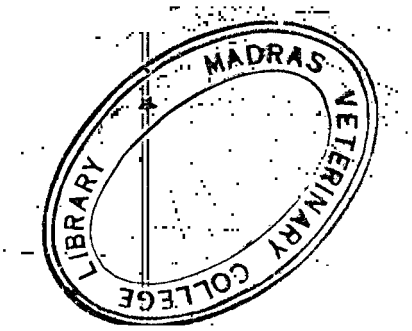

APPLICATION OF ELECTROENCEPHALOGRAPHY IN CANINE VIRAL ENCEPHALITIS

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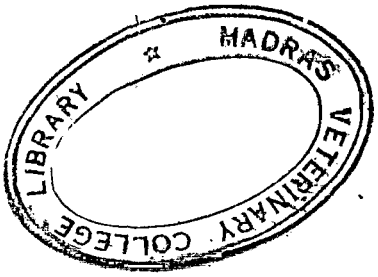

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1993

CERTIFICATE

This is to certify that the thesis entitled, "APPLICATION OF ELECTROENCEPHALOGRAPHY IN CANINE VIRAL ENCEPHALITIS", submitted in part fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY (VETERINARY)** in **CLINICAL MEDICINE AND THERAPEUTICS** to the Tamil Nadu Veterinary and Animal Sciences University, Madras is a bonafide research work carried out by **P.C.ALEX**, under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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ABSTRACT

ALEX, P.C. 1993. Application of electroencephalography in Canine viral Encephalitis. Ph.D. (P.DHANAPALAN).

A study was designed to ascertain the usefulness of Electroencephalography in the diagnosis and prognosis of canine viral encephalitis. The efficacy of an antiviral drug, Ribavirin and Vitamin C in the treatment of Distemper encephalitis were also assessed. Ten apparently healthy dogs, six experimental dogs and two hundred and twenty seven clinical cases were taken up for the study. Provisionally diagnosed cases of distemper encephalitis were subjected to detailed investigations such as ophthalmologic examinations, cerebrospinal fluid analysis, agar gel immunodiffusion test, counter immunoelectrophoresis and electroencephalography. Incidence of distemper encephalitis was worked out to be 0.87 per cent in the local canine population in the present study. Maximum occurrence was in the age group of 1-5 years. The incidence was maximum during the winter months and least during summer months.

The major clinical signs observed were fits, myoclonus and deficits in postural reactions. Ophthalmoscopic examination revealed changes in the fundus. Agar gel immuno diffusion test and counter immuno electrophoresis could be used only with lot of scepticism because of false negative results. Cerebrospinal fluid analysis showed significant increase in cell count, total protein and creatine phosphokinase and was sufficient for the diagnosis of CDE in majority of the cases.

Electroencephalographic pattern changes were observed in distemper encephalitis and could be used in the diagnosis and differential diagnosis of distemper encephalitis in conjunction with other data. Slow waves, low voltage runs, spike and wave, asymmetry, slowing and spikes were observed. The serial EEG recordings of the experimentally infected dog revealed characteristic pattern changes such as asymmetry and spikes.

Histopathological examination of brain revealed neuronal necrosis, gliosis and inclusion bodies.

Oral administration of Ribavirin and intravenous administration of vitamin C had no significant effect on the outcome of distemper encephalitis cases.

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CHAPTER I

INTRODUCTION

Electroencephalography (EEG) is the study of small, constantly changing electrical potentials from the brain which can be recorded from the scalp. Animal electroencephalography began in 1875 when Richard Caton reported that minute electrical currents of varying polarity could be detected from the brains of cats, monkeys and rabbits (Redding and Knecht, 1984).

Although EEG has been used as a research procedure in animals for several decades its use in clinical cases has only been practiced since 1963 (Redding, 1964a). It is a field which has come in for strong criticism. However, any limitations on the usefulness of the EEG which may exist today are not necessarily inherent limitations of the EEG, but may result from our own inability to assess its full meaning (Klemm, 1969).

In 30 years of clinical electroencephalography an increasing cast of veterinary clinical scientists have added greatly to the state of knowledge. Inspite of the indications for EEG and the burgeoning knowledge and experience, usage is presently limited. It is unfortunate that the EEG is still regarded with considerable skepticism by some members of the veterinary profession. Such doubts usually arise through unfamiliarity with the equipment and an unreasonable expectation of its function (Skerritt, 1984).

The EEG may contribute valuable diagnostic information but that is not a substitute for more conventional lines of investigation (Klemm, 1969; Redding, 1978). The EEG can frequently tell the clinician whether or not the brain is diseased, if the disease is focal in nature; if the disease is acute or chronic; if the disease process is inflammatory or degenerative; the extent of the damage and if the disease is progressing, stabilized or showing some improvement. This information, when combined with that obtained from careful case history and a complete physical and neurologic examination, is of great value in making a differential diagnosis and prognosis in diseases of the central nervous system (Redding and Knecht, 1975). The EEG is one of the most useful tools in the diagnosis of distemper encephalitis (CDE) (Redding ***et al.,*** 1966).

Canine distemper (CD) remains one of the most important clinical problems in canine medicine. Canine distemper virus (CDV) causes more morbidity and mortality than any other virus that infects dogs. Distemper virus is classified in the paramyxoviridae family and is closely related antigenically and biophysically to measles and rinderpest virus. It is a pantropic virus that causes a multisystemic disease in dogs often with severe neurologic signs. The virulence of the viral strain, the age and the immunocompetence of the dog determine the course and outcome of the disease. The prognosis of nervous distemper is generally poor although dogs can recover from this disease (Tipold ***et al.,*** 1992).

There are no antiviral drugs or chemotherapeutic agents of practical value for specific treatment of distemper in dogs. Hence treatment is largely supportive and symptomatic (Swango, 1989).

Ribavirin is a broadspectrum antiviral agent with **in vitro** activity against a number of viruses capable of causing encephalitis. It has been shown to cross blood-brain barrier in both mice and humans (Crumpacker ***et al.,*** 1986; Ogle ***et al.,*** 1989). Treatment of measles with ribavirin reduces both severity and duration of the clinical manifestations of the disease as well as the complications usually associated with this viral disease (Fernandez ***et al.,*** 1986).

The authors concluded their review on ribavirin with the following statement.

"We believe it is appropriate to conclude that ribavirin has made a significant impact on the medical and scientific communities. From AIDS to Zoster, the drug has been the subject of over 500 publications and has been studied in more than 50 clinical trials. Ribavirin's potential to reduce human suffering and death is close to being realized".

With these background informations in mind, this study on viral encephalitis (distemper encephalitis) was undertaken with the following objectives:

1. To study the incidence of distemper encephalitis among the dog in and around Madras City.
2. To study the clinico-pathological changes in blood and cerebrospinal fluid (CSF) in distemper encephalitis.
3. To standardize the EEG technique in dogs and to evaluate the utility of EEG in the diagnosis, prognosis and evaluation of therapy.
4. To study the effect of 'Ribavirin' in distemper encephalitis.

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

2.1 ELECTROENCEPHALOGRAPHY

The Electroencephalogram (EEG) is a graphic representation of the electric activity of the cerebral cortex and of the influence that subcortical structures have upon the cerebral cortex. It is a record of more or less rhythmic fluctuations in the electric potentials that occur in the brain (Redding and Knecht, 1975). The EEG is a graph of voltage against time and is shown as series of biphasic waves, possessing both positive and negative polarities (Skerritt, 1984).

2.1.1 History of EEG

Animal electroencephalography began in 1875 when minute electrical currents of varying polarity were detected from the brains of cats, monkeys and rabbit by a British physiologist, Richard Caton (Redding and Knecht, 1984). After 1914, numerous reports of both animal and human EEG have been published. Some significant scientific articles were by Croft (1962), Redding (1964a) and Klemm (1969).

The history of Veterinary electroencephalography, in the strictly applied clinical sense is short. In 1963, a book was published on the development of cerebral function of the dog. In the same year, a British practitioner specialising in neurology

began a series of clinical studies mostly in dogs. Later a French investigator studied the normal EEG of almost all domestic animals (Klemm and Hall, 1974).

2.1.2 Genesis of EEG

Dempsey ***et al.*** (1941) identified a non-specific thalamic nucleus and suggested it as the probable pace maker region for the wave generation.

Microelectrode studies by Brock ***et al.*** (1952) demonstrated that extra-cellular membrane potential fluctuations diminished greatly within a short distance from the neuron of origin.

On the other hand, EEG activity was recorded in decorticated, animals especially over temporal and occipital regions by Cobb and Sears (1956).

These principles concerning surface attenuation of deep seated voltages led to the conclusion that the upper most granular layer of cortex probably made the greatest contribution to the surface recorded EEG. Activity from deeper structures was too attenuated at the surface to be recorded by the EEG. Deeper sited pathological processes might alter the regulatory influence of the subcortical structures which would result in changes recorded at the scalp (Klemm, 1969).

The view of Redding (1978) that the EEG resulted from the sum of changing potential differences from all the sources such as cerebral cortical cells, their synapses and processes seemed, realistic. Further more, it was suggested that the rhythmicity

of this activity was conferred by the subcortical nuclei and the medullary reticular formation (Skerritt, 1984).

2.1.3 The electroencephalograph

The electroencephalograph (EEG machine) is a mechanical device specifically designed to record electrical potentials from the scalp (Redding and Knecht, 1984). The machine consists of an input board, selector switches, differential variable amplifiers, filters and a time-based pen writing system. The operational controls include the one for paper speed, sensitivity and frequency filter.

2.1.4 Electrodes

Electrodes generally transduce the ionic flow in tissue to electron flow in wiring that supplies the input of amplifiers. Therefore the interface between the subject and electrode should introduce the minimum possible resistance into the input circuit. A good electrode should have qualities like easy attachment, secure to the skin over the cranium and low resistance. The electrodes were mainly divided into two categories based on their site of application, viz. surface electrodes and intracerebral electrodes (Klemm, 1969).

Redding (1964b) described the use of clip electrodes attached to the scalp that would grasp a portion of the skin firmly. He compared it with electrodes surgically implanted in the skull resting on dural surface. The only difference noted in EEG were voltage magnitude reduction and damping of high frequency component seen in electrocorticogram (ECoG).

Klemm (1969) described the method of application of electrodes on scalp. He suggested to place a chlorided silver disc electrode on electrode paste that has been applied to scalp after clipping of excess hair and thorough cleaning of skin with alcohol. He found disc electrode satisfactory if animal movements were controlled.

The scalp of man is markedly different from that of canines. The human scalp is relatively adherent to the skull in contrast to the dog and cat where the scalp is loose and freely movable. Therefore, needle electrodes on small heads and clip electrodes on large heads are suitable for dogs and cats. However comparative recordings made on animals with copper clip, needle and silver disc electrodes yielded equal quality tracings (Redding and Knecht, 1984).

Redding and Knecht (1984) have listed the different types of EEG electrodes namely (1) metal mini cup (silver) with a central hole, (2) metal disc (silver), (3) metal cup (gold) with central hole (4) subdermal needle (platinum or silver), (5) large metal clip electrode and (6) small metal (copper) clip electrode.

2.1.5 Placement of electrodes

Klemm (1968 b) proposed an eight electrode system and number scheme for clinical electroencephalography in dogs. He proposed two frontal (F_1, F_2), two temporal (T_1 and T_2) three central (C_1, C_z, C_2) and two occipital (O_1, O_2) electrodes placement on head.

Klemm (1969) recommended that for reproducibility, comparison or detection of focal abnormalities, scalp electrodes need to be placed in standardised positions. Electrode positions should be measured from skull land marks and there should be adequate coverage of all parts of head including homologous regions on the two sides of the head.

Redding (1978) described five electrodes system consisting of two electrodes on the frontoparietal areas (LF, RF), two over the occipital areas (LO, RO) and one over the vertex (V). A ground electrode was attached to the skin over the external occipital protuberance.

2.1.6 Techniques of recording

Combination of a number of derivations from electrodes is known as montages. There are basically three different types of montages viz. bipolar, unipolar or monopolar and average reference. Each montage has its own merits and each individual electroencephalographer has his own frame of reference for detecting abnormality (Kiloh ***et al.,*** 1972).

Monopolar recording refers to registration of the potential difference between one 'active' electrode on the head and another 'inactive' electrode placed over an area that is relatively less active electrically. However, inactive reference electrode also picks up certain amount of electrical activity from the nearest part of the brain and may be contaminated by a variety of non-cerebral potentials. There was no perfectly suitable reference point for the inactive electrode. Another disadvantage of reference

recording was that an artifact potential at the reference electrode would cause the artifact to appear in all recording channels (Klemm, 1969).

In 'average reference electrode' technique, the basic concept is to connect all the electrodes together through equal parallel resistances of 0.5-2 megohms to serve as a reference electrode. This is a modification of reference technique which is aimed at reducing the effect of activity at the reference electrode (Klemm, 1969).

In bipolar recording, each channel is connected between a pair of 'active' electrodes on the scalp and the potential difference between them is recorded. Here the differential amplifier discriminates against inphase activity at its two grids and amplify only differential voltage. Therefore, a given wave will have the largest amplitude in the bipolar lead nearest to the region where that wave originated. However, it may give an underestimate of amplitude because of cancellation of the common component at the electrode from which the signal is derived. If a localized discharge occurs at or near one of the electrodes common to two channels, the corresponding pens will deflect in opposite directions, giving rise to phase reversal (Klemm, 1969 and Kiloh ***et al.,*** 1972).

When small time differences existed, between the occurrence of a widespread discharge of a number of electrodes, unipolar recording would demonstrate these differences much more clearly (Kiloh ***et al.,*** 1972).

Currently, there are considerable variations in the techniques utilised to obtain electroencephalograms. Different techniques for recording the EEG in dogs have been described by Croft (1962), Redding (1964), Klemm (1965) and Fox (1967).

Most of the neurologists prefer a five electrode montage for recording the EEG (Redding and Knecht, 1984).

2.1.7 Restraint of Patient

Proper restraint of the patient is necessary to obtain a good EEG picture without artifacts. Different methods of restraints are being practised which come under two main categories - Chemical restraint and physical restraint.

2.1.7.1 Chemical restraint

Different anaesthetics have been studied for their beneficial and adverse effects on the EEG by many scientists.

Fox (1964) studied the effect of pentobarbitol on the development of electrocortical activity of maturing dogs and found that barbiturate anaesthesia had a paradoxical effect on the EEG activity of the brain in relation to the age of the subject.

Based on the comparative studies of EEG of anaesthetized and unanaesthetized dogs, Klemm and Mallo (1966) opined that anaesthesia provided considerable advantages in restraint and in reduction of artifacts in addition to the fact that normal wave forms were not enhanced in unanesthetized state.

Klemm and Hall (1970) reported electroencephalographic seizures in anaesthetized dogs with neurologic disorders and found that a wide variety of neurological disorders could be diagnosed clinically.

On the other hand, it has been found that chlorpromazine when administrated, though reduced the muscle activity, influenced the basic activity of EEG (Kalab ***et al.,*** 1977).

Knecht ***et al.*** (1980) reported that succinyl choline caused a low amplitude dominant activity with increased moderate amplitude low frequency waves in dogs.

The sedative with least effect on the EEG was ethchlorvynol (Redding and Knecht, 1984).

Predominant EEG patterns under xylazine sedation were quite similar to that of natural light sleep stage in dogs (Tourai ***et al.,*** 1985). Katherman ***et al.*** (1985) studied the effects of fentanyl citrate and droperidol on EEG findings and reported that the only changes observed were a slight increase in amplitude of the dominant activity and an increase in spindle like activity.

Acetyl promazine had little effect on the EEG recording whereas xylazine exerted a substantial effect (Mysinger ***et al.,*** 1985).

2.1.7.2 Physical restraint

The physical restraint method consists of muzzling, taping the forelimbs and hind limbs together, placing the animal on a padded table equipped with seat belt type straps to hold the animal securely and covering the eyes (Redding and Knecht, 1984).

The same authors reported that the recordings from physically restrained animals were most useful for clinical diagnosis. The major disadvantage of physical restraint was the likelihood of movement and muscle potential artifacts. These could be minimized by carrying out the EEG examination in a comfortable environment. Any visible movements were carefully noted on the chart so that artifacts were easily identified (Skerritt, 1984).

2.1.8 Descriptive Terminology

The principle objective criteria by which a EEG record is assessed are based upon the frequency, amplitude and shape of waves of which it is composed and upon their spatial and temporal distributions. The list is based on the various published glossaries of the international federation of societies of electroencephalography and clinical neurophysiology as reported by Klemm (1969), Scott (1976) and Redding and Knecht (1984).

The distinguishing features of an EEG include wave form, or shape, repetition, frequency, amplitude, distribution, phase relations, timing, persistence and reactivity (Redding and Knecht, 1984).

2.1.8.1 Wave form or shape

It defines the configuration of a wave.

Wave : All changes in electrical potential between recording electrodes are called waves.

Activity	:	Any wave or sequence of waves is termed as activity
Monophasic wave	:	Waves with single deflection up or down from the base line.
Diphasic wave	:	Wave with two components, one on either side of the baseline. Synonym, 'biphasic'.
Regular Wave	:	Wave having a fairly uniform symmetrical appearance.
Irregular or random wave	:	Wave having uneven shapes and durations.
Sinusidal Wave	:	Some regular waves similar to sine waves are called sinusidal waves.
Transient wave	:	It is a single wave or complex sequence of two or more waves (spike, sharp wave, etc.) that stands out from the back ground activity.
Sharp transient	:	Wave having pointed peaks when recorded at 30mm/sec paper speed.
Spike	:	Wave which last 70-200 m.sec

Spike and wave complex	:	A spike followed by a slow wave is called a spike and wave complex. Two or more spikes in sequence are termed multiple spike complexes or polyspike complexes

2.1.8.2 Frequency

The number of times a wave occurs per second is the frequency and is expressed in Hertz (Hz)/Sec or Cycle (c)/Sec. The wave length is the reciprocal of the frequency. By tradition the frequency of EEG waves is often divided into following frequency bands.

Delta	:	Less than 4 Hz
Theta	:	From 4Hz to less than 8 Hz
Alpha	:	From 8 Hz to 13 Hz inclusive
Beta	:	More than 13 Hz

The boundaries of the above frequency bands are arbitrary and for a matter of convenience only. In animal EEGs these boundaries are frequently exceeded as the normal background activity is faster than man. Frequency more than 13 Hz/Sec is fast activity and less than 8Hz/sec is slow activity.

The activity which is more or less general and continuous in nature in contrast with paroxysmal and focal activities is known as background activity. The term is used for the dominant activity in a particular region.

2.1.8.3 Amplitude

Amplitude is voltage of EEG waves usually expressed in microvolts and determined by measuring the total vertical size (nadir to peak) of a wave and comparing it with the height of a standard calibration signal recorded at the same gain and filter settings. The calibration in Veterinary EEGs is set at 50 μv = 10 mm.

The amplitude is reported as low (under 20 μv), medium or moderate (20-50 μv) or high (over 50 μv) and used only to describe certain waves in relation to other waves.

Comparison of amplitude in corresponding parts or sides of the head is frequently referred to for symmetry (asymmetry) of amplitude.

2.1.8.4 Distribution

Distribution refers to the occurrence of electrical activity recorded by electrodes over various parts of the head, not in the time base of recording.

Activity that occurs at the same time over most parts or all of the head is widespread, diffuse or generalized. Activity involving only one side of head is known as lateralized activity. Focal activity is restricted to a given area of the head.

2.1.8.5 Phase

Polarity relations of different parts of a wave or waves as seen in single trace or of wave or waves as recorded simultaneously in different channels are known as phase.

Waves with troughs and peaks occurring in different channels at the same time are said to be "in phase". When peaks and troughs do not coincide, they are termed "out of phase".

Phase reversal is the occurrence of opposed peaks and troughs in different channels, created by the particular way that electrodes are connected in bipolar recording. It indicates the origin of EEG potentials in bipolar recordings.

2.1.8.6 Timing

Synchrony implies that the event is the same and simultany indicates the events appear to coincide within the limits of the EEG recording.

Waves that occur exactly at the same time on the two sides of the head are termed bilaterally synchronous and the waves need not be in phase in the same hemisphere. Those waves which occur in different channels without a constant time relationship are termed as asynchronous waves. Waves that occur in one area at one time and other areas at another time are independent.

2.1.8.7 Persistence

Persistence describes how often the wave or pattern occurs during the recording. They may occur irregularly or infrequently and be called sporadic, intermittent or with high, moderate or low persistence.

2.1.9 Factors influencing EEG

2.1.9.1 Age

The brain of a new born animal is neither structurally nor functionally fully developed.

Frequent isoelectric periods were observed during the first week of life, followed by progressive development of activity with age. A mature EEG pattern was observed at about five months, consisting of posterior 6-8/sec waves interrupted by 12-14/Sec rhythm and fast irregular activity. EEG responses to stimuli developed after first three to three and a half weeks of age. Sleep spindles began to appear at about two months of age (Pampiglione, 1963).

The stage of maturation of the cerebral cortex and other subcortical structures of the CNS influenced the EEG pattern (Redding, 1966).

Fox (1967) summarised the post-natal development of the EEG in dog from birth to thirty six days of age.

Redding (1978) reported an adult alert pattern of approximate 8-38 Hz with voltages from 3 to 15 μv.

Rhythmic waves which were occasionally seen at first post-natal day increased with age and appeared constantly at seventh day. Wakefulness and sleep were distinguished at two weeks of age. At fifth week of age all wave patterns or wave forms were observed and the amplitude of these waves increased upto 12 weeks of age and then gradually reduced. Maturation of EEG was observed between 20-30 weeks of age (Senba ***et al.***, 1984).

2.1.9.2 Level of consciousness

Awareness produced high frequency, low voltage waves and relaxed but not somnolent state produced high voltage low frequency pattern. Drowsiness was characterised by even lower frequency and higher voltage. The amplitude increase and the frequency decreases with the depth of sleep (Fox, 1967). The authors opined that deep sleep patterns were seldom recorded under clinical conditions.

Klemm (1969) described the EEG of all higher animals as low voltage fast activity (LVFA) during alertness and high voltage slow activity (HVSA) during drowsiness. He stated that in a given animal, EEG voltages and frequencies were very consistent under consistent environmental conditions. When a relaxed animal was presented with a biologically significant stimulus, the cortical EEG changed from HVSA to LVFA. Such response was known as "arousal response".

2.1.9.3 Light and sound

In a study, it was found that light or sound stimuli produced fast waves with a decreased occurrence of slow waves and paradoxical sleep (Sugawara and Kuriso, 1974).

2.1.9.4 Internal influences

2.1.9.4.1 Oxygen-carbon dioxide

Anoxia produced high voltage slow activity (Gellhorn and Heymans, 1948). High level of carbon dioxide initially caused HVSA which was later followed by isoelectric activity and deep anaesthesia (Klemm, 1964).

2.1.9.4.2 Temperature

EEG frequency increased as temperature rose from 20°C to 38°C and decreased above that. Amplitude also increased with increasing temperature upto about 34°C after which they decreased (Gaenshirt ***et al.***, 1954).

2.1.9.4.3 Cations

The two important ions affecting the EEG were potassium and calcium. Increased potassium levels usually increased EEG frequencies whereas calcium had the opposite effect (Klemm, 1969).

2.1.9.4.4 Glucose

Glucose is the major energy source for brain function and hence changes in glucose levels can cause profound changes in the neuronal function and EEG (Klemm, 1969). Sandhya (1992) recorded abnormal wave forms, the frequency and amplitude of which varied with the degree of hypoglycemia

2.1.9.4.5 Others

Klemm (1969) reported that various hormones and drugs exerted their influence on brain and hence the EEG. They include thyroid and adrenal cortex hormones, tranquilizers, antidepressants, atropine, hallucinogens and anaesthetics.

2.1.10 Artifacts

In electroencephalography, an artifact is any recorded electrical potential that does not originate in the brain (Redding and Knecht, 1984).

Klemm (1969) and Redding and Knecht (1984) have listed some of the common sources of artifacts as follows :

1. Source noise (from the animal)
2. *Instrument noise*
3. Electrostatic-Electromagnetic interference-Sixty Hz noise
4. Physiologic artifacts-Skin potentials, ECG signals, muscle potentials
5. Motion artifacts-Respiration, Eye movements, General movements. Ballisto-cardiographic motion.

2.1.11 Uses of EEG

Klemm and Hall (1972) analysed the EEG pattern of over 550 dogs with neurologic disorders and classified them according to various abnormalities. The electroencephalogram could be used for the differential diagnosis of the following conditions - encephalitis, hydrocephalus, trauma, space occupying masses, abnormal vascular structures, vascular disorders, metabolic diseases, toxaemias, bacterial infections, emotional disorders and seizure disorders (Oliver and Greene, 1983).

The EEG was also useful in determining an animal's ability to hear, the depth of anaesthesia (Tonuma, 1967; Prynn and Redding, 1968; Kadono and Nakagawa, 1974) and whether an animal was capable of receiving sensory stimulation, that is, whether there was transmission of information from the spinal cord to the brain especially, in spinal cord diseases (Parker ***et al.,*** 1974).

Klemm and Hall (1974) stated that the fundamental changes in EEG in any disease was in frequency and amplitude. Of the two kinds of changes, amplitude changes were the least reliable because, in normal animals, it could vary as much as two to two and half fold. Frequencies on the other hand, are consistent within normal dogs and cats.

The same authors summarized the clinical value of EEG as follows :

1. Brain disease was indicated by changes in the frequency and amplitude of brain waves.
2. LVFA and spikes indicated an ongoing irritative process due to variety of causes.

3. HVSA - if persistent - indicated death of many neurons due to variety of causes.
4. LVFA and HVSA were not pathognomonic of any given disease.
5. Local EEG abnormalities indicated a cortical rather than subcortical lesion.
6. Common causes of local cortical lesions were vascular (infarct, haemorrhage) and early tumour or focal necrosis.
7. Generalised EEG abnormalities could indicate that the lesion was either generalised in the cortex or focal subcortically.
8. Common causes of generalised lesions were :
   a. infection (distemper, bacterial encephalitis),
   b. trauma,
   c. Space occupying lesions (tumour, hydrocephalus) or
   d. idiopathic epilepsy.
9. Serial recordings during illness could indicate effectiveness of therapy and progress of the disease.

Unfortunately, a normal appearing EEG did not always mean normal brain function nor an abnormal tracing necessarily meant abnormal brain function (Redding and Knecht, 1984).

Fox (1967) stated that, as such, the EEG was only a diagnostic indication or technique employed in clinical evaluation and without supportive data from other parameters, had little value.

Skerritt (1984) suggested that EEG should not be regarded as the ultimate diagnostic tool for disorders of the brain but only as an ancillary diagnostic aid.

2.1.12 Normal EEG

The normal EEG differed among species and between breeds within species. Part of the difference was due to the variation in head size and configuration. The interposition of a large cranium and thick temporal muscles resulted in a lower amplitude wave in large breed dogs. The EEG in dogs with smaller heads and minimal temporal muscle mass had higher amplitude and somewhat slower wave forms. Therefore, the normal EEG depended on species, breed, age and method of restraint. EEGs were reported in frequency and amplitude, with frequency on the abscissa and the amplitude on the ordinate of the graph (Redding and Knecht, 1984).

2.1.13 EEG in disease

Klemm (1968a) reported that nervous system diseases caused changes in either frequency or amplitude or both.

The interpreter of an EEG must have a clear mental picture of the normal pattern variations that will serve as a reference for comparison with EEGs from animals with suspected brain disease (Redding and Knecht, 1984).

2.1.13.1 Encephalitides

Croft (1965b) reported that EEG from dogs with encephalitis consisted of continuous low voltage or of slow waves combined with runs of low voltage activity.

Low voltage records were observed in distemper cases that progressed to the "chorea" stage.

In encephalopathies, where degeneration of cerebral neurons has occurred, but the classical signs of inflammation are absent, EEGs showed a marked and continuous suppression and slowing of cerebral activity (Croft, 1965a).

The EEG was one of the most useful tools in the diagnosis of distemper. encephalitis (Redding ***et al.,*** 1966). The authors reported three distinguishable EEG patterns associated with viral encephalitis.

Early encephalitis	:	Slow frequency 3-6 CPS, 5-75 μv activity superimposed by high frequency spikes (20-30 Hz) and low (5-20 μv) amplitude.
Acute encephalitis	:	Very high voltage of 100-200 μv with 1-3 Hz slow activity.
Late encephalitis	:	10-75 μv amplitude and 4-7 Hz frequency.

The patterns were found to be relatively consistent and could be altered for only a few seconds by either auditory or visual stimulation after which it returned to the characteristic pattern for particular stage of the disease.

The most common correlate of encephalitis in anesthetized dogs was excessive and generalized HVSA which reflected the fact that the disease was well established before the animal was presented for evaluation.

EEG could supply valuable information in cases of encephalitis and assisted greatly in differential diagnosis, prognosis and assessment of progress (Croft, 1970).

Redding (1978) reported that residual slow wave activity of the EEG was present in post-encephalitic patients for as long as one year after clinical recovery. The clinical signs of recovery could be correlated with lessening of slow wave activity and the return to normal frequencies.

2.2 VIRAL ENCEPHALITIS

The most important and common viral diseases affecting the central nervous system are canine distemper and rabies.

2.2.1 Canine distemper

Canine distemper is a highly contagious febrile disease of dogs and other carnivores with a worldwide distribution. It is the most prevalent viral disease of dogs. The morbidity ranges from 25 to 75 per cent and the fatality is often as high as 50 to 90 per cent (Swango, 1989).

Swango (1989) continued that canine distemper virus is transmitted primarily by aerosol and infective droplets from body secretions of infected animals. Some strains of CDV were mildly virulent and usually caused inapparent infections. Some strains caused acute disease with a high frequency of encephalitis and high mortality.

Immunosuppression from viral replication in lymphoid tissue during the incubation period was an important factor in determining the outcome of infection

with CDV. Signs typical of distemper usually occurred only in dogs that were immunosuppressed by CDV. Secondary bacterial infections due to the immunosuppressive effects of CDV were often responsible for many of the clinical signs associated with distemper and they contributed to increased mortality. (Swango, 1989).

2.2.1.1 Epizootiology of canine distemper

Ganesan (1983) surveyed the occurrence of canine distemper in and around Madras City using Agar gel immunodiffusion test and found that 363 samples out of 504 suspected samples (72 per cent) were positive for distemper antibodies. The differences in incidence among various breeds were not significant. Dogs below three months of age showed 35.2 per cent incidence, dogs of 3-6 months of age showed 45.2 per cent, dogs between 6-12 months of age showed 52.1 per cent incidence and dogs above one year showed 61.8 per cent of incidence by immunodiffusion test. Out of 67 apparently healthy dogs screened for distemper antibodies 25 showed positive reaction, giving a percentage of 37.3 as subclinical or carrier of CDV. There was a slight increase in the incidence of disease during the months of July, August and January.

Gouveia ***et al.*** (1987) studied the occurrence of Canine distemper in vaccinated animals and age group distribution and reported that out of 3193 canine cases, 6.1 per cent showed clinical signs and/or laboratory results positive for CD.

Distemper is considered as an epizootic infectious disease primarily affecting young dogs and spread by contact with clinical and subclinical cases or symptomless

carriers. The ratio of clinical to subclinical cases could not be determined by the prevalence of cases, using clinical or pathological findings as a basis (Swango, 1989).

Out of 71 cases of CD, 34 per cent were in young animals (2.5-12 months) and 1.5 per cent of cases were in over 5 years old dogs (Pop ***et al.*** 1991)

2.2.1.2 Distemper encephalitis

CDV was responsible for some if not most of the sporadic cases of encephalitis in dogs (Lincoln ***et al.***, 1971).

It has been suggested that nervous symptoms occurred in at least 50 per cent of clinical distemper cases. (Gorden, 1971). The nature of the clinical signs depend on the area of brain involved.

Nervous manifestations might be delayed for months or years after a clinical episode of generalised distemper. Severe neurologic disturbances could develop quite suddenly in dogs which have had no history of systemic distemper (Wright ***et al.***, 1974). Some dogs might recover after brain infection (Mc Cullough ***et al.***, 1974).

2.2.1.3 Pathogenesis of distemper encephalitis

Virus replication started in the lymphoid tissues. Ten to 14 days after inoculation the virus invaded various epithelial tissues and the CNS. CDV crossed the blood brain barrier by way of infected lymphoid cells or entered the brain parenchyma through the CSF pathways (Higgins ***et al.***, 1982, Summers ***et al.***, 1979).

In the CNS it replicated in neurons and in glial cells, resulting in grey matter and white matter lesions with demyelination.

Distemper virus might directly injure neuronal elements or secondarily alter the cell membrane so that it was destroyed by host immune responses. CDV might also damage myelin-producing cells or elicit an immune-mediated myelinolysis. Specific antimyelin antibody has been found in the sera of dogs with distemper encephalitis. The role of antimyelin antibodies in the pathogenesis of CNS lesions was controversial (Greene and Braund, 1989).

Mechanism of demyelination in CDV induced encephalitis involved expression of viral gene products at the lesion site (Mitchell ***et al.***, 1991).

Tipold ***et al.*** (1992) reviewed the neurological manifestations of CDV infection and made the following observations. Dogs that responded immunologically early to CDV infection would recover with little or no clinical signs. Dogs with a delayed immune response tend to develop a chronic neurological disease.

CDV was able to persist in the CNS despite the presence of an intrathecal immune response providing a continuous source of viral antigen to maintain such tissue damaging reactions.

2.2.1.4 Clinical signs

The commonest clinical manifestations of distemper encephalitis were epileptiform convulsions and chorea. The mildest convulsion was manifested by rapid

clonic spasms of the muscles of mastication with hyper-salivation and sharp jerky nodding or side to side movements of the head without loss of balance. In severe form, balance was lost and the affected dog fell on its side, paddled its limbs and frequently defaecated and micturaited involuntarily. The muscular spasms regressed in a few minutes. The interval between epileptiform convulsions was highly variable, from minutes to days and tended to become shorter as the disease progressed. Hysterical fits were common in the earlier stage of illness. Involuntary rhythmic clonic spasm of a muscle or muscle group, known as chorea, was seen as a sequel to distemper infection. Whining often accompanied the onset of chorea. Following epileptiform convulsions and chorea, in order of incidence of nervous complications, comes posterior inco-ordination. Rarer nervous complications were blindness, deafness and self mutilation. The temperature tended to be above normal but constant high temperature was unusual (Lauder ***et al.***, 1954).

Gillespie and Rickard (1956) recorded the following signs in experimentally produced distemper cases: depression, myalgia, myoclonus, inco-ordination, circling, epileptiform convulsions, coma and death.

Palmer (1976) reported that after the febrile stage, there was a delay of a few days to several weeks before the onset of overt nervous signs. These might be diverse in character and distribution. There might be a slight visual deficiency to total blindness, vestibular signs and paresis. There was loss of body condition and muscles might atrophy.

On occasions, the first sign might be the so called 'canine chorea'. In mature dogs, distemper was associated with nervous signs that were difficult to be distinguished from intracranial neoplasia.

Several clinical syndromes of CDE have been recognized in dogs. They included canine distemper encephalitis in immature animals, multifocal encephalitis secondary to CDV in mature animals and disseminated encephalomyelitis in mature dogs or old dog encephalitis (Braund, 1986).

Distemper could occur together with other infections in the CNS (Greene and Appel, 1990). Tipold ***et al.***, (1992) reviewed the clinical signs of CDE extensively, after studying about 100 well documented cases of nervous distemper. The massive immunosuppression caused by CDV could lead to activation of a latent protozoal infection such as Toxoplasmosis and Neosporosis (Vandevelde and Cachin, 1992).

2.2.1.5 Laboratory findings

Hematologic and biochemical changes including electrophoresis were nonspecific (Greene and Braund, 1989).

A frequent hematological finding was lymphopenia, sometimes combined with leucopenia or leucocytosis with left shift, anaemia, monocytosis and rarely thrombocytopenia (Greene and Appel, 1990).

Examination of CSF could be a very useful diagnostic procedure in CDE. CSF analysis might reveal a moderate pleocytosis (15-60 leucocytes/mm^3) of mononuclear cells and elevated r-globulins (Cutler, 1969).

The demonstration of an elevated IgG index was very helpful to detect the presence of inflammation of the CNS but was not specific for distemper (Tipold ***et al.***, 1992).

2.2.1.6 Diagnosis

2.2.1.6.1 Laboratory diagnosis in the live animal

Having established a preliminary diagnosis based on history and clinical signs, a number of steps could be taken to confirm the diagnosis in the live animal (Wright ***et al.***, 1974; Ganesan 1983; Braund, 1986).

They included :

1. Smear preparations
   a. Histological examination for inclusion bodies.
   b. Immunofluorescence
   c. Immunoelectrophoresis
   d. Immunodiffusion
2. Virus isolation
3. Serum antibody Neutralization test
4. CSF analysis
5. Ophthalmoscopy

2.2.1.6.2 Laboratory diagnosis following necropsy

1. Pathology
   a. Macroscopic findings
   b. Microscopic findings
2. Immunofluorescence test
3. Virus isolation

2.2.1.7 CSF Analysis

2.2.1.7.1 Collection technique

CSF was collected by puncture of the subarachnoid space at the level of cisterna magna or in the lumbar region. The technique of CSF collection has been described by several authors (de Lahunta, 1977; Wright, 1978).

2.2.1.7.2 Physical characteristics

Normal CSF was a clear and colourless fluid, resembling distilled water. A red tinge was indicative of recent hemorrhage. Xanthochromia (Yellow tinge) was caused by previous subarachnoid hemorrhage. CSF turbidity or cloudiness was caused by increased cellularity greater than 500/mm^3. If turbidity was present, the sample should be cultured and smears examined using routine stains. Coagulation of CSF occurred if the protein levels were greatly increased, as in acute suppurative meningitis or profuse hemorrhage (Wright, 1978, Coles, 1980).

2.2.1.7.3 Cellularity

Determination of total and differential leucocyte counts were the most important parts of CSF examination

Pleocytosis in CSF might be the result of an inflammatory lesion in the meninges, brain, spinal cord and in association with neoplasms, spinal cord abscessation, encephalitis, chronic inflammation, and toxic conditions (Coles, 1967).

Roszel ***et al.*** (1972) and Vandevelde and Spano (1977) reported the conditions in which different cells predominate in CSF.

Wilson and Stevens (1977) commented that blood contamination had little effect on the number of leucocytes or protein content of CSF sample.

The number of cells in normal CSF did not exceed eight and were mainly lymphocytes and rarely mononuclear phagocytes (Vandevelde and Spano, 1977). Alleman ***et al.*** (1992) described intracytoplasmic inclusions with large mononuclear cells in the CSF of a dog with CD. The inclusions were detected using routine cytologic techniques and might be significant in determining the prognosis of the disease.

