



12-1977

Effects of Anticonvulsant Drugs on the Morphological and Neurophysiological Aspects of an Experimental Model of Epilepsy

Linda D. Compton
Western Michigan University

Follow this and additional works at: https://scholarworks.wmich.edu/masters_theses



Part of the Biology Commons

Recommended Citation

Compton, Linda D., "Effects of Anticonvulsant Drugs on the Morphological and Neurophysiological Aspects of an Experimental Model of Epilepsy" (1977). *Masters Theses*. 2169.

https://scholarworks.wmich.edu/masters_theses/2169

This Masters Thesis-Open Access is brought to you for free and open access by the Graduate College at ScholarWorks at WMU. It has been accepted for inclusion in Masters Theses by an authorized administrator of ScholarWorks at WMU. For more information, please contact wmu-scholarworks@wmich.edu.



EFFECTS OF ANTICONVULSANT DRUGS ON THE
MORPHOLOGICAL AND NEUROPHYSIOLOGICAL ASPECTS OF AN
EXPERIMENTAL MODEL OF EPILEPSY

by

Linda D. Compton

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment
of the
Degree of Master of Arts

Western Michigan University
Kalamazoo, Michigan
December 1977

ACKNOWLEDGEMENTS

Dr. Jean Lawrence, Dr. Robert Straw, Dr. Arthur Diani, Dr. Fred Gault, and Dr. Cecil McIntire have been of tremendous help in designing and finishing this thesis. I want to thank them for supporting my work in the challenging and significant area of epilepsy research.

Linda D. Compton

INFORMATION TO USERS

This material was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.
2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.
3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again — beginning below the first row and continuing on until complete.
4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.
5. PLEASE NOTE: Some pages may have indistinct print. Filmed as received.

Xerox University Microfilms

300 North Zeeb Road
Ann Arbor, Michigan 48106

MASTERS THESIS

13-10,037

COMPTON, Linda Darlene

EFFECTS OF ANTICONVULSANT DRUGS ON
THE MORPHOLOGICAL AND NEUROPHYSIOLOGICAL
ASPECTS OF AN EXPERIMENTAL MODEL
OF EPILEPSY.

Western Michigan University,
M.A., 1977
Biology

University Microfilms International, Ann Arbor, Michigan 48106

TABLE OF CONTENTS

Section	Page
I INTRODUCTION	1
II LITERATURE REVIEW	5
Focal Models of Epilepsy	5
Morphology of the Cobalt Lesion	6
Secondary Epileptogenic Lesions	13
Anticonvulsant Drugs	15
III MATERIALS AND METHODS	23
Operative Techniques	23
Experimental Procedure	25
Data Evaluation	27
Histological Examination	28
IV RESULTS	29
Evaluation of Baseline EEG Activity	29
Evaluation of Drug Induced Changes in the EEG	31
Histological Results	33
V DISCUSSION	53
VI CONCLUSIONS	60
VII LITERATURE CITED	61

List of Tables

	Page
Table 1. Changes in Microvolts Amplitude After Anticonvulsant Drug Administration	36
Table 2. Changes in Spiking Rate After Anti- convulsant Drug Administration	38

List of Figures

	Page
Fig. 1 Percent Change from Baseline EEG Microvolts Amplitude	40
Fig. 2 Percent Change from Baseline EEG Spikes and Spike Bursts per Minute	41
Fig. 3 EEG Tracing Before and After Phenobarbital . . .	42
Fig. 4 EEG Tracing Before and After Diazepam	43
Fig. 5 EEG Tracing Before and After Trimethadione . . .	44
Fig. 6 EEG Tracing Before and After Phenytoin	45
Fig. 7 Cobalt Implanted Brain at Eleven Days	46
Fig. 8 Contralateral Hemisphere at Eleven Days	46
Fig. 9 Cobalt Implanted Brain at 65 Days	47
Fig. 10 Contralateral Hemisphere at 65 Days	47
Fig. 11 Glass Implanted Brain at 91 Days	48
Fig. 12 Contralateral Hemisphere at 91 Days	48
Fig. 13 Cobalt Implanted Brain at 142 Days	49
Fig. 14 Contralateral Hemisphere at 142 Days	49
Fig. 15 Cobalt Implanted Brain at 142 Days	50
Fig. 16 Cobalt Implanted Brain at 178 Days	51
Fig. 17 Contralateral Hemisphere at 178 Days	51
Fig. 18 Cobalt Implanted Brain at 178 Days	52
Fig. 19 Contralateral Hemisphere at 178 Days	52

INTRODUCTION

Epilepsy affects at least fifteen million people in the world today. Early descriptions of epileptic seizures date from 2000 B.C. The most widely accepted general definition of epilepsy was proposed almost a century ago by Hughlings Jackson, who proposed that seizures were caused by an "occasional, sudden, excessive, rapid and local discharge of grey matter" which spread from an abnormal focus to normal brain tissue (Jackson, 1931). Introduction of the electroencephalogram (EEG) in the early 1930s was a significant development enabling scientists to describe precisely and evaluate different types of epilepsy. There are many ways to classify epilepsy. The following classification has been chosen to best typify types for this paper.

The first classification of epilepsy is focal epilepsy, where a seizure originates in a local area of grey matter, and convulsions may be confined to a single limb or muscle group. This type of epilepsy might develop after a head injury or abscess resulting in pressure of scar tissue forming on the cortex of the brain. If the patient is conscious, an aura or sensation may occur which can help in localizing the origin of the epileptic discharge. Usually, focal epilepsy is considered to consist of seizures originating in the cerebral cortex, and not in underlying structures. Electrically, in focal epilepsy, there are usually interictal discharges present which can be detected with an EEG in the period between overt seizures. Focal epilepsy may or may not develop into a generalized convulsive or grand mal seizure.

The second major type of epilepsy is characterized by petit mal

(absence) seizures. These brief generalized seizures usually occur in children between eight and fourteen years. There are no interictal discharges in this class. The overt seizure is usually limited to abrupt loss of consciousness which is rapidly regained, usually in five to ninety seconds. This type of epilepsy is suspected when no cortical focus can be detected. The three per second spike and wave abnormal discharge seems to originate in the brain stem and subsequently spreads to both cerebral hemispheres. Changes in body chemistry such as hyperventilation may initiate a petit mal seizure. Drugs effective here may not be effective in any other type of epilepsy.

The third category of epilepsy is generalized tonic-clonic or grand mal. This class includes Jacksonian epilepsy which is characterized by seizures originally local in nature which subsequently spread to the entire brain. This is a less distinctive class which may be initiated by generalized biochemical or metabolic disturbance in the brain such as in progressive degenerative diseases of the brain. Deep brain structures as well as the cortex are involved. The convulsions are major, usually with tonic spasm of all muscles followed by synchronous clonic jerking and prolonged depression of all central functions. Unlike focal epilepsy, there are multiple areas of susceptibility to seizures involving large areas of both hemispheres, or sometimes the entire brain. Status epilepticus is a series of rapidly repeated epileptic convulsions without any periods of consciousness in between. The EEG shows diffuse continuous spiking.

The science of understanding and treating the disease of epilepsy was greatly advanced by the acceptance of the use of animal models for

research. Intravenous injections of certain drugs or various forms of cortical insult create, in laboratory animals, a variety of symptoms which behaviorally, morphologically, or electrophysiologically appear similar to characteristic seizure phenomena in man. In the present study an experimental model of chronic focal epilepsy was created by placing a tiny piece of cobalt metal on the surface of one hemisphere of the cerebral cortex of rats.

The presence of the cobalt metal, by some mechanism not well understood, promotes development of spontaneously epileptic nervous tissue in the area of the implant. The abnormal, but discrete, section of the cortex from which random bursts of electrical discharge originate, is a chronic epileptogenic focus. Following implantation, the electrical activity of the cortical area adjacent to the site of implant, and of the corresponding area of the opposite undisturbed cerebral hemisphere, was recorded with cortical electrodes. The progressive development of the epileptogenic focus with certain cortical neurons discharging in a random, irregular manner with abnormally high bursts of electrical activity was monitored. Once the presence of the focus had been established, four drugs commonly used in the treatment of epilepsy in humans were selected and their effect on the electrical activity of the brain was observed.

There were four major objectives of this study. The first was to observe any physiological change in the brain tissue. This was measured electro-physiologically with an electroencephalogram (EEG), and recordings were made from forty-eight hours to 178 days after implantation of the cobalt.

The second objective was to watch for the spread and development of a secondary focus on the "mirror" side or undisturbed cerebral hemisphere of the brain. A secondary focus would be indicated by abnormal spontaneous electrical discharges originating on the undisturbed side of the brain, or areas of abnormal morphology in the appearance of the cerebral cortex in sections of the undisturbed hemisphere.

The third objective was to observe the effect of four anticonvulsant drugs, diazepam, phenobarbital, phenytoin, and trimethadione, on the electrophysiological state of the primary focus. The electroencephalogram was recorded before and after drug administration, and any changes were recorded.

The final or fourth objective was a morphological study of the lesions. The affected areas of the brain were removed and appropriate histological and histochemical stains were used to compare the structure and composition of the epileptogenic tissue to that of control animals. The changes in the tissue over a period of time from eleven days to 178 days were observed.

LITERATURE REVIEW

Focal Models of Epilepsy

Various techniques for the development of a model of epilepsy have been studied. While many of these methods are useful in simulating behavioral and electrical events in epilepsy, the entire brain is involved, the seizure is of a short duration and, unlike clinical epilepsy, does not recur.

Chronic models have been developed to aid in the study of the mechanism by which damaged or diseased neurons generate spontaneous chronic clinical epilepsy. These models of epilepsy are created by damaging or irritating a portion of the brain tissue. Also, repetitive bursts of photo stimulation, ten to fifteen hertz, are used for induction of seizure in animals that have a lower convulsive threshold than normal animals, e.g. animals with experimentally produced epilepsy. The epileptic models produced in these animals are similar to behavioral and electrical manifestations of clinical epilepsy in man. Furthermore, there is convincing evidence that makes the further utilization of these models valuable as a tool for research. A review of these models can be found in Ward, 1969.

Implantation of heavy metals on the cortex of the brain to produce experimental epilepsy, has been extensively studied. The most successful model has been created by the implantation of cobalt powder or wire, and will therefore be the only model reviewed here. The cobalt model of experimental epilepsy is not well understood. Spontaneous generalized

convulsive attacks are rare, but animals implanted with this heavy metal become sensitive to sub-threshold convulsive stimuli which would not affect normal animals. On the fourth or fifth day after implantation on the cortical surface, seizure activity can be monitored with an electroencephalogram, but usually no clinical or behavioral seizures are evident (Henjyoyi and Dow, 1965). A delay between cortical insult and initiation of spiking was observed, but any behavioral event such as a muscle or whisker twitch always occurred simultaneously with a spike in the EEG. The seizure threshold, tested by electroshock and Metrazol (pentylenetetrazol) challenge, were lower in cobalt implanted animals. Prodding or stressful handling did not produce clinical (behavioral) or electrical seizures. Spontaneous electrical seizures were observed, however, and strong independent focal spike activity was observed on both sides of the brain after eight days (Kopeloff, 1960). Anatomically, large lesions were present in all rats within one week after cobalt implant, but the size of the lesions did not correspond to the amount of primary electrical activity (Engel, 1968). The behavior of the rats was normal at all times. Rats, implanted in the frontal lobe, developed spiking on the mirror side of the brain before the primary side, after which spikes were recorded from the primary side (Dow et al., 1972). In the parietal lobe, any signs of focus development consistently disappeared, whereas the abnormal activity in the frontal lobe lasted three to nine months after implantation.

Morphology of the Cobalt Lesion

Kopeloff (1960) reported that several heavy metals, in particular

cobalt, had epileptogenic properties when applied to the brain of rats and mice. Payan (1967) implanted several cats with copper, brass, and silver metals, and observed mild gliosis, but no lowered epileptic convulsive threshold, or chronic epileptic focus. Cobalt, both in powder and solid form, produced edema, congestion, inflammatory response, fibrous tissue reaction around the lesion, gliosis, neuronal damage, iron and calcium deposits, scarring of the meninges and an increased presence in many subacute inflammatory cells. Inflammatory infiltrates were more diffuse in powder-implanted rats. The crater in the brain of rats implanted with a metallic sliver of cobalt, was more effectively localized by formation of a fibroglial scar. The crater left by the cobalt powder was filled with cell debris and inflammatory cells, whereas the crater formed by the cobalt rod was virtually empty (Engel, 1968).

Although other metals caused necrosis without lowering the convulsive threshold, the severe vascularity and the calcium deposit may have been involved in the mechanism by which the convulsive threshold was lowered. Heavy metals are thought to exhibit convulsant action because of interference with enzymatic processes of the cell rather than by mechanical effect of its presence, or the secondary scarring which follows. Dow et al. (1972) concluded that epileptic activity can not be related to the scar or reactive gliosis. Epileptic activity sometimes decreases one to two weeks after implant, when the reaction is strong, but no scar is formed.

Dow et al. (1972) reported that the implantation of solid cobalt made a discrete lesion, and that the contralateral hemisphere was always normal. The cobalt lesion was two to three millimeters in diameter, and

was necrotic with varying degrees of fibrosis. The central lesion area was surrounded by a layer of calcium deposit and gliosis. The adjacent brain tissue had increased vascularity with neuronal degeneration. A slight pial thickening was observed at the site of each screw electrode on the brain, probably due to pressure from the screws on the brain. Glass implants produced no necrotic reaction or any evidence of abnormal electrical activity. Dow et al. (1962) suggested that the epileptic activity probably depends on cobalt's chemical influence on the neurons of the cortex which at first shrink then disappear. Payan in 1967 examined the epileptogenic effect of cobalt. He found that cobalt slivers worked as well as cobalt powder, and therefore, the effectiveness could not be related to the solubility of the powder, furthermore, cobaltous nitrate, a soluble powder was not effective. A breakdown of the blood brain barrier was not involved, because other metallic implants of the same size and shape and in the same location did not lower pharmo-convulsive thresholds. The epileptic foci were not caused by the necrotizing effect, gliosis, or fibrosis because other metals and compounds produced similar histological response without epileptic activity. Payan concluded that severe vascularity around lesions and heavy calcium deposits, or some inherent power or molecular conformation of the cobalt could possibly initiate the epileptogenic state.

