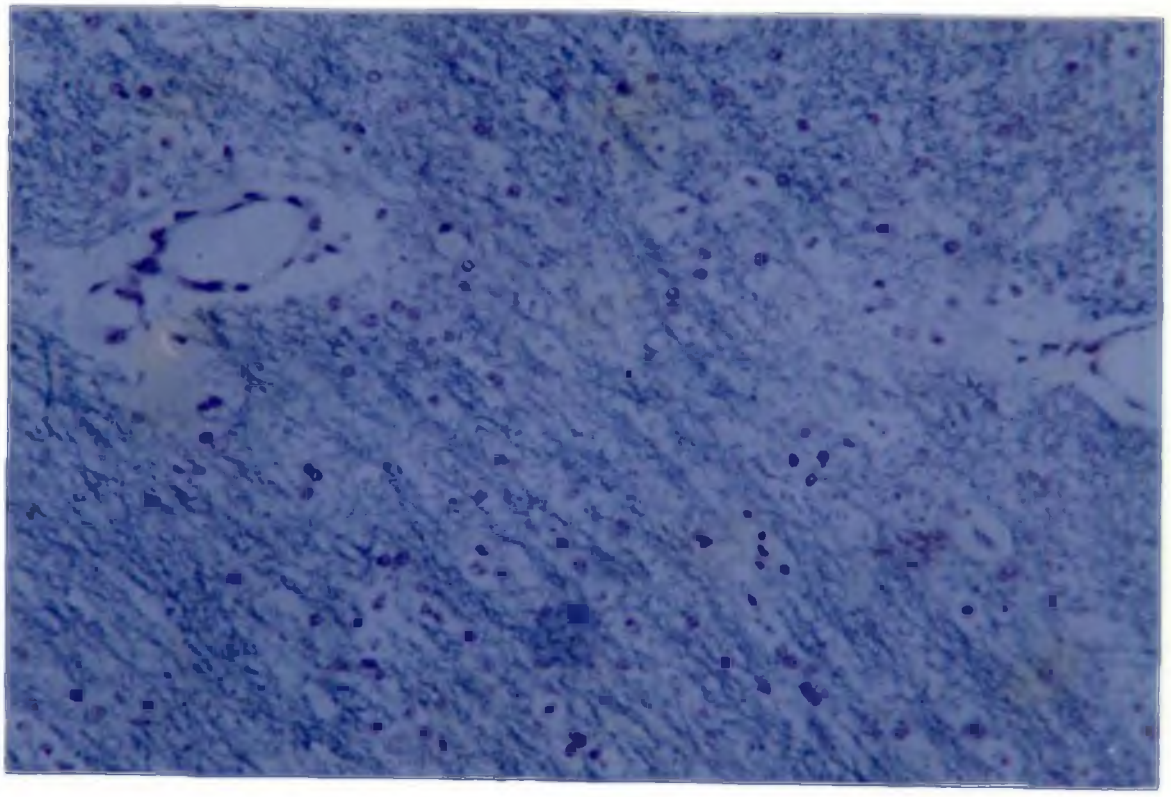
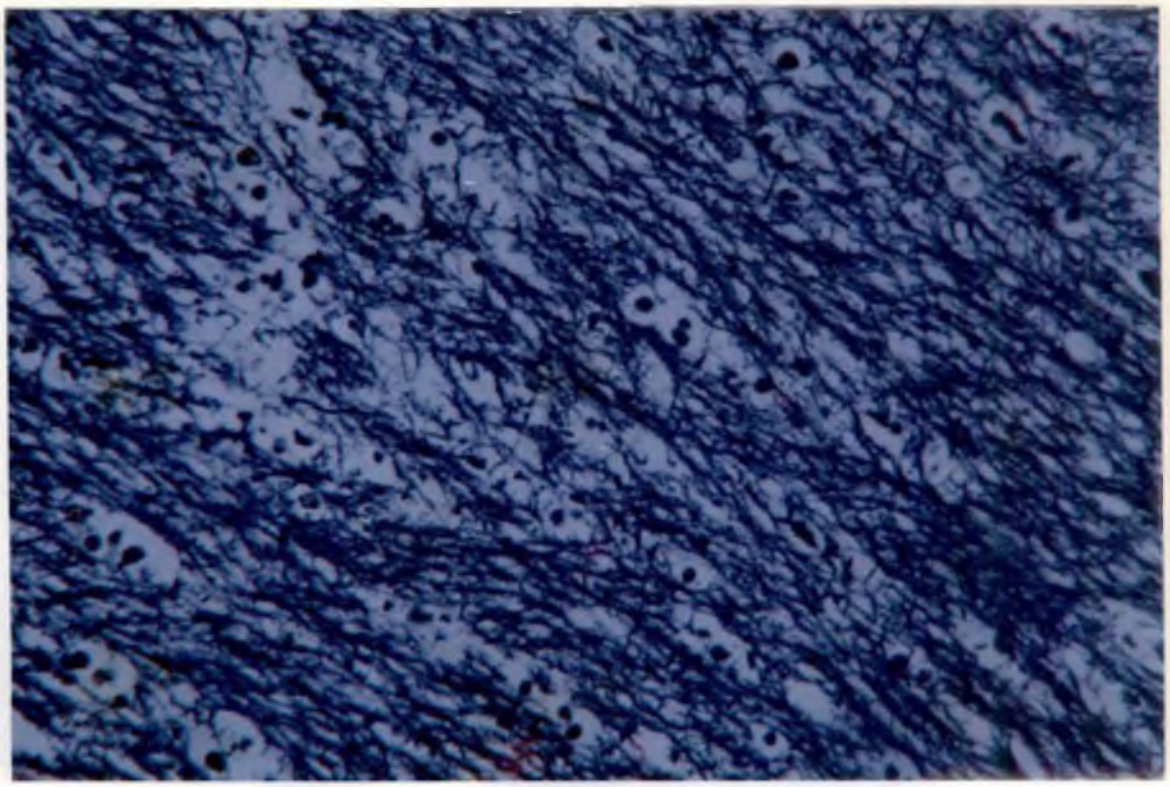


Fig.53. Cerebral white matter - demyelination, broken fibers and less impregnation with silver. Glees and Marasland's modified silver stain x 400

Fig.54. Spinal cord - subdural haemorrhage x 250



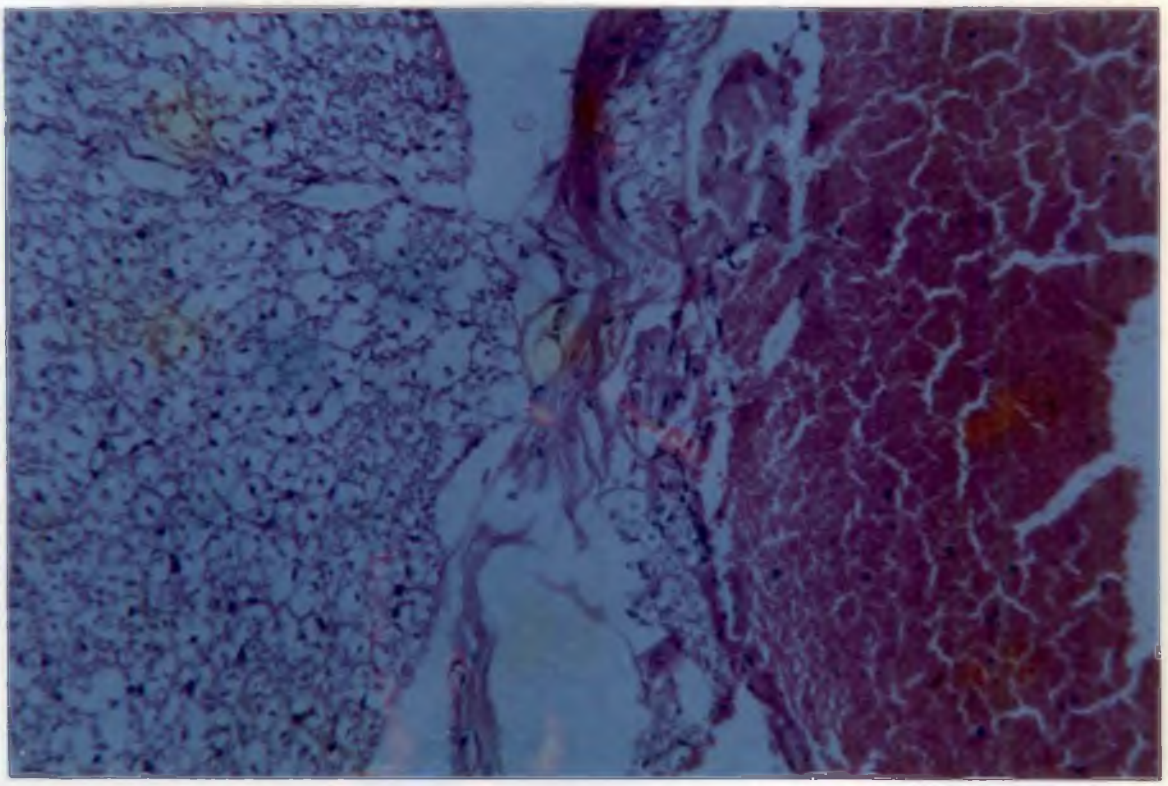
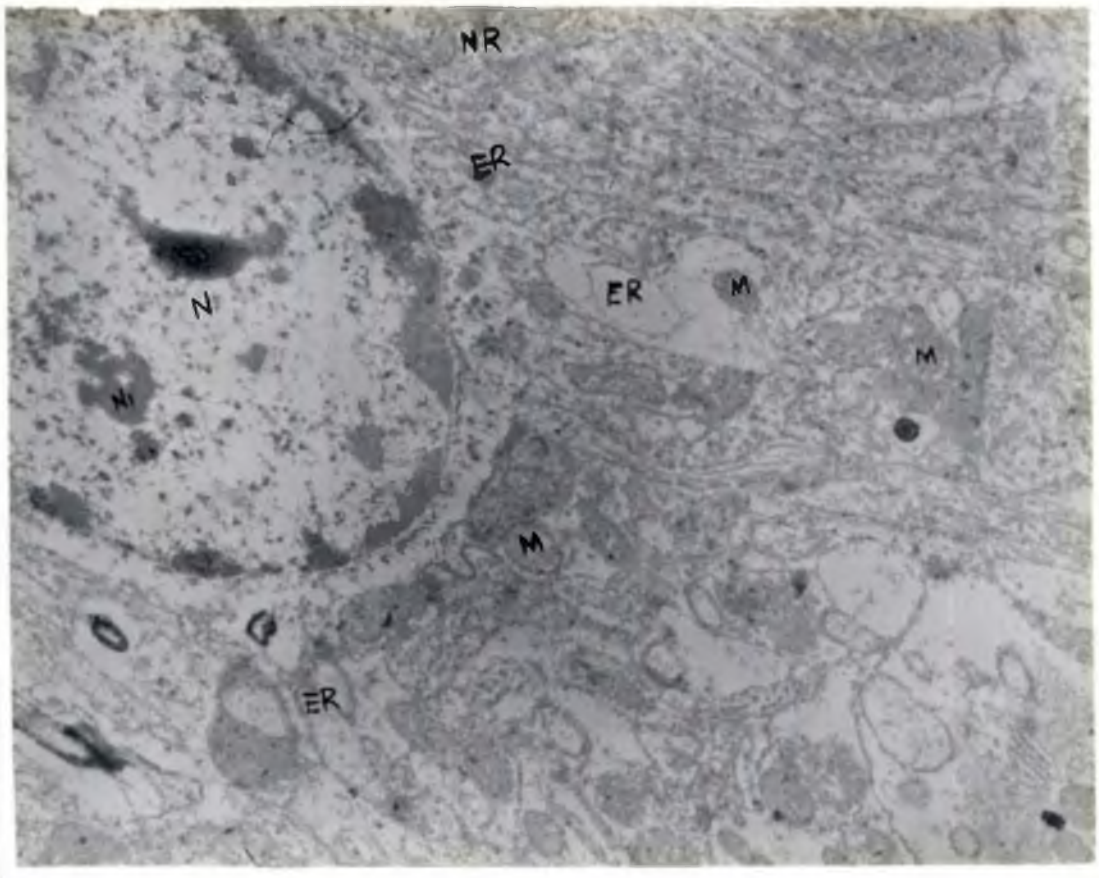


Fig.55. E/m: Neuron (NR) showing swollen nucleus (N) with a lucent Karyoplasm. Dilatation of endoplasmic reticulum (ER), vacuole formation and lytic areas are noticed. Mitochondria (M) in different stages of destruction NI-Nucleolus x 20000

Fig.56. E/m: Mid cortex - The microglia (MS) shows sparse cytoplasm and a nucleus (N) with prominent nucleolus (NL). The cytosol of neurons (NR) is either swollen or condensed, many showing lysis of membrane components. Swollen non-myelinated axons (NAX) seen x 16000


NR
ER
N
ER
M
M
NI
M
ER

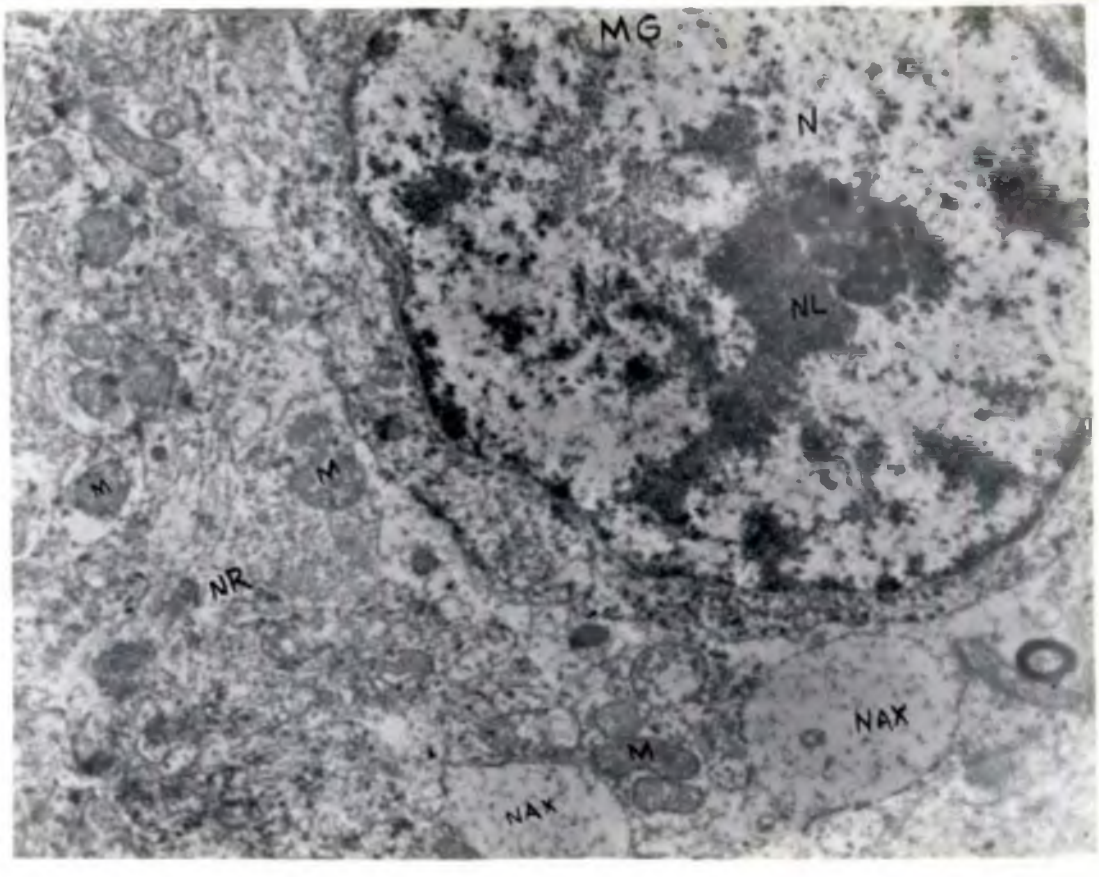
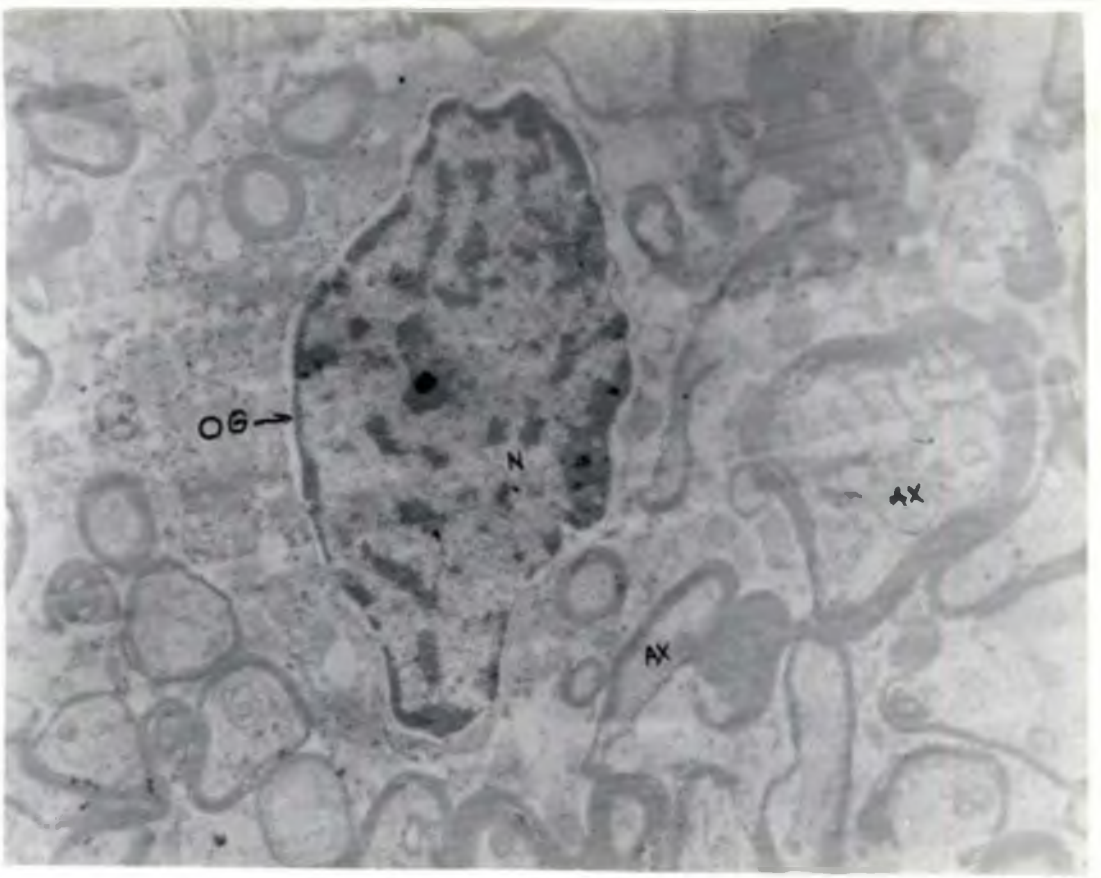

MG
N
NL
M
M
NR
NAX
M
NAX

Fig.57. E/m: An oligodendroglia (OG) showing amorphous cytoplasm and loss of organelle structure. Axoplasma shows swelling and necrosis. Note severe destructive changes in the myelin sheath x 32000

Fig.58. E/m: Cerebral cortex - oedematous neuropil with separation and lysis of structures. Swollen non-myelinated axons (NAX) and partially damaged mitochondria (M) are noticed. Also see a capillary with swollen endothelium (Er) and loosening from basement membrane. x 16000


OG
N
AX
AX

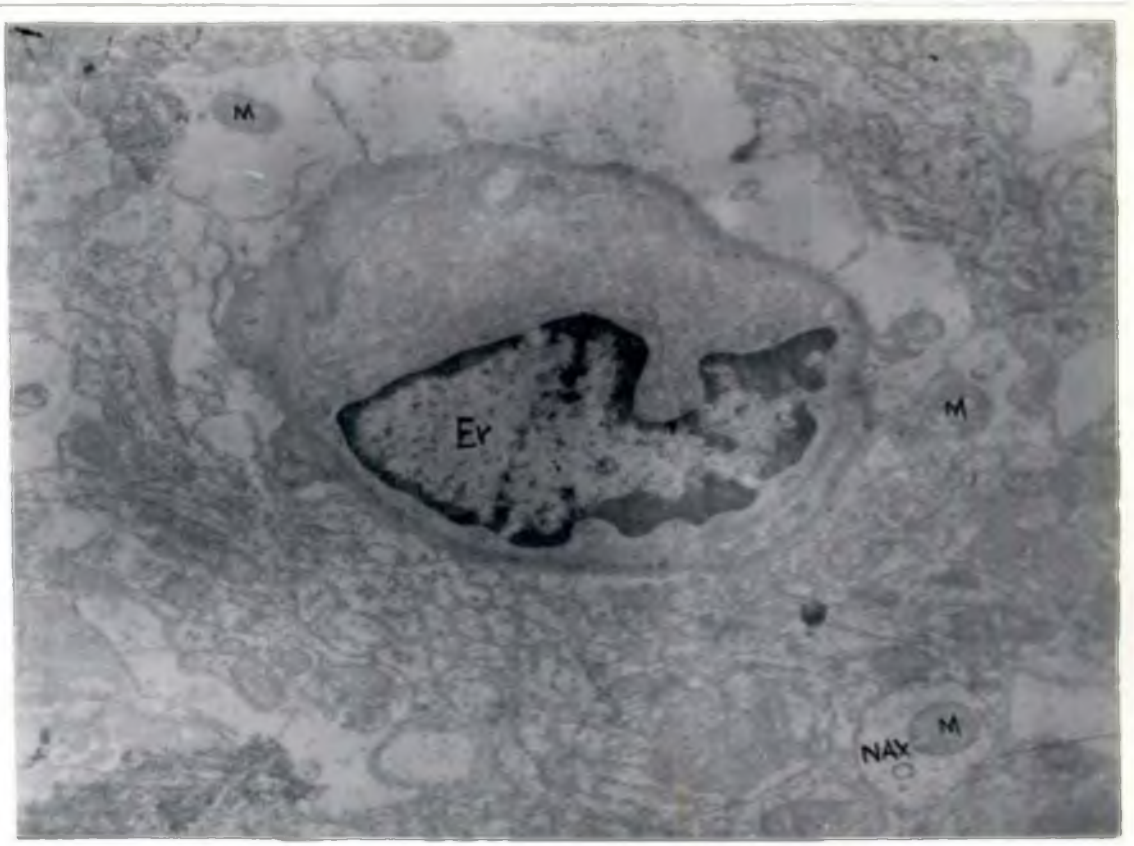
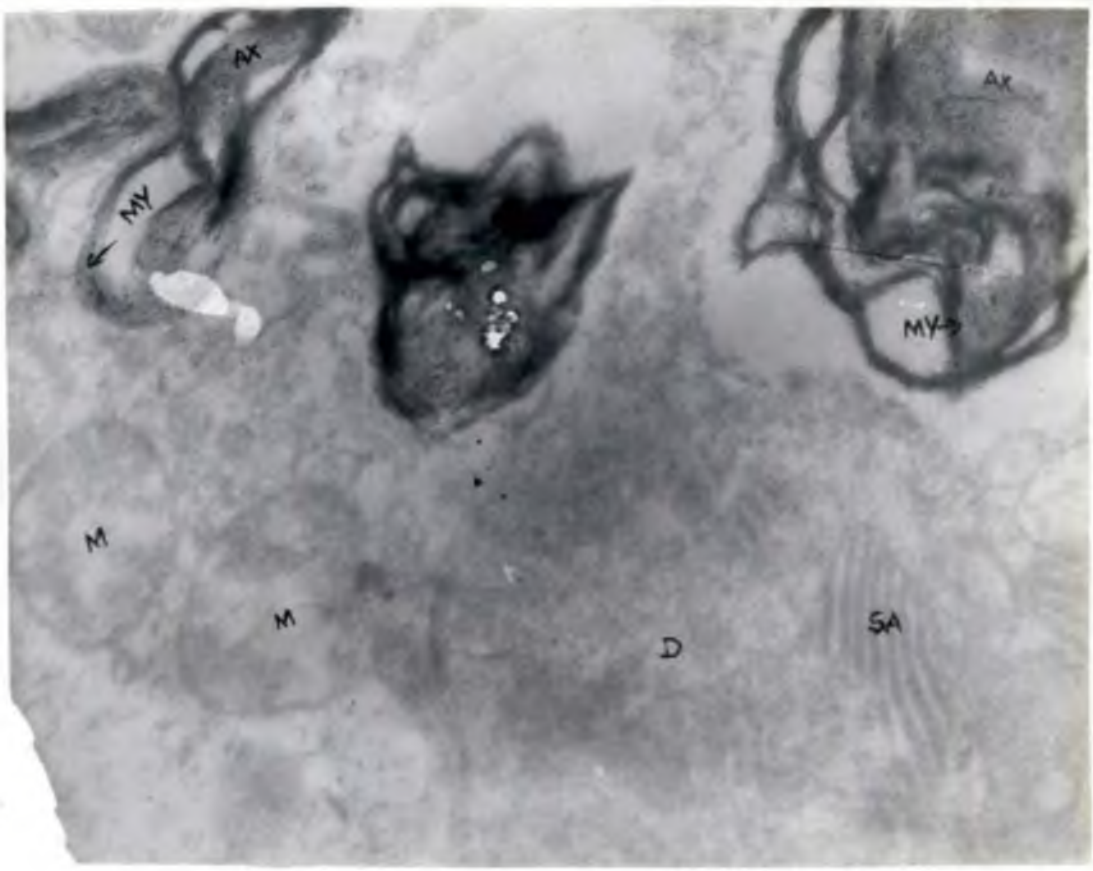

M
Ey
M
NAX
M

Fig.59. E/m: Portion of neuron (NR) with swollen dendrite (D) myelin (MY) sheath of axons (AX) shows splitting and cleft formation. Mitochondria(M) are swollen with loss of cristae and matrix. Dendritic spine with spine apparatus (SA) and with prominent dense laminae are seen x 50,000


AX
MY
AX
MY
M
M
D
SA

Group F (Chela - *Ficus tsiela* Roxb)

4.7 Clinical signs

All the chela leaf fed kids except one remained active till the end of the experiment. One had occasional depression. Though the animal ate and relished the plant, remained sleepy more or less towards the end. All the animals were sacrificed on the 45th day.

4.7.1 Weight of the animals

Though the average body weight progressively increased (Table 3) the weight as compared to the control remained low and was significant statistically (Fig.26).

4.7.2 Cerebrospinal fluid

The pretreatment and post-exposure protein concentration of the CSF did not show any significant difference as compared to the control (table 4 and Fig.27).

4.7.3 Weight of the brain

There was no significant variation of the weight of the brain as compared to the controls (Table 5).

4.7.4 Autofluorescence

None of segments of the cerebral hemispheres showed any fluorescence.

4.7.5 Gross lesions

In one animal which was dull and sleepy, congestion of the brain was observed. Dark brown pin head sized spots could be detected in the brain stem. Cerebral haemorrhage was seen. Sub pial vessels were highly engorged. Scattered dark brown spots throughout the white matter were observed. The brain of the other animals did not show any gross changes. All other organs appeared normal.

4.7.6 Histopathology

Neuropathological evaluation of the different segments of the cerebrum, cerebellum, pons, spinal cord and medulla oblongata were made in all the cases. Changes were observed in all the cases starting from the cortical neuronal shrinkage and cavitation to congestion, haemorrhage and oedema. In the animal which was dull, meningeal haemorrhage was observed which extended deep into the molecular layer. The vessels were engorged and the endothelium was damaged. An increased glial cell reaction

was seen at the site of the haemorrhage in the molecular layer.

Diffuse neuronal degeneration in the cerebral cortex characterized by neuronal shrinkage and pericellular and perivascular cavitation were observed. Swollen astrocytes were also seen in the vicinity, mostly surrounding or close to the vessels and damaged neurons. In another region of the cerebral cortex at the middle laminar part, numerous shrunken, elongated to angular neurons with potential space around them could be seen. Interspersed in the neuropil were many oligodendrocytes and microglia (Fig.60). Astrocyte reaction was less prominant. The lesions described above werediffuse. Deeper lamina did not show much change. The cerebral white matter had multiple diffuse vacuolations. Circumscribed, elliptical to coalesced vacuoles in the interfascicular areas could be observed. Many of such vacuoles contained microglial cells and damaged oligodendroglia (Fig.61). The lining cells of the ventricle had many layers. Extensive haemorrhage was seen close to the ependymal layer. Congestion and collections of the glial cells and lymphocytes could be observed in the subependymal area. Such type of collection was not noticed in others. Satellite cells were mostly shrunken in the depth of the sulcus.

Astrocytes were swollen, but appeared few in number in certain regions. In a few of the neurons, the cytoplasm appeared homogenous and pink staining.

Congestion of vessels, endothelial cell swelling and accumulation of glial cells/lymphocytes were seen surrounding the vessels in the white matter. Neurons in some areas were vacuolated. A small rim of nuclear membrane remained and the internal structures were lacking. In some of the cerebral gyri and sulci gliosis could be seen. Nucleus of some of the spherical neurons had prominant nucleoli, but these were present within the vacuolar remnants of the neurons. Satellite neurons were mostly shrunken in the depth of the sulcus.

Molecular layer of the cerebellum showed few vacuoles. Purkinje cells were few and swollen. Granule layer revealed less cellularity. White matter of the cerebellum appeared intact.

The pons, medulla oblongata and spinal cord did not show any micro and macroscopic lesions.

4.7.7 Ultrastructural pathology

Neurons of the animals which showed periodical dullness appeared swollen (Fig.62) in few of the

cerebrocortical gyri. There was an involvement of neurons of both the external granular and internal fusiform layer. The nuclear membrane appeared either compact in some or irregularly notched in a few other neurons. The external and internal membranes were discernible. The perineuronal space at focal areas appeared distended. The euchromatin appeared electronlucent and was sparse. Heterochromatin remained as condensed and appeared as a narrow rim along the nucleoplasmic side of the inner membrane. Nucleolus appeared compact and sometimes appeared displaced to one side.

The cytoplasm of the neurons contained all the organellar profiles. There was a loosening of the cytoplasmic matrix. The RER appeared degranulated at focal areas (Fig.62). Focal collections of polysomes were seen. SER profiles were predominant. Mitochondria remained moderately swollen with intact membranes. Cristae in some showed moderate swelling. The mitochondrial matrix was electrondense. Dispersed glycogen granules appeared in the cytoplasm.

Glial cells appeared compact with well preserved membrane structures and cytoplasmic organelles and were seen uniformly distributed. The neurons of the cerebellum

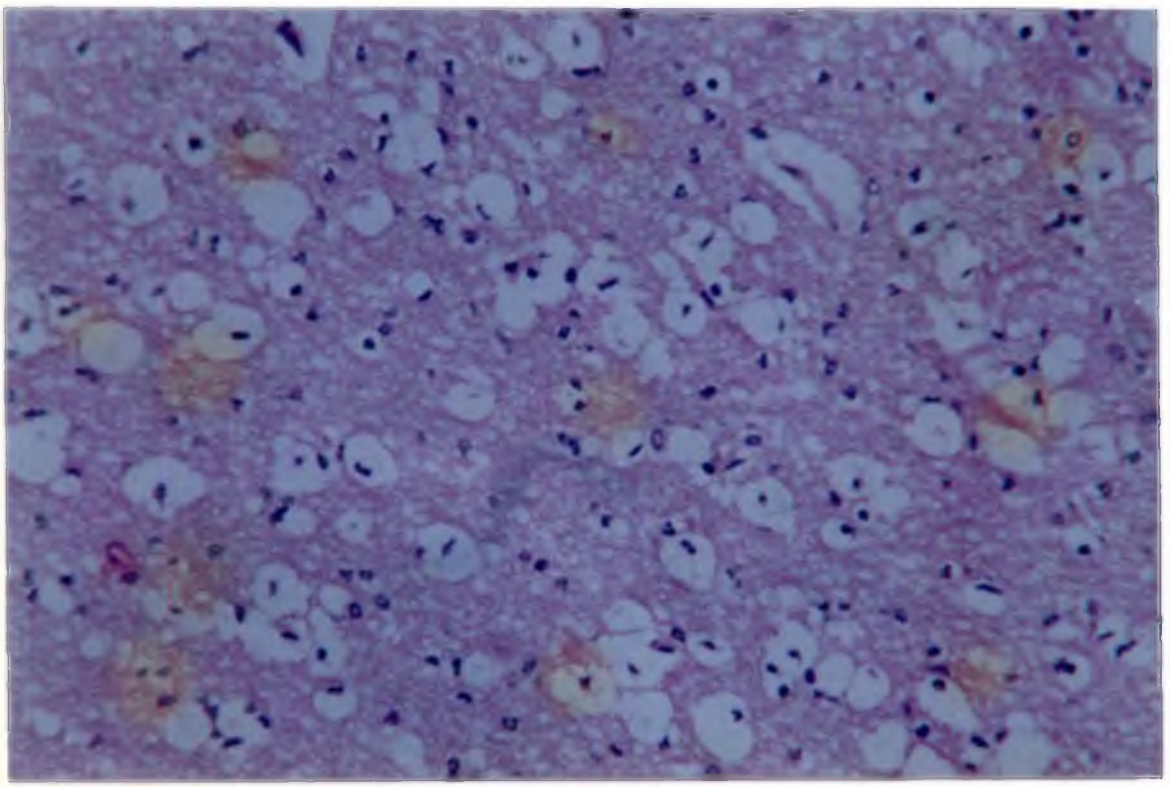
remained normal. Purkinje cells had regular nuclear in foldings and the cytoplasm appeared compact.

There was loosening of the white matter. Myelin in those areas appeared irregularly fragmented and intramyelinic vacuoles were present. There was occasional swellings of axons and the mitcochondria of such axons showed pleomorphism. Interaxonal oedema was prominent in some locations (Fig.62). There was clumping of neurofilaments in few of the swollen axons. Axonolysis was prominant (Fig.63). Increased density of the axoplasm and myelinated axons were intermittently separated from the myelin sheath by an electronlucent space. Clumping of the microtubules in the dendrites were seen. Synapses appeared well preserved and normal.

The ultrastructural features of the cells in the brain of the other animals in this group did not manifest any significant changes.

Fig.60. Group. F (*Ficus tsiela* Roxb) Cerebrum - middle cortex elongated and angular shrunken neurons with potential space around them. The neuropil contains many microglia and oligodendroglia. H&E x 400

Fig.61. Cerebrum - white matter inter fascicular vacuolation. H&E x 250



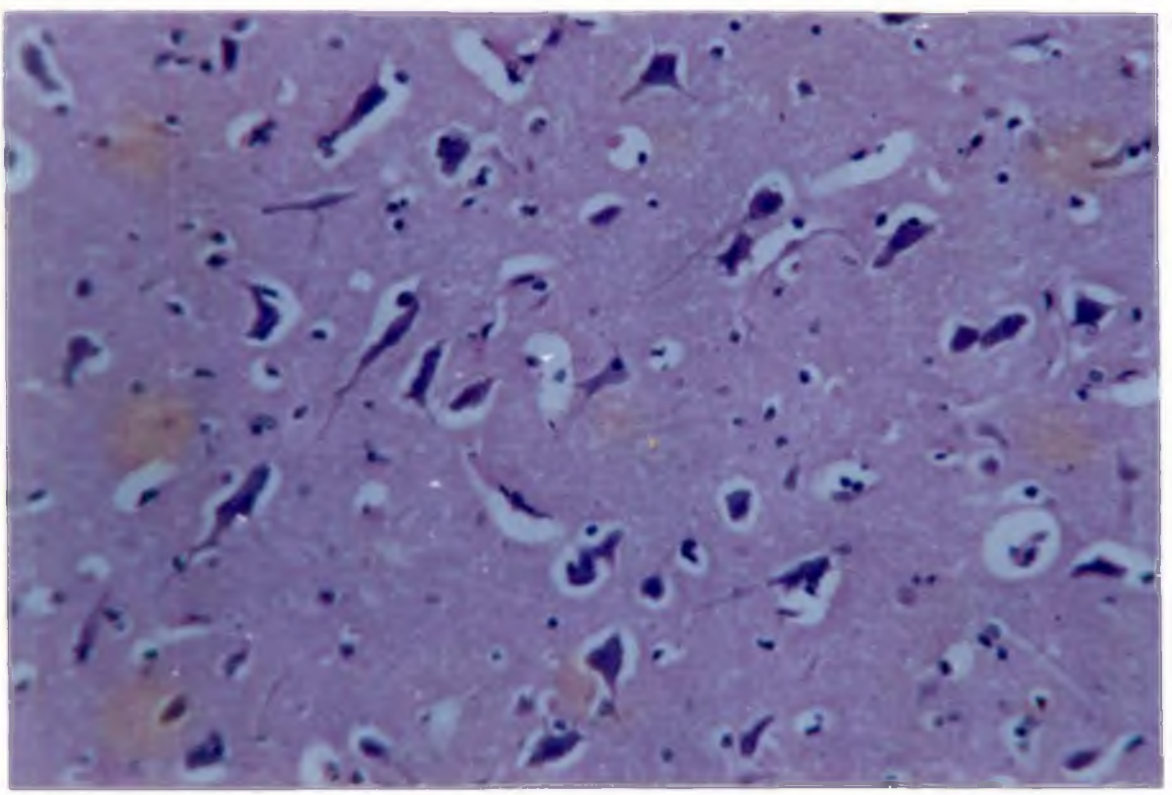
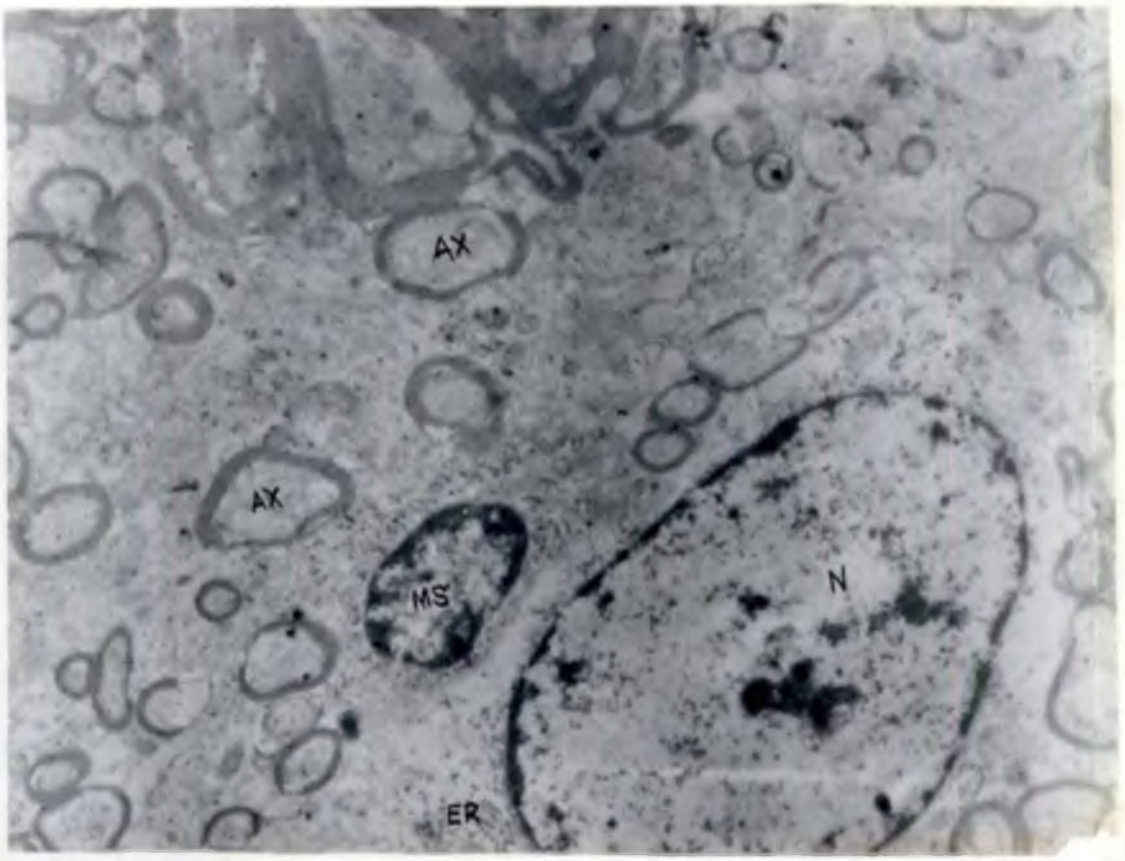
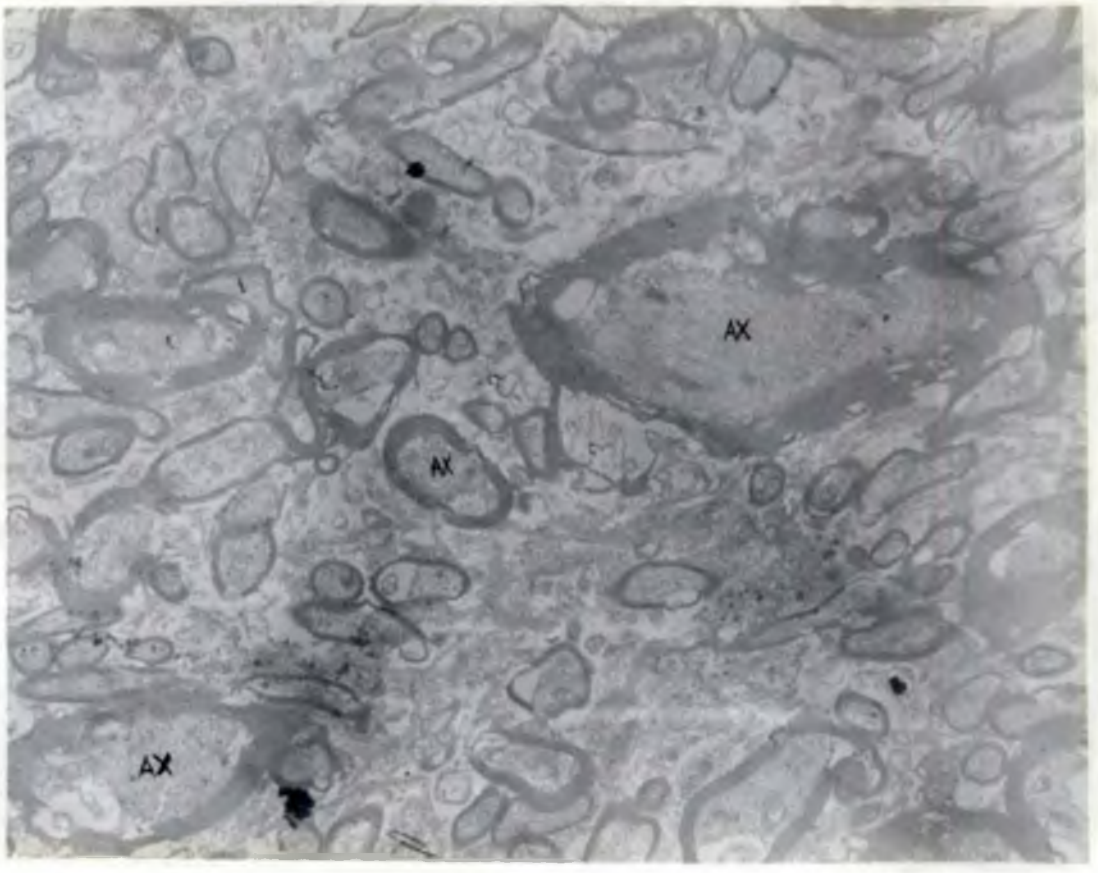


Fig.62. E/m: Cerebral cortex: A neuron (NR) with swollen nucleus (N) and partially degranulated endoplasmic reticulum (ER) A microglial cell (MS) and sections of axons (AX) are widely separated by oedema. x 10000

Fig.63. E/m: Neuropil showing axons (AX) with degenerative changes in the myelin. Some of the axoplasm are partially lytic and free of ribosomes. Interaxonal oedema noticed. x 8000


AX
AX
MS
N
ER


AX
AX
AX

Group G (Control)

4.8 Body weight, CSF and brain weight

The body weight and CSF concentration are given in the Table 3 and 4 and also depicted in the Fig.3 and 4. The weight of the brain is shown in the table. The average mean brain weight in relation to the body weight was 0.00696 ± 0.00041 (Table 5). The body weight showed a consistent increase.

4.8.1 Clinical signs and histopathology

None of the animals showed any symptoms of any disease. They appeared active and normal. The brain on histopathological examination did not show any lesions. The pattern of arrangement of neurons, the structure and consistency of neurons, glial cells, the inter neuronal neuropil and other structures appeared normal and well preserved.

4.8.2 Electronmicroscopy

Neurons, glial cells, neuropil and the white matter in all parts of the central nervous system appeared normal and did not show any significant structural changes.

The nucleus of neurons was spherical to oval and had compact and smooth nuclear membrane. A uniform perinuclear cisternae and a nucleoplasm of uniform granularity and density were seen. The nucleolus appeared normal and the granular and filamentous structures were well preserved. The cytoplasm of most of the neurons contained large numbers of well preserved mitochondria. Glycogen, lipid and other vesiculo cisternal structures such as RER, SER and Golgi apparatus were present. The Nissl, ribosomes appeared well preserved. The Purkinje cells of the cerebellum appeared compact with regular nuclear membrane infoldings. The granule cell layer was intact.

The astrocytes in the cerebral cortex had well preserved nucleus with profiles of RER in the cytoplasm. The RER was dotted with ribosomes. The mitochondria of varying size were seen in the cytoplasm. Microglia were seen occasionally in the cerebral cortex.

Myelinated and non myelinated fibers of compact nature were seen in the cortex. Myelin and neuropil of the cerebral cortex appeared compact. The oligodendroglia appeared along the course of the fibers were well preserved. The white matter appeared compact.

All the synapses appeared normal with well dispersed uniform vesicles. The axons appeared compact with normal mitochondria, tubules and filaments. The several layers of myelin surrounding the axons appeared compact.

Group H (Natural cases)

The Veterinary Officers all over the state of Kerala were requested to provide goats suspected to be ailing from PEM. Four cases were referred by field Veterinary Officers. The details of the animals are given below.

No.	Age (Approx)	Date	Male/Female	Place
1.	2 years	22.4.95	Female	Chalakkudy
2.	4-4½ m	29.9.95	Female	Prima Feeds Ernakulam
3.	4-4½ m	29.9.95	Female	"
4.	5-6 m	11.4.96	Female	Kolenchery

4.9 Clinical signs and gross lesions

Animal number one was brought on the 14th day of illness. It was under treatment by the local Veterinarian. The animal had generalised ataxia, reduced postural reflex, seizures, myoclones and reduced vision and was being treated with thiamine, anti inflammatory drugs and antibiotics. The animal did not respond.

The goat was under observation for three days at the pathology department. The symptoms observed included

anorexia and opisthotonous. The animal could not bear weight on the limbs and preferred to lie down. Occasional nystagmus, shivering of the whole body and finally paralysis of the limbs were observed. Animal pressed the head against hard objects and there was loss of eye preservation reflex. The animal was sacrificed by jugular exsanguination on the third day.

There was extensive meningeal congestion, diffuse yellowish discolouration of the cerebral grey matter gelatinsation. The gyri were moderately swollen and th was flattening of the sulci and they were imperceptible the occipital part. There was congestion of the spinal mater. Dark brown pin head sized spots of necrosis w seen in the grey and white matter.

The liver was moderately enlarged and pale yellow. Petechiae were seen in the kidney cortex and there was emphysema of the lungs.

Animals (2) and (3) had similar symptoms and lesions. Besides there was diarrhoea, restlessness, staggering gait, salivation, protrusion of the tongue, opisthotonous, and blindness. The blindness was unilateral (Fig.64 and 65). Frequent urination and paralysis of the limbs were observed. The animals preferred to lie on one side. Since

the symptoms were extensive and the animals were recumbent, they were sacrificed on the day they were brought. The animals were brought on the 7th day of illness.

The brain had a cooked appearance. There was diffuse yellowish discolouration of the brain. The vessels were prominent in many of the sulci. The yellowish discolouration was extensive in the cerebral grey matter. The whole brain was swollen and the gyri at the rostral portion of the hemispheres on both the sides were swollen and the sulci imperceptible (Fig.66). Both grey and white matter on incision showed dark coloured spots diffusely distributed in the parenchyma. Dark brown spots were also seen on the surface of the gyri. There was congestion of the cerebellar folia. The pons, medulla oblongata and spinal cord did not reveal any gross lesions. The dura was relatively more thick.

Pulmonary collapse, congestion of the liver with patchy pale white areas of necrosis, distended gall bladder, focal pale foci in the kidney were the other lesions observed. The intestinal tract in both the animals contained few segments of *Monezia expansa*. The intestinal mucosa was pale.

The fourth animal was showing staggering gait, nystagmus, shivering and salivation. It often pressed the forehead over the wall. The animal did not respond to thiamine therapy by the veterinary Officer. The animal was reported to have consumed some plant leaves which the owner could not identify. The animal was brought on the third day of illness for ruling out rabies. The animal died on the way.

A detailed autopsy was conducted. The whole brain was swollen and flattening of the gyri was evident. The meninges were highly congested. The duramater was thick and was found closely adhering to the brain. The cerebral cortex contained diffuse dark brown spots and there was focal petechiae. Distension of the lateral ventricles and congestion of the caudate nucleus were observed. Petechiae were present in the hippocampus. Focal diffuse pin point cavitation was observed on the cut surface of the cerebral hemisphere especially in the grey matter.

Extensive congestion of the spinal dura was also observed. Grossly visible petechial haemorrhages were found in the brain stem, cerebral cortex and cerebellar vermis.

Pulmonary oedema, moderate hepatomegaly, diffuse congestion of the liver and scattered pale areas of necrosis, focal pale white patches in the cortex of the kidney, hydropericardium and ruminal tympany were the other lesions seen.

4.9.1 Body weight and weight of the brain

The body weight and weight of the brain were recorded at the time of sacrifice/death. The body weight of animal numbers one to four were 16 kg, 5.1 kg. 6.2 kg and 8.7 kg respectively. The corresponding weight of the brain of the animals one to four respectively were 98 g, 52.2 g, 58 g and 82 g.

4.9.2 CSF changes

The CSF was collected from the animal one, two and three. The results of qualitative and quantitative analysis are shown in Table 6.

Table 6. Qualitative and quantitative analysis of CSF

Animal No.	CSF flow	Colour	Turbidity	Coagulation	Total protein (mg/dl)
1.	Rapid	Light grey	Turbid	Absent	48.82
2.	Slow	Light grey	Turbid	Absent	36.31
3.	Slow	Colourless but blood mixed	Clear on centri-fugation	Absent	23.23
4.	Not examined				

4.9.3 Auofluorescence

Buffered formalin fixed brain tissues from all the four cases were examined. Slices from the different segments of the cerebral hemispheres were exposed to 365 nm UV light. Autofluorescence varying from feeble blue green flourescence to focal creamy flourescence were observed in the cerebral cortical areas in three cases. Animal numbers one, two and four showed visible flourescence, but no fluorescence was detected in the brain of the third animal. The fluorescence was observed mostly at the crown of the gyri and sulcal depressions, close to the superficial part of the grey matter of the cerebral cortex. Creamy white

fluorescence was also noticed in the white matter at focal areas in the animal numbers one and four.

4.9.4 Histopathology

The rostral, middle and caudal cerebral segments, the sylvian, presylvian and suprasylvian gyri, the hippocampus, the lateral and third ventricular areas, the caudate nuclei, cerebellar folia including the culmen, declive, tuber vermis, and other areas, the pons, medulla oblongata and cervical spinal segment were examined in detail in all the four cases.

Considerable differences existed in the histopathological lesions which varied from region to region and from gyrus to gyrus. The molecular layer of the cerebral cortex remained intact in two cases (animal two and three) whereas in the other two diffuse vacuolation of the neuropil of the molecular layer could be seen. This malacic foci were extensive in the presylvian and suprasylvian gyral part. The vacuoles contained one or two microglial cells. There was considerable sprouting of the capillaries in animal number two in the molecular layer. The capillary structures were well discerned. The intersulcal vessels close to and attached to the piamater revealed hypertrophy and proliferation of endothelial

cells, thickening of the adventitial structures, congestion and thrombus formation. This was evident in animal one, three and four. Swelling and vacuolation of endothelial cells, proliferation, perivascular oedema and aggregates of inflammatory cells were seen in the perivascular areas in animal one and four. The neuropil surrounding this vessel had a very cellular appearance indicating gliosis (Fig.67). A few lymphocytes were seen within the lumen. Extensive meningeal congestion was observed in the animal number one.

The lesions in the cerebral cortical grey matter varied from region to region and gyrus to gyrus. This included gliosis, degeneration and necrosis of neurons, angularity of neurons to neuronal loss in some segments. The cell distribution and cytoarchitecture were analysed by staining with toluidine blue. This revealed irregular pattern of arrangement of cells in many of the gyri, loss of neurons, crowding, swelling and gliosis (Fig.68). In the first animal the neurons in the third, fourth and fifth laminae of the cerebral cortex namely external pyramidal, internal granular and internal pyramidal as well as the external granular layer in some of the gyri revealed ischaemic necrosis. The ischaemic neurons appeared dark blue staining with shrunken cell body in Phosphotungstic acid haematoxylin stained sections (Fig.69). The dendrites

appeared detached and discontinuous in most of the neurons. The cytoplasm appeared shrunken, deeply eosinophilic and often triangular in outline with small dark staining nucleus. Most of such neurons were surrounded by microglia and oligodendroglia having elongated and round compact nucleus respectively. In both superficial (external granular) and deep cortical (multiform or fusiform) layers of the cerebrum in all the cases, individual or groups of neurons became elongated, contracted with intensely acidophilic cytoplasm and pyknotic basophilic nucleus with progressive nuclear karyorrhexis and cytoplasmic pallor (Fig.70). Gliosis was evident in the neuropil surrounding such neurons. Swelling of the endothelial cells and dilatation of the perivascular space surrounded by astrocytes were also observed.

The neurons in the internal granular, pyramidal and fusiform layer of the caudal cerebral region were shrunken and remained as short globular and angular structures. There was marked cavitation of the neuropil in affected areas of the cortex, especially around blood vessels and necrotic neurons (FIg.71). Microglial reaction was extensive. Oligodendroglia were also seen in groups within the spaces. The dendritic process in a few appeared hypertrophic. The astrocytic reaction in the area varied

from degeneration to mild hypertrophy. In certain gyri there was an overall reduction in the number of neurons and glial cells. Vascular proliferation, perivascular oedema astrogliosis along with prominent oligodendroglial reaction were present in the middle cortical grey matter of the animal number four.

The caudal cerebral grey matter in the animal two and three and middle cerebral grey matter in animal one showed neuronal necrosis and neuronal loss and such areas were seen infiltrated by gitter cells in the form of nodules. This type of crowding and diffuse accumulation of cells were seen in the superficial laminae of the cerebral cortex, fusiform layer of the cerebral cortex (Fig.72), ependymal and sub-ependymal regions of the ventricles, close to the fusiform layer of the hippocampus and also within the hippocampal layer. Crowding of cells was also seen around dilated blood vessels in the superficial cortex. Chromatolysis and nucleolar loss were observed in a few neurons around these lesions.

Marked loss of neurons in the polymorphic layer of the hippocampus and necrosis of the cells of the hippocampus were seen in animals one and four. The large neurons in the sub hippocampal areas appeared necrotic and surrounded by

spaces. Proliferation of microglia and crowding of oligodendroglia were also seen. There was moderate neuropil vacuolation. Necrosis of the large pyramidal neurons was more patchy in the animal number one. Many of the neurons showed central chromatolysis and necrosis. Occasional multinucleated astrocytes were present in these areas.

Most of the astrocytes in the middle cerebral cortex were surrounded by vacuoles. In most of the astrocytes there was development of visible cytoplasm. Most of them appeared broad and polygonal with the nucleus at the margins forming reactive gemistocytes. Cytoplasm was smooth and eosinophilic and many had small vacuoles at the margin. Many of the neurons in these areas appeared granular with loss of nucleolus. Cytoplasm appeared condensed around such neurons. The vessels close to the astrocytes had distended perivascular space.

In the animal number four the grey matter of the cerebrum in most of the region had scanty neurons (Fig.73) and glial cells. Cortex at the entire depth showed marked cavitationof the neuropil with pronounced widening of the perivascular and perineuronal space. Degenerated and necrosed neuronal remnants appeared as compact,

hyperchromatic to eosinophilic condensed structures in the vacuoles (Fig.74). In the crypt of few sulci, the neurons were highly condensed and hyperchromatic. Some of the neurons had enlarged vesicular nucleus with fading of the staining intensity of the cell. The malacic foci in the grey matter showed reactive astrocytes, neuronophagia and satellitosis in focal areas. A few of the neurons showed central as well as peripheral chromatolysis. Gemistocytic astrocytes with extensive cytoplasm and peripherally located nucleus were prominent in certain gyri where there was neuronal damage and neuropil vacuolation. In certain gyri there was an increase in the width of the grey column. But most of the neurons appeared shrunken and hyperchromatic in these areas. In animal number one certain gyri especially the presylvian and sylvian, the grey matter was thin.

Vascular congestion was a predominant changes in all the layers of the brain in kid numbers two and three. This was observed in the molecular layer, cortical grey matter and also in the white matter in all the regions. Proliferation of the vessel was evident represented by increased number of capillaries. Endothelial cell proliferation was predominant and there was thickening of the vessels . Cortical layer in certain gyri at the depth

of the sulcus and superficial cortex close to the molecular layer showed extensive collections of microglia and oligodendroglia in the form of nodules as in Fig.72. This type of reaction was prominent in animal numbers one, two and three. Stratified sub-ependymal infiltrates and microglial nodules were also present below the ependyma of the lateral ventricles. Neurons in the middle cortical laminae in these animals showed total necrosis. They appeared shrunken and homogeneous. The necrotic neurons were surrounded by a few satellite cells. Intraneuronal vacuolation was predominant in most of the mid and deeper laminar areas in the animal one and three. There was perineuronal oedema.

Cerebral cortical white matter in all the cases showed varying degree of malacic changes characterized by multiple and diffuse vacuoles of varying size and shape. Both inter and intrafasicular vacuolation were observed. The vacuoles were seen in the cerebral white matter, mid brain, pons and also immediately lateral to the main thalamic nuclei. The vacuolation was extensive in animal number one. It was observed along the course of the axons. The vacuoles appeared large and ovoid running parallel to the direction of axons and such spaces were seen traversed by delicate strands separating them into chambers (Fig.75) like the

digestion chambers of the peripheral nerves. Such areas on staining by Glees and Marsland's modified silver stain revealed loss of fibers indicated by discontinuity and cavitation (Fig.76) and the area appeared non impregnated with silver. The fibers were seen running in different directions and branched. Occasional neurons and damaged oligodendroglia were seen amidst spaces which are not impregnated (Fig.77). Luxol fast blue stained sections showed oedema as there was myelin loss indicated by pale staining areas in between regularly appearing blue stained myelinated tracts (Fig.78). Damaged lightly stained as well as pyknotic oligodendroglial cells were seen either individually or as clumps within the vacuoles. There was congestion of vessels in those malacic foci. Moderate infiltration of gitter cells and lymphocytes were seen in those malacic areas and also in the perivascular areas (Fig.79).

In all the other animals mild to moderate vacuolation were present which appeared in the form of circumscribed vacuoles, very small to large ones. Most of the vessels in these zones were congested and the endothelial cells were less prominent.

In the animal number one severe dilatation of the vessels were seen with endothelial discontinuity and damage. The vessels were congested and showed oedema. Cells were seen infiltrating in the vicinity of such vessels and even they were seen invading the damaged capillaries. The cells were mostly gitter cells.

Extensive haemorrhage was seen in the white matter in the subependymal layer of the lateral ventricles in animal numbers two and three. Subependymal oedema was also observed. Ependymal cells were mostly the non ciliated tannycytes.

Vascular congestion, endothelial damage and perivascular cuffing with lymphocytes, gitter cells and stray plasma cells were seen in the white matter of the cerebral cortex in the animal number four (Fig.80)

Cerebellum showed extensive changes in animal number one. In this case there was Purkinje cell degeneration and these cells appeared as homogenous, pink staining structures. The Purkinje cell layer was discontinuous in most of the cerebellar folia. The granule layer appeared less cellular with loss of glomerular pattern of arrangement. The cerebellar folial white matter showed extensive cavitation and breakage of fibers. Myelinated

tract was discontinuous. There was oedema and demyelination as revealed by luxol fast blue staining (Fig.81). Basket cell fibres were predominant in those areas where there was loss of Purkinje cells.

Many areas of the midbrain were affected to varying degree with the substantia nigra and caudal colliculi being most regularly affected which included degeneration, necrosis and congestion. This was noticed only in animal number one.

There was extensive congestion, haemorrhage and perivascular accumulations of lymphocytes in the pons and spinal cord grey and white columns in the animal number one and four. The affected areas were sprinkled with fresh hameorrhages and contained prominent capillaries. These vessels had swollen endothelial cells.

There was disseminated accumulation of lymphocytes, plasma cells and gitter cells around the blood vessels and within the substance of the brain in different locations. Both grey and white matter areas were affected. These type of changes were prominent in the pons, spinal cord grey and white column (Fig.82, 83 and 84). Some of the large neurons of the grey column of the spinal cord showed chromatolysis, karyorrhexis and gliosis (Fig.85).

Nucleolar loss, Nissl clumping and satellitosis were also observed as revealed by toluidine blue staining (Fig.86). Cavitation of the neuropil of the pons was observed in the first animal and the neurons within this showed degeneration and chromatolysis. Collection of inflammatory cells was also observed in the grey and white column of the spinal cord in animal number three. Glial cells were also seen crowded around blood vessels.

Spinal dura was thickened in animal number one and extensive haemorrhage into the dura could be seen. Gliosis of the grey column, degeneration of the ependymal cells lining the central canal and occasional axonal swelling were observed in animal one and four. The neurons in the anterolateral areas of the dorsal horn showed clumping. Occasional axonal swelling was observed in the medulla oblongata in all the cases.

4.9.5 Electronmicroscopy

The ultrastructural pattern of alternations in the brain of the affected goats was basically similar but with a wide range of intensity within and between cases. The changes were more or less comparable in different parts of the brain. In many of the cerebral gyri structural changes were pronounced both in the neuropil and cell body of the

neurons. In certain other gyri, changes were predominant in the neuropil. At times neurons of normal structure appeared amidst the damaged ones and in certain other locations, the satellite cells were found to be affected. Focal laminar and diffuse spongy changes were observed in the cerebral cortex in one case, whereas the spongy changes were confined to the neuropil in the other two. Degeneration to frank destruction of structural elements could be observed in such malacic areas. The transition to necrosis of most of the cortical elements appeared to follow a uniform pattern.

4.9.5.1 Neurons

Moderate to severe neuronal necrosis could be observed in the cortex at various locations. Most of the neurons appeared swollen with prominent perinuclear cisternae and heterochromatin along the inner nuclear membrane (Fig.87).

The perinuclear cisternae in some contained electrondense material. Neurons appeared clumped or condensed in certain regions. Most of them were seen as electrondense angular bodies amidst myelinated and non myelinated fibers. Swelling of the cell body, dispersion of central Nissl substances and peripheral displacement of nucleus were observed in the mid cortical areas in one

case. Nuclear membrane disruption, breakage, irregularity and even loss and dispersal of chromatin contents were observed in some of the gyri.

Euchromatin was sparse in some cells. Heterochromatin appeared as a narrow rim along the inner nuclear membrane (Fig.88) or occasionally as clumps in the nucleoplasm at times. In many of the neurons the nuclear matrix appeared electronlucent. The cortical neurons in certain gyri, the chromatin was seen dispersed along with glycogen granules wherein both the membranes of the nuclei were not discernible. The nucleolus appeared condensed in some (Fig.88) whereas in others it appeared highly engorged and amorphous (Fig.87). Nucleolus associated chromatin appeared in few of the neurons. Partial segregation of the granular and filamentous components was occasionally seen. In many of the neuronal outer membranes the ribosomes were sparse and in a few cases there was a clumping of such structures in focal areas of the disrupted membrane.

The cytoplasm of most of the neurons was swollen and there was an increase in the volume indicated by its electronlucent appearance. The Nissl ribosomes had lost their polygonal configuration. In some other neurons there was absence and complete lysis of Nissl substances from

around the nucleus. In one of the animals the aggregate of Nissl substance could be seen as dispersed granular particles towards the periphery of the cytoplasm in focal areas. The cytoplasm surrounding most of the damaged neurons had an expanded volume and the organelles were found within loose and faintly electronlucent matrix. Clumping and condensation of organelles which were found displaced to certain pockets of the cytoplasm could be seen in some. Very extensive irregular vacuolation of the cytoplasm presenting a moth eaten appearance was found in a few cases (Fig.89). Fine granular electron dense materials were seen in such vacuoles in a few cases.

RER showed various degrees of dilatation, clumping fragmentation and cisternal collapse. Cytoplasm in many locations were found devoid of RER. The dilated cisternae of RER presented irregular distribution. The fine structure of ribosomes at times well preserved, but at times showed various degree of disorganization with irregular pattern of distribution and variable degree of reduction in number. Partial degranulation of the ribosomes with RER was observed in some (Fig.87) whereas in some other neurons there was complete degranulation and clumps of ribosomes were found intermingled in the fragmented RER.

There was extensive arborization of SER in some of the neurons in one animal. This appeared dilated irregular structures devoid of any contents inside.

In most of the damaged neurons the mitochondria appeared pleomorphic. This was evident in many of the neurons of the mid laminar and inner fusiform layer. Some appeared as highly condensed circular profiles whereas others appeared elongated and curved.

Elongated mitochondriae with central constriction were also observed. A few of the mitochondria appeared circular. The mitochondria appeared swollen in a few cases with partial loss of cristae and electrondense matrix (Fig.89). There was occasional separation of the outer and inner membranes. The separated areas either appeared clear or with an electronlucent material. The mitochondrial cristae showed various alterations. Cristae in few of the cases appeared swollen and detached from the inner membrane. There was partial lysis of cristae and there was retention of remnants of cristae in the matrix. Complete cristolysis was observed in many mitochondria of the pyramidal neurons. The cristae appeared preserved in few of the mitochondria, though there was a moderate swelling.

A few of the mitochondria had wooly electrondense structures in the matrix.

Most of the capillaries of the cerebral cortex had swollen endothelial cells (Fig.90 and 91).

4.9.5.2 The Glial cells

The cell bodies of the glial cells were generally smaller than those of neurons. They frequently gave of long processes making it difficult to ascertain the limits of the cell bodies. The nuclei were generally oval in shape and were more electron dense than those of the neurons.

The astrocytes appeared either shrunken in few cases and swollen in some other cases. There was swelling of the nuclei of astrocytes in many. The outer nuclear membranes of most of the astrocytes were not discernible. Heterochromatin clumping was observed in a comparatively clear nucleoplasm. Euchromatin in most of them appeared finely granular. Swollen astrocytes especially had their nucleoplasm expanded and electronlucent and sometimes clear. The cytoplasmic organelles were found clumped and in most of the cases formed a necklace pattern around the outer membrane. In some, the neuropil was widely separated from the cytoplasm and the cytoplasmic boundary was

imperceptible. Disorganized arrays of RER and disaggregation of ribosomes were seen. The cytoplasm of astrocytes in many appeared loose. There was lysis of the processes of astrocytes in focal areas and such areas appeared vacant in the neuropil. Fragmentation of RER occurred at times and ribosomes were seen attached to such fragments. Mitochondria were either condensed or hardly discernible. Golgi apparatus and other structures were hardly visible. Some of the astrocytes were hypertrophied and contained abundant intermediate filaments, oedematous cytosol, dilated cytocavitary systems and abnormal mitochondria. Astrocytes with markedly elongated cell bodies had oedematous cytosol and moderate proliferation of haphazardly arranged glial filaments. An increase in the glycogen granules was observed within swollen astrocytic processes.

Microglial reaction was seen in the cortical areas and most of them appeared swollen (Fig.91). Most of them were found to be close to the damaged neurons. Some of them had assumed the morphology of typical tissue macrophage with many lysosomal bodies and vacuoles (Fig.92). The nuclear membrane appeared irregular and invaginated in most of the cases. The chromatin appeared condensed and highly electrondense. Pseudopod like cytoplasmic evaginations

were observed in a few such cells. The neuropil around such cells had organelles of varying shape and configuration. There was extensive lysis and fragmentation of neuropil. The capillaries with endothelial cells showed focal separation from basement membrane (Fig.92). Most of the microglia were devoid of any ingested material within, that too in the mid laminar areas. The oligodendrocytes were generally preserved in the expanded cerebral cortex.

Purkinje cells were observed to have invaginated nuclear membranes. The cytoplsmic organelles such as RER appeared fragmented. Partial degranulation of the ribosomes was seen. Mitochondrial matrix appeared highly electron dense.

The cerebellar granule cells showed varying degree of degeneration. The nuclei were swollen. The chromatin of the nuclei was condensed in some or they were mainly euchromatin in nature (Fig.93). Few other granule cells showed vacuolated appearance. Cytoplasmic structures at times appeared distorted and bit of dilated cisternae of RER were evident in some.

In pons and medulla oblongata, the nuclei of neurons remained more or less compact with well preserved organelles.

Occasionally the myelinated fibres showed myelin separation.

The horn cells of the spinal cord of one of the animals had distorted nuclei. Mitochondria were swollen with distorted cristae and there was cristolysis in many.

Vascular endothelial swelling was evident in many vessels of the cerebrum as well as cerebellum. The perivascular spaces were found to be membrane bound and many contained mitochondria and RER and occasional clumps of glial fibrils and glycogen. They were considered to be astrocytic end feets. Blebbing of the basement membranes of the vessels was observed occasionally. Most of the vessels were packed with RBCs.

4.9.5.3 Neuropil and white matter

The neuropil and white matter had expended volume and there was oedema with loosening of structures (Fig.91). Many myelinated axons were swollen (Fig.88 and 90). There was swelling of axoplasm and homogenisation, splitting and lysis of myelin (Fig.94). In some the axoplasm contained many swollen and condensed mitochondria. The axoplasm appeared electrondense in many. Many of the myelinated axons showed collapse of the axonal membrane and the myelin appeared separated. Occasional axonolysis was a feature.

The lamellar nature of the myelin was maintained in many, but occasionally there was ballooning of the myelin and intramyelinic vacuoles of varying size. Wide separation of myelin was seen in one. There was clumping of neurofilaments in many of the axons.

The synapses appeared swollen and the lumen contained only few vesicles. In some the vesicles appeared fused with one another. Separation of the synaptic cleft was seen occasionally. The oligodendrocytes in the white matter and corticomedullary areas appeared with granular cytoplasm and swollen karyoplasm (Fig.94). An apparent increase in the extracellular space because of the rupture of the neuronal and glial processes was evident. Occasionally axons exhibited an increased number of vesicular profiles and mitochondria. Elongated to circular mitochondria were seen in some. Both myelinated and non myelinated axons were found to be highly distended with homogenised internal electron dense contents.

Myelin ballooning and circumscribed vacuoles were present in the myelinated axons of cerebellum. Many of the axons had markedly reduced number of myelin lamellae. Occasional axons were surrounded by one or two lamellae of loose or compact myelin. Electrondense subaxolemmal

material was seen in myelinated fibres. There was no evidence of macrophage infiltration. Endoneurial collagen was increased and electrondense hypertrophied endoneurial fibroblasts were sometimes seen.

The dendritic processes showed varying degree of swelling. There was clumping of neurotubules in many. The dendritic spine was not discernible. But occasionally sac like cisterns separated by irregular electron dense laminae were seen scattered in a few of the processes. Most of the processes contained distorted mitochondria.

Fig.64. Natural case : PEM- weakness and opisthotonos

Fig.65. Natural case : unilateral evulsion of the conjunctiva

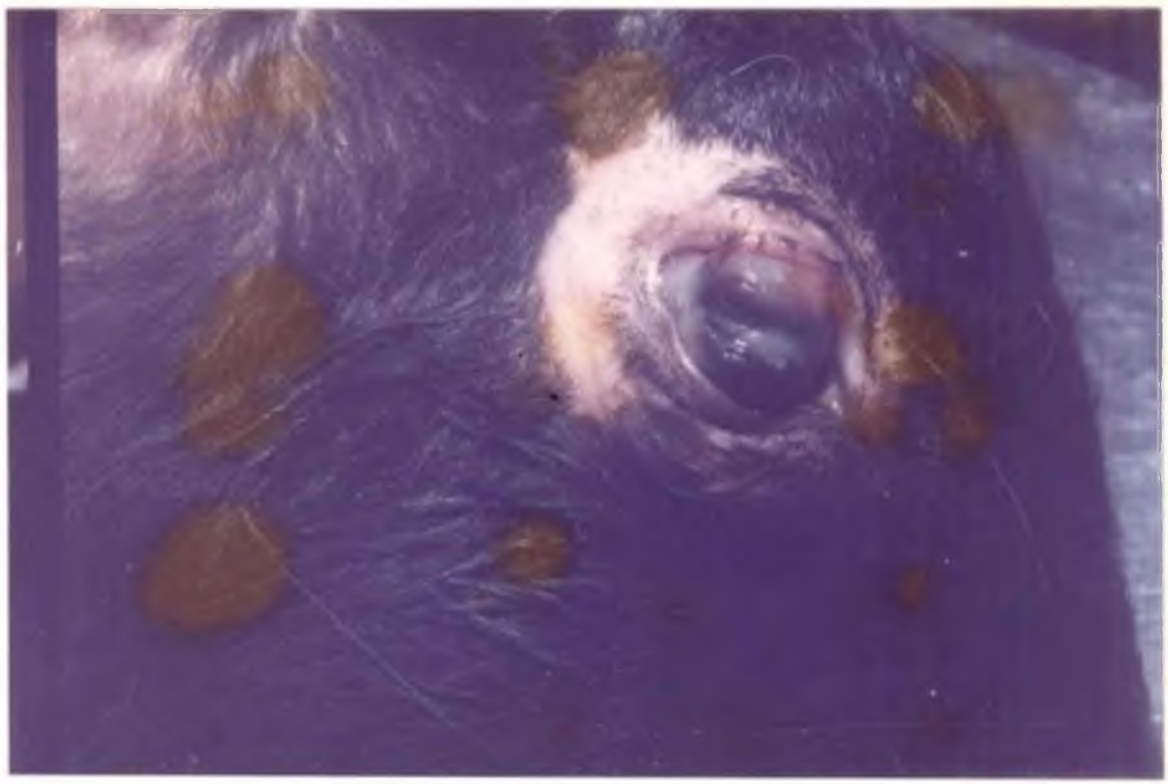


Fig.66. Natural case Brain - yellowish, flattened and swollen gyri in the rostral region and congestion of vessels.

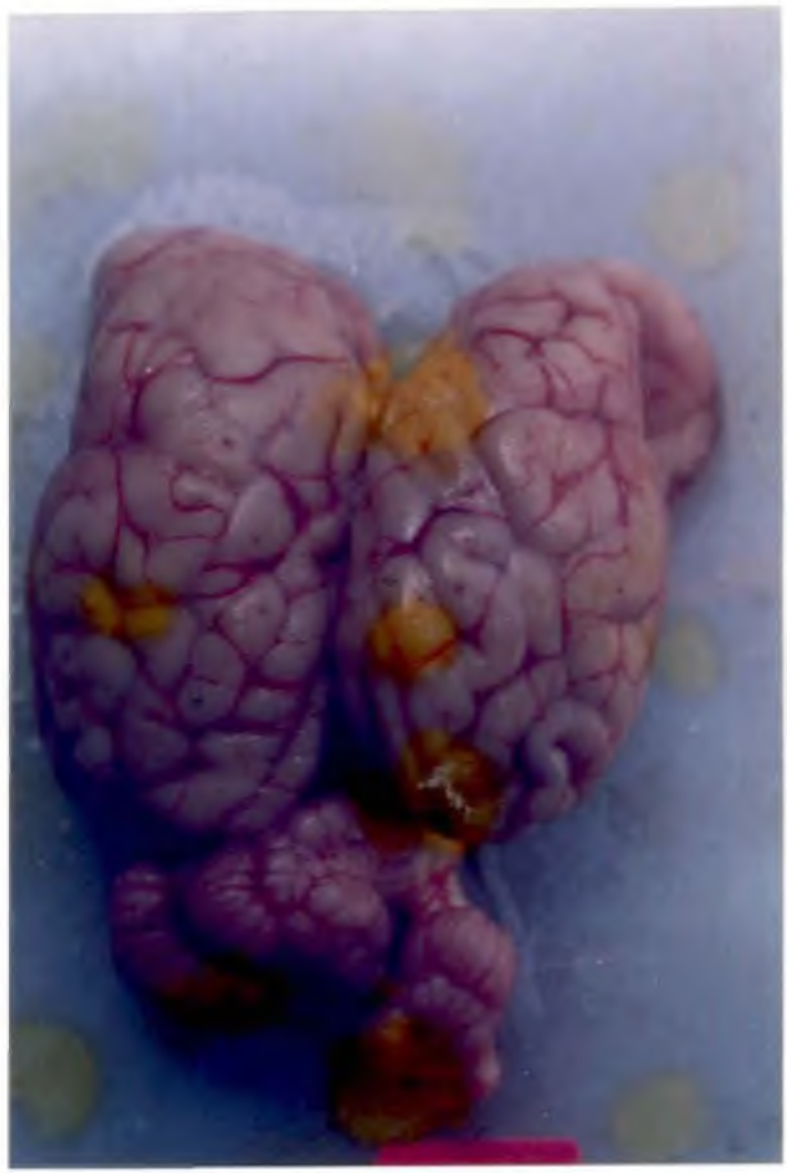
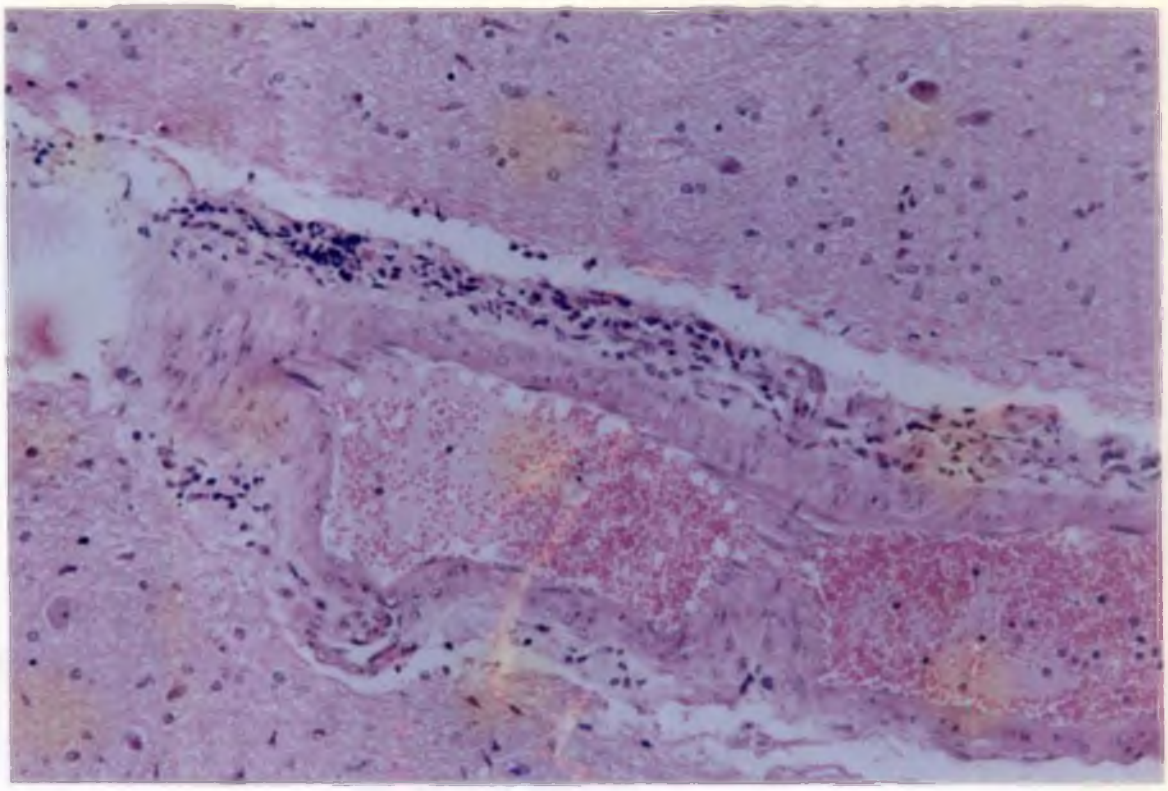


Fig.67. Cerebral cortex - inersulcal vessel - perivascular infiltration of lymphocytes and glosis of the neuropil. (Animal No. 1 and 4). H&E x 250

Fig.68. Cerebral cortex - Gliosis, necrosis of neurons, neuronal loss and irregular pattern of arrangement of cells. Toluidine blue stain. x 400



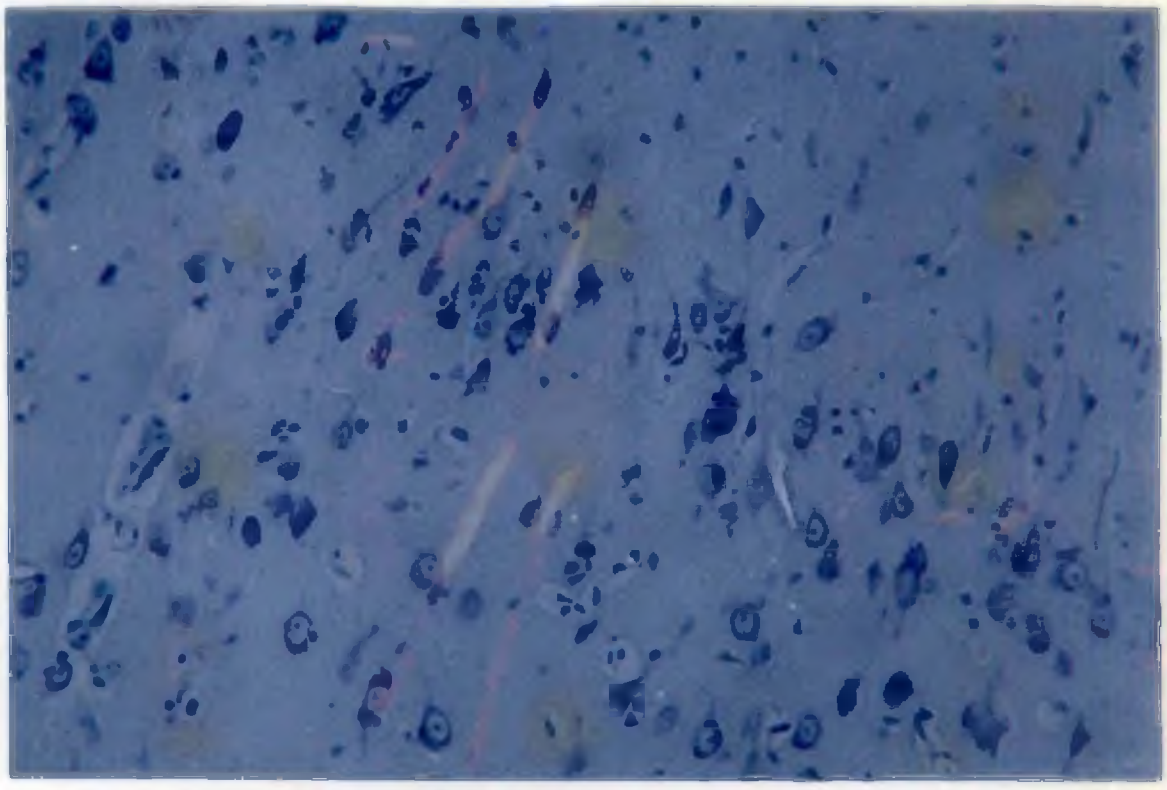
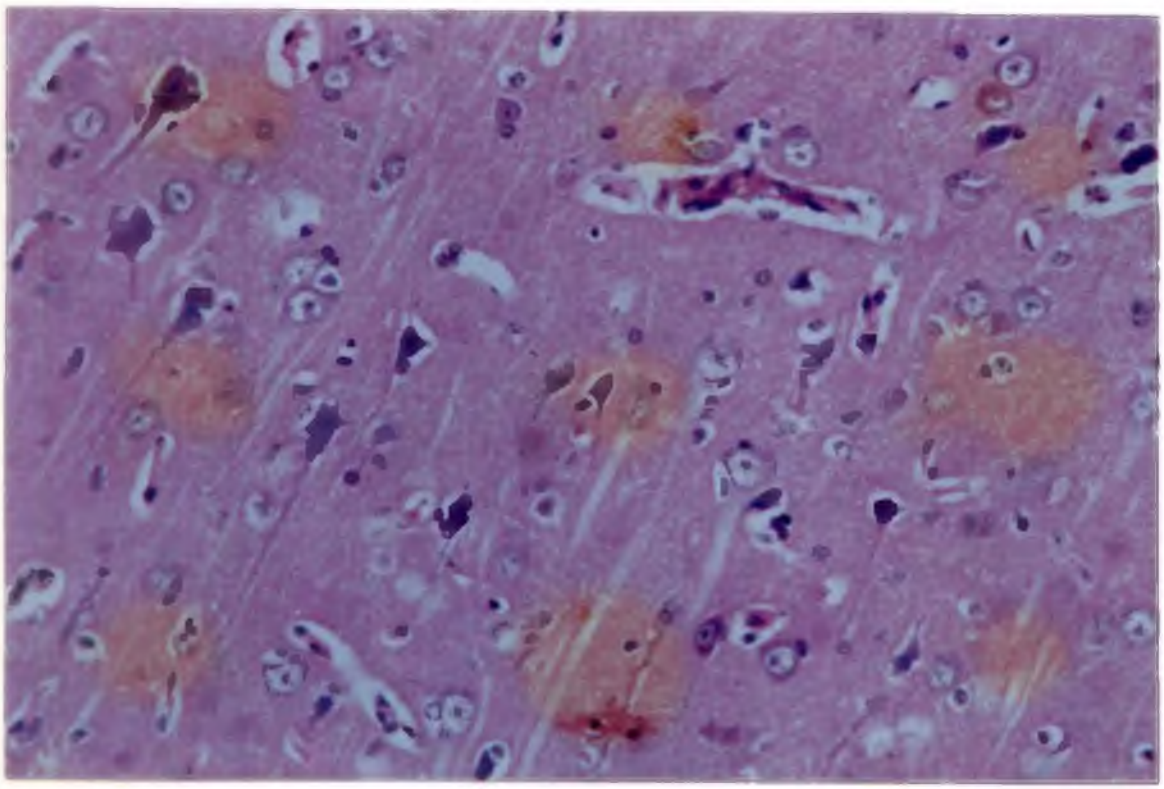


Fig.69. Cerebral cortex - dark blue staining and shrunken neurons, broken and discontinuous fibrils. PTAH x 400

Fig.70. Cerebrum : fusiform layer - shrunken neurons, eosinophilic cytoplasm, perineuronal oedema and pervascular astrocytic reaction. H&E x 400



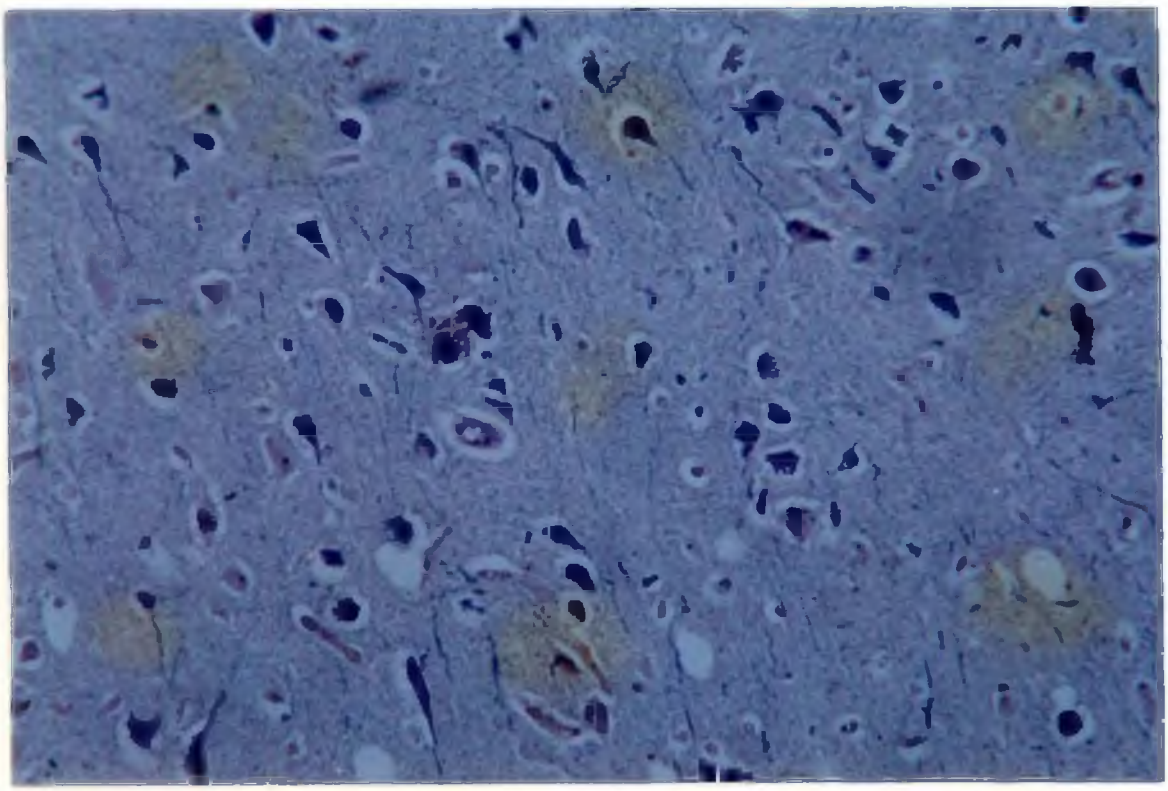
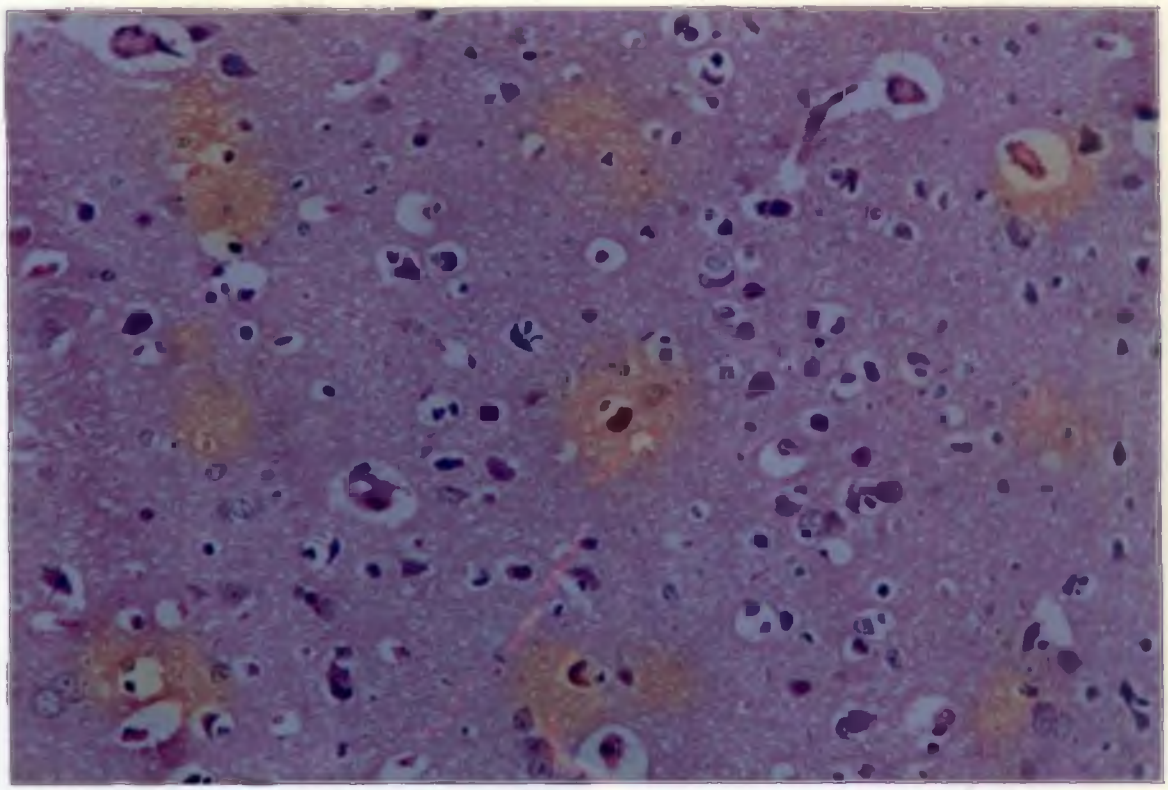


Fig.71. Cerebrum - internal granular, pyramidal and fusiform layer - neuronal necrosis, micro and oligodendroglial reaction and vacuolation of neuropil. Vacuolation around vessels and necrosed neurons. H&E x 250

Fig.72. Cerebrum - crowding of gitter cells and microglia in the fusiform layer, around the vessels and neuronal necrosis. (Animal 1,2 and 3). H&E x 400



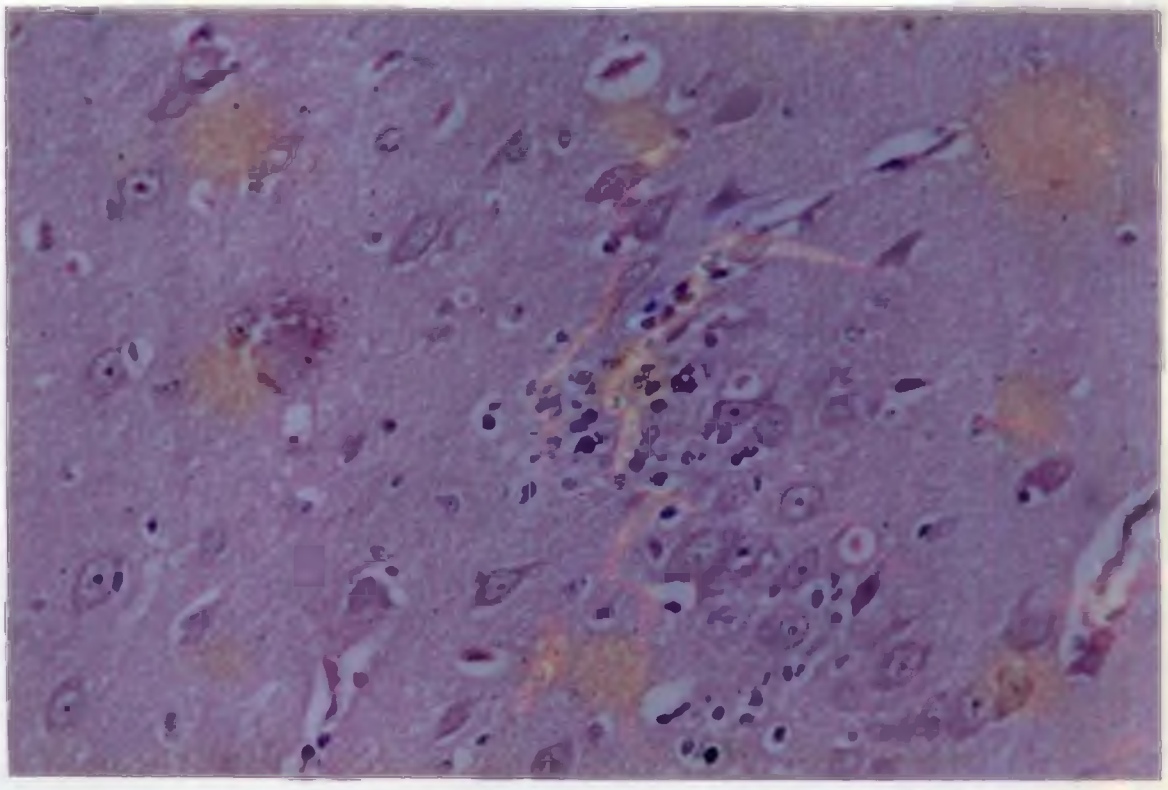
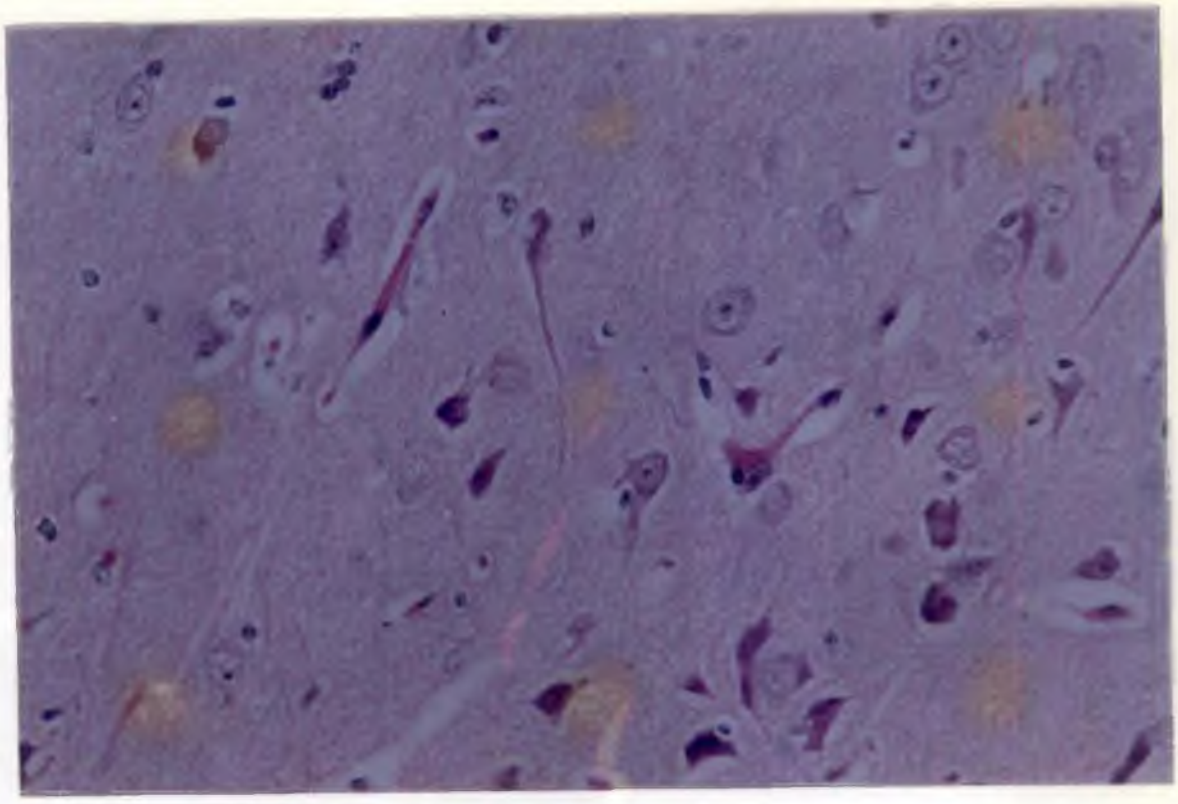


Fig.73. Cerebrum - grey matter - scanty neurons, eosinophilic shrunken neurons and faint neurons disappearing into the neuropil (Animal No.4). H&E x 400

Fig.74. Natural case - animal No. 4 cerebral cortex- neuropil vacuolation, widening of perivascular and perineuronal space, necrosed neurons, microglial reaction and fading of the staining intensity of neurons. H&E x 400



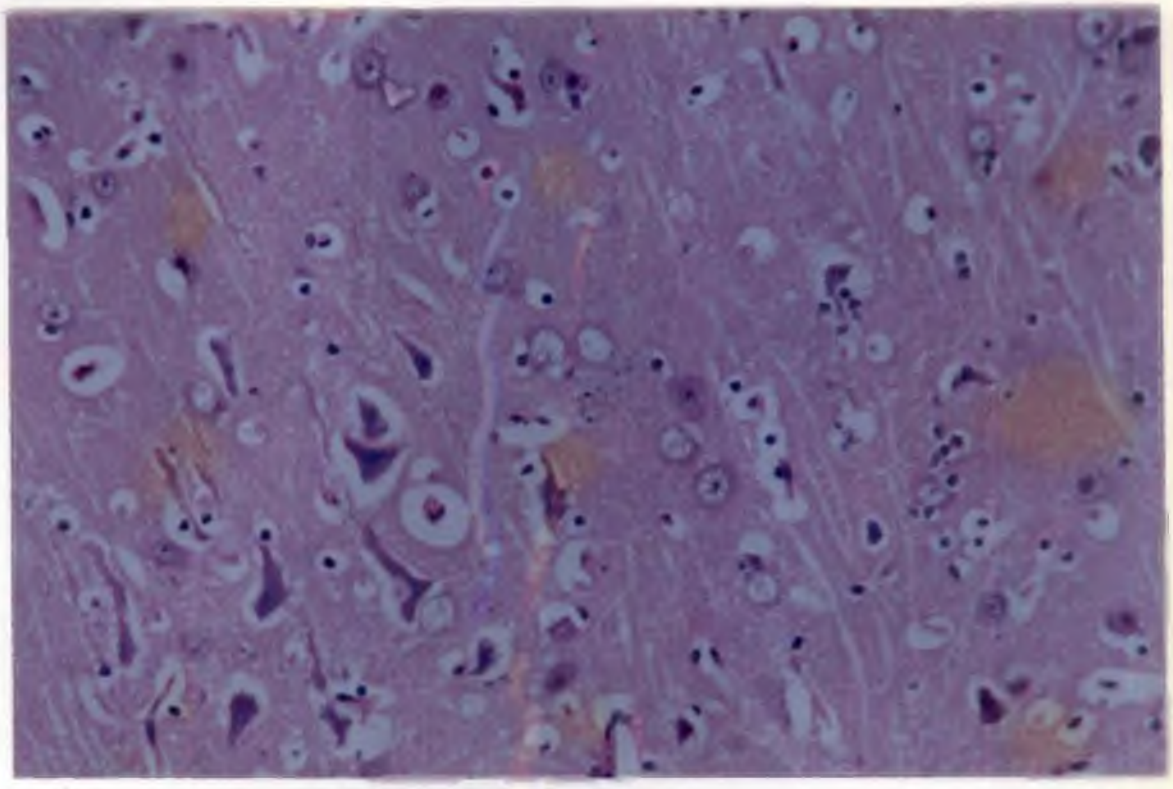
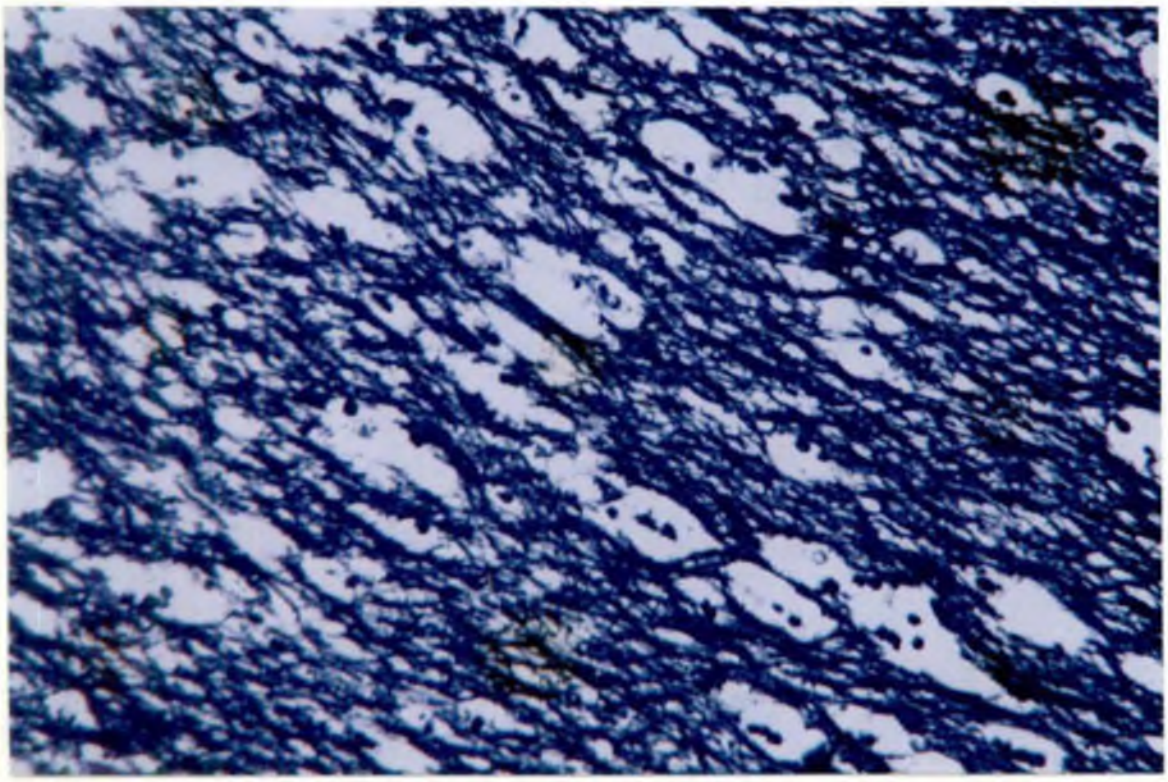


Fig.75. Natural case - white matter - inter and intrafascicular vacuolation running parallel to the direction of axons. H&E x 250

Fig.76. Natural case 1 and 4 : Cerebral white matter : Discontinuity and cavitation of axons separated by regularly appearing black fibers.

Glees and Marsland's modified silver stain x 400



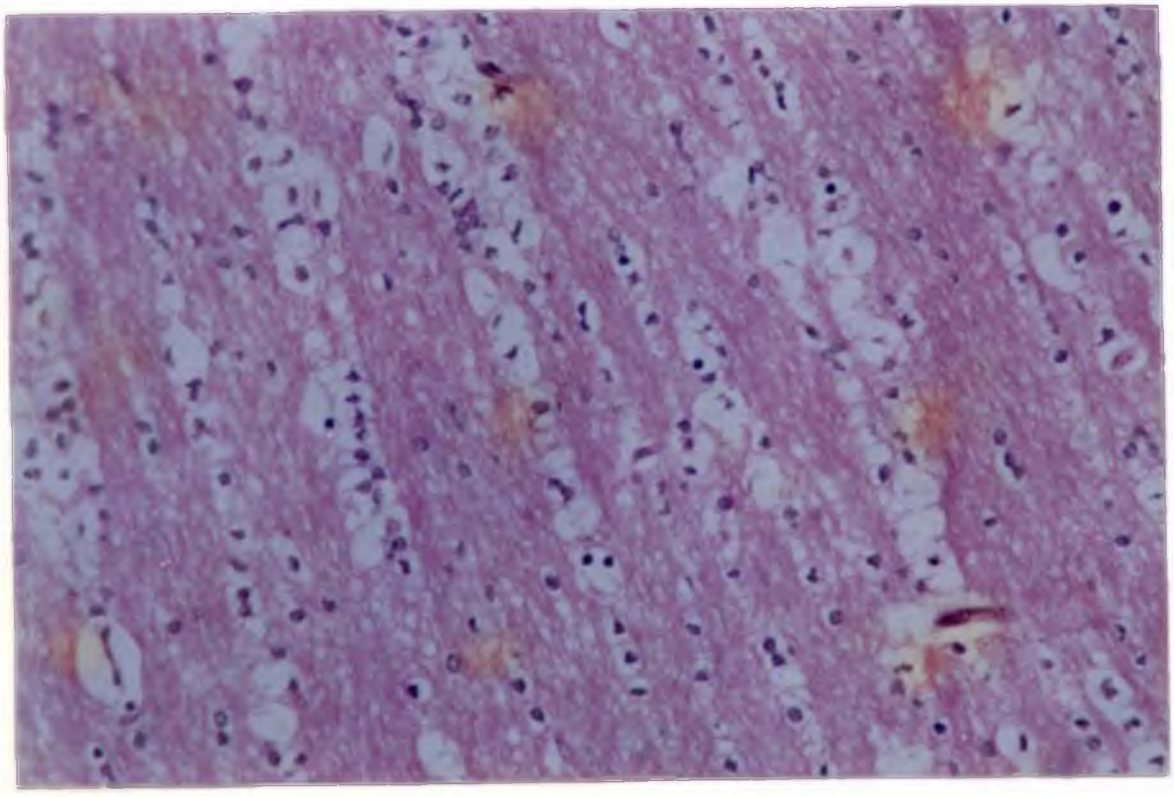
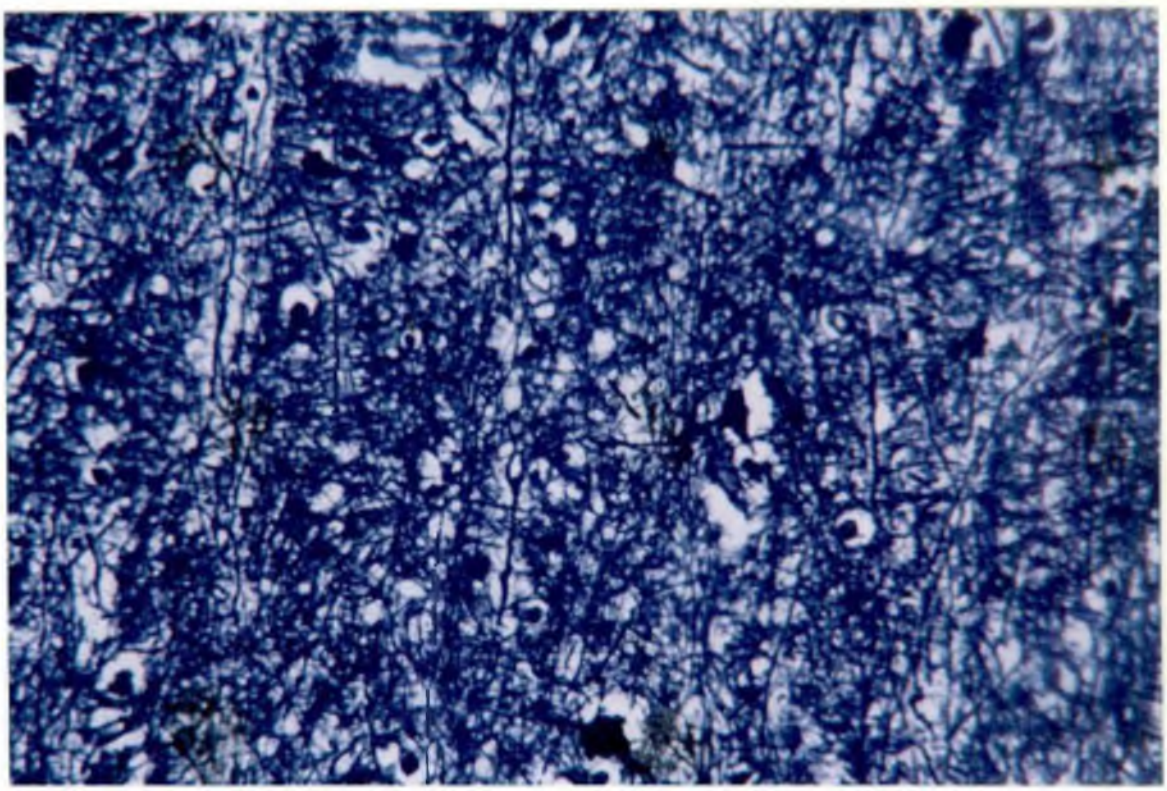


Fig.77. Cerebral cortex : white matter; diffuse discontinuity and cavitation, fibers running in different directions and branching along with damaged oligodendroglia.

Glees and Marasland's modified silver stain x 400

Fig.78. Cerebral white matter - vacuolation and oedema of myelin, myelin loss amidst regularly appearing green to blue stained myelinated tracts

Kluver Barrera Luxol fast blue stain x 400



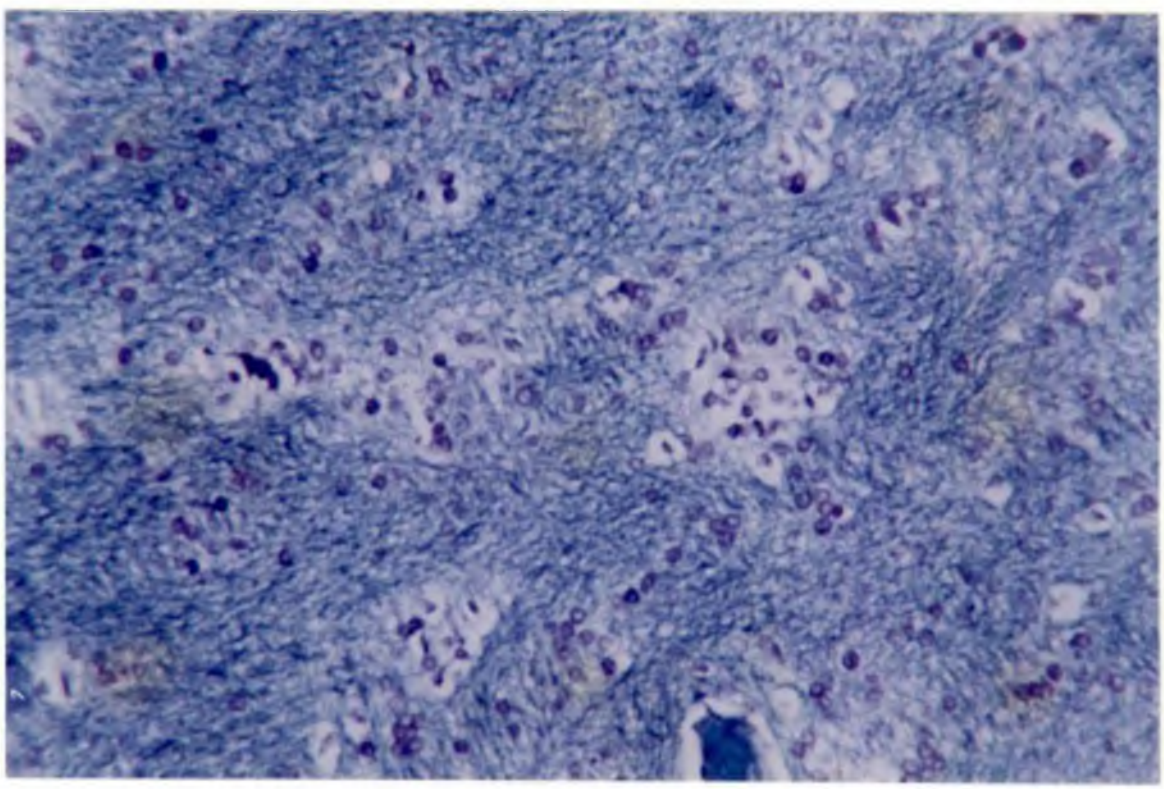
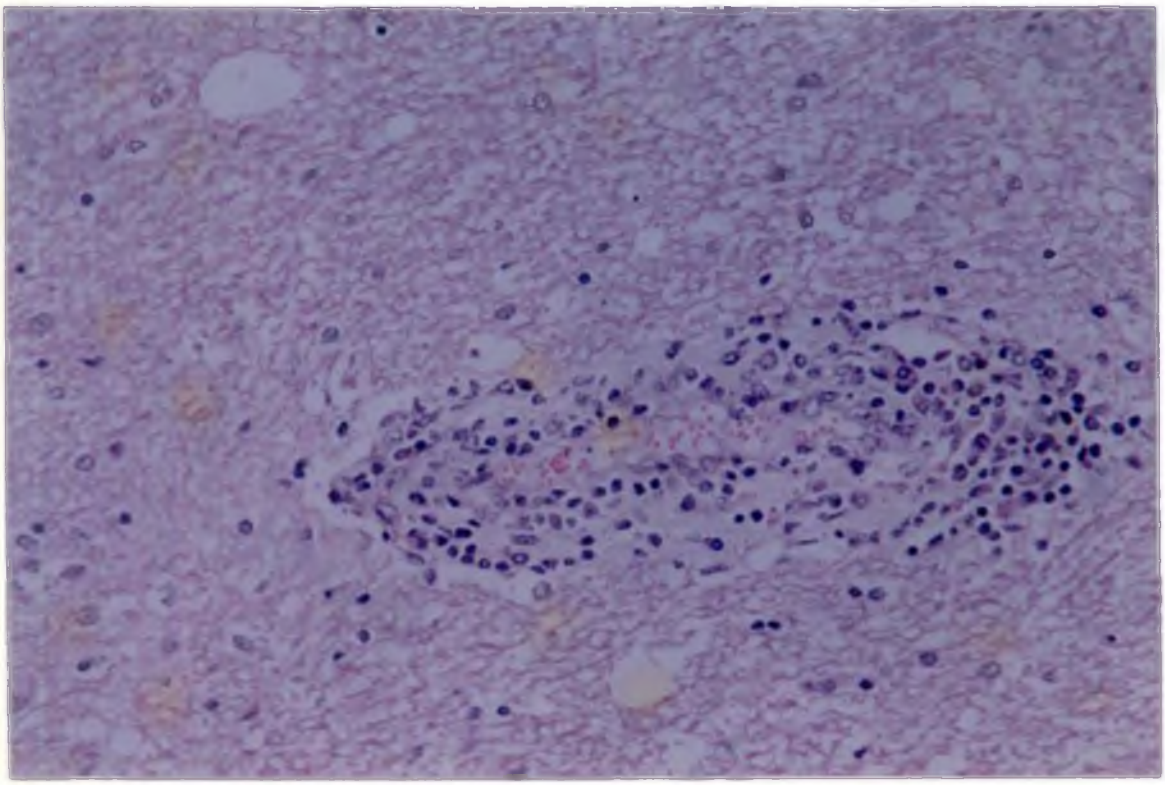


Fig.79. Cerebral white matter : malacic changes, accumulation of gitter cells and lymphocytes. H&E x 250

Fig.80. Cerebral white matter (Animal No.4) congestion of vessels, perivascular lymphoid cuffing, gitter cells and plasma cells. H&E x 250



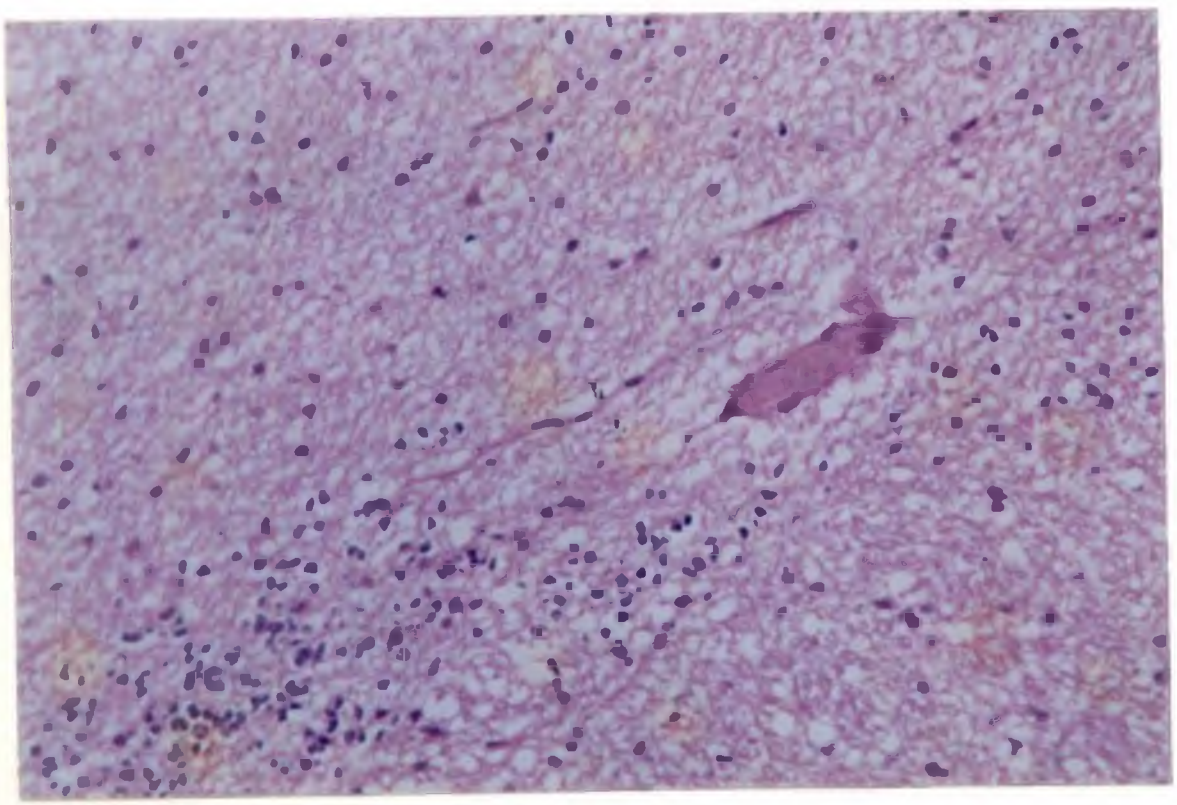
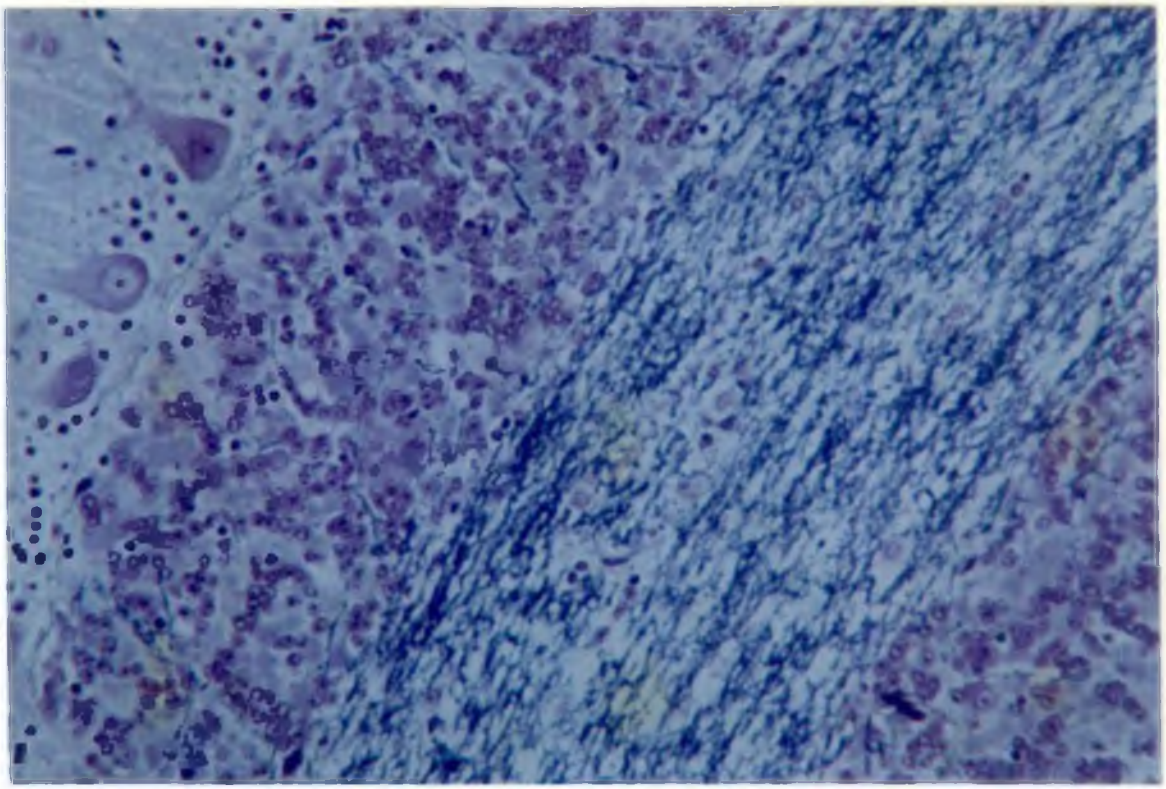


Fig.81. Cerebellum - Demyelination and loss of staining of myelin. Kluver Barrera Luxol fast blue stain x 400.

Fig.82. Pons - lymphocytes, gitter cells and plasma cell infiltration in to the tissues. H&E x 250



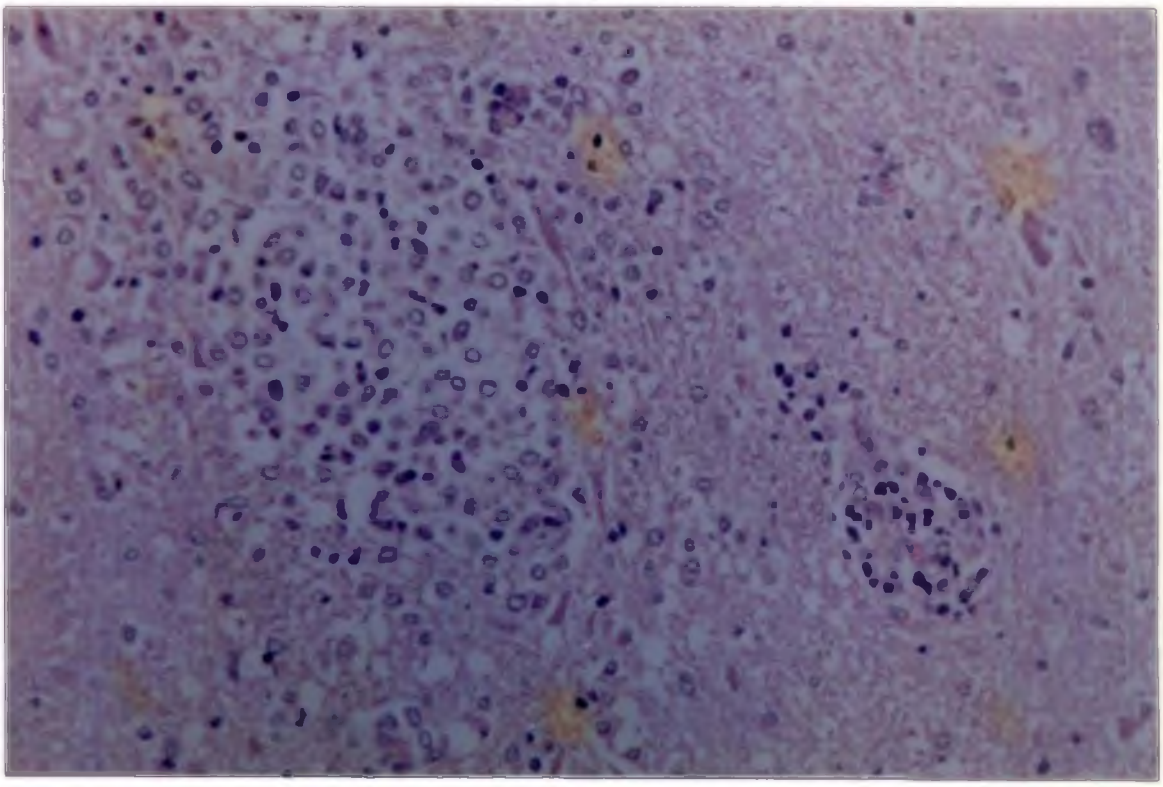
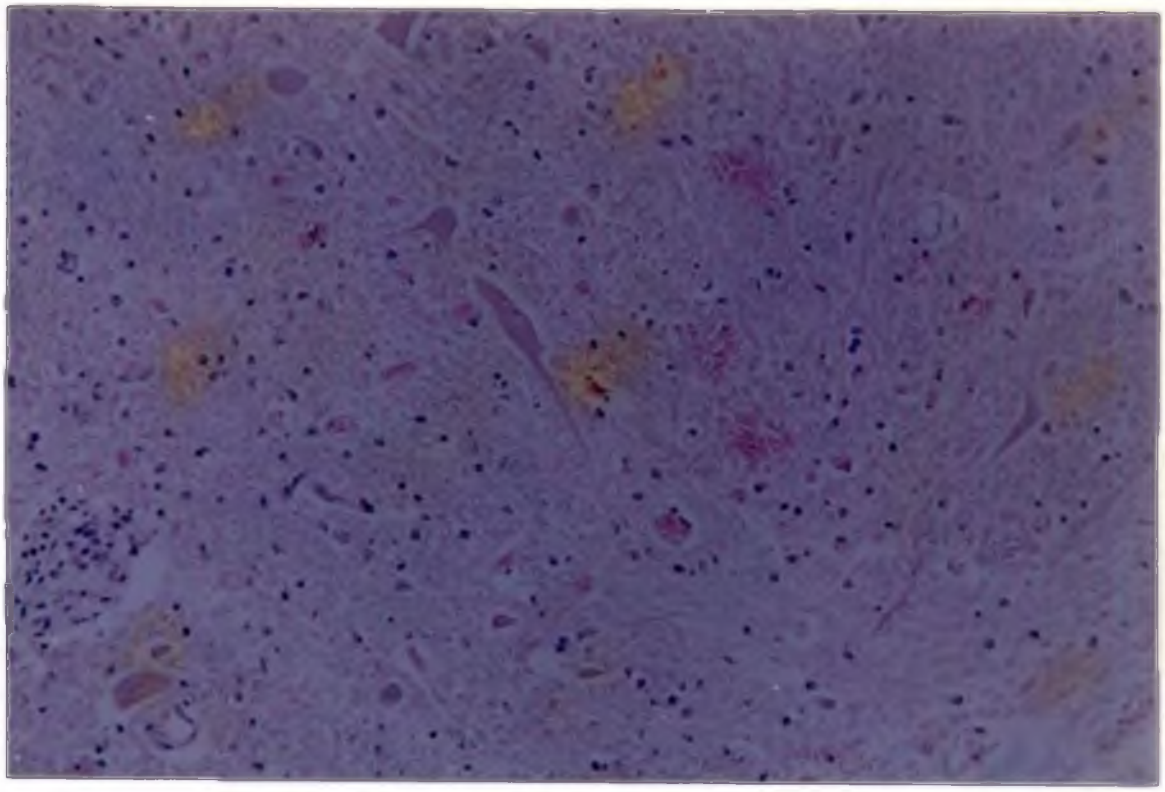


Fig.83. Spinal cord - grey column - congestion, haemorrhage, perivascular cuffing and gliosis. H&E x 250

Fig.84. Spinal cord - grey column- perivascular lymphocyte, plasma cell and gitter cell infiltration, 5 to 10 cell thick, infiltration in to the tissues and cavitation of white matter. H&E x 250



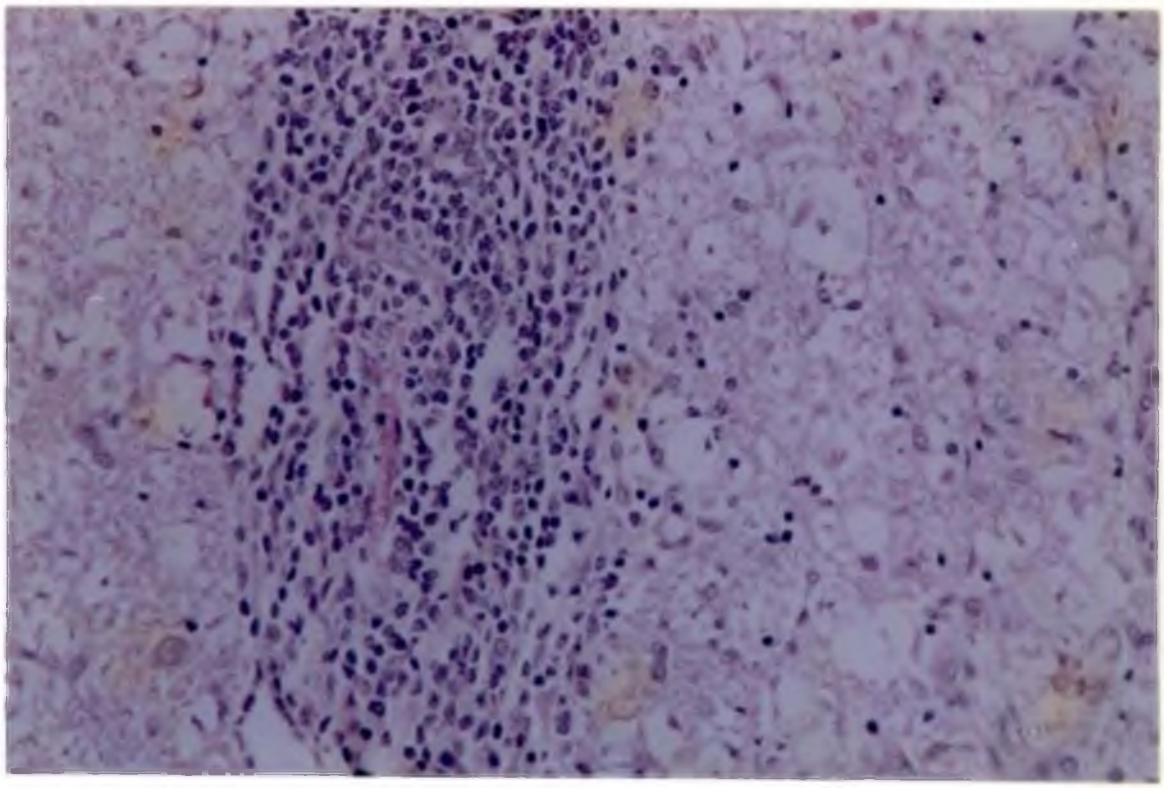
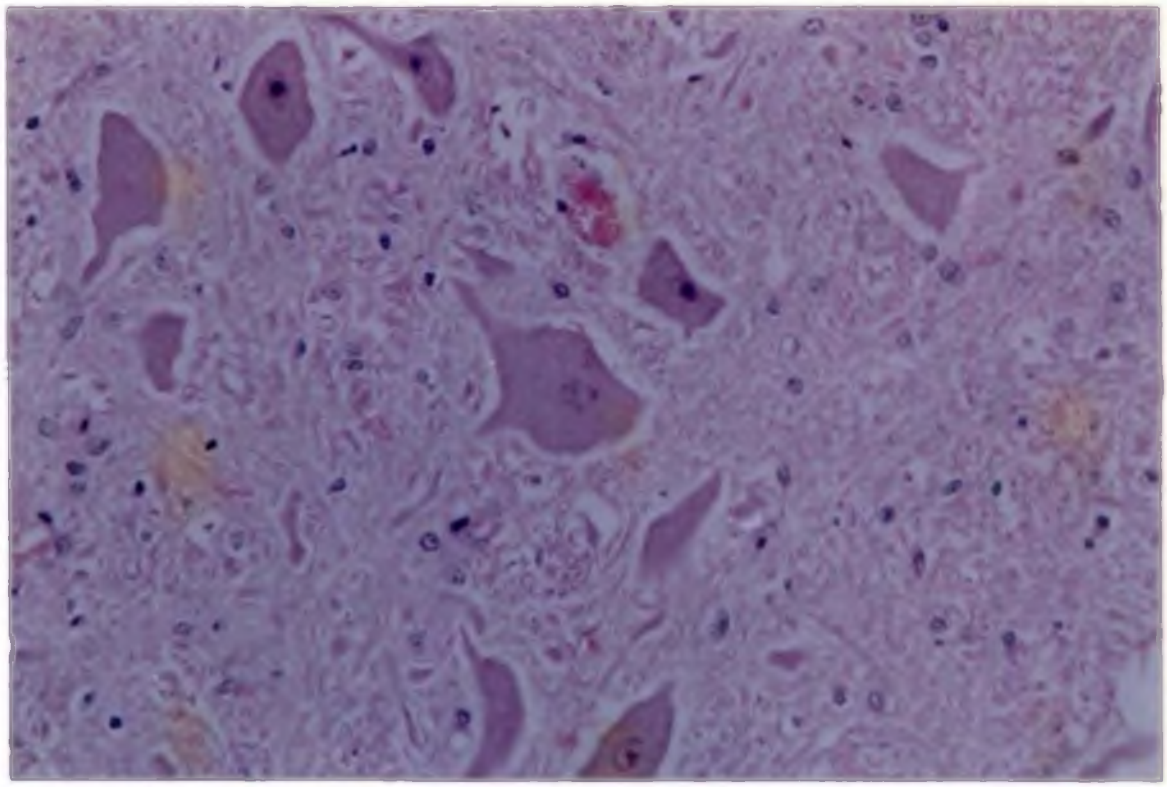


Fig.85. Spinal cord : grey column - neuronal damage, chromatolysis, Karryorrhexis, congestion and gliosis.

Fig.86. Spinal cord - Nucleolar loss and Nissl clumping in the horn cells. Toluidine blue stain x 400.



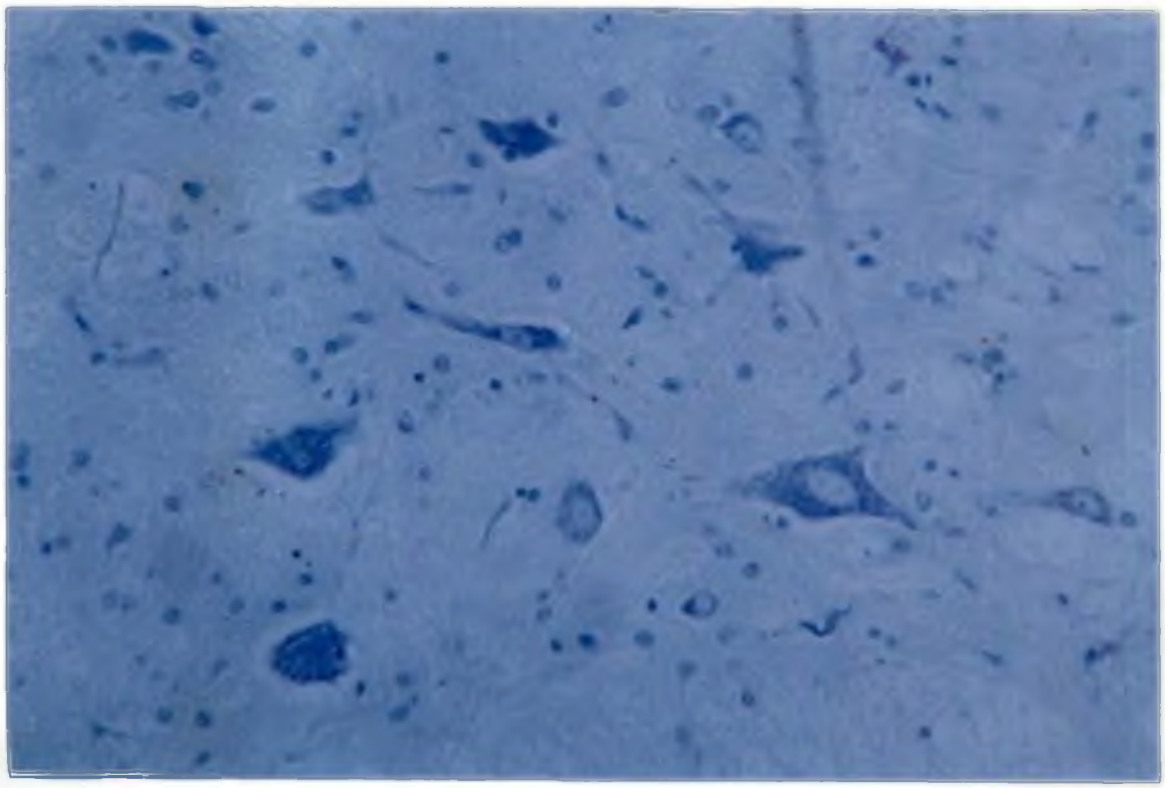
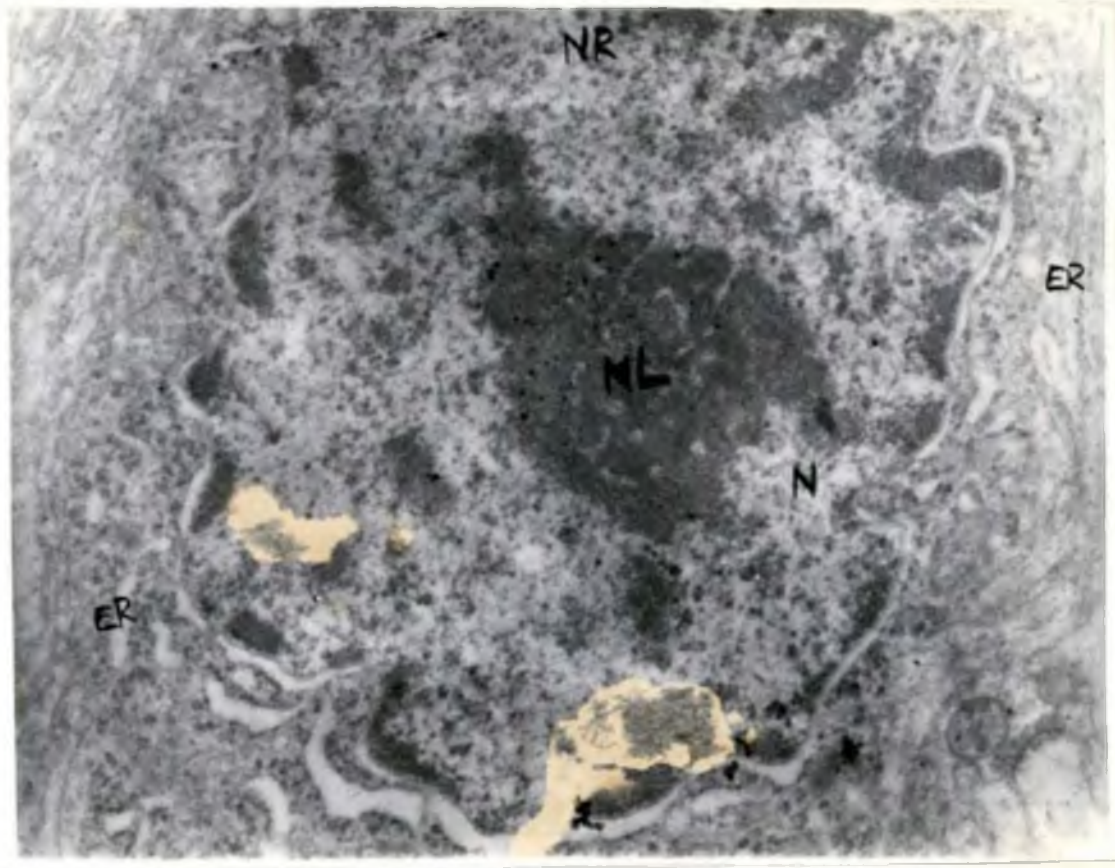


Fig.87. E/m: Natural case : Neuron (NR) showing nucleus (N) with prominent perinuclear cisternae and heterochromatin along the inner nuclear membrane. Large amorphous nucleolus (NL) and partially degranulated swollen rough endoplasmic reticulum (ER) noticed. x 60000

Fig.88. E/m: Cerebral cortex - white matter showing part of a neuron (NR) and sections of axons. Nucleus (N) swollen with aggregated chromatin along inner nuclear membrane. Note condensed nucleolus (NL). Swelling and partial lysis of axons (AX) seen. x 12500


NR
ER
NL
N
ER

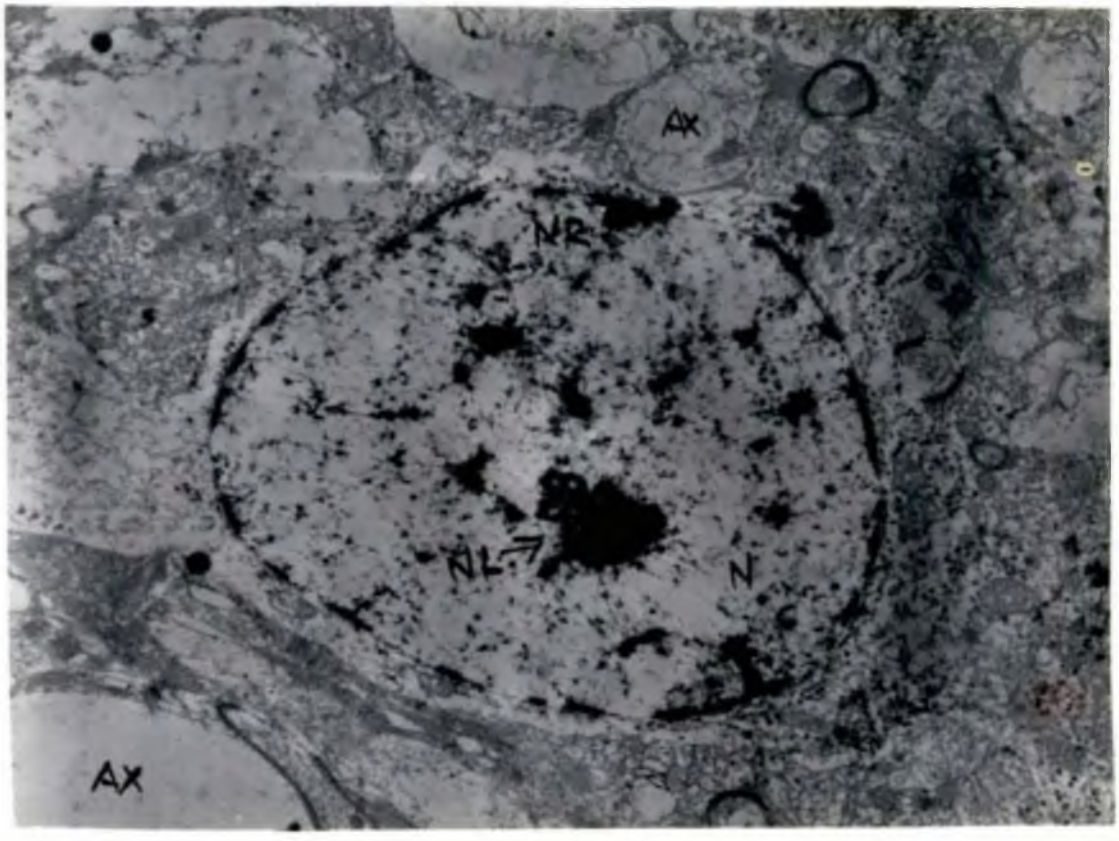
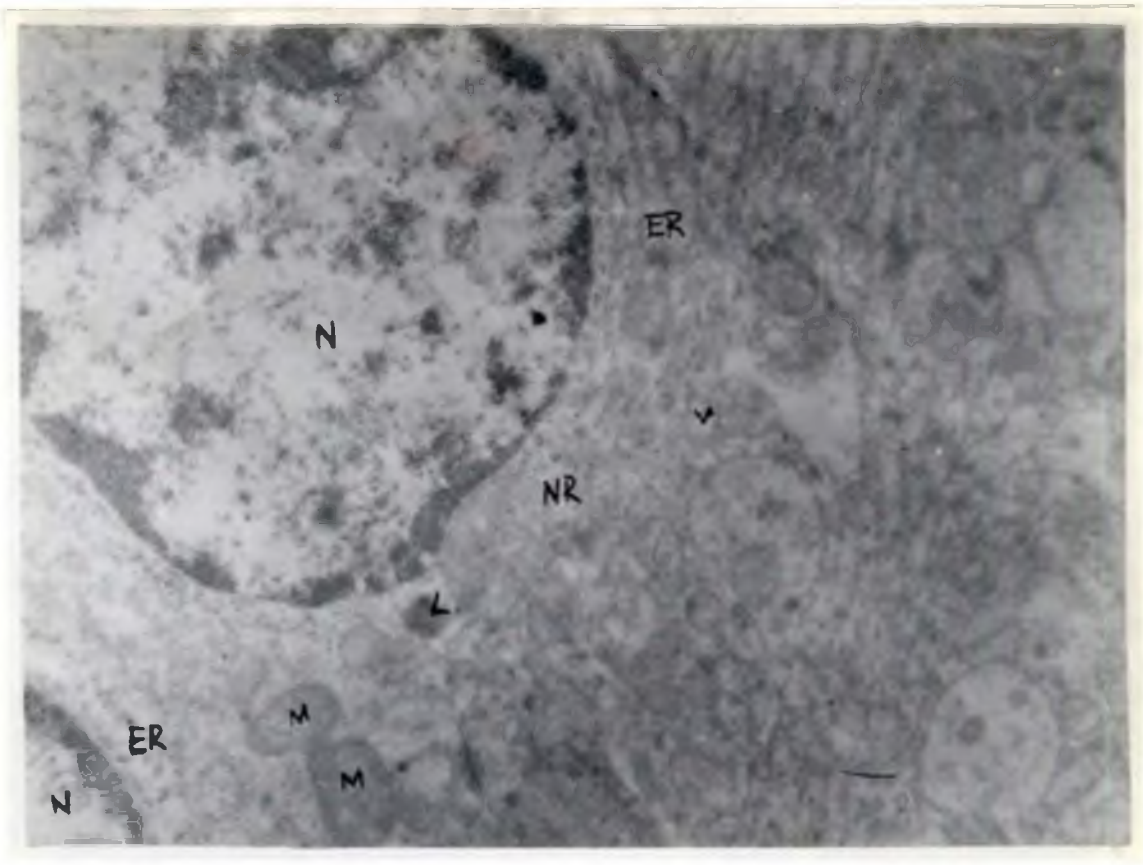

AX
NR
NL
N
AX

Fig.89. E/m: Two adjacent neurons (NR) in the cerebral cortex. Note numerous vacuoles (V) and dilated endoplasmic reticulum (ER) Mitochondria (M) are swollen with partial loss of cristae and electron dense matrix. L-lysosome, N - Nucleus x 50000

Fig.90. E/m: Cerebral cortex : white matter a capillary with swollen endothelial cell (EN). Note fragmentation and loosening of neuropil with numerous vesicles. Mitochondria (M) with swollen cristae. There is swelling of myelinated axons (AX) with partial lysis of myelin, ER. (Erythrocyte) x 18000


ER
N
V
NR
L
M
ER
M
N

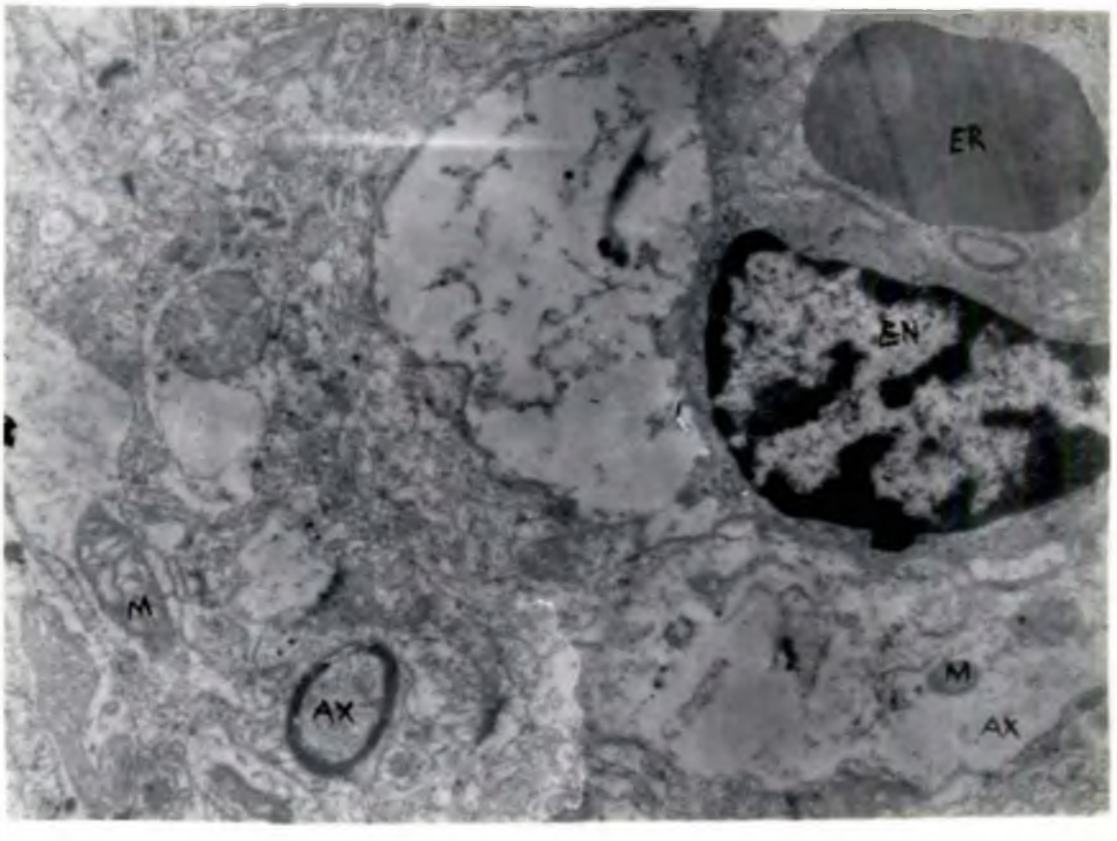
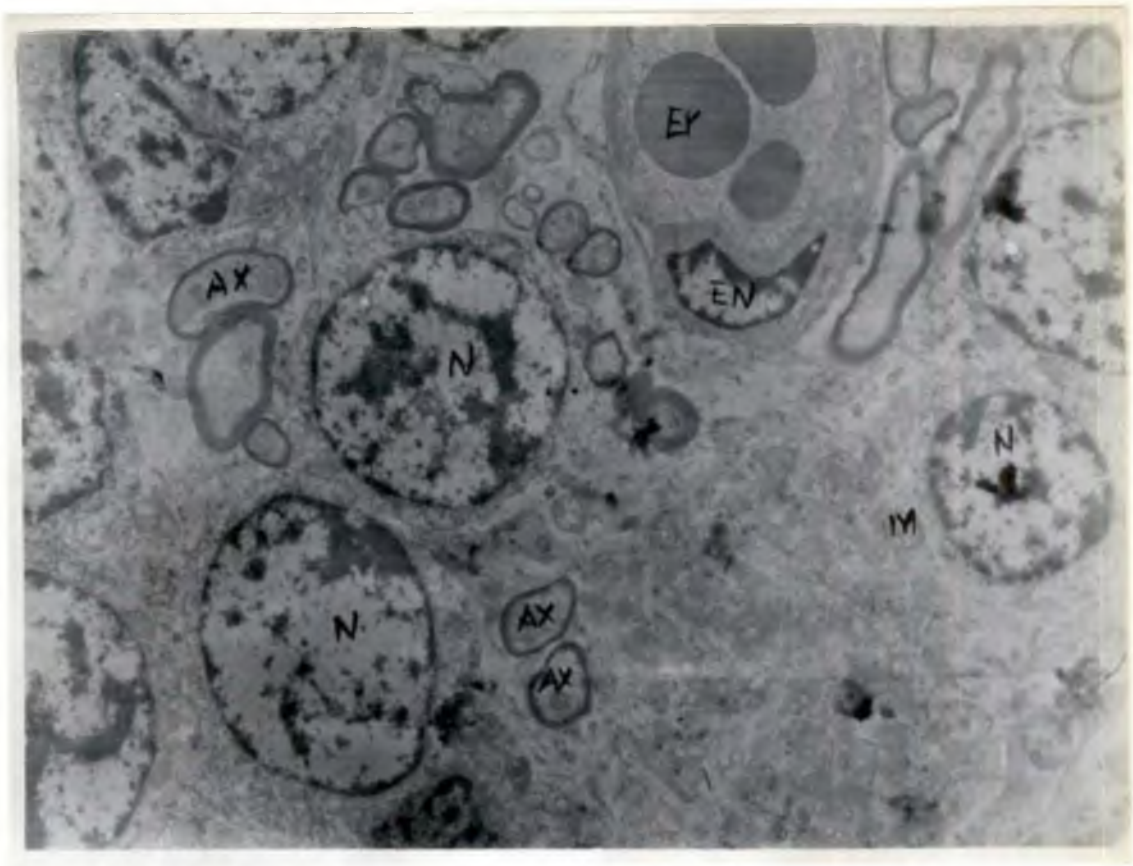

ER
EN
M
AX
M
AX

Fig.91. E/m: Swollen microglial cells, axons (AX) in different stages of degeneration and lysis and section of a capillary with swollen endothelial cells (EN). Neuropil is oedematous with loosening of structures x 14000

Fig.92. E/m: Cerebral cortical area showing extensive lysis and fragmentation of neuropil. A macrophage (MA) is present with many lysosomal (L) bodies and vacuoles. Capillary with endothelial cells (EN) showing focal separation from basement membrane. x 8000


EY
AX
EN
N
N
IM
N
AX
AX

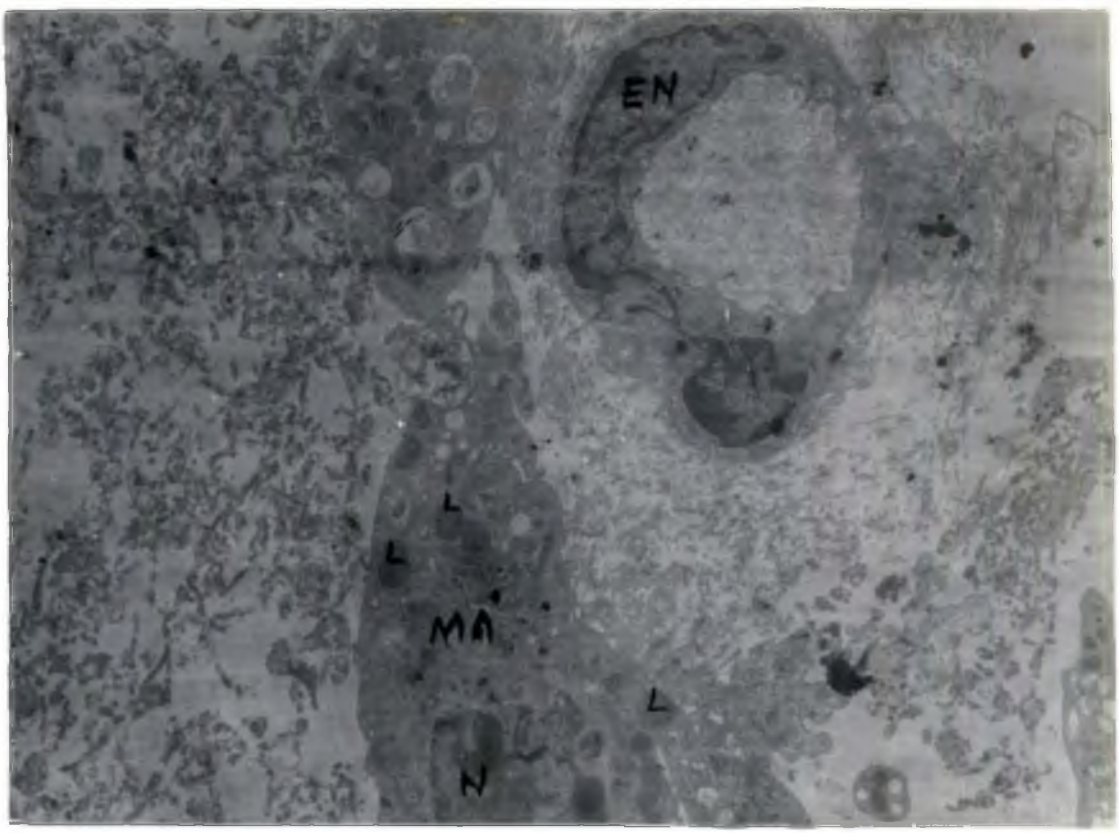
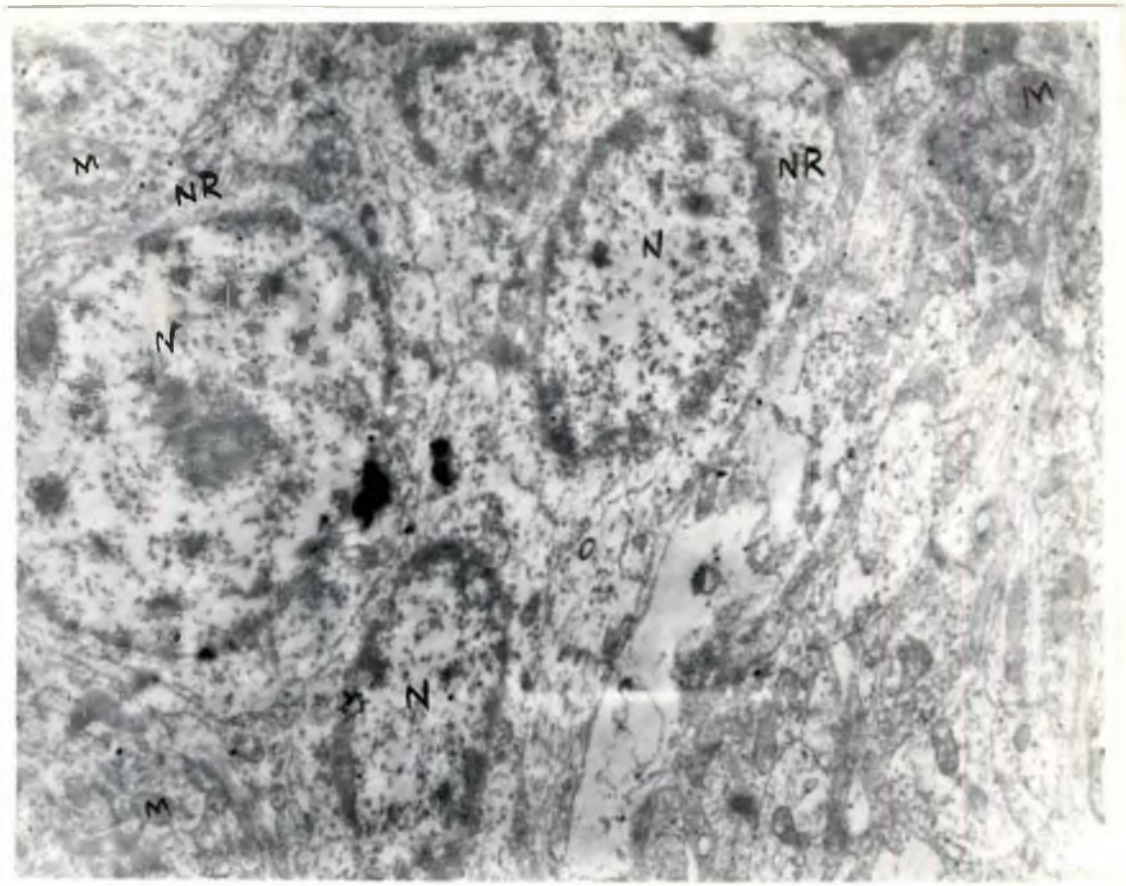
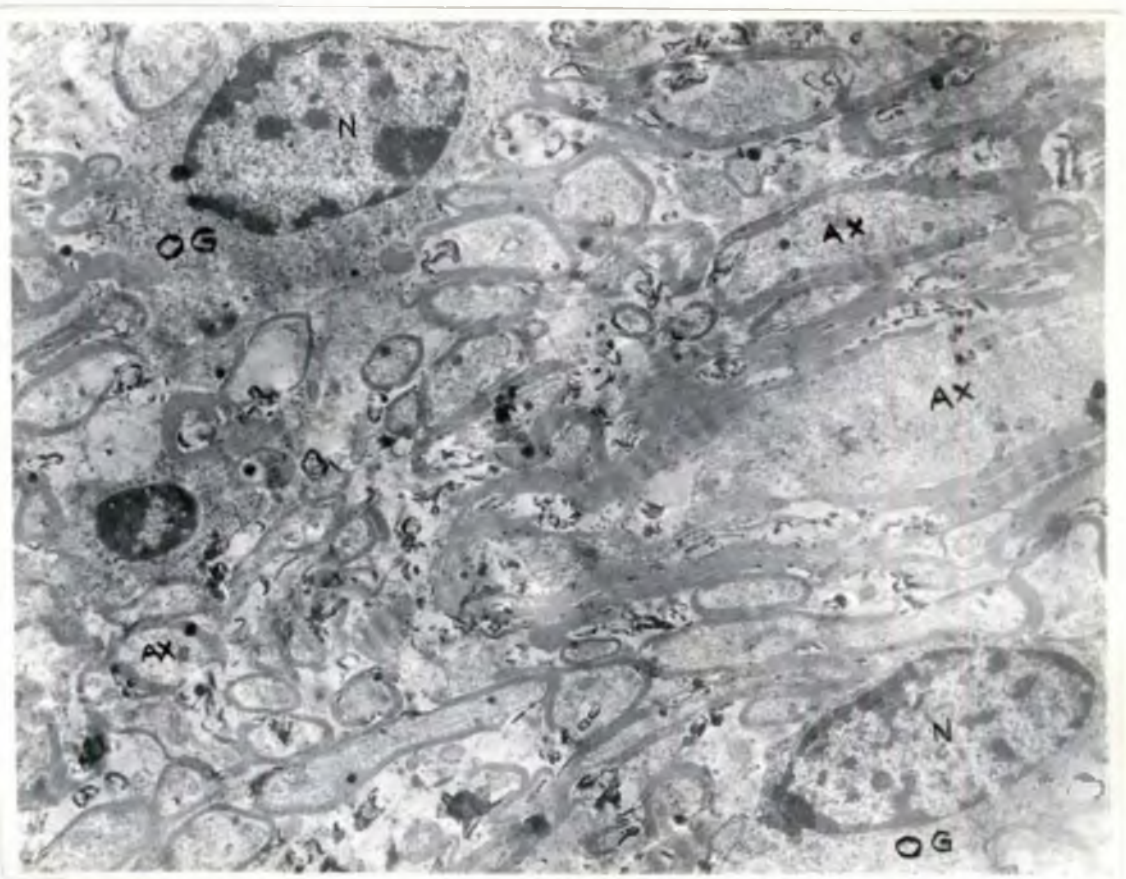

EN
L
L
MA
L
N

Fig.93. E/m: Cerebellum - granule cells. portions of neurons (NR) with swollen nucleus containing mostly euchromatin. Cytoplasm with scanty organelles and free ribosomes. Neuropil oedematous with fragmentation of fibers. Mitochondria (M) show condensation with loss of cristae x 12000

Fig.94. E/m: Corticomedullary areas with many myelinated axons (AX). There is swelling of axoplasm and homogenisation, splitting and lysis of myelin. Notice an oligodendroglia (OG) with granular cytoplasm and swollen karyoplasm. x 8000


M
NR
N
NR
M
N
N
M


N
OG
AX
AX
AX
N
OG

Discussion

DISCUSSION

It was the considered view of the Veterinarians of the state who have encountered many cases of PEM to go in to the aetiopathological aspects of the disease in detail and to suggest treatment modalities based on the observations. They had also observed that the incriminating agents are varied and need elucidation based on experimentation. This study was therefore undertaken to assess the extent of prevalence of the problem and to evaluate whether certain suggested incriminating agents can induce PEM at certain dose levels and if so describe the pathological features in comparison with the natural cases.

The prevalence of the condition was assessed based on the data collected through a proforma sent to the Veterinary Surgeons of selected Veterinary Hospitals of the state. Though 200 Veterinary hospitals were included, data were received only from 76 spreading all the fourteen districts. The data collected from the 76 hospitals for a period from 1991-1994 revealed an increasing trend in the occurrence of the disease. This points out to the importance of the problem and clarified that the disease continues to exist with an increasing trend of prevalence.

Considering the whole state, the data collected actually do not give a true picture as it represents only a small cross section of the population. In many of the hospitals diagnosis is not precisely made and data are not properly recorded in a systematic manner. This is a serious limitation for an epidemiological investigator. The situation has to considerably improve. However, it is understood that efforts are being made by the Department of Animal Husbandry to systematise and computerize the data on animal disease occurrence. This has to be done on a priority basis so that a well organised primary data will be available to the investigators.

The distribution of the condition was also different in various districts. The district-wise distribution revealed a highest rate of prevalence in Malappuram followed by Ernakulam, Kottayam and Calicut. It is relevant to observe that this distribution directly correlates with the goat population in these districts.

The disease was recorded throughout the year. Month-wise prevalence of the disease on analysis revealed the distribution in almost all the months. But a significantly high incidence was observed in the first five months of which the peak incidence was observed in the month of

April. Being the summer peak, fodder scarcity can be expected and the animals have to depend upon available vegetation which are not consumed normally. Some of these might contain toxic and blocking factors of vitamins like thiamine leading to neurological disorders. Comparatively low incidence was observed in the month of June, July, November and December which may be due to the availability of fresh greens. The difference in the incidence occurred could thus be generally attributed to the climatic variations and the type of feed available, but it is pertinent to note that this is not a disease which is always confined to a particular season as occurrence was recorded throughout the year. Similar observations were made by Tanwar (1987). He observed the occurrence more in January, February and mid summer. Hamlen *et al.* (1993) recorded more cases during late autumn and winter or early spring, a time of great concentrate feeding. Lonkar *et al.* (1993) observed the disease throughout the year.

Experimental studies were conducted utilizing Amprolium, Amprolium and rice gruel, rice gruel alone, BHC, sodium sulphate and *Ficus tsiela* Roxb.

Amprolium is a well-known coccidiostat which has been shown to inhibit the uptake of thiamine or a thiamine

antagonist causing thiamine deficiency and PEM. Thiamine deficiency has been attributed as the cause of PEM in many cases. (Edwin and Jackman, 1970; Edwin and Lewis, 1971). Attempts have been made by different research workers to induce PEM by thiamine blocking agents. But it has not been always possible to induce the same (Spicer and Horton, 1981; Fakhruddin *et al.* 1987; Loew *et al.*, 1975 and Rammell and Hill, 1986). Therefore, seven times the treatment dose was used to clarify whether it could produce a deficiency state and induce PEM.

Rice gruel forms a major component of the concentrate fed to goats in this state. At times of availability large quantities of this will be fed and many veterinarians suggested it to be the cause of PEM by way of altering the rumen P^H and hence to elucidate this rice gruel was prepared and experiment conducted.

Amprolium and rice gruel were together fed in order to produce an aggressive state. Feeding of rice gruel induces rumen disorders such as acidity and subsequent changes of flora and fauna resulting in the production of substances blocking the availability of vitamins.

BHC is an organochlorine compound which has wide use in veterinary practice to control lice and mite infestation

and also has been used in agriculture as a crop protectant against pests. Though banned now, till recently it was used indiscriminately in plants and the animals had chances to ingest those contaminated plants. This is a CNS stimulant which can lead to various nervous disorders. No systematic study has so far been conducted to evaluate the neuropathological changes associated with this and to assess whether the brain changes produced have some resemblance to those seen in PEM, the BHC was used as an experimental chemical.

Many medium and large scale industries are present in Kerala which act as potential source of contamination of the environment. The effluents discharged includes sulphates, sulphur dioxide, chlorides, mercury, fluorides and others (Cheeran *et al.* 1987). In many instances the effluents are discharged through open drains resulting in contamination of sub soil water through sweepage. The contaminated drinking water therefore is a potential source of these chemicals to animals. The accumulation of toxicants often exceeds the permissible limits. The sulphate concentration in the water in many areas of the state was found to be very high. Sulphate have been shown to produce PEM and many research workers have produced PEM with various combination of sulphates (Raisbeck, 1982;

Sadler *et al.* 1983; Beke and Hironaka, 1991; Olkowski *et al.* 1992; Hamlen *et al.* 1993; Gould *et al.* 1997). Against this background sodium sulphate was utilized for this study.

The plant *Ficus tsiela* Roxb. locally known as "chela" was demonstrated to cause nervous disorders in cattle (Divakaran Nair *et al.*, 1985 and 1987; Rajan *et al.*, 1986). They demonstrated that this plant cause leucoencephalomalacia and cortical neuronal necrosis. This plant was therefore utilized in this investigation in goats to clarify whether it can bring about similar neurological disorders and could be one of the causes of PEM induced seizures in goats.

Goats showing neurological disorders and provisionally diagnosed as PEM by Vets on the basis of symptoms were procured and observed further. The symptoms and lesions produced were evaluated and compared with the experimental ones and controls. This was done to find out whether there is any similarity of symptoms and lesions in the experimental and natural cases.

Out of the six sets of experiments, animals in three sets did not show any nervous symptoms throughout the experimental period. The groups included the A (Amprolium),

C (Rice gruel) and F (*Ficus tsiela* roxb.). A few animals in the other groups showed nervous signs which were much indistinguishable from the natural cases of PEM except for minor differences. The number of animals which had nervous symptoms were two in group B (Rice gruel and amprolium), four in group D (BHC) and three in group E (sodium sulphate). The absence of any nervous symptoms in three sets of experimental groups and manifestation of symptoms only by a few animals in other groups is an indication of the high tolerance and adaptability of the goats to various chemicals.

The manifestations of symptoms appeared at different latent periods and this included lethargy, depression, anorexia, knuckling at the fetlock, lameness, tremors, opisthotonos, loss of eye preservation reflex and head pressing. These symptoms were predominant in the sodium sulphate fed group. In the BHC group, opisthotonos was not observed. In fact the animals had intermittent remissions of symptoms which were not progressive. Eye preservation reflex was present. In extreme cases there was rigidity and spasms, anorexia and facial oedema in the BHC fed group. In the group B (Amprolium and rice gruel) though the above mentioned symptoms were occasionally observed were not progressive. In fact the animals had diarrhoea,

distension of abdomen and intermittent convulsions. Eye preservation reflex was present. There was absence of opisthotonos and the animals recovered from the symptoms within few hours. Only sodium sulphate fed animals had more or less similar symptoms as in natural cases. Opisthotonos and blindness were observed in natural cases besides the other symptoms described above.

The clinical signs were either not progressive or only slowly progressive. They diminished in intensity after sometime and this could be due to development of tolerance or a functional compensation, though such spontaneous compensation was not reported in natural cases. This could be due to the facts that at the hospitals only progressive cases were brought for treatment. The animals which had clinical signs had certainly lesions in the CNS. Clinical signs like lameness, tremors, opisthotonos and intermittent convulsions also indicated disturbances in cerebellar function. The cerebellum is a central point in the CNS for the organization of movement (Llinas, 1975). Any change in the configuration and degeneration of the granule cell layer and Purkinje cells of the cerebellum usually adversely affects the harmonious action of synergic muscle groups. The demonstration of neuronal damage in many parts of the cerebral cortex and cerebellum and also

demyelination and cavitation in the white matter clarified the nature of the clinical signs and delineated the pathobiological basis of the symptomatology.

Progression from myelin vacuolation to demyelination has been reported in a number of experimental intoxications, but it was only infrequently associated with naturally occurring diseases (Prozesky *et al.*, 1994). Involvement of the deep cerebral and cerebellar white matter may explain the development of ataxia and tremors. Progressive hind limb paralysis observed in the natural cases could be explained by the presence of diffuse spinal cord vacuolization and inflammatory changes.

Various were the symptoms in PEM. Eventhough there were varied manifestation of symptoms, opisthotonos and blindness were consistently observed in all the reported cases (Fakhruddin *et al.*, 1987; Tanwar, 1987; Sager *et al.*, 1990; Pugh, 1993; Moro *et al.*, 1994 and Tanwar and Malik, 1995). In this investigation, only in one natural case there was blindness. None of the experimental cases showed blindness except in group E (sodium sulphate). In this group two had loss of eye preservation reflex. The blindness could be due to a lesion in the visual cortex. There was histological lesions in all the layers of the

cerebral cortex and perforce visual cortex would also had been affected. It may be based on the degree of involvement that the symptoms appeared in the form of nystagmus, loss of eye preservation reflex and finally blindness.

In the group A (Amprolium), the animals did not manifest any symptoms. There was however, moderate degree of degenerative changes in few of the neurons of the cerebral cortex. It would appear that the dosage of amprolium administered was not sufficient enough to induce PEM and associated neuropathological changes. Lonkar and Prasad (1994) observed wide individual variations regarding the total quantity of amprolium required and the time required to produce PEM.

Experimentally, PEM was produced by feeding extremely high dose levels of amprolium (Fakhruddin *et al.*, 1987; Itabisashi *et al.*, 1990; Strain *et al.*, 1992). The dose of amprolium used by these investigators was 4.5 g/day, 600 mg/kg and 1 g/kg body weight respectively. This was three to 10 times higher than the dose used in this investigation. Such very high dose of amprolium is very unlikely to be consumed by the animals naturally. It

would, therefore, appear that amprolium might not be a cause of PEM in natural situations.

The group C (Rice gruel) animals also did not manifest any symptoms and lesions except in one. In this animal there was depression and abdominal distension which occurred towards the end of the experiment. These symptoms along with other changes after consumption of rice gruel are an indications of the development of acidosis and probably this animal might have consumed a large quantity of the rice gruel on the day when it showed such symptoms. The animal showed degenerative changes in the cortical neurons and moderate spongiform changes of white matter. For others the consumption everyday might have resulted in a state of tolerance and it is therefore pertinent to point out that rice gruel everyday as a component of concentrate feed in goats might not cause any detrimental effects. But one should be cautious not to overfeed the animals only with rice gruel. The Veterinarians observation that feeding of rice gruel is a cause of PEM appear to have relevance only if the animals are overloaded with rice gruel suddenly on a single day.

The body weight of the animal in group A, B, d, E and F remained low as compared to the control throughout the

experimental period. In group C, though the weight remained low on the 15th day, all the animals showed a weight gain on the 30th and 45th day. The reduction in the weight in all the other cases can be attributed to reduced feed intake and the anorexia that the animal developed during the course of the experiment. In the animals fed rice gruel, there was a gain in weight which indicated that rice gruel is not detrimental to the health. Since it is carbohydrate rich diet and easily digestible, the system might have used this as the source of energy and there was no protein and fat depletion to produce a state of weight loss.

The presence of fluorescence is an indication of necrosis in the brain (McAllister *et al.* 1992, Sager *et al.* 1996). Autofluorescence was not consistently observed in all the affected animals. Two animals which were in the group E (sodium sulphate group) and three of the natural cases revealed autofluorescence. The fluorescence which ranged from focal to diffuse blue green to creamy was present mostly in the crown of the gyri and sulcal depressions. Also it has been observed focally in the white matter. Since PEM is characterized by laminar cerebro-cortical necrosis, autofluorescence can be expected. The cause of autofluorescence of necrotic tissue

when illuminated with UV light was attributed to accumulation of lipid peroxidationproducts as a result of the degradation of the lipoidal material within the macrophages (Lee and Little, 1980). They also opined that it could also be related to high molecular weight collagen like material. The presence of microglia with ingested materials and lysosomes as observed by electronmicroscopy in the area of degeneration and necrosis in the experimental and natural cases in the present study directly correlated with the degradation processes and accumulation of lipoidal material. Autofluorescent foci were also observed by various investigators (Gould *et al.*, 1991; McAllister *et al.*, 1992; Tanwar *et al.* 1993 and Sager *et al.* 1996). The autofluorescence observed by them varied from creamy white, blue green to greenish yellow in the cortical areas. From the observations made in this investigation it appears that autofluorescence of the cerebral cortex is not specific as majority of the brain did not show any fluorescence. In this context it is pertinent to point out the observation made by Rehncrona *et al.* (1980) that autofluorescence is not specific to the necrotic lesions of PEM or even to the brain tissue. This test is only a supportive and confirmative one and has no primary diagnostic value.

The CSF protein concentrations in the experimental goats with or without nervous disorders were always within or below the expected range and was not significantly different as compared to the control. But the pre and post exposure protein concentration in group A (amprolium treated) was significantly low and different from the control. Surprisingly symptoms or lesions were not observed in this group. The moderate decrease in protein concentration appears to be non-specific and might be as a result of the dietary change. The protein concentration in the CSF in the natural cases was high in all the three animals examined and the concentration in animal number 1, 2 and 3 was 48.82 mg/dl, 36.31 mg/dl and 23.23 mg/dl respectively. This changes could be attributed to disturbances in the blood-brain barrier. Sorjonen (1987) opined that the albumin quota and total CSF albumin values could be used as indicators of blood brain barrier disturbances. Sunder *et al.* (1992) observed a significant elevation of protein in the CSF in PEM affected calves. Scott (1992) observed significant increase in the group mean CSF protein concentration in sheep with meningitis, listeriosis and spinal abscesses, but not in PEM. Sargison *et al.* (1994) observed normal CSF values in lambs with PEM.

The histopathological and ultrastructural lesions present in the experimental animals and natural cases were comparable in different parts of the brain although there was variation in the intensity. There was also variation in the intensity of reaction noticed between different segments and from gyrus to gyrus. The selective vulnerability of different neuronal groups within the CNS in various conditions is a well established phenomenon (Cork, 1994). The vulnerability is usually associated with the neurotransmitter phenotype of the neuron or the expression of particular receptors on the surface of these neurons. Immunocytochemical reagents directed against specific neurotransmitters, cytoskeletal proteins or other neuronal proteins may provide proof to the fact that injury to a selective population of neurons has occurred. The use of glial fibrillary acidic protein assays in conjunction with the traditional histopathological examination as well as immunocyto-chemical analysis of the CNS structural proteins should make it possible to localize and quantify areas of induced neuronal damage (Cork, 1994). In this investigation efforts were made to analyse the structural damage associated with various possible etiological agents employed in this investigation through histopathological and ultrastructural studies. The swelling and vacuolar

degeneration of the neurons and glial cells resulting in the death of these cells in the cerebral cortex were the main pathological features consistently observed in the experimental and natural cases clarifying that the changes were mainly degenerative neuropathy. Although, found in various parts of the CNS, the degeneration and necrosis of the neurons were most prominant and wide spread in the cerebral cortical laminae and in the cerebellar granule cell layer. All the cases studied as a whole gave a general picture of various stages in the development of degenerative neuronal lesions. The initial stage was identified to be a variation in the staining affinity of the cells with localized swelling in some areas. This was followed by a marked swelling of the cell and necrosis. Necrotic cells sometimes disappeared and were seen replaced by glial nodules as observed in natural cases and those treated with sodium sulphate. The disappearance of these neurons together with a diminution of the cells in the cerebral cortex resulted in a reduction of the total mass of the cerebral cortex in some of the gyri which were well evident by Toluidine blue and Phosphotungstic acid and haematoxylin stained sections.

Gliosis and perivascular lymphocytic and gitter cell infiltrates are features usually associated with infections

or allergic encephalitis. The presence of these features in the brains of one of the animals which was fed *Ficus tsiela* and sodium sulphate treated cases is rather a relatively uncommon finding. In natural cases also there was such lesions in various segments of the brain. Glial cell proliferation and inflammatory cell infiltrates have been described in plant and fungal toxicosis such as equine leucoencephalomalacia (Marasas *et al.*, 1976). The cell infiltrates in these conditions were clearly associated with malacia and it could be surmised that this was secondary manifestations following degenerative neuronal lesions. Severe malacic changes were observed in the cortical neuropil and also in the white matter.

In the group A (Amprolium) though the animals remained clinically normal, occasional neuronal necrosis was evident in the cerebral cortex with perineuronal and perivascular oedema and generalised congestion and haemorrhage in some of the segments of brain. They were ultrastructurally characterized by swollen neurons, shrunken astrocytes and disaggregation of polyribosomes.

Morgan *et al.* (1974) stated that a haemorrhagic diathesis resulting from thrombocytopenia may occur in amprolium poisoning of pre-ruminant lambs. They observed

this when lambs were dosed with amprolium at the rate of 280 mg/kg for three weeks. Though many investigators have experimentally produced PEM in goats using amprolium at different dose levels, they have not reported any haemorrhagic lesions (Spicer and Horton, 1981; Fakhruddin *et al.* 1987; Itabisashi *et al.* 1990; Lonkar and Prasad, 1994). The congestion and haemorrhages seen in this case also can be attributed to thrombocytopenia as the dose level used was more (350 mg/kg) as compared to 280 mg/kg used by Morgan *et al.* (1974). Enumeration of thrombocytes was not undertaken in this study and thrombocytopenia could not be established.

In the group B (rice gruel and amprolium), diffuse degeneration of the cerebrocortical laminae, swollen neurons, congestion and reactive astrocytes were seen together with extensive neuropil and white matter cavitation in two animals. Besides this, degeneration and necrosis of the hippocampus and congestion and haemorrhages in the cerebellum were seen. More or less similar lesions were recorded in Amprolium induced PEM by Spicer and Horton (1981) in goats and they attributed the lesions to thiamine deficiency and subsequent metabolic derangement. Electronmicroscopic observations revealed swollen neurons with disruption of membrane systems, prominent

perichromatin granules, mitochondrial changes, cytoplasmic enlargement of astrocytes alongwith myelin splitting. According to Derenzinie and Moyne (1978) an increase in the number of perichromatin granules may be an indicator of aberration of protein synthesis activity and accumulation in the nucleus reflects a state of suppressed protein synthesis.

Though the animals were clinically normal in group C (rice gruel), only one developed depression and distension of the abdomen. The brain of that animal revealed neuronal degeneration which were ultrastructurally characterized by chromatin condensation, partial degranulation of ribosomes, nuclear swelling and mitochondrial changes. The other animals in this group did not show any brain lesions.

Amprolium is a thiamine antagonist and rice gruel when consumed in excess can lead to acidosis. Both these agents might have induced a state of thiamine deficiency. The adult goats meet their thiamine requirement via intraruminal microbial synthesis. Feeding on rice gruel leads to lactic acidosis which can predispose thiamine deficiency in several ways. Bacilli that are potent thiaminase producers increase in the acidic medium (Boyd and Halton, 1977; Morgan and Lawson, 1974; Shreeve and

Edwin, 1974). The pH decreases to near the optimum for action of bacterial thiaminase 1 and histamine accumulate becoming a co-substrate for thiaminase action. Ruminal osmotic pressure increases ruminal water uptake and causes haemoconcentration and disturbances in the electrolyte balance which are well evident in this study from the damage to the membrane systems of the neurons. This clearly indicated that in the rice fed animals a state of thiamine deficiency might have occurred and this would have led to nervous lesions. The intensity of the nervous lesions was more in the amprolium and rice gruel fed animals and two of the animals developed nervous symptoms. This would have been due to the combined effect of the substances used.

Elam (1976) reported that acidosis in cattle was caused by excessive ingestion of feeds which were rich in readily available carbohydrates. Rice a carbohydrate rich diet at times of surplus availability at home forms the main component of concentrate ration for goats in Kerala. It is true that some times widely different responses are obtained with animals fed the same diet and given the same treatment. The ruminant animals response to consumption of readily fermentable diets may vary from that of giving good productivity to death of the animal on the other extreme.

The most important factor towards a favourable response may be a control of animals feed intake until the animal is completely adapted to the situation. This might account for the absence of symptoms and lesions in the other animals in the groups. However, it is possible that even in an adapted animal over eating can cause digestive disturbances and this might have been the case with the one animal in the rice gruel given group which developed nervous symptoms.

In the group D dosed with BHC astrogliosis, vascular changes and spongiosis in the neuropil and white matter in different segments of the brain were predominant. The spongiosis was confirmed as demyelination and oedema based on the reaction to Luxol fast blue staining.

Electronmicroscopic examination revealed neuronal swelling, mitochondrial pleomorphism and membrane system damage in the cerebral cortex. Besides this splitting of myelin at multiple sites, rupture of surface membranes of axons and axonolysis were observed. No literature is available on the histopathology and ultrastructure of BHC induced CNS changes in goats or other species. The available literature did not reveal any neuropathological lesions in BHC toxicity. However, Ward *et al.* (1973)

reported focal anoxic-ischemic cerebrocortical lesions in dogs poisoned with hexachlorophene, a related chemical. The animals in this group showed repeated episodes of convulsions. The symptoms even started on the 17th day in some, but was not progressive. Fennie and Hooper (1984) observed that repeated convulsive activity caused exhaustion of the metabolic activity of neurons and associated structures and this resulted in degeneration and necrosis.

In the sodium sulphate treated groups (Group E) histopathological examination revealed severe vascular damage including multiple haemorrahges. Neuronal changes were extensive and most of them had perineuronal oedema. Microglial nodules in the cortex along with subependymal accumulation of lymphocytes, monocytes and Kolmer cells were characteristic. White matter vacuolation was confirmed as oedema and demyelination by luxol fast blue staining which again were comparable with electronmicroscopic changes of myelin splitting and fragmentation. Subdural haemorrhage in the spinal cord was a feature. The lesions observed were comparable to the lesions produced in sodium sulphate induced PEM (Olkowski *et al.* 1992) and in lead encephalopathy in goats (Christian and Tryphonas, 1971). Cerebrocortical and subcortical necrosis with microvascular

fibrinoid necrosis predominantly in the thalamic region were reported to be the characteristic lesions in sulphate induced PEM in goats (Olkowski *et al.* 1992). Though vascular changes were present in different segments, fibrinoid necrosis was not observed in the experimental cases in this investigation.

The ultrastructural changes observed in this group clearly demonstrated well defined organellar changes supporting the toxic degenerative neurological changes. The changes gave clue to the histological changes and clarified the nature of pathogenesis of the histological lesions. These histological and ultrastructural changes can certainly lead to sufficient functional changes manifesting clinical symptoms. The basic malacic nature of the lesion was clarified by the ultrastructural changes.

Nicholls (1975) pointed out that sulphates on bacterial action will be converted to sulphides in the rumen. Sulphides inhibit cellular respiration by blocking activity of cytochrome C oxidase in the electron transport chain leading to a histotoxic hypoxia. Sulphide also inhibits superoxide dismutase and glutathione peroxidase which are required for controlling oxidant injury (Khan *et al.* 1987). It appears that the onset of nervous signs

may be heralded by an abrupt burst of sulphide generation or accumulation. PEM with characteristic neuropathological changes has been reported in sheep fed diets high in sulphates (Hibbs and Thilsted, 1983; Sadler *et al.* 1983; Gooneratne *et al.* 1989; Gould *et al.* 1991 and Gould *et al.* 1997).

Since the rumen microbes are capable of efficiently reducing sulphate to sulphide, high sulphate given could be expected to contribute to production of increased rumen sulphide. It is also possible that sulphate can produce PEM by a mechanism similar to ruminal thiaminase. It has been shown by Raisbeck (1987) that the intermediate product produced when sulphate is reduced to sulphide is sulphite which can degrade thiamine *in vitro*. It may be possible that sulphite produced from sulphate by microbes destroy ruminal thiamine causing PEM.

The interaction of several dietary factors determines the sulphide toxicity. Dietary content of copper, zinc, iron and molybdenum have important modifying effect on sulphur toxicosis (Gooneratne *et al.* 1989). Molybdenum and copper can combine with sulphur to form insoluble copper thiomolybdate. Copper, zinc and iron form insoluble salts with sulphide and their expected effect would be to

decrease the bioavailability of sulphide formed in the rumen. Conversely low or deficient dietary content of these metals could be prerequisite for excess absorption of sulphide to occur. So it becomes essential to simultaneously evaluate the copper, zinc, molybdenum and iron content of animals dosed with sulphate as out of six animals, only three developed symptoms and lesions. The protective effect of these metals in those animals can not be ruled out.

Loosening of white matter, fragmentation of myelin, intramyelinic vacuoles, swelling of axons and moderate neuronal swellings were the electronmicroscopic lesions observed in the group F fed *Ficus tsiela*. The neuropathological changes were seen only in one animal which clinically showed dullness. This is a significant observation and points out the capability of goats to survive unconventional fodders. This becomes more relevent particularly in the context of the report that calves are very susceptible to Ficus leaf poisoning (Divakaran Nair *et al*. 1995). The variations in the pathological changes between affected animals in the same group can be explained only as individual idiosyncracy. It would appear that goats are very much tolerant to Ficus leaf toxicity. When compared to other species of animals goats in general can

detoxify many of the compounds and the browsing habit is possibly a manifestation of this potential. They sustain on a variety of plants which other species of animals can not make use of. The changes observed in one goat in this investigation was similar to those reported in Ficus leaf poisoning in calves by Divakaran Nair *et al.* (1995). They could induce neuropathological lesions in all the experimental calves fed Ficus leaf. From this investigation variation in the susceptibility of different species to Ficus leaf toxicity has been brought to light.

The histological changes like swelling of endothelial cells, perivascular oedema, degeneration and necrosis of neurons, cavitation of neuropil, an overall reduction in the neuronal population, microglial and oligodendroglial accumulations in the form of nodules were seen in the natural cases (Group H). The histopathological lesions of similar types were described by various workers in animals affected with PEM and lead encephalopathy (Fakhruddin *et al.* 1987; Sager *et al.*, 1990; Christian and Tryphonas, 1971). The white matter showed extensive haemorrhage, endothelial damage, perivascular cuffing with lymphocytes, gitter cells and plasma cells. These lesions are suggestive of an immunological reaction. In this context the observation of Wekerie *et al.* (1986) that myelin

breakdown products acting as antigen might induce an immunological response is relevant. In the natural case one of the animals had the history of consuming a plant which the owner could not identify. There was severe malacia of the white matter and this would support a toxic neuropathic change. Infiltrations were also seen in the pons, medulla oblongata and spinal cord and these generalised lesions are indicative of an infections etiology. However, there is no further proof either clinical, epidemiological or aetiological involvement of any infectious agent. The absence of any biological agents in ultrastructural observation suggest absence of an infectious etiology and points out only to a degeneration neuropathy of a toxic nature. This type of reaction can no doubt also be considered as a secondary inflammatory response following extensive degenerative neuropathological lesions. Electronmicroscopically structural changes were more pronounced in the neuropil and in the cell body of the neurons. Spongy changes and neuronal necrosis were prominent in the cortex and within the CNS. Normally the cells and their processes which form the neuropil are closely packed and the surface membranes of the neighbouring elements are separated by a constant distance. According to Summers *et al.* (1995) this results in a

network of continuous intercellular gaps and the extracellular space is limited to these gaps. Following necrosis of the neuropil as happened in the natural as well as in experimental cases, a dissociation of these surface membranes occurred. This loosening and separation of the surface membranes is a major factor and serves to explain the softening or spongy changes of the neuropil of the cerebral cortex.

Ultrastructurally vesiculation of the cytoplasm and moth eaten appearance were observed in the neurons which indicated a complete dissolution of the ctyoplasmic organelles. Pleomorphic mitochondria, lysis of nuclear membrane, partial degranulation of ribosomes and swelling of astrocytes with expanded cytoplasm were seen. These changes in the organelles point out to a toxicological insult to the neurons and explains the basis of histological changes observed under the light microscope. The white matter had a loose texture and there was balooning of myelin, intramyelinic vauoles and separation of myelin.

In this investigation, ultrastructurally the spongioses observed were found to be due to swelling of the protoplasmic astrocytes in deep cortical grey matter and an

intramyelinic vacuoles and polycavitation in subcortical white matter. In some the astrocytes were shrunken and others hypertrophied. The various astrocytic changes also probably represent a continuum of degenerative changes which evoked as a result of direct injury.

Spongy degeneration of the neuropil of the CNS was reported to result from an astrocytic biochemical abnormality of unknown cause especially in children with Canavan Van Bogaert sponginess (Adachi *et al.* 1973). According to them this abnormality results in excessive fluid accumulation in astrocytes and myelin lamellae and secondary separation of lamellae and loss of myelin.

In the natural as well as experimental cases myelin changes were predominant and there was extensive white matter vacuolation. There was separation, lysis, segmental loss and vacuolation of myelin. The vacuolation of myelin resulted from the separation of lamellae along the intraperiod line thereby reopening the extracellular space originally obliterated by the spiralling process of the myelinated cell. Protein zero (PO) is a major structural protein responsible for the compaction of myelin lamellae acting as structural cement or adhesive. Since PO is located at the intraperiod line, any alteration in its

composition or expression may result in splitting in this location (U Urso *et al.* 1990 and Filbin *et al.* 1990). Splitting and lysis observed in the experimental and natural cases indicated some defects in this protein. According to Dorman *et al.* (1992) white matter vacuolization occurs through the uncoupling of mitochondrial oxidative phosphorylation. This lead to diminished sodium and potassium adenosine 5'-triphosphatase dependent ion channel pump activity resulting in intracellular fluid accumulation.

In general the nature and distribution of the brain lesions in the experimental as well as natural cases indicated a primary metabolic insult leading to the organellar changes. Therefore, this appears to be the basis of the neuropathological changes. The structural changes of the cell injury become visible only after some critical biochemical system within the cell has been deranged. This inturn induces structural changes at the ultrastructural level. The most important structural changes were swelling of neurons and glial cells, damage to the membrane systems and extensive white matter vacuolation. These changes observed indicated the interaction of the agents with the physico-chemical homeokinesis of the intra and extra cellular electrolytes

which in turn is dependent on the morpho-chemical integrity of the membranes of the cellular and intracellular structures. This definitely showed that there was a total inhibition of the ATPase dependent sodium ion channel pump activity as the basic primary alteration. Moreover the defect in the protein synthesis as indicated ultrastructurally by the presence of perichromatin granules and segregation of granular and filamentous components of nucleolus resulted in a reduction in the intracellular osmotic colloidal pressure allowing the entry of fluid. The cells so damaged liberate lot of potassium which inturn injures neurons by inhibiting anaerobic glycolysis and deprives the cells of their last source of energy ultimately ending up in a continuous state of hypoxia. The rate of consumption of ATP is high in the brain because of the high surface/volume ratio of the cerebral neurons in which membrane bound ATPase utilizes ATP with maximum rapidity. Depletion of ATP is followed by irreparable disorganisation of cell membranes and consequent biochemical lesions.

In this investigation the neuropathological changes in the brain in spontaneous cases of PEM was delineated and the basic mechanism involved was elucidated by ultrastructural changes. The changes were indicative of

toxic degenerative neuropathy. In experimental studies similar pathological changes were observed in sodium sulphate, BHC and amprolium + rice gruel toxicity. However, in each group there was much variation in the manifestation of symptoms and lesions. Although sodiumsulphate, BHC, rice gruel and amprolium could induce neuropathological lesions simulating spontaneous cases of PEM, the changes were not consistent and mathematically reproducible. Hence, it could be said that these agents can be initiating agents under certain circumstances. It was not possible to specifically pin point one single factor as the aetiology. It would appear that PEM has a multifactorial aetiology and no single cause seems to be responsible for PEM.

Summary

SUMMARY

This investigation was undertaken to assess the prevalence and to delineate the pathological features of PEM in goats and to evaluate the role of certain selected incriminating agents in the causation of PEM.

The data collected from 76 Veterinary hospitals for a period of four years from 1991 to 1994 revealed an increasing trend for the occurrence of the disease. The district-wise distribution revealed a highest rate of prevalence at Malappuram which directly correlated with the goat population. The disease was found to occur throughout the year. A significantly high incidence was observed in the first five months and the peak incidence was observed in the month of April. Comparatively low incidence was observed in the months of June, July, November and December.

Experimental studies were conducted taking goat as the model utilizing Amprolium, Amprolium and rice gruel, rice gruel alone, BHC, sodium sulphate and *Ficus tsiela* Roxb.

Goats suspected to be suffering from PEM based on the symptoms were procured and the symptoms and lesions were studied.

The animals in groups A (Aprolium), C (Rice gruel) and F (*Ficus tsiela*) did not manifest any nervous symptoms throughout the experimental period. However, two animals in group B, four in group D and three in group E showed clinical symptoms.

The symptoms appeared at different periods and this consisted of lethargy, depression, anorexia, knuckling at the fetlock, lameness, tremors, opisthotonos and loss of eye preservation reflex. These symptoms were predominant in group E (sodium sulphate). Intermittent remission of symptoms were observed in group D (BHC) and the symptoms were not progressive in group B. Only sodium sulphate treated animals (group E) had more or less similar symptoms as in spontaneous cases. Opisthotonos and blindness were observed in spontaneous cases besides the other symptoms described.

The body weight of the animals in group A, B, D, E and F remained low as compared to the control. In group C, all the animals showed a weight gain on the 30th and 45th day.

Brain autofluorescence was not consistently observed in all the affected animals. Two animals in group E and three animals with spontaneous PEM revealed diffuse blue

green to creamy fluorescence in the crown of the gyri and sulcal depressions indicating necrosis. This test was demonstrated to have no primary value in the diagnosis of PEM.

The CSF protein concentration in the experimental goats with or without nervous disorders was always within the expected range and was not significantly different as compared to the control. There was an increase in the CSF protein concentration in all the three spontaneous cases which indicated a disturbance in the blood brain barrier.

The brain:body weight ratio did not vary significantly and proved not useful for evaluating the gross brain lesions.

The histopathological and ultrastructural lesions observed in the experimental animals and in spontaneous cases were comparable in different segments of the brain. However, they varied in their intensity. The lesions were much pronounced and extensive in the natural cases.

In the group A, though the animals remained clinically normal, occasional neuronal necrosis with perineuronal and perivascular oedema were evident in certain segments of the brain and ultrastructurally there was neuronal swelling and disaggregation of polyribosomes. It was not possible to

induce PEM with seven times the treatment dose of amprolium and it was clarified that the amprolium treatment is not a cause for PEM. Diffuse degeneration of the cerebro-cortical laminae, reactive astrocytes and neuropil vacuolation associated with extensive cavitation of the white matter were the histopathological lesions observed in group B. Electronmicroscopic observations revealed swollen neurons with disruption of membrane systems, fragmentation of RER and mitochondrial changes. Myelin splitting, segmental loss and axonolysis were observed which accounted for the occasional symptoms.

The animals remained clinically normal in group C. But one developed depression and distension of the abdomen and the brain revealed occasional neuronal degeneration which were ultrastructurally characterized by chromatin condensation, partial degranulation of ribosomes with RER and mitochondrial changes. The observations indicated that the rice gruel consumption everyday resulted in a state of tolerance. It is, therefore, pertinent to point out that rice gruel everyday as a component of concentrate feed in goats might not cause any detrimental effects.

In the group D dosed with BHC, astrogliosis, various vascular changes, neuropil vacuolation, demyelination and

intramyelinic oedema were observed along with malacic changes in the cerebellum, mid-brain, medulla oblongata and pons. Nuclear swelling, chromatin condensation, mitochondrial, pleomorphism, SER proliferation, loosening and fragmentation of the surface membranes of the neuropil, glial cell changes and multiple myelin splitting at the intraperiod lines were the electronmicroscopic changes observed.

In the sodium sulphate treated group (Group E), histopathological examination revealed vascular changes, perineuronal oedema, vacuolation of astrocytes and microglial nodules alongwith lymphocytes in the cerebral cortex. Lymphocytic, monocytic and Kolmer cell accumulation were seen in the subependymal areas of the lateral ventricle. White matter revealed inter and intra fascicular vacuolation. Swelling of neurons, cytocavitation with organellar loss, nuclear membrane lysis, pleomorphism of mitochondria, fragmentation of RER, accumulation of perichromatin granules, axonal swelling, multiple splitting and segmental loss of myelin were evident ultrastructurally. It was demonstrated that sodium sulphate at the dose rate of 500 mg/kg body weight could induce PEM in goats and individual idiosyncracy plays an important role in the manifestation of the disease.

In the group F, the neuropathologocal changes were seen only in one animal which clinically showed dullness. Vascular damage and occasional neuronal degeneration were seen histologically and ultrastructurally loosening of white matter, fragmentation of myelin and intramyelinic vacuoles were observed. By this investigation it was clarified that there is great variation in the susceptibility of different species to *Ficus tsiela* toxicity. Goats were shown to be highly resistant to *Ficus tsiela* toxicity.

Degeneration and necrosis of the neurons, perivascular oedema and various vascular damages, neuropil spongiosis, microglial and oligodendroglial response in the form of nodules, white matter haemorrhage, perivascular cuffing with lymphocytes, gitter cells and plasma cells with vacuolation of white matter were seen in spontaneous cases.

Electromicroscopic examination revealed pronounced changes in the neuropil and in the cell body of neurons. Spongy changes were predominant in the neuropil. Nuclear membrane lysis, segregation of granular and filamentous component of nucleolus, nuclear membrane lysis, fragmentation of RER, mitochondrial pleomorphism, astrocyte swelling and clumping of organelles, endothelial swelling

and bleb formation, myelin ballooning, separation of myelin and myelin splitting were the ultrastructural lesions. The basis of the symptomatology was clearly identified and related to these histological and ultrastructural changes.

The histopathology and ultrastructural changes observed both in the experimental and spontaneous cases pointed to a primary biochemical insult which resulted in the derangement of the volume control mechanisms of the neurons especially the ATPase dependent sodium pump mechanism.

It was concluded that the vascular damage seen in the experimental as well as spontaneous cases added to the cellular destruction by creating a state of ischaemic hypoxia leading to mitochondrial changes.

By this investigation it was clarified that toxic degenerative neuropathy was the basic change. The neuropathological changes in the brain in spontaneous cases of PEM and those in sodium sulphate, amprolium and rice gruel and BHC toxicity were similar. However, in each group there was variations in the manifestation of symptoms and lesions and the changes were not consistent. It appeared that PEM is of multifactorial etiology and no single cause seems to be responsible for PEM.

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* Originals not seen

PREVALENCE AND PATHOLOGY OF POLIOENCEPHALOMALACIA IN GOATS

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ABSTRACT OF A THESIS

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ABSTRACT

Polioencephalomalacia (PEM) is a significant emerging diseases problem in goats. Although, PEM in goats was recognized and reported as early as 1956, its etiology is poorly understood and symptomatology, pathological features and therapeutic approach have not been well defined and documented.

Hence an investigation was undertaken to assess the prevalence of the disease based on the data available from 76 Veterinary hospitals of the state for a period from 1991 to 1994. This data documented revealed an increasing trend in the occurrence of the disease and significantly high incidence was recorded in the first five months of which the peak incidence was observed in the month of April.

Spontaneous cases of the disease were studied in detail and experiments were conducted taking goat as a model using selected incriminating agents such as Amprolium (350 mg/kg body weight), Amprolium and rice gruel (350 mg/kg, and *ad libitum* rice gruel), rice gruel (*ad libitum*), sodium sulphate (150 mg/kg body weight followed by 500 mg/kg body weight on the 10th day) BHC (2.5 mg/kg followed by 5 mg/kg body weight on the 10th day) and *Ficus tsiela* Roxb. The experiment was for a period of 45 days.

Symptomatology, weight of the animals at fortnightly intervals, weight of the brain, CSF protein concentration, brain autofluorescence, gross and histopathological alterations of the brain and ultrastructural pathology were the markers utilized for evaluating the disease processes.

The sodium sulphate, BHC and Amprolium and rice gruel treated group showed symptoms and lesions more or less similar to the spontaneous cases. Only few animals in each group developed the disease such as two in amprolium and rice gruel treated group, four in BHC treated group and three in sodium sulphate group. This showed that individual idiosyncracy plays an important role in the manifestation of the disease.

The symptoms developed at different latent periods were not progressive as compared to the spontaneous cases where the symptoms were progressive. The symptoms included lethargy, depression, knuckling at the fetlock, frequent tremors, opisthotonos and loss of eye preservation reflex. Blindness was seen in one of the natural cases.

The histological lesions of the brain in all the cases were comparable in different segments of the brain. Mostly it was characterized by diffuse laminar cortical degeneration and necrosis, occasional neuronal swelling,

glial cell reaction and white matter vacuolation. Vascular changes predominated in the sodium sulphate group and also in the natural cases. There was glial cell response in the form of nodules in sodium sulphate group and natural cases. A predominant perivascular and neuropil accumulation of lymphocytes, gitter cells and monocytes were seen in the natural cases. These were considered as secondary deposition following a toxic degenerative neuropathy. The necrotic focicould well be delineated in few of these cases by the bluish or creamy autofluroscence of the affected brain, but was not found to be of any primary diagnostic value as all the affected brain did not show fluorescence.

Ultrastructural investigation revealed the basic reaction of the brain tissue to be similar in both the experimental and natural cases except for their intensity. Ultrastructural lesions were characterized by neuronal swelling, membrane lysis, segregation of the filamentous and granular component of nucleolus, cytoplasmic organellar damage such as fragmentation of RER, partial degranulation of ribosomes, mitochondrial swelling, cristolysis and complete disappearance of organelle. Neuropil spongiosis and splitting of myelin at the intraperiod line and formation of multiple vacuolations of the white matter were characteristic. From this observations it was clearly

delineated that the primary insult was a biochemical one which caused much damage to the volume control mechanism of the cell and subsequent cellular damage.

The CSF protein evaluation revealed high protein level in the spontaneous cases whereas in the experimental cases, the concentration remained within the normal range indicating that it has no diagnostic value.

In this investigation it has not been possible to induce PEM with Amprolium even at a dose rate of 350 mg/kg body weight and it was proved that amprolium is not a cause for PEM. Rice gruel *ad libitum* was found to be tolerated by the animal except one which showed dullness and abdominal distension towards the end of the experiment. Diffuse neuronal degeneration was observed in the brain of this animal. Based on this observation it was concluded that rice gruel consumption every day as a component of the concentrate feed in goats might not cause any detrimental effects and the problem comes only when it is fed in large quantities on a single day.

Ficus tsiela Roxb. though produced vascular damage and diffuse neuronal degeneration in one of the experimental animals, goats were found to be highly resistant to *Ficus tsiela* Roxb. toxicity and the variation in the

susceptibility of different species to this toxicity was brought to light.

From this investigation it was also clarified that sodium sulphate, BHC, rice and amprolium could be initiating agents of PEM under certain circumstances and no single cause seems to be responsible for PEM.