DENERVATION SUPERSENSITIVITY IN THE CORTEX: A POSSIBLE BASIS OF FOCAL EPILEPSY

A Thesis Presented to

The University of Manitoba

In Partial Fulfillment of

the Requirements for the Degree of

Doctor of Philosophy

by

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October, 1964

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ABSTRACT

The hypothesis that focal cortical epilepsy is due to the development of denervation supersensitivity was tested in chronically isolated (denervated) slabs of cerebral cortex. The slabs were examined for changes in anatomical and physiological properties which might be expected to occur if the increased epileptogenesis of cortex after chronic isolation was due to a mechanism other than denervation supersensitivity.

Considerable degeneration of neurones in the chronic slabs was seen, but there was no evidence of "dendritic distortion" which might cause a generator potential in the dendrites. Nor did it appear likely that there was any axon-collateral proliferation or increase in axo-dendritic synapses within chronic slabs. The chronic slabs did not have nervous connections with the surrounding cortex which could be responsible for the long duration of the discharges in the chronic slabs.

The thresholds of all parameters of electrical stimulation except the stimulus frequency were the same in chronic and acute slabs. No change in the minimum electrical threshold (current x pulse-width) of the cortical neurones resulted from chronic isolation. The reduction in threshold stimulus frequency may be accounted for by increased effect of synaptic activation of the cells during the intervals between stimulus pulses at the low frequencies. The same minimum area of cortex must be

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stimulated to initiate an afterdischarge in chronic and acute slabs. For areas above this minimum, the threshold density of stimulated cells appeared to be the same, since the radial potentials developed in small areas of cortex following just sub- or just suprathreshold epileptogenic electrical stimulation were similar in acute and chronic slabs, as were the voltage (current density) thresholds for various stimulating electrode areas above the minimum. Discharges do not seem to be critically dependent on a "differential repolarization" mechanism for initiation, nor does the maintenance of discharges appear to be dependent on the radial potential gradients of the cortex in either acute or chronic slabs. The radial potential gradients are more likely to be produced by, rather than cause, the paroxysmal activity of the cortex. It follows that the differences in epileptogenesis of chronic and acute slabs cannot be ascribed to differences in the magnitude of the potentials produced by the cortex in each slab.

The chronically denervated cortex does become supersensitive to ACh (perhaps due to a reduction in cortical cholinesterase). However, cholinergic pathways do not seem to be necessary for the production or maintenance of afterdischarges, since doses of atropine which block all ACh-induced activity do not affect electrically induced afterdischarge. These observations are compatible with the original hypothesis, and it is suggested that the major functional difference between normal and epileptic cortex is the greatly increased maintenance and propagation of the seizure in the latter because of more effective recurrent synaptic activation due to denervation supersensitivity.

ACKNOWLEDGEMENTS

The author is indebted to many persons for their help during the preparation of this thesis, especially to:

DR. G.B. FRANK for his supervision and criticism during the course of this investigation.

DR. C. PINSKY for hours of helpful discussion and for many suggestions which have been incorporated into the manuscript.

MRS. DIANE STEPHENS, MR. HARALD STROM and MISS YETTA SHATKIN for their assistance with the surgery and during the experiments.

MR. CASEY BIALEK for the maintenance of the electronic equipment.

MRS. OLIVE NICHOLSON and MISS J.M. MELVILLE for the preparation of the histological sections.

MR. JOHN KIEKUSH for his careful maintenance of the chronic animals.

MR. E.J. BISHOP of Glaxo-Allenburys (Canada) Ltd. for the supply of antibiotics used during this study.

MESSRS. ERNEST MORANETZ and JOHN DELAIVE for the photography.

MR. W. ALLEN DRYBURGH of General Office Machines Ltd., Winnipeg, for his kindness in loaning the electric typewriter on which the thesis was typed.

MRS. SYLVIA WHITE, MISS ALMA WALBERG and MR. RUDY ZATYLNY for their help in the preparation of the manuscript, and in assembling the thesis.

MRS. ANNE SWARDFAGER for her careful typing of this thesis.

My wife, JOAN, for her patience and confidence.

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I. PREFACE

It has been estimated that between 0.5 and 3.0% of the population are epileptics. While modern therapeutics have removed much of the physical distress caused by the disease, it might be said that epileptics still suffer from society's ignorance. A majority of the laity still associate epilepsy with mental deficiency or insanity (Caveness, 1954), with the result that epileptics are discriminated against, both without and within the law. To those whose seizures are not completely controllable or who admit to epilepsy, many aspects of life, from employment to marriage, may be closed to them. The United States, for example, does not allow immigration of epileptics. Some states even include epileptics along with mental defectives in eugenic laws ordering sterilization or prohibiting marriage (Barrow and Fabing, 1956). Generally these laws take no cognizance of the fact that a majority of epileptics can be well controlled medically, or that inheritance is unlikely, especially if the seizures are acquired rather than idiopathic. While these latter laws are rarely applied, there are still many epileptics who are subjected to other varieties of persecution. Although a determined effort is being made to educate the public and to alter these unjust laws (Barrow and Fabing, 1956), the ultimate solution to the problem lies in research into the cause and effective therapy. The fundamental lack of knowledge about the physiological origin of epilepsy has made this search a rather hit-or-miss process. The present study was undertaken in the hope that a contribution could be made to the understanding of the pathophysiology of the disorder so that eventually a rational approach to treatment, and perhaps a cure, will be found.

II. HISTORICAL REVIEW

A. TWENTY CENTURIES OF SUPERSTITION

Epilepsy has for many centuries been recognized as a separate clinical entity. Accurate descriptions of the disease exist which date as far back as 400 B.C. (Harrell, 1961). The primary medical description is the Hippocratic book On the Sacred Disease. According to this work, the popular belief of the time was that epilepsy was a form of retribution from the gods (particularly Pan and Hekate), a view which Hippocrates vigorously opposed. He tried to point out that epilepsy was an organic disease of the brain, explainable in terms of natural causes, and not divine in origin. Such a clear concept as to the nature of epilepsy does not appear again until the nineteenth century. Hippocrates also left his mark on our present terminology. He used the term "the great disease", which comes to us by way of French as "grand mal" (Temkin, 1945). The word "epilepsy" is also Greek in origin, being derived from "to seize".

Certainly the ancient Greeks left excellent descriptions of acute clinical fits, and knew of many conditions predisposing to fits, such as heredity, age (i.e., they are particularly prevalent in young children and at puberty), physical and mental strain, drunkenness, and injuries to the skull. Most of these observations have been verified only in the last century. They recorded attacks starting in one limb, the particular variety that Jackson was to analyze in great detail twenty centuries later. They knew of photic activation of epilepsy, since the common test for epilepsy was to have the patient watch a spinning potter's wheel (Temkin, 1945). The course and prognosis were

also quite accurately described by the medical writers of the time, although they had no effective treatment for the condition.

Although the ancient Greek physicians attempted physical explanations for psychic as well as functional disturbances, the laity was no so enlightened. Because of the popular belief that sufferers of epilepsy ("the falling disease") were in disfavour with the gods, epileptics were regarded with disgust, and were generally outcasts. The Sophoclian tragedy The Philoctetes, first performed in 409 B.C., shows the attitude of the Greeks at that time. Philoctetes, because he had the falling sickness, was marooned on the island of Lemnos so that his influence would not interfere with the religious rites conducted in preparation for the seige of Troy (Harrell, 1961). In Roman times also, the ill-omen of the presence of an epileptic prevented business from being conducted at public meetings. The demons of the epileptic also appear to have been considered contagious, since Pliny noted the habit of spitting at epileptics to "throw back the contagion". It is certain that epileptics were not regarded as prophets, or divine seers. The Apology by Apuleius, a Roman, makes it evident that epileptics were considered to be unclean and not fit to be mouthpieces of the gods (Temkin, 1945).

The physical theories developed by the Greeks and Romans to explain epilepsy were all varients of the "humors" and "spirits" theories which were typical of Galen's (131-201 A.D.) writings on all disease processes. For example Aretaeus the Cappodocian suggested that a "phlegm" was responsible, since he observed the frothing at the mouth during seizures. Galen himself believed that epilepsy was caused by accumulation of a thick humor in the ventricles of the brain, blocking

the free passage of "psychic pneuma" and thus interrupting sensation. The nerves were then supposed to shake themselves loose, causing the convulsions. This theory was to stand virtually unchallenged well into the 17th and 18th centuries.

Galen did make some observations which can be transposed almost directly into modern theory, making allowances for his scheme of physiology. For example, he thought that there were two types of epilepsy: idiopathic, by which was meant a disease of the brain itself, and sympathetic, in which the brain involvement was secondary and the disease originated in the periphery (Temkin, 1945). He divided the latter into cases in which the attack began with a motor effect, or a visceral aura. The sympathetic epilepsy with initial motor manifestations we would today term "Jacksonian". In any case he thought the attack originated in the peripheral structure first affected. This lead to ligating limbs to prevent the spread of the attack to the rest of the body, a procedure which has been performed even in the late 19th century. It is to a patient of Galen's that we owe the term "aura", which literally means "breeze". The patient described the progression of the attack up his leg as a "cold breeze blowing upwards".

From the time of Galen until the 18th century little progress was made in theoretical medicine, and this was particularly the case with epilepsy. Partly this was due to an unquestioning acceptance of Galen's work, and partly because demonological theories became firmly entrenched once more. Both were direct results of the influence of the Christian church. The former was due to acceptance of Galenic medicine by the Church, while the latter arose because of the story of Christ exorcing an "evil spirit" or "demon" from what was an obviously epileptic

boy (St. Mark 9, 14-29; St. Matthew 17, 14-20; and St. Luke 9, 37-43) and the further statement that "this kind [of demon] can be brought forth by nothing but prayer and fasting". What had up to that time been a popular, non-medical belief in demonological causes of epilepsy suddenly became religious dogma and for the next sixteen hundred years virtually the only treatment of epilepsy was the rites of the Church.

This had another great and unfortunate effect on the study of epilepsy. Epilepsy became associated with and submerged in many psychic conditions which were also considered to be lunacy or demoniac possessions. Thus what was a clearly defined entity for the Greeks became ill-defined, poorly understood, and a matter of much confusion. In France during the middle ages epilepsy was called "le mal Saint-Jean", but the name of St. John was also associated with the "dancing mania" which swept Europe many times from the 12th to the 15th centuries (Garrison, 1929). The dancing mania later became known as St. Vitus's Dance, and is now known as Sydenham's chorea. In England St. Willibrord was also connected with epilepsy via the dancing mania, while in Germany the falling sickness was also called St. Valentine's (St. Veltins-Sucht) disease, which name, according to Luther (Temkin, 1945) was applied only because of the phonetic similarity of the saint's name to the German for "fallen". The name "falling sickness" was applied to any condition whose symptoms included falling, among them being apoplexy, fainting, and a large variety of others. Patron saints of many "falling" diseases were associated also with epilepsy (Murphy, 1959). This was both caused by, and perpetuated the confusion in terms. Certainly there was no distinction made by the laity, and it is difficult to determine whether writers of the period mean epilepsy when referring to the falling sickness,

unless the symptoms are described.

While on the subject of the influence of the Christian Church on the concepts concerning epilepsy, it may be noted that it has been suggested that St. Paul was an epileptic (Black, 1962) on the basis of the conversion story (Acts 9, 3-9) and his own comment on the "thorn in in the flesh, the messenger of Satan to buffet me..." (II Corinthians 12, 7). In view of the disgust with which epileptics were regarded, it is doubtful if he, as an epileptic, could have wielded the influence that he did, or obtained the protection of the Roman courts. A cerebral vascular accident might better explain the visual hallucinations and the subsequent temporary blindness.

Certainly it is remarkable that, in the face of the theory of demonology officially approved by the Church, the medical profession maintained any trace of an organic theory of epilepsy at all. But during the middle ages there were many such theories advanced. Almost all of these however were variations of the Galenic theory of spirits and humors, since Galen's theories were accepted by the Church, and the Church largely controlled medical education. The idea that blockage of the ventricles at the base of the brain was responsible for epilepsy was very popular. Perhaps this was because of postmortem observations of hydrocephalics, and because of the belief that epilepsy following skull injuries was caused by compression. Even Thomas Willis (1621-75) was not able to persuade himself that infliction of epilepsy by the devil and by witches could be excluded, even though the revolt against the theological concepts of this disease was begun years before by Joannes Fernelius (1485-1558) who proposed a poisonous vapour theory of epilepsy (Bunker, 1947) without resorting to demons as a final cause.