Cesa-Bianchi et al. (1967) applied cobalt powder to the cortex of 50 cats and observed that nerve cells involved in the lesion were modified in structure with less stainable nissl zones. The glial reaction was much more severe after three to four weeks. Electroclinical manifestations induced by powder faded away as the perifocal glial

reaction became more intense. This suggested that the epileptogenic effect is due to direct action of powder on neurons located at the implant site. He suggested that alteration of cellular oxidative processes may cause a direct massive, and abnormally excitatory action on nerve cells in the lesion site. Payan et al. (1965) found that rats with extradural or extracranial cobalt implants were epileptic, but had only meningeal adhesions, local depressions of the cortical surface, minimal subadjacent necrosis, and granulation of tissues. He therefore concluded that cobalt epilepsy can be produced without concurrent severe necrosis.

Fischer et al. (1968), observed that scarring and EEG abnormalities occurred at the same time. In animals with strong inflammatory reaction or abscess, no epileptic activity could be found in the EEG. Engle (1968) found that six days after cobalt implantation the primary epileptic focus was poorly developed, whereas after eight days, it was well developed with dependent mirror focus activity. Eleven days after cobalt implantation, the rat cortex was electrically depressed and no epileptiform activity appeared to propagate from the mirror focus to the primary site. The primary focus in the powdered cobalt rat developed on the anterior edge of a large lesion, several millimeters anterior to the implant. By day 18, the cobalt implanted rat had spike activity several millimeters anterior the site of implantation and a mirror focus homotopic and contralateral to the primary focus rather than to the site of implantation. The primary focus was active at day 18. At day 24 the focus activity was depressed, and Engle found that the primary focus activity was absent by the thirty-second day after implant.

There are several excellent histological descriptions (Payan, 1971; Dow et al., 1962; Cesa-Bianchi et al., 1967; Fischer et al., 1968; and Henjyoyi and Dow, 1965) of the development of the epileptogenic lesion in the brain of rats. These reviews are briefly combined here to give an overview of the morphological aspects of cobalt implantation of the cortex of the brain.

During the first through third days after implantation, Payan (1971), described edema, congestion, necrosis in rats implanted with cobalt. Eight hours after implant, there was minimal edema or necrosis around the lesion, and the tissue adjacent to the lesion showed vacuolar degeneration with a few anoxic neurons. There was little demyelination and blood vessels appeared normal. From one to six days after implant, Payan (1971) and Cesa-Bianchi et al. (1967) saw a similar picture with gradual exaggeration of the tissue response. Size of the lesion gradually increased. Edema and necrosis increased, minimal hemorrhage was noted, and spongy degeneration was more extensive. Anoxia and neuronal degeneration was seen around the lesion with moderate proliferation of astrocytes and blood vessels and fibroblasts. Bodian, cresyl violet and silver stained tissue, showed granular substance adjacent to the necrotic center of the lesion. Some distortion and disruption of axons were noted, and the number of cells in the adjacent cortex was reduced.

Between the first and second week after implant, Henjyoyi and Dow, 1965, found inflammatory and phagocytic cells at the borders of the lesion. Payan (1971) and Dow et al. (1972) observed an exaggeration of most of the tissue responses seen in the first week after cobalt implantation in rats. A calcium deposit gradually appeared around the

lesion within the granular area and became more intense with time.

Occasional small necrotic foci were noted a few millimeters away from the main lesion with granules near the center similar to those found in the main lesion.

Cesa-Bianchi et al. (1967) found that the glial reaction was more pronounced when the animal was sacrificed three to four weeks after the implant. Nerve cells involved were modified in structure with less stainable nissl zones. Henjyoyi and Dow (1965) found a fibrous menin-gocerebral scar at the site of the cobalt implant. Payan (1971) observed a marked proliferation of fibroblasts, histiocytes, astrocytes and blood vessels. The intensity of the edema and vacuolar degeneration was reduced from the third week to the sixth. The fibroblastic and histiocytic reaction was prominent with an occasional giant cell. The calcium deposit was heavier, and a minimal iron deposit was noticed in some animals. Pronounced proliferation of blood vessels and a reticular network was still seen adjacent to the lesion in some sections stained by the reticular and Bodian methods. Meninges showed severe fibrosis. Some studies showed perivascular lymphocytic cuffing a few millimeters from the primary lesion. On the sixteenth day, Dow et al. (1962) found that the reaction totally surrounded the lesion and the lesion was increased in size, deepened and more circular. The center was pale and the outer part deeply stained because of striking cellular infiltration.

One to three months after cobalt implant in rats, Payan (1971) reported that the tissue reaction had lessened after a few months, whereas the calcium deposit and the blood vessel proliferation increased. Some animals again showed perivascular cuffing and psammoma bodies

(small brain tumors with calcareous particles). Vacuolar degeneration of the cortical lesions occurred from seven to ten weeks after implant, with occasional neurons calcifying around the main lesion. The fibroblastic and histiocytic reaction was still active, although myelin was not affected. The surrounding tissue was vascular with occasional distant lesions characterized by calcification and necrotic areas. A few animals showed lateral ventricle dilation on the same side as the implant. No direct connection between the cobalt lesion and the dilated ventricle was hypothesized. After two months, Dow et al. (1962) noticed the lesion was larger, and an inflammatory reaction extended from peripheral parts of the lesion into the central area.

Three to six months after implant, Payan (1971) reported reactions similar to those described previously, with moderate rather than severe intensity. Exceptions were the calcium deposit and the vascular proliferation which were quite intense. The calcium completely engulfed the lesion. After six to twelve months, Dow et al. (1962) observed that the central core of the lesion had disappeared, but there was a dense sclerotic reaction surrounding the site of the cobalt application. Payan (1971) noted a decreasing intensity of the inflammatory processes, but the heavy calcium deposit actually interfered with sectioning of the tissue. Many animals had a solid band of calcium around the lesion. Vascularity of adjacent brain tissue was intense, but all other reactions were markedly reduced. Twelve months after implant Payan (1971) reported that some of the rats were still epileptic.

Fischer et al., in 1968, published an excellent review of zone histology, describing the development of the lesion as changes occurred

in the layers or zones of brain tissue below the surface of the cortex. Electron microscopic studies of epileptic lesions are presented in a paper by Westrum et al. (1964).

Secondary Epileptogenic Lesions

There is much controversy surrounding the appearance and physiology of secondary epileptogenic lesions. Guerrero-Figueroa et al. (1964) described the secondary lesion as a ganglionic network in which spontaneous and evoked activity have been chronically altered by continuous epileptiform bombardment, causing a lasting metabolic aberration to take place in the cells at the secondary site. These authors, in contrast to others, reported that no structural change took place at the secondary site.

Morrell et al. (1959), using a method of topical freezing with ethyl chloride, observed that there was always an anatomical connection between the primary and secondary foci. This connection was at least one synapse away, and appeared to be callosally mediated. The secondary spike was always an evoked potential at first, and later became independent. Payan (1970) observed that the secondary or mirror focus takes over when the primary focus has been surgically removed, and has independent activity. Ischemia on one or both sides of the brain increased the convulsive threshold by the same amount. Engle (1968) agreed that the secondary epileptogenesis observed in rats appeared similar to the mirror focus described in other animals. Dense cells arranged in discrete nests were seen in the contralateral cortex of epileptic rats, and these corresponded topographically to the borders of the primary lesion.

These nests contained darkly stained fibrillar neurons. The basophilic properties of the cells with their densely stained dendrites resembled mirror epileptic cortex, as described by Morrell et al. (1959). Similar basophilic neurons were observed in control animals but throughout the entire cortex, whereas the secondary epileptogenic cortex contained discrete nests which were either anterior, posterior, medial or lateral to points homotropic and contralateral to the original site of implant.

Morrell, 1969, found that the secondary focus could be readily identified by the nests of pyronine-dense cells, stained with methyl green pyronine, aqueous azure B bromide, or gallocyanin at acid pH. The secondary focus was thirty-five to fifty percent increased over controls in azure B binding. This basophilic substance was RNA. Increased dye binding was limited to neurons and the glia were not altered. Jasper discussed three possible hypotheses concerning the presence of an increased RNA concentration in the secondary focus site. The first hypothesis assumed that the cells might be dying or dehydrated and the increased RNA concentration was a function of decreased cell size rather than increased RNA concentration. Perhaps the continuous bombardment from the primary focus across the callosal pathways was sufficient to cause damage and eventual death of the neurons in the mirror focus. The second hypothesis suggests that the dense cells might be inactive. Studies were reported suggesting that physiological inhibition of activity on a neuron would cause decreased RNA consumption and result in dense chromophilic cells due to accumulation of this substance intracellularly. The third hypothesis suggested that the hypersynchrony and rapid unit discharge could result from intracellular alterations in

RNA. Unfolding the RNA molecule would result in total increase in closely spaced atomic charges. Potassium concentration near the membrane would change and an increase in the intracellular potassium would occur, resulting in hyperpolarization. This would make the neuron less responsive until specific synchronizing input from the primary foci or some other source was received.

Anticonvulsant Drugs

There are three mechanisms by which anticonvulsant drugs might affect seizures (Goodman and Gilman, 1975). First is an effect on non-neuronal lesions, such as normalization of the ischemic blood supply of typical cortical seizure foci. The second exerts effects confined to pathologically altered neurons of the seizure focus, to prevent their excessive discharge. The third category of anticonvulsant drugs affects normal neurons to prevent their discharge by the seizure focus. Dow et al. (1973) suggested that most antiepileptic drugs in current clinical use probably exert their effect on normal neurons by preventing spread of depolarization from the seizure focus. The ideal anticonvulsant would have effects confined to abnormal neurons of the focus to prevent excessive discharge. This ideal anticonvulsant drug would not necessarily show potent anticonvulsant activity in the standard laboratory animal tests where no chronic pathological state exists. Electroshock and administration of convulsive drugs like Metrazol, are examples of commonly used models of seizure activity which produce no chronically altered or damaged neurons. Swinyard (1969) proposed that drugs which modify electrically and chemically induced generalized seizures, and

raise the seizure threshold for low-frequency electroshock in animals are those which are clinically effective against grand mal seizures. Boyer (1966) had three theories for forecasting clinical drug usefulness. The first, similar to that of Swinyard, was that drugs which block seizures induced by maximal electroshock should be effective against grand mal in man. Secondly, those which inhibit minimal electroshock might benefit patients with psychomotor seizures. Lastly, protection against Metrazol-induced seizures will be seen with drugs which are clinically effective against petit mal epilepsy.

Four anticonvulsant drugs, representative of four major types of anticonvulsants in common clinical use, were tested in this study. The first drug studied was diazepam (Valium). This drug is characteristic of a group of drugs called the benzodiazepines. These were first introduced as tranquilizing agents, and possess varying degrees of anticonvulsant action against grand mal, petit mal, and psychomotor epilepsy (Boyer, 1966). Diazepam is surprisingly nontoxic and large single doses calm patients. Therapeutic doses cause a low voltage, fast activity EEG tracing lasting a week after the drug is discontinued in humans (Boyer, 1966). Bell (1970) observed that the effect of diazepam on the EEG resembles that of rapidly acting barbiturates. An intravenous dose large enough to impair consciousness generates activity similar to barbiturate fast activity, within two minutes. In status epilepticus seizure discharge is reduced or gone (Bell, 1970).

In experimental models of epilepsy, benzodiazepines suppress the spread of seizure activity produced by epileptogenic foci in the cortex but do not abolish the abnormal discharge of the focus (Goodman and

Gilman, 1975). Van Duijn and Visser (1972) using cobalt-powder implanted cat brains, found that diazepam increased the amount of focal EEG epileptic activity. Stark et al. (1974) concluded that diazepam 0.5 mg/kg and 1 mg/kg was anticonvulsant in cats with penicillin-induced foci. Randall et al. (1961), and Spehlmann and Colley (1968), agreed that unmistakable protection against chemical shock and maximal electroshock was observed, but an unequivocal effect on focal epilepsy has not been demonstrated (Guerreo-Figueroa et al., 1968 and Spehlmann and Colley, 1968).

Phenobarbital (Luminal), the second drug studied here, was the first effective organic antiepileptic agent (Hauptman, 1912). Phenobarbital has been found to be clinically effective in treatment of generalized tonic-clonic (grand mal) and cortical focal seizures and in status epilepticus. Its effects are very general and nonspecific compared to phenytoin and trimethadione. Phenobarbital has more side effects and is slower acting than diazepam. Phenobarbital limits spread of seizure activity and also elevates seizure threshold (Goodman and Gilman, 1975). Phenobarbital is often the first medication tried for grand mal and focal seizures (Woodbury et al., 1972) but is not effective in temporal lobe epilepsy, and may actually make petit mal seizures worse. Sedation is common in initial treatments, but this disappears after continued use. Phenobarbital possesses a significant antiepileptic effect at dose levels that do not cause sedation. Abrupt withdrawal may increase seizure frequency or precipitate status epilepticus.

Dow et al. (1973) demonstrated that phenobarbital given acutely to

cobalt-epileptic rats increased EEG spiking, while phenytoin given acutely or chronically had no abnormal effect on spiking activity. Van Duijn and Visser (1972) found no significant effect on the EEG in cats implanted with cobalt powder, which were treated with phenobarbital. There is a threshold raising effect for chemical shock and electroshock. Some experimenters assume it suppresses focal cortical epileptic discharges (Millichap, 1969), whereas others observed activation of the focus (Morrell et al., 1959). Roldan et al. (1971) found that in cobalt implanted rats, the presence of sleep cycles after the low dose make measurement of duration of effect difficult. Spikes around the focus increased after barbiturate administration and during slow wave sleep. Low doses depressed spontaneous seven to nine per second spike bursts, ten to fifteen minutes after injections. High doses of barbiturates suppressed spike and spike bursts for several hours. Guerreo-Figueroa et al. (1964) found that phenobarbital inhibited development of a secondary focus. Rats receiving fifty milligrams phenobarbital daily, beginning seven days after implantation of alumina, did not develop a secondary focus until three weeks after drug discontinuation. Daily administration of fifty milligrams phenobarbital, begun three months after alumina implantation, suppressed or reduced the frequency of clinical convulsive seizures, and the previously established primary and secondary foci were diminished in amplitude and frequency of discharge. Therefore, it appears that phenobarbital retards the establishment of secondary epileptiform discharges but has little effect on the primary focus.