2.2.1.7.4 Chemical composition

Normal CSF protein was in the range of 40 to 150 mg/dl in dogs (Fankhauser, 1962). Increase in CSF protein could occur in any condition causing altered capillary permeability, especially inflammatory and infectious diseases. The normal CSF has

small quantity of protein almost entirely of albumin. The presence of globulin was therefore of chief interest which increased in pathological conditions (Coles, 1967).

CSF globulin level increased in dogs suffering from encephalitis (Cutler and Averill, 1969).

Bullmore and Sevedge (1978) recorded increase in CSF protein, total cell count and neutrophil in cases of meningo-encephalitis in dogs. They opined that early stages of viral infection might be characterized by presence of neutrophils and later stages with lymphocytic pleocytosis.

Wright (1978) reported that CSF protein increased in inflammatory conditions like encephalitis, brain or spinal cord abscessation, toxoplasmosis and meningitis. Greater alterations usually occurred in meningeal disease. When total cell count and protein values were greatly increased involvement of meninges could be suspected.

Increased CSF protein level in CDE was due to globulin (IgG and (IgM) with specific anticanine distemper virus activity. Specific neutralizing antibody in CSF was the most definitive evidence for CDE without CNS disease (Greene and Braund, 1989).

2.2.1.7.5 Creatine phosphokinase (CPK) activity

Plasma CPK does not normally cross the 'blood brain barrier' and normal CSF CPK activity appears to be derived from the CNS (Sherwin ***et al.***, 1969).

Culebras and Richards (1971) could detect increase in CSF CPK activity in only two of 30 patients within 48 hours of generalized seizures.

CPK was found in measurable quantities in CSF of man and other animal species. The normal CPK level in dog was reported to be less than one sigma unit (SU) per millilitre (Wilson and Wiltrout, 1976; Wilson, 1977).

Values greater than one SU were observed in dogs with neoplasia, degenerative diseases, inflammatory diseases including toxoplasmosis and distemper (Wilson, 1977).

Indrieri ***et al.*** (1980) reported normal CSF CPK concentration in the range of 0.0 to 10.0 IU/L with an average of 3.17 $\pm$ 2.85 IU/L, in dogs with neurological problems.

Wright (1980) reported CSF CPK activity in CSF samples in normal and dogs with neurological problem to be 1.2 $\pm$ 2.7 and 0 to 28 IU/L respectively.

2.2.1.8 Ocular findings

Fischer (1971) studied the retinal and retinochoroidal lesions in early neuropathic canine distemper and reported large, easily identified areas of advanced retinochoroidal degeneration in peripheral nontapetal fundus.

Martin (1982) reported that conjunctivitis was usually mucopurulent and occur invariably with typical forms of distemper. Dogs with distemper often developed a multifocal chorioretinitis. The chorioretinal lesions could be small or large and located

anywhere in the ocular fundus. Indistinct border and haziness over the lesion were the main signs to indicate that the lesion was active. Sharply demarcated hyper-reflective lesions in the tapetum or depigmented lesions in the nontapetal areas indicated inactive scars. Canine distemper would result in acute blindness due to bilateral optic neuritis. Acute papillitis was reflected by dilated tortuous retinal vessels and elevation of the optic disc. This was accompanied by surrounding and overlying haziness and hemorrhages on or adjacent to the optic disc. In chronic cases, optic disc atrophy occurred.

2.2.1.9 Pathology

2.2.1.9.1 Macroscopic findings

The principal gross findings in the CNS were hyperaemia of the meningeal and deep vessels, a variable excess of CSF and a gelatinous fluid in the subarachnoid space especially in the region of the chiasma and around the third and fourth ventricles. (Lauder ***et al.***, 1954).

2.2.1.9.2 Microscopic findings

Lauder ***et al.*** (1954) reported that the earliest changes were most prominent in the tissues adjacent to the ventricular system of the brain, in the pia-arachnoid, and sub-pial glial mat of the brain stem and consisted mainly of reactive changes by microglia and astrocytes. Phloxinophilic cytoplasmic inclusion bodies were found in the glia, ependymal cells, fixed and free meningeal cells and occasionally in neurons. Progression to the stage of demyelination often occurred especially in the deep and superficial parts of the cerebellar white matter and in areas of the brain where highly

myelinated tissue was closely related to the meninges. Gliosis was found in many parts of the brain. In the later stages of the disease reactive changes were prominent in and around the blood vascular system of brain and cord.

Histological examination of the brain in puppies showed early demyelination particularly around the ventricular system and in the pontine, medullary and cerebellar white matter. Glial cells proliferated and intracytoplasmic inclusion bodies could be demonstrated in glial cells and neurons. These changes were found whether or not the dog was showing clinical signs (Wright ***et al.***, 1974).

Young puppies dying of acute fatal encephalitis could have acute non-inflammatory neuronal and myelin degeneration or demyelination. Immunocompetent animals developed widespread perivascular lymphocytic infiltrates, which might progress to sclerosis in more chronically affected animals. Intranuclear and intracytoplasmic inclusions could be detected in most cases of CDE. Atypical chronic forms of distemper infections have been recognized in the CNS. Multifocal perivascular infiltration with demyelination and necrosis progressing to sclerosing panencephalitis was noticed in these cases (Greene and Braund, 1989).

2.2.1.10 Prognosis

Croft (1965 a) opined that fits due to encephalitis were more difficult to control and there was always a danger that the brain damage might be progressive.

Fewer lymphocytes in CSF as well as elevated CSF albumin were associated with a poor prognosis (Johnson ***et al.***, 1987)

The prognosis of CDV infection with neurological signs was generally guarded. Seizures in CDE were an unfavourable prognostic sign because they were generally difficult to be controlled with anticonvulsants. Since dogs had a chance to recover from the disease, supportive treatment was recommended for one or two weeks to study the course of the condition (Tipold et al., 1992)

2.2.1.11 Treatment

Cortisone was administered to a number of dogs with CDE and an improvement in the clinical condition sometimes coincided with the treatment. If cortisone was used, side effects should be watched carefully (Croft, 1965a).

There was no treatment for CDE and dogs with progressive neurological signs leading to incapacitation need to be euthanatized (Braund, 1986).

Therapy for neurologic disturbances in CD was unrewarding, as progressive encephalitis often led to tetraplegia, semicoma and incapacitation such that euthanasia was recommended. Seizures, myoclonus and optic neuritis could be tolerated if the signs were non-progressive. No drug has been shown to control myoclonus. Anticonvulsants might suppress foci from causing seizures (Greene and Braund, 1989).

Broad-spectrum antibiotics were indicated to control secondary bacterial infections, and fluids, electrolytes, B vitamins and nutritional supplements were indicated for supportive therapy. Vitamin C and diethyl ether have been claimed to

be benefit but there was a lack of controlled studies. Dexamethasone has been reported to be of some value in treating dogs with post-distemper neurologic signs (Swango, 1989).

Tipold ***et al.*** (1992) summarized the general principles of treatment in CDE as follows:

The lack of an effective antiviral treatment for CDV infection required the need for supportive care and symptomatic treatment. Parenteral nutrition, fluids, antibiotics, and vitamin B were indicated. In patients with seizures, anticonvulsants such as phenobarbitone should be administered. Corticosteroids were used but its immunosuppressive effect could be a disadvantage. Passive administration of canine hyperimmune serum might be beneficial to combat viraemia, but probably not useful in CDE. Antioxidants such as vitamin E, vitamin C, superoxide dismutase and iron chelators should perhaps be used therapeutically.

2.2.1.11.1 Specific antiviral therapy

2.2.1.11.1.1 Ribavirin

Ribavirin is a synthetic nucleoside designated chemically as 1-beta-D-ribofuranosyl-1,2,4,-triazole-3,-carboxamide.

First synthesized in 1970 by Robins and his colleagues, it has since been shown to be active **in vitro** against at least 20 different RNA viruses (Witkowski ***et al.***, 1972; Sidwell ***et al.***, 1972). **In vivo** evidence corroborated these findings, with 11 RNA viruses and 8 DNA viruses showing marked susceptibility (Sidwell ***et al.***, 1973;

khare ***et al.***, 1973; Decamps and Declercq., 1978, Burlington ***et al.***, 1983. The susceptible viruses include, herpes, cytomegalo, varicella, respiratory syncytial, influenza A and B, arena, rubeola and oncorna viruses.

Ribavirin has been administered by oral, parenteral and aerosol routes (Connor ***et al.***, 1984).

Fernandez ***et al.*** (1986) suggested that one or both of the following actions of ribavirin might account for its observed broad-spectrum antiviral potency.

1. Perturbation of intracellular nucleoside triphosphate pools.
2. Competitive inhibition of both guanyl-transferase and methyl-transferase enzymes.

Three double blind, placebo controlled studies, were conducted to evaluate the efficacy and safety of ribavirin in the treatment of measles virus infections. Oral ribavirin was administered at a dose of 10 mg/kg/day for 5-7 days. Although transient anemias and elevated bilirubin levels have been reported for the oral or intravenous administration of ribavirin, these side effects have not been observed with small particle aerosol treatment (Gilbert and knight, 1986).

In humans, oral ribavirin therapy for subacute sclerosing panencephalitis failed to show clear improvement in the neurological status of patients (Ogle ***et al.***, 1989)

Ribavirin administered by small-particle aerosol (SPA) reached the brain and achieved concentrations which should be sufficient for antiviral effectiveness (Gilbert ***et al.***, 1991).

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

The study was conducted in the Department of Clinical Medicine and Therapeutics, Madras Veterinary College, Madras. Clinical cases attending the Small Animal Clinic out-patient medical unit of Madras Veterinary College Hospital were screened for a period of eighteen months (January, 1992 to June, 1993) and were utilized for this study. The dogs for the experimental study were selected from the Madras City Corporation Lethal chambers.

3.1 DESIGN OF STUDY

3.1.1 Study on healthy animals

Ten apparently healthy animals belonging to different breeds and ages brought to Small Animal Clinic were selected as control animals for obtaining normal data including EEG for comparison with clinical cases for the parameters under study.

3.1.2 Clinical study

The animals brought to the Small Animal Clinic Medical Unit of Madras Veterinary College with clinical signs suggestive of encephalitis were provisionally selected. Ultimate inclusion of a case in the study depended on the fulfillment of certain minimum criteria.

The diagnosis of distemper encephalitis was made in clinical cases which were positive for any five of the characters studied. Animals that were diagnosed to be in the initial stages of nervous form of canine distemper were selected for the various drug trials. Although advanced cases were not included in the drug trial they were also subjected to detailed physical, neurological and laboratory investigations.

3.1.3 Experimental study

A trial was conducted to reproduce canine distemper encephalitis experimentally, in six apparently healthy adult mongrel animals. All the dogs were kept under observation for a period of sixty days.

3.2 CHARACTERS STUDIED

The number of animals studied under each character are indicated in appropriate places.

3.2.1 Signalment

3.2.2 History

3.2.3 Clinical signs & Leucogram

3.2.4 Ocular findings

3.2.5 CSF Analysis

3.2.6 EEG

3.2.7 Serological tests

3.2.8 Post-mortem and histopathological studies

3.3 DETAILS OF CASE STUDY

3.3.1 Clinical cases

The dogs in the present study were subjected to general clinical and physical examinations as described by Boddie (1962) and neurological examination as per Chrisman (1982) (Appendix, Plate 1A).

Detailed anamnesis including vaccination history of the animal was recorded to know the immune status of the animal.

3.3.1.1 Signalment

Age, Breed and Sex of the patient were noted.

3.3.1.2 Clinical signs and Leucogram

The symptoms exhibited by the animal during the observation period and the total leucocyte count in blood were recorded.

3.3.1.3 Ocular findings

The eyes were examined macroscopically and with the help of a direct ophthalmoscope and the findings were recorded.

The direct ophthalmoscope is an instrument that directs a beam of artificial light into the patient's eye, and the observer's eye is placed in the correct position to view the reflected beam and details of the interior eye. It is termed 'direct' because

PLATE 1

A. Neurological examination of a canine distemper encephalitis case

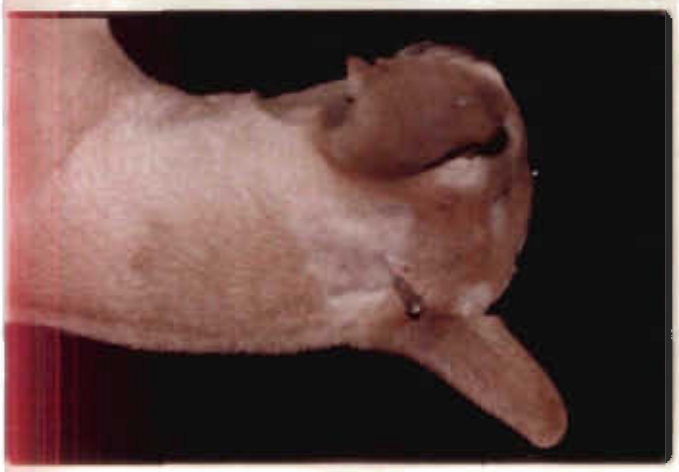
B. Puncture at Cisterna Magna for CSF Collection

C. Scalp electrode arrangement for EEG recording

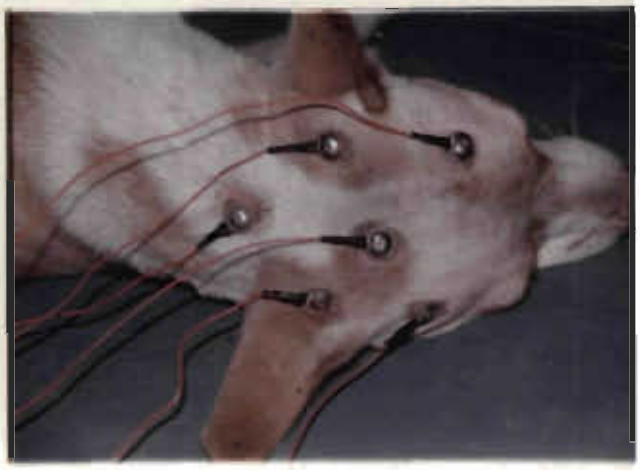
D. EEG recording in progress

PLATE 1

A



B



C

D

the fundus is viewed directly, as against the image of the fundus provided by the indirect ophthalmoscope (Slatter, 1981).

Ophthalmoscopic examination was performed as outlined by Magrane (1971) and Gelatt (1981) in a dark room maintained for that purpose.

3.3.1.4 Cerebrospinal fluid (CSF) analysis

3.3.1.4.1 Technique of collection

The technique as suggested by Wright (1978) was adopted for cerebrospinal fluid (CSF) collection (Plate 1B). Xylazine at the rate of 1.0 to 1.5 mg per kg body weight intramuscularly was used for sedating the dogs instead of general anaesthesia towards later part of the study.

3.3.1.4.2 Cytology

Total cell count was performed within half an hour of CSF collection as per the technique of Coles, (1980).

A differential cell count was carried out in all cases of pleocytosis as suggested by Hoerlein (1978).

3.3.1.4.3 Total Protein

Total protein was determined using modified method of Biuret and Dumas as described by Varley ***et al.*** (1980).

3.3.1.4.4 Creatine Phosphokinase (CPK)

The creatine phosphokinase estimation was carried out based on the modified method of Huges as described by Varley ***et al.*** (1980).

3.3.1.5 ELECTROENCEPHALOGRAPHY

3.3.1.5.1 Electroencephalograph

The electroencephalograph used in this study was eight channel 'Medicare' electroencephalograph machine manufactured by Recorders and Medicare Systems, Chandigarh. An addition of two more electrode selector point to existing master electrode selector switch were made, suitable to canine requirement of five and nine electrodes combinations.

Electroencephalograph consists of an input board, selector switches, differential variable amplifiers, filters and a time based pen-writing system. The input board serves to connect electrode on animal's head to the input selector switches used to select two electrodes as inputs to each amplifier.

The amplifier increases the strength of electrical signals detected between the two electrodes on the scalp. The differential amplification system rejects common mode signals and help in amplification of cerebral potentials only.

3.3.1.5.2 Preparation of Animal

For reduction of artifacts and better EEG tracings, following procedure was adopted.

Scalp was defatted by rubbing vigorously with acetone after clipping the hair over the area of electrode placement. The skin, subcutaneous tissue and skeletal musculature of head immediately under the point of application of electrodes were infiltrated with long acting Lidocaine 0.5 per cent solution to eliminate the muscle potential artifact.

3.3.1.5.3 Restraint of Animal

The EEG recording was carried out in quiet, comfortable air cooled room. The pectoral limbs and pelvic limbs were separately tied with tape. The eyes were covered to prevent visual stimuli from affecting the cortical potentials. The auditory perception to animal was minimised by placing a large pledget of cotton in each external auditory canal. An assistant was asked to hold the animal in a lateral recumbent position for some time till the animal was relaxed and co-operative.

3.3.1.5.4 Electrodes Placement

Surface electrodes in the form of small chlorided silver disc electrodes were used in this study. A montage consisting of bipolar electrodes and referential electrodes has been designed to utilize eight channels of the standard EEG machine. The electrodes were placed on scalp as suggested by Redding (1978) at a equal distance from each other over following areas (Plate 1C).

Fronto-parietal areas	:	2 electrodes (one each on left and right hemisphere)

Occipital areas	:	2 electrodes (one each on left and right hemisphere)
Vertex	:	1 electrode
Skin over the external occipital protuberance	:	1 Ground electrode

The disc electrodes were fixed to the place with bentonite paste. With these five electrodes recording points, simultaneous recording of following areas was made.

Sl. No.		Corresponding EEG Channel	
1	Four areas of the cerebral hemisphere against the vertex lead as a common reference	LO-V RO-V LF-V RF-V	1 2 3 4
2	Transoccipital Leads	LO-RO	7
3	Transfrontal leads	LF-RF	8
4	Left hemispheric leads occipital to frontal	LO-LF	5
5	Right hemispheric leads occipital to frontal	RO-RF	6

3.3.1.5.5 General Operative Procedure

For recording EEG, both G_1 and G_2 electrode selector knobs were turned to M/S on all channels. The all channel sensitivity, high filter and low filter switches were set at 0,70 and 1 respectively. All individual amplifier controls were set as follows :

Sensitivity at μV/mm and

Low linear frequency at 1

High linear frequency at 70

50 Hz filter to its IN position

OFF STANDBY ON switch to ON position

Chart paper speed at 30 mm/sec.

3.3.1.5.6 Calibration Curve

Calibration consists of recording the instrument response to a square wave of known value in order to compare the value of signal recorded from patient. The shape of square wave response is useful in determining any defect in the instrument. To check the time constant (frequency response), a voltage is applied to the amplifiers by a single short push on the calibration button with a paper speed of 30 mm/sec.The signal should give a 10 mm amplitude. The time taken for this amplitude to fall 37 per cent of its original value is the time constant.

All channels were tested simultaneously and proper adjustments were made on the amplifier panels.

In the present study, the calibration was marked at 50 μ V per cm at a chart paper speed of 30 mm/sec.

3.3.1.5.7 Biocalibration

Recording the same pair of electrode from the patient over all the channels is known as biocalibration. The identical waves on all channels indicate uniform and smooth functioning of all the channels. This is important when comparing the EEG

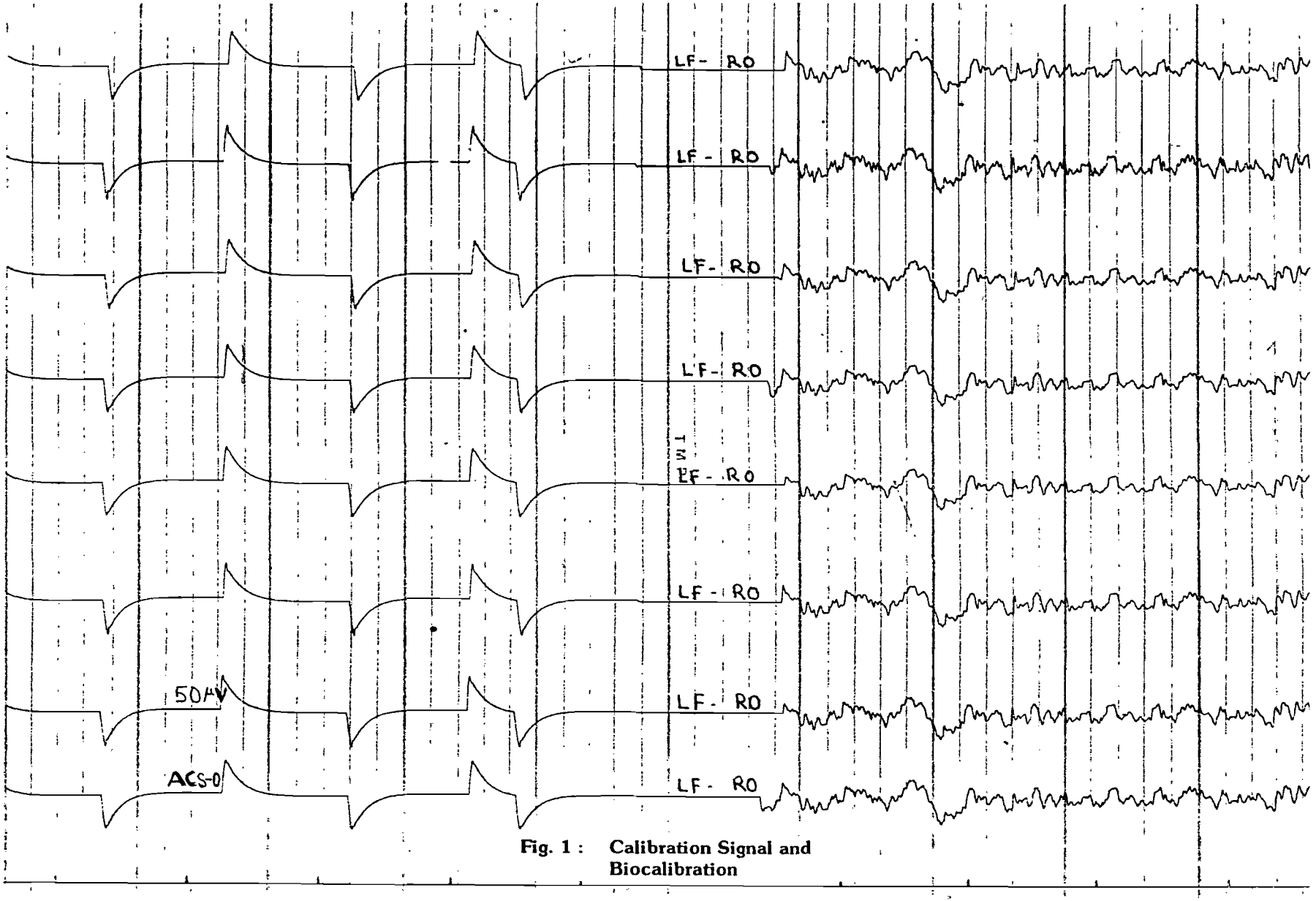


Fig. 1 : Calibration Signal and Biocalibration

tracings of different channels recorded simultaneously. The biocalibration in the present study was set at LF-RO (Figure 1).

3.3.1.5.8 Recording

Bipolar recording using two active electrodes was carried out for routine work in the present study (Plate 1D).

Electrodes connected to the corresponding jacks on the electrode board were applied to scalp on their respective positions with the help of Bentonite paste. The electrode resistance was checked with built-in resistance indicator. After obtaining the calibration curve, biocalibration was carried out by turning master electrode switch to test (T) position. Once an identical record is obtained on all eight channels, electrode selection for desired montages used in this study was obtained by turning master electrode switch to 'A' position.

Recording was carried out for 10-15 minutes. Artifacts associated with body movement, eye movement, panting etc., were noted accordingly on the tracings during recording and given due weightage at the time of interpretation.

Changes made in master control or in individual amplifier during recording were noted on the chart accordingly and were brought to normal position on completion of recording.

The breed, age and level of consciousness and co-operation from the patient at the time of recording were noted.

3.3.1.5.9 Reading Electroencephalogram

Visual analysis was carried out as suggested by Klemm, (1969), Kiloh ***et al.*** (1972) and Redding and Knecht (1982).

The number of complete waves of a rhythm in one second is known as frequency per second and is expressed as Hertz (Hz) per second. A visual average estimate of wave frequency was made after going through the whole EEG record. Any deviation from the background activity was noted. Depending on nature and distribution of waves, the wave activity was termed as diffuse, focal, persistent or paroxysmal.

Average amplitude of a wave was determined by measuring the vertical size (Nadir to peak) of a wave and comparing it with the height of standard calibration curve at 50 μ V at normal all channel sensitivity.

Serial EEG recording were carried out at frequent intervals in as many cases as possible to study the pattern of electrical activity of brain during various stages of distemper encephalitis.

EEGs were recorded from other clinical cases with nervous signs also in order to study the various EEG patterns which might help in differential diagnosis.

3.3.1.6 Serological tests for detection of canine distemper virus antigen

Conjunctival suspensions from dogs showing clinical signs suggestive of canine distemper encephalitis were collected in 4-5 ml of 0.85 per cent normal saline and were subjected to AGID and or CIE.

3.3.1.6.1 Preparation of hyperimmune serum in rabbits

The hyperimmune serum was raised in rabbits by the method suggested by Scott ***et al.*** (1986).

Canine distemper vaccine obtained from the Institute of Veterinary Preventive Medicine, Ranipet was used to produce hyperimmune serum against canine distemper in rabbits.

Rabbits were injected intramuscularly with two millilitres of canine distemper vaccine with Freund's adjuvant. Totally four injections were given at intervals of one week. Freund's complete adjuvant was used for the first injection and Freund's incomplete adjuvant was used for subsequent injections. The rabbits were bled through cardiac puncture seven days after the last injection. The collected serum was pooled and inactivated at 56°C for 30 minutes and stored after adding 1 in 10000 merthiolate solution as preservative at -20°C.

3.3.1.6.2 Agar gel immunodiffusion (AGID) test

Immunodiffusion test was conducted as per the method of Williams and Chase (1971) with a few modifications.

3.3.1.6.3 Counterimmunoelectrophoresis (CIE)

Immunoelectrophoresis was carried out as per the method of Williams and Chase (1971) with some modifications.

3.3.1.7 Post-mortem Examination

A general post mortem examination was conducted in animals that were available for autopsy. The gross changes noticed in organs such as brain, lungs, heart, liver, kidneys and intestines were noted.

3.3.1.7.1 Histopathology

Brain specimens were collected from all the dogs autopsied and were stored in 10 per cent formal saline. The samples were processed and thin paraffin sections of five microns were made as suggested by Gardon and Bradbury (1977).

Sections were stained with hematoxylin and eosine to study the tissue changes.

Histopathological sections of the tissues were examined under the low power and high power of the light microscope and changes in the cells were recorded.

3.3.1.8 Experimental design for drug trial

A summary of the experimental design adopted to study the efficacy of Ribavirin and vitamin C in distemper encephalitis is given below.

	No. of units in each trial	Name of the drugs	Dosage of the drugs	Mode of administration of the drugs
Trial I	26	Ribavirin[a] & supportive therapy	10 mg/kg/day (at 6 hour intervals) for ten days	Oral
Trial II	12	Vitamin C[b] & supportive therapy	500 mg/day	Intravenous
Trial III	10	Ribavirin,	10mg/kg/day for ten days	Oral
		Vitamin C, & supportive therapy[c]	500mg/day	Intravenous

[a] Lupin laboratories Ltd.
[b] Roche Products Ltd./N.I. Pharmaceutical Works (Pvt.) Ltd.
[c] Fluids, Electrolytes, Antibiotics, Anticonvulsants and B Complex Vitamins according to the clinical condition of the animal.

All the animals were observed from the date of admission to the date of recovery/death/euthanasia.

3.3.2 Experimental Study

An experimental study was undertaken to study the EEG changes in distemper encephalitis from the early stages onwards.

Virulent canine distemper virus (Strain A 75-17) was procured from James A. Baker Institute for Animal Health, U.S.A. The titre of the virus was $10^{5.7}$/ml in dog lymphocyte culture. All the dogs were observed for a period of two months for any signs of infection. The selected parameters were studied in animals that developed

signs of encephalitis. As the success rate in the experimental reproduction of distemper encephalitis is reported to be low, three routes of infection were tried viz. intravenous, intrathecal, (Cisterna magna) and intracerebral.

3.3.2.1 Technique adopted for the inoculation of canine distemper virus

Intracerebral

0.5 ml of CDV was inoculated intracerebrally as per the technique of Gillespie and Rickard (1956) in two animals.

Intrathecal

0.5 ml of CDV was injected into the cisterna magna as per the technique of Wright (1978) in two animals.

Intravenous

0.5 ml of CDV was injected intravenously in two animals.

3.4 STATISTICAL ANALYSIS

The data collected were analyzed and reported as per Snedecor and Cochran (1967).

Results

CHAPTER IV

RESULTS

4.1 CLINICAL STUDY

A total of 227 clinical cases which exhibited clinical signs suggestive of canine distemper encephalitis (CDE) were provisionally selected over a period of eighteen months for the study. These cases were subjected to detailed neurological examination and 175 cases were confirmed to be suffering from CDE. 48 dogs that were diagnosed to be in the initial stages of the disease were selected for the drug trial.

Ten apparently healthy animals were utilized as control animals and the data collected from them on various parameters were taken as control values.

4.1.1 Incidence

Every case brought to SAC (M), Madras Veterinary College Hospital with clinical signs, which from the past experience were regarded as diagnostic of CDE, was provisionally selected and was included for the study.

The total number of dogs that were brought to the hospital irrespective of the nature of disease, were taken from the hospital records over a period of 18 months.

Total number of dogs presented in the hospital during the study period and the number of CDE cases are shown in Table 1. It indicated that CDE cases were more from January to April when compared to those from May to August and September to December.

The total number of dogs brought to SAC (M), Madras Veterinary College Hospital during the period were 20087 and only 227 animals were suspected to be affected with canine distemper encephalitis and 175 animals (0.87 percent) were confirmed to be suffering from CDE. Among these, there were 105 males and 70 females.

To find out the incidence of CDE in different age groups, the animals were grouped into three categories, viz. less than one year, 1 to 5 yrs. and more than five years. The number of animals in each group were 72, (41.14, per cent) 82 (46.86 per cent) and 21 (12 per cent) respectively.

The highest incidence was seen in Non-descript dogs (35.43 per cent), followed by Spitz (29.15 per cent) and Doberman (12.57 per cent). Rest of the breeds represented only a small percentage.

Breed and age distribution of CDE in dogs is represented in Table 2 and 3.

4.1.2 History and Clinical Signs

Majority of the pure bred animals (80 per cent) were vaccinated against canine distemper at least once whereas majority of the non-descript animals (76 per cent)

TABLE 1

INCIDENCE OF CANINE DISTEMPER ENCEPHALITIS

Period/ Season	1992		1993	
	Total number of dogs registered	Percentage of CDE affected dogs	Total number of dogs registered	Percentage of CDE affected dogs
January to April (Winter and the following period)	4236	1.35	4567	1.25
May to August (Summer and the following period)	4629	0.37	2383 (May and June)	0.34
September to December (Monsoon period)	4272	0.84	-	-

TABLE 2

BREED-WISE INCIDENCE OF CANINE DISTEMPER ENCEPHALITIS

Breed	Percentage
Non-descript	35.43
Spitz	29.15
Doberman	12.57
German shepherd	6.86
Mixed breed	5.71
Others	10.28

TABLE 3

AGE-WISE INCIDENCE OF CANINE DISTEMPER ENCEPHALITIS

Age	Percentage
Less than 1 year	41.14
1-5 years	46.86
More than 5 years	12.00

TABLE 4

MAJOR CLINICAL SIGNS OBSERVED IN CDE CASES

Signs	Percentage occurrence
Fits	61.71
Myoclonus	40.57
Abnormalities in postural reactions and spinal reflexes	25.14
Respiratory signs	24.57

Note : Some cases exhibited more than one clinical sign.

were never protected against the disease. A few animals (9 per cent) were regularly vaccinated against canine distemper. In many cases owners of the animals did not observe any clinical signs prior to the development of nervous signs, whereas in many other cases they reported mild gastro-intestinal and/or respiratory involvement prior to the development of nervous signs.

The common clinical signs noticed at the time of presentation of the cases were fits, (61.71 per cent) flexor spasms (myoclonus) (40.57 per cent), abnormalities in postural reactions and spinal reflexes (25.14 per cent) and respiratory disturbances, (24.57%), (Table 4). Many animals were having inappetence. Occasionally, gastrointestinal disturbances such as vomiting and diarrhoea were recorded. Many animals were suffering from conjunctivitis. In mature dogs, weakness of the pelvic limbs, generalized inco-ordination and occasional falling were also noticed. Some animals had head tilt and nystagmus. Visual impairment was noticed in many dogs. Myoclonus involving mostly the muscles of the limbs and the head was noticed approximately in 40 per cent of the cases. Hyperkeratosis of the pads and nose were noticed only in four cases. Whining, especially in the night hours, was noticed in many serious cases.

Ten cases were found to be concurrently suffering from Ehrlichiosis.

4.1.3 Leucogram

The mean total leucocyte count of control animals were 9330 $\pm$ 345.79 per c.mm In the clinical cases of encephalitis it was found to be 10350.83 $\pm$ 282.26 per

c.mm. There was no statistically significant difference between the control and sick animals. (Table 5).

4.1.4 Ocular changes

Conjunctivitis and purulent ocular discharge were noticed in more than 80 percent of cases. The margin of the eye-lids were inflamed occasionally. Diffuse keratitis and corneal ulceration were noticed in certain cases. Pupillary reflexes were poor in a number of cases.

Ophthalmoscopic examination revealed signs of chorio-retinitis with grey to pink irregular areas of degeneration on either the tapetal or nontapetal fundus in 40 per cent of the cases. Areas of hyperreflectivity appearing as brightly coloured lesions were observed in many cases (10 per cent). Hazy areas with indistinct margins were noticed in the tapetal area in two cases.

4.1.5 Examination of conjunctival swabs

The conjunctival swabs collected were subjected to Agar gel immuno-diffusion test and/or counterimmuno electrophoresis. Out of 227 samples tested, 51 samples (22.47 per cent) were found to be positive either by AGID or CIE. Many typical cases of CDE (61 per cent) gave only negative results to CIE and AGID (Plate 2C,D).

TABLE 5

MEAN ± S.E. VALUES OF TOTAL LEUCOCYTE COUNT IN BLOOD, AND CELL COUNT, TOTAL PROTEIN AND CREATINE PHOSPHOKINASE IN CEREBROSPINAL FLUID OF ENCEPHALITIS CASES

Groups	Blood	Cerebrospinal Fluid			
	Total leucocyte count per c.mm	Cell count (No)	Type of cells	Total protein mg %	Creatine phosphokinase IU/L
Control (n=10)	9330±345.79	3.6±0.65	Lymphocytes	17.0±2.14	4.5±0.56
Clinical Encephalitis (n=41)	10350.83±282.26	37.88±7.99*	Lymphocytes Neutrophils (5 cases)	69.76±4.58**	51.66±7.79**

* $P \leq 0.05$ ** $P \leq 0.01$

TABLE 6

ANALYSIS OF EEG PATTERNS OBSERVED IN CANINE DISTEMPER ENCEPHALITIS

EEG Character	Percentage occurrence
Spikes	26.35
Asymmetry	25.00
Low voltage runs	14.19
Slow waves	13.51
Slowing	10.81
Spike and wave	6.76
Sinusoidal waves	6.76
Low voltage	3.38
No characteristic abnormal pattern	18.92

Note : Many of the EEGs showed more than one character.

PLATE 2

A. CSF Smear: Note the lymphocytes

Giemsa - Leishman x 1000

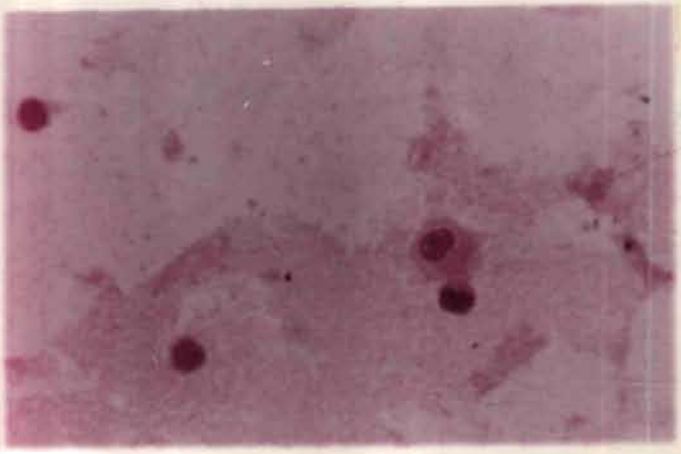
B. Lung: Hyperaemia and Cellular Infiltration

H & E x 400

C. Slide showing positive reaction by CIE

D. Slide showing positive reaction by AGID

PLATE - 2



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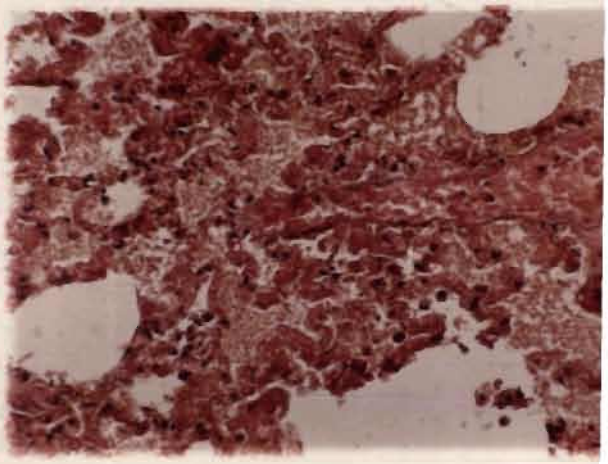

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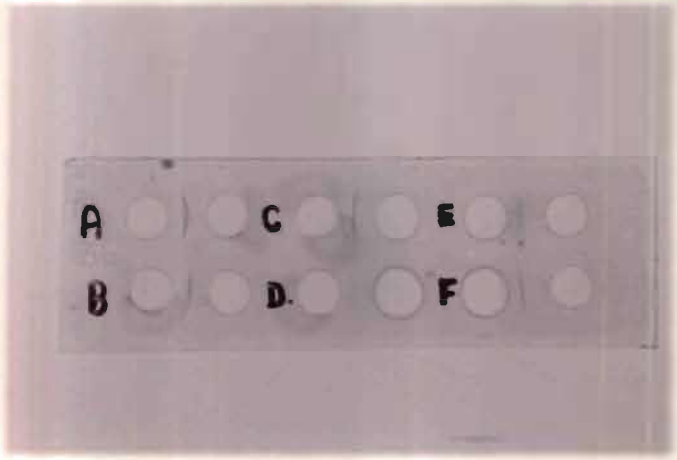

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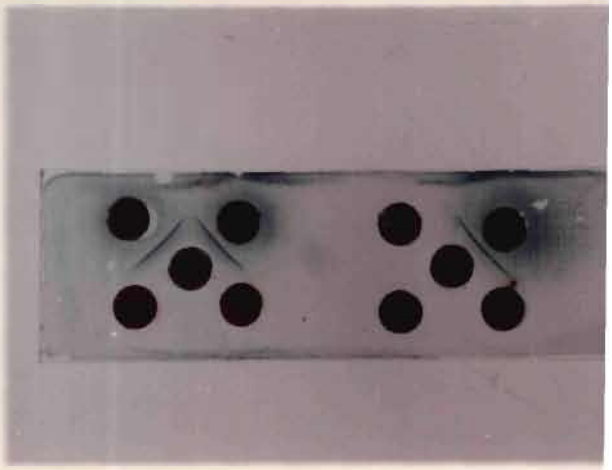

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4.1.6 Cerebrospinal Fluid (CSF)

4.1.6.1 Technique of Collection

CSF was collected from 46 animals. Collection of CSF from Cisterna magna using a 1½ inch long, 21 gauge disposable needle was found to be a safe and relatively easy procedure. General anaesthesia was used in the initial stages of the study. Xylazine @ 1 to 1.5 mg/kg body weight intramuscularly was found to be sufficient for sedating the dog for collection of CSF. In a few cases (10.87 per cent) CSF got contaminated with blood during collection and was discarded. No complication was noticed in any animal after collection of CSF.