It was really not until the 17th century that epilepsy came to be discussed as a separate entity again, still suffering however from a confusion of definition. Probably this advance resulted from an increased number of case reports, and the increasing correlation between epilepsy and gross pathology such as produced by head wounds and syphlitic abcesses in the brain. Steeghuis (c.1600) actually differentiated between "symptomatic" and "idiopathic" epilepsy, the former being the direct result or complication of some other disease process. This distinction is still made today. Charles de Pois (1563-1636) was one of the first to refute the Galenic concept of "sympathetic" epilepsy, and stated that all epilepsy originated in the brain. Both Fernelius and Jean Taxil (c.1600) had found pathological changes in the brain in cases of epilepsy, and both thought that the damage produced the epilepsy by irritation (Temkin, 1945). Fernelius blamed the mercury used in the treatment of symphilis as the primary cause. The concept of irritation was foremost among the theories of the time. The Galenic theory of sympathetic epilepsy was in fact that influences from the periphery irritated the brain, but the cause and effect relationship was undoubtedly confused by the teleological arguments of the time, which reasoned that the convulsion was the body's attempt to rid itself of a noxious influence. It was only in the 17th century that physicians began to take the mechanistic view that the fit was just a consequence of a pathological process rather than a purposeful act of the body. Pathological investigation in the 18th century supported the concept of irritation; for example boney concrescences were often found in the dura, and were said to have irritated the brain surface.

Among the theories proposed during the 17th century are two which deserve a brief mention. These were proposed by Franciscus

Deleboe Sylvius (1614-1672) and Thomas Willis, who because of their general approach to disease, may be regarded as more sophisticated successors to the alchemical school of Paracelsus. Both proposed an alchemical system, in which chemical reactions took place between spirits and humors. According to Willis, "a strong spasmodic copula distilled from the blood into the brain affecting the animal spirits which be in the middle of the brain, causing an explosion" (Gastaut and Fischer-Williams, 1959). It is tempting to regard Willis' "explosion" in the sense of a massive, synchronous nerve discharge, but Willis meant the term quite literally. He believed that muscle movement for example, was due to an explosion within the muscle, analogous to the explosion of gunpowder. These physical theories can be traced to the concepts of such people as Borelli (1608-79), whose belief that animal spirits twitched the beginnings of nerves and thus caused a release of nervous juice at the muscles is very similar to modern ideas of neuromuscular transmission, Malpighi (1628-1694), and Baglivi (1666-1707) who thought that the brain pushed nervous juice down the nerves.

Hermann Boerhaave (1668-1736) is an example of a great teacher and observer. Like many others of his time, he tended to put emphasis on obvious causes which could be observed on the dissecting table, and to dispense with consideration of the mechanics of epileptic attacks. He believed in particular that birth injuries could result in epilepsy. In general, until late in the 19th century, investigation was centered around inquiry as to the predisposing and provoking causes. Simon-André Tissot (1728-1797), for example, frankly admitted that the basic understanding of mechanisms of the brain was so poor that the theories on epilepsy were only wild conjectures. The latter half of the 18th century

might be considered a period of observation, in which pathological examination played an important role. It was also a period of sorting out of the nosological confusion which had been inherited from the middle ages, a process which continued for over a century. Great strides were taken in this direction early in the 19th century. Two factors were responsible. The first was the establishment of hospitals for the insane and the epileptic (instead of incarcerating such persons in prisons) where more accurate observations could be made, and the other was the advances made in basic neurophysiology, especially in the middle of the century. Some of this work on epilepsy suffers from confusion of epilepsy with psychiatric problems. This is not surprising since epileptics were mostly confined in lunatic asylums. Hysteria and many other conditions were discussed as manifestations of epilepsy, in a manner reminiscent of the situation in the middle ages and the Renaissance. It was even thought necessary to show that epilepsy had nothing to do with the phases of the moon (Leuret, quoted by Temkin, 1945). There was controversy as to whether unilateral convulsions, and convulsions without loss of consciousness were truly epilepsy. This argument had considerable effect on the thinking and terminology of Hughlings Jackson later on. Another controversy concerned the exact part of the nervous system responsible for epilepsy, i.e., medulla, midbrain or cerebral cortex. This controversy will be examined further to give the background for Hughlings Jackson's development of the physiology of epilepsy.

B. THE JACKSONIAN ERA, AND THE DEVELOPMENT OF THE CONCEPT OF FOCAL CORTICAL EPILEPSY

John Hughlings Jackson above all others can be regarded as being responsible for the modern developments of investigation of epilepsy. Some of Jackson's contributions, and the background against which he did his work, are discussed below.

Albrecht von Haller (1708-1777) did experiments to test the concept of irritability, which had been in existence since Galen as a philosophical theory. He attempted to discover which parts of the nervous system showed irritability. Von Haller said that the cerebral cortex was not irritable, because mechanical stimulation could not provoke convulsions; rather stimulation of the medulla could do so. The latter had been shown a century before by Charles Drélincourt (1633-1694) who poked the fourth ventricle of a dog with a needle. Much later (1823) Flourens (1794-1867) found that stimulation of the cerebrum and cerebellum produced no effects, and that the medulla, spinal cord and corpora quadrigemina were the only areas of the central nervous system where mechanical stimulation caused movement (Temkin, 1945).

The concept of a medullary origin of epilepsy received support from Marshall Hall (1790-1857) who developed the idea of reflexes, which he thought were a property of the medulla and spinal cord alone. He felt that epilepsy could be divided into two types: that which started in the medulla itself, and that in which the cause acted on peripheral nerves which in turn acted on the brain. He coined the term "reflex epilepsy" for this type, and intended that it should replace the Galenic concept of "sympathetic epilepsy". His term is still in use today to denote cases in which acute epileptic fits can be precipitated by sensory stimulation (e.g., audiogenic seizures). Hall's theory had one major

defect which was obvious immediately, that is, that it failed to account for loss of consciousness during an epileptic fit; heightened reflex excitability seemed incompatible with loss of consciousness. Much of Brown-Sequard's work led him also to believe in the reflex nature of epilepsy. He added the concept that the loss of consciousness resulted from cerebral vascular spasm and a reduction of the blood flow to the brain. This concept greatly influenced Jackson's early thinking, and has directed some of the early investigations of Penfield and his group in this century. It was known from the work of Astley Cooper (1768-1841) that central anoxia could cause convulsions. There were several variations of the nutritional aspect of this theory proposed. Russell Reynolds (1828-1896) believed that the basic defect was an inherited faulty medullary nutrition, but that cerebral angiospasm could precipitate an attack (Temkin, 1945). Kussmaul and Tenner also believed that sudden arrests in nutrition would produce cellular alteration in the brain structure leading to epilepsy. They were of the opinion that this change should be considered to be physiological rather than anatomical. They also suggested that the convulsive poisons (such as strychnine) acted by interfering with normal nutrition (quoted by Jackson, 1958). Thus by the middle of the 19th century there were three theories of epilepsy (i.e., reflex epilepsy, initiation of fits by cerebral angiospasm, and altered molecular state of the brain caused by malnutrition or poisoning) which were more or less interconnected by various authors. Almost all who wrote on this subject believed that the origin of the fits was the medulla.

On the other hand there was evidence that the cerebral cortex might be involved in the production of epilepsy. Charles Bell (1774-

1842) had proposed in his book of 1811, Idea of a New Anatomy of the Brain, that the motor pathway originated from the cerebral cortex, and he believed that the seat of intellectual function was also in the cortex (Temkin, 1945). This led to a dichotomy in any attempt to explain epilepsy according to his anatomical viewpoint, for one had to explain how intellectual function could be obtunded and motor function be enhanced by a single disease process in the cortex. There was also a large accumulation of pathological evidence that associated cerebral lesions with epilepsy. It is important to note, however, that most physicians of the time did not regard such symptomatic epilepsy as "true" epilepsy. There was, however, a school which, on the basis of anatomical evidence, believed that there was a synapse in the corpora striata and hence this latter was viewed as the beginning of the motor pathway and the origin of movement, rather than the cerebral cortex.

Jackson studied many cases of epilepsy which were unilateral, at least to start with, and in which quite frequently consciousness was not lost. This type of seizure had been described before, but again the opinion of the time was that this was not a form of epilepsy. Jackson, on the other hand, thought that this type represented a simple form of epileptic seizure and although he called such fits "epileptiform" seizures, he stated many times that the difference was a matter of degree rather than type. Jackson's first hypothesis (1864) was that the cause of this type of seizure was an embolus lodged in the middle cerebral artery (Jackson, 1958). This early stage of his career was also marked by his gradual rejection of the concept of a primary medullary involvement in epilepsy in favour of the corpus striatum. He continued to hold the view that the corpus striatum was the structure primarily responsible for

epilepsy until at least 1860, although, as he said later (Jackson, 1958), he did not at any time exclude involvement of the cerebral cortex in the genesis of epilepsy. Gradually his emphasis shifted from the corpus striatum to the cerebral cortex, but he often referred to the involvement of the corpus striatum, noting that both it and the area of cortex that he thought responsible for epilepsy were both supplied by the middle cerebral artery.

The turning point was provided in 1870 when G. Fritsch and E. Hitzig demonstrated a motor area in dogs by the use of electrical stimulation, and also that when this area was strongly stimulated convulsions were produced which began in a localized group of muscles and then became typical generalized epileptic attacks (Gastaut and Fischer-Williams, 1959). The concept of localization of function of the cerebral cortex, and the observation that the cortex could be involved in epileptic attacks, which developed from this work both proved useful to Jackson. They offered Jackson a method of explaining the frequent start of seizures in a localized group of muscles such as those of the toe or thumb and forefinger on the basis that a localized area of the motor cortex was involved in the start of the attack. In other cases he had observed there was an aura of some sensation which suggested to him that the seizure started in the area of cortex which subserved this sensory function. The high frequency of involvement of thumb and forefinger was easy for Jackson to explain. He was familiar with the work of the psychologist Herbert Spencer, who had advanced the theory of localization of function in the central nervous system on theoretical grounds. Spencer suggested that the area of brain subserving a particular function would be related to the complexity of the function, thus the thumb and forefinger would have

a much greater representation in the motor cortex than would the muscles of the trunk. This theory, proposed first in 1855, has been amply proven by Penfield and his co-workers in this century (Penfield and Jasper, 1954).

Thus Jackson clearly established the concept of epilepsies due to focal cortical "discharging lesions", and although he differentiated between epilepsy and epileptiform convulsions, he believed that they were both cortical in origin. He believed that the motor or sensory aura were indications of the localization of the discharging lesions (Jackson, 1874, 1876, 1880, 1958). Moreover, he thought that even cases of epilepsy in which loss of consciousness was the first symptom of an attack were cortical in origin (Jackson, 1886).

Even while Jackson was propounding the theory of cortical epilepsy, there was still some disagreement in the literature as to whether the cortex alone could maintain such a discharge. For example, Franck and Pitres (1883, quoted by Gastaut and Fischer-Williams, 1959) found that they could not stop an existing epileptic afterdischarge by ablating the original focus and concluded that the discharge was maintained by other cortical areas and subcortical structures. However, earlier work by Munk (quoted by Bubnoff and Heidenhain, 1881) had shown that convulsions could be prevented if the ablation was done immediately, but that shortly thereafter the discharge spread to other regions of the cortex. At this later time extirpation of a local motor area would stop the convulsions in the appropriate extremity, but if the ablation was delayed still longer even the involved extremity continued to be active. This was probably the first experimental demonstration of the progressive involvement of the rest of the brain in a seizure of focal cortical

origin. It has since been shown that the cortex by itself can maintain epileptiform discharges without the necessity of subcortical structures being involved (Kristiansen and Courtois, 1949; Burns, 1951; Pinsky and Burns, 1962). There is, however, also a considerable body of evidence that subcortical structures do play a significant role in the clinical manifestations of the epileptic discharge, especially in focal epilepsies which become bilateral during a seizure (Erickson, 1940; Hayashi, 1952; Walker, Poggio and Andy, 1956). The question as to whether all epilepsy is cortical in origin is quite another problem. Although Jackson did not really believe his own distinction between generalized epilepsy (bilateral convulsions beginning with loss of consciousness) and partial epilepsies or "epileptiform" convulsions, (one-sided convulsions, at least at the start, with or without subsequent loss of consciousness) the terms still remain in use. Jackson regarded both of these as cortical in origin. An extensive review of the current literature led Gastaut and Fischer-Williams (1959) to regard seizures which begin with loss of consciousness as subcortical in origin. In large measure, their opinion rests on experimental seizures produced by anoxia or by various convulsant drugs. Electroencephalographically at least, these seizures are not necessarily similar to the naturally occurring seizures. There is no doubt, however, that discharges in a variety of subcortical nuclei can cause epileptiform discharges in the corresponding cortical projection areas (e.g., Goldring and O'Leary, 1951; Rakic, Buchwald and Wyers, 1962). The caudate nucleus also can act to inhibit cortically induced motor activity (Rakic et al., 1962). These facts appear to form the basis for the theory offered by Gastaut and Fischer-Williams (1959) that the thalamocaudate inhibitory system is responsible for the tonic-clonic

electroencephalographic and muscle movement patterns during seizures. Their concept is that the activation discharge builds up an inhibitory discharge in these subcortical structures. This inhibitory discharge then shuts off the tonic discharge temporarily. Thus one might say that the clonic phase is merely repeated interruptions of the tonic discharge. This theory does not seem to be an adequate explanation because a clonic discharge pattern can be produced in the isolated cortical slab (Kristiansen and Courtois, 1949; Burns, 1951, 1958; Pinsky and Burns, 1962).