Another drug tested was phenytoin, also known as diphenylhydantoin

(Dilantin) which is of the hydantoin family and is a primary drug for all types of epilepsy except petit mal. Of the four drugs tested, Boyer (1966) found phenytoin to be less effective than diazepam, trimethadione or phenobarbital, in elevation of threshold for seizures produced by convulsant drugs like Metrazol but was second only to diazepam in protection against maximal electroshock. Phenytoin was less effective than phenobarbital or diazepam against minimal electroshock, but better than trimethadione. Unlike phenobarbital, phenytoin is effective against temporal lobe epilepsy. Van Duijn and Visser (1972) found phenytoin suppressed electrocortical focal epileptogenic activity in cats with cobalt foci. In rat studies conducted by Guerrero-Figueroa et al. (1964), phenytoin was given one week before alumina implant, and three weeks after. The primary and secondary foci developed with characteristic EEG. Ten mg/kg intraperitoneally daily resulted in no inhibition of the EEG but a toxic effect was observed. From subcortical structures, spindles were observed, preceded by epileptiform discharge with high amplitude voltage. Phenytoin applied to alumina foci in the hippocampus was followed by decreased amplitude of voltage in abnormal discharges in the primary and secondary foci, or only on the treated side when applied unilaterally. As with phenobarbital, the effect only lasted a few days after drug application. Strobos and Spudis, 1960, found that phenytoin elevated the threshold for excitation and decreased the duration of after discharges, but did not alter the spread of discharges. Guerrero-Figueroa et al. (1964) found, in contrast, that intracerebral phenytoin blocked primary and secondary discharges, but intraperitoneally or intramuscularly produced only slight suppression of

epileptiform discharges. High doses (10-40 mg/kg) facilitated discharges from both foci.

Phenytoin did not prevent development of focal spiking in the rat but did reduce the discharge frequency of the focus (Musgrave, 1963). Secondary foci developed by paroxysmal activity from these foci was less conspicuous in phenytoin treated animals. The effect of phenytoin on the primary focus differed from a barbiturate effect. Phenytoin reduced pacemaker potentiality of old injured neurons, caused rapid repolarization of elements involved in initial trauma, and suppressed subsequent rhythmically recurring nonsynaptically induced fluctuations in neuronal excitability.

Stark et al. (1974) found fifteen mg/kg intravenous phenytoin, to be anticonvulsant in cats with penicillin-induced foci, but the effect lasted only thirty minutes. Jasper (1969) observed that phenytoin did not raise the threshold of seizures induced by photo-stimulation or when challenged by strychnine, and other convulsant drugs and in fact potentiated seizures induced by pentylenetetrazol, salicylate, and carbon dioxide inhalation. Phenytoin slightly elevates those seizures initiated by electroshock, cortisone or thyroxin. Phenytoin inhibits inhibitory neurons as well as excitatory ones possibly accounting for the increase. Dow et al. (1973) concluded that although barbiturates increase the number of spikes arising from the cobalt induced epileptic foci in the rat, phenytoin has no effect. Craig et al. (1976) also found that phenytoin had no effect on seizure frequency, but observed that unlike trimethadione, withdrawal of the phenytoin resulted in a marked enhancement of seizure activity.

The effects on seizure activity found by Craig et al. (1976), after chronic treatment of cobalt epileptic rats with either phenytoin or trimethadione, were not dose related. The dose of phenytoin is very important, because if it exceeds the effective amount and enters the toxic range, the mechanism for arresting focal convulsive seizures might be inhibited. Therefore even though a low dose inhibits seizures, a high dose may potentiate them. High doses facilitate primary and secondary discharges, and are likely to cause sedation, nausea, weight loss, constipation, ataxia and nystagmus (Guerreo-Figueroa et al., 1964).

Trimethadione (Tridione), the fourth drug used in this study, is effective primarily in petit mal seizures. It is the only oxazolidinedione used extensively in the United States today. The chemical structure is different from most of the other anticonvulsant drugs (Goodman and Gilman, 1975).

Trimethadione in large doses produces CNS depression, analgesia, sedation and a form of photophobia, hemeralopia (a visual disturbance consisting of a glare or white halo around objects). The threshold for chemical and electrically induced seizures is raised after trimethadione administration. It is better than phenytoin in protecting against pentylenetetrazol-induced seizures, but not as good in modifying maximal electroshock patterns.

Craig et al. (1976) suggested that trimethadione may enhance spiking activity originating from the cobalt focus in rats, but limit the subsequent spread that leads to the development of grand mal convulsions. Morrell et al. (1959) also observed that trimethadione depressed

projection of seizure activity from cortical foci to the thalamus while leaving cortical spread relatively unaffected. The opposite is true of phenytoin. The thalamocortical system is very important in the genesis of petit mal epilepsy, and the selectivity of the thalamus to trimethadione may account for much of its therapeutic effectiveness in this type of epilepsy. There is little information in the literature about this drug in chronic animal models of epilepsy.

MATERIALS AND METHODS

Operative Techniques

Thirty-six male albino Sprague Dawley origin, specific pathogen free, rats from the Upjohn colony, weighing between 250 and 300 grams each, were used in this investigation. Twenty-four rats were in the experimental group and had a one millimeter length of cobalt wire placed on the surface of one cerebral hemisphere. The other twelve rats received glass implants of the same size as the cobalt wire, which served as a space occupying, but non-epileptogenic lesion. The rats were maintained on a diet of Purina Laboratory Chow and water which were always available.

Following anesthetization with forty mg/kg sodium pentobarbital intraperitoneally, a two centimeter midsagittal incision was made through the shaved scalp from directly behind the eyes to between the ears. The periosteum was scraped aside and retracted with hemostats to expose the skull. The naso-frontal, coronal, lambdoidal and sagittal sutures were located and used as coordinates to consistently place cobalt wire or glass implants approximately three millimeters right of the sagittal suture, and three millimeters behind the coronal suture. A two millimeter hole was drilled with a Foredom dental drill, series EE. Figure 3 illustrates electrode placement, and headpiece construction. Stereotaxic placement of the glass or cobalt in a precise location was not needed because the lesion was on the cortical surface and did not penetrate into the brain. Adequate precision was possible by visual

mapping of the skull using the highly visible sutures in the skull.

Rats weighing less than 250 grams were not used because the skull had not completely calcified, and had a spongy, porous texture. These small rats hemorrhaged more frequently which made locating the sutures, and drying the surface of the skull to adhere the cap, more difficult. The skull of larger rats rarely bled, and dried quickly.

A one to one-and-one half millimeter length of one millimeter diameter cobalt wire with oxidized metal removed by scraping, was gently pushed through the dura and to the top of the cortical surface. The hole was filled with bone wax, and the skull roughened with a scalpel to provide a more abrasive surface for the cap. Five one millimeter holes were drilled in the skull for placement of four recording electrodes and one ground electrode. One pair of holes was located one millimeter anterior to the coronal suture and spaced three millimeters to either side of the sagittal suture. The next pair of holes was placed four millimeters behind the implant site, spaced three millimeters to either side of the sagittal suture. A hole for the ground electrode was drilled five millimeters in front of the coronal suture on the left side of the sagittal suture.

Electrodes were constructed by soldering single-strand, insulated stainless steel wire to three millimeter flat end screws. The recording electrodes were screwed into the holes in the skull until the head was flush with the surface of the skull. The tips of the screws rested against the dural or cortical surface but did not penetrate into the brain. These screws were used to record the EEG, and apparently did not disrupt normal electrical activity of the brain. The ground electrode

was a five millimeter pointed-end screw with deeper threading. This was screwed down until the head was three millimeters above the skull and the tip was on the cortical surface, to achieve better anchorage of the cap. Cold-cure acrylic resin was painted thickly around the screw heads and allowed to harden. The hardened dental acrylic cap shielded the wires and screws and gave the cap mechanical strength as well as helping adhere the headpiece more firmly to the skull. After the first application of acrylic, only the wire leads from the electrodes were visible, projecting out of the top of the cap. An eight-lead female continental connector was soldered to the exposed ends of the electrode wires. The excess wire was tucked under the headpiece and an additional layer of acrylic cement immobilized the wires and the headpiece. This procedure smoothed the sides, and lessened the danger of catching the headpiece on the wire cages.

The animals were ready for experimental use forty-eight hours after implanting the cobalt. The entire operative procedure took thirty to sixty minutes, and the headpiece was functional for as long as seven months.

Experimental Procedure

Electroencephalogram recordings were begun no sooner than forty-eight hours after the surgery, continuing for as long as 178 days after implant. Wires from the male continental connector lead into a 7P511 amplifier and paper recordings were made by a Grass Model 79 polygraph. Two hand screws on the male plug allowed the headpiece to be firmly fitted to the female plug in the rat's headpiece. Two channels of EEG

were recorded. The montage used is shown in the inset in Figure 3. Bipolar recordings were made at least three times with each rat after development of the epileptic focus. A four liter beaker served as a recording chamber. A cardboard box with an observation window provided protection from environmental distraction. The rat could move freely in the chamber restrained only by the electrode leads. The wire leads from the headpiece were bound in woven steel thread insulator mesh, and brought through an opening in the top of the chamber to the Grass polygraph.

The rats were assigned to four experimental groups with six cobalt implanted rats, and three glass implanted rats in each group. Each group received a different anticonvulsant drug with each rat tested at least once at each of three dose levels. The order in which the rat received the dose levels was determined by a Latin square design. The anticonvulsant drugs tested were selected as representative of major classifications of anti-convulsive drugs. The doses were chosen after reviewing several journal articles using rat models of experimental epilepsy, and picking frequently used low, high, and mid-range doses. The first experimental group received phenobarbital at 5, 10, and 20 mg/kg. The second group was given diazepam at 5, 15, and 30 mg/kg. Group three got 25, 100, and 200 mg/kg phenytoin, and the fourth group received trimethadione 40, 100, and 300 mg/kg. All drugs were dissolved or suspended in physiological saline immediately before use. The volume did not exceed 1.5 ml and was given as a bolus injection intraperitoneally.

The EEG was recorded for forty-five minutes prior to drug administration, and for three to three-and-one-half hours after. Five cobalt-

implanted rats and two glass-implanted rats were given sham injections of physiological saline, and the EEG was recorded in the same fashion.

Data Evaluation

EEG recordings were examined visually by the experimenter, and were quantified according to the number of spikes per minute, and the general amplitude of electrical activity in microvolts. A spike and/or spike burst was defined as an abnormally large burst of electrical activity either appearing singly or grouped in the EEG record. These spikes varied in duration and voltage between subjects and between recording sessions in one subject, but were usually consistent in appearance within one recording session. Electrical activity across each cortical hemisphere of the brain was recorded, and rated separately. No differentiation was made between spikes and spike bursts in the evaluation. Each peak exceeding twice the EEG background electrical activity level was counted as one unit. The number of units per minute was counted, and an estimate of mean background amplitude was made. These two parameters served as a means of quantifying the EEG activity.

The data was grouped in fifteen minute blocks and then the average number of units per minute and average microvolts of electrical activity for the following specific time intervals were computed. To facilitate data evaluation, fifteen minute averages were further grouped into five periods, baseline was measured one hour before drug administration, and zero to thirty, thirty to ninety, ninety to 150, and 150 to 210 minutes after drug administration.

Histological Examination

Brains were examined at days 11, 30, 65, 91, 142 and 178 after the implantation of glass or cobalt in the frontal cortex. The animals were sacrificed by cervical dislocation, and the appropriate section of brain immediately removed. Tissue sections from the implanted side and the control mirror side were fixed in Bouin's solution prior to paraffin embedding procedures (Luna, 1968). Eight micron thick sections of the brain tissue were made. Stains used were Bodian method for myelinated and unmyelinated nerve fibers, neurofibrillae, and neuron cell bodies; Phosphotungstic acid-Haematoxylin (PTAH) for neural glial fibers, myelin sheaths and collagen; Luxol Fast Blue for myelin sheaths, nuclei, and nissl substance, and Kossa's method for calcium salts (Luna, 1968). Appropriate photomicrographs were taken in cooperation with The Upjohn Company.

RESULTS

Several parameters of the EEG tracings were evaluated to provide means of comparing drug effects. The frequency of spiking, measured in peaks per minute, and the mean amplitude of electrical activity per minute, were measured from recordings made across the implant as well as across the contralateral hemisphere of the brain. The EEG of the glass implanted animals was recorded and evaluated in the same way. The mean amplitude of the EEG and spiking rate was measured over five time intervals. The first interval was thirty minutes, measured immediately prior to the drug administration. Thirty minutes following the injection was chosen as the second interval, with the third interval covering thirty to ninety minutes after the drug. The fourth interval included ninety to 150 minutes after the drug challenge. Finally 150 to 210 minutes post-drug was evaluated.

Evaluation of Baseline EEG Activity

Because the volume of data generated by this system of measurement was too cumbersome to evaluate, an abbreviated method of measurement was sought. There was almost no difference between the implant side of the brain and the contralateral hemisphere with respect to spiking rate or amplitude, therefore it was not possible to demonstrate spread of the abnormal activity from the implant side to the contralateral side. The first appearance of the focus was seen simultaneously in recordings from both halves of the brain. Because there was no difference in the effect

of the four drugs studied on spiking rate or amplitude, only the implant side will be discussed.

The presence of the abnormal epileptic activity or focus, was clearly established within the first week after implant. The appearance of the abnormal EEG activity varied between rats and in one rat between recording sessions. Some animals exhibited a sudden burst of large spikes, sometimes accompanied by a facial twitch or hesitation. Some animals had random large single spikes in the EEG, and never had spike bursts. Most animals did not exhibit overt behavioral seizure activity. Some of the glass implanted controls also were observed to have an abnormal EEG, similar in appearance, but not as severe as some of the cobalt implanted rats. Based upon histological confirmation, this unexpected observation in the controls could probably be due to surgical trauma. Because of this trauma these animals were not considered to be adequate controls.

The second major goal of this study was to observe spread of the focus to the other areas of the brain. When abnormal spiking appeared in the area of the cortex around the lesion it appeared in the EEG tracing on the contralateral side as well. With the corpus callosum intact, electrophysiological spread between the implant side and the undamaged hemisphere took place too quickly for a general EEG recording to differentiate. There are therefore, no quantifiable results reported from this objective. The effect of anticonvulsive drugs on the focus was evaluated on the basis of changes in spiking rate and EEG amplitude from the pre-drug baseline.

The control animals receiving saline in place of one of the four

anticonvulsant drugs, had variable EEG activity changes. If the rat was sleeping before the injection was made, the EEG tracing reflected a change from slow wave sleep, to smaller amplitude, faster wave, alert EEG stages. Cobalt-implanted rats with large spike and spike-bursts continued to show the abnormal EEG activity after the saline administration intermittently throughout the recording session. Glass-implanted rats in this group had no spike or spike-burst complexes, before or after the saline injection.

Evaluation of Drug Induced Changes in the EEG

Using a nonparametric Friedman two-way analysis of variance by ranks (Siegel, 1956), there was no significant difference at the 5% significance level between any of the dose levels of any drug tested, so the three dose levels have been combined, and one mean value calculated for each drug. Tables 1 and 2 contain the baseline EEG amplitude and spiking rate and the percent changes in activity over the four previously described time intervals.