4.1.6.2 Physical Appearance

The CSF collected was clear and colourless in 80 per cent of the cases. Slight turbidity observed in a few cases (10 per cent) was found to be due to the presence of erythrocytes.

4.1.6.3 Cell Count

Control group had a mean of 3.6 $\pm$ 0.65 cells consisting of lymphocytes in CSF. The clinical cases had an average of 37.88 $\pm$ 7.99 cells, consisting mainly of lymphocytes. The values obtained could be grouped into three categories such as 0-4, 5-50 and more than 50. The frequency obtained for those categories were 4, 29 and 8 respectively. The increase in cell count was found to be significant (Table 5 & Plate 2A).

4.1.6.4 Total Protein

According to the CSF total protein values, the clinical cases studied were divided into three categories namely those having values between 0-17, 18-80 and above 80 mg per cent and the frequency were found to be 0, 30 and 11 respectively. The values were found to be significantly high in the clinical group when compared to the control values of 17.0 $\pm$ 2.14 (Table 5).

4.1.6.5 Creatine phosphokinase (CPK)

The mean CPK enzyme level in control animals was found to be 4.5 $\pm$ 0.56 IU/L. The values for the same in the clinical cases of CDE were found to be 51.66 $\pm$ 7.79 IU/L. The CPK values were grouped into three categories viz. 0-5, 6-50 and above 50 and the frequency obtained for these groups were found to be 0, 30 and 11 respectively. The CPK enzyme level in the clinical cases was found to be significantly higher than the control (Table 5).

Pearsonian correlation coefficient of CSF cell count, total protein and CPK were worked out and it was found that there was no significant correlation between these parameters. The values obtained for cell count and total protein, cell count and CPK and total protein and CPK were less than 0.111, 0.28 and 0.100 respectively.

4.1.7 Electroencephalography

Electroencephalography was undertaken in 148 dogs. Out of this, 120 (81.08 per cent) animals were co-operative throughout the EEG recording and required only

physical restraint. 10 dogs (6.76 per cent) were apprehensive and were not co-operative during the initial stages but co-operated towards later stages. 18 animals (12.16 per cent) were not co-operative and required sedation/anaesthesia for the EEG recording.

4.1.8 Electroencephalogram

The normal adult EEG in alert condition was found to be low voltage fast activity of 10-20 μv, 15-20 Hz. The animals with thick musculature on the head showed myographic artifacts frequently, especially on right occipital region. The EEG in relaxed state was characterized by slow waves of high amplitude. The normal adult pattern was more or less consistent in all the control animals. High voltage slow waves were noticed in younger animals. Adult pattern was generally seen in animals that were more than 8 months of age. The level of consciousness was found to affect the EEG pattern. The normal EEG patterns in various breeds and age groups are shown in Figures 2 to 11. The artifacts commonly encountered were motion artifacts, physiologic artifacts and electrostatic-electromagnetic interference. The common artifacts encountered are shown in Figures 12 to 21.

The EEG patterns associated with CDE were recorded and are presented (Table 6) and Figures 24 to 46. The patterns were slow waves, low voltage runs, spike and wave, low voltage, asymmetry, slowing, spikes, sinusoidal waves, high-voltage slow waves and spindles. Many of the EEGs showed more than one character. Repeat recordings were done in certain cases. Spikes (26.35%) asymmetry (25%), slow waves (13.5%), low voltage runs (14.19%) were the most common patterns. In certain cases,

the patterns were consistent and could not be altered by either auditory visual or tactile stimuli. 28 EEGs (18.9%) did not show any characteristic deviation from the normal EEG pattern.

EEGs from other clinical conditions with neurological signs were also recorded for the purpose of differential diagnosis (Figure 47 and 48).

4.1.9 Post-mortem findings

4.1.9.1 Macroscopic findings

Post-mortem examination was performed in 19 animals. The dogs were examined by autopsy within four hours of death. The post-mortem changes were often variable and inconclusive. Diffuse pulmonary oedema and pneumonia were observed in seven cases. Enteritis was observed in three cases. Heart, liver and kidneys did not reveal any macroscopic changes. Congestion of the meningeal vessels of the brain was observed in three cases.

4.1.9.2 Histopathology

All the brain samples subjected to histopathological examination revealed varying degrees of encephalitis. Congestion and hyperemia were consistent. Neuronal necrosis and degeneration, spongiosis, Gliosis, eosinophilic intranuclear inclusion bodies in the astrocytes, neuronophagia, lipid accumulation and satellitosis were observed in the cerebrum, cerebellum and medulla (Plate 2B, Plate 3).

PLATE 3

A. **Cerebellum: Spongiosis**

H & E x 125

B. **Brain: Inclusion body in an astrocyte**

H & E x 600

C. **Cerebrum: Necrosis of Neurons**

H & E x 600

D. **Cerebellum : Spongiosis and necrosis of Purkinje cells**

H & E x 320

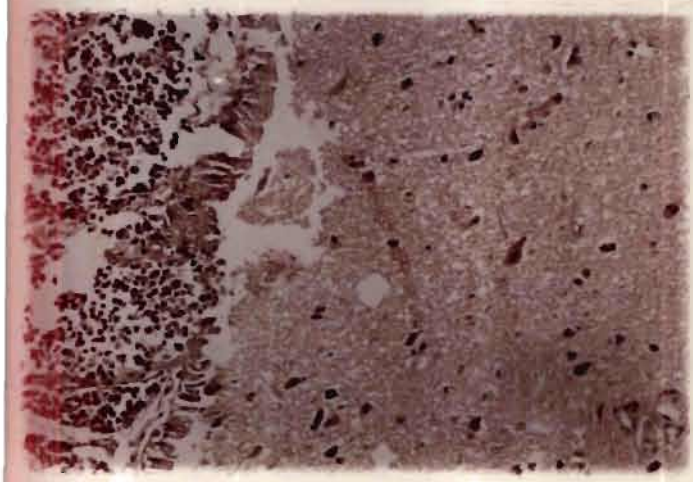
E. **Cerebrum : Neuronal Necrosis and mild Gliosis (Experimental CD**

H & E x 1000

F. **Intestine showing Catarrhal Changes**

H & E x 100

PLATE - 3



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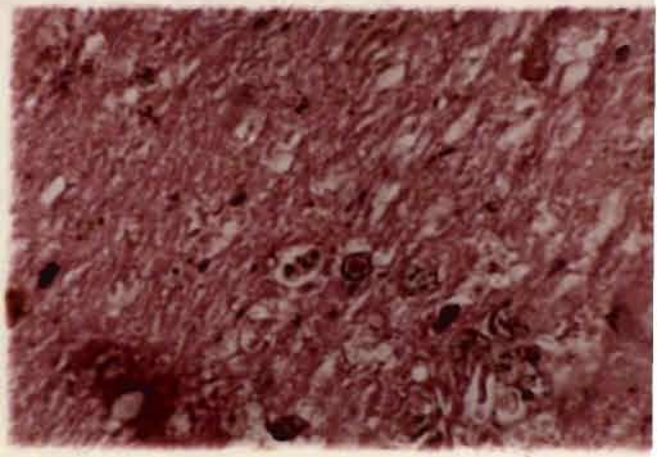

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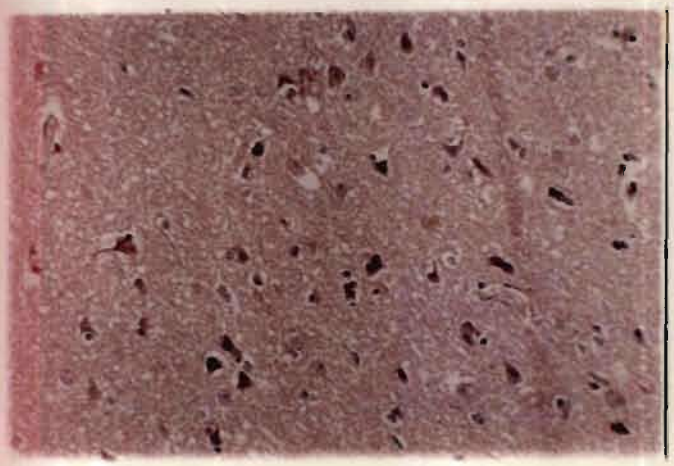

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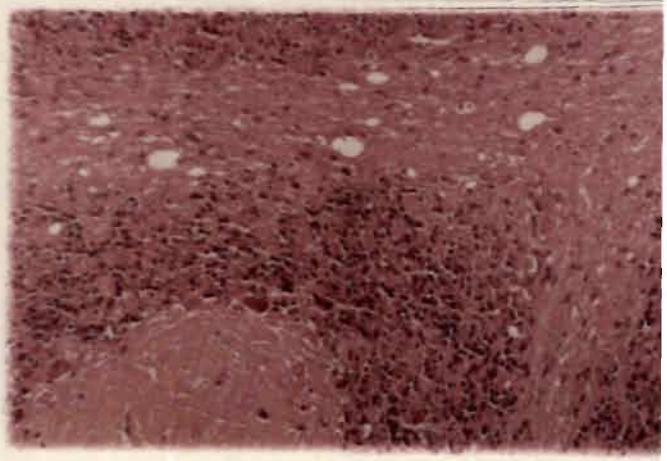

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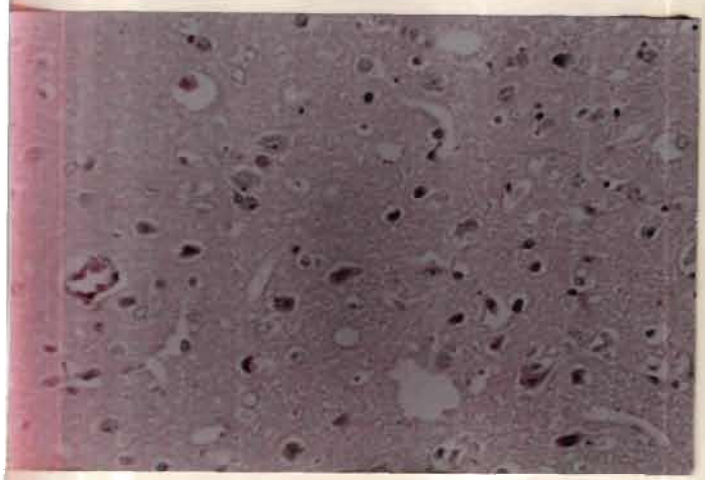

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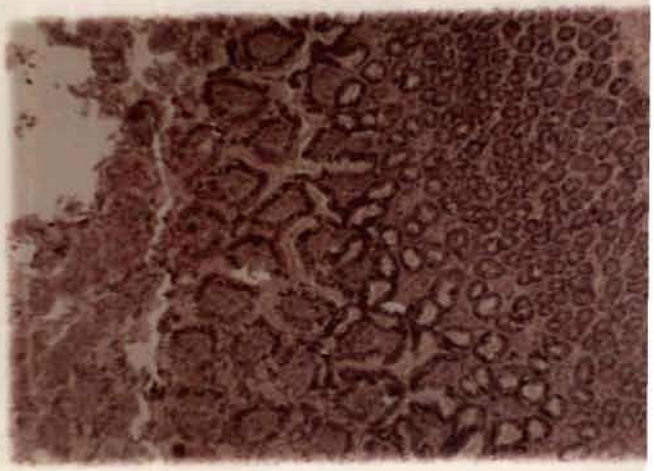

F

4.1.10 Effect of drug trials

4.1.10.1 Ribavirin

The effect of oral administration of Ribavirin at the rate of 10 mg/kg body weight/day, divided into four doses, was assessed in 26 animals belonging to various breeds and age groups suffering from early stages of encephalitis (Table 7). Out of those animals, only one non-descript dog recovered from the illness. It was one year old and was given Ribavirin and supportive therapy for ten days. Though the animal recovered, it had myoclonus. All other animals died or were euthanized as per the request of the owner.

4.1.10.2 Vitamin C

The effect of Vitamin C-500 mg/day intravenously was assessed in 12 animals (Table 7). Out of these animals, one cocker-spaniel dog aged 3 years, which was vaccinated regularly against CD, showed marked clinical improvement after one week and recovered. Another German shepherd dog aged seven years and a non-descript dog aged 2 years showed improvement in the initial stages of treatment but later succumbed to the disease. A two year old non-descript dog recovered from the infection after two weeks treatment but myoclonus of the muscles of the head persisted. All other animals were dead within ten days of treatment or were euthanized at the owner's request. However, the severity of the clinical signs in this group was apparently less when compared to the earlier group.

TABLE 7

EFFECT OF THE TREATMENT TRIALS ON THE SURVIVAL RATE OF CDE AFFECTED DOGS

Drug	Number of animals included in the trial	Survival rate (per cent)	Remarks
Ribavirin	26	3.85	-
Vitamin C	12	16.67	Two animals showed clinical improvement initially but later died. One recovered animal had regular vaccinations.
Ribavirin and Vitamin C	10	20.00	One recovered animal had regular vaccination.

4.1.10.3 Ribavirin and Vitamin C

Ten animals belonging to various breeds and age groups were included in this trial. One non-descript dog aged six years showed improvement during the treatment and recovered. Another Doberman pup aged 5 months, which was vaccinated against CD, survived the infection but 'chewing gum fits' were noticed as a sequelae. All other animals succumbed to the disease within 12 days or were euthanized at the owner's request. The details are given in Table 7.

4.2 EXPERIMENTAL STUDY

Two experimental adult mongrel dogs that were given the CDV intravenously were observed for a period of two months. They did not develop any signs of infection during the observation period.

The animals that were given the virus intrathecally also did not develop any signs of the disease during the two months observation period.

Out of two animals that were given the CDV intracerebrally one animal developed pyrexia (104.6° F) after four days. It was dull and inactive during the remaining days and died after 23 days, without showing any typical nervous signs. Total leucocyte count at the time of development of pyrexia was found to be 8850 per cubic milli metre. No ophthalmologic changes were detected. Conjunctival swabs were negative for CDV antigen by AGID and CIE. CSF analysis during the period of illness revealed cell count of 9, total protein level as 37 mg percent and CPK level 8

IU/L. Serial EEGs taken from this animal revealed spikes and asymmetry from the 15th day of infection (Fig.49 to 52).

Histopathological examination of the brain revealed mild signs of encephalitis.

The other animal did not develop any signs of infection during the observation period of two months.

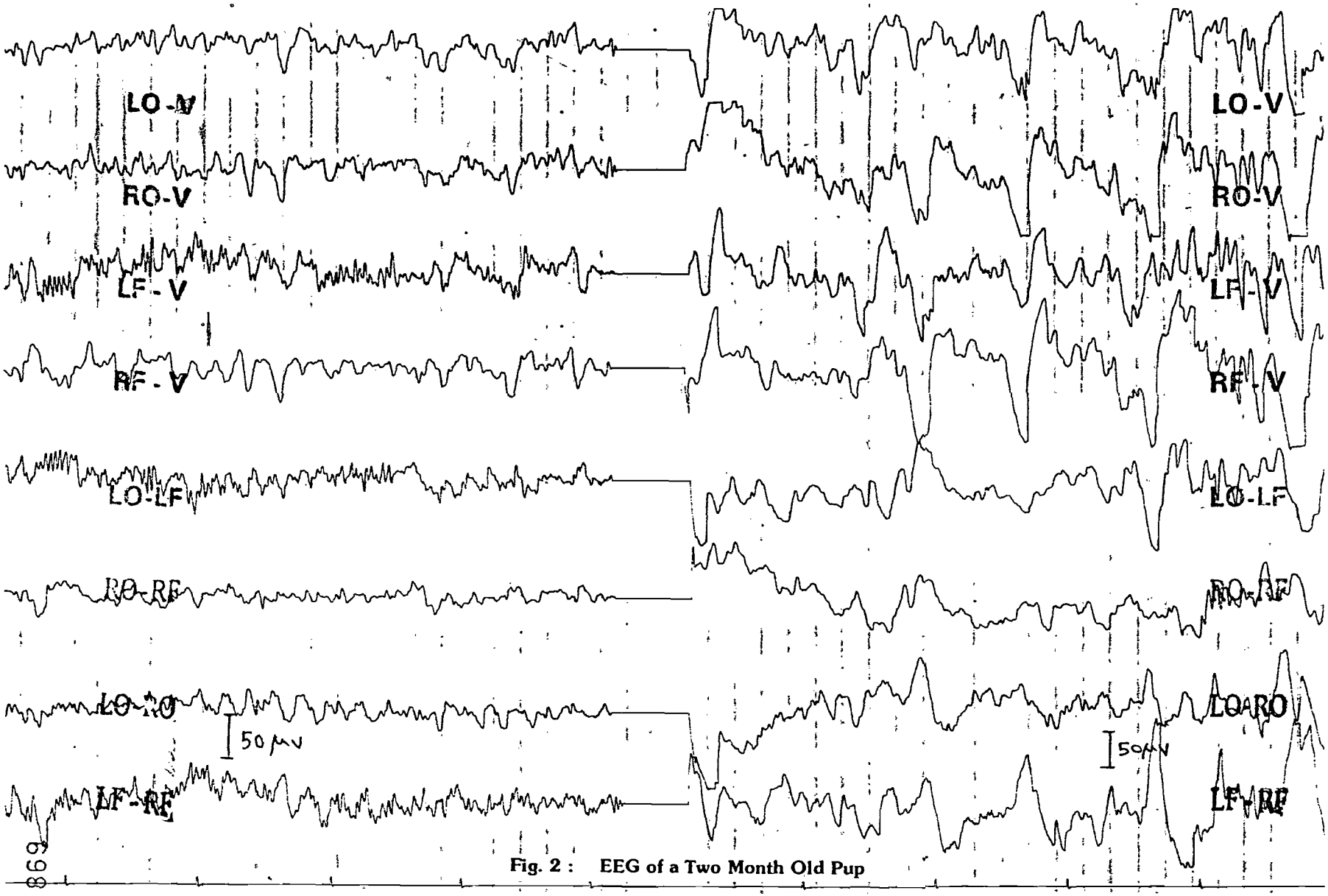

Fig. 2 : EEG of a Two Month Old Pup

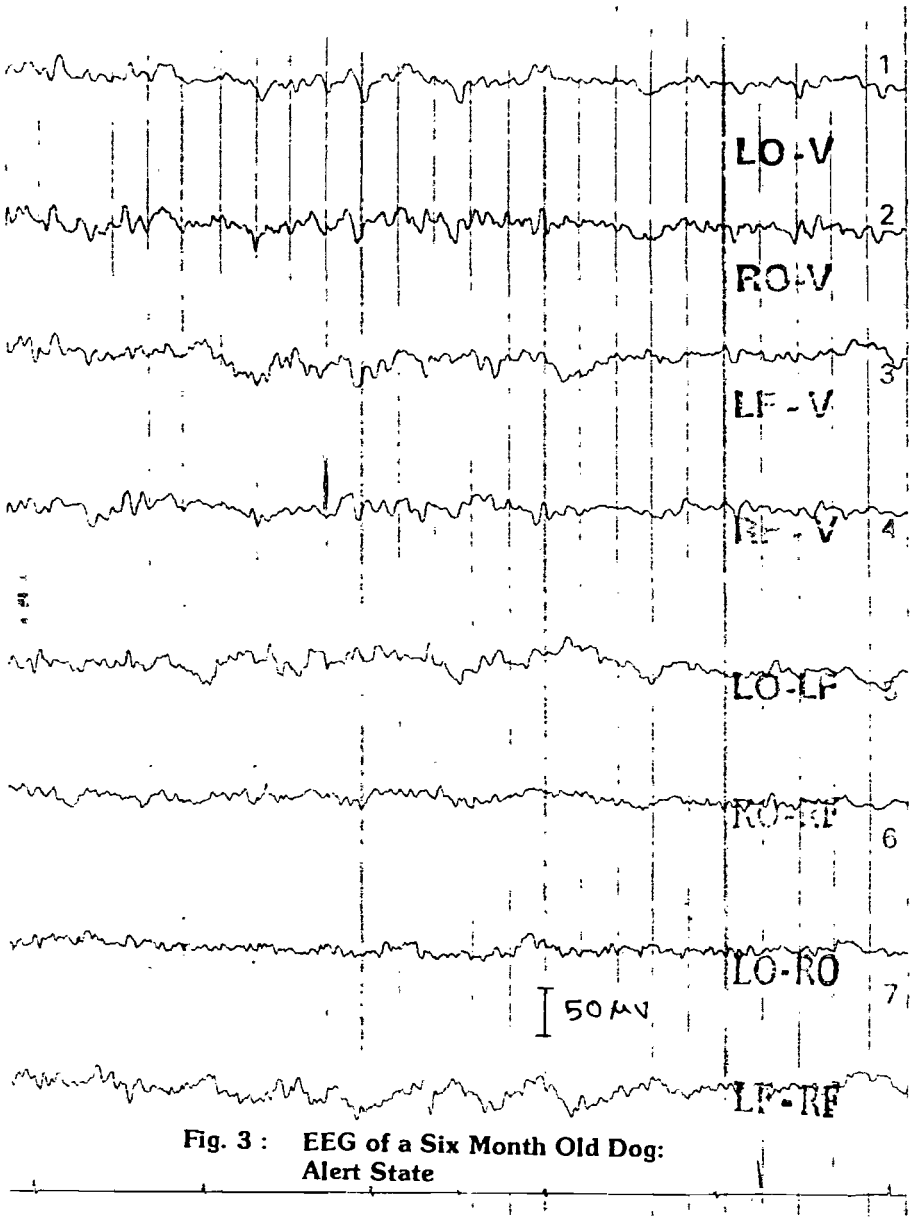

Fig. 3 : EEG of a Six Month Old Dog: Alert State

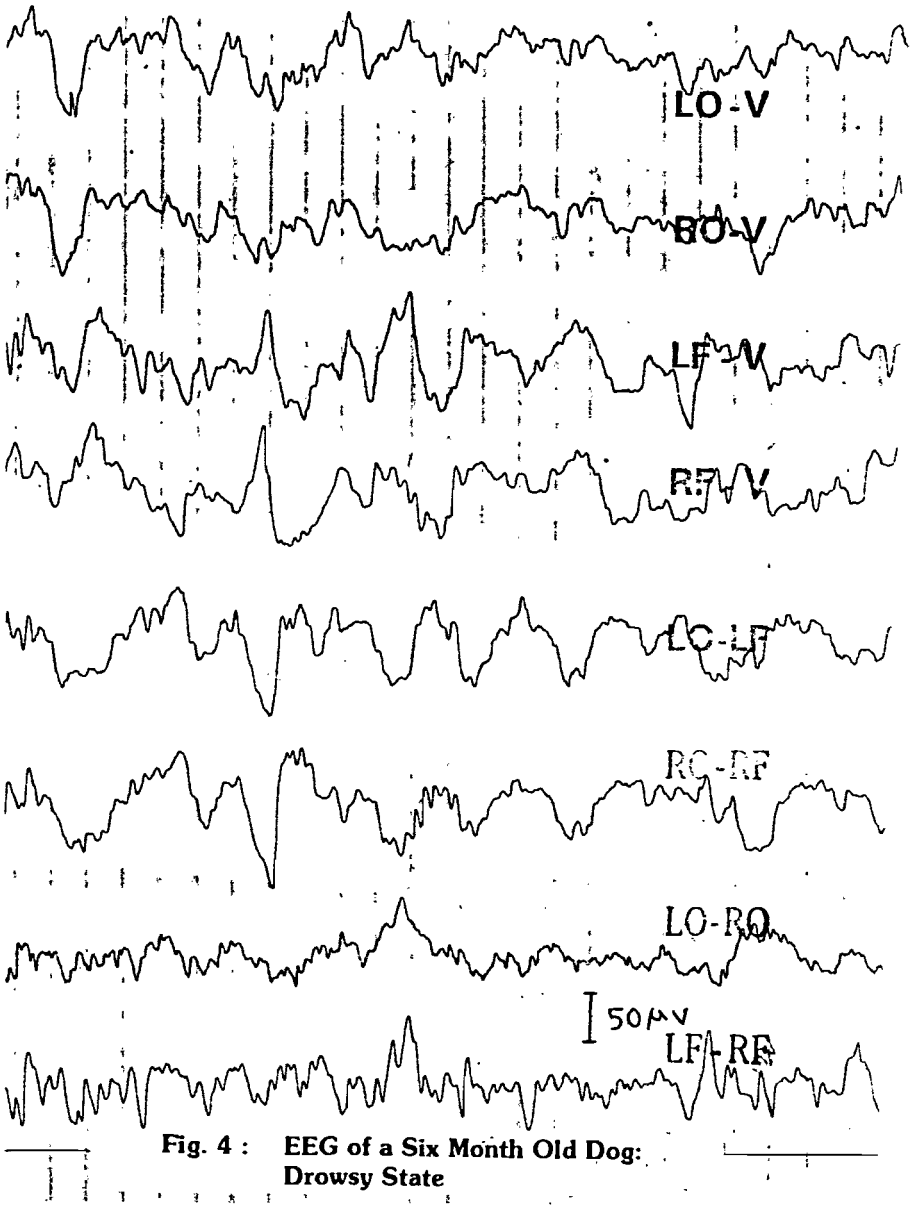

Fig. 4 : EEG of a Six Month Old Dog: Drowsy State

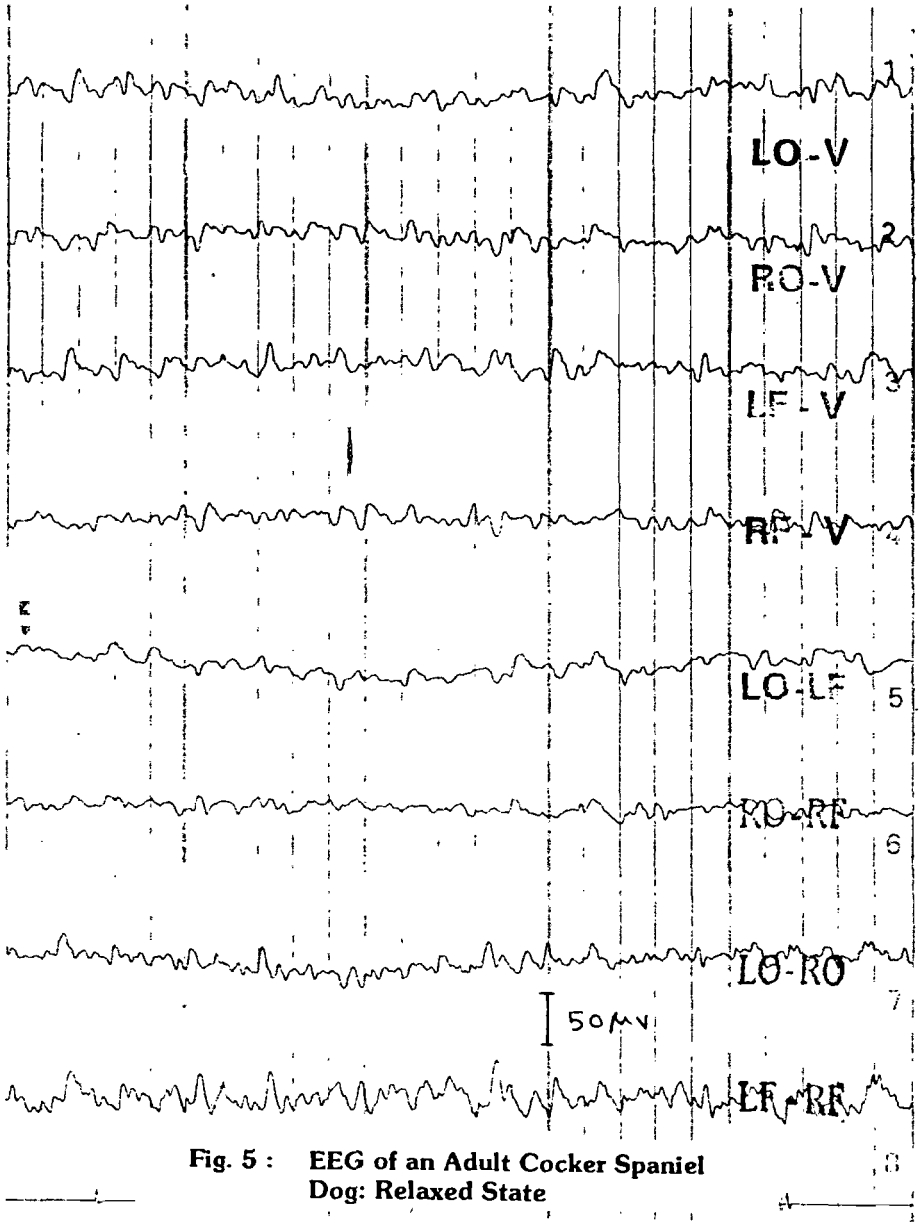

Fig. 5 : EEG of an Adult Cocker Spaniel Dog: Relaxed State

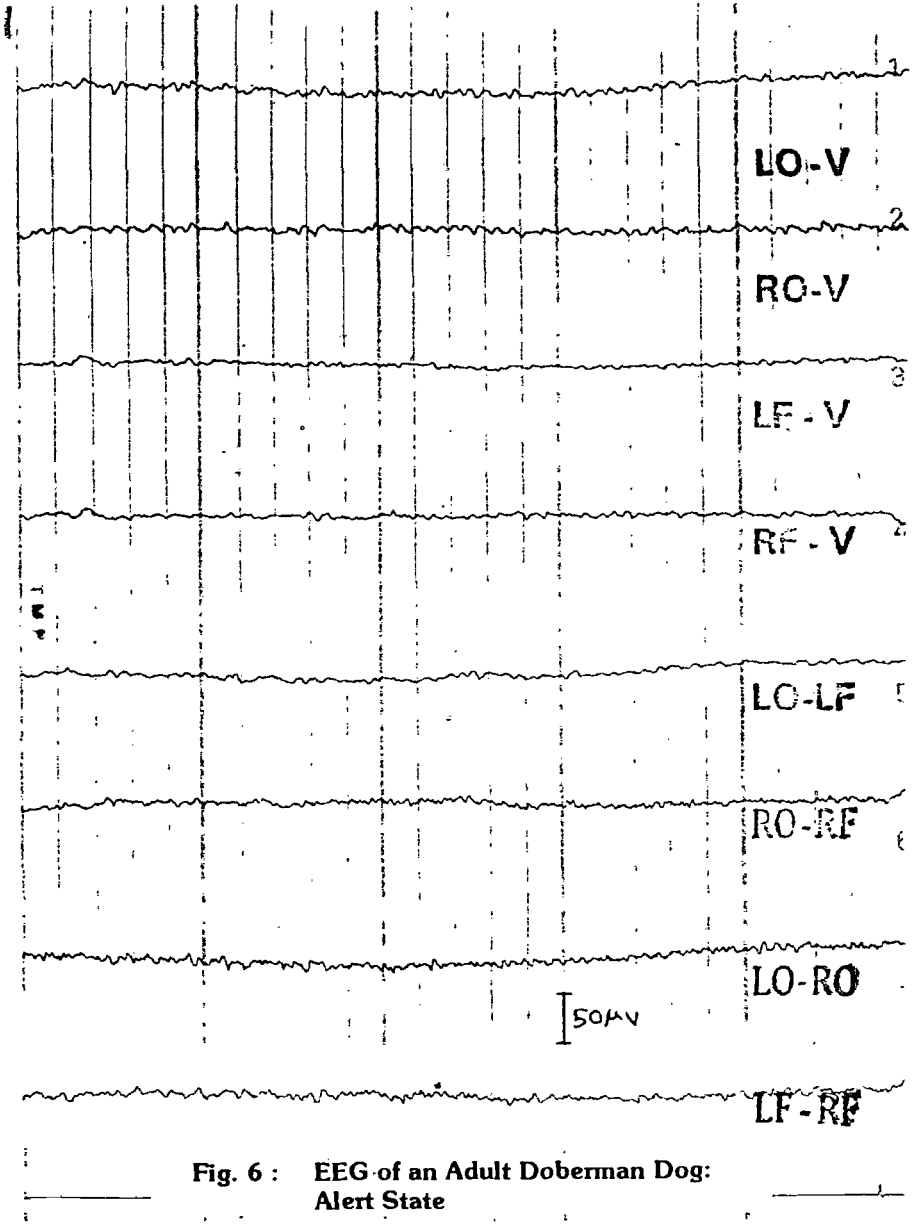

Fig. 6 : EEG of an Adult Doberman Dog: Alert State

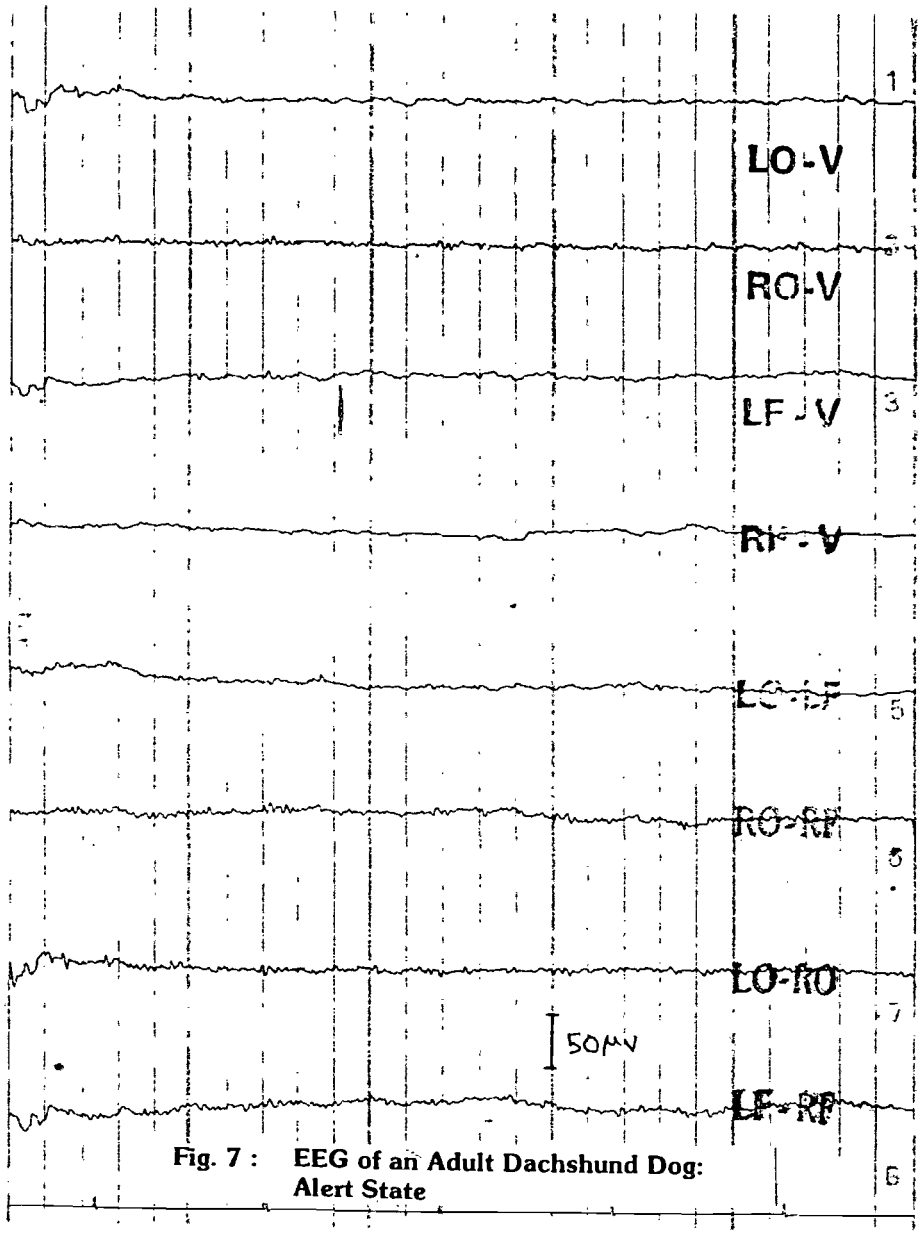

Fig. 7 : EEG of an Adult Dachshund Dog: Alert State

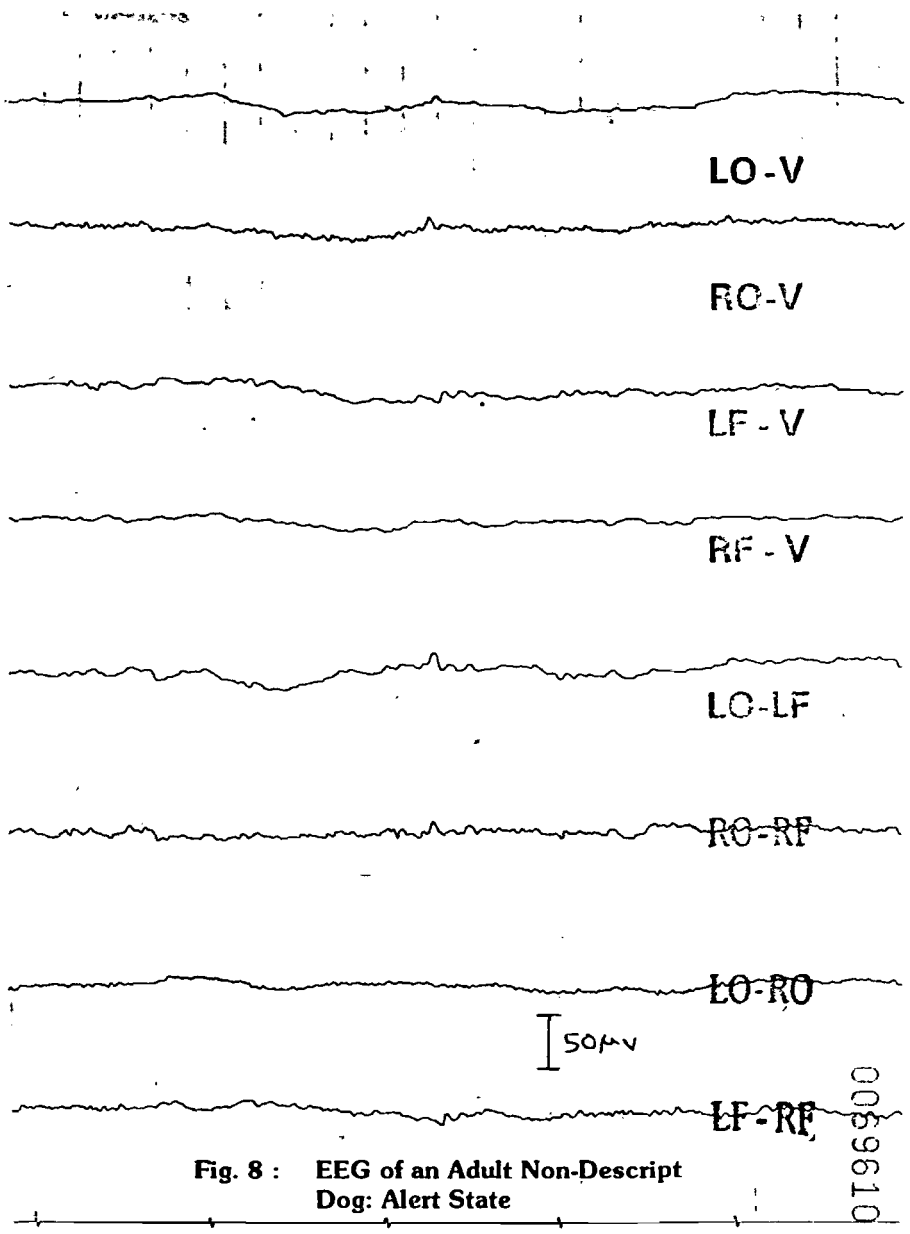

Fig. 8 : EEG of an Adult Non-Descript Dog: Alert State

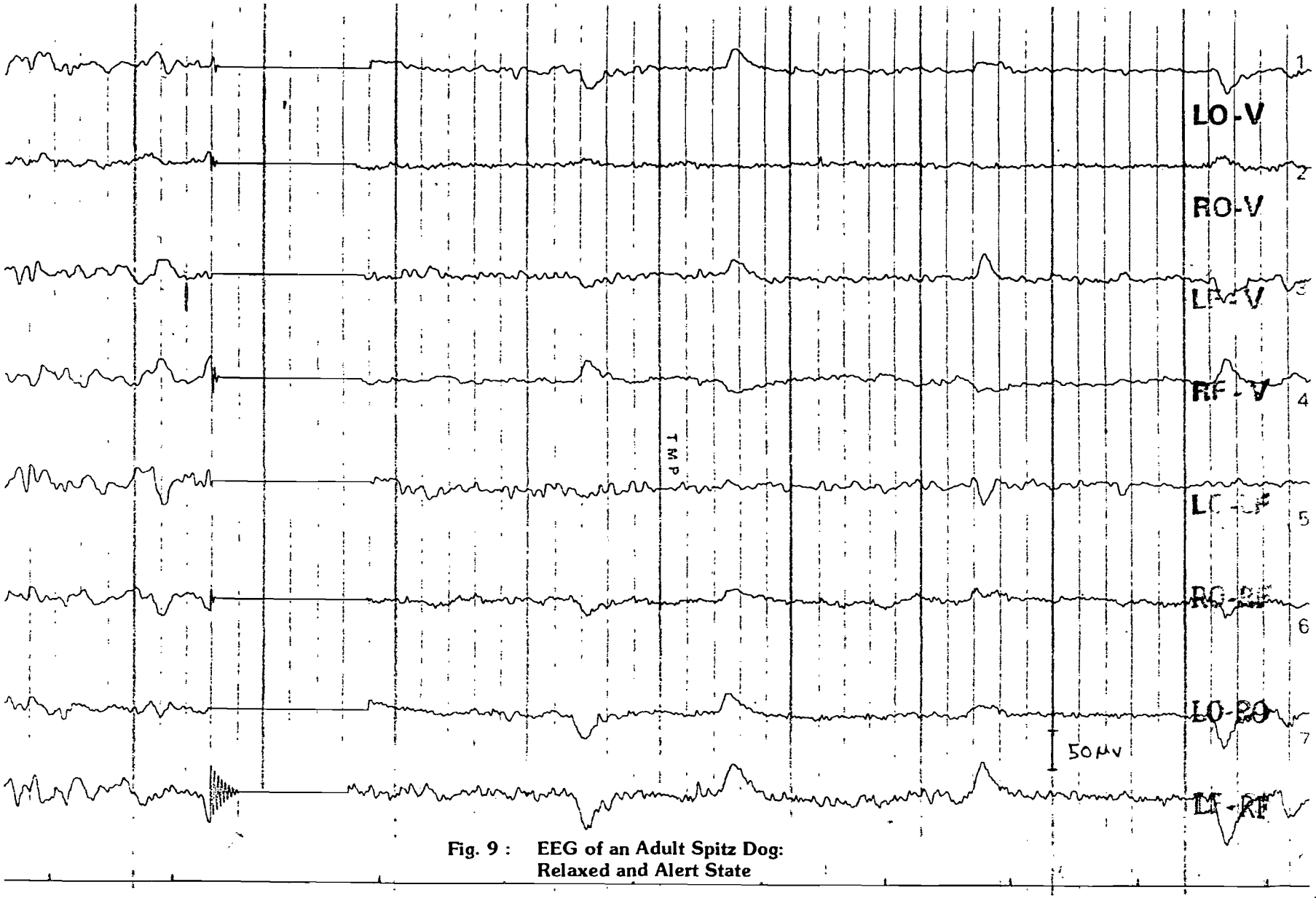

Fig. 9 : EEG of an Adult Spitz Dog: Relaxed and Alert State

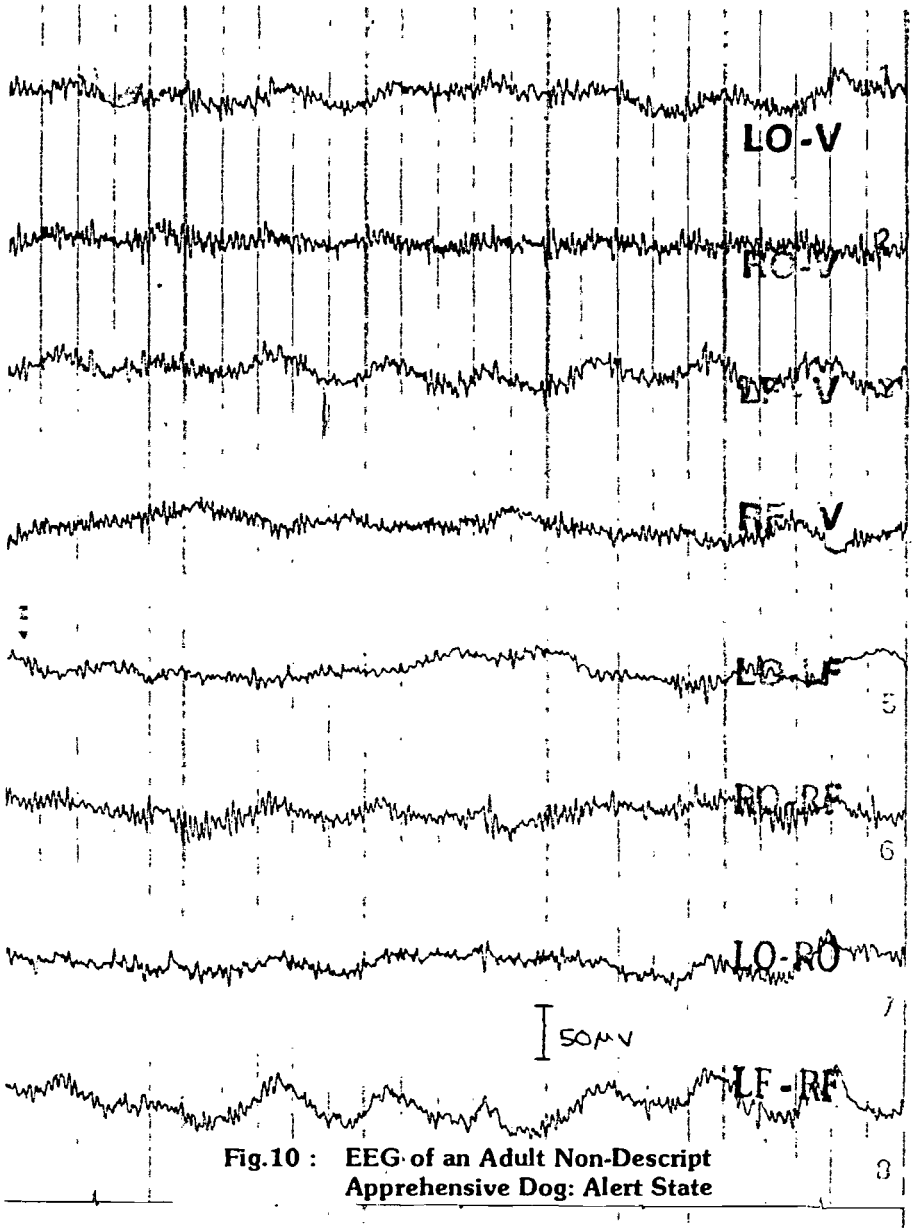

Fig.10 : EEG of an Adult Non-Descript Apprehensive Dog: Alert State

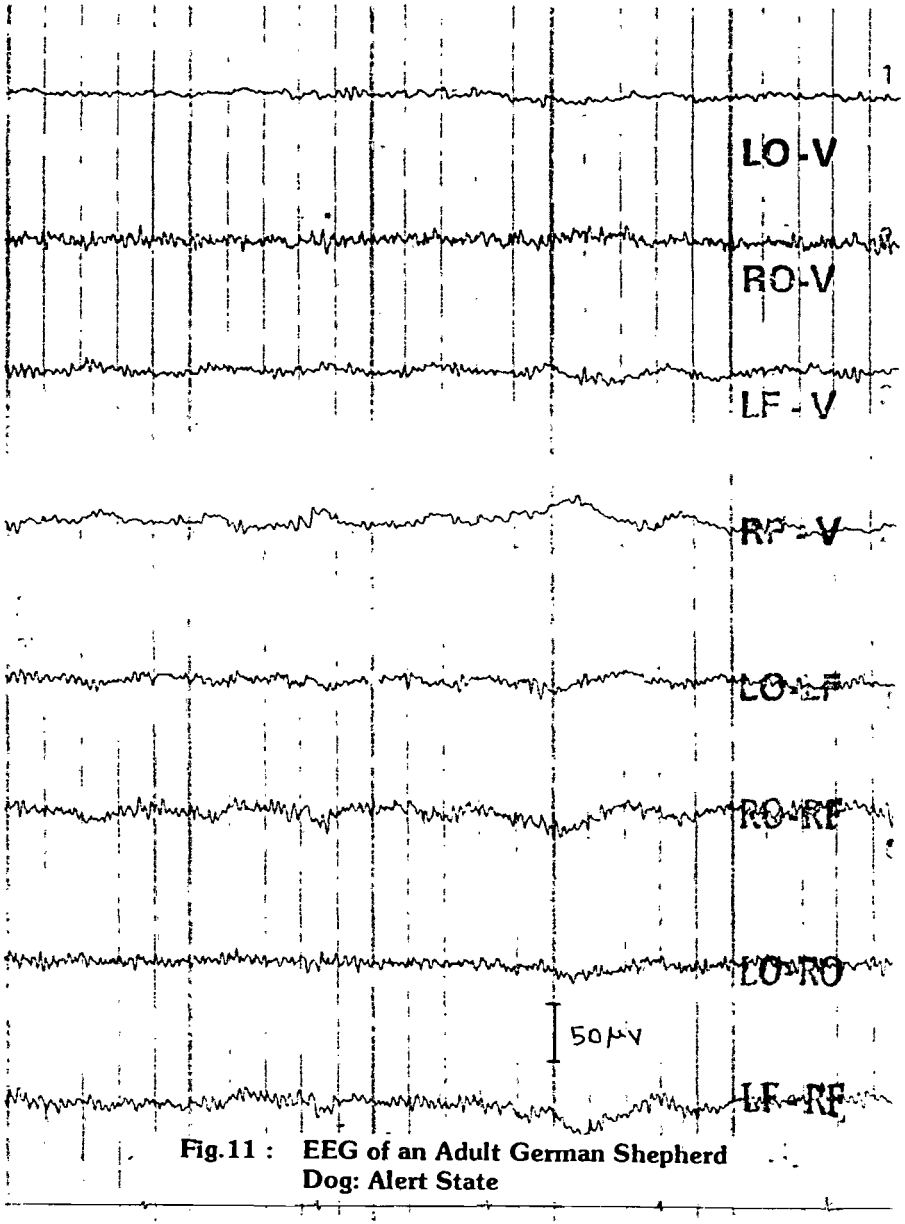

Fig.11 : EEG of an Adult German Shepherd Dog: Alert State

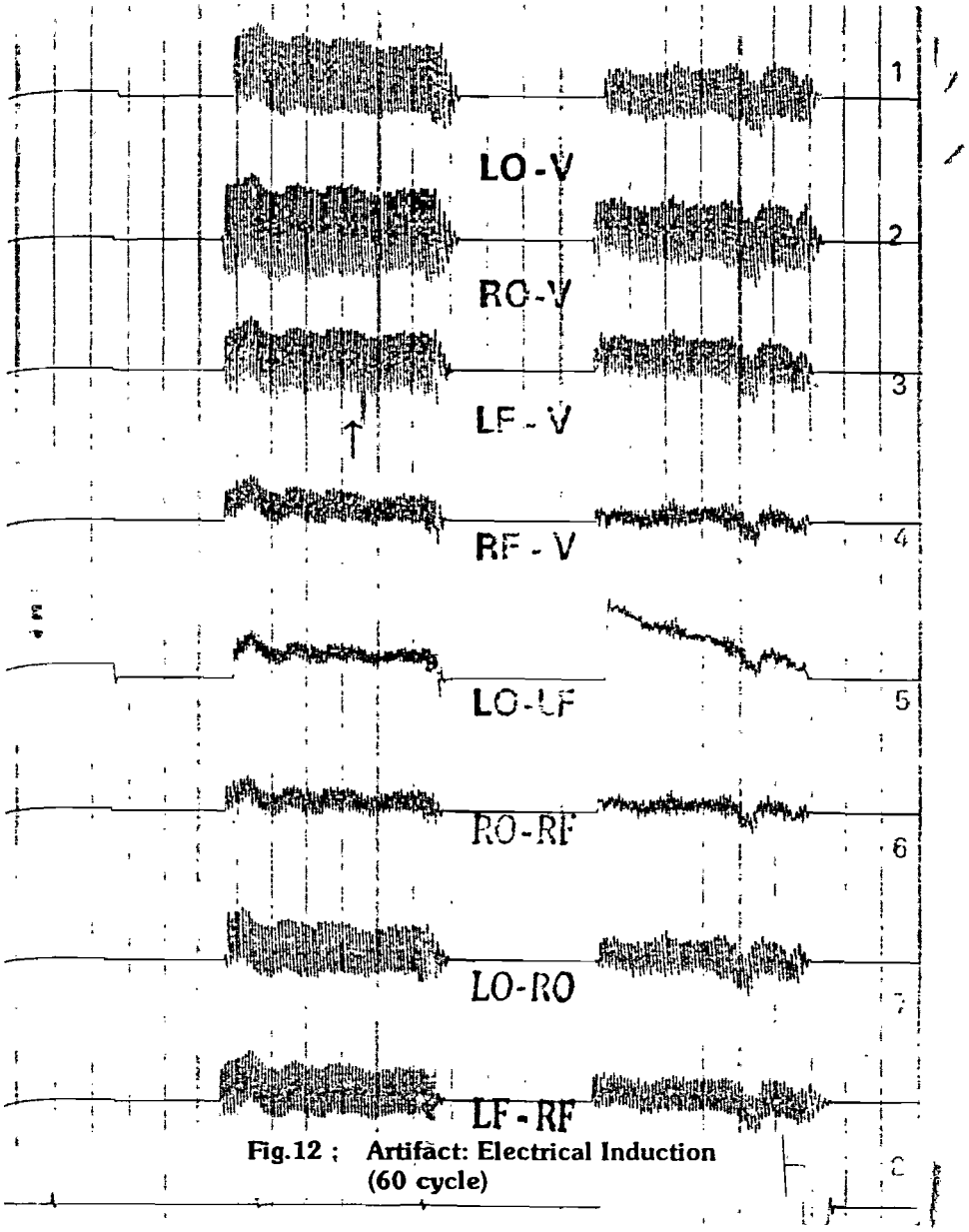

Fig.12 ; Artifact: Electrical Induction (60 cycle)

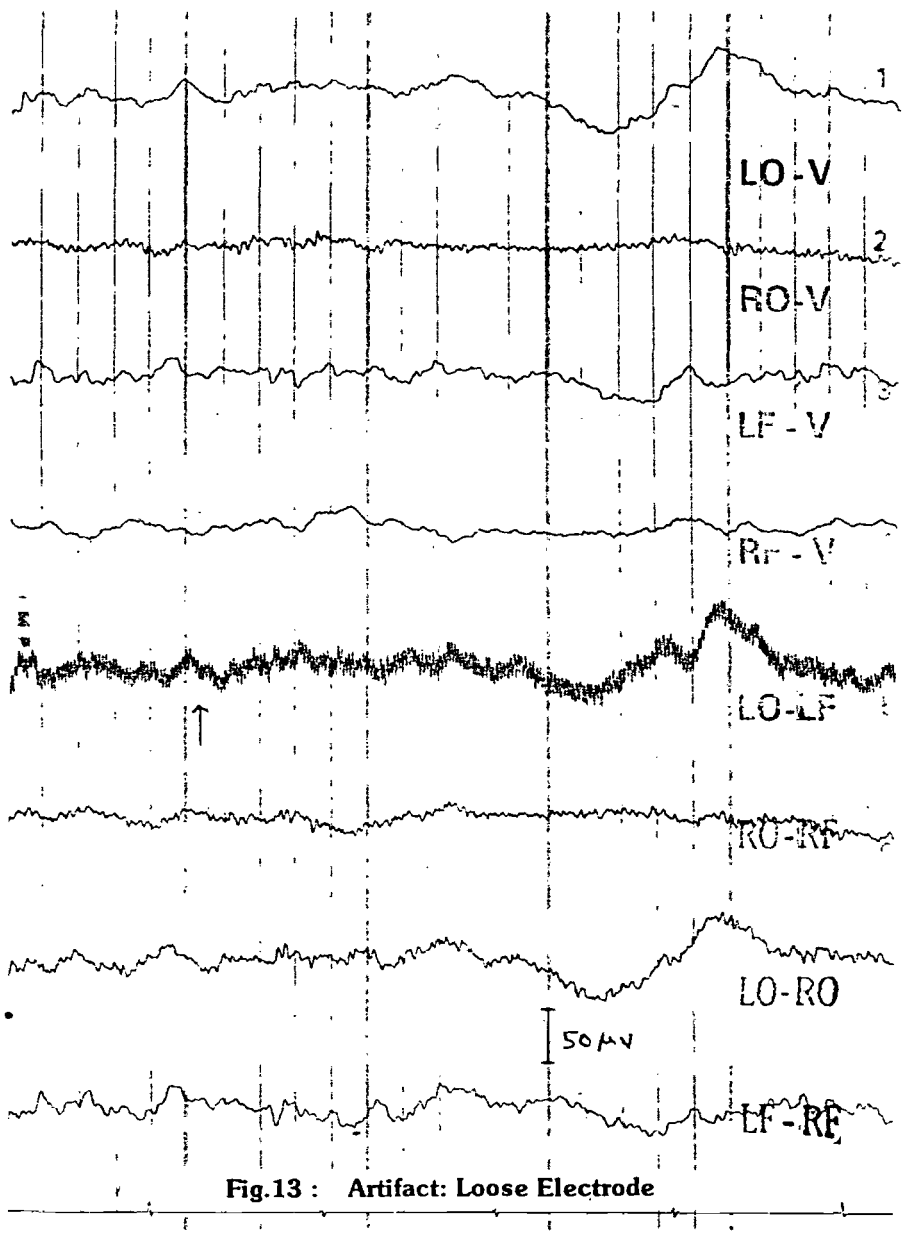

Fig.13 : Artifact: Loose Electrode

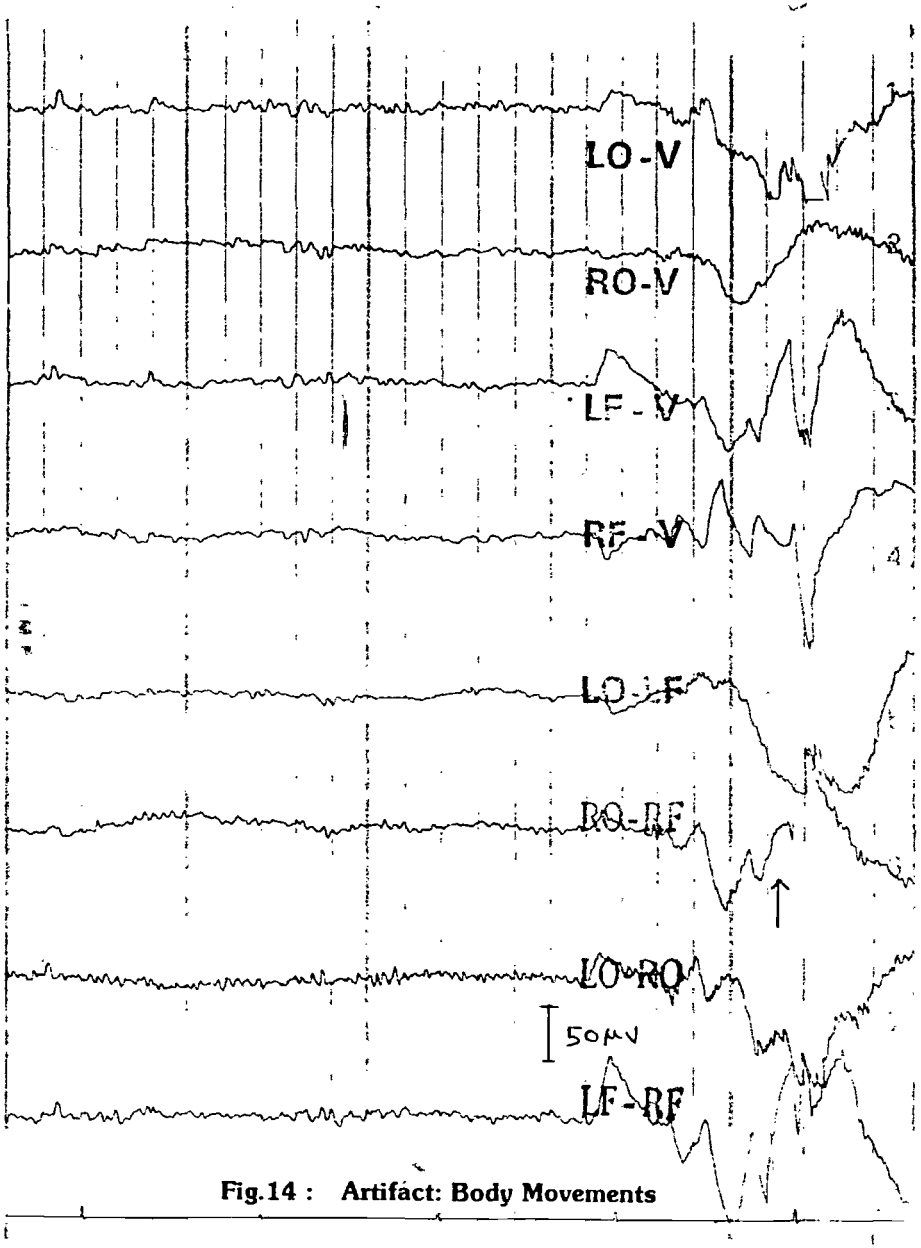

Fig.14 : Artifact: Body Movements

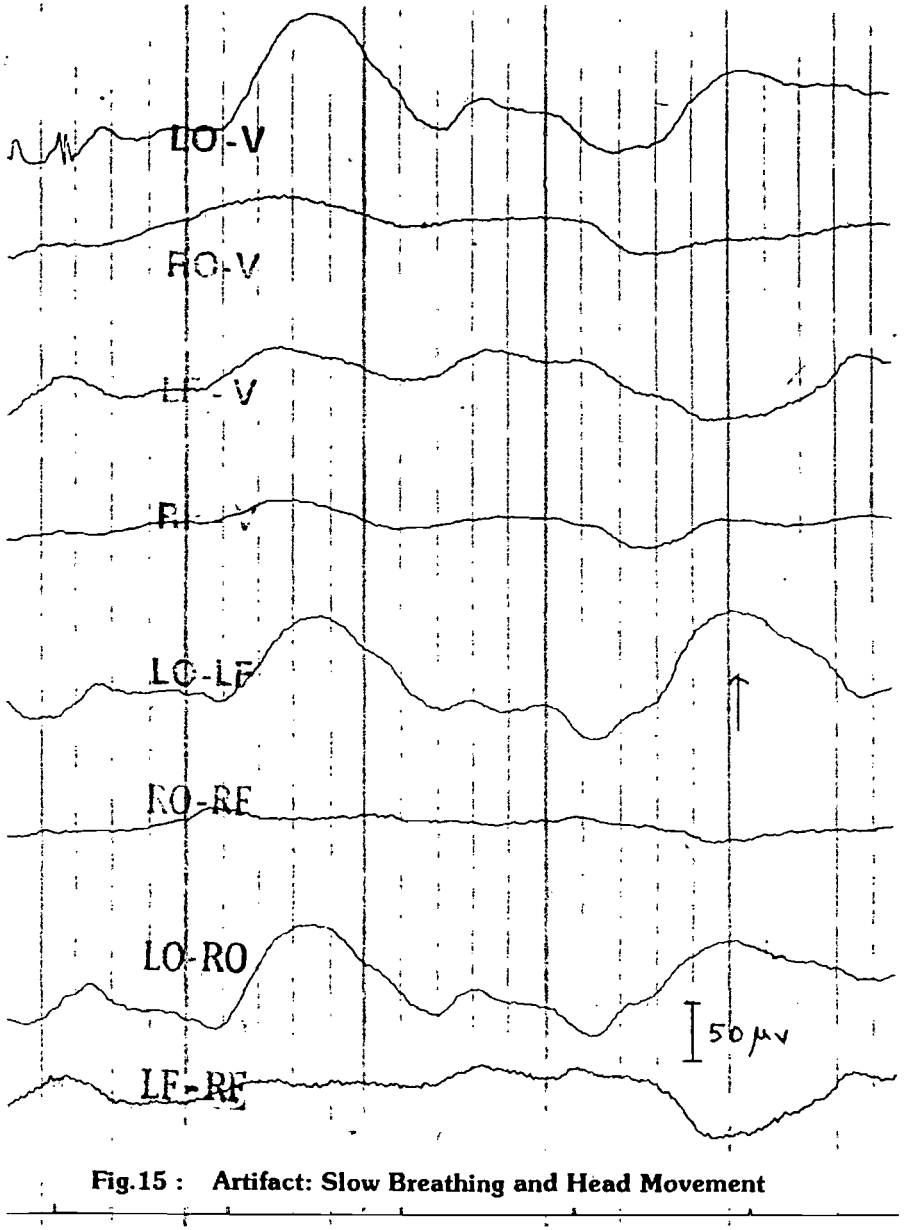

Fig.15 : Artifact: Slow Breathing and Head Movement

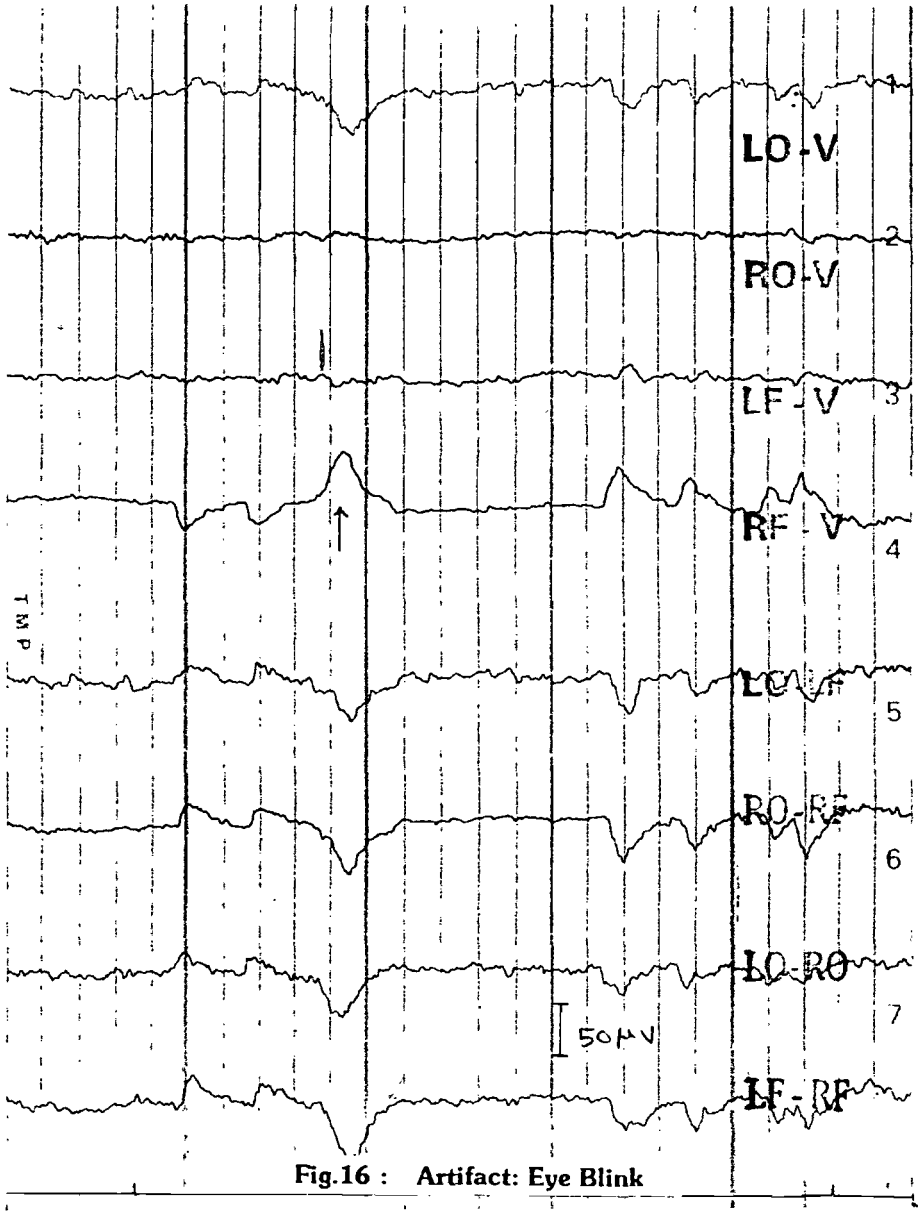

Fig.16 : Artifact: Eye Blink

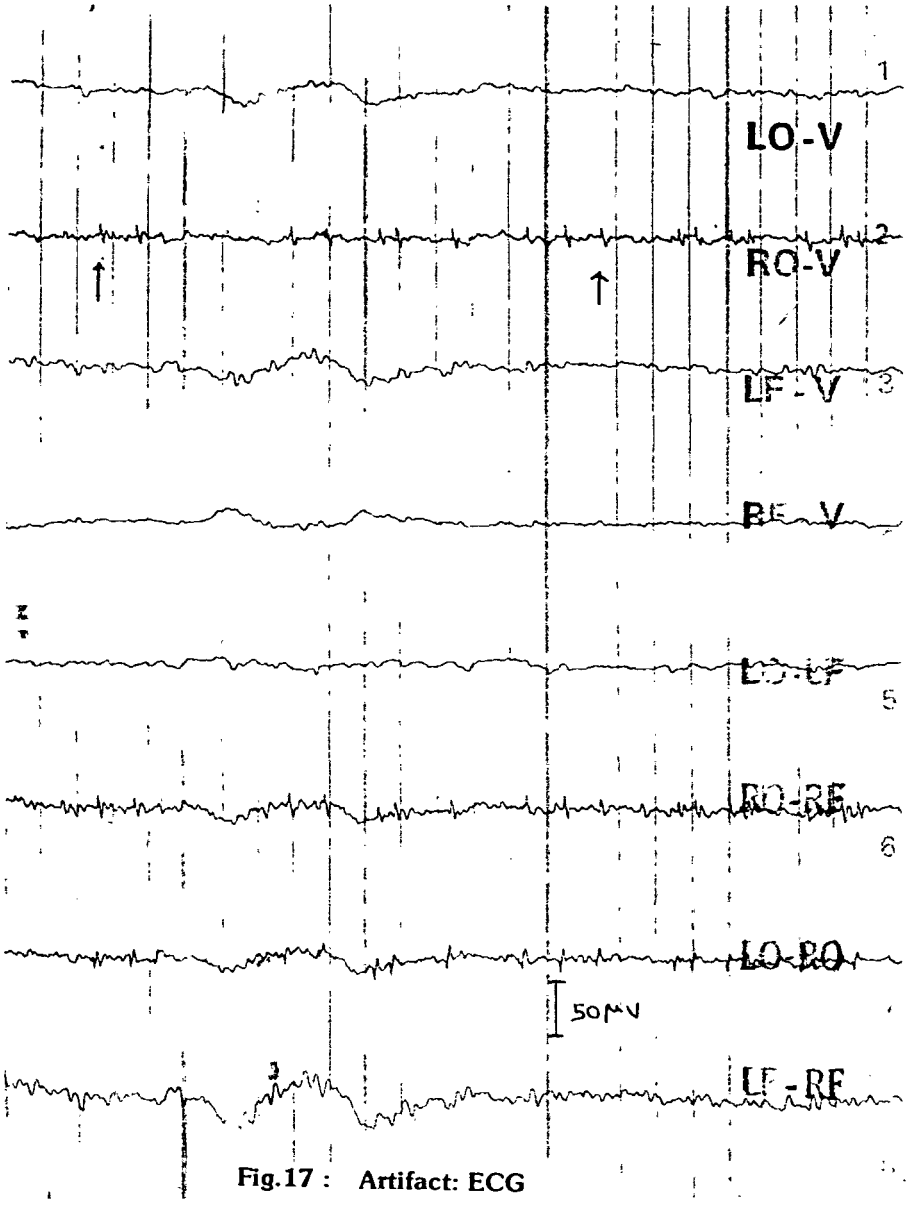

Fig.17 : Artifact: ECG

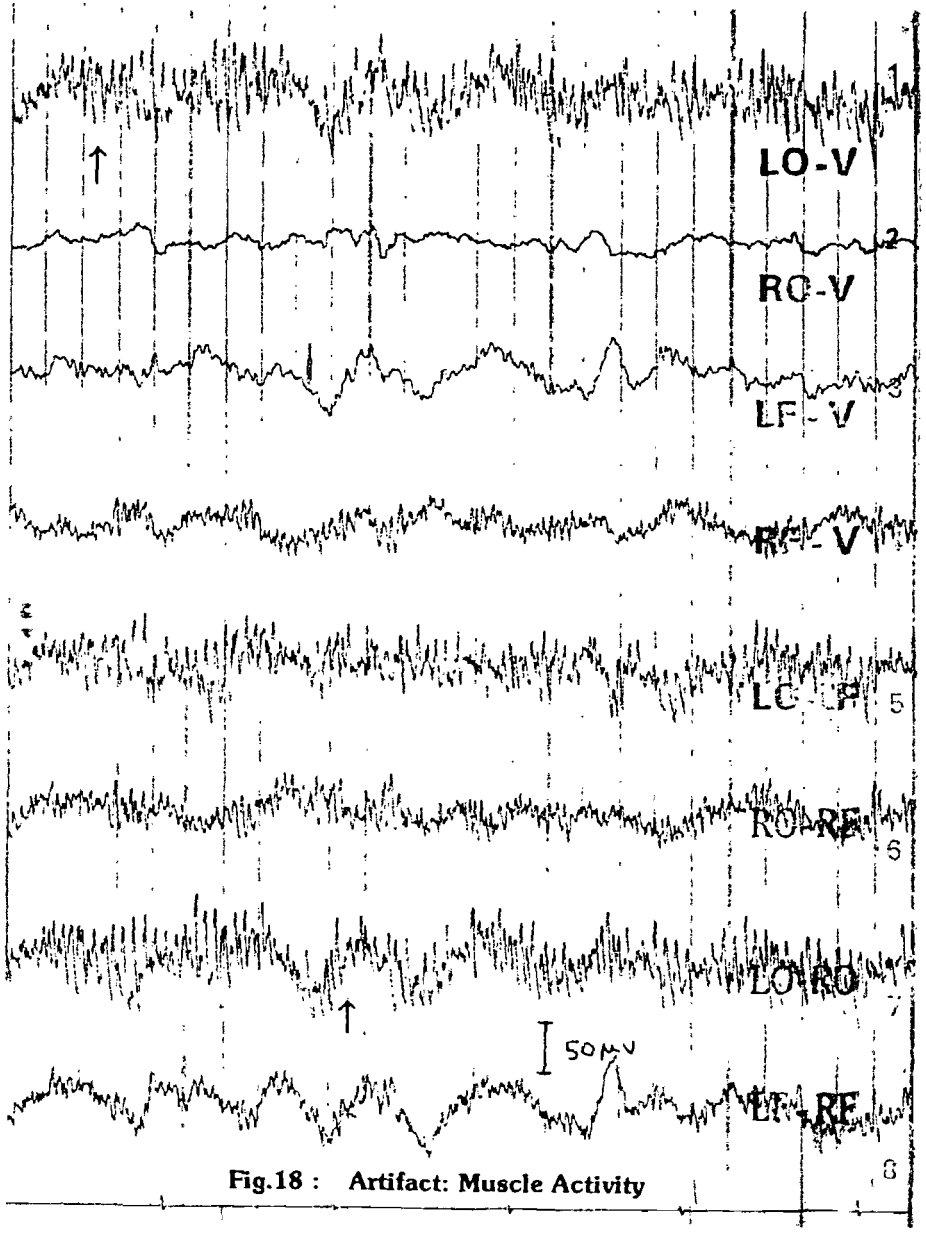

Fig.18 : Artifact: Muscle Activity

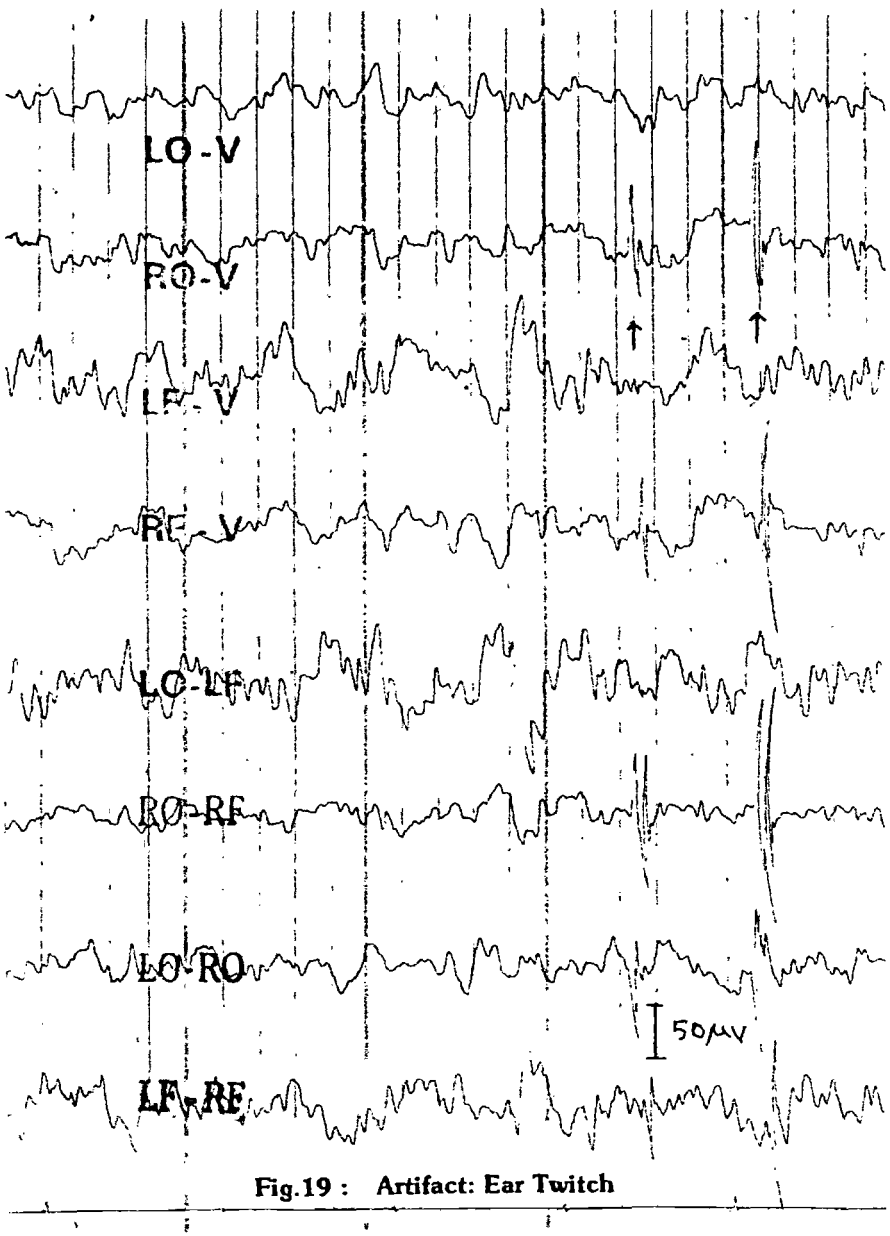

Fig.19 : Artifact: Ear Twitch

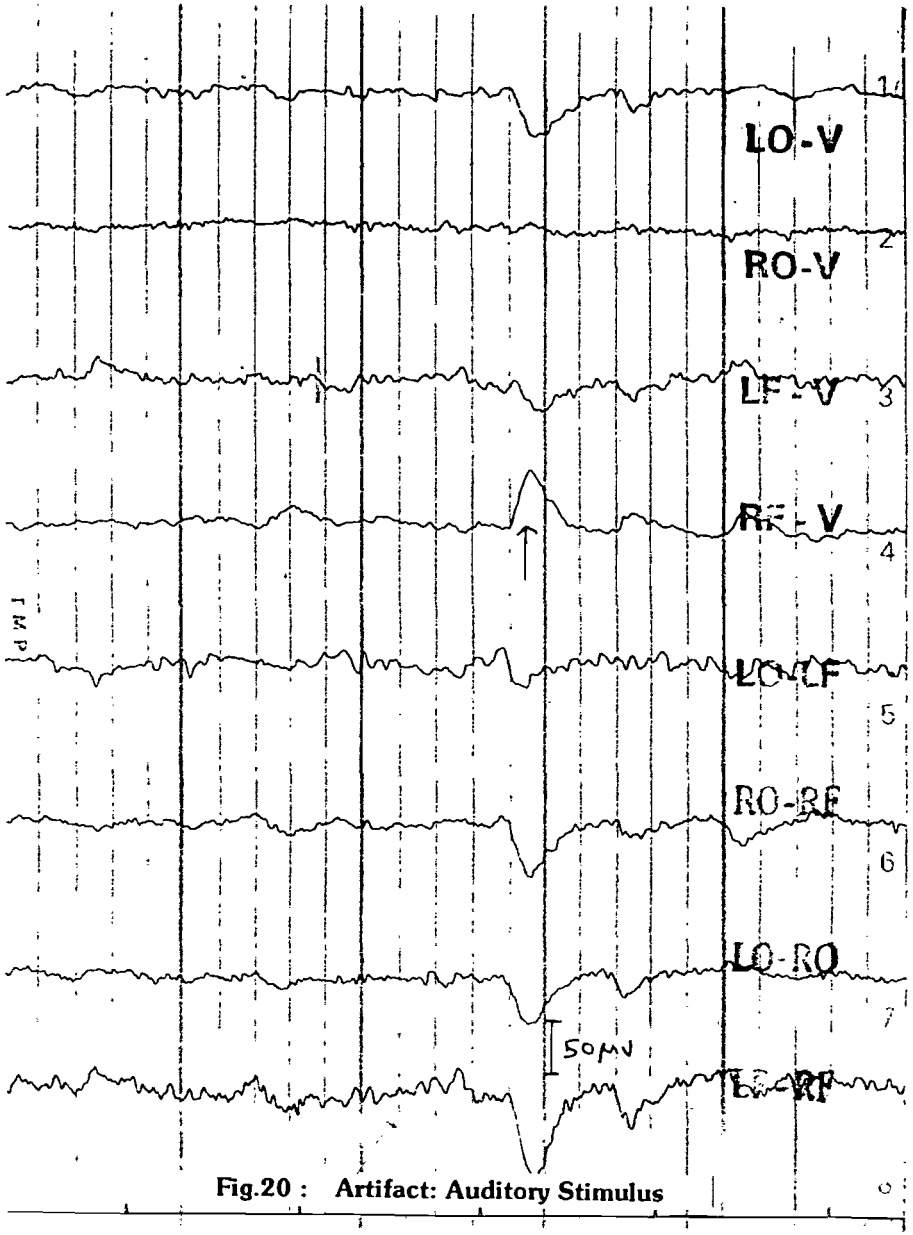

Fig.20 : Artifact: Auditory Stimulus

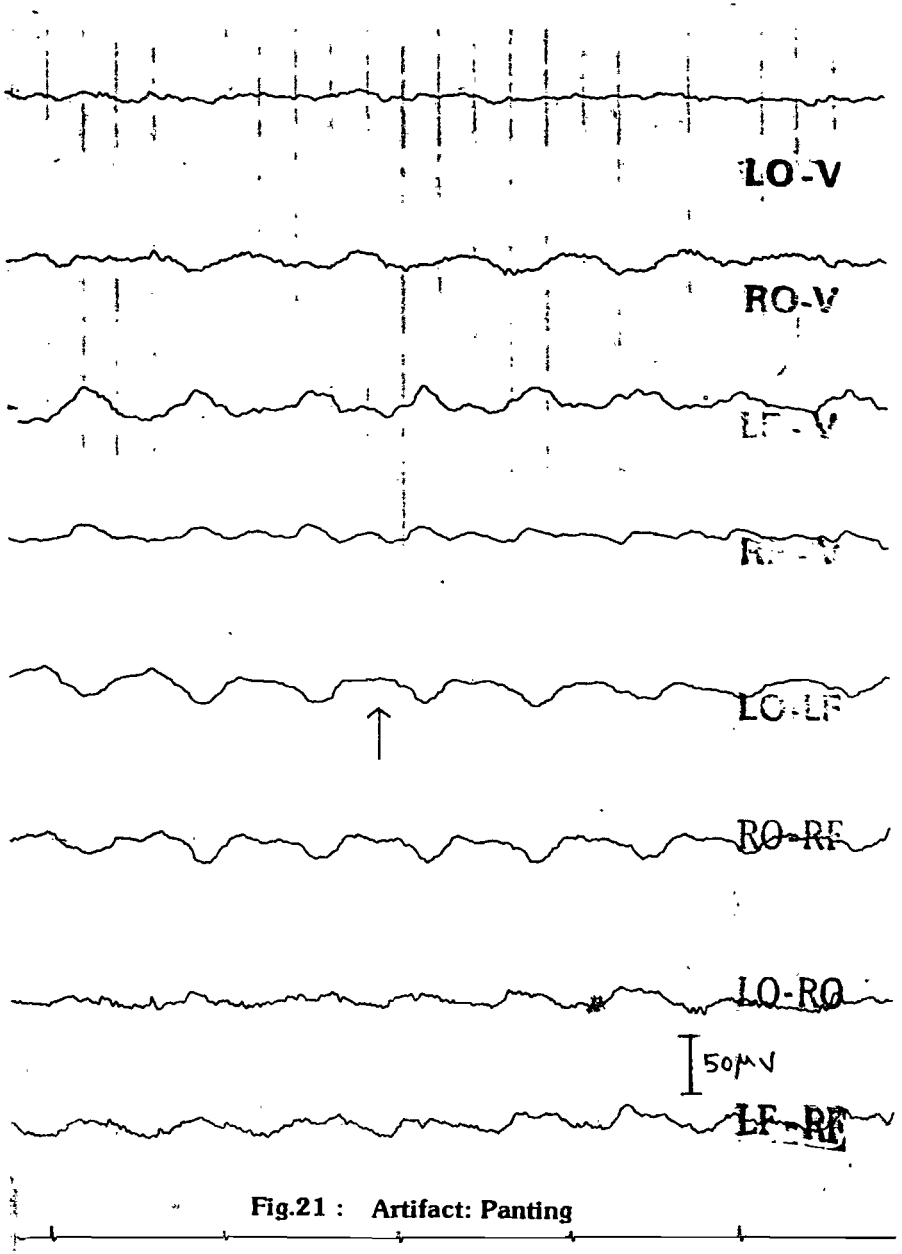

Fig.21 : Artifact: Panting

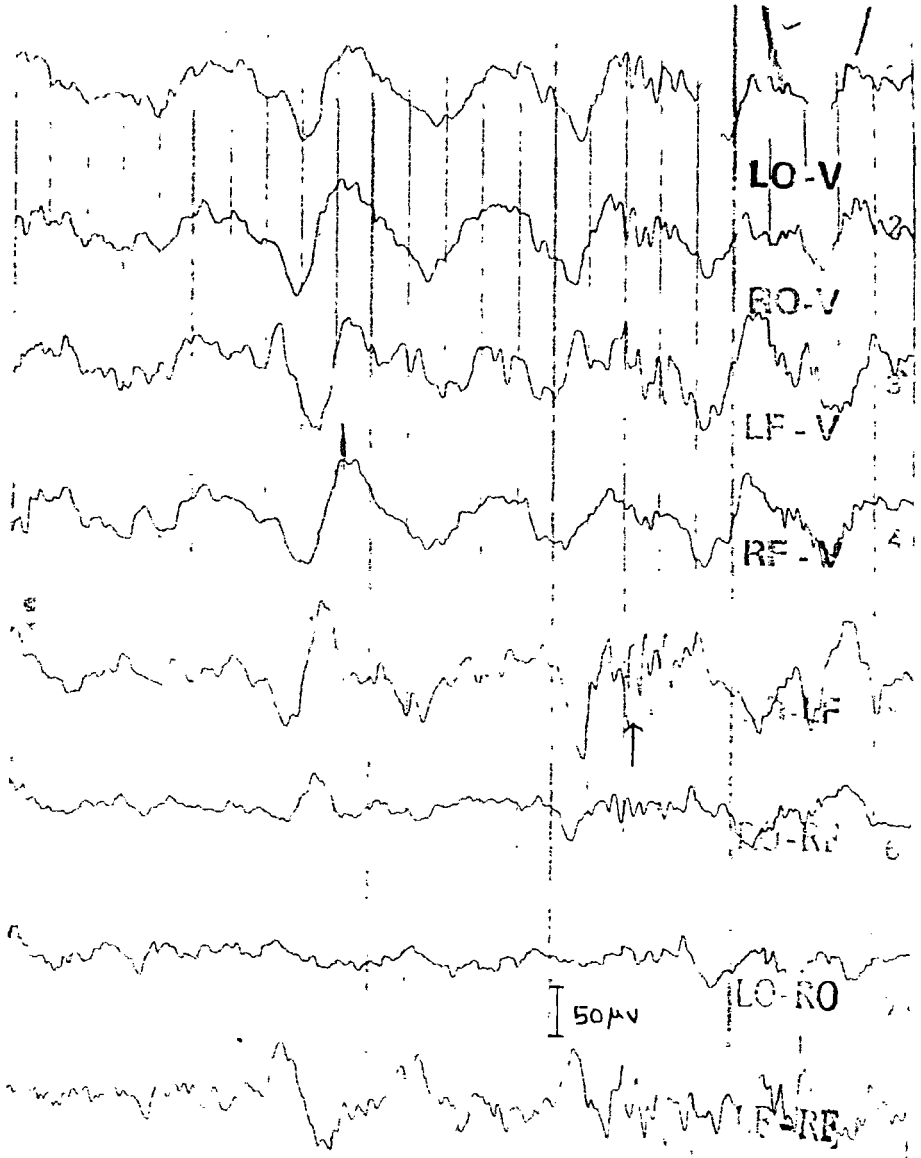

Fig.22 : Effect of Xylazine Sedation

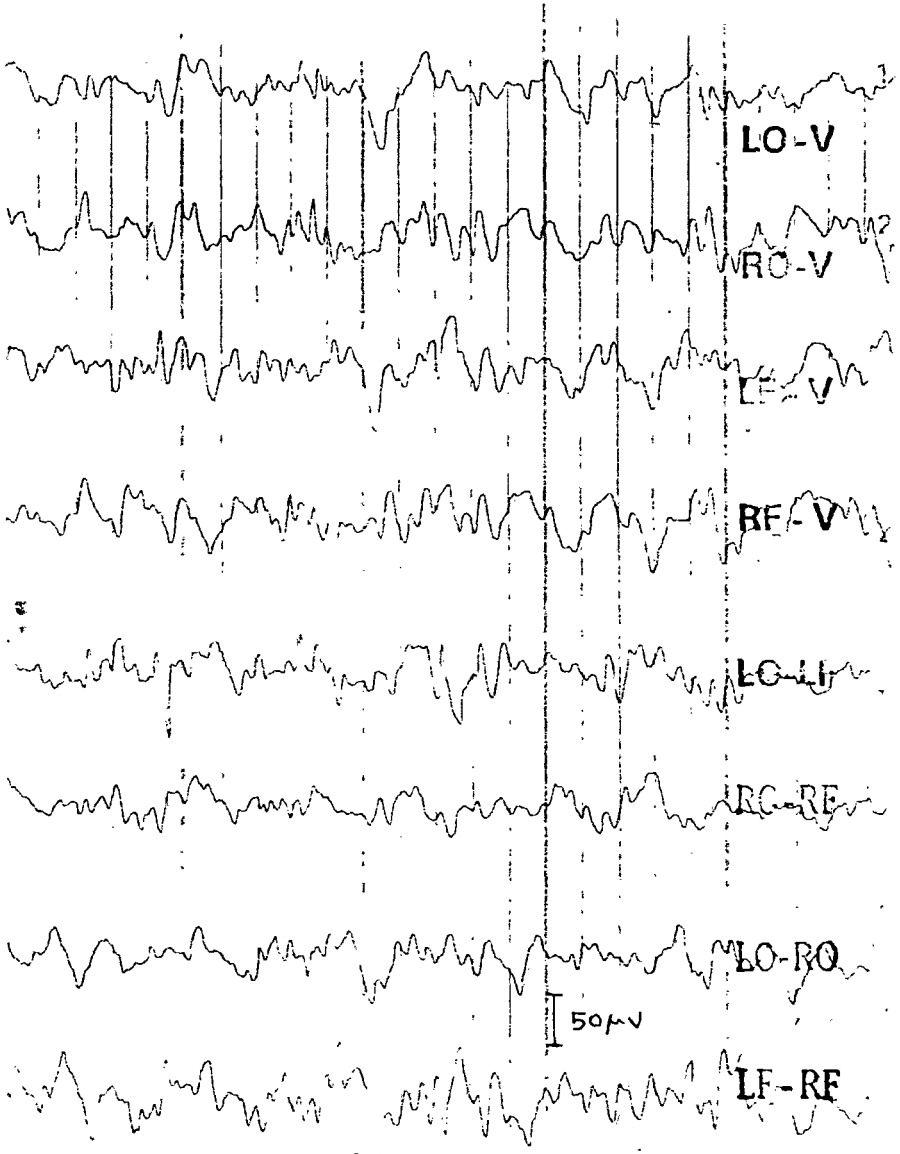

Fig.23 : Effect of Barbiturate Anaesthesia

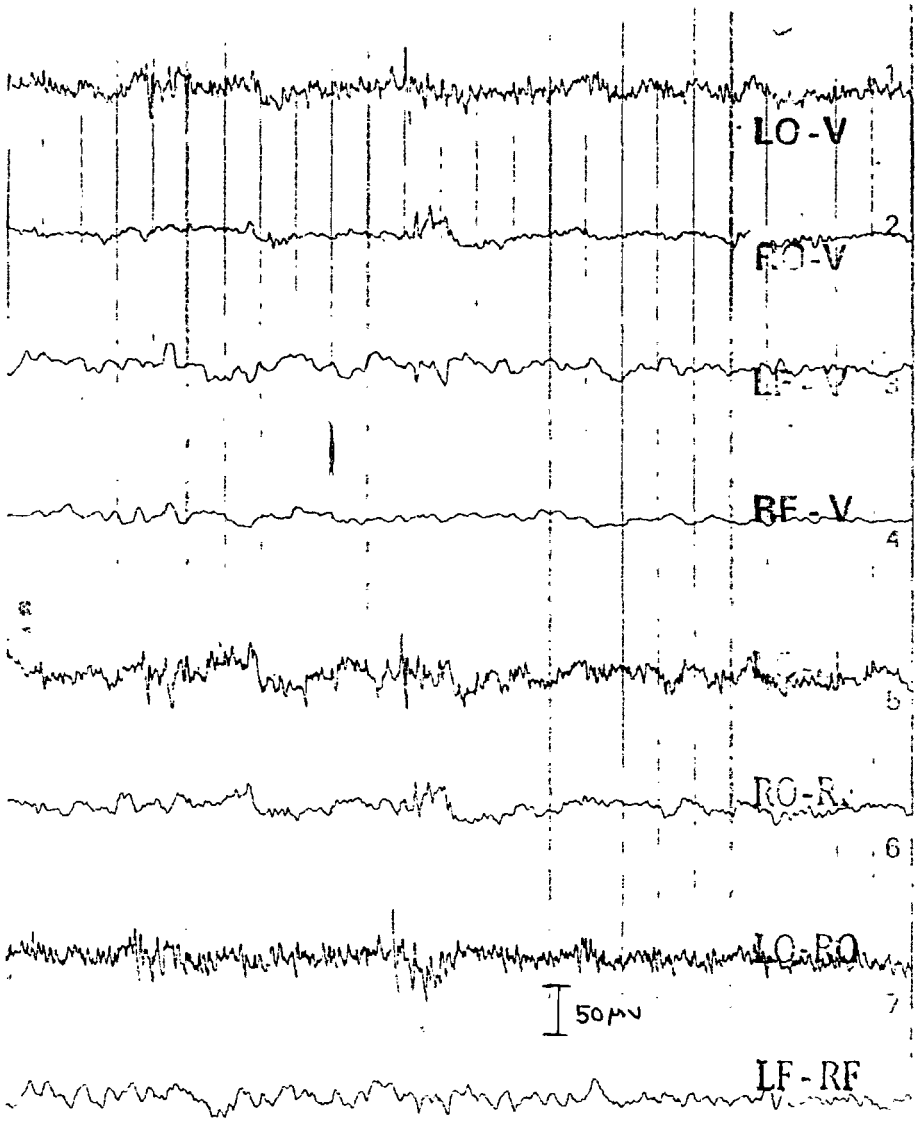

Fig.24 : EEG in CDE: Spitz, 2 years, Asymmetry and spikes

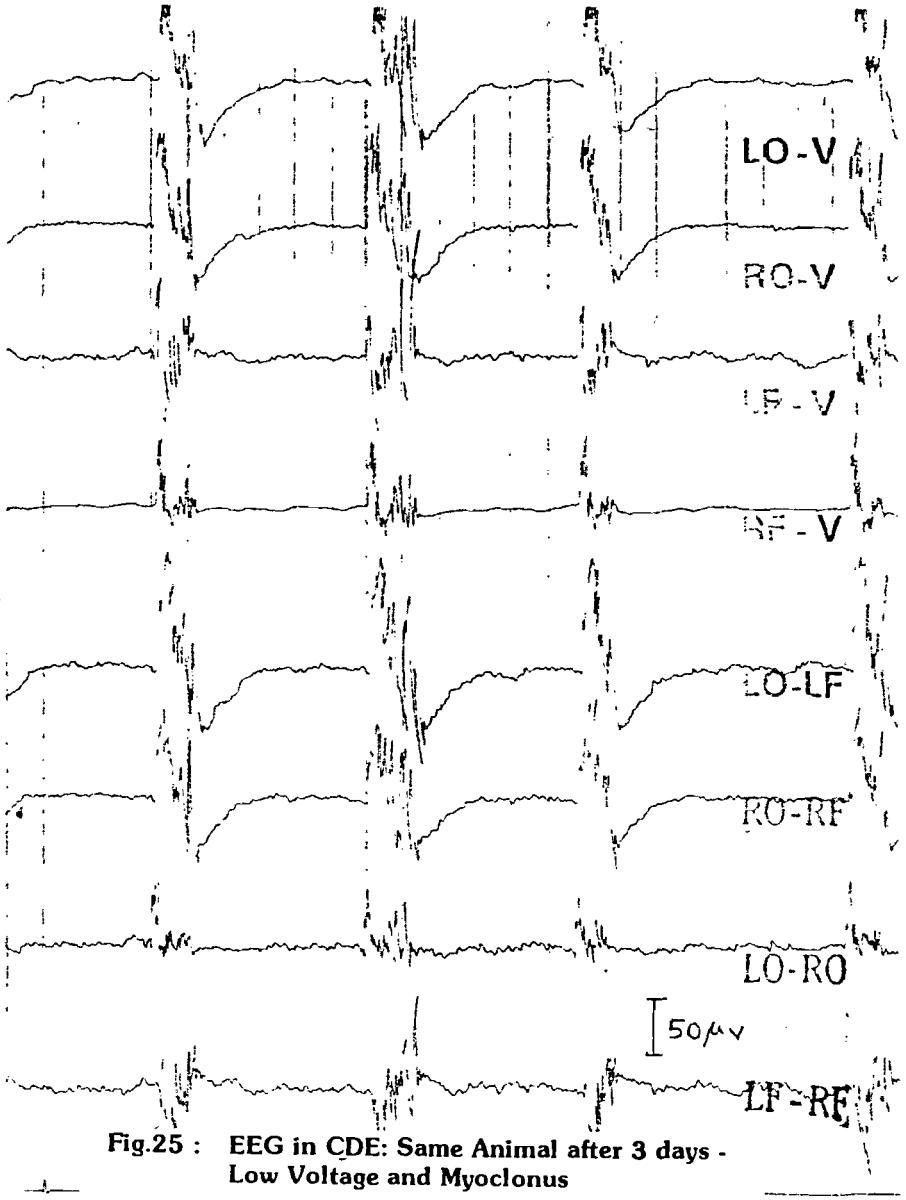

Fig.25 : EEG in CDE: Same Animal after 3 days - Low Voltage and Myoclonus

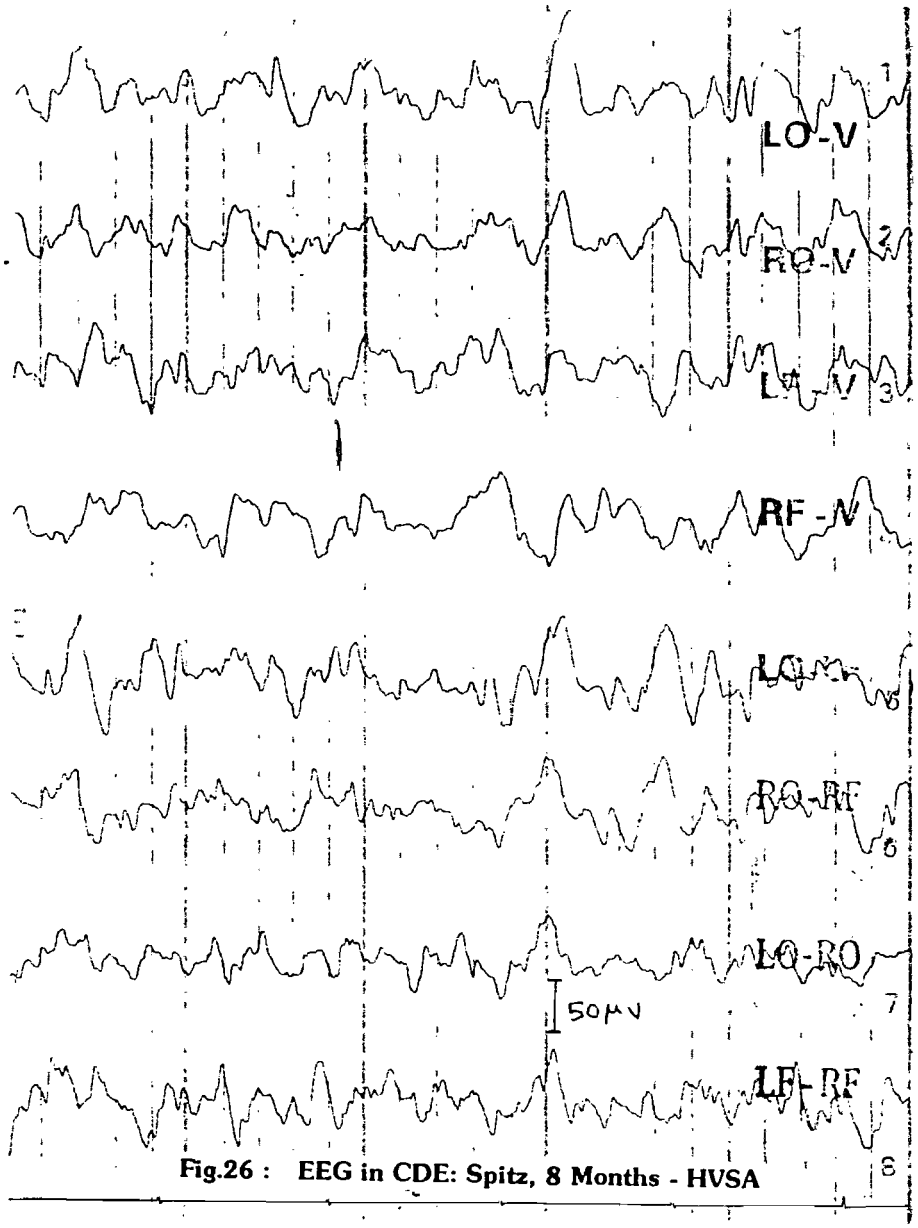

Fig.26 : EEG in CDE: Spitz, 8 Months - HVSA

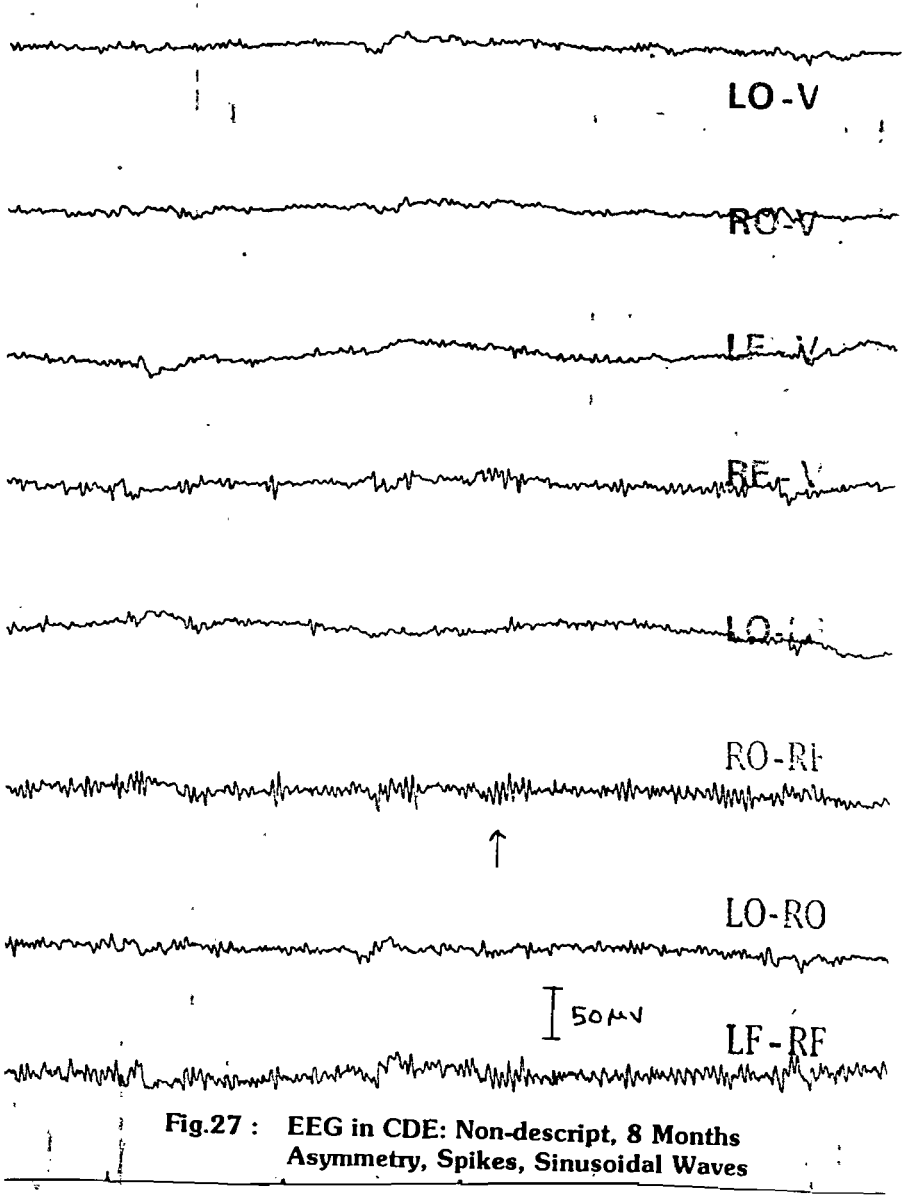

Fig.27 : EEG in CDE: Non-descript, 8 Months
Asymmetry, Spikes, Sinusoidal Waves

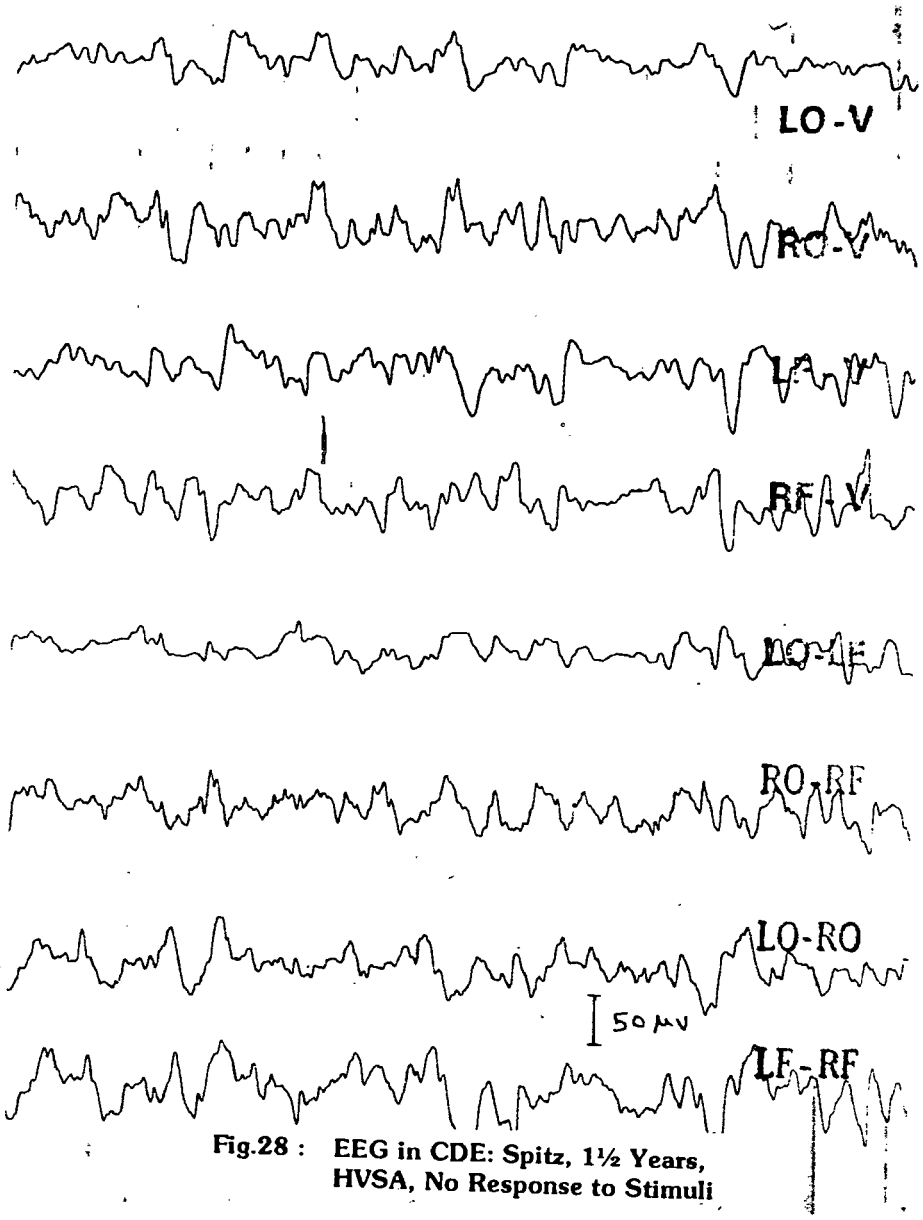

Fig.28 : EEG in CDE: Spitz, 1½ Years, HVSA, No Response to Stimuli

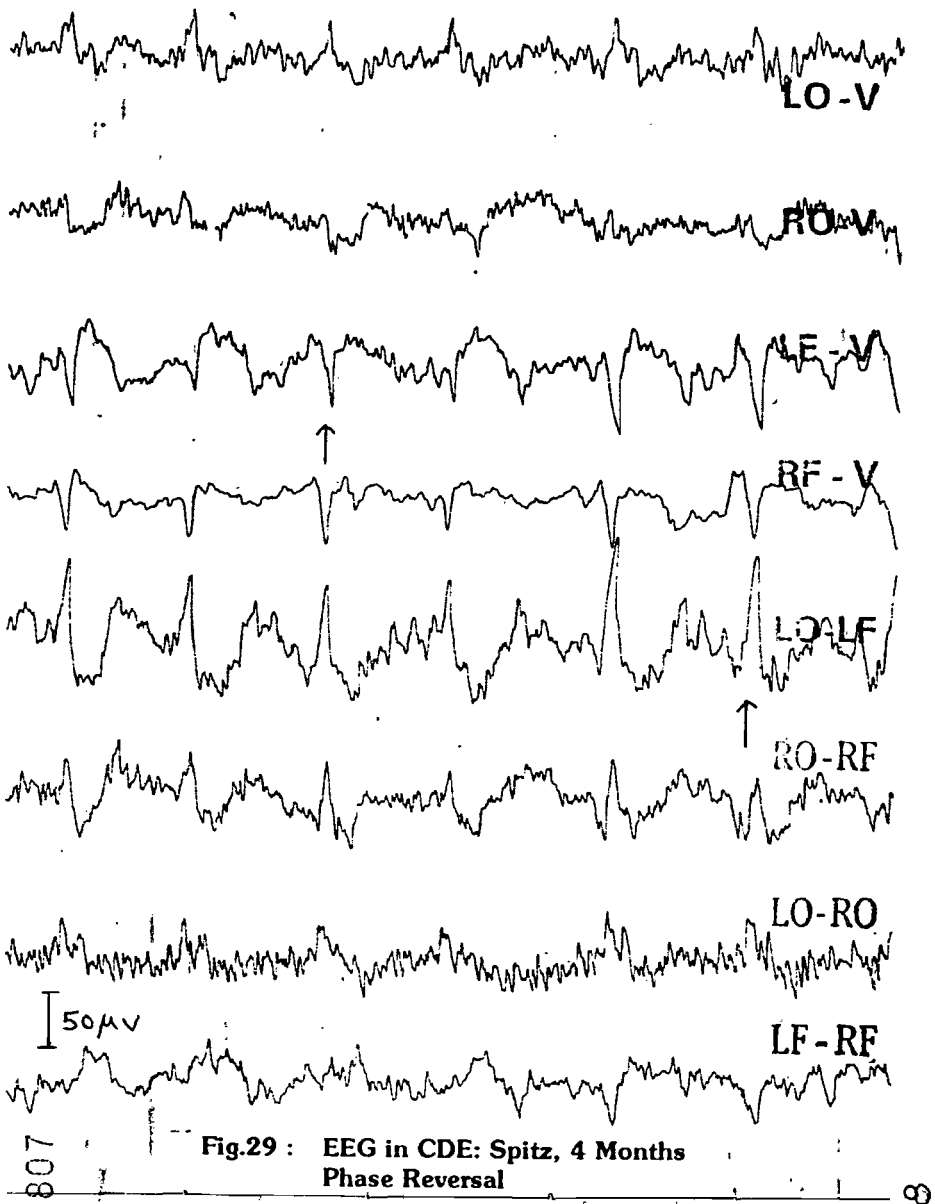

Fig.29 : EEG in CDE: Spitz, 4 Months Phase Reversal

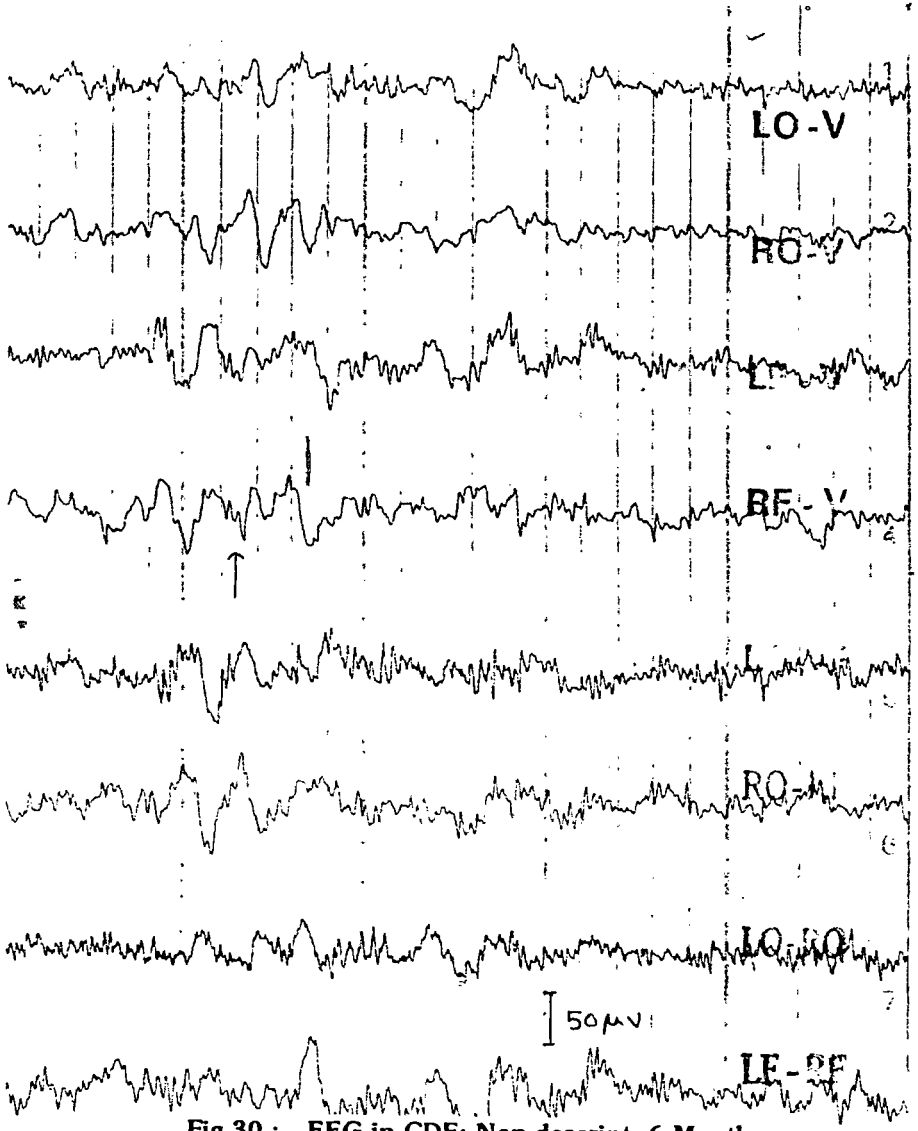

Fig.30 : EEG in CDE: Non-descript, 6 Months Asymmetry, Sinusoidal Waves and Slowing

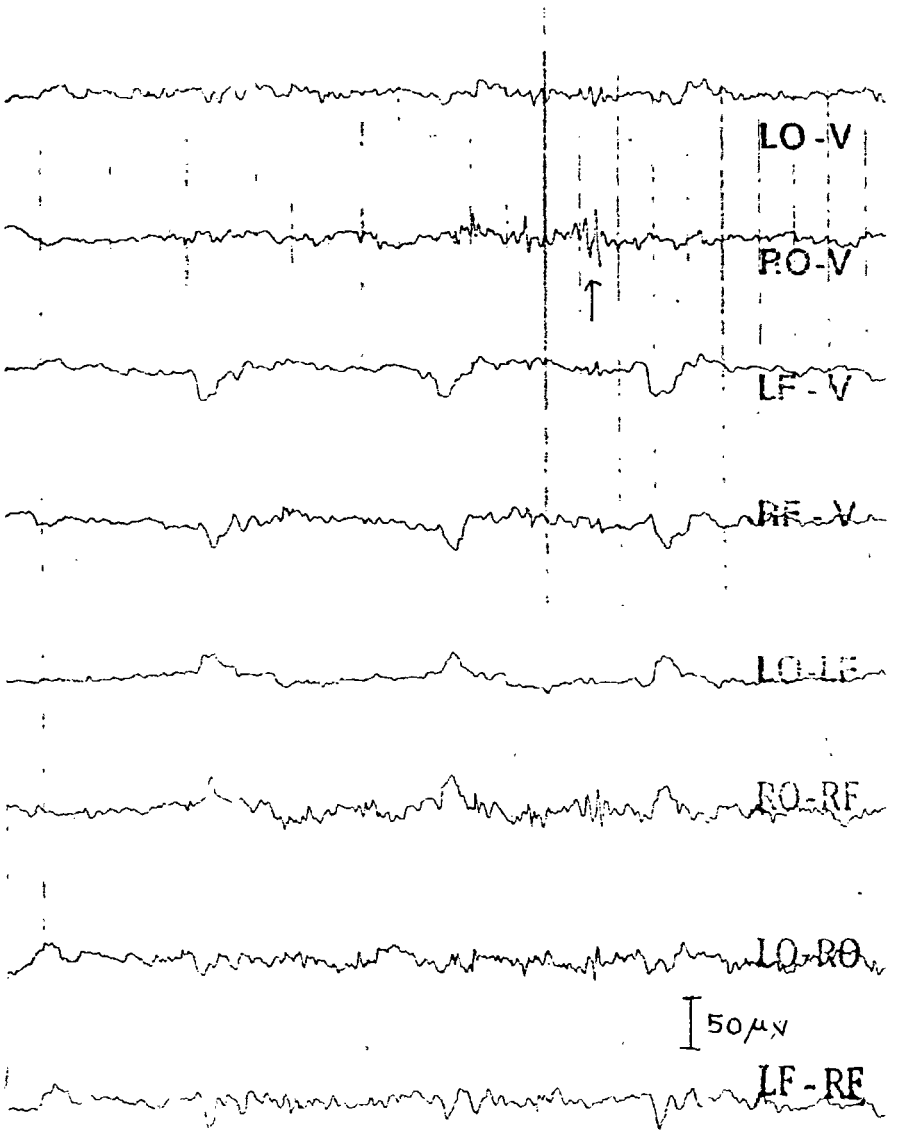

Fig.31 : EEG in CDE: Dalmation, 3 Years, Asymmetry and Spikes

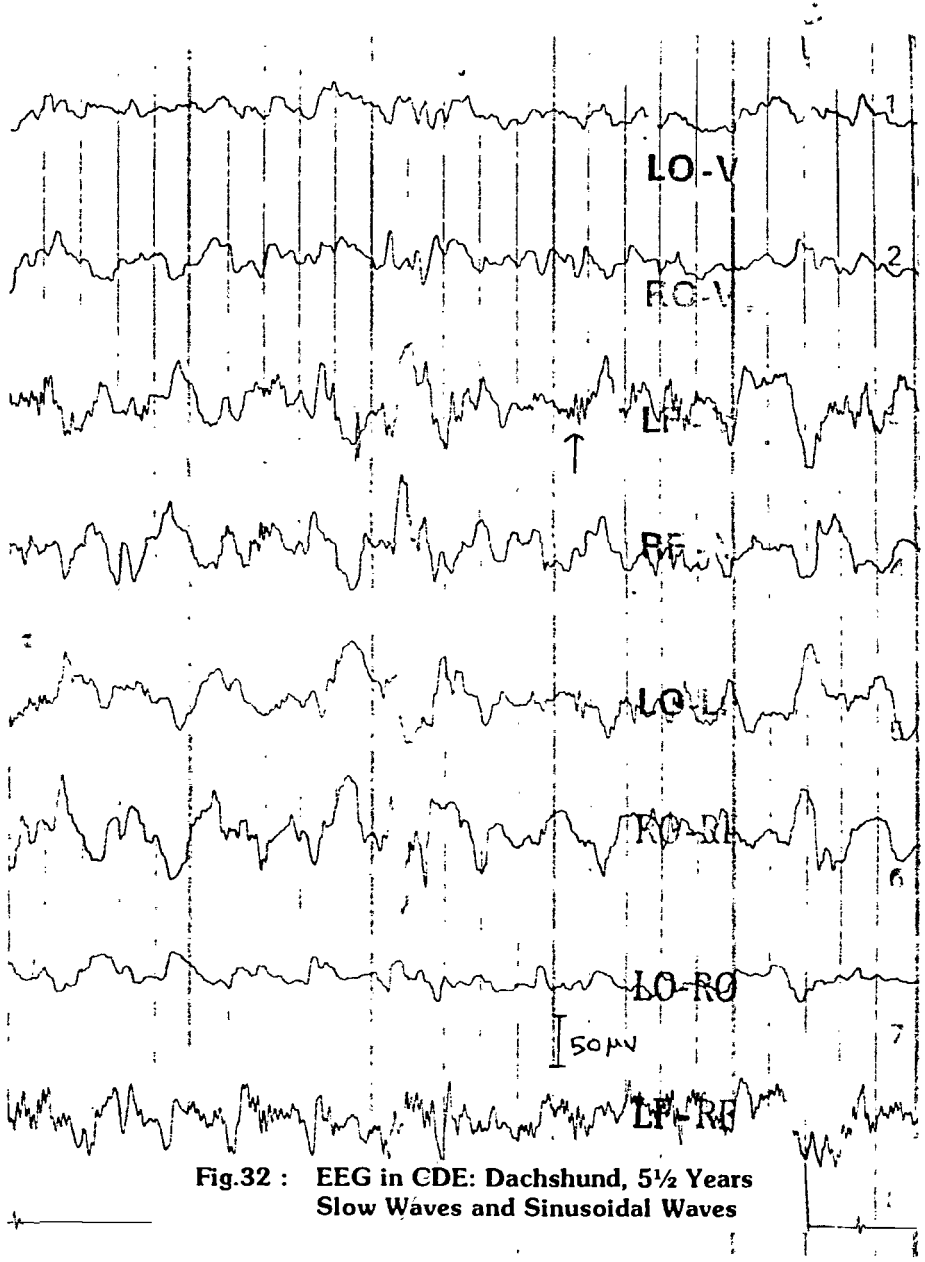

Fig.32 : EEG in CDE: Dachshund, 5½ Years
Slow Waves and Sinusoidal Waves

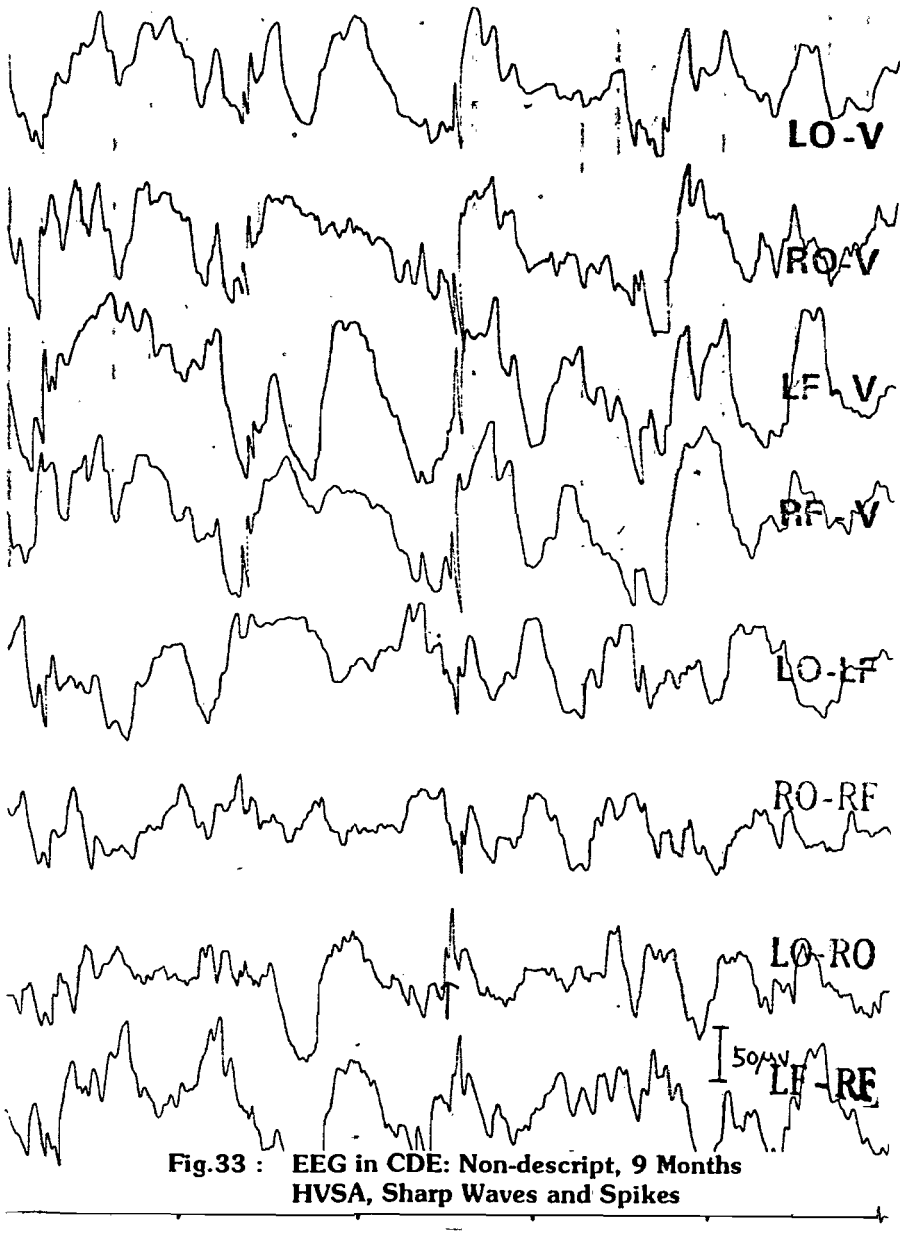

Fig.33 : EEG in CDE: Non-descript, 9 Months
HVSA, Sharp Waves and Spikes

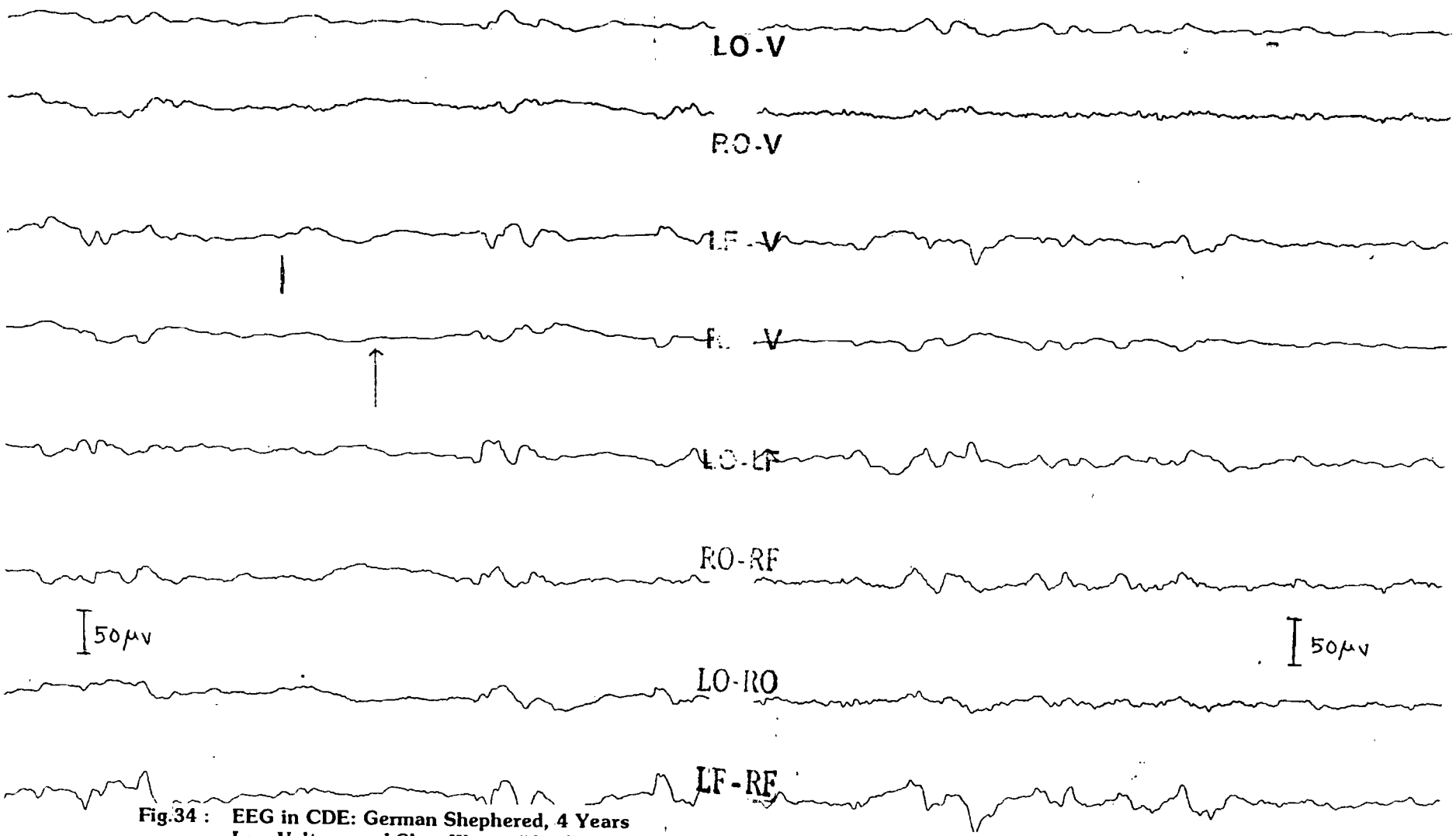

Fig.34 : EEG in CDE: German Shephered, 4 Years Low Voltage and Slow Waves (Needle Electrode Recording)

Fig.35 : EEG in CDE: Same Animal as in Fig.34 (Disc Electrode Recording)

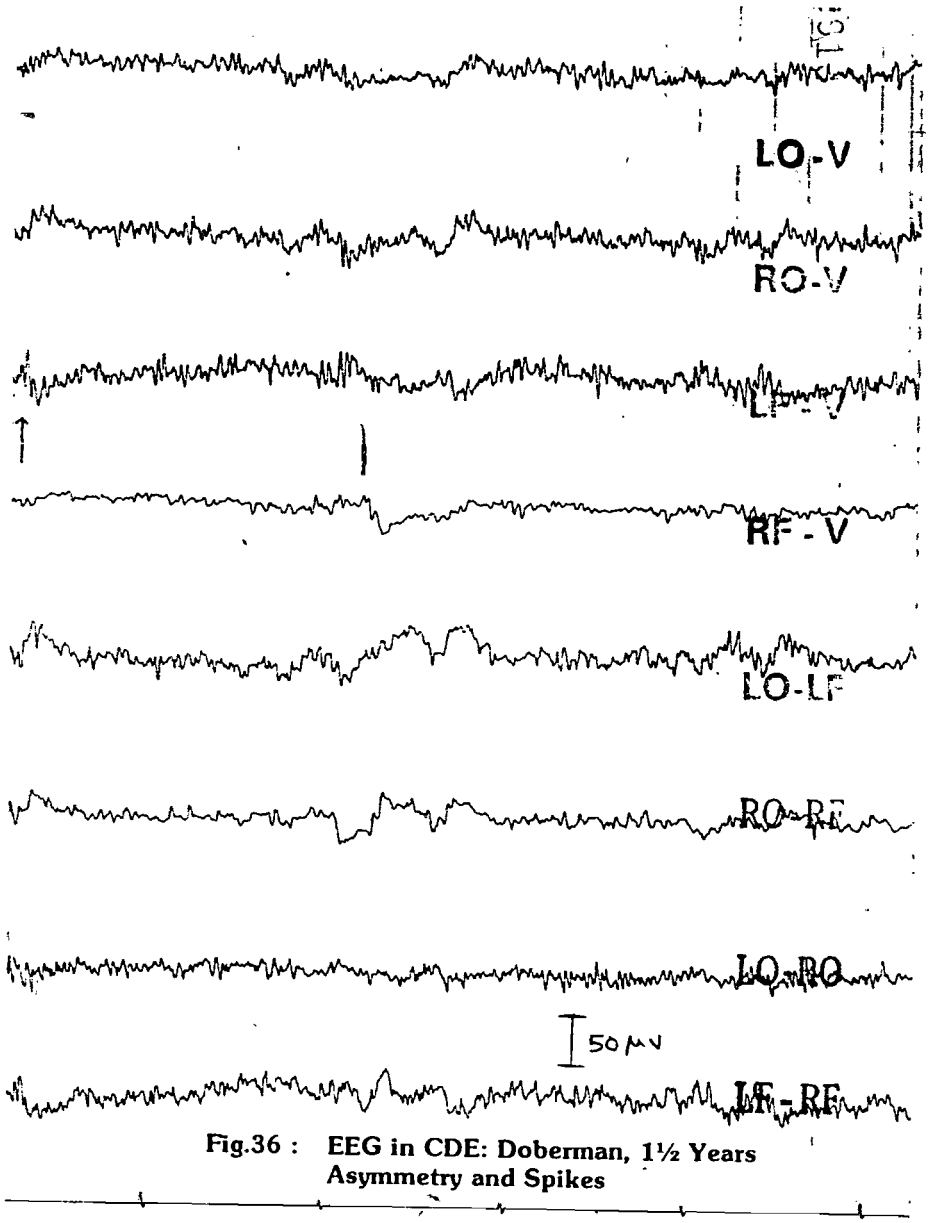

Fig.36 : EEG in CDE: Doberman, 1½ Years
Asymmetry and Spikes

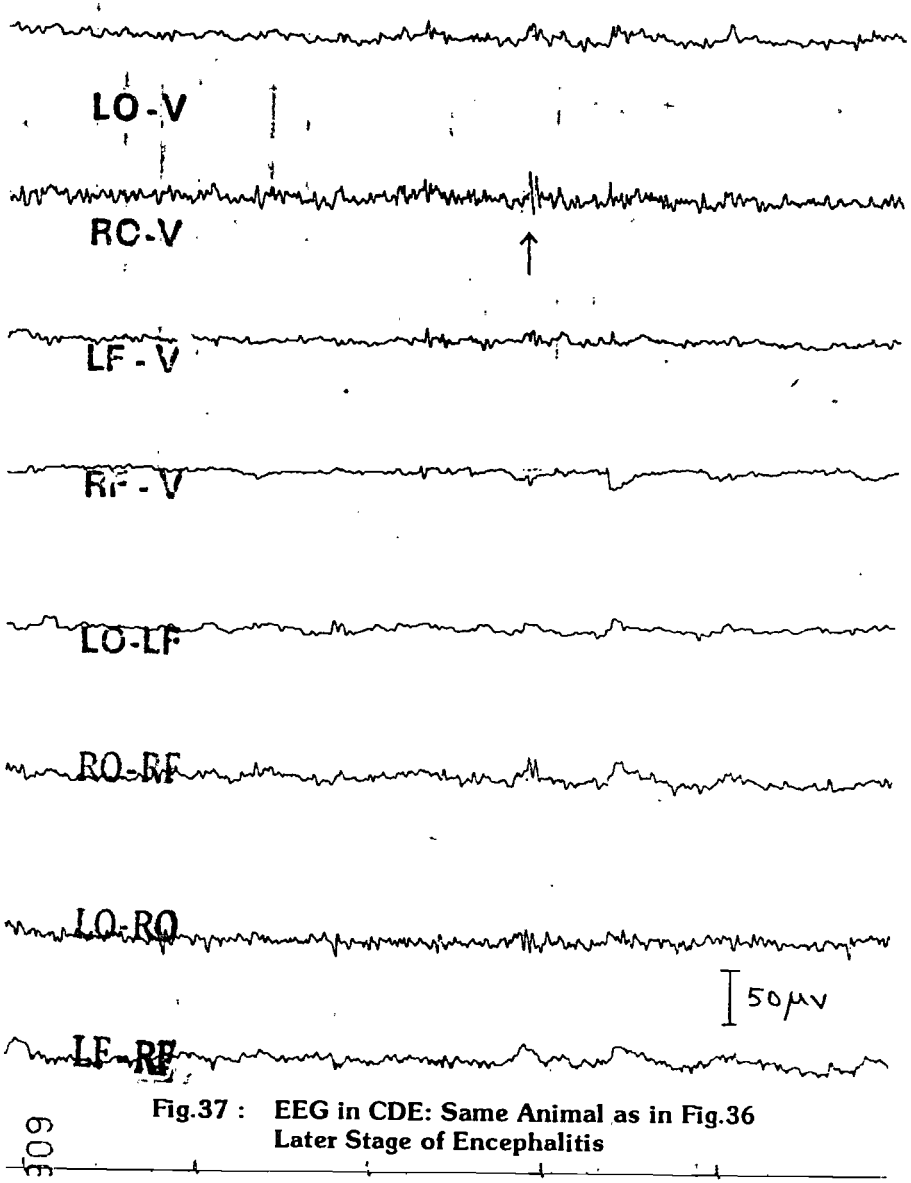

Fig.37 : EEG in CDE: Same Animal as in Fig.36
Later Stage of Encephalitis

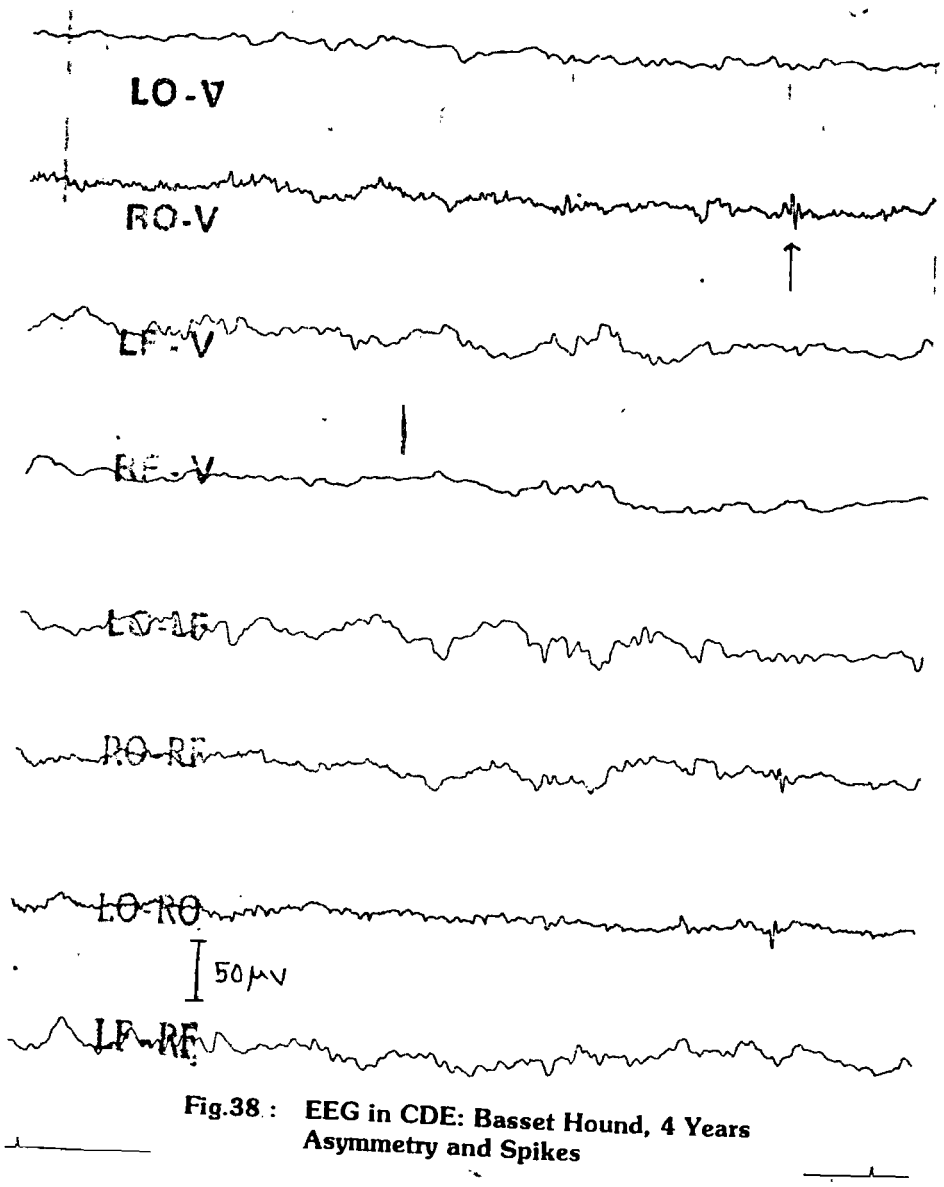

Fig.38 : EEG in CDE: Basset Hound, 4 Years
Asymmetry and Spikes

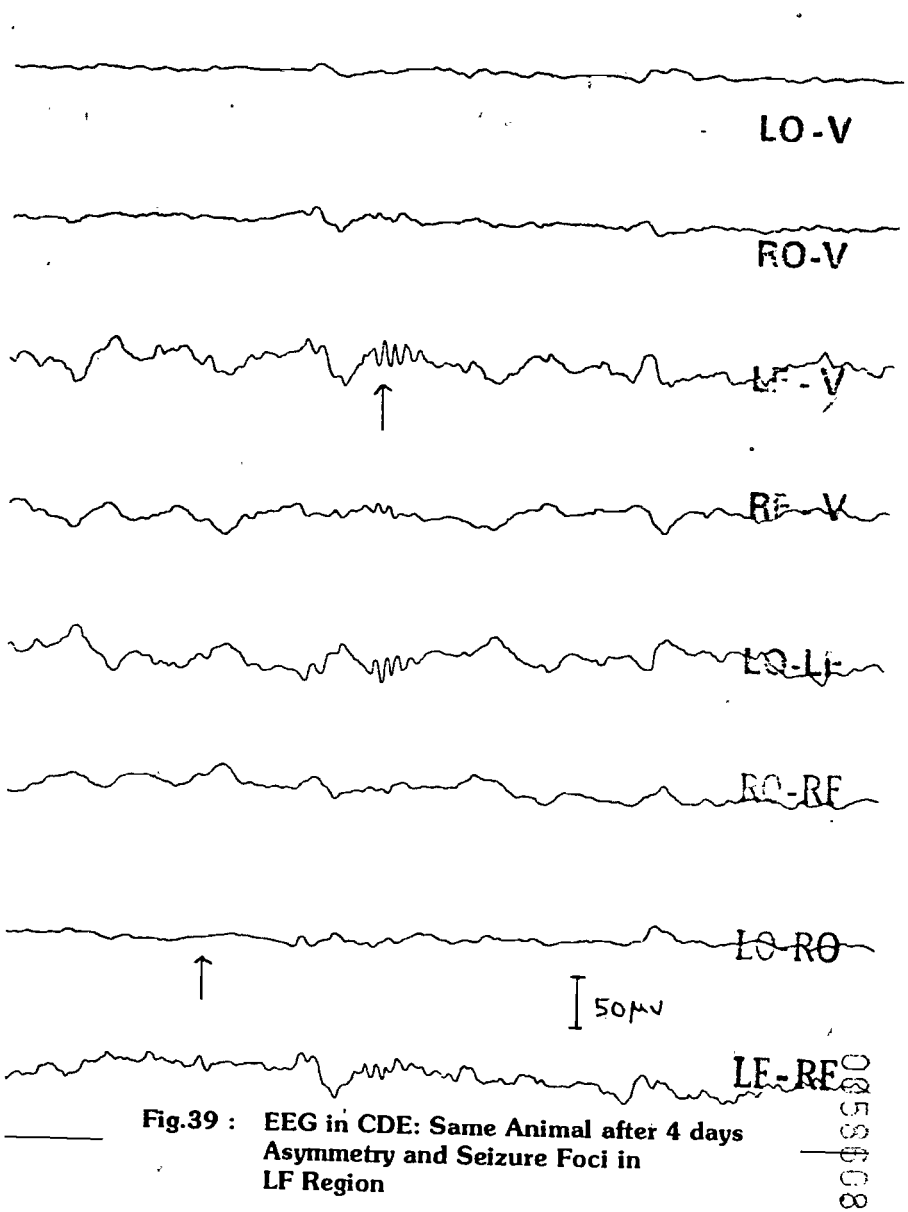

Fig.39 : EEG in CDE: Same Animal after 4 days
Asymmetry and Seizure Foci in
LF Region

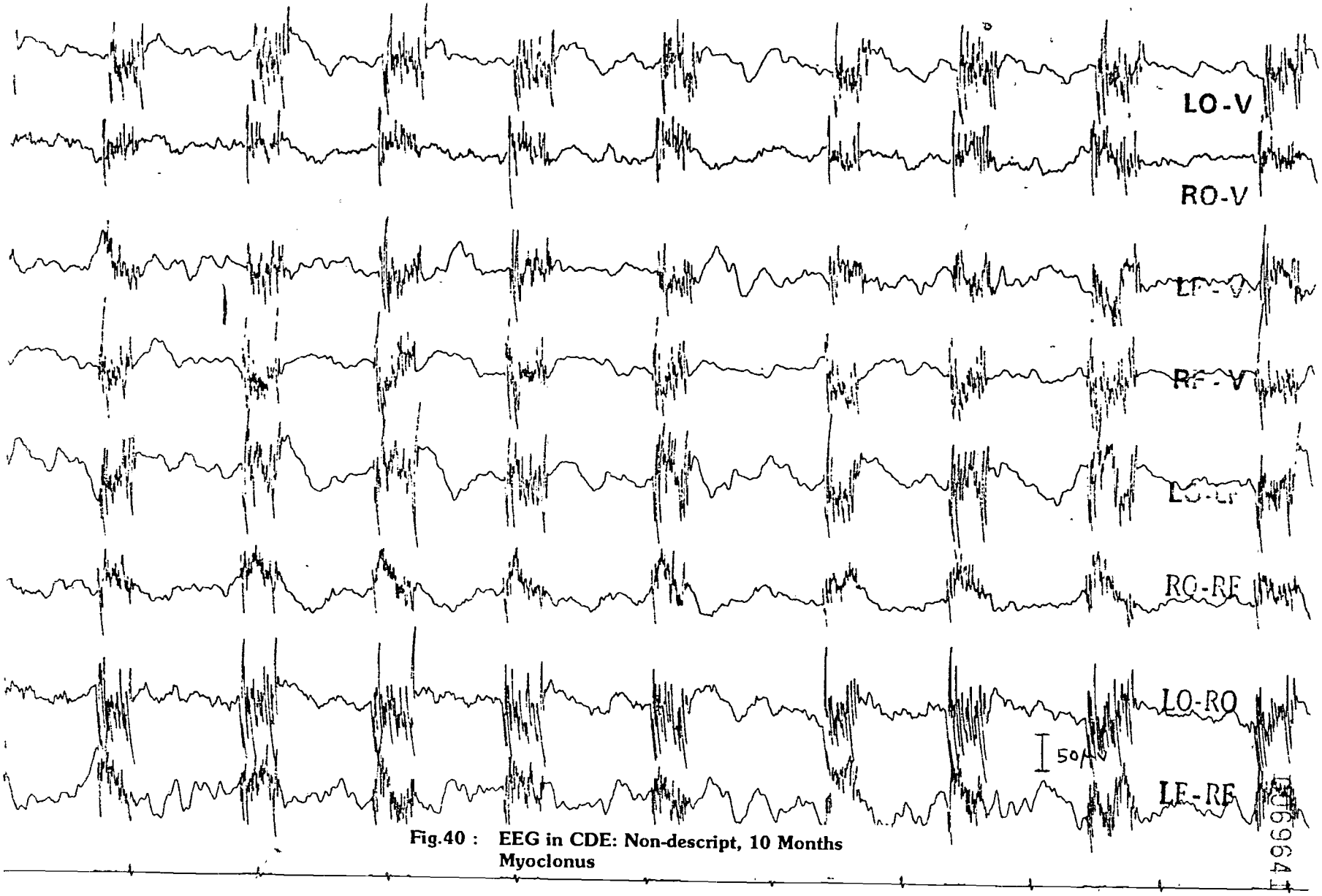

Fig.40 : EEG in CDE: Non-descript, 10 Months Myoclonus

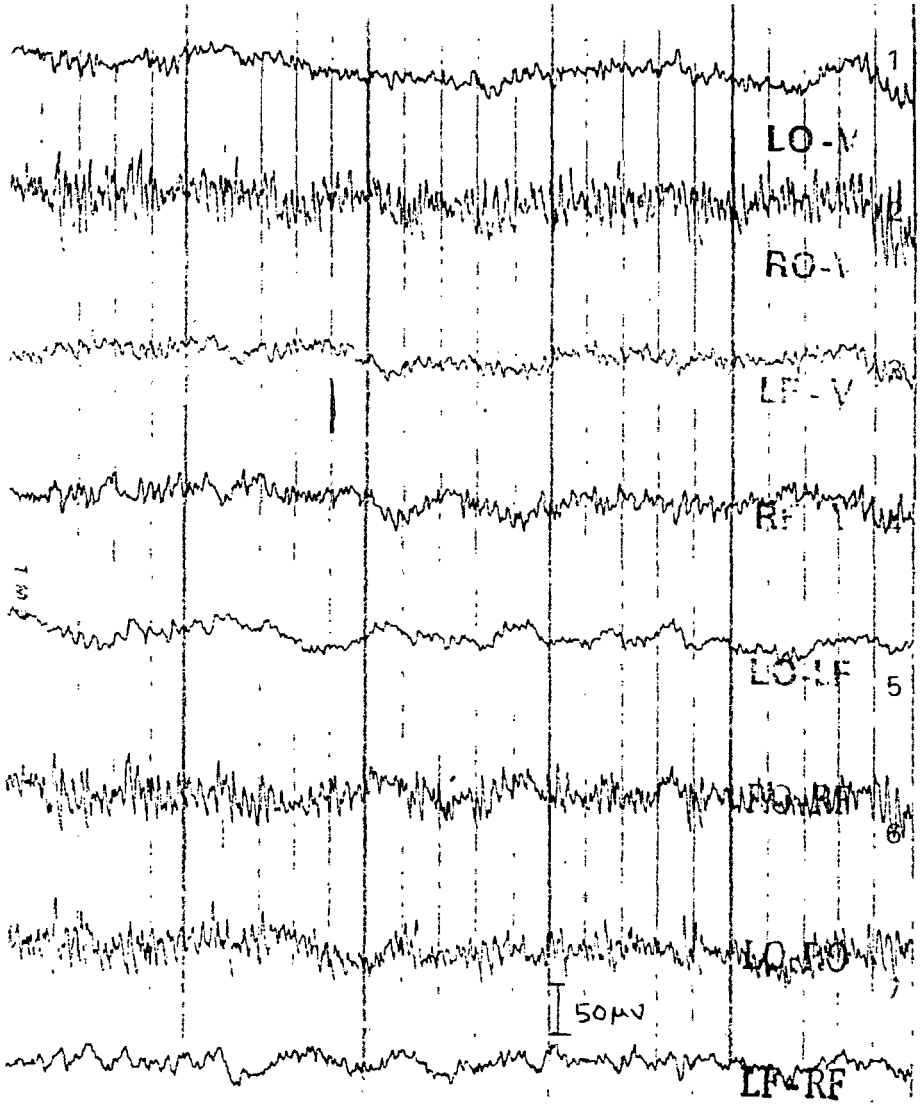

Fig.41 : EEG in CDE: Labrador, 10 Months (Ehrlichiosis) Asymmetry and spikes

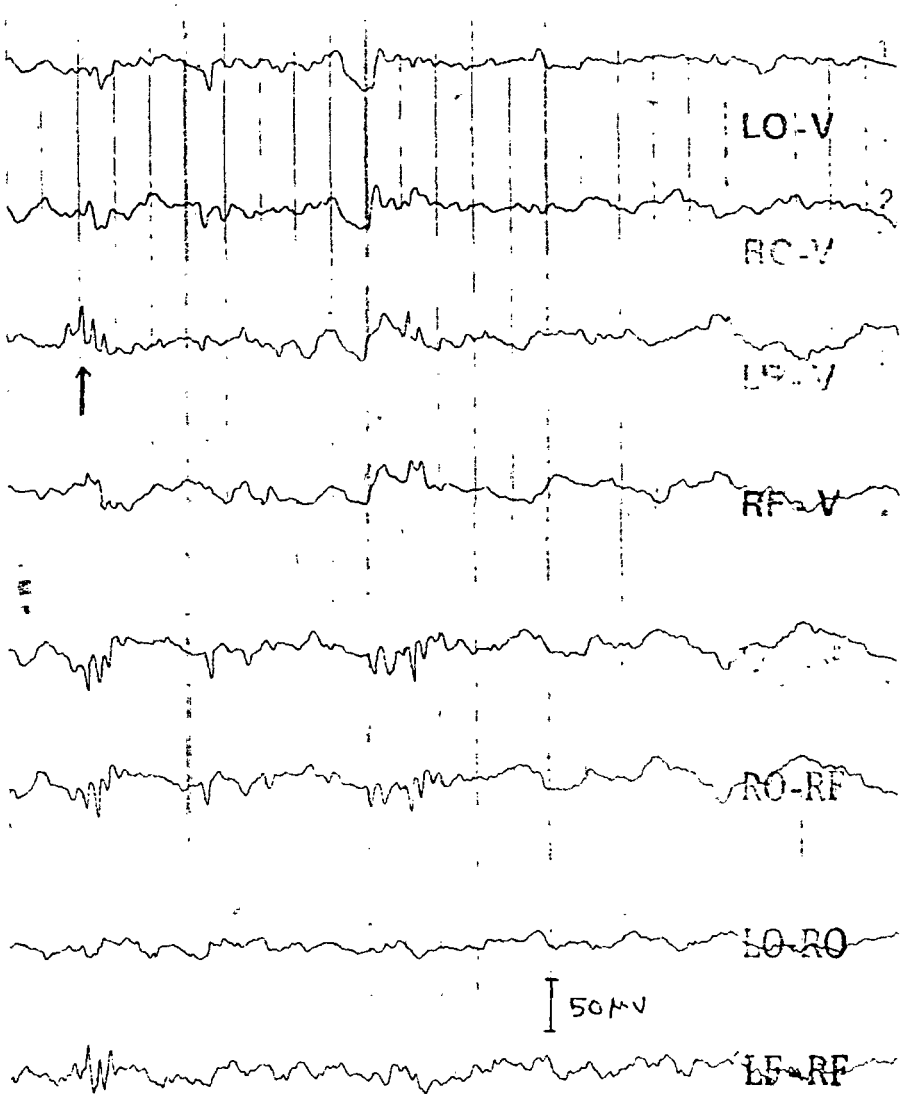

Fig.42 : EEG in CDE: German Shephered, 1 Year Polyspikes

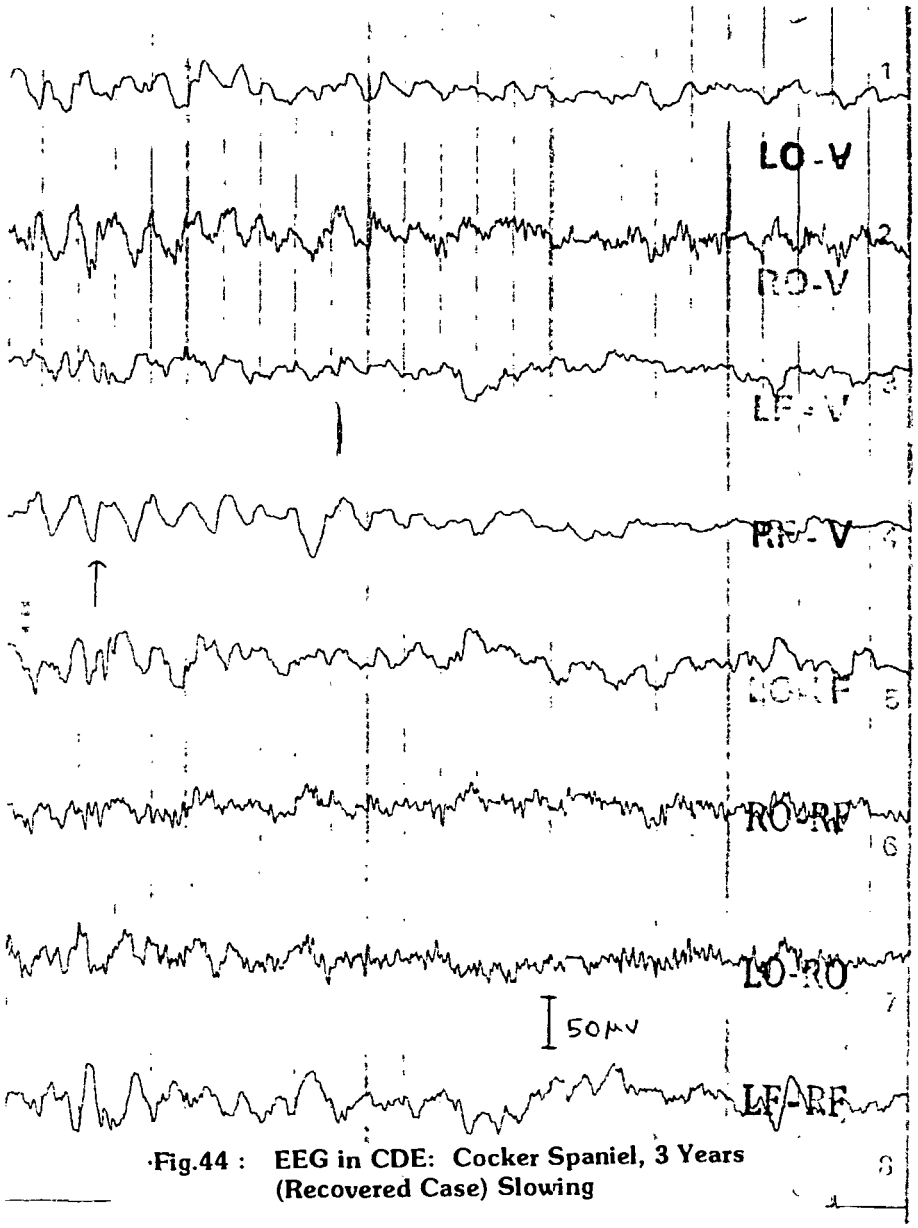

Fig.44 : EEG in CDE: Cocker Spaniel, 3 Years (Recovered Case) Slowing

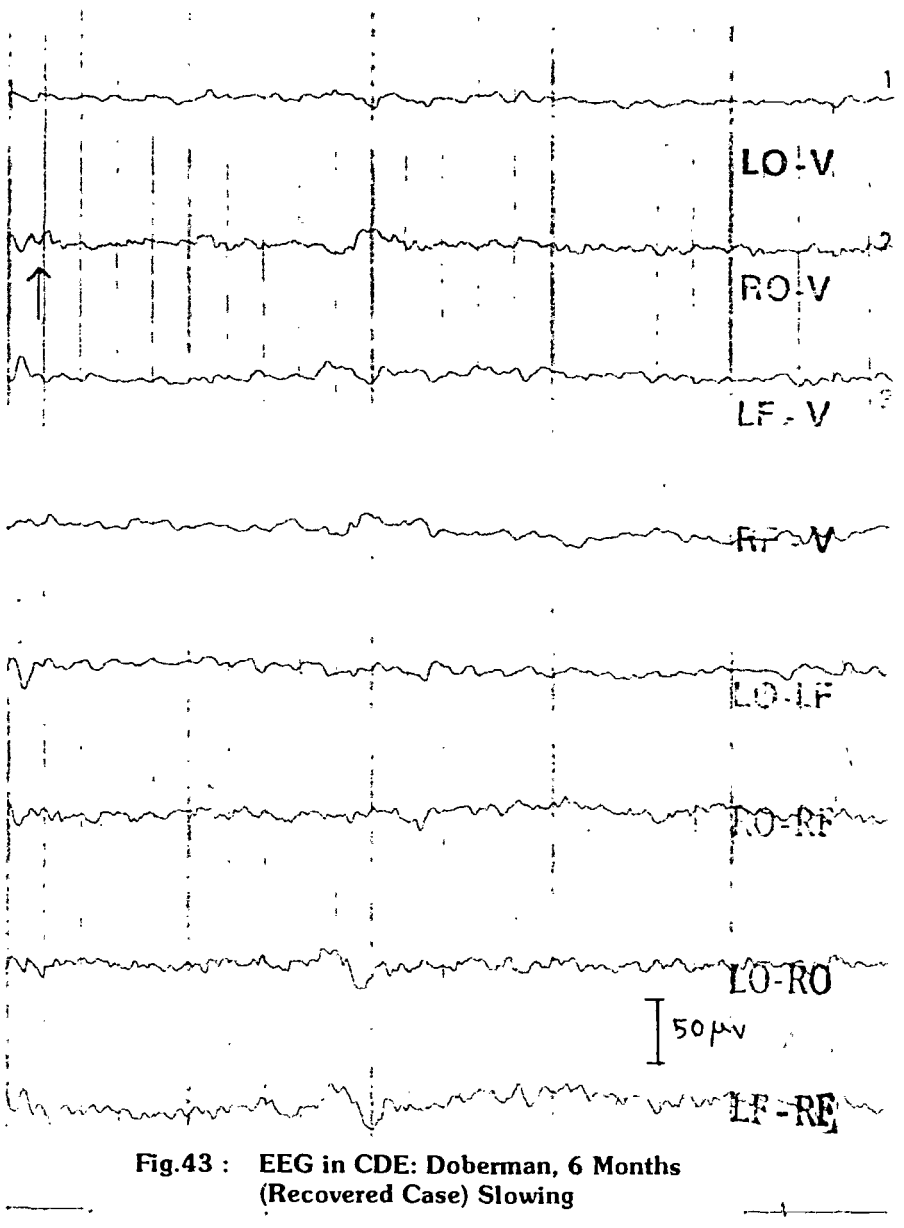

Fig.43 : EEG in CDE: Doberman, 6 Months (Recovered Case) Slowing

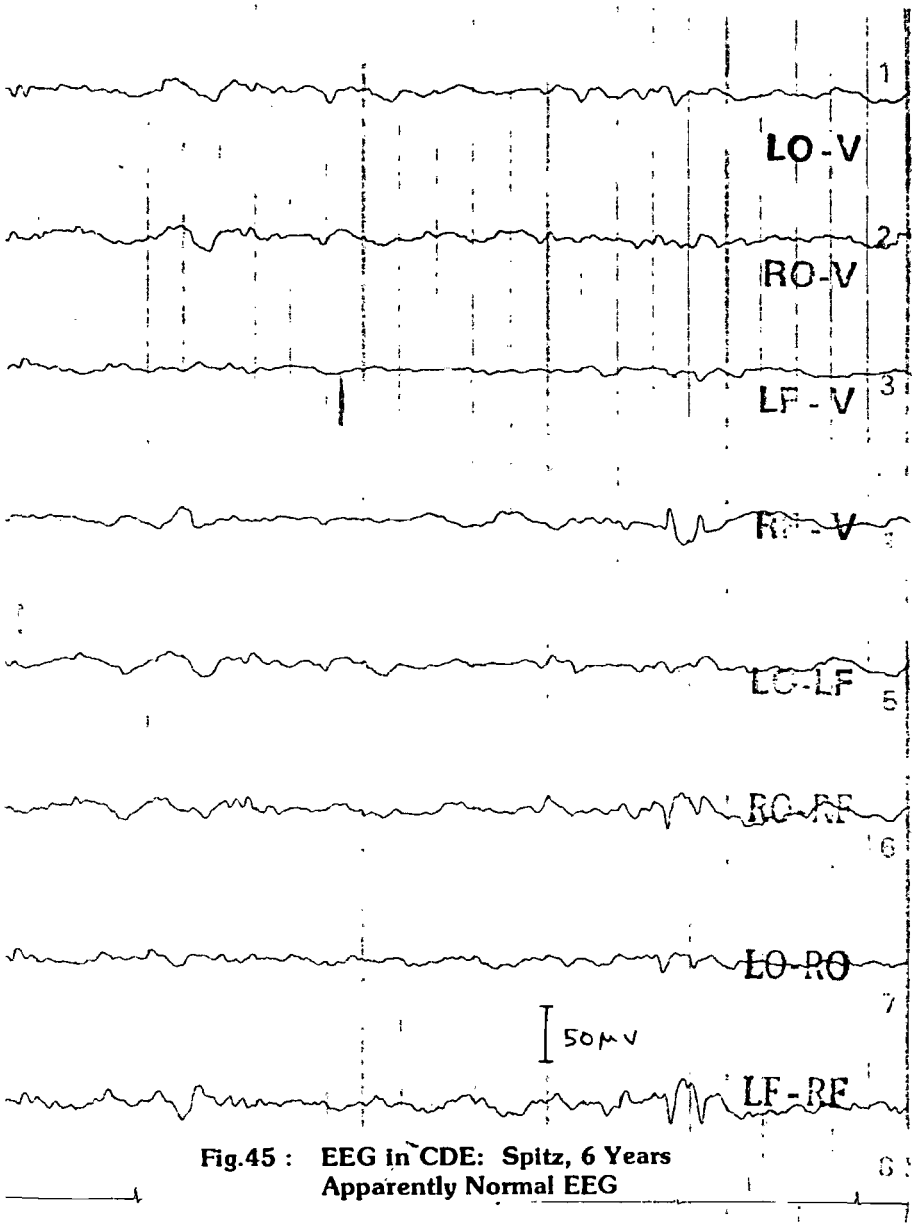

Fig.45 : EEG in CDE: Spitz, 6 Years
Apparently Normal EEG

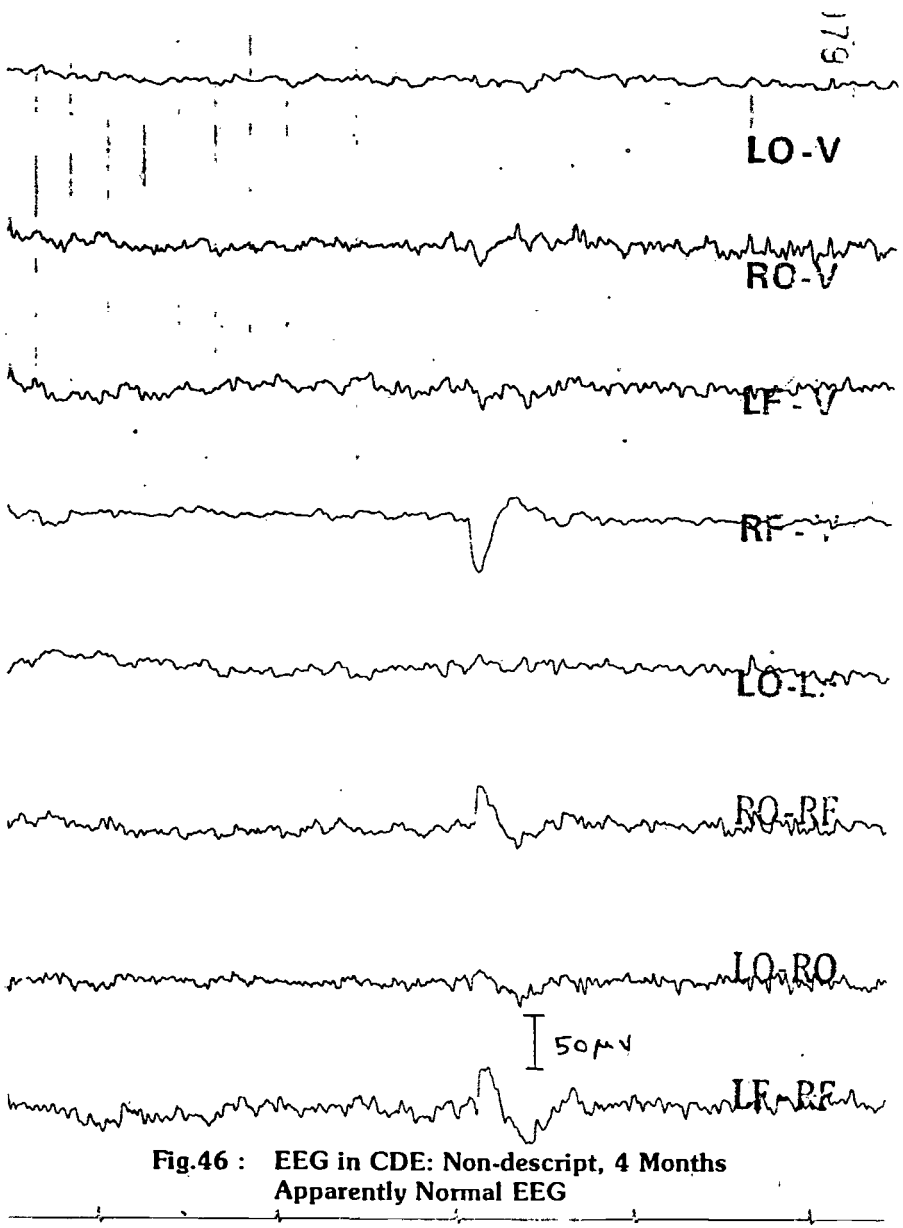

Fig.46 : EEG in CDE: Non-descript, 4 Months
Apparently Normal EEG

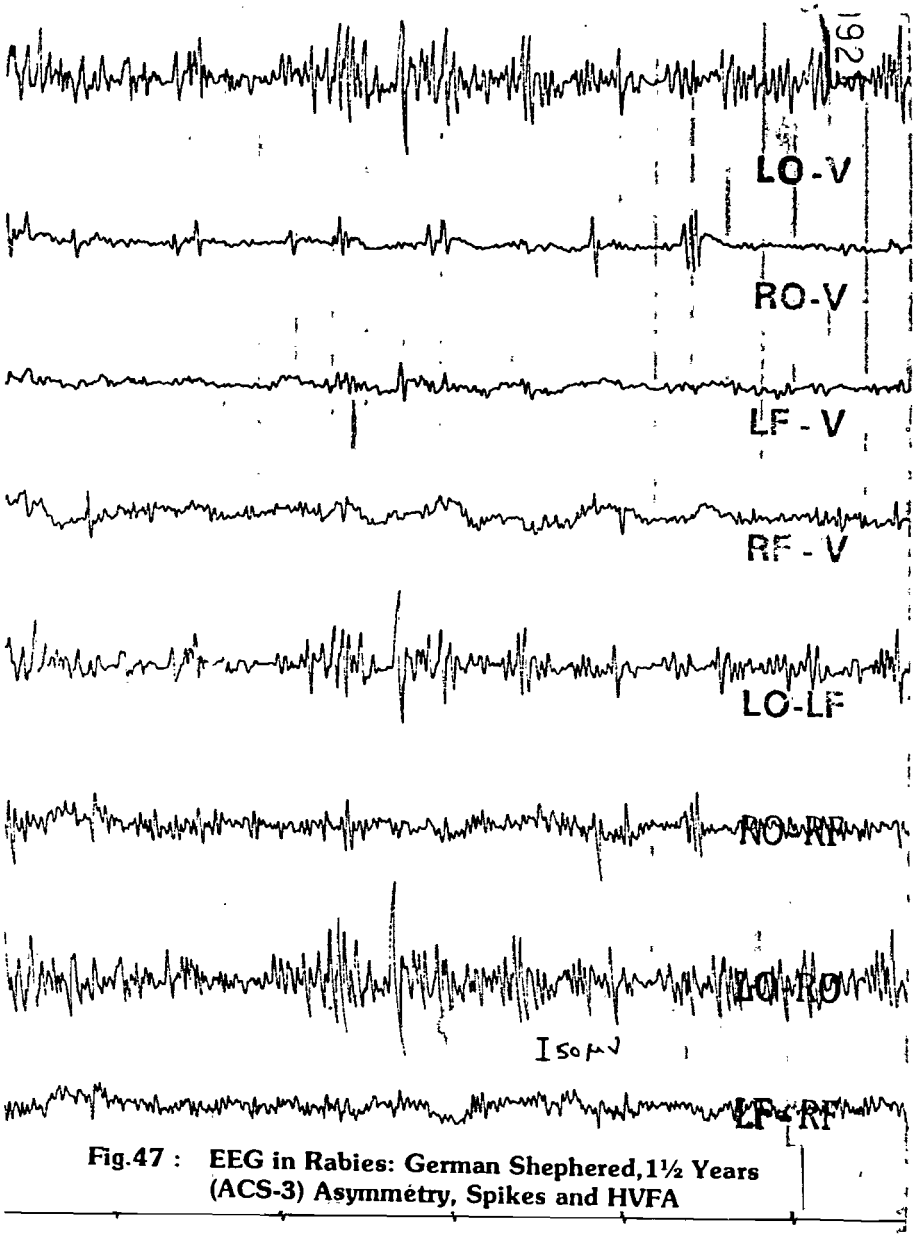

Fig.47 : EEG in Rabies: German Shephered, 1½ Years (ACS-3) Asymmetry, Spikes and HVFA

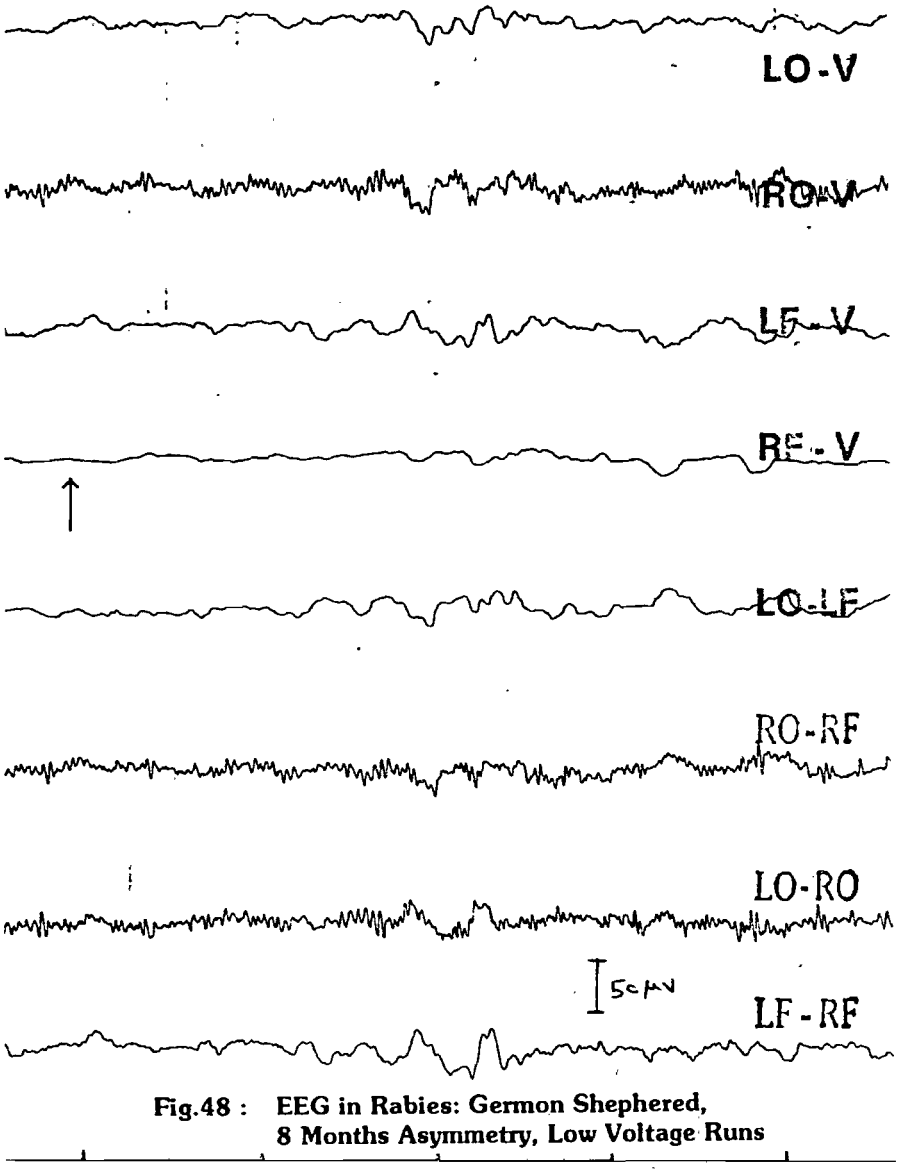

Fig.48 : EEG in Rabies: Germon Shephered, 8 Months Asymmetry, Low Voltage Runs

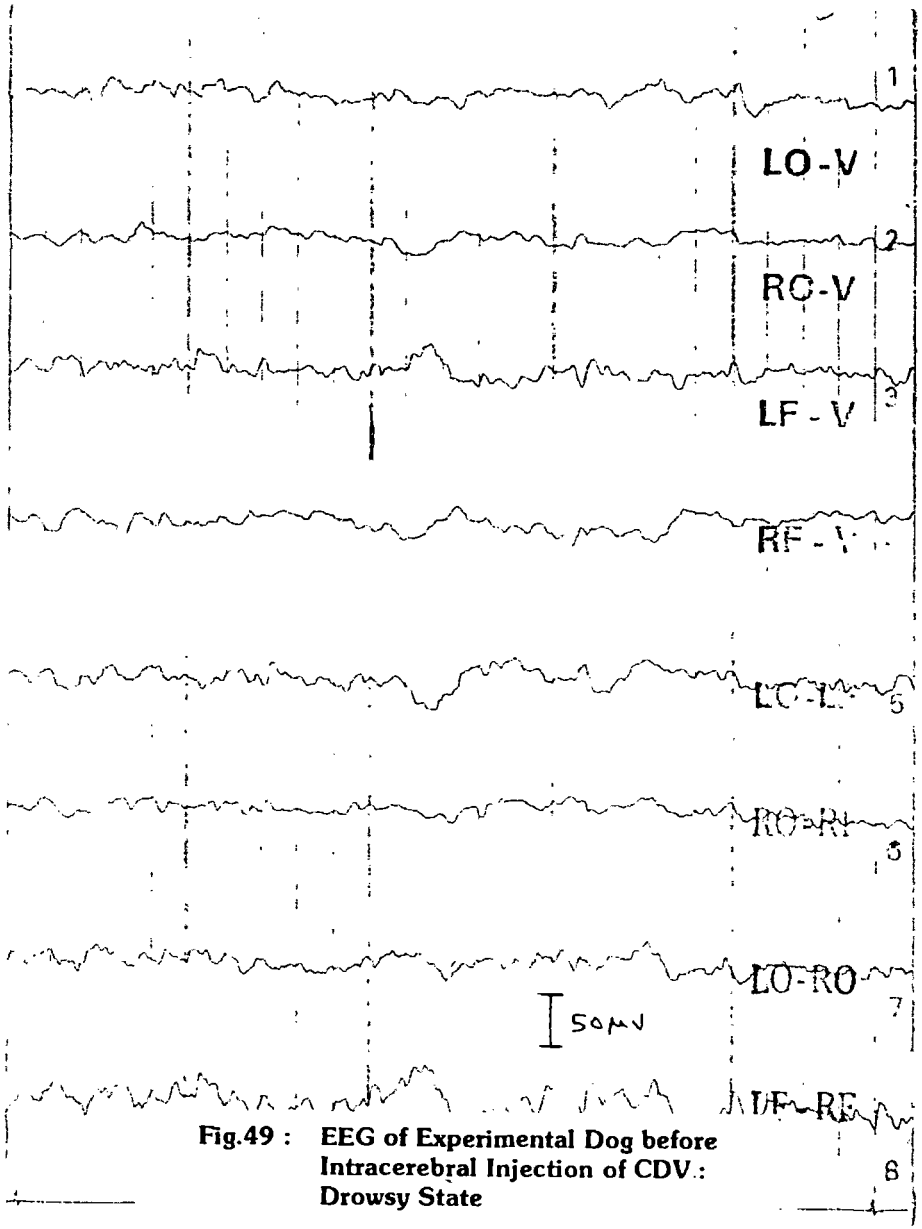

Fig.49 : EEG of Experimental Dog before Intracerebral Injection of CDV.: Drowsy State

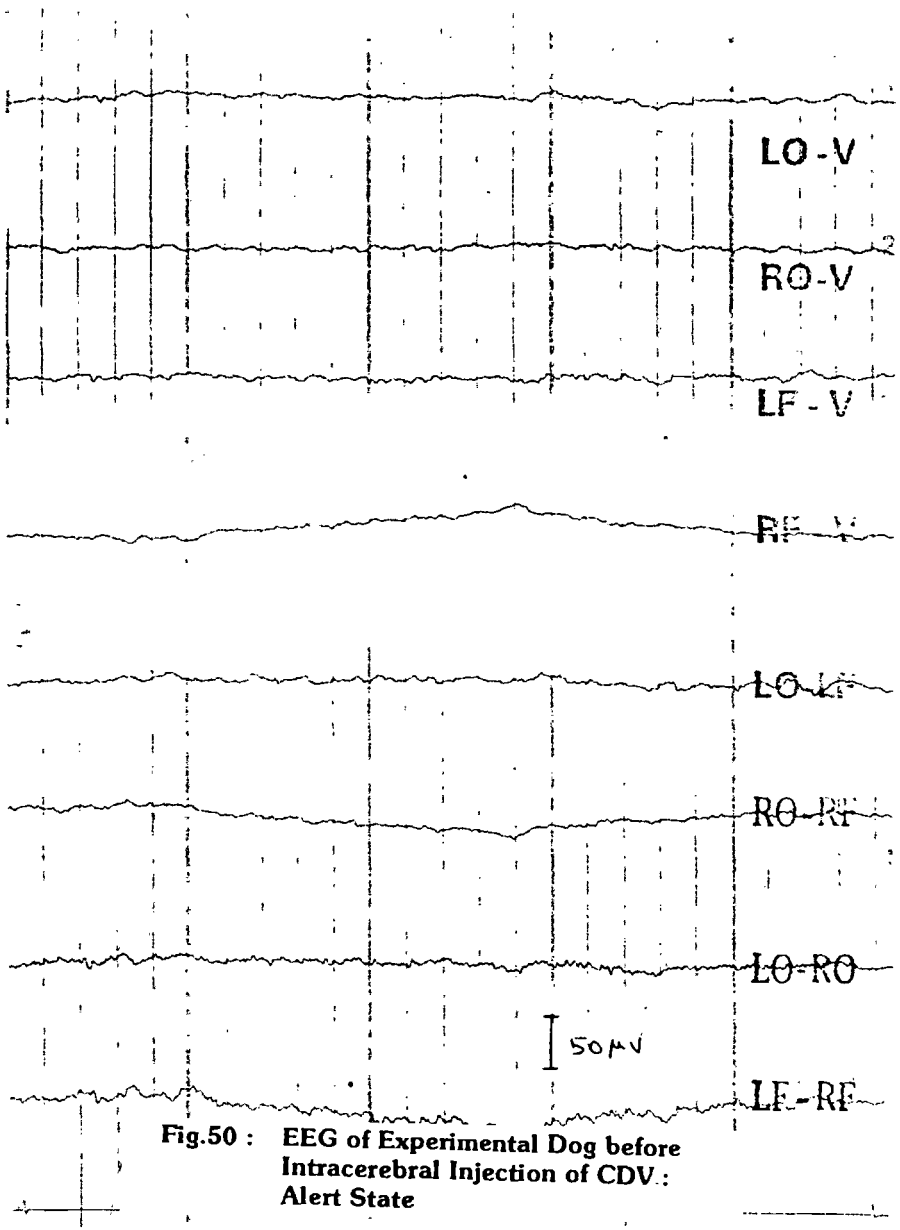

Fig.50 : EEG of Experimental Dog before Intracerebral Injection of CDV.: Alert State

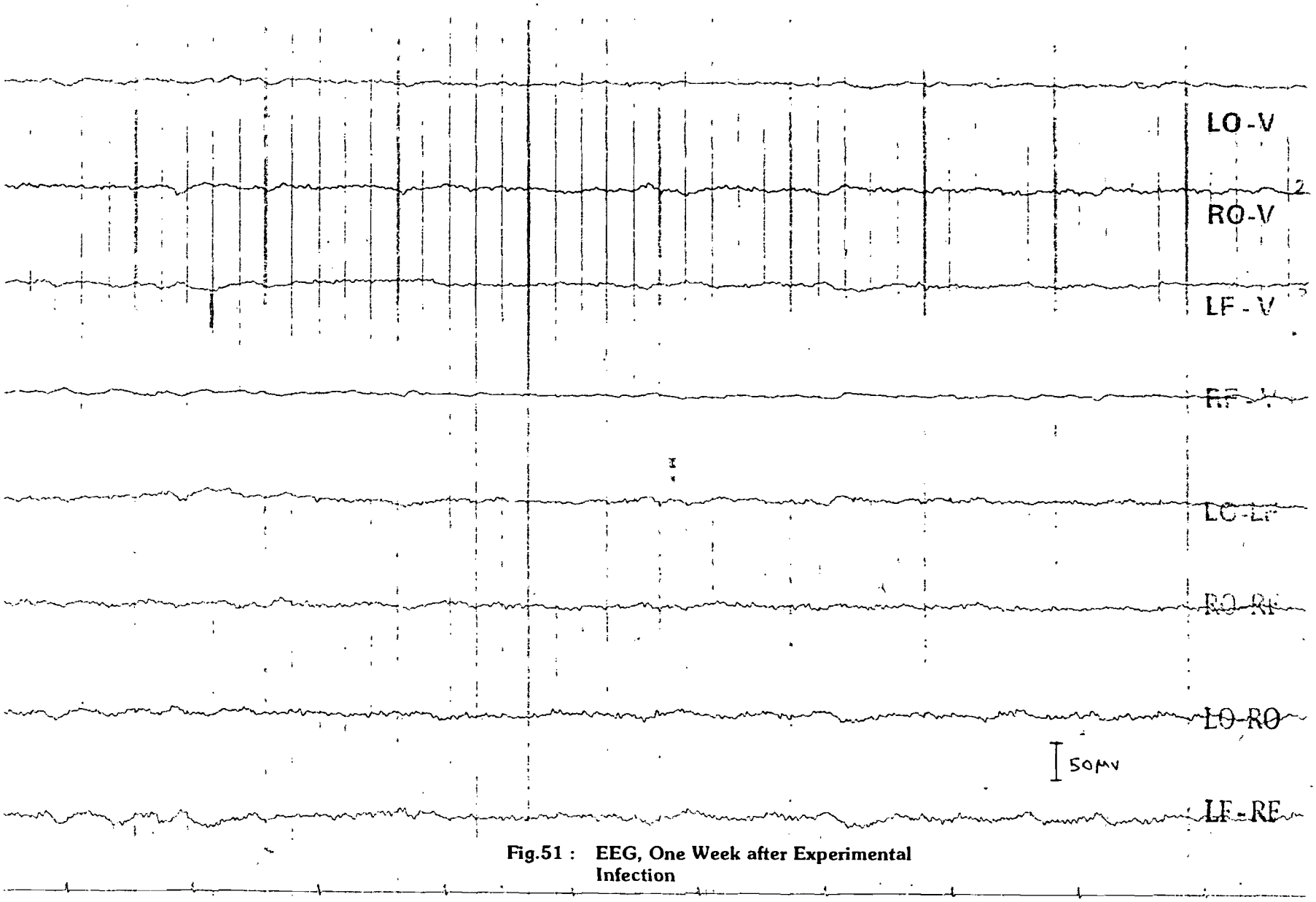

Fig.51 : EEG, One Week after Experimental Infection

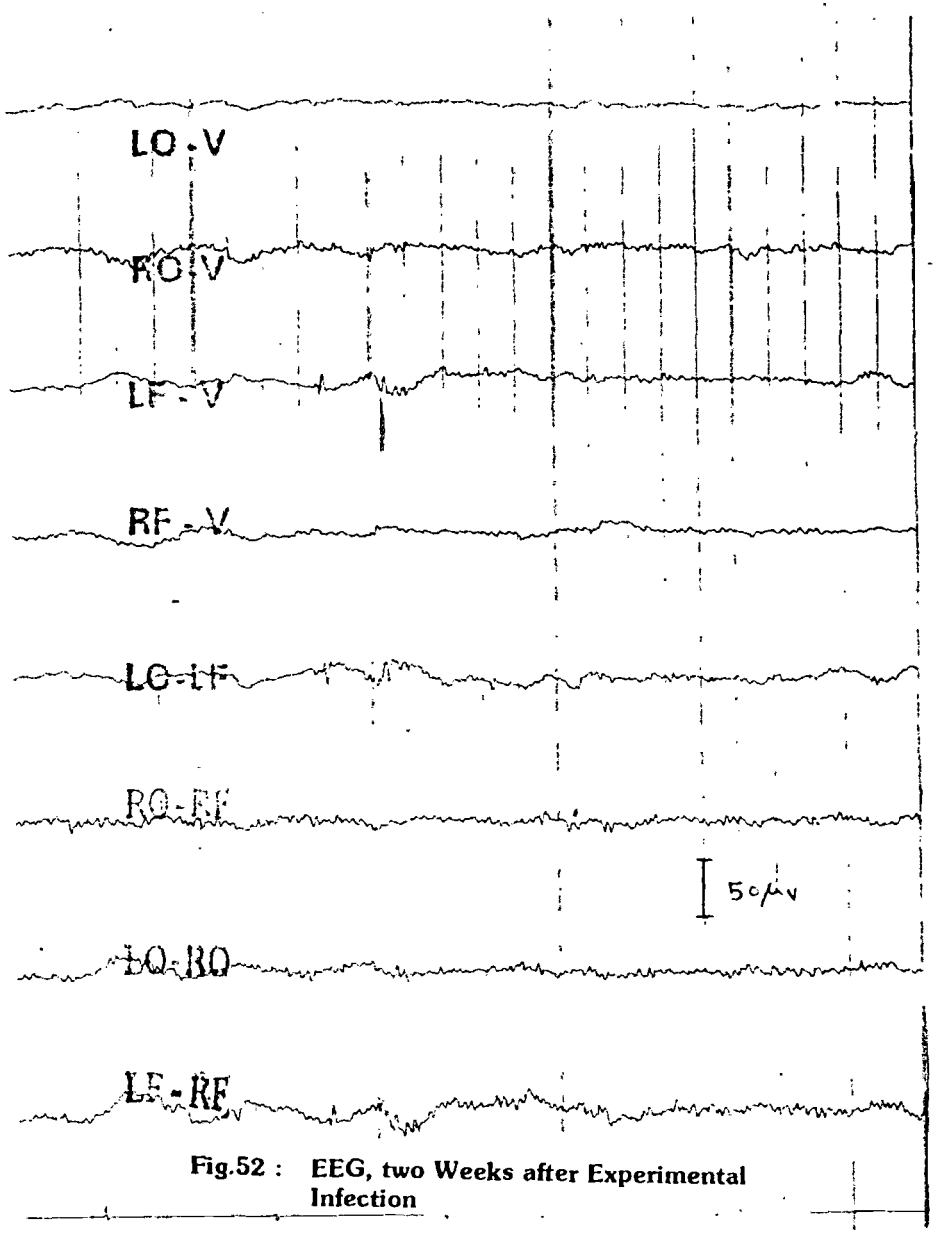

Fig.52 : EEG, two Weeks after Experimental Infection

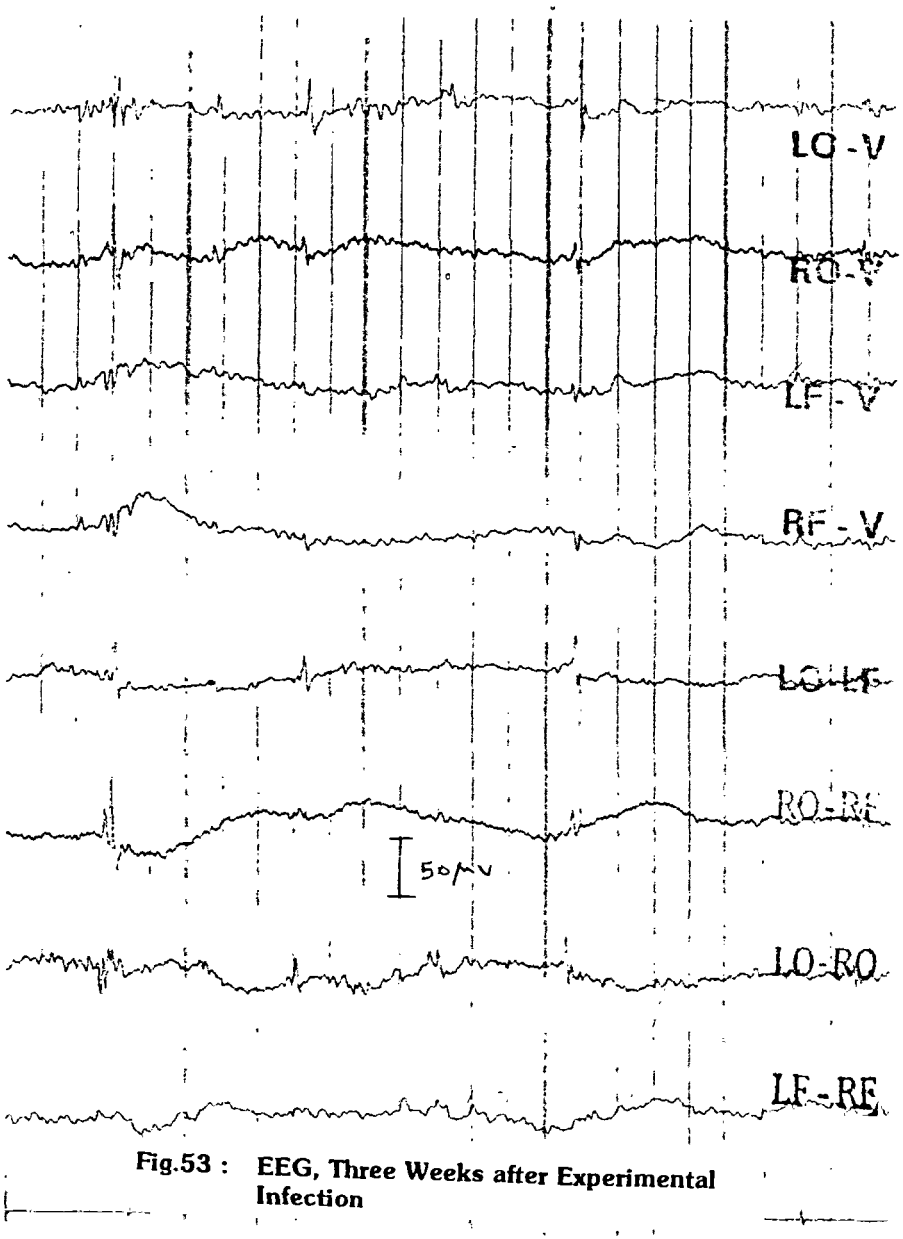

Fig.53 : EEG, Three Weeks after Experimental Infection

Discussion

CHAPTER V

DISCUSSION

The diseases of the Central nervous system are difficult to be diagnosed and treated. One reason neurological disorders are such an enigma is that the clinician has only limited diagnostic tools. Basically, he is restricted to history taking and a detailed neurologic physical examination. Crucial as these are, too often they are not enough (Klemm and Hall, 1974).

The important neurotropic viruses affecting dogs were Rabies virus, canine distemper virus, canine parainfluenza virus, canine herpes virus and Pseudorabies virus (Greene, 1984).

Greene (1984) was of the view that canine distemper has more historical and clinical significance than any other disease of dogs. It is a pantropic virus and enters the nervous system of many viremic CDV-infected dogs, whether or not neurologic sings are observed. The type of the lesion produced and the course of infection within the CNS depends upon a number of factors, including the age and immunocompetence of the host and the neurotropic and immunosuppressive properties of the virus. Either acute or chronic encephalitis could occur, and acute phase lesions might progress to those of the chronic form in animals that survived. Neurologic complications of canine distemper were the most significant factors concerning prognosis and recovery from infection.

5.1 CLINICAL STUDY

5.1.1 Incidence

The incidence of canine distemper encephalitis was found to be 0.87 per cent in the present study (Table 1,2, and 3). The male to female ratio was 1.5:1. The three commonest breeds affected were non-descript mongrel dogs, Spitz and Doberman, as these were the common breeds brought to the hospital. While the age incidence ranged from 2 months to 13 years, the maximum occurrence (46.86 per cent) was noticed in the age group of 1 to 5 yrs, followed by less than one year (41.14 per cent) age group.

No particular importance was attached to these observations as no detailed demographic study was undertaken. However, the popular belief that CD is a disease of young dogs does not seem to be correct in the light of present findings.

Lauder *et al.* (1954) reported that the three commonest breeds affected were Fox terrier, collie and Alsatian, as these were the commonest breeds attending the clinic. He further observed that approximately two-thirds were male and most dogs were affected before they reached six months of age. In susceptible, isolated populations of dogs, the disease was severe and widespread, affecting all ages. Increased susceptibility among breeds has been suspected but not proved (Greene, 1984).

Pop *et al.* (1991) reported that out of 71 cases of canine distemper, 34 per cent were in young animals (2.5 to 12 months) and 1.5 per cent were in over five years old dogs and their findings generally concurred with the present study.

The present study indicated that maximum incidence of distemper encephalitis occurred during January to April (winter months and the immediately following period). The least incidence was during May to August (summer months and immediately following period). This variation could be attributed to the climatic conditions. Storage and survival times of CDV were longer at colder temperature. CDV was extremely susceptible to ultraviolet light, heat and drying. In warm climates CDV would not persist in kennels after infected dogs have been removed. (Greene, 1984). The increased occurrence of CDE in the month of April could be attributed to the fact that neurological signs might develop only after weeks or months after the infection (Swango, 1989).

5.1.2 History and Clinical Signs

The results obtained in this study indicated that both vaccinated and unvaccinated animals suffered from the disease. But the animals that were regularly vaccinated seldom suffered from serious disease.

Although immunity to canine distemper was prolonged, it was not necessarily solid or lifelong. Dogs might lose their protection in the absence of vaccination and become infected following stress, immunosuppression or contact with diseased individuals (Green, 1984).

Gouveia *et al.* 1987 reported that the percentage of animals that had distemper although previously vaccinated one or more times was relatively high (22.3 per cent) suggesting that breakdowns in immunity were present. Factors contributing to this situation might be inherent in the antigen (low viral titres, inadequate storage,

use of weakly immunogenic strains, possible interference between unrelated antigens in polyvalent vaccines) or inherent in the animal (concurrent diseases, parasites, hyperthermia and nutritional deficiencies) reducing the capability of the animal to synthesize antibodies or inherent in the vaccination (level of passive antibodies in the animal at the time of injection of the vaccine). Adelus-Neveu (1991) while reporting an outbreak of canine distemper in France suggested poor population immunity as the cause due to low number of dogs being vaccinated against the disease.

Owners of many dogs (35 per cent) did not notice any clinical signs prior to the development of nervous signs in this study which supported the view of Lauder *et al.* (1954) that about 25 per cent of the cases had been ill for a longer time than the clinical histories indicated. Mature and immune dogs could develop nervous signs without prior history of systemic disease (Greene, 1984). Neurologic signs might occur with delayed onset, weeks or months after recovery from inapparent infections (Swango, 1989).

The major clinical signs noticed in this (Table 4) study were fits, flexor spasms (myoclonus) and deficits in postural reactions (Table 4). Myoclonus and/or chewing gum fits were noticed in a large number of cases and were the only signs in certain cases. 'Chewing gum type of seizures' occurred in dogs that developed polioencephalomalacia of the temporal lobes (Greene, 1984). Myoclonus, a forceful involuntary twitching of muscles, reflected irritation or loss of inhibition to the particular lower motor neuron segment innervating the muscle group. The neural mechanism for myoclonus originated in the area of spinal cord or brain stem motor neurons and was modified by higher centers. Neurologic signs were variable and

could be correlated to the area of involvement. The duration of neurological disease varied from a few days to more than one month and chronic relapsing course was rare (Tipold *et al.* 1992).

All the symptoms noticed in this study were reported by earlier workers (Lauder *et al.* 1954; Greene, 1984; Braund, 1986; Tipold, 1992).

Severe multisystemic signs were rather rare in non-descript dogs in the present study. Myoclonus and chewing gum seizures were the most common signs in non-descript dogs. Ott *et al.* (1954) suggested that many unvaccinated urban dogs have, at some time or other, had a symptomless or mild attack of distemper which has stimulated the production of antibodies.

In the present study, Ehrlichia organisms were detected in the blood smear of ten cases of CDE and was found to be complicating the clinical condition. Immunosuppression caused by distemper virus might make concurrent infections more virulent (Greene, 1984).

5.1.3 Leucogram

Hematologic changes in distemper encephalitis cases were found to be non specific. Gillespie and Rickard (1956) reported that leucopenia was found in most experimentally infected dogs and the leucocyte count remained low throughout the illness. However, Brian (1975) reported that the initial leucopenia was seldom detected in clinical conditions. The finding of the present study (Table 5) is in agreement with that of the above worker.

5.1.4 Ocular findings

The ocular changes noticed in the present study were conjunctivitis, epiphora, keratitis, grey to pink irregular areas of degeneration on the fundus, areas of hyperreflectivity and hazy areas in the tapetal area. One or another type of ocular changes were seen in majority (80 per cent) of the clinical cases. These observations are in agreement with Jubb *et al.* (1957), Fischer, (1971) and Martin, (1984). Complete ophthalmic examination in dogs with generalized infections could facilitate diagnosis and should be part of general physical examination (Peiffer, 1981).

5.1.5 Examination of Conjunctival Swabs

All the suspected cases of canine distemper encephalitis were tested by agar gel immunodiffusion test and/or counterimmuno electrophoresis. Out of 227 samples, 22.47 per cent were found to be positive for canine distemper. Counter immuno electrophoresis was found to be not having any significant advantage over Agar gel immunodiffusion test. As AGID test was more simple, less expensive and did not require any sophisticated equipment, it could be used as a regular screening test. 61 per cent clinical cases of distemper encephalitis, which were confirmed by other means did not give positive reaction to the above tests. Viral antigen was difficult to detect in extraneural tissues of dogs with canine distemper encephalitis (Vandevelde and Cachin, 1992). Blood leucocytes and conjunctival specimens became free of virus ten or more days after exposure in those dogs that were able to develop antibody early and progressively. These dogs represented about 50 per cent of those exposed (Appel, 1970).

5.1.6 Cerebrospinal Fluid

Fankhauser (1962) did not see any need for anaesthetizing the dog for collection of CSF and the present study is in agreement with the above author. As general anaesthesia is more risky in diseased animals, takes longer recovery time and likely to cause more concern to the owners, xylazine could be used by field veterinarians for collection of CSF. It was found to be a relatively easy and safe procedure for neurological investigations.

5.1.6.1 Physical Appearance

The appearance of CSF collected was clear and colourless in majority (80 percent) of the CDE cases which agreed with the findings of Anonymous (1965). They reported that in CD and viral infections, CSF appeared normal and turbidity suggested bacterial infection. Thus CSF colour might help in differential diagnosis. The turbid appearance seen in ten per cent of the CDE cases in the present study, could be attributed to large increase in the cells as suggested by parker (1972).

5.1.6.2 Cell Count

Normal CSF cell count has been reported to range from 0-25 per cmm (Coles, 1967; Chandler *et al.* 1979). The values in the control group in the present study ranged between 1 to 8 with a mean value of 3.6 $\pm$ 0.6 5 (Table 5).

The normal cell count observed in four CDE cases in the present study could be explained in the light of the observation made by Bichsel *et al.* (1984) that during

the acute demyelinating stage of the disease, inflammatory reactions would be lacking and protein and cell content of the CSF might be normal. In chronic stage of the disease, pleocytosis due to mononuclear cells was frequently found.

In the present study, the cell count was in the range of 2 to 237 cells/cmm. Ninety per cent of the cases showed higher values than the control and majority of the cases (70.73 per cent) had a cell count between 5 and 50. Vandevelde and Spano (1977) reported moderate pleocytosis due to lymphocytes and suggested that a differential diagnosis of encephalitis could be attempted based on the cyto-morphologic examination of CSF. The findings of the present study also suggested that field veterinarians could use CSF cytology as a reliable test in majority of CDE suspected cases.

Five cases of CDE in the present study had neutrophils along with lymphocytes, Bullmore and Sevedge (1978) reported that in early stages of viral infections, neutrophils could be detected and later lymphocytic pleocytosis.

5.1.6.3 Total Protein

The values of the control group were 17.0 $\pm$ 2.14 mg per cent which was in agreement with Parker (1972) and Greene (1984).

The significantly higher level of total Protein (69.76 $\pm$ 4.58) in encephalitis cases in the present study agreed with the findings of Greene (1984) and Braund (1986). All the individual CDE cases showed higher values than the control.

Four animals only showed high increase (more than 100 mg per cent) in the protein level which suggested that there was no meningeal involvement in majority of the CDE cases studied. Increased protein concentration (>100 mg/dl) was typical of meningeal inflammation. Globulin and albumin increase occurred when the Blood CSF barrier was disrupted by inflammatory or compressive processes. Local *de novo* synthesis of immunoglobulin in the CNS and CSF could occur in chronic CNS infections such as CDE (Greene, 1984). There was no correlation between the cell count and total protein in this study and the findings agreed with de Lahunta (1977). The present study concurred with the view of Greene (1984) that total protein and cell counts were adequate for the diagnosis of most CNS diseases.

5.1.6.4 Creatine Phosphokinase (CPK)

The normal CSF CPK level was reported to be 3.1 IU/L with a high of 6.0 IU/L (Indrieri *et al.* 1980). The CPK level noticed in the control group of this study was 4.5 $\pm$ 0.56 IU/L. This is in agreement with the above author. The CPK level in CDE ranged from 10 to 259 IU/L with a mean of 51.66 $\pm$ 7.79 IU/L in the present study. The CPK level was not having any correlation with cell count or total Protein level. (Table 5).

Indrieri et al. (1980) critically evaluated the role of CPK level in CSF of dogs with neurologic disorders and commented that measurement of CPK concentration on a routine basis, would not provide any diagnostic advantage in all the cases of CDE as 25 per cent of the dogs with progressive, neurological diseases might have normal CSF CPK concentrations. They recorded that half of the dogs with CDE had

CSF CPK concentration of 0.0 IU/L. Contrary to their findings, in the present study, CSF CPK was elevated in all the individual cases of CDE. The reasons for increased CSF CPK activity were, cell dysfunction or death and increased entry of plasma CPK into the CSF due to altered blood - brain permeability. (Wilson, 1977). Jadhav (1992) found significantly elevated CSF CPK values in CDE.

Indrieri *et al.* (1980) further observed that CSF CPK concentration more than 20.0 IU/L might indicate a poor prognosis. Considering the present observations, it could be stated that CDE cases having any rise in CPK level, carried poor prognosis. The high rate of mortalilty and lack of response to various treatment trials in the present study could be explained in the light of these higher values of CPK.

The abnormally high values of 259 IU/L, 177 IU/L and 175 IU/L observed in three cases could be due to contamination by blood although it was not detectable at the time of collection. Wright (1980) observed that at the time of cisternal puncture, inclusion of a small quantity of blood could occur without it being realized.

5.1.7 Electroencephalography

Although EEG has been widely used in man for the diagnosis and localization of neurological disease, application of the technique to animals has been limited. Thick muscles on the head and lack of co-operation from the patient were two problems faced by Veterinary clinicians. The inherent problems and failure to recognize artifactual activity have brought the technique into disrepute. However, in the hands of an experienced clinician, EEG could be a valuable aid to diagnosis in

some instances, but it must always be regarded as an ancillary test in the light of other clinical findings (Palmer, 1976).

Redding (1978) stated that once the patterns seen in an EEG tracing had been learnt, visual analysis of the EEG became a relatively simple task of recognition very much like learning to read a new language with a unfamiliar alphabet and different kind of script. Practice was necessary to gain proficiency in interpreting electroencephalograms. The interpreter must have had a clear picture of what was considered normal before he could interpret the tracing with any degree of accuracy.

In the present study, physical restraint was used in majority (81.08 per cent) of the cases which agreed with the findings of Redding and Knecht (1984) and Skerritt (1984). They reported that the major disadvantage of physical restraint was the likelihood of movement and muscle potential artifacts. It was suggested that it could be minimized by carrying out the EEG examination in a comfortable environment. The owner was present during the examination and all the procedure were carried out in a quiet and unhurried manner. Most patients when restrained physically struggled only for a short time, then relaxed so that a recording could be made.

Redding and Knecht (1984) opined that the EEG changes resulting from chemical restraint were difficult to be interpreted because these changes closely resembled patterns observed in encephalopathy and they preferred not to sedate the patient. Nevertheless, chemical restraint might be necessary and should be recognized as a useful adjunct to EEG recording. Thiopental Sodium and xylazine produced

relatively higher amplitude slow waves and spindles almost similar to deep sleep in the Present study and these findings are in agreement with that of the above authors (Fig.22 and 23).

The hair over the scalp were clipped in the present study to assure good electric contact although Redding (1978) felt it was not necessary.

Chlorided silver discs were used in the present study as it caused only minimum discomfort to the animal. Needle electrodes were tried in two animals and it was found to be not having any advantage over disc electrodes. Redding and Knecht(1984) also recorded equal quality tracings with clip, needle and silver disc electrodes (Fig.34 & 35).

5.1.7.1 Encephalitis

The EEG tracings obtained from the confirmed cases of encephalitis are presented in Figures 24 to 46 and are discussed. No single character was pathognomonic for encephalitis (Table 6) and confirmatory diagnosis always depended on results of various investigations.

The most common abnormalities seen in the present study were spikes and asymmetry followed by low voltage runs and slow waves (Table 6). The EEG in encephalopathy caused by inflammation or degeneration was marked by irregular slow waves, paroxysmal bursts, repeated spikes or general spikiness, and seizural patterns, sinusoidal or saw-toothed, were strong indicators of inflammation. High frequency activity seen early in encephalitis disappear in the later stages of the

disease. The random slow waves of higher amplitude indicating parenchymal brain damage were also characteristic of encephalopathies (Redding and Knecht,1984). However Skerritt (1984) opined that in all cases of EEG-recorded abnormality, diagnosis could only be made in the light of further clinical information.

Spikes (26.35 per cent) were easily recognized and in many cases were not found in all leads, suggesting that the abnormal activity was not generalized in the brain. In the present study spikes were detected before the development of typical clinical signs of CDE (Fig.41). Spikes indicated the presence of an irritative lesion and suggest that an acute inflammatory process is taking place and carried a poor prognosis.

Asymmetry was apparent in 25 percent of the cases and in certain cases it was the only obvious abnormality. The clinical signs exhibited by the animals were not necessarily unilateral. Asymmetry was seen in the early and late stages of encephalitis in the present study.

High voltage slow waves were typical of a acute attack of encephalitis with clinical deterioration whereas random slow waves, were more common in mild encephalitis. A marked and continuous suppression and slowing suggested suppressed cerebral activity as in encephalopathy. Dogs showing EEGs with more marked abnormalities such as asymmetry, continuous low voltage or slow waves combined with runs of low voltage activity appeared to have a more severe encephalitis and carried a worse prognosis. Slowing and sinusoidal waves were detected in the early stages or recovery stages of encephalitis.

All the above observations were in agreement with that of Croft (1965a) and Croft (1970) who analysed the EEG patterns of 41 cases of confirmed CDE. They reported slow waves, low voltage runs, spike and wave and low voltage at a frequency of 24,12,11 and 9 respectively. They further observed that the slow waves were more common in severe encephalitis and low voltage runs were more common in mild encephalitis. EEGs with spike and wave carried a poor prognosis. Slowing was attributed to mild cerebral anemia and occurred as an early sign in a normal animal and carried a comparatively a better prognosis. In the present study, except for slowing, all other patterns carried a poor prognosis.

In the present study, only 14 per cent of CDE affected dogs showed low voltage runs and about 11 per cent dogs exhibited slowing in their EEGs. Hence it could be suggested that by the time majority of the animals were brought to the clinic and the condition diagnosed the disease was well established and hence carried a grave prognosis.

28 cases (18.92%) did not show any characteristic abnormal patterns of EEG and was of no use in diagnosing these cases of encephalitis. Croft (1970) and Redding and Knecht (1984) suggested that a normal EEG did not entirely exclude the presence of a lesion, if this was small, mild and remote from the recording electrodes. A cortical lesion might show abnormal EEG changes that last only a few weeks, then disappear even though the animal has some clinical deficits. Hence, a normal appearing EEG did not always mean normal brain function.

Some cases of encephalitis recover completely, whilst others, despite medical treatment, deteriorate severely that euthanasia was inevitable. Serial EEGs could be used to determine the improvement or deterioration, in cerebral activity. EEGs are valuable tools in the study of the effects of drugs and should certainly be used in clinical trials of new, potentially useful agents. (Croft, 1970). The findings of the present study reinforced the above opinion.

On a comparison, it was found that when CSF analysis diagnosed 100 per cent of CDE cases, EEG could diagnose only (81.08 per cent) of CDE cases.

The EEG patterns observed in two cases of Rabies (Fig.47 & 48) were asymmetry, high amplitude spikes,HVFA and low voltage runs. Spikes and HVFA observed in one case suggested acute irritative foci in the brain. Asymmetry and low voltage seen in the other cases suggested severe encephalitis (Croft, 1970) and the clinical signs observed were dullness and staggering gait.

5.1.8 Post-Mortem findings

In the present study, changes were mainly noticed in the lungs and intestine. Occasional meningeal congestion was also noticed. The findings were in agreement with that of Lauder *et al.* (1954) and Greene, (1984) and supported the view of Wright *et al.* (1974) that post mortem findings in CDE were often inconclusive.

5.1.8.1 Histopathology

The purpose of histopathology in this study was to confirm the presence of encephalitis in those clinical cases that were subjected to electroencephalography. The important changes noticed in the cerebrum were hyperemia, neuronal necrosis and degeneration, gliosis, spongiosis, neuronophagia and intranuclear inclusion bodies in astrocytes. Similar changes were noticed in the cerebellum also. The findings in the present study confirmed the findings of Lauder *et al.* (1954), Gillespie and Rickard (1956), Campbell (1957), and Braund (1986). All the clinically diagnosed cases, which were subjected to histopathology were found to be positive for encephalitis suggesting that CDE cases could be diagnosed with almost certainty ante-mortem. As the number of animals studied were small, detailed studies are needed before arriving at a final opinion on this regard.

5.1.9 Effect of drug trials

Four criteria were adopted to assess the efficacy of the drug trials (1) qualitative improvement in clinical signs, (2) Number of days the animal survived since the start of the treatment (3) Number of animals survived (4) Serial EEG tracings.

5.1.9.1 Ribavirin

Ribavirin is a purine nucleoside analog that inhibits the replication in vitro of a wide range of RNA and DNA viruses. The oral bio-availability of ribavirin is about 45 per cent (Douglas, 1991). Crumpacker *et al.* (1986) reported that significant

amounts of ribavirin might cross the blood-brain barrier even in the absence of neurological symptoms.

As the CDV affected the ependymal and glial cell initially and neurons were affected last (Alleman *et al.* 1992) it could reasonably be assumed that Ribaviran might be of use in the treatment of CDE, if it is used in the initial stages. CDV has been included in the conventional slow virus group because it could produce a chronic progressive encephalomyelitis (Greene, 1984).

In the present study, 26 clinical cases of distemper encephalitis were treated with ribavirin (Table 7). All the animals were given supportive treatment also. The clinical recovery of one non-descript dog aged 1 year with minor neurological deficits could be considered as a natural recovery. Such recoveries have been reported by Palmer (1976) and Greene (1984). It appeared that ribavirin had no effect on any of the criteria adopted for assessing the effect of the treatment.

Ribavirin triacetate has been shown to be more effective than ribavirin in the treatment of dengue virus and colorado tick fever virus encephalitis in experimental animals as the triacetate form allows ribavirin to remain longer in tissues and to cross the blood-brain barrier. Ribavirin could inhibit virus replication in the respiratory tract but that an intact immune system is important for sustained recovery (Gilbert, and Knight, 1986). But in CDE, immunosuppression from viral replication in lymphoid tissue, was an important factor in determining the outcome of the disease. Typical signs usually occurred only in immuno-suppressed dog (Swango, 1989).

Gilbert *et al.* (1991) compared conventional route of administration of ribavirin with aerosol and reported that ribavirin was not effective in treating viral encephalitis when administered by conventional routes and recommended administration of ribavirin by small particle aerosol (SPA). Therefore, before forming a final opinion on the efficacy of ribavirin in canine distemper cases a detailed experimental study using aerosol administration and ribavirin triacetate is warranted.

5.1.9.2 Vitamin C

In canine distemper encephalitis, antiviral antibodies could stimulate macrophages with secretion of toxic factors such as free radicals of oxygen. It has been shown that these radicals are highly toxic to oligodendrocytes, the myelin producing cells,Burge *et al.* (1989) and Griot *et al.* (1990) and Botteron *et al.* (1992). Tipold *et al.* (1992) suggested that as macrophages and their products especially free radicals of oxygen were important in the induction of tissue damage in distemper, anti-oxidants such as vitamin E and vitamin C should perhaps be used therapeutically.

Three injections of 200 mg ascorbic acid intravenously at 24 hour intervals has given favourable results in CD (Belfield. 1967). In contrary Swango (1989) and Greene (1984) reported that controlled studies were lacking to support the claims of Vitamin C and in his opinion it was of no value. In the present study (Table 7) there was apparently no difference between the Ribavirin treated group and Vitamin C treated group as far as the final outcome was concerned, though it was felt that there

was a qualitative improvement in the clinical signs exhibited by the affected animals, in Vitamin C trial.

5.1.9.3 Ribavirin and Vitamin C

Ten animals belonging to various breeds and age groups (4 months to 6 years) were included in this trial (Table 7). All the dogs except two succumbed to the disease or were euthanatized within a period of 5 to 14 days. The Survival of one doberman dog with nervous deficits viz. chewing gum fits could be attributed to the immunity attained by this animal due to earlier vaccination. The other one was a non-descript adult dog.

From the present therapeutic trial, it might be summarised that neither oral administration of Ribavirin nor Vitamin C had any significant influence on the course of distemper encephalitis. The dogs treated with Vitamin C showed some improvement in clinical symptoms although it did not affect the final outcome. Most of the cases of canine distemper encephalitis are well established by the time it is admitted in the hospital. As the antiviral drugs interfere only at the stage of viral replication, acute viral infections are difficult to treat because a diagnosis cannot be made before the course of infection is terminated.

5.2 EXPERIMENTAL STUDY

Although canine distemper virus was injected into six animals, through various routes, viz. intravenous, intrathecal and intracerebral in two animals each, only one animal (which was infected intracerebrally), developed the disease. The observations

made by the following authors might explain the reasons for the failure in experimental reproduction of CDE in majority of the animals. Ott *et al.* (1955) detected high titres of neutralizing antibodies in dogs subjected to the exposures of an urban environment and suggested that many dog, might have already suffered from subclinical form of distemper.

Gillespie and Rickard (1956) conducted detailed studies on experimental reproduction of distemper encephalitis. A strain of virus, designated as snyder Hill, from a dog showing signs of generalized distemper, was transferred in series by intracerebral inoculation into other dogs. Beginning with the fifth passage and in all subsequent transfers, neurological signs have appeared regularly six to 16 days after inoculation and histopathology revealed encephalitis. In initial passages, some inoculated dogs developed epileptiform convulsions after more than 22 days or died without observed neurological signs. Only a fourth of the dogs that were inoculated by other routes (intravenous, subcutaneous, intranasal and contact) showed nervous signs. He further observed that dogs recovered from the generalized form of distemper induced by the Snyder Hill strain or by an attenuated egg strain were fully immune to intracerebral inoculations with the Snyder Hill strain CDV. Campbell (1957) stated that the interplay between a virus and the tissues of the host was complex and might well be influenced on the one hand by such factors as variation in viral strains, infective dose, and route of inoculation; on the other by age, breed, plane of nutrition and immunity.

CSF analysis revealed slight increase in cell count (9 cells per cmm) moderate increase in total protein (37 mg per cent) and slight increase in CPK (8 IU/L). The

serial EEG recordings from the experimentally infected dog exhibited spikes and asymmetry from the 14th day of inoculation onwards (Figure 49 to 52). These findings confirmed the experimental reproduction of encephalitis.

As spikes and asymmetry were the first and only pattern changes noticed in the serial EEGs during the study it could be suggested that these were the initial abnormal patterns noticed in the early stages of encephalitis. Low voltage runs, which was reported to be the pattern in the initial stages of clinical distemper encephalitis (Croft , 1970) were not detected in this study. As the sample size was low no definite conclusion could be arrived at.

Spikes and asymmetry, the common abnormal patterns observed in the initial stages of the disease, as suggested by the present experimental work, were observed in 26.35 per cent and 25 per cent of the clinical cases respectively in the present study. Detailed experimental study is required to throw further light on the matter as the sample size was small.

Summary

CHAPTER VI

SUMMARY

A study was designed to assess the utility of Electroencephalography in the diagnosis, prognosis and treatment of encephalitis in dogs.

It included 175 clinical cases of canine distemper encephalitis and ten apparently healthy dogs (control) and six experimental cases.

CLINICAL STUDY

The provisionally selected Canine distemper cases were subjected to detailed neurological examination. The following characters were studied in the clinical cases:

1. History
2. Clinical signs and Leucogram
3. Ocular findings
4. Conjunctival swabs : Agar gel immunodiffusion test
   : Counter immunoelectrophoresis
5. Cerebrospinal fluid analysis: Physical appearance, Cell count, total protein and CPK
6. Electroencephalography: Amplitude, Frequency and abnormal discharges and their distribution.
7. Post-mortem findings.

The diagnosis of distemper encephalitis was made in clinical cases which fulfilled any five of the above criteria. 48 animals that were considered to be in the early stages of canine distemper encephalitis were selected for the drug trials, viz. Ribavirin, and Vitamin C. Ribavirin was administered orally at the rate of 10 mg/kg body weight/day, divided into four doses and Vitamin C at the rate of 500 mg/day intravenously. All the animals were given other supportive treatment also, as the case warranted.

The study revealed that the prevalence rate of CDE in Madras city was 0.87 percent. The condition was most common in non-descript dogs (35.42 per cent), followed by Spitz (29.14 per cent) and Doberman (12.57 per cent). The incidence was maximum in the age group of 1-5 years closely followed by less than 1 year age group. The maximum incidence was during winter months and least during summer months.

The disease was observed both in vaccinated and non vaccinated animals. Owners of the many affected dogs had not observed any major systemic signs prior to the development of nervous signs. The common clinical signs observed at the time of presentation of cases were fits, myoclonus, deficits in postural reactions and respiratory disturbances. 'Chewing-gum fits' and myoclonus involving muscles of the limbs and the head were prominent clinical signs especially in non-descript dogs.

The mean total leucocyte count of affected dogs was 10350.83 $\pm$ 282.26 per c.mm and was found to be of no use in the diagnosis of CDE.

Ophthalmologic examination of the clinical cases, revealed conjunctivitis, ocular discharge, keratitis and corneal ulcers. Signs of chorioretinitis with areas of degeneration on the fundus were observed in certain cases.

Examination of the conjunctival swabs by Agar gel immuno diffusion test and counter immuno electrophoresis was found to be not that sensitive, as only 22.47 per cent of the provisionally diagnosed cases only gave positive results. The negative results could be attributed to the absence of the viral antigen in the secretions and excretions of the dog towards later stages of the disease.

Collection of cerebrospinal fluid from the cisterna magna was found to be a relatively easy and safe procedure. Sedation of the dog with xylazine was found to be sufficient for collection of CSF and this technique is recommended for use in the field.

CSF collected from the clinical cases was clear and colourless in 80 per cent of the cases. The normal values of cell count, total protein and creatine phosphokinase in apparently healthy animals was found to be 3.6 ± 0.65 cells, 17.0 ± 2.14 mg per cent and 4.5 ± 0.56 IU/L respectively. In clinical cases of CDE, these values ranged from 2-237 cells, 20-156 mg per cent, and 5 to 259 IU/L respectively. The results of CSF analysis were found to be highly significant and were enough for diagnosing clinical cases of encephalitis. Hence field veterinarians can use this technique for the diagnosis of viral encephalitis.

81.08 per cent dogs were found to be co-operative during electroencephalography and physical restraint alone was sufficient. The normal adult

EEG pattern was low voltage fast activity of 10-20 μV and 15-20 Hz. The animals with thick musculature on the head frequently showed myographic artifacts. EEG in relaxed state was characterized by slow waves of high amplitude. High voltage slow waves were noticed in young animals. Adult pattern was seen generally in animals that were more than eight months of age. The artifacts commonly encountered were motion artifacts and physiologic artifacts. The EEG patterns associated with canine distemper encephalitis were slow waves, low voltage runs, low voltage, asymmetry, slowing, spike and wave and spikes. No characteristic EEG pattern could be ascribed to distemper encephalitis. As EEG is a non-invasive technique and successfully diagnosed more than 80 percent of the cases, it could be recommended as an ancillary diagnostic aid.

The post-mortem macroscopic findings were often inconclusive. Histopathological examination of brain revealed neuronal necrosis, spongiosis, gliosis and inclusion bodies.

The various treatments tried viz. Ribavirin and Vitamin C were found to be having no apparent effect on the survival rate of affected dogs. Administration of Vitamin C intravenously resulted in qualitative reduction in the severity of clinical signs.

6.2 EXPERIMENTAL STUDY

Experimental reproduction of CDE in mongrel adult dogs was found to be a difficult task. Intravenous and intrathecal administration of the virus did not result in the disease. Intracerebral injection of the virus produced mild signs of disease in one

dog out of two dogs. The EEG pattern of the affected dog showed asymmetry and spikes and post mortem histopathological studies confirmed encephalitis.

6.3 CONCLUSION

Following conclusions could be drawn from the present study.

1. Ophthalmoscopy, Agar gel immunodiffusion test, cerebrospinal fluid analysis and electroencephalography can be considered ancillary diagnostic tests which may supplement mutually in the diagnosis of distemper encephalitis.

2. Cerebrospinal fluid collection technique is easy and safe to practice. CSF analysis may throw sufficient light on the nature and severity of the neurological disorder and hence recommended for field veterinarians.

3. Oral administration of Ribavirin is not effective in treating canine distemper encephalitis.

4. More than 80 per cent clinical cases of canine distemper encephalitis could be diagnosed from EEG and hence recommended for institutions.

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Appendix

APPENDIX 1

MADRAS VETERINARY COLLEGE
DEPARTMENT OF CLINICAL MEDICINE AND THERAPEUTICS

DATA SHEET

Serial Number :

Case Number :

Date :

Name of the owner :

Address :

Telephone Number :

Signalment

Breed :

Sex :

Age :

Colour :

Body weight :

Owners Complaint :

History :

Present history :

Past history :

Immunization done :

General Inspection

Behaviour and general appearance :

Body condition :

Respiration :

Abdomen :

Posture and gait :

External surfaces of body :

Abnormal acts :

General Clinical Examination

Body temperature :

Pulse/min :

Respiration/min :

Mucous membrane :

Eyes :

Detailed examination for Canine Distemper

Eyes

Eye sight :

Ocular discharge :

Respiratory System

Snout :

Nasal discharge :

Cough :

Dyspnoea :

Auscultation findings :

Digestive system

Appetite :

Vomiting :

Diarrhoea :

Skin

Dehydration :

Pustules :

Appearance of foot pads :

Detailed examination for nervous involvement

Behaviour :

Posture :

Gait :

Seizures :

Myoclonus :

Posterior paralysis :

Ocular manifestations (Ophthalmoscopy) :

Examination of cranial nerves :

Postural reactions :

Tonic neck responses :

Placing reaction :

Proprioceptive positioning :

Extensor postural thrust :

Wheel barrowing :

Hemi walking

Hopping

Spinal reflex examination

Thoracic limb

Pelvic limb

Perineal reflex

Panniculus reflex

Physical examination of muscles

Laboratory investigations

CSF

Physical

Cell count

Protein

CPK

Haematology

Electroencephalography

Others

Diagnosis

Prognosis

Therapy

Results

Remarks