The vast amount of clinical literature currently available is the result of the revival, in 1929, and popularization by Hans Berger of the original observation by Caton and by Beck of what is now called the electroencephalogram (EEG) (Brazier, 1959). The spontaneous rhythmic electrical activity reported earlier by both Caton and Beck was forgotten because of the wide interest in their demonstration of sensorily evoked cortical potentials and the acrimonious public debate about these evoked potentials with Von Marxow (Brazier, 1959). In contrast, Berger's work was quickly put into use by such people as Lennox (Gibbs, Davis and Lennox, 1935) who demonstrated the abnormal EEG pattern of petit mal epilepsy and Grey Walter (1936) who showed how to localize brain tumors by determining the origin of the slow waves in the EEG's of such patients. This technique developed by Grey Walter has been successfully used to locate cortical epileptic foci, enabling their clinical identification, investigation and surgical removal (Penfield and Jasper, 1954). In large measure, the modern era of epilepsy investigation dates from this demonstration of cortical electrical activity associated with the convulsion. Much of the large volume of clinical literature, primarily dealing with diagnosis and treatment, that has since accumulated is not relevant to the

problem of mechanism of epileptic discharge. Even less of this work is concerned with the basic cellular defect which is responsible for the spontaneous origin and generation of seizures. Gross pathology has, of course, been observed, but the relation of such pathology to convulsions has been recognized for centuries. However, a large number of cases are still classified as idiopathic, for even with modern electrographic localization techniques no lesions are apparent. This is especially so in cases of loss of consciousness with generalized convulsions (Brain, 1962).

The first experimental work of the modern era was done by Adrian and Matthews (1934) and by Adrian (1936) who experimentally confirmed the work of Berger, Lennox and of Grey Walter, and demonstrated the spread of convulsive electrical activity in the cerebral cortex.

C. MODERN THEORIES OF THE CELLULAR MECHANISMS OF EPILEPSY

First of all it is obvious that there must be some cellular abnormality in cases of epilepsy, since some such patients respond abnormally to simple stimuli such as a light flashing in the eyes. The cells in the epileptic focus are generally regarded as hyperexcitable or more susceptible than normal neurones to convulsive activity (Alpers and Mancall, 1961). In recent years there have been many theories postulated to account for this difference between normal and epileptic neurones. Most of them may be classified into three groups: biochemical and metabolic disorders, alterations to the physiological properties of the neurones, and anatomical changes. These will now be reviewed in some detail. The various theories are not meant as distinct alternates and the suggestions of most workers have combined several theories in explaining the imbalance of excitatory and inhibitory influences in epileptogenic cortex.

(a) Biochemical and metabolic disorders.

(i) General metabolic and anoxic theories. Metabolic deficiencies could arise from either an intrinsic cause, such as genetically controlled absence of various enzymes, or from an extrinsic cause, such as deficient blood flow. The latter is the concept originally held by Penfield (Penfield and Humphreys, 1940), who believed that the epileptic focus was an area of intermittent ischaemia. The intermittent hypoxia was believed to cause the focus, and the periodic nature of the incidence of seizures (Penfield and Jasper, 1954). Implicit in this theory is the suggestion that the periodic hypoxia could be the indirect irritating factor for starting a seizure in the cortex.

There seems to be general agreement that there is no basis for

suggesting any intrinsic general metabolic inadequacy in epileptic tissue (Tower, 1960). Elliott and Penfield (1948) and Pappius and Elliott (1954) demonstrated that oxygen utilization and glycolysis of epileptic foci are not different from those of normal cortical tissue in vitro. While the metabolic poisons fluoroacetate and fluorobutyrate cause epileptiform convulsions, Hendershot and Chenoweth (1955) demonstrated that the activity of metabolic pathways and genesis of epileptiform discharges are independent. They showed that the convulsive effects of fluoroacetate and fluorobutyrate could be blocked by structural analogues (glycerol monoacetate and glycerol monobutyrate) without altering the metabolic inhibition produced by these fluoro compounds.

It is well known that during and following epileptic discharge there is an increase in brain O_2 consumption, pCO_2 and blood flow (Jasper and Erickson, 1941; Schmidt, Kety and Pennes, 1945; Ingevar, Lübbers and Seisjö, 1962), but these appear to be secondary to the seizure. The role that pH plays is still uncertain. Hyperventilation can activate seizures in some patients. Pope, Morris, Jasper, Elliott and Penfield (1946) have shown that the pH of epileptic focal tissue is essentially normal. Changes in cortical pH develop during a seizure (Dusser de Barenne, McCulloch and Nims, 1937; Jasper and Erickson, 1941) but like the blood flow, pO_2 and pCO_2 changes, the pH changes seem to be the passive result of the increased cellular activity that occurs during the seizure (Tower, 1960). There is an acidosis at the beginning of the seizures but this disappears as the blood flow increases. In any event, an acidosis would tend to make the brain less excitable. Pappius and Elliott (1954) also reported that ATP-ase, Na+ and K+ of epileptogenic cortex was normal.

(ii) Abnormalities of acetylcholine metabolism. Interest in acetylcholine (ACh) metabolism has been high since Miller, Stavraky and Woonton (1940) and Brenner and Merritt (1942) demonstrated that ACh can exert a convulsant effect on cerebral cortex.

Pope et al. (1946) reported increased cholinesterase (ChE) in epileptogenic cortex. Tower and McEachern (1949b) confirmed this and found that the cerebrospinal fluid (CSF) of epileptic patients contained ACh. The causal relationship is not clear, for they also reported (1949a) the presence of ACh in the CSF of patients receiving electroshock therapy. Tower and Elliott (1952) reported that total ACh content and rate of ACh synthesis (in vitro in high potassium) was normal in cortical tissue from epileptic foci, but that such tissue had a reduced ability to store or bind ACh, and postulated an increased ACh release in vivo in epileptic foci as the cause of seizures. In 1953 Tower and Elliott reported that the defect in ACh-binding in epileptic cortex could be corrected in vitro by adding glutamine or asparagine (but not glutamate) to the incubating medium. This concept was extended to in vivo studies in nine patients, with apparent success (Tower, 1955). Much of this theory now appears doubtful for Pappius and Elliott (1958) reported they could not duplicate the earlier in vitro observations: they found that the bound ACh was normal in epileptic tissue, that free ACh was lower than normal, and that neither the presence nor production of ACh, free or bound, was altered by addition of glutamine in vitro. Other conflicting evidence also exists. Human epileptic foci have increased ACh content (Tower, 1958). In cerebral cortex made sensitive to epileptogenesis by chronic neuronal isolation (Grafstein and Sastry, 1957) the ACh content is depleted (Sastry, 1956), and the ChE activity is

reduced (Echlin and Battista, 1962a,b). Choline acetylase activity, as well as ChE, is reduced in chronically undercut cortex (Hebb, Krnjević and Silver, 1963).

(iii) Disturbances of gamma-amino butyric acid metabolism. Defects in inhibitory mechanisms have been suggested to play a role in the genesis of epileptic foci. The main interest has centered on gamma-amino butyric acid (GABA), and the possibility that decreased GABA is the major defect in focal discharging lesions. GABA has been shown to inhibit a variety of responses of the cerebral cortex, including the spiking activity of cortical "epileptic" foci induced by local freezing of the cortex (Purpura, Girado, Smith and Gomez, 1958a,b). However, at the time that the spiking started GABA was still normal in these lesions, while instead glutamic acid, glutamine and glutathione were reduced (Berl, Purpura, Girado and Waelsch, 1959). Administration of GABA returned the concentrations of the latter three amines to normal. Marrazzi, Hart and Rodriguez (1958) suggest that diphenylhydantoin has its action by increasing cortical GABA content, but Maynert and Kaji (1962) found opposite results.

Attempts to modify the cortical content of GABA have also led to conflicting results. Killam and Bain (1957) showed that development of seizures in response to the hydrazides (e.g., thiosemicarbazide) was accompanied by an inhibition of glutamic acid decarboxylase and a decrease in GABA. They suggested a competition with pyridoxal phosphate (vitamin B_6) was responsible. However, Maynert and Kaji (1962) found, in contrast with Roberts, Baxter and Eidelberg (1960), that the ability of the hydrazides to cause convulsions was independent of their ability to reduce cortical GABA concentration. Roberts et al. (1960) and

Maynert and Kaji (1962) are also in disagreement about the ability of hydroxylamine-induced increases in cortex GABA concentrations to protect against hydrazide convulsions. The latter also found that pyridoxine and pyridoxal also are ineffective in antagonizing the hydrazides. Thus the experimental evidence linking epileptogenesis to cortical GABA content is tenuous at best.

There is some clinical evidence which definitely links pyridoxine to convulsions. There was an epidemic of convulsions among infants in the United States in the early 1950's which was traced to avitaminosis-B_6 due to use of a commercial infant feeding formula which was deficient in pyridoxine. These infants exhibited diffusely abnormal EEG's, and seizures which in their severest form resembled grand mal. All responded to phenobarbital medication, change of diet or to vitamin B_6 (Coursin, 1954, 1955; Molony and Parmelee, 1954). It is speculated that the ultimate lesion in these infants was a deficiency in GABA, since pyridoxine is a necessary coenzyme for GABA synthesis. There are a few reports of rare cases who normally require a large amount of vitamin B_6 to stay free of convulsions, and in addition it had been shown experimentally that pyridoxine deficiency produced convulsions in chicks (Lerkovsky and Kratzer, 1942), rats (Daniel, Kline and Tolle, 1942; Chick, El Sadr and Worden, 1940) and pigs (Hughes and Squibb, 1942).

(b) Changes in physiological properties of neurones.

(i) Denervation supersensitivity. Cannon (1939) first suggested that denervation supersensitivity might play a role in focal cortical epilepsy. Stavraky was the first to investigate the possibilities of denervation supersensitivity in the central nervous system (Stavraky, 1961) although as Sharpless (1964) has pointed out, Stavraky

included many conditions which would not be analogous to denervation supersensitivity in the peripheral nervous system as outlined by Cannon and Rosenblueth (1949).

This concept has been investigated in the cerebral cortex by a number of workers (Echlin, 1959; Grafstein and Sastry, 1957; Sharpless and Halpern, 1962; Halpern, 1960, 1962) using the neuronally isolated cerebral cortex slab preparation developed by Kristiansen and Courtois (1949) and Burns (1949, 1950). It was found that chronic neuronal isolation, or chronic partial denervation caused the appearance of electrical activity in the slab that was similar to discharges found in spontaneous and other experimental epilepsies (Echlin, 1959). The chronically isolated slabs were more sensitive to electrical stimulation (Echlin, McDonald, Duke and Peck, 1953; Grafstein and Sastry, 1957) and to pentylenetetrazol (Echlin et al., 1953) than was normal cortex. Echlin (1954, 1956, 1959) also reported that these chronic slabs were supersensitive to ACh. The epileptiform discharges of chronically isolated slabs also last much longer than those of acutely cut slabs, or local discharges in intact cortex (Echlin, 1959; Grafstein and Sastry, 1957; Sharpless and Halpern, 1962). These changes develop over a minimum period of 3 weeks (Echlin, 1959; Sharpless and Halpern, 1962) or about the same time-course as for peripheral supersensitivity (Cannon and Rosenblueth, 1949). Echlin and Battista (1961) have reported that epileptiform discharges can be evoked in chronically partially isolated slabs by peripheral nerve stimulation, thus connecting the abnormal discharges of denervated cortex with the clinical entity of epileptiform seizures.

There are a number of other reports in the literature which show an increased responsitivity of various parts of the central nervous

system and an increased susceptibility to epileptogenic stimuli following a wide variety of lesions, ablations, and other forms of partial denervation. For example, Kennard (1957) found that temporal pole ablations in monkeys led to an increase in spontaneous seizures. Morrell (1959) and Eidelberg and French (1961) reported that production of an epileptic focus by application of aluminium hydroxide gel to one cerebral hemisphere led to the appearance of a "mirror image" focus in the opposite hemisphere. This had the same time course as the development sensitization of isolated slabs. Furthermore, this secondary focus remained after ablation of the original focus. Franken and Desmedt (1957) produced greatly increased sensory evoked responses in the suprasylvian gyrus association area by chronically depriving it of its transcallosal input by removal of the opposite suprasylvian gyrus 2 to 4 months previously.

(ii) Electrophysiological theories of epilepsy. Analysis of electrical potentials of the cortex in terms of cerebral substructure was first done by Renshaw, Forbes and Morison (1940), applying volume conductor theory developed by Lorente de Nó (1939). Subsequently identification of the cellular elements responsible for various cortical potentials has been attempted by Chang (1951), Eccles (1951, 1957), Burns and Grafstein (1952), Clare and Bishop (1955a,b) and Purpura and Grundfest (1956b). In investigations of the mechanism of epilepsy, and the abnormalities of epileptic cortex, at the cellular level, the surface potentials (electrocorticogram or ECG), radial transcortical potentials, and the activity of single cells have all been utilized. Few studies, however, have directly compared the difference between seizures of normal and abnormal cortex.

Analysis of ECG alone (e.g., Rosenblueth and Cannon, 1942;

Ralston, 1958; or Blum, Magnes, Bental and Liban, 1961) is probably relatively ineffective in elucidating cellular mechanisms of epileptiform discharges, for the surface waves often show no correlation with cell discharge immediately below the surface recording site (Jasper, 1961). Some success has been obtained, however, by relating other phenomena to the development of seizures, and by studying the slow potential changes of the ECG during seizures. For example, the surface-positive burst response of acutely isolated cortex might be regarded as a single epileptic paroxysm, however, according to Burns (1958) the positive burst response meets the criteria for being due to reverberating chains of neurones (Forbes, 1929), while the epileptiform discharge does not. The surface negative response (direct cortical response, DCR) has also been implicated in epileptogenesis. Natural or chemically induced epileptic foci develop DCR-like waves between seizures (e.g., Ralston, 1958; Konigsmark, Abdullah and French, 1958; Rech and Domino, 1960). Both the DCR and these focal waves (but not overt seizures) are suppressed by gamma-aminobutyric acid (Purpura and Grundfest, 1956b; Goldring, O'Leary and Shi, 1958; Rech and Domino, 1960; Berl, Takagaki and Purpura, 1961). Eidelberg, Konigsmark and French, (1959) found that the distribution of amplitude of the DCR over the cortex is identical to the distribution of susceptibility to electro-seizures. Experimental and clinical foci also have augmented DCR's (Eidelberg et al., 1959; White, Eidelberg and French, 1959, 1960; Goldring, Jerva, Holmes and O'Leary, 1960; Smith and Purpura, 1960; Eidelberg and French, 1961). This suggests that increased electrical excitability of neurones may be a factor in development of epileptic foci, providing it is assumed that the DCR is a presynaptic response (Chang, 1951; Burns, 1958). However, if the DCR is a postsynaptic

response (Purpura and Grundfest, 1956b; Frank and Pinsky, 1964), then increased chemical sensitivity could be inferred. In contrast, however, Grafstein and Sastry (1957) and Goldring, O'Leary, Holmes and Jerva (1961) found that the threshold for the DCR, was increased or unchanged in cortex made epileptogenic by chronic denervation.

D-C potentials. Ever since the work of Libet and Gerard (1941) considerable attention has been paid to the relation of the mean surface potential and the transcortical potential to convulsive, and other activity of the cortex(O'Leary and Goldring, 1964). Burns (1953), Goldring and O'Leary (1951, 1957), Goldring, O'Leary and King (1958) and O'Leary and Goldring (1960) have observed that normal cortex became relatively surface-positive during seizures, suggesting that the deep ends of radially arranged structures were depolarized. Pinsky (1961, 1963) found a brief (1-2 sec) focal, negative shift of the transcortical potential following subconvulsive electrical stimulation. This shift is maximum between 0.8 and 1.0 mm deep in the cortex. The amplitude of this "negative after-deflection" appears closely correlated with stimulus intensity, and critically related to threshold. Burns (1953), Pinsky (1961) and Pinsky and Burns (1962) postulated that convulsive activity in normal cortex is initiated by relatively sustained somatic depolarization resulting from differential repolarization in soma and dendrites. A similar process is proposed for afterdischarge in veratrine-treated muscle (Burns, Frank and Salmoiraghi, 1955). Gloor, Vera, Sperti and Ray (1961), Gloor, Sperti and Vera (1962) and Gloor (1962) also measured radial potential changes and unit activity associated with production and maintenance of afterdischarges, and have reached similar conclusions, namely that a critical potential gradient between apical dendrites and soma must be

generated before epileptiform activity occurs, that the somatic depolarization fires the cell repetitively, and that each burst of activity "resets" the polarization to allow the discharge to continue until cathodal block or synaptic inhibition occurs. Goldring and O'Leary (1951) and Gloor et al. (1961) found that artificial surface-positive polarization of the cortex caused, or enhanced, epileptiform discharges, while the opposite polarization inhibited seizures.

The pial surfaces of penicillin and aluminium hydroxide lesions have been found to be negative with respect to the deeper cortical layers or the pia of surrounding normal cortex, and an enduring dendritic depolarization is thought to be the cause (Ward, Thomas and Schmidt, 1956; Ward and Mahnke, 1960; Morrell, 1960a; Esberard, 1961; Mahnke and Ward, 1961; Ward, 1961). They postulate a mechanism of seizure initiation similar to that suggested above for normal cortex, except that the cell potential gradient is reversed, with the depolarized dendrites acting as a current sink for the soma. Seizure discharge breaks out when the current is enough to fire the cell. It is not clear, however, whether this relative polarity persists during the actual seizure, for Matsumoto and Ajmone-Marsan (1964b) have found that there is a negative shift in the deeper layers during seizures (just as occurs in normal cortex) indicating relative somatic depolarization. However, they also found an inconsistent correlation of this radial potential shift with the degree of cellular activity. In another study (Purpura, Goldensohn and Musgrave, 1963) no relation was found between the D-C surface potential and onset of epileptic activity in freezing lesions of the cortex.

Extracellular and intracellular unit activity. In normal cortex, unit activity of cells during electrically and chemically induced

seizures has been recorded extracellularly by Li and Jasper (1953), Jung (1953), Enomoto and Ajmone-Marsan (1959), Gerin (1960) and Ajmone-Marsan (1961), while intracellular recordings have been made by Li (1959), Kandel and Spencer (1961,a,b,d) and Sawa, Maruyama and Kaji (1963). Generally, the results of these studies support the theories of somatic depolarization proposed by Burns, Pinsky, and by Gloor et al. (op. cit.). Repetitive stimulation progressively depolarizes the cell below the firing level, and cell spikes grow smaller and stop. Repolarization allows soma firing again. Most cells are subjected to large depolarizing waves (probably summed excitatory postsynaptic potentials (EPSP's)) approximately concurrently with the waves in the ECG, during which the cell fires rapidly. Sometimes the waves exceed the critical firing level and the cell ceases to fire temporarily. During this time individual EPSP's may be seen. It has been inferred from the predominance of these potentials of synaptic origin, that, in contrast to the concept of Pinsky and Burns (1962), synaptic activation plays a large role in maintenance of seizures. Some cells stop firing during the ECG waves, and these have been shown to hyperpolarize due to synaptic inhibition (IPSP's). While most investigators found no consistent temporal relationship between bursts of cell firing and the ECG waves, individual cells usually maintained a constant relationship with the ECG. The surface waves are considered to be temporal summations of all the IPSP's and EPSP's. It is uncertain how the synchrony is achieved, but Moruzzi (1953) suggested that ephaptic interactions could play a role. Anderson and Eccles (1962) propose that brain rhythms may be determined by a resonance frequency of neuronal chains related to recovery times of individual neurones.

In epileptic cortex, studies of unit activity have been made

by Thomas, Schmidt and Ward (1955), Ward, Schmidt and Thomas (1956), Ward, Thomas and Schmidt (1956), Enomoto and Ajmone-Marsan (1959), Schmidt, Thomas and Ward (1959), Ajmone-Marsan (1961), Morrell (1961), Ward (1961), Ward and Schmidt (1961), and Matsumoto and Ajmone-Marsan (1964c) with extracellular electrodes; intracellular investigations have been done by Atkinson, Macs and Ward (1961), Goldensohn and Purpura (1963) and Matsumoto and Ajmone-Marsan (1964a,b). A special case is that of Kandel and Spencer (1961a,d) who studied intracellular potentials in hippocampus of deafferented fornix preparations. As is the case in normal cortex, firing of cells in epileptic cortex was only approximately in phase with the ECG. Some disagreement exists concerning the presence of cellular depolarizations corresponding to EPSP's. Atkinson et al. (1961) found that bursts of spikes were not superimposed on EPSP's. The other investigators have found the same pattern of events as in normal cortex with regard to EPSP's, depolarizations, and cell firing (Goldensohn and Purpura, 1963; Matsumoto and Ajmone-Marsan, 1964b). Some cells evidently are maintained in a partly depolarized state throughout most of the seizure, a few others hyperpolarize and do not fire during ECG activity. The pyramidal cells studied by Kandel and Spencer (1961a,d) were predominantly this latter type. The evidence suggests that cell participation in seizures of epileptic cortex is by depolarization, maintained by synaptic activity. Schmidt et al. (1959) found that there was no attenuation of spikes during high frequency activity or bursts, and therefore suggested that spike initiation is not occurring at the axon hillock (Phillips, 1956b), supporting their concept of dendritic depolarization in epileptic foci, rather than the soma-depolarization theory proposed for normal cortex. Schmidt et al. (1959) observed that

most spikes were small, and concluded that the participating neurones were small in size. However, the small spike size could result from maintained partial depolarization of the soma. Maintained partial depolarization of some part of the cell could explain the observation that epileptic lesions usually have a low electroconvulsive threshold (Penfield and Erickson, 1941), and provide a mechanism for the suggestion that epileptic foci have an increased electrical excitability (Fessard, quoted by Gastaut and Fischer-Williams, 1959).

Resting activity (interictal) of single cells in epileptic foci is much greater than that of normal neurones (Ward, et al., 1956; Schmidt et al., 1959; Enomoto and Ajmone-Marsan, 1959; Ward and Schmidt, 1961; Ajmone-Marsan, 1961; and Matsumoto and Ajmone-Marsan, 1964a). Normal cortical neurones fire single spikes at about 15/sec. Epileptic cells fire in bursts of high frequency spikes, or at high frequency (100-400/sec) interrupted with bursts of higher frequency (800-1000/sec) spiking or brief silences. Neither normal nor epileptic firing is related to the ECG, although some bursting of epileptic cortex is related to paroxysmal waves in the ECG. These bursts of spikes are due to massive EPSP depolarizations of the cell. A greater proportion of cells in epileptic foci than in normal cortex appear to be "spontaneously" active between seizures. These observations also appear to hold during seizures, where more cells are active, and at higher frequencies, in epileptic cortex compared to normal. Fewer cells are active in driven seizures in normal cortex than in the original epileptic focus (Ajmone-Marsan, 1963). Schmidt et al. (1959) concluded that the capacity for sustained autonomous discharge is a fundamental property of the epileptic neurone.

Repetitive discharge in nerve terminals. While the majority of

investigators believe the primary site of the repetitive activity in an epileptic seizure is the cell body, it has been suggested, on pharmacologic grounds that the primary event is repetitive activity in the nerve terminals. Post-tetanic potentiation is due to repetitive discharge of nerve terminals. Failure of repolarization in the nerve terminals is suggested to induce the repetitive activity in nerve endings in the same manner as do generator potentials of afferent nerves (Standaert, 1963). Diphenylhydantoin suppresses the post-tetanic potentiation and the post-tetanic repetitive activity without affecting normal transmission (Esplin, 1957; and Paris and Raines, 1963). Paris and Raines (1963) suggested that this action of diphenylhydantoin might be the explanation of the drug's anticonvulsant activity. This may be so in electroconvulsions of normal cortex where the cortex is stimulated tetanically, but there is, as yet, nothing to indicate how such repetitive activity could be spontaneously induced in epileptic cortex.

(c) Anatomical theories of epilepsy.

(i) Mechanical irritation by glial scar. Ward et al. (1956), Schmidt et al. (1959), Ward and Mahnke (1960) and Mahnke and Ward (1961) proposed that the persistent depolarization of dendrites, and the spontaneous high frequency discharge of neurones in epileptic foci were due to distortion of the apical dendrites by glial scar cells. Ward (1961) reported that he had found anatomical evidence of destruction and distortion of apical dendrites. He suggested that the mechanism whereby distortion produced the depolarization and firing of the cell was analogous to production of impulses by sensory end-organs, such as Paccinian corpuscles, which passively depolarize in proportion to the degree of deformation.

(ii) Multiplication of excitatory synapses. A re-organization and increase in excitatory synapses by collateral sprouting of axons has been proposed as the cause of the increased epileptogenesis in chronically denervated cortical slabs, (Purpura, 1961; Purpura and Housepian, 1961). This investigation was performed on immature cortex, and it is unknown if the results could apply to mature cortex. Ward (1961) and Echlin (1959) observed a loss of apical dendrites and destruction of large pyramidal cells, which suggest a decreased number of synapses.

(iii) Failure of inhibition. Strychnine-treatment of the cortex has long been used as a model of epilepsy (Gastaut and Fischer-Williams, 1959) since strychninisation mimics many features of clinical and other experimental epilepsies. There is no a priori reason for the action of strychnine on the cortex to differ from its action elsewhere in the central nervous system (Eccles, Fatt and Koketsu, 1954), and some evidence that it does indeed block inhibitory synapses in the cortex (Wright, Andrew and Jacobson, 1954; Purpura and Grundfest, 1956a,b). Thus block of inhibitory mechanisms can cause epileptiform convulsions. Several reviewers (e.g., Symonds, 1959; and Fessard, quoted by Gastaut and Fischer-Williams, 1959) have suggested that dysfunction or destruction of inhibitory mechanisms may play a role in epilepsy.

There is no reason to suspect that the inhibitory mechanisms are any more labile than excitatory processes, although this has been suggested by Goodman and Gilman (1958), except perhaps in the following case. Recurrent inhibitory pathways to cortical pyramidal cells are well established (Phillips, 1956a; Kandel, Spencer and Brinley, 1961). Destruction of part of the pathways mediating this recurrent inhibition might be responsible for the epileptogenesis of isolated cortex, as when

large pyramidal cells (and their recurrent collaterals) are destroyed (Sastry, 1956; Echlin, 1959). Blum (1962) found that motor cortex seizures could be inhibited by antidromic pyramidal stimulation, however, this may have been occlusion rather than inhibition. Moreover, Kandel and Spencer (1961a,d) have shown that the pyramidal cells can be very strongly inhibited without affecting the overall seizure. Thus, it is not certain if removal of this particular pathway would contribute to seizure genesis.

III. STATEMENT OF THE PROBLEM

The possibilities for explaining the basic defect of focal epileptic cortex may be considered in two main categories. The electrical properties of the cortical cells may have changed in such a way that permits excessive cell firing, or else anatomical changes may have disturbed the balance between excitation and inhibition of the cortical neurones. Among the latter, the possibility that chronic partial denervation of the cortex could produce a denervation supersensitivity seemed to be the most probable explanation, and the problem has been approached with this bias.

The preparation chosen, the denervated (isolated) slab of cerebral cortex, could be reasonably expected to develop denervation supersensitivity, and was known to become epileptogenic after a few months of isolation. The cortical slab preparation also offered the advantage that the properties of the epileptic focus could be studied without the influences of the rest of the nervous system, and where all effects seen could be referred to the surgery. The experimental objective was to demonstrate that denervation supersensitivity could be responsible for the increased epileptogenesis. This was approached indirectly by examination of the properties of the isolated slabs other than the sensitivity to possible chemical transmitters, and elimination of other possibilities. Anatomical changes, the electrical sensitivity, and the radial potentials of acute and chronic slabs were examined.

IV. GENERAL METHODS

A. PREPARATION OF CHRONICALLY ISOLATED SLABS OF CEREBRAL CORTEX

Cats predominantly male, weighing between 1.8 and 5 kg, were used. Surgery was done under pentobarbital (35 mg/kg/intraperitoneally) anaesthesia. The operative technique was clean, but not aseptic. Following the induction of anaesthesia the animals were held in a Czermak small animal head-holder (C.P. Palmer Co.) during all procedures.

The scalp was incised in the midline and the left temporal muscle reflected laterally uncovering the skull. The temporal eminence of the skull was trephined and the bone over the suprasylvian gyrus and two millimetres laterally and medially to it was removed by nibbling with mastoid rongeurs. "Bone-wax" (beeswax and phenol) was used to seal the opened sinuses in the skull. The cerebral cortex was exposed by a single incision in the dura mater along the length of the exposed area.

During the removal of the skull, and during all subsequent steps in the procedure, the operative field was kept under constant irrigation with a warm (37° C) saline (0.9%). This facilitated removal of blood from the field, prevented entry of air into the venous sinuses of the skull until they were sealed with bone-wax and kept the exposed cortex moist.

An isolated slab of cerebral cortex was then prepared approximately according to the method of Burns and Grafstein (1952). A small area (approximately 3 x 3 mm) at the posterior end of the suprasylvian gyrus was made bloodless (killed) by electrocautery (Birtcher Hyfrecator). In this region a small hole, 2 mm in diameter, from the surface of the cortex to the lateral ventricle was made by suction. A flat blunt knife (3 x 25 mm, made from a razor blade (Fig. IV - 1a) was inserted

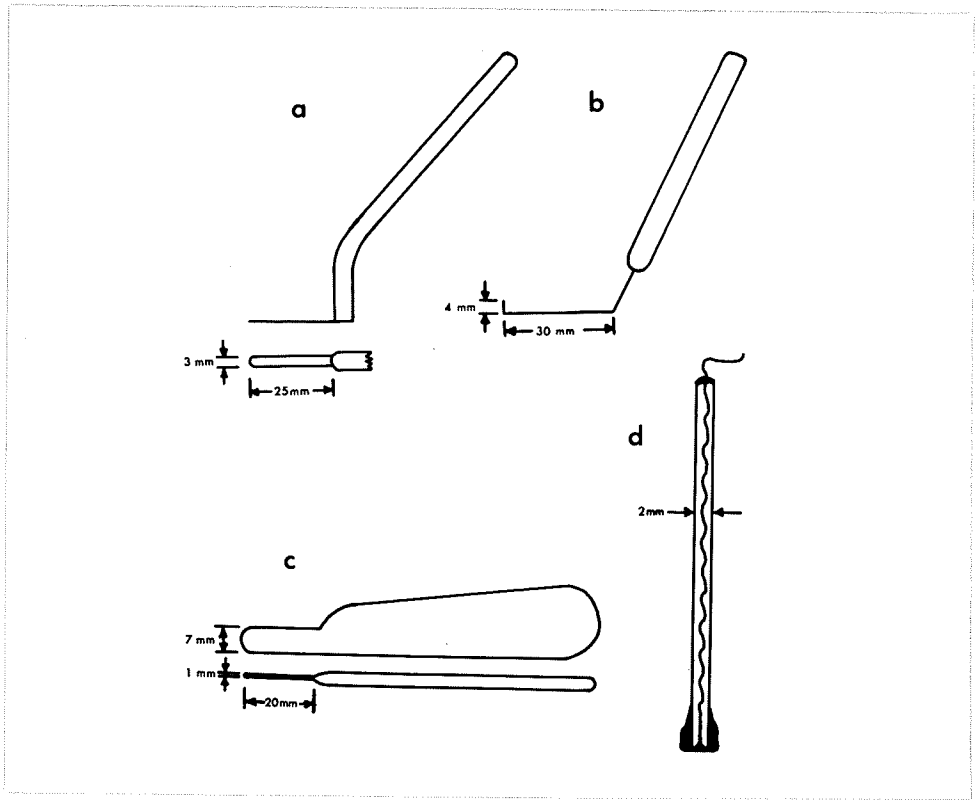

Fig. IV - 1

Instruments

(a) Undercutting knife; blade cut from razor blade.
(b) Wire knife; tip rounded.
(c) Decerebration spatula (plastic).
(d) Construction of monopolar electrodes. Shaft is glass tube. Platinum plate is at bottom of tube. Plastic insulation applied near tip, except on the bottom surface of the platinum.

in the hole and passed under the suprasylvian cortex, parallel to and 4 mm deep from the surface. A wire knife, bent at right angles 4 mm from the end and polished at the tip (Fig. IV - 1b), was then inserted through the hole and passed around the margin of the area to be isolated, with the terminal 4 mm of the knife perpendicular to the cortical surface, and with the tip of the knife just visible through the pia. Care was taken to sever all neuronal connections with the slab without tearing the pia mater or damaging blood vessels in the arachnoid. Thus a piece of cortex approximately 3 x 4 x 12 mm was neuronally isolated from the rest of the brain, yet retained an intact blood supply.

A piece of polyethylene film was placed immediately over the exposed cortex (to prevent adhesions of the dura and muscles to the cortex, the cut edges of the dura sewn together, and the temporal muscle and scalp sutured back into place. Ethilon^{R} 6-0 dermal nylon sutures were used on the dura mater, and 00 dermal nylon for the muscle. Michel wound clips were used on the scalp. In some animals 1 to 2 ml of cerebrospinal fluid was removed from the foremen magnum causing the surface of the brain to fall several millimetres. This facilitated apposition of the edges of the dura mater. The whole procedure took 50-60 minutes.

The animals were kept in a warm environment (28^{o} C) and under close observation during recovery from the anaesthetic and for the subsequent two days before being placed in the animal colony for long term maintenance. Because of the septic procedure, antibiotics were administered about a half hour after the end of surgery, and once a day for the next 3 to 5 days: up to 0.5 gm streptomycin and/or up to 400,000 units of penicillin (Seclomycin^{R}, Glaxo; Crystapen^{R}, Glaxo) intramuscularly in the thigh. The animals were maintained 2 to 18 months before testing,

either in cages in the animal colony, or when kept for longer periods, they were boarded out at a commercially operated kennel. The overt behaviour of the animals appeared the same before and after surgery, except that they became more docile after being handled in the animal colony for several weeks. Some weight was gained by most animals, probably because of their relative lack of exercise.

B. PREPARATION OF THE ANIMALS FOR TESTING

The preparation was done under ether anaesthesia. The anaesthetic was induced with the animals in an "ether box" (closed box, 38 cm cube, containing cotton wool soaked in ether). After removing the cat from the box its trachea was cannulated and ether was administered from a variable-bypass ether bottle which could be adjusted to maintain an even level of anaesthesia as judged by reflexes and the breathing pattern.

A midline incision was made in the scalp, and scalp and temporal muscle were reflected. After being clamped as close as possible to its insertion, the temporal muscle was removed. The skull bone remaining on the left side was removed with rongeurs, exposing a triangular area of the hemisphere posterior to the sigmoid gyrus, as far as the posterior border of the tentorium, and to within 2 mm of the midline (i.e., parietal and temporal bone). Bleeding from the bone was controlled with "bone wax". The dura mater was then removed (with especial care over the slab area, which tended to have adhesions despite previous precautions) and a new hole down to the lateral ventricle was prepared, either in the posterior corner of the ectosylvian gyrus or in the suprasylvian gyrus lateral to the previous hole. This hole was necessary for drainage of cerebrospinal fluid following decerebration.

When the hemisphere was exposed, a mid-collicular decerebration was done and the anaesthetic administration discontinued. A plastic spatula, was used to decerebrate (Fig. IV - 1c). The brainstem was severed with several slow stabbing motions using the anterior margin of the tentorium as a guide for the spatula, care being taken not to drag the spatula across the base of the skull and so sever the vertebral arteries. See Fig. IV - 2a for saggital view, and Fig. IV - 2b for

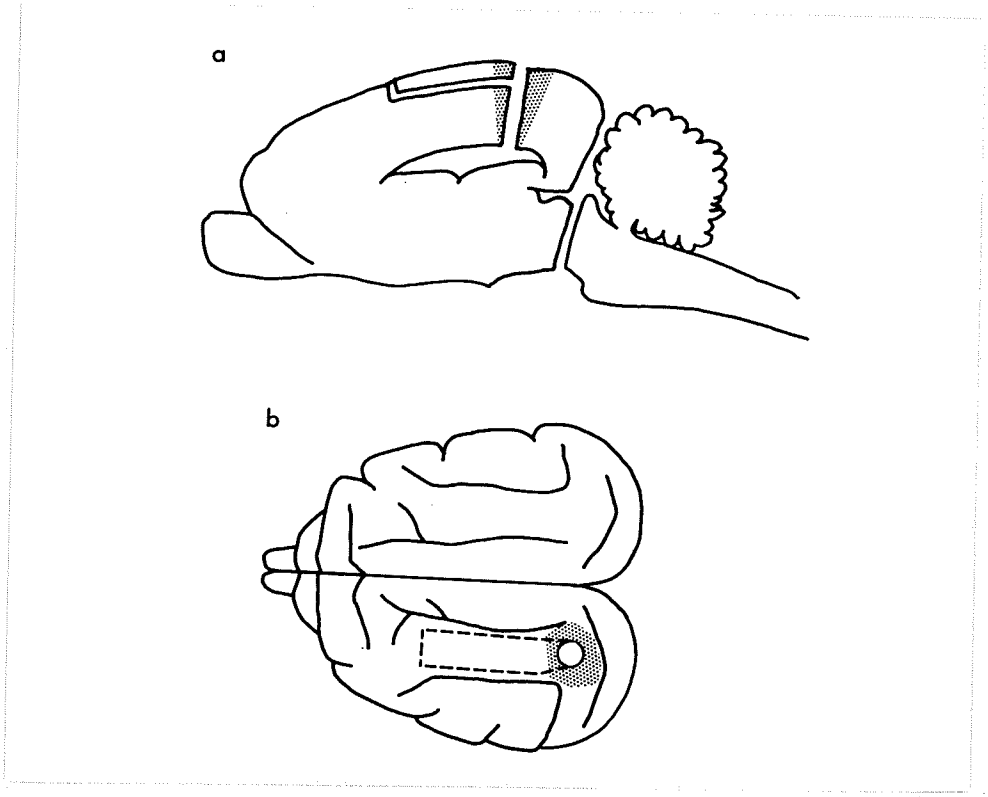

Fig. IV - 2 Diagrams of the preparation

(a) Saggital view of the preparation. The isolated slab and hole to the ventricle are shown. The stippled region indicates the area killed by electrocautery. The decerebration cut is also shown.

(b) Dorsal view of the preparation. The margins of the isolated slab are indicated by the dashed line. The electrocauterised region (stippling) surrounds the drainage hole.

dorsal view of the preparation.

Usually several large blood vessels running from the posterior part of the hemisphere to the tentorium had to be sealed and severed by electrocautery before introduction of the decerebration spatula. The two most common of these blood vessels were a large branch of the superior petrosal sinus running from the centre of the posterior composite gyrus to the dura next to the dorsal surface of the tentorium, and a small branch of the transverse sinus, running from the posterolateral edge of the hemisphere to the posterior edge of the tentorium. A large branch of the transverse sinus, draining the posteromedial surface of the hemisphere and running from the posterior end of the marginal gyrus, was left intact if it would not interfere with the decerebration.

As in the initial preparation of the chronically isolated slab, the operative field was constantly irrigated with warm saline during all the above procedures. The animals were left for three hours following discontinuation of the anaesthetic to allow the ether to be breathed off. During this time a large piece of saline-soaked cotton wool was placed over the exposed cortex. This was found to substantially reduce any cortical swelling which might have occurred during surgery. The scalp edges were tied to a ring so as to form a well around the exposed cortex, and the well was filled with warm liquid petrolatum (U.S.P.). A wad of saline-soaked cotton was placed in the animal's mouth around the tooth-bar of the head-holder to provide a grounding point for the animal.

During the course of the experiment the rectal temperature of the animals was maintained at 36-37° C using a heating pad and a fan,

which were automatically controlled with a thermostat (YSI Thermistemp temperature controller No. 71 with a Thermistor probe No. 402) in many cases.

For control comparisons, in some of the chronically prepared animals the right hemisphere was exposed and a cortical slab acutely prepared in the suprasylvian gyrus in a manner identical to the original preparation of the chronic slab. In all other cases, control experiments were done in animals with only acutely prepared slabs. The method of preparation of these animals was similar to the above, the slab being cut in the left suprasylvian gyrus following exposure of the left hemisphere and decerebration from the left side. Experimental procedure in these animals was matched as closely as possible to their chronically prepared counterparts; identical stimulation was used and testing done in the same sequence.

C. ELECTRICAL RECORDING

(a) Monopolar surface recording

These recording electrodes were silk wicks embedded in a 1% agar-in-0.9%-NaCl gel. Connection to recording instruments was by Ag-AgCl electrodes (coiled to provide greater length of contact with the agar and hence greater stability). One electrode was placed on dead cortex (the electrocauterized area) and is referred to as the "reference" electrode. Another electrode was placed on the cortical slab and is termed the "active" electrode. Potentials so recorded are referred to as being tangential to the cortical surface. In general the electrodes were placed so that the recording electrodes were at right angles to the arrangement of the stimulating electrodes, with the active recording electrode equidistant from both stimulating electrodes, so as to minimize the stimulus artifact (Fig. IV - 3T).

(b) Bipolar depth recording

Recording of extracellular potentials at various depths in the cortex was done with glass micropipettes having a barrel diameter of 40-150 mu and a cylindrical tip with parallel sides of 2-8 mu diameter. These were filled with a 90% saturated solution of NaCl. Such micropipettes had a resistance of 150 K ohms to 1 M ohm. An Ag-AgCl wire provided connection between electrode and recording apparatus. The other recording electrode was a wick electrode as described above. The wick was placed on the cortical surface as close as was possible to the site where the microelectrode punctured the pia mater (Fig. IV - 3R). Potentials so recorded are referred to as radial to the cortical surface. These electrodes were placed directly between the stimulating electrodes so as to be at the focus of activity.

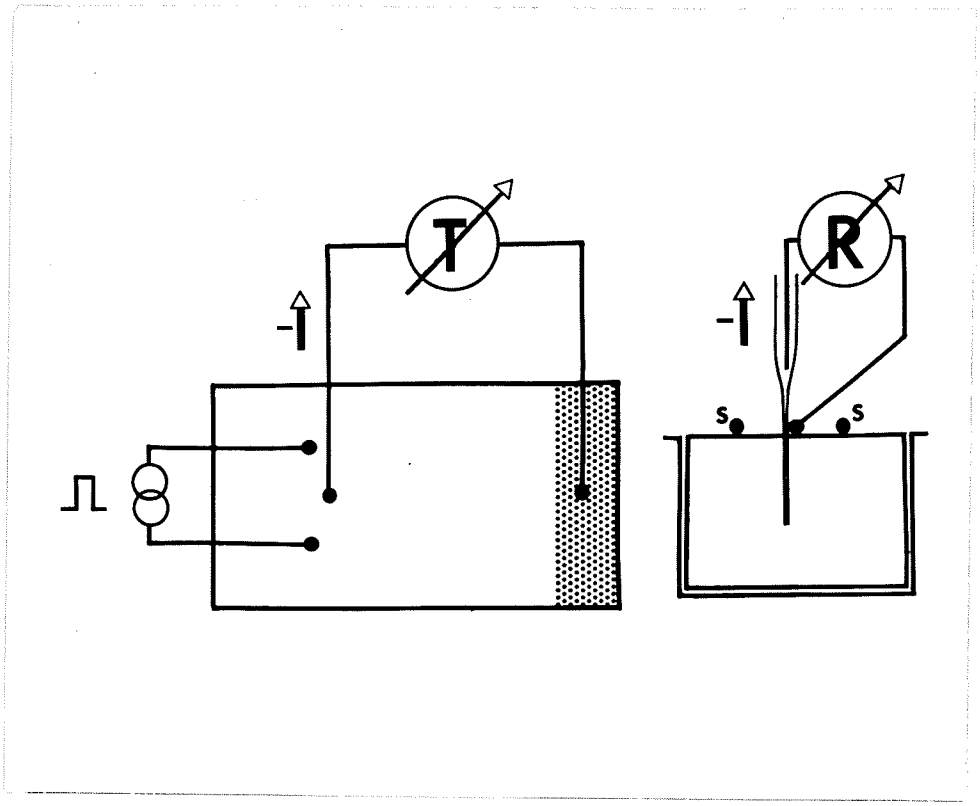

Fig. IV - 3 Stimulating and recording arrangements

Left: Arrangement for recording tangential potentials (T), dorsal view.
Negativity at active recording electrode is indicated by upward deflection in records.
Stimulating electrodes are arranged in a line at right angles to the line of the recording electrodes.
Reference recording electrode is on electrocauterised area (stippled).

Right: Arrangement for recording radial potentials (R), saggital section through slab.
Negativity at the microelectrode tip is indicated by upward deflection in the records.
The stimulating electrodes (S) straddle the recording electrodes.

Gross movement of all electrodes was controlled with mechanical micromanipulators (W.R. Prior Co.) and fine vertical movement of the micropipette was provided for by a hydraulic micromanipulator. The latter was constructed of two syringes, a 1 ml tuberculin syringe whose plunger was advanced by a micrometer screw, and a 5 ml syringe which was mounted on a Prior micromanipulator and connected to the 1 ml syringe by several feet of polyethylene tubing filled with fine oil. The microelectrode holder was mounted on the plunger of the 5 ml syringe. The long length of the tubing between the syringes afforded a degree of "remote" control, yet the time constant of response of the system was still under a second. Movement of the microelectrode could be controlled to $\pm$ 0.00069 mm, however, the minimum increment actually used was 0.069 mm.

(c) Recording systems

Two direct-coupled amplifier systems, both with cathode follower inputs, were used at various times. These were the Princeton Science associates' Model TA-2 transistorized amplifier with an HP-2 cathode follower probe, and the Grass Instrument Company P-6 amplifier with cathode follower probe. Records were taken photographically with moving 35 millimetre film from the face of a 5-inch oscilloscope (Tektronix 502) and activity was directly observed on the screen of another slave oscilloscope. Alternately, permanent records were obtained using an Offner RP Dynograph (Beckman Instruments Inc.) with either curvilinear ink recording or rectilinear heat recording. Film recordings could be measured to $\pm$ 0.02 mV and paper records to $\pm$ 0.01 mV under the conditions and amplification used.

A loudspeaker unit with a gated oscillator was used to provide an audible monitor of the potentials being recorded, and to give an

audible signal when the microelectrode made contact with and pierced the pia.

D. ELECTRICAL STIMULATION

(a) Bipolar stimulation

Electrical stimulation was done using a system of waveform and pulse generators (Tektronix 161 and 162) which allowed production of trains of rectangular impulses of which the pulse strength, pulse width, frequency and total number of pulses could be independently varied.

This stimulus was isolated from ground either by a Hammond #835, 1-to-1 transformer, or a General Radio Co. Type 578-B transformer with a 4-to-1 turns ratio. In the former case, the loading provided by the cortex (approximately 5 K ohms with the bipolar stimulating electrodes) reduced the voltage actually applied across the cortex to approximately 2/5 the dial reading. Furthermore, under experimental conditions the relationship between dial voltage and delivered voltage was not linear. In part, this probably arose because the stimulating effect of the higher stimulus voltages increases the conductance of the cortex, thus reducing the load resistance across the transformer and the proportion of the total voltage appearing across the load. Except when using monopolar stimulation described below, this departure from linearity has been neglected in order to arrive at an approximate comparison between the effects of stimulus voltage in the different experiments. Voltages are quoted in terms of those expected at the cortex for a given dial reading and coupling transformer (i.e., 1/4 dial voltage in the case of the G.R. Co. transformer, and 2/5-dial voltage in the case of the Hammond transformer).

The stimulus was applied to the cortex via bipolar platinum or platinum-iridium electrodes with beaded tips, the tips being approximately

2 mm apart on the cortex.

(b) Monopolar stimulation

Monopolar stimulation was done with a single electrode placed on the surface of the cortex and an indifferent electrode in the mouth of the animal. The pulse voltage across the cortex was measured and the current delivered was calculated from the voltage drop measured across a 10 ohm resistor in series with the indifferent electrode. The transformer output voltage, and IR drop across the 10 ohm resistor for the first pulse of each train of stimulating pulses was recorded photographically from the face of an oscilloscope and measurements made from the film (Fig. IV - 4).

The stimulating electrodes were constructed from small plates of platinum and varied in area from 0.17 mm^2 to 16.75 mm^2. The electrodes (Fig. IV - 1d) were mounted at the end of glass tubes, and all surfaces of the platinum except the one to contact the cortex were coated with an insulating layer of plastic, in order to reduce spread of the current through leucocytes and other conducting material on the surface of the cortex. The contact surfaces of all except the largest were flat and circular. The largest was rectangular so that it did not overlap onto intact cortex on either side of the slab.

Stimulus pulses were provided from the Tektronix system and Hammond transformer as outlined above.

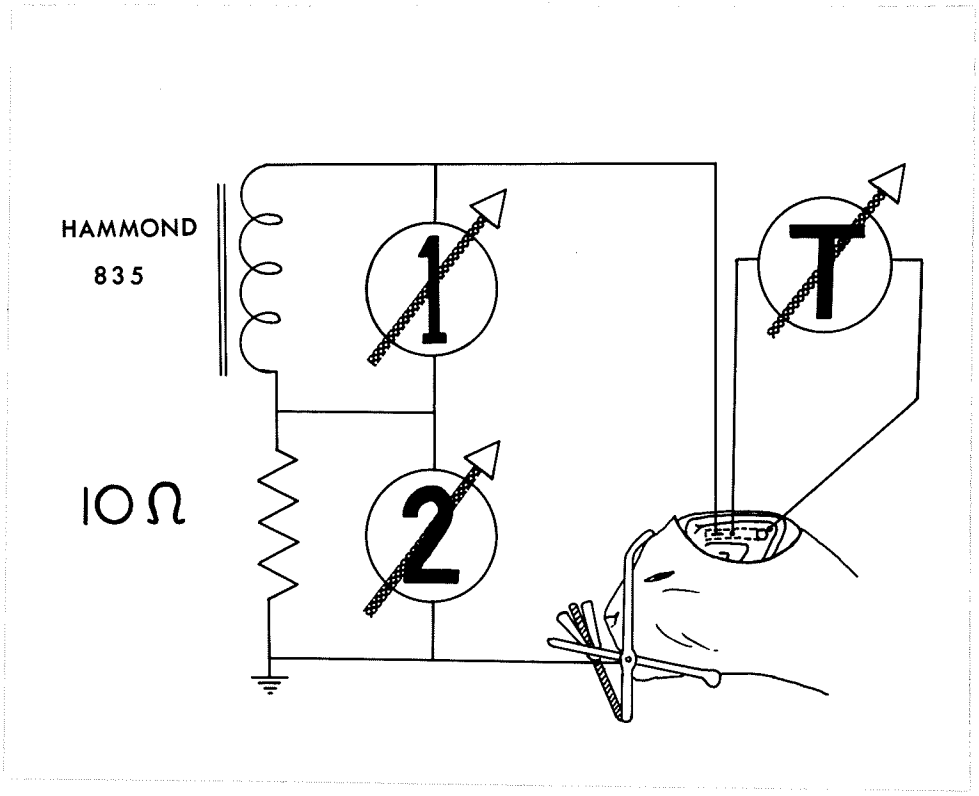

Fig. IV - 4 Arrangement for recording the effect of variations in stimulated area.

See text for details of use.

E. TOPICAL APPLICATION OF DRUGS

Local, topical application of drugs to the cerebral cortex were done in the following manner. A solution containing the drug was added dropwise from syringe and 27-gauge hypodermic needle to a small (1 x 5 mm) piece of filter paper which had been previously moistened in saline and placed on the cortex. The filter paper was positioned either between the stimulating electrodes, underneath the active surface recording electrode, or at a distance from both but still on the isolated area.

F. EXPERIMENTAL PROCEDURE AND ANALYSIS

At least 5 minutes was allowed after a stimulus which produced an epileptiform afterdischarge and the next test stimulus; one to two minutes following subthreshold stimuli.

Occasionally strong stimulation produced spreading depression (Leao, 1944; Grafstein, 1956) which renders the cortex temporarily inexcitable. This phenomenon is characterized by a large surface negative swing, lasting about 30 seconds, followed by a prolonged lower voltage surface positivity. 20 to 30 minutes was allowed after the occurrence of spreading depression.

Because of the strong suggestion that the ease with which spreading depression is elicited is directly correlated with recent asphyxial damage to the cortex (Van Harreveld and Stamm 1953, 1954), slabs which showed a lower threshold to spreading depression than to epileptiform afterdischarge were arbitrarily rejected. This was never found necessary in chronic slabs, but about one quarter of acute slabs prepared were so rejected.

Duration of afterdischarges were measured from the last stimulus pulse to the end of the last paroxysmal burst. Thresholds of various stimulus parameters are quoted as a range between the highest subthreshold stimulus and the lowest suprathreshold stimulus tested. Occasionally, with stimuli near the threshold, a short burst of spikes (about one second) was seen. As this was seen only when the recording electrodes were within a millimetre of the stimulated focus, these bursts appeared not to be propagated, and thus were not considered as epileptiform afterdischarges.

In most cases the frequencies or numbers of pulses used were

those directly available from calibrated positions on the Tektronix waveform generators (i.e., frequencies of 2, 2.5, 3.3, 4, 5, 7.7, 10 etc. pulses/sec and 8, 10, 13, 16, 20, 25, 32, 40, 50, 63 etc. pulses). While this did not allow very accurate determinations of threshold at higher values, it was decided to take this risk of a type two error; furthermore preliminary results suggested that most pulse thresholds would be between 16 and 40, where the range between tested values was not great; also it was expected that the threshold might vary 10-15% during the course of the experiment. Sometimes thresholds did change upwards during the course of the experiment, however testing was continued until the preparation was unresponsive, or for 24 hours. The lowest threshold obtained is quoted in the results. Such increases in threshold were believed to be signs of deterioration of the preparation. Decreases in duration of the afterdischarges after several tests have been reported by Sharpless and Halpern (1962) who attributed it to a desensitization of supersensitive neurones by the induced activity.

In experiments where threshold values of the "negative afterdeflection" (subthreshold radial cortical potential gradient; Pinsky, 1963) were measured, finer variation of the number of pulses used was obtained using a scaler (CMC Company, Model 314A) to count pulses when settings of the Tektronix instruments were off the calibrated positions. Generally thresholds were obtained to $\pm$ 2 pulses or better under these conditions.

V. HISTOLOGICAL EXAMINATION OF THE CHRONICALLY ISOLATED CORTEX.

A. INTRODUCTION

The anatomical effects of undercutting the cerebral cortex was first extensively studied by Cajal (1929). He observed extreme degeneration of neurones above the cut within 14 days. In addition to those neurones which disintegrated, many others developed chromatolysis (eccentric nuclei, swollen axons, and loss of Nissl substance). There was an apparent shift in cell types from a predominance of Golgi Type I (pyramidal cells with axons descending into white matter) to Golgi Type II cells ("arciform" cells with recurrent axon and axon collaterals which ramify entirely within the cortex).

Cajal offered several explanations for the alteration in cell types. He noticed that cells whose axons were severed proximal to their axon collaterals appeared to eventually die, whereas in cases where the axon was cut distal to collaterals, the axon disintegrated retrogradely only as far as the level of the collaterals. Such cells eventually recovered (after $1\frac{1}{2}$ - 3 months there were no chromatolytic neurones) and their axons appeared to have started to regrow, often ending in free axonic balls. Cajal suggested that the prominence of recurrent axon collaterals resulted from collateral sprouting during the initial period of regrowth of the axon. Collateral sprouting is well demonstrated in the peripheral nervous system (Edds, 1953), and has been suggested to occur in the spinal cord (McCouch, Austin, Liu and Liu, 1958). Cajal also pointed out it was not necessary to invoke collateral sprouting as an explanation. Simple degeneration of cells whose axons did not have collaterals to start with, or whose axons were severed proximal to their collaterals, would leave only those cells that had collaterals originally.

He did see small collaterals on wound-damaged axons, but he was unsure if they represented new or degenerating collaterals.

In the immediate region of the cut, there was extensive degeneration and lysis of all elements, nervous and glial, within 24 hours. The glial scar along the cut was fully developed in 30 days.

Sastry (1956) studied chronically completely isolated cerebral cortex. He found that the number of cells in the slabs decreased in the first 2 weeks after isolation. However there was an increase in the cell density after 8 weeks of denervation. He did not specify the types of cells involved. The most striking effect seen was the loss of the large pyramidal cells normally seen in layer V (Grafstein, personal communication). Echlin (1959) also noted that there was a dropout of the large pyramidal cells. The only other effect of chronic isolation on the cortex which he reported was a persistence of chromatolysis. Cajal (1929) had also noted that the large cells were the most affected by undercutting: "Giant or Betz cells ..... alone disappear; the association neurones, whose axon has undergone no mutilations, or has suffered them a great distance from the soma are largely intact".

Electron microscope studies of cortical slabs isolated for 1 to 15 days (Colonnier and Gray, 1962) have shown that a rapid and widespread destruction of presynaptic endings occurs. Phagocytosis of <u>both</u> pre- and post-synaptic elements of cortical synapses occurs within 5 days.

Purpura and Housepian (1961) and Purpura (1961) also observed axon-collateral sprouting in chronically isolated, immature, developing cat cortex. They believe that the increase in excitatory synapses resulting from this prolific collateral sprouting is the cause of increased epileptogenesis in the chronically isolated immature neocortex.

Referring to Cajal's (1929) observations, they postulated that collateral sprouting was also the cause of the increased epileptogenesis in chronically isolated mature cortex.

Ward (1961) observed that the dendrites of many neurones in aluminium hydroxide (epileptic) cortical lesions appeared bent and twisted. The lesions also were a region of relative surface negativity, and the neurones in the lesions had a high resting frequency of discharge. He suggested that the distortion of neurones by glia would cause chronic depolarization of these dendrites, the cells then firing continuously and rapidly in the manner of stimulated peripheral stretch receptors.

B. METHODS

At the termination of most experiments, the chronic slabs and the contralateral homotopic cortex (which in some cases had an acute slab cut in it) were removed for histological study. Removal was effected by section through adjacent gyri, and wide undercutting. The samples were washed free of blood, and cut into 4 mm thick cross-sections.

One or two sections of each sample were fixed and stained with mercury according to a Golgi-Cox technique, as modified by Sholl (1953). This technique completely stains the cells body and dendrites, however only about 1½% of all neurones are stained, and even fewer of their axons become stained (Sholl, 1956). 100 mu thick sections were cut to allow cellular organization and dendritic ramifications to be seen, as well as to determine cell packing density.

The remaining cross-sections of the samples were fixed with formalin, and cut into 6 mu sections. Nissl stains (cresyl violet) were done to allow cells counts of both neural and glial elements.

Two cell-counting techniques were used. The mercury (Golgi-Cox) stained samples were counted under low power, the microscope eyepiece having a grid graticule which outlined a 1 mm square area on the sample. The square was aligned so that one edge was at the deepest margin of layer I, parallel to the pia, and in the middle of the slab, so that layers II to V of acute slabs were included in the square, and a count of all cells in this square was made. The focusing of the microscope was continuously adjusted up and down so that cells at all depths in the 100 mu thick sample were included in the count. To be considered a neurone, stained objects had to have at least one clear dendritic process.

Because a much greater number of cells are stained with the

cresyl violet technique, and differentiation of cells impossible under low power, sampling of the cell population could not be accurately done over such a large area as above. Therefore the following procedure was adopted. Micrographs of a small area of cortex were made and all cell counts were made from prints, enlarged to a final magnification of 173X. The area to be counted was selected by centering the middle of the low-power (10X objective) field of the microscope in the center of the slab cortex. The objective was then changed to a higher power so that an area of about 0.58 x 0.46 mm was photographed. This area included layers IV, V, and part of layer III in the acute slabs. Negatives were made on 35 mm "Kodak Fine Grain Positive"; a green filter was used over the source light to increase contrast. A 4-by-4 grid inscribed on polyethylene was laid over the final positives to aid in counting. The grid rectangles outlined roughly the field of the 40X microscope objective. The cells were then counted from the prints, with a cell-by-cell comparison to the original slide, using the 40X objective, to identify the cell type. Criteria for differentiation of neurones, oligodendroglia, astroglia and microglia in Nissl preparations were as described by Glees (1955) and Maximow and Bloom (1940).

Width and depth of chronic and acute slabs, the depth of the cortex, and the width of the supresylvian gyri were measured to give a gross indication of the extent of degeneration which had occurred. Of course where the control sample was intact suprasylvian cortex, only the width of the gyrus and the depth of the cortex could be compared. Measurements were made to the nearest 0.1 mm using a 7X jewelers loupe with graticule, except for measurements of depth of the cortex in the Golgi-Cox stains, where a microscope and vernier micrometer stage was

used. The points where the measurements were made are shown in Figure V-1 by the fine double-headed arrows superimposed on the drawings.

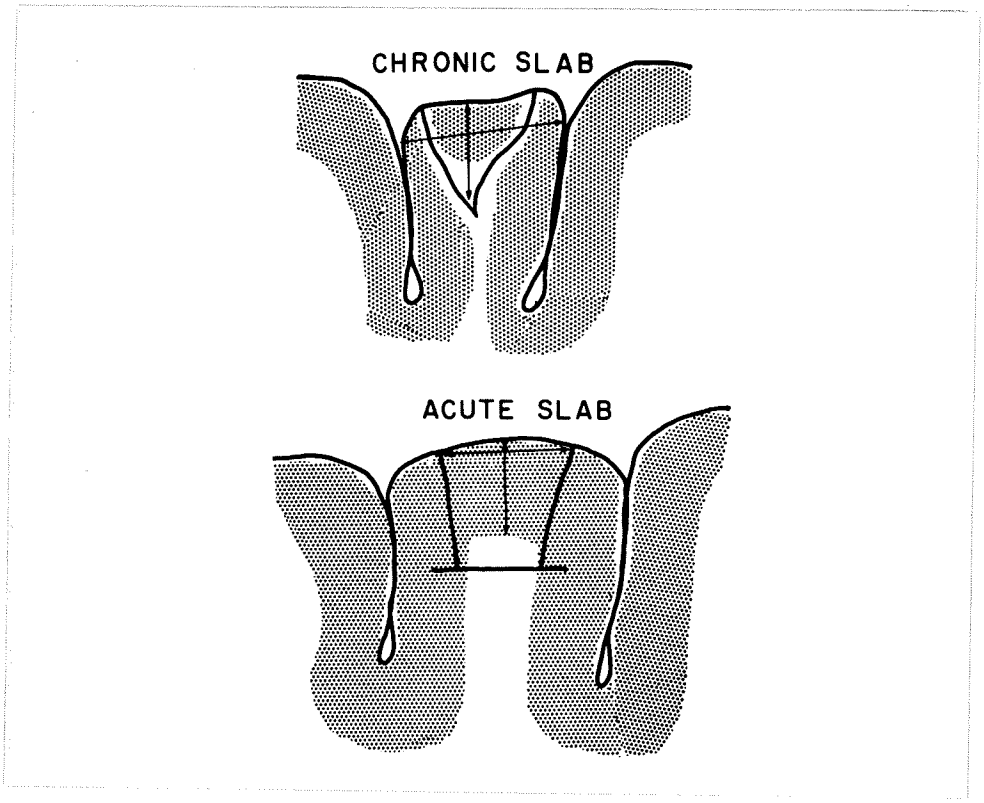

Fig. V - 1 "Camera lucida" drawings of slabs

Cross-sectional drawings of gyri containing chronic and acute slabs.
The small, double-headed arrows indicate the measurements made of various dimensions (see text): "gyrus width" and "slab depth" are indicated in the chronic slab drawing, "slab width" and "cortex depth" in the acute slab.

C. RESULTS

(a) Gross appearance

Blood supply to the chronic slabs appeared normal. The original pial blood vessels remained; the slabs appeared adequately vascularized and were the same colour as the surrounding cortex. There was no sign of anoxia. A large number of capillaries were seen during histological examination of the cortex of both acute and chronic slabs. Both the slabs and surrounding cortex became white after death.

The surface of the gyri with chronic slabs appeared flat or concave instead of the normal convex appearance. The slabs and gyri appeared considerably narrower than they were after the slabs were first cut. The slab outline was not always clearly visible and so quite often the depression in the gyrus was used as a guide for locating the slab to place the electrodes during the course of the experiment. Occasionally there was a clear separation between the slab and surrounding cortex which was evidently filled with cerebro-spinal fluid. When removed at post-mortem, these slabs appeared to be attached to the surrounding cortex only by the pia. The pia of the chronic slabs was tougher and thicker than normal.

(b) Dimensions of the slabs

The impression of slab and gyrus shrinkage obtained from the gross appearance of the slabs was confirmed by measurement of the fixed preparations. Examination of both Nissl and Golgi-Cox preparations showed that all dimensions measured significantly less in the chronic slab preparations than in the acute slab or intact cortex preparations (Table V-1). Measured values were greater in the Golgi-Cox preparations; apparently there was less shrinkage for this method than in the formalin

Table V-1

DIMENSIONS OF CORTICAL SLABS

Sample Number	Weeks Isolation	Slab Width A	Slab Width C	Slab Depth A	Slab Depth C	Gyrus Width A	Gyrus Width C	Cortex Depth A	Cortex Depth C
				A. Nissl Stain					
1	8	4.5	2.6	3.0	2.7	6.4	5.2	1.4	0.9
3	9	3.2	2.7	4.8	2.5	6.0	5.0	1.4	1.0
5	11	2.7	2.3	3.3	2.2	4.5	4.8	1.4	1.0
8	13	3.5	2.0	3.1	1.0	6.5	3.0	1.5	1.0
9	13	-	2.5	-	2.5	5.3	4.0	1.7	1.2
10	15	3.0	2.1	4.0	3.2	6.0	3.8	1.8	1.5
11	17	3.0	1.0	3.2	1.2	5.2	3.0	1.6	0.9
12	20	-	2.7	-	1.0	5.9	4.5	1.9	0.7
13	20	-	1.8	-	1.4	5.0	3.5	1.6	1.0
15	26	-	1.3	-	1.4	4.1	3.2	1.1	0.8
17	46	3.6	2.5	3.4	3.0	5.8	4.5	1.5	1.5
18	60	2.5	1.7	3.0	1.4	4.8	3.2	1.7	1.1
Averages:		3.25	2.10	3.47	1.96	5.46	3.95	1.55	1.05
C as % of A:			64.6		56.4		69.5		67.7
"p" less than:		0.01		0.01		0.001		0.001	
				B. Golgi-Cox Stain					
1	8	3.6	2.8	3.9	2.9	6.6	5.4	1.6	1.2
2	8	-	2.4	-	2.6	5.9	4.9	2.1	1.4
6	12	3.3	2.1	3.5	2.1	5.2	4.4	1.6	1.3
7	12	3.9	3.0	5.4	2.9	7.0	4.9	1.8	1.5
8	13	3.8	3.2	3.6	2.7	8.6	4.6	1.7	1.7
9	13	-	2.7	-	3.4	5.7	5.2	1.8	1.4
11	17	4.2	2.4	4.0	3.1	6.5	4.5	2.0	1.8
14	23	-	1.5	-	2.0	5.0	4.6	1.7	1.4
17	46	4.0	3.1	4.5	2.8	7.0	4.8	1.8	1.6
18	60	3.3	2.1	3.5	2.2	6.0	4.3	1.6	1.2
19	71	2.4	2.3	3.7	2.9	6.0	4.4	1.5	1.5
20	77	3.2	2.5	4.2	2.1	7.0	4.7	1.8	1.2
Averages:		3.52	2.51	4.03	2.64	6.38	4.72	1.75	1.43
C as % of A:			71.1		65.2		73.9		81.7
"p" less than:		0.001		0.001		0.001		0.001	

A = acute slab or intact cortex contralateral to chronic slab; absence of data in acute colums indicates no slab.
C = chronic slab
Probability (p) calculated by Student's test for paired data; ie. chronic slab data was not used where there was no acute slab data.
"Sample Number" s provide cross reference to "Expt. Nbr." s in Table V-2. They are numbered in sequence according to the duration of isolation of the chronic slabs.

fixation. Moreover, the chronic slabs appear to have shrunk more than the acute slabs: in the Golgi-Cox preparations, the cortex of chronic slabs was 81.7% of the thickness of acute slabs, but 67.7% in the Nissl preparations. Although only half of the samples were common to both staining groups, this sample difference was not the cause of the difference in values. The extreme difference in depths of the cortex may have been in part due to differences in the method of determining where the bottom limit of the chronic slabs was, but the increased shrinkage of the chronic slabs in formalin was also evident in other measurements.

The chronic slabs became roughly triangular in cross-section (Fig. V-1). This was mainly due to a massive loss of white matter, both above and below the undercutting lesion. Probable very little axonal material was left in these regions, the area being almost totally given over to glial cells. The white matter left under the lesion appeared to be that of the gyrus remaining outside the slab. It is also likely that some of the axons of cells outside the slab were also damaged by the undercutting.

(c) Density of cell population

The estimates of cell population density showed that there was no difference in the neurone density between chronic and acute slabs (Table V-2). The Nissl stain showed an easily visible increase in cell density in many animals (Plates I and II); however this increase was solely an increase in the number of glial cells (primarily astrocytes and microglia). The results were subjected to analysis of significance by the "Student's t-test", however there may have been a bias in the categorizing of cells in the chronic slabs, particularly in differentiating between neurones and astrocytes, in the Nissl stains. Although in gross

Table V-2

CELL POPULATION DENSITY

A. Nissl Stain

Exper. No.	Weeks Isolation	Neurones A	Neurones C	Astroglia A	Astroglia C	Oligodendroglia A	Oligodendroglia C	Microglia A	Microglia C
1	8	156	163	56	78	107	135	86	172
3	9	182	129	62	85	93	94	132	136
5	11	131	110	65	82	127	137	84	200
8	13	149	129	47	37	109	60	148	61
9*	13	142	152	47	84	67	96	72	114
10	15	95	85	30	31	52	70	45	97
11	17	161	142	48	40	78	80	98	168
12*	20	122	234	39	120	75	102	29	65
13*	20	179	175	33	78	69	109	38	48
15*	26	224	307	66	156	102	124	136	144
17	46	137	138	43	73	127	127	81	83
18	60	156	129	42	89	104	133	54	111
Averages:		153	158	48	79	93	106	84	117
"t" test:		$p > 0.05$		$p < 0.05$		$p > 0.05$		$p < 0.05$	

B. Golgi-Cox Stain

Exper. No.	Weeks Isolation	Large Neurones A	Large Neurones C	Small Neurones A	Small Neurones C	Astroglia A	Astroglia C
1	8	24	9	350	226	6	22
2*	8	11	1	300	214	0	17
4+	10	13	3	126	159	1	22
6	12	26	11	258	215	8	26
7	12	11	5	256	288	1	4
8	13	17	6	375	395	7	4
9*	13	25	4	381	442	2	8
11	17	8	2	235	201	2	1
14*	23	15	9	312	343	6	9
16+	29	10	8	266	245	3	14
17	46	12	7	461	469	13	44
18	60	15	3	391	367	5	15
19	71	19	3	347	349	5	16
20	77	14	4	267	344	2	28
Averages:		15.7	5.4	309	304	4.4	16.4
"t" test:		$p < 0.001$		$p > 0.05$		$p < 0.001$	

Exper. No.'s" are a cross reference to sample numbers in Table V-1. They are numbered in sequence according to duration ofisolation of the chronic slabs.

* = A- counts are done on intact cortex from contralateral hemisphere.

+ = Acute slabs not removed; acute counts done on intact cortex beside chronic slab.

All counts in Part B are the sum of counts on two slides (see text).

appearance, the Nissl-stained chronic slabs were often darker than the acute, the cytoplasm of neurones in the chronic slabs was reduced in volume and not stained as darkly (Plate III C & D). Since criteria for identification of nuclei as belonging to neurones were dark cytoplasm (Nissl substance) and clearly stained dendritic processes, it was often extremely difficult to classify medium-sized nuclei with only a very small bit of lightly stained cytoplasm as astrocyte or neurone nuclei. Choice was then made on the assumption that the neurone nuclei would stain darker, contain only one nucleolar body, and be more nearly oval in outline. Even these criteria did not satisfactorily differentiate many nuclei. Size of the nuclei was of no aid in differentiation since small neurone nuclei are about the same size as astrocyte nuclei. In addition, astrocyte nuclei are known to swell under prolonged unfavourable conditions (such as chronic hypoxia) and may even become binucleate (Greenfield, Blackwood, McMenemey, Mayer and Norman, 1958). Moreover it was evident from the Golgi-Cox preparations that there was an astrocytosis in the chronic slabs. These may have caused a bias to assign doubtful cells to the astroglial category.

Unfortunately the Golgi-Cox technique appeared to give very variable results. A number of preparations proved impossible to analyse, either because they showed a complete absence of any stained structures, or because the background was opaque. These occurred in both acute and chronic slabs; often serial sections of a sample would show such variability, with a 200-300% variability in the number of neurones which could be seen. For this reason at least 5 sections of each of the Golgi-Cox stains were counted for each sample, and the two greatest counts summed and entered in the table (Table V-2B). There is no way of knowing

if a similar proportion of all neurones stain in acute and chronic slabs, however there was good agreement between staining techniques in that there was no difference in the neurone counts of chronic and acute slabs for both techniques.

An easily visible effect of chronic isolation was the almost complete loss of large pyramidal cells (15 mu or greater in their smallest diameter) (Plate IIIA) which are normally found in layer V (Plate IIA & B). There were only one third as many large neurones in the chronic slabs as in the acute slabs (Table V-2B), and the ones which were left in the chronic slabs were almost entirely in the more superficial layers (III and IV). A similar lack of large cells was seen in Nissl stains (e.g. Plate X-IA & B) but no differential counts were made.

In the cortex of most chronic slabs there were many large irregularly shaped masses, 10-40 mu in diameter. They were found in all layers, but predominantly in the deeper layers (Plate IIB). These appeared to be fibrous astrocytes. Very few astrocytes are normally seen in the cortex with Golgi's stain. With this technique there were 4 times as many astrocytes within the area counted in chronic slabs compared with acute (Table V-2B). There was only a 2-fold increase in astrocytes in the Nissl counts, however these counts did not include as much of the deeper layers (where most of the astrocytes were located) as did the counts of the Golgi-Cox preparations because of the difference in counting technique used.

(d) Cortical organization in chronic slabs

(i) Cortical layering. Because of the absence of a major landmark, the large pyramidal cells of layer V, from the chronic slabs, it was difficult to estimate the locations of the various layers in the

chronic slabs. A consequence of the difficulty in identification of cell layers was the difficulty in determining from which layers the dropout of cells responsible for the loss of cortical depth occurred. In the normal suprasylvian gyrus, layer VI appears to make up 25-30% of the cortex. Although the loss of cortical depth was quite close to this value, it was apparent that not all the loss of cells occurred from this layer. Layer VI could be identified in chronic slabs from the characteristic pattern of cellular organization in the suprasylvian gyrus: axons and apical dendrites are not all arranged radially as in