Rats injected with phenytoin were observed to have a thirty-five percent increase in electrical amplitude over the baseline amplitude, from an initial level of around 166 microvolts to more than 200 microvolts two hours after the drug (see Figure 1). The spiking rate increased slightly from around nineteen spikes per minute to almost twenty-one spikes per minute, immediately after phenytoin, and after two hours was about twenty-four spikes per minute (Table 2). A characteristic tracing illustrating the effect of phenytoin is presented in Figure 3.

Diazepam caused an eighteen percent increase in the spiking rate

immediately after administration, which peaked and began to recover after ninety minutes. During the 150-210 minute interval, the spiking rate remained eleven and seven-tenths percent above the initial baseline (Table 2). Figure 2 shows the results graphically. The amplitude of the electrical activity rose seventeen percent over control after diazepam (Figure 1). After 210 minutes the amplitude was only thirteen percent higher than the pre-drug baseline. Figure 4 is a segment of characteristic EEG tracing before and after diazepam administration.

Figure 5 illustrates the effect of trimethadione on the EEG of an epileptic rat. Trimethadione lowered the spiking rate eleven and one-half percent in the first thirty post-drug minutes (Figure 2). In the following hour, the suppressed rate continued, but was only about six percent below the pre-drug baseline. After 150 minutes, the rate became level at approximately fourteen spikes per minute (see Table 1). Tridione was the only drug which lowered the mean spiking rate below the baseline level. Electrical amplitude increased three and four-tenths percent in the first thirty minutes but continued to rise to a peak increase of seventeen percent after 150 minutes. Figure 5 illustrates the drop in spiking rate, but increased amplitude.

The final drug tested, phenobarbital, caused a tremendous increase in electrical amplitude of the EEG (Figure 6). A thirty and one-half percent increase thirty to ninety minutes after drug administration was observed (Figure 1). Figure 2 illustrates the almost eighty percent increase in spiking rate thirty to ninety minutes after the drug. This returned to only forty-nine percent increased over baseline after 210 minutes.

Histological Results

One to two weeks after cobalt implantation a definite lesion was present with the formation of some scar tissue. Blood vessels were numerous and some intracellular vacuoles were observed in the lesion site. Figure 7 shows a phosphotungstic acid hematoxylin (PTAH) stain of a lesion at 40X magnification eleven days after implant, showing massive damage caused initially by the cobalt. The contralateral side of the brain is shown in Figure 8. This tissue was normal, healthy, had a moderate number of blood vessels and the nerve fibers, and pial membranes were intact.

Two months after the cobalt implant an increased number of blood vessels and glial cells were observed. In Figure 9, dark spots around the periphery of the lesion were seen in this Luxol Blue stain. These resembled the secondary lesions reported by Engle (1968), and Morrell et al. (1959). The contralateral half of the brain was normal (Figure 10).

The glass implanted brains also had a definite lesion site, but a less severe vascular reaction was seen than was observed after the cobalt lesions. Figure 11 is a 250X magnification of a glass implanted brain, ninety-one days after implantation. Glial cell fibers and some vacuoles were present in the localized lesion. In the contralateral side of the brain shown in Figure 12, evenly spaced neurons and glial cells were noted and no disturbance was evident.

Four months after the cobalt implant there was a marked proliferation of blood vessels, glial cells, and collagen. Fibroblasts were present in greater number, while fewer neurons were visible in the

lesion site. Figure 13 shows the cobalt lesion 142 days after the implant. The blood vessels in the lesion were very obvious. The opaque area near the lesion site is probably scar tissue and fluid. Figure 14 shows the normal, healthy, mirror half of the brain. Kossa stain for calcium salts was used to look for traces of calcium deposits near the cobalt lesions. The dark spots evident in the center of Figure 15 were only slightly positive for calcium.

After six months no nerve cell fibers were evident in the area of the cobalt lesions, but glial cell fibers were present. Many densely packed small cells were observed in the lesion itself. Outside of the immediate area of the lesion, nerve cell bodies and fibers were normal. The tremendous infiltration of blood vessels in the lesion are shown in Figure 16 in a 178 day old cobalt implant at 100X, stained by the Bodian method for nerve fibers. The clear areas appear to be myelin. Many neurons and a moderate number of myelinated and unmyelinated nerve fibers are present in the contralateral half of the brain (Figure 17). Some round cells with nuclei and no nissl substance were observed. Multi-polar neurons looked normal as did the blood supply.

The lesion site described above is shown again in Figure 18 as it appeared stained with Luxol blue at 250X magnification. Numerous small vacuoles were present around blood vessels in the lesion with some myelination easily visible in the implant areas. There was a definite increase in the number of glial cells in the lesion, along with membranous bone fragments. The bone probably splintered from the skull during implant. Figure 19 shows one row of rounded cells uniform in size,

density, and cell type. The pia mater is undamaged and few blood vessels are present. Neurons are evenly scattered throughout the entire section.

Table I
Changes in Microvolts Amplitude After
Anticonvulsant Drug Administration

Diazepam				
Dose (n=6)	5 mg/kg	15 mg/kg	30 mg/kg	Mean of Three Doses
Mean baseline \pm SE (microvolts amplitude)	136.2 \pm 12.4	140.1 \pm 12.2	142.5 \pm 15.1	139.6 \pm 7.2
Minutes Post-Drug	Mean Percent Change from Baseline \pm SE			
0-30	8.2 \pm 8.0	3.7 \pm 3.3	12.9 \pm 16.4	8.2 \pm 5.9
30-90	17.1 \pm 13.5	11.2 \pm 6.5	12.8 \pm 6.0	13.7 \pm 5.1
90-150	19.1 \pm 13.1	9.7 \pm 9.3	22.7 \pm 14.5	17.2 \pm 6.9
150-210*	21.3 \pm 14.9	-2.9 \pm 21.6	21.3 \pm 11.5	13.2 \pm 9.3
Phenytoin				
Dose (n=6)	25 mg/kg	100 mg/kg	200 mg/kg	Mean of Three Doses
Mean baseline \pm SE (microvolts amplitude)	172.4 \pm 9.1	160.5 \pm 16.0	166.3 \pm 14.1	166.4 \pm 19.0
Minutes Post-Drug	Mean Percent Change from Baseline \pm SE			
0-30	9.6 \pm 3.1	7.3 \pm 5.6	14.1 \pm 5.6	10.3 \pm 3.0
30-90	7.3 \pm 7.9	26.8 \pm 11.0	17.9 \pm 11.1	17.3 \pm 5.8
90-150	12.4 \pm 4.9	27.8 \pm 12.5	30.9 \pm 7.5	23.7 \pm 5.2
150-210*	26.5 \pm 7.7	46.7 \pm 16.5	32.6 \pm 9.1	35.3 \pm 6.7

Table I

Changes in Microvolts Amplitude After
Anticonvulsant Drug Administration

Phenobarbital				
Dose (n=6)	5 mg/kg	10 mg/kg	20 mg/kg	Mean of Three Doses
Mean baseline \pm SE (microvolts amplitude)	170.2 \pm 26.8	171.4 \pm 16.0	166.3 \pm 15.1	169.3 \pm 10.3
Minutes Post-Drug	Mean Percent Change from Baseline \pm SE			
0-30	11.7 \pm 8.8	1.2 \pm 7.6	19.7 \pm 8.4	10.8 \pm 4.8
30-90	34.1 \pm 15.6	17.8 \pm 15.1	39.5 \pm 10.6	30.5 \pm 7.9
90-150	42.0 \pm 17.4	16.0 \pm 8.0	32.8 \pm 10.2	28.3 \pm 7.7
150-210**	41.3 \pm 22.8	12.5 \pm 10.1	22.5 \pm 5.8	25.5 \pm 8.5
Trimethadione				
Dose (n=5)	40 mg/kg	100 mg/kg	300 mg/kg	Mean of Three Doses
Mean baseline \pm SE (microvolts amplitude)	157.8 \pm 24.8	123.0 \pm 11.4	152.7 \pm 16.6	144.5 \pm 10.7
Minutes Post-Drug	Mean Percent Change from Baseline \pm SE			
0-30	-0.1 \pm 2.7	11.4 \pm 5.4	-1.02 \pm 5.7	3.4 \pm 3.0
30-90	3.0 \pm 3.7	19.8 \pm 8.3	3.6 \pm 6.9	8.8 \pm 4.1
90-150	15.2 \pm 5.5	30.2 \pm 13.1	4.3 \pm 4.1	17.2 \pm 5.4
150-210	5.8 \pm 4.1	21.3 \pm 8.2	9.5 \pm 7.4	12.2 \pm 4.0

*Means calculated on basis of 4 rats because of missing data.

**Means calculated on basis of 3 rats because of missing data.

Table II
Changes in Spiking Rate After
Anticonvulsant Drug Administration

Diazepam				
Dose (n=6)	5 mg/kg	15 mg/kg	30 mg/kg	Mean of Three Doses
Mean baseline \pm SE (spikes/min)	20.6 \pm 6.3	23.9 \pm 4.3	18.4 \pm 3.3	21.0 \pm 2.7
Minutes Post-Drug	Mean Percent Change from Baseline \pm SE			
0-30	10.5 \pm 18.4	-9.9 \pm 16.5	54.7 \pm 55.4	18.4 \pm 20.1
30-90	41.6 \pm 32.5	-0.7 \pm 16.6	21.4 \pm 21.8	20.8 \pm 14.0
90-150	77.1 \pm 40.0	-4.5 \pm 13.8	40.3 \pm 25.9	27.6 \pm 16.6
150-210*	17.2 \pm 37.5	-28.9 \pm 26.2	21.3 \pm 11.5	11.7 \pm 18.4
Phenytoin				
Dose (n=6)	25 mg/kg	100 mg/kg	200 mg/kg	Mean of Three Doses
Mean baseline \pm SE (spikes/min)	26.8 \pm 14.0	16.0 \pm 4.0	14.1 \pm 3.8	19.0 \pm 4.6
Minutes Post-Drug	Mean Percent Change from Baseline \pm SE			
0-30	17.5 \pm 6.8	16.2 \pm 11.5	-6.5 \pm 9.6	9.1 \pm 5.8
30-90	22.4 \pm 17.6	13.7 \pm 16.1	19.1 \pm 10.9	18.4 \pm 8.3
90-150	38.2 \pm 15.6	-9.2 \pm 10.4	34.6 \pm 20.7	21.2 \pm 10.2
150-210**	16.4 \pm 48.0	-2.2 \pm 16.2	63.0 \pm 30.3	25.7 \pm 19.6

Table II
Changes in Spiking Rate After
Anticonvulsant Drug Administration

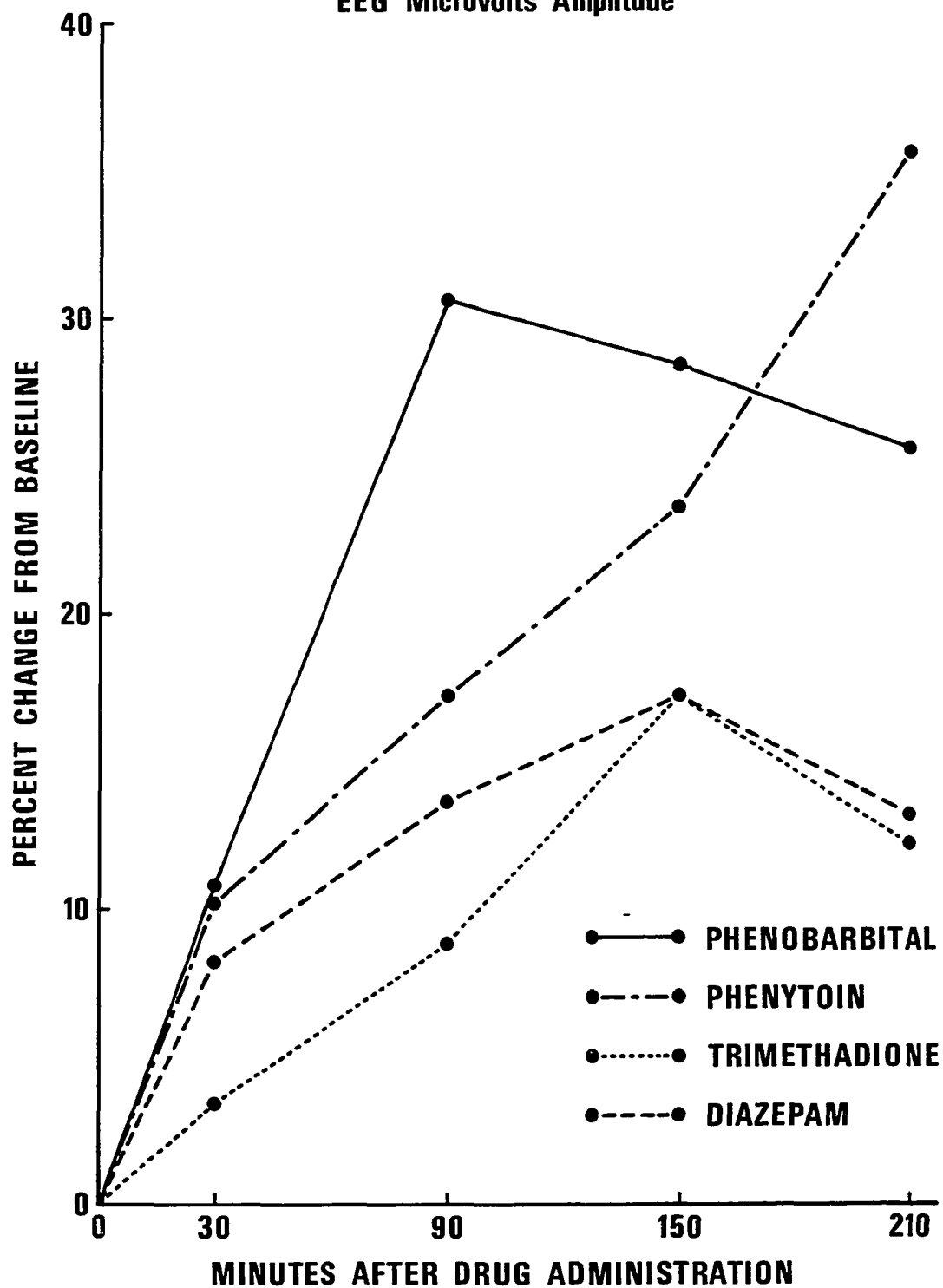
Phenobarbital				
Dose (n=6)	5 mg/kg	10 mg/kg	20 mg/kg	Mean of Three Doses
Mean baseline \pm SE (spikes/min)	30.4 \pm 7.8	23.2 \pm 6.7	31.7 \pm 8.7	28.4 \pm 4.3
Minutes Post-Drug	Mean Percent Change from Baseline \pm SE			
0-30	22.8 \pm 5.9	-4.0 \pm 16.0	39.0 \pm 20.1	18.3 \pm 9.4
30-90	65.7 \pm 24.4	55.6 \pm 40.3	115.3 \pm 39.2	78.8 \pm 20.2
90-150	52.7 \pm 24.2	19.3 \pm 20.7	99.9 \pm 19.6	47.3 \pm 14.2
150-210**	50.2 \pm 17.6	38.5 \pm 48.7	58.4 \pm 37.7	49.0 \pm 18.7

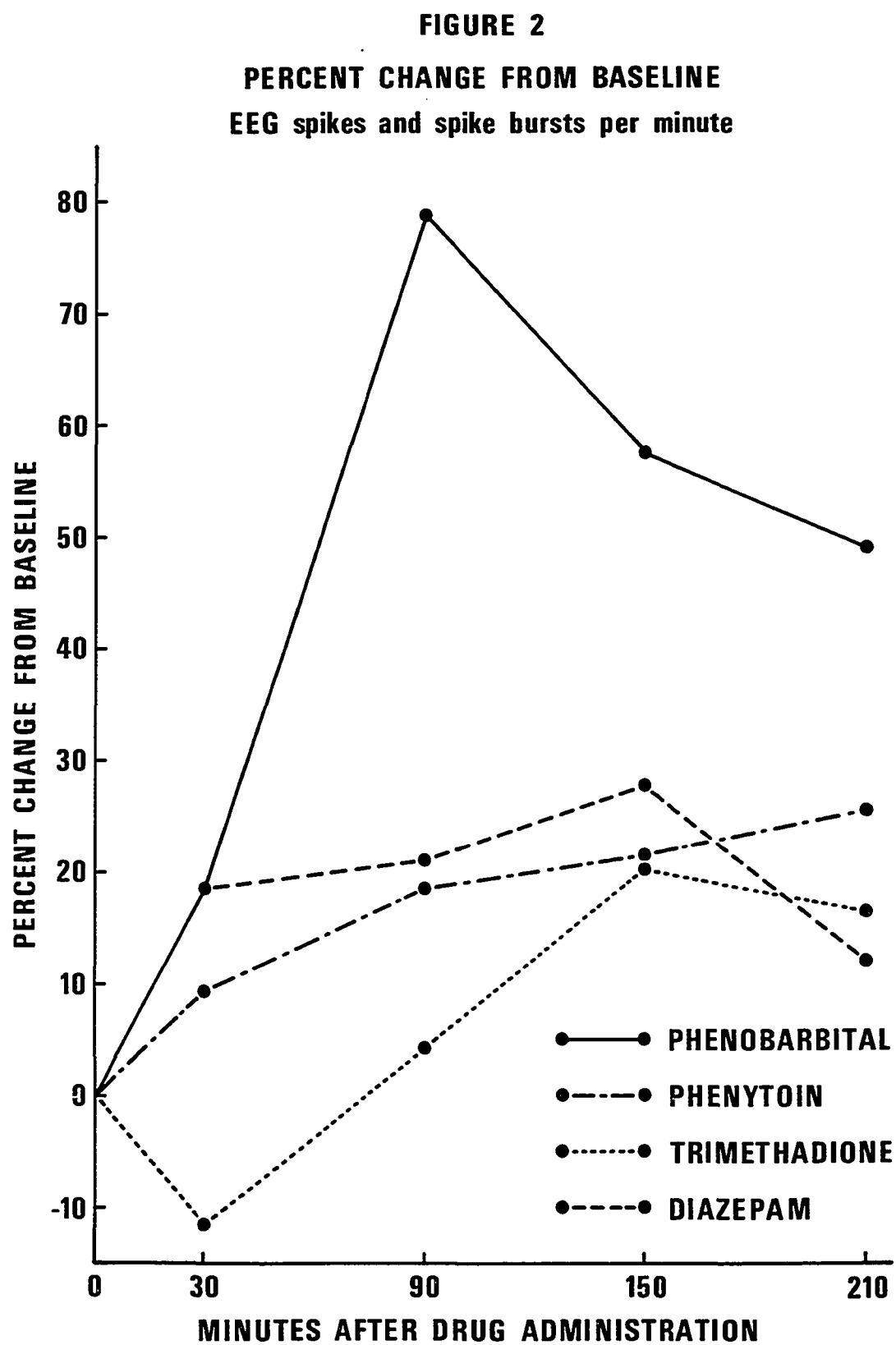
Trimethadione				
Dose (n=5)	40 mg/kg	100 mg/kg	300 mg/kg	Mean of Three Doses
Mean baseline \pm SE (spikes/min)	11.9 \pm 3.3	9.6 \pm 2.3	13.9 \pm 3.2	11.8 \pm 1.7
Minutes Post-Drug	Mean Percent Change from Baseline \pm SE			
0-30	-5.5 \pm 4.6	-13.7 \pm 8.9	-15.1 \pm 19.5	-11.5 \pm 6.9
30-90	-5.3 \pm 11.1	-9.7 \pm 12.7	-2.8 \pm 14.5	-5.9 \pm 6.9
90-150	16.7 \pm 5.9	23.6 \pm 6.3	20.5 \pm 17.5	20.2 \pm 6.9
150-210	5.0 \pm 8.6	35.4 \pm 33.8	8.9 \pm 9.7	16.5 \pm 11.7

*Means calculated on basis of 4 rats because of missing data.

**Means calculated on basis of 3 rats because of missing data.

FIGURE 1
PERCENT CHANGE FROM BASELINE
EEG Microvolts Amplitude





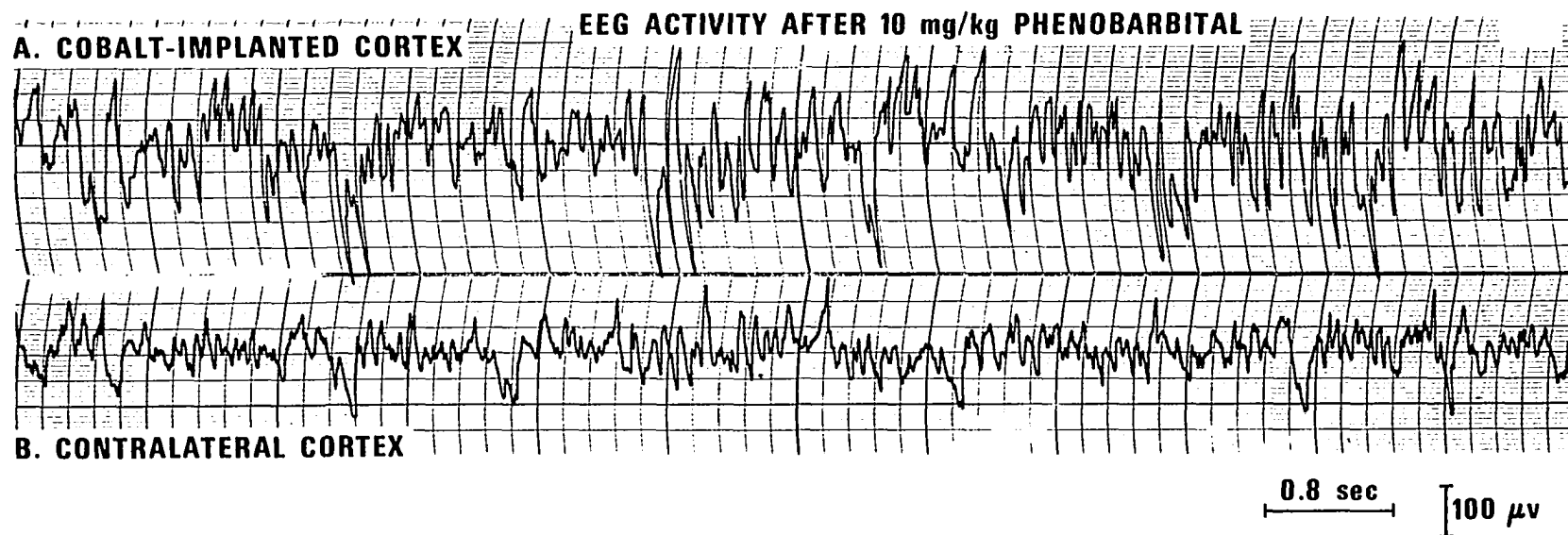
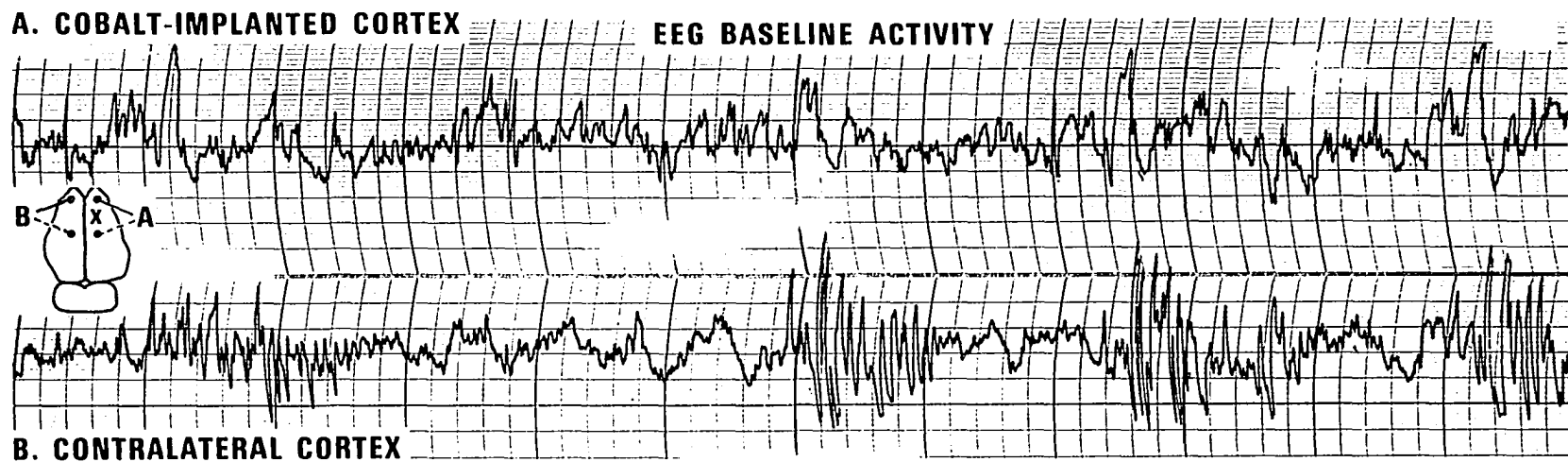
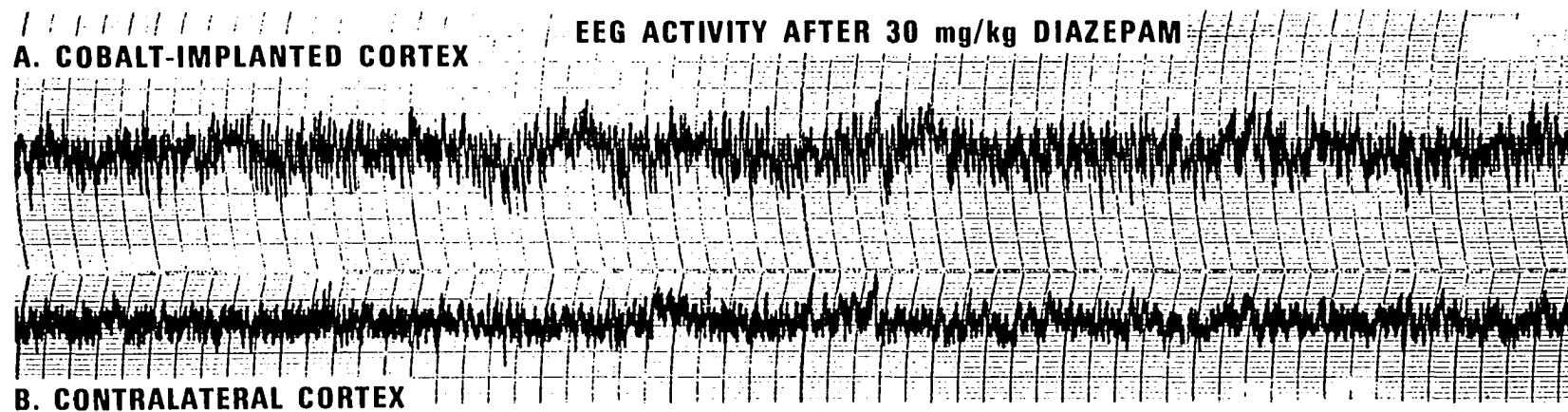
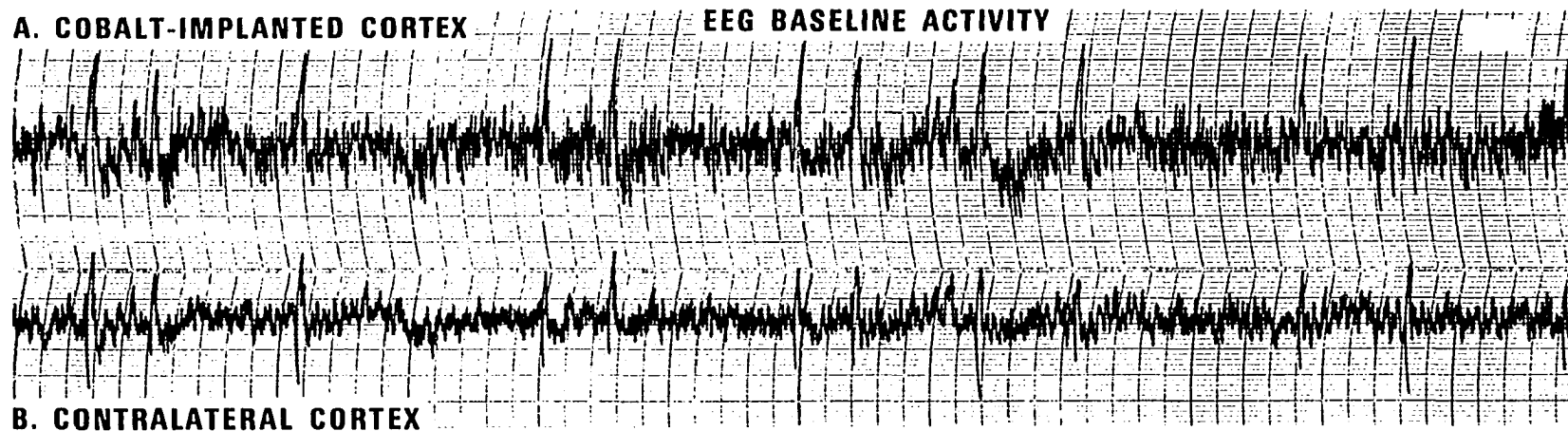


FIGURE 3. EEG TRACINGS FROM A COBALT-IMPLANTED RAT, EIGHT DAYS AFTER IMPLANT.



5 sec \downarrow 100 μ v

FIGURE 4. EEG TRACINGS FROM A COBALT-IMPLANTED RAT, EIGHT DAYS AFTER IMPLANT.

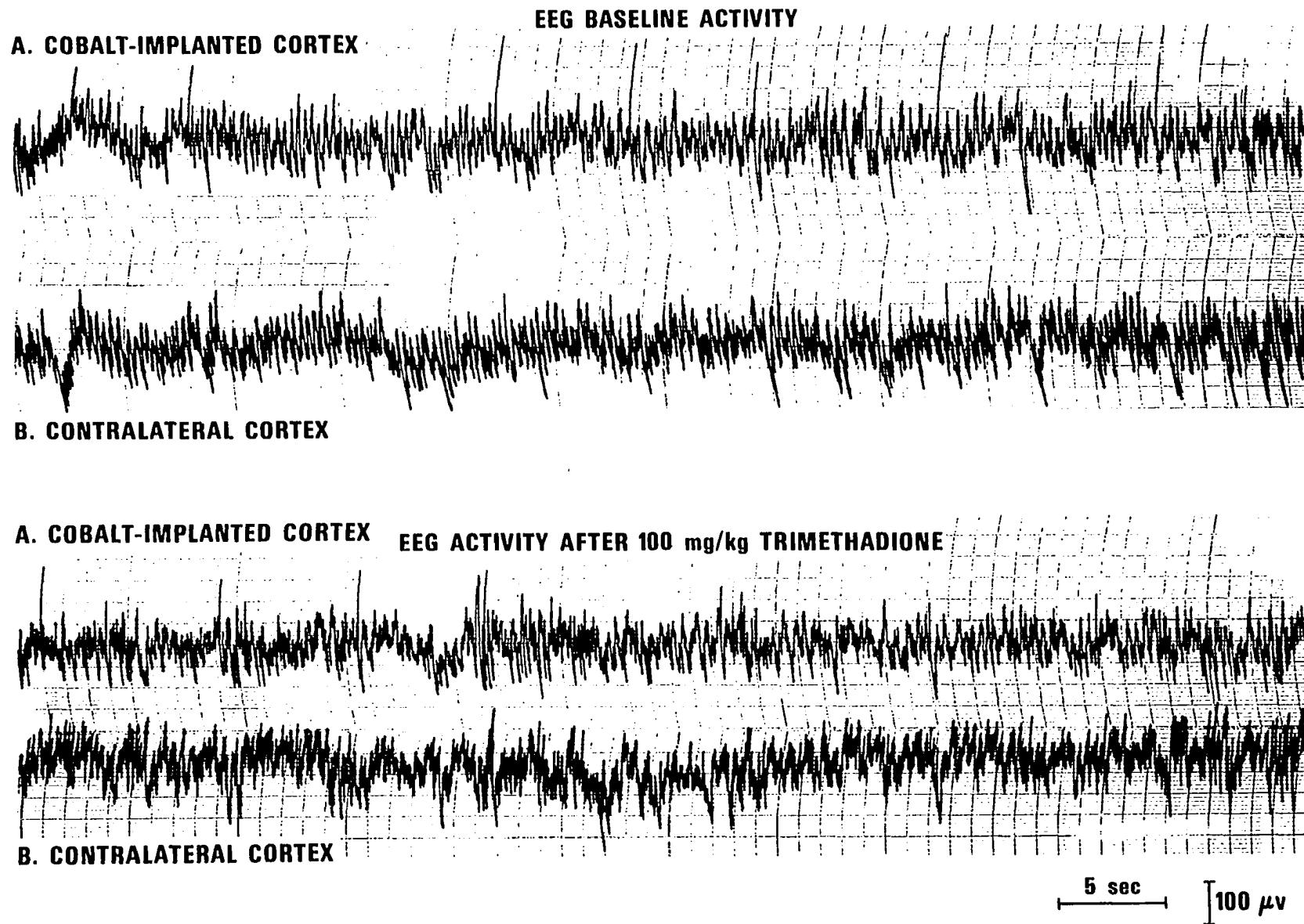


FIGURE 5. EEG TRACINGS FROM A COBALT-IMPLANTED RAT, SIX DAYS AFTER IMPLANT.

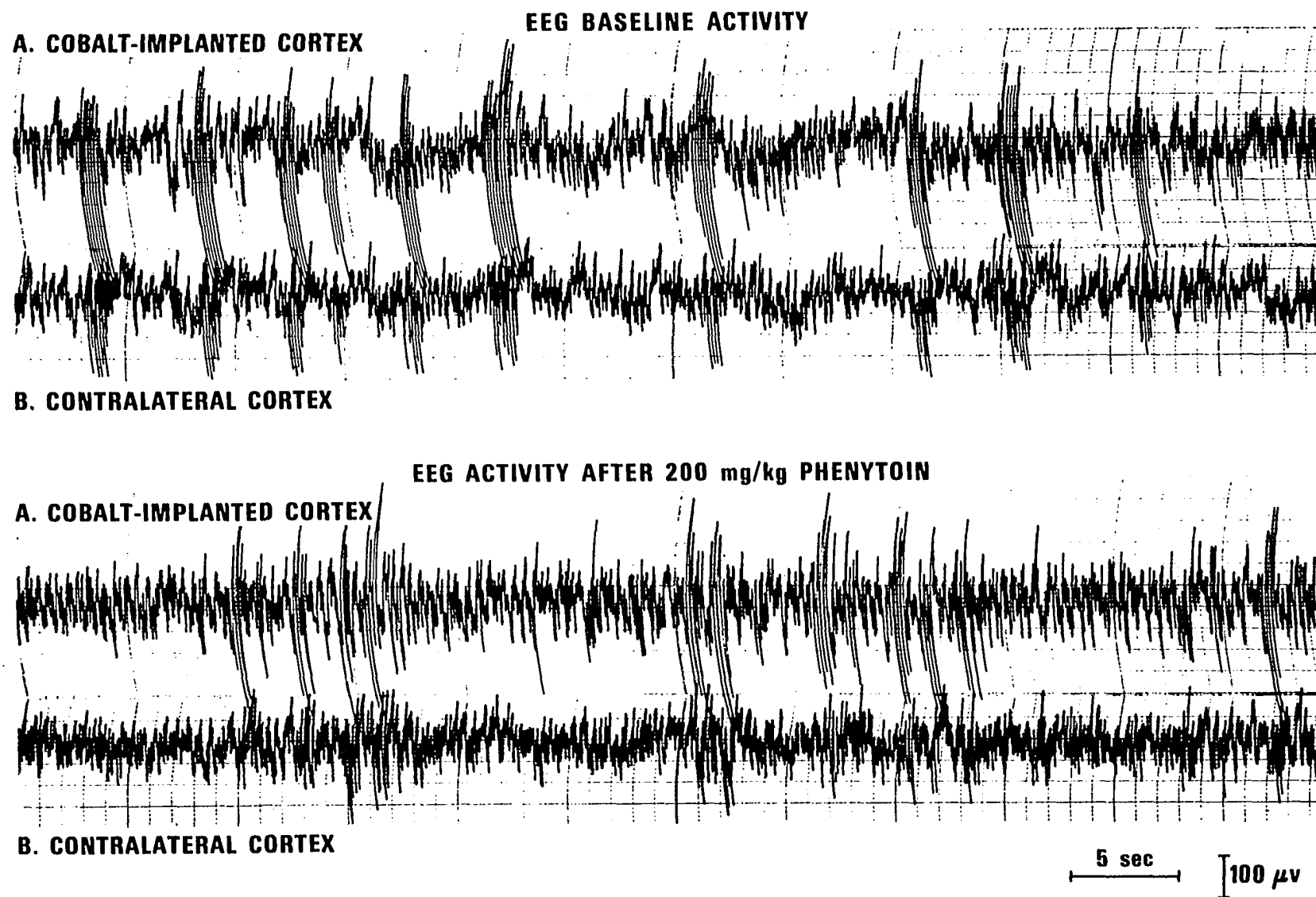


FIGURE 6. EEG TRACINGS FROM A COBALT-IMPLANTED RAT, FORTY-SIX DAYS AFTER IMPLANT.



Fig. 7. (Top) Cobalt implanted brain section, eleven days after implant. PTAH stain (40X). Lesion with vacuoles, blood vessel proliferation and scar tissue.

Fig. 8. (Bottom) Contralateral brain hemisphere. PTAH stain (40X). Normal tissue with meninges intact, and evenly spaced, plentiful neurons.

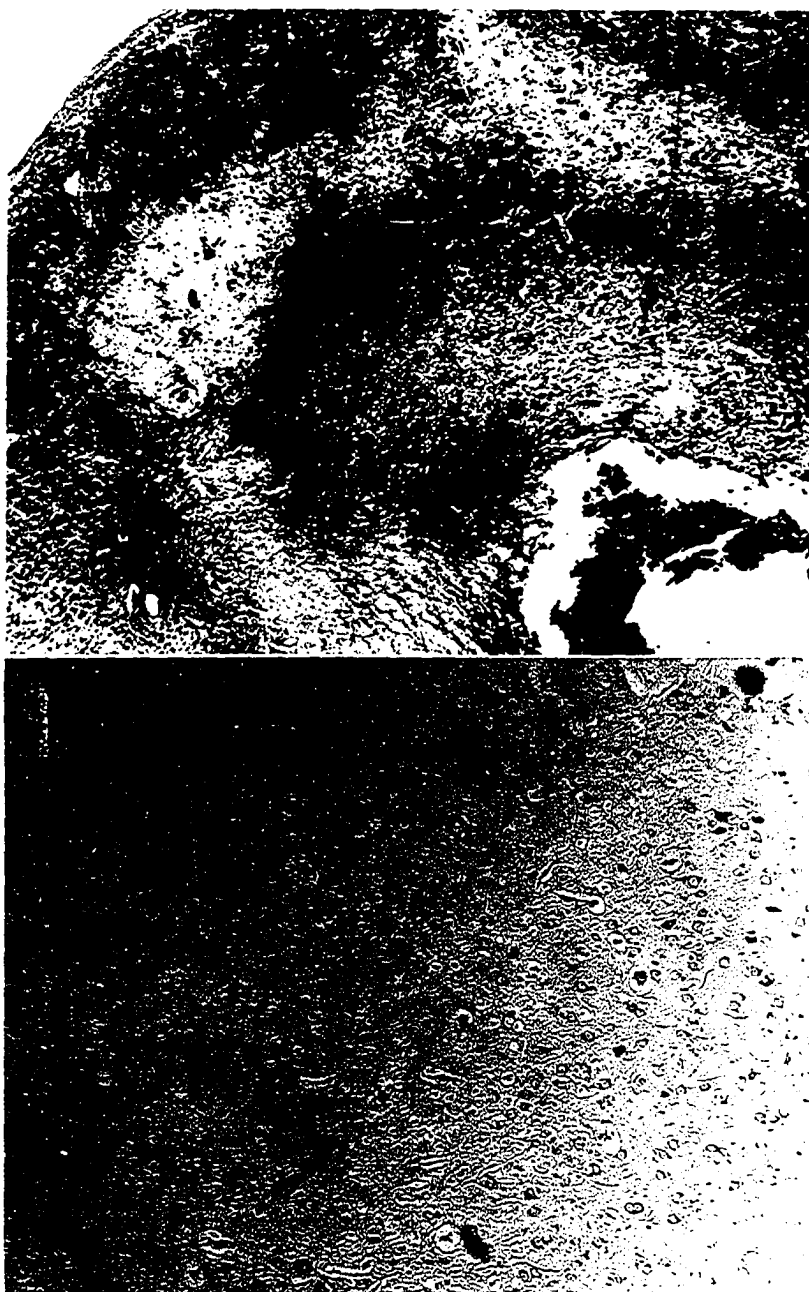


Fig. 9. (Top) Cobalt implanted brain section, sixty days after implant, Luxol blue stain (40X). Removal of cobalt rod resulted in crater in lower right corner of photograph.

Fig. 10. (Bottom) Contralateral brain hemisphere, Luxol blue stain (100X). Normal tissue.

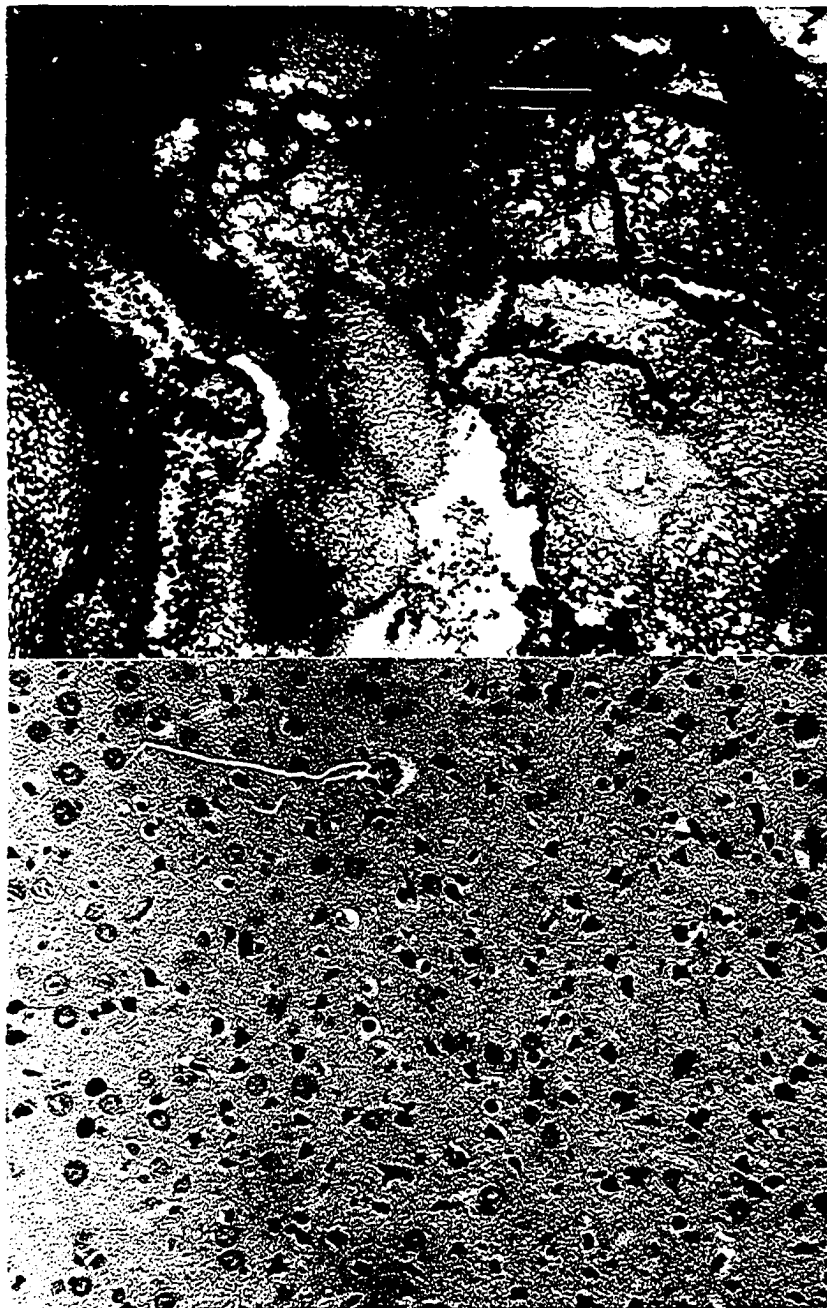


Fig. 11. (Top) Glass implanted brain hemisphere, ninety days after implant. PTAH stain (250X). Extensive lesion with vacuoles, fibers, scarring, and cellular debris.

Fig. 12. (Bottom) Contralateral brain hemisphere. PTAH stain (250X). Normal tissue with nerve fibers, glial cells, and blood vessels.



Fig. 13. (Top) Cobalt implanted brain section, 142 days after implant. PTAH stain (40X). Massive disruption of normal cortical structure.

Fig. 14. (Bottom) Contralateral brain hemisphere. PTAH stain (40X). Normal tissue.

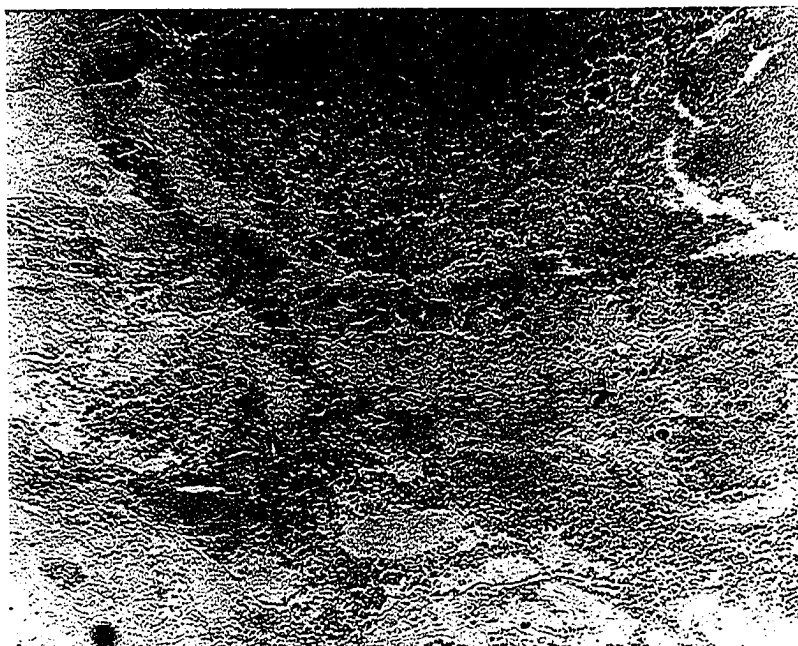


Fig. 15. Cobalt implanted brain section, 142 days after implant.
Kossa's stain for calcium (40X).

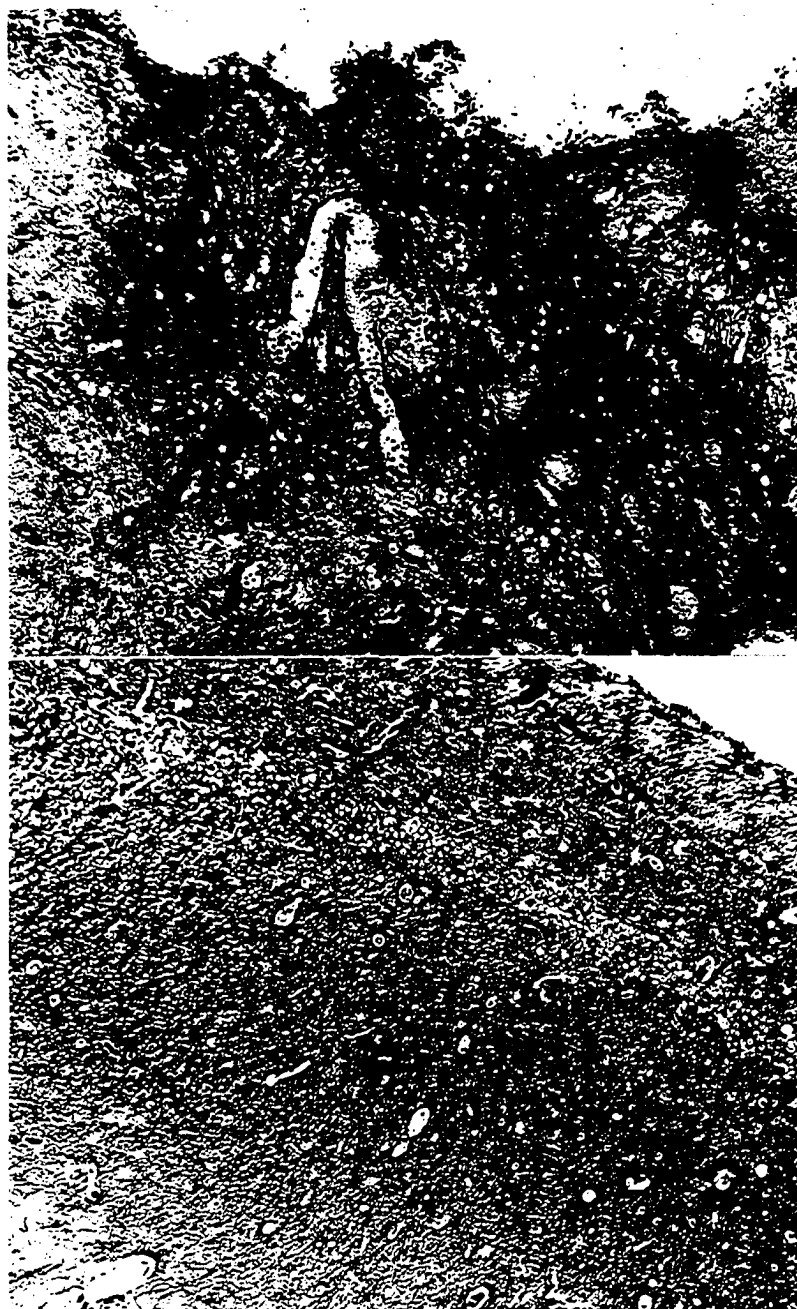


Fig. 16. (Top) Cobalt implanted brain section, 178 days after implant. Bodian stain for nerve fibers (100X). Lesion in center, normal tissue at periphery. Cellular debris and increased vascularity in lesion site.

Fig. 17. (Bottom) Contralateral brain hemisphere. Bodian stain for nerve fibers (100X). Many nerve cells and fibers, normal blood supply, and pia mater intact.

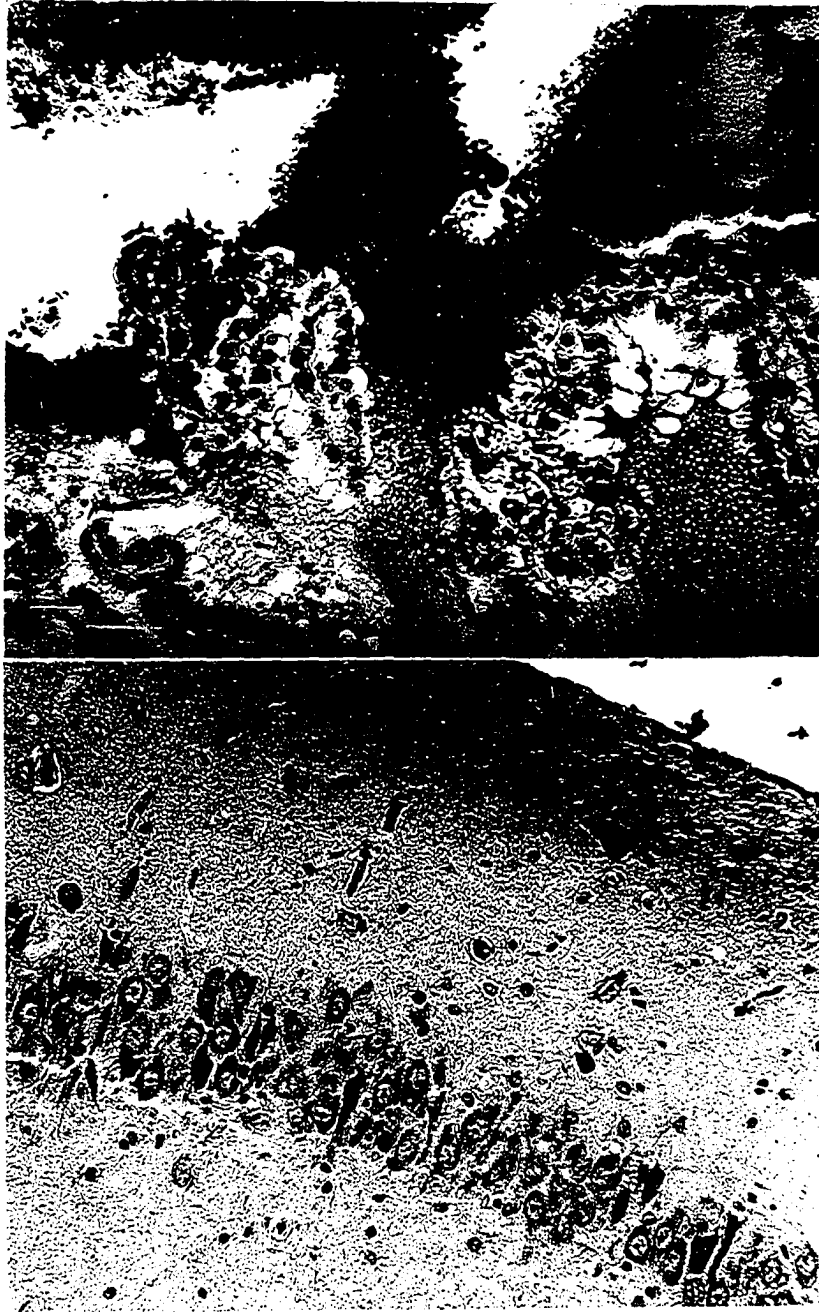


Fig. 18. (Top) Cobalt implanted brain section, 178 days after implant. Luxol blue stain (250X). Numerous intracellular vacuoles, necrosis, and cellular debris are evident.

Fig. 19. (Bottom) Contralateral brain hemisphere. Luxol blue stain (250X). Pia mater intact, evenly scattered neurons, few blood vessels are seen throughout the cortex.

DISCUSSION

The cobalt model of experimental epilepsy is widely accepted as an investigational tool for studying focal epilepsy (Van Duijn and Visser, 1972). Development of a primary epileptic foci was evident within the first week after cobalt implant, which confirmed the observations of Henjyoyi and Dow (1965).

A delay between implant and development of electrical abnormality, described by Dow et al. (1972), was not observed. EEG recordings were not made until at least three days after cobalt implant to insure adequate recovery from surgery, and a delay may have been missed. Little decrease in activity three months after implant was observed, contrary to reported attenuation after scar formation in studies in rat frontal cortex (Dow et al., 1972). Active epileptogenic foci were observed in some rats in this study five months after cobalt implant.

The presence of an independent mirror focus or a secondary epileptogenic lesion was not established. This confirmed work by Dow et al. (1972). The contralateral cortex was not histologically abnormal up to six months after cobalt implantation. Some instances of spike bursts occurred on the hemisphere of the brain contralateral to the cobalt implant. In other EEG recordings, abnormal spike bursts were observed in both sides of the brain, but were not synchronized. Those few EEG tracings are not in themselves conclusive evidence of a mirror focus.

Certain idiosyncracies of the cobalt model should be acknowledged when interpreting results of these studies. Most cobalt implanted rats did have unequivocal electrical evidence of abnormal discharges in the

cortical region surrounding the lesion. Few rats had any overt behavioral twitches or seizures. In animals with observable seizures, the EEG spiking was always in distinct rhythmic spike bursts. These seizures consisted of whisker twitches, forelimb jerks, or head nodding, all more pronounced on one side of the rat. Some of the glass-implanted rats also developed abnormal cortical discharges, similar in appearance to the cobalt induced spike bursts. Histological examination of the glass-implanted brains, suggested that the abnormal discharges could have been initiated by surgical trauma or scar formation adjacent to the glass implant. The presence of a large necrotic lesion in the glass controls is in contrast to results reported by Dow et al. (1972). These non-cobalt electrical foci usually disappeared more quickly than the cobalt-induced foci, although the lesion remained.

Another problem in data interpretation arose from the difficulty in defining a spike. No absolute system has been accepted for differentiating a single spike from a spike burst. In this study, where it was possible, each individual peak of EEG tracing was counted as a spike if it exceeded twice the background level of electrical amplitude. This flexible definition of spiking took into account the baseline of activity for each individual subject, and changes in the baseline influenced by non-epileptic parameters such as sleep stages. This study is in agreement with Roldán et al. (1971), that in EEG tracings with sleep cycles evident, it was difficult to differentiate between a sleep spindle and a spike burst. In tracings with more than 350 microvolts of electrical amplitude, the spikes are obscured by high voltage, fast or slow electrical activity, which could be seizure, drug, or sleep in-

duced, or a combination of all three. This confusion over drug induced spiking opposed to cobalt-focus induced spiking was discussed by Dow et al. (1973), and Roldán et al. (1971). Swisher (1962) proposed that some of the atypical rat EEG patterns could be due to activated sleep, also called paradoxical sleep. These fast, low voltage six to eight hertz waves are theorized to be part of the normal sleep-wakefulness cycle of many animals.

The four anticonvulsant drugs tested in this study were representative of four major types of anticonvulsants, the benzodiazepines, oxazolidinediones, barbiturates, and hydantoins. This cross-selection was chosen in an attempt to show differences in drug effects on the cobalt epileptogenic focus. This portion of the study was not successful, due to the high degree of variability between animals. No significant differences were found between any drug or any dose, on either spiking rate or electrical amplitude with Friedman's non-parametric two-way analysis of variance (Siegel, 1956). Several studies have reported similar large variation, and only limited dose relationships (Stark et al., 1974; Kutt et al., 1968; Louis et al., 1968). Edmonds et al. (1974) found phenytoin, phenobarbital and diazepam to be relatively ineffective against penicillin-induced foci. This work produced similar conclusions, with the addition of trimethadione as a virtually ineffective anticonvulsant on cobalt-induced epilepsy. These results are in contrast with Louis et al. (1968), Sharer et al. (1971), Roldán et al. (1971), and Stark et al. (1974) who all reported some degree of anticonvulsant activity in one or all of the four compounds in chronic animal models of epilepsy.

Phenobarbital caused the greatest increase in spiking rate of any of the anticonvulsant drugs tested. Ninety minutes after intraperitoneal administration the spiking rate had increased almost eighty percent over the pre-drug baseline. The electrical amplitude had increased thirty percent, ninety minutes after phenobarbital. Marked sedation was observed in all of the rats, especially at the highest dose tested. Since this tremendous increase in focus activity was also seen in the glass implanted controls, the increases probably reflected an increase in high amplitude, slow wave sleep with sleep spindles, rather than increased epileptic discharge frequency or amplitude. Chocholová et al. (1970), Dow et al. (1973), and Morrell et al. (1959), reported increased incidence of epileptiform spiking after barbiturates, but also postulated the increases were due to slow wave sleep or toxicity. Ectors (1955) and Millichap (1969) found a suppression of focal cortical epileptic discharges, which was not confirmed in this study. Edmonds et al. (1974) found no marked change in spiking rate after 15 or 30 mg/kg phenobarbital, orally, in penicillin-induced epileptic rats. He also reported that the higher dose was toxic. The EEG spiking rate and increased amplitude dropped slightly from the ninety minute peak, but was still elevated fifty percent over the baseline spiking rate and twenty-five percent over the baseline amplitude after 210 minutes post-drug.

The only anticonvulsant drug tested which had a greater percent increase in EEG electrical amplitude than phenobarbital, was phenytoin. Phenytoin produced a continuous rise in amplitude which had not peaked after 210 minutes post-drug. This thirty-five percent increase was

larger than the maximum twenty-five percent phenobarbital induced percent change. This prolonged increase could be indicative of a longer latent period before the effect of the drug is evident, or a longer duration of action of the drug, possibly due to a slower metabolism. The spiking frequency also increased, although unlike the phenobarbital effect, only a twenty-five percent change, comparable to the percent change of amplitude, was observed. This relatively mild change in spiking rate confirms results of Edmonds et al. (1974), Craig et al. (1976), and Dow et al. (1975). The observations of Van Duijn and Visser (1972), and Stark et al. (1974), that phenytoin was anticonvulsant after fifteen mg/kg intravenous in the cat, was not confirmed. The effect of phenytoin on the EEG amplitude most closely resembled that of phenobarbital whereas the effect on the spiking rate was similar to diazepam.

Trimethadione produced an immediate eleven and one-half percent decrease from baseline in mean spiking rate. This was the only anticonvulsant tested which consistently decreased the spiking rate for ninety minutes after drug administration. After 150 minutes, the spiking rate had increased twenty percent over the baseline rate where it leveled. This second phase increase was similar to an enhancement of spiking activity observed by Craig et al. (1976) in cobalt-epileptic rats. Unlike the other drugs tested, very little sedation was observed even at the high dose. The effect of trimethadione on the EEG amplitude closely resembled diazepam. Goth (1976) postulated that diazepam and trimethadione exert an effect primarily on potassium permeability in neurons, whereas phenytoin influences sodium permeability. Drugs effective in the treatment of petit mal tend to decrease potassium perme-

ability.

Diazepam produced slight increases in spiking rate and amplitude which peaked at 150 minutes and returned to only about 12 percent greater than baseline activity level. This increase confirms data presented by Edmonds et al. (1974) who found that at 30 mg/kg diazepam induced a variable increase in spiking frequency. This is in contrast to anticonvulsant activity reported by Stark et al. (1974), with 0.5 mg/kg and 1 mg/kg in penicillin epileptic cats, and by Edmonds et al. (1974) with 15 mg/kg in penicillin epileptic rats. Bell (1970) and Camerman and Camerman (1970) observed low voltage, delta fast waves in the EEG tracings after diazepam. This may be similar to the activated, or paroxysmal sleep discussed by Swisher (1962). Stretches of low voltage fast waves were occasionally observed after diazepam, but not enough for conclusive evidence of altered EEG activity.

Presence of calcium deposits in the lesion site, that was extensively discussed in the literature, were confirmed. The calcium precipitate observed, however, was not as intense as that observed by Payan (1971). Myelin was observed in the lesion site after six months which agrees with Payan's description of myelin present three months after cobalt implant. Presence of edema, inflammatory cell infiltration, necrosis, and fibrous scarring also confirmed histological changes previously discussed in the literature review. Lesions in the glass implanted animals probably resulted from surgical trauma and infection precipitated by the non-sterile, sharp edged glass. Large lesions were evident soon after cobalt implant, but no correlation between lesion size and magnitude of electrical discharges was seen, which is com-

patible with observations made by Engle (1968). The depression in the cortical surface was littered with mineral deposits, bone splinters and inflammatory cells, and was also necrotic. This was in contrast to Engle's description of an empty crater left by the cobalt rod.

The dark spots observed at the periphery of the lesion in some of the two months, cobalt-implanted brains, resembled the secondary lesions reported by Engle (1968) and Morrell et al. (1959). Much additional investigation is needed before this observation can be attributed to anything other than artifacts in preparation of the stained tissue section. Another possible explanation could be that a branch of the primary lesion was jutting out into the adjacent undisturbed cortex, possibly with subcortical connections.

CONCLUSIONS

A chronic, long-lasting, epileptogenic lesion was created by implanting cobalt on the cortex of rat brains. Few of the seizures were accompanied by behavioral manifestations such as muscle jerks or whisker twitches. However, electrical evidence of abnormalities in the EEG in the form of either spikes or spike bursts were evident.

Trimethadione was the only anticonvulsant drug tested which lowered the spiking rate. Phenytoin, diazepam, and phenobarbital caused increases in the number of spikes or spike bursts. Administration of all four compounds resulted in an increase in EEG microvolts amplitude. This probably reflected an increase in drug-induced high amplitude, slow wave sleep, or drowsiness, rather than activation of the epileptic focus. Although the decreased spiking rate after trimethadione was temporary, this drug has been shown to be the most successful in reducing cobalt-induced EEG abnormalities without producing drowsiness or sleep.

Large lesions were detected histologically in both the cobalt and glass implanted animals. Presence of edema, inflammatory cell infiltration necrosis, scarring, neuronal degeneration, and calcium precipitate at the site of the cobalt lesions were observed. Where lesions occurred in glass implanted animals, they were shown to be due to surgical trauma and infections resulting from non-sterile implants.

LITERATURE CITED

- Bell, D. S. 1970. The effect of diazepam on the EEG of status epilepticus. J. Neurol. Neurosurg. Psychiat. 33:231-237.
- Boyer, P. A. 1966. Anticonvulsant properties of benzodiazepines. Dis. Nerv. Syst. 27:35-42.
- Camerman, A. and N. Camerman. 1970. Diphenylhydantoin and diazepam: molecular structure similarities and steric basis of anticonvulsant activity. Science 168:1457-1458.
- Cesa-Bianchi, M. G., M. Mancina and R. Mutani. 1967. Experimental epilepsy induced by cobalt powder in lower brain-stem and thalamic structures. Electroenceph. Clin. Neurophysiol. 22:525-536.
- Chocholová, L. and T. Radil-Weiss. 1970. The level of vigilance and the EEG manifestations of cortical cobalt foci in rats. Physiol. bohemoslov. 19:385-386.
- Craig, C. R., P. Chiu and B. K. Colasanti. 1976. Effects of diphenylhydantoin and trimethadione on seizure activity during cobalt experimental epilepsy in the rat. Neuropharmacology 15:485-489.
- Dow, R. C., J. C. Forfar and J. K. McQueen. 1973. The effects of some anticonvulsant drugs on cobalt-induced epilepsy. Epilepsia (Amst.) 14:203-212.
- Dow, R. C., J. K. McQueen and H. R. A. Townsend. 1972. The production and detection of epileptogenic lesions in rat cerebral cortex. Epilepsia (Amst.) 13:459-465.
- Dow, R. S., A. Fernández-Guardiola and E. Manni. 1962. The production of cobalt experimental epilepsy in the rat. Electroenceph. Clin. Neurophysiol. 14:399-407.
- Ectors, L. 1955. Barbiturate action upon cortical epilepsy. Electroenceph. Clin. Neurophysiol. 8:149.
- Edmonds, H. L., L. G. Stark and M. A. Hollinger. 1974. The effects of diphenylhydantoin, phenobarbital, and diazepam on the penicillin-induced epileptogenic focus in the rat. Experimental Neurology 45: 377-386.
- Engel, J. 1968. Secondary Epileptogenesis in rats. Electroenceph. Clin. Neurophysiol. 25:494-498.
- Escueta, A. V. and E. L. Reilly. 1971. The effects of diphenylhydantoin on potassium transport within synaptic terminals of the

epileptogenic foci. Neurology (Minneap.)21:418.

Fischer, J., J. Holubár and V. Malík. 1968. Neurohistological study of the development of experimental epileptogenic cortical cobalt-gelatine foci in rats and their correlation with the onset of epileptic electrical activity. Acta. Neuropath. (Berlin) 11:45-54.

Goodman, L. S. and A. Gilman. 1975. Drugs effective in the therapy of the epilepsies. pp. 201-208. The Pharmacological Basis of Therapeutics. Macmillan Publishing Co., Inc., New York.

Goth, A. 1976. Antiepileptic Drugs. pp. 275-281. Medical Pharmacology (Eighth Edition). Mosby, St. Louis.

Guerrero-Figueroa, R., A. Barros, R. G. Heath and G. Gonzalez. 1964. Experimental subcortical epileptiform focus. Epilepsia (Amst.) 5:112-139.

Hauptmann, A. 1912. Luminal bei epilepsie. Munch. med. Wschr. 59:1907-1909.

Henjyoji, E. Y. and R. S. Dow. 1965. Cobalt-induced seizures in the cat. Electroenceph. Clin. Neurophysiol. 19:152-161.

Jackson, J. H. 1931. Neurological Fragments. pp. 362. Selected Writings of John Hughlings Jackson. J. Taylor (Ed.) Hodder and Stroughton, London.

Kopeloff, L. M. 1960. Experimental epilepsy in the mouse. Proc. Soc. Exp. Biol. (N.Y.) 104:500-504.

Kutt, H., S. Louis and F. McDowell. 1968. Intravenous diphenylhydantoin in experimental seizures - I. Correlation of dose, blood and tissue level in cats. Arch. Neurol. 18:465-471.

Louis, S., H. Kutt and F. McDowell. 1968. Intravenous diphenylhydantoin in experimental seizures - II. Effect of penicillin-induced seizures in the cat. Arch. Neurol. 18:472-477.

Luna, L. (Ed.) 1968. Armed Forces Institute of Pathology: Manual of Histologic and Special Staining Techniques. pp. vii-258. McGraw-Hill, New York.

Millichap, J. G. 1969. Relation of laboratory evaluation to clinical effectiveness of antiepileptic drugs. Epilepsia (Amst.) 10: 315-328.

Morrell, F., W. Bradley and H. Ptashne. 1959. Effect of drugs on discharge characteristics of chronic epileptogenic lesions. Neurology 9:492-498.

- Morrell, F. W. 1969. Physiology and Histochemistry of the Mirror Focus. pp. 357-370. Basic Mechanisms of the Epilepsies. H. H. Jasper, A. A. Ward, Jr. and A. Pope (Eds.). Little, Brown and Co., Boston.
- Musgrave, E. S. and D. P. Purpura. 1963. Effects of dilantin on focal epileptogenic activity in rat neocortex. Electroenceph. Clin. Neurophysiol. 15:923-931.
- Payan, H. M. 1967. Cerebral lesions produced in rats by various implants: epileptogenic effect of cobalt. J. Neurosurg. 28:146-152.
- Payan, H. M. 1970. Cobalt experimental epilepsy in gerbils. Exp. Med. Surg. 28:163-168.
- Payan, H. M. 1971. Morphology of cobalt experimental epilepsy in rats. Exp. Mol. Pathol. 15:312-319.
- Payan, H., R. Strebel and S. Levine. 1965. Epileptogenic effect of extradural and extracranial cobalt. Nature 208:792-793.
- Randall, L. O., G. A. Heise, W. Schallek, R. E. Bagdon, R. F. Banziger, A. Boris, R. A. Moe and W. B. Abrams. 1961. Pharmacological and clinical studies on Valium a new psychotherapeutic agent of the benzodiazepines class. Curr. Therap. Res. 3:405-425.
- Roldán, E., T. Radil-Weiss and L. Chocholová. 1971. The influence of barbiturates on paroxysmal EEG activity induced by hippocampal and/or thalamic cobalt foci. Psychopharmacologia (Berl.) 19:273-281.
- Sharer, L. and H. Kutt. 1971. Intravenous administration of diazepam. Arch. Neurol. 24:169-175.
- Siegel, S. 1956. Nonparametric Statistics for the Behavioral Sciences. pp. 166-172. McGraw-Hill Book Company, Inc., New York.
- Sphelmann, R. and B. Colley. 1968. Effect of diazepam (Valium®) on experimental seizures in unanesthetized cat. Neurology (Minneap.) 18:52-60.
- Stark, L. E., H. L. Edmonds and P. Keesling. 1974. Penicillin-induced epileptogenic foci - I. Time course and the anticonvulsant effects of diphenylhydantoin and diazepam. Neuropharmacology 13: 261-267.
- Strobos, R. R. J. and E. V. Spudis. 1960. Effect of anticonvulsant drugs on cortical and subcortical seizure discharges in the cat. Arch. Neurol. (Chic.) 2:399.
- Swinyard, E. A. 1969. Laboratory evaluation of antiepileptic drugs. Epilepsia (Amst.) 10:107-119.
- Swisher, J. E. 1962. Manifestations of "activated" sleep in the

rat. Science 138:1110.

VanDuijn, H. and S. L. Visser. 1972. The action of some anticonvulsant drugs on cobalt-induced epilepsy and on the bemegride threshold in alert cats. Epilepsia (Amst.) 13:409-420.

Ward, A. A. 1969. The epileptic neuron: chronic foci in animals and man. pp. 263-288. Basic Mechanisms of the Epilepsies. H. H. Jasper, A. A. Ward, Jr. and A. Pope (Eds.). Little, Brown and Co., Boston.

Westrum, L. E., L. E. White and A. A. Ward. 1964. Morphology of the experimental epileptic focus. J. Neurosurg. 21:1033-1046.

Woodbury, D. M., J. K. Perry and R. P. Schmidt (Eds.). 1972. Antiepileptic Drugs. pp. xxiii-536. Raven Press, New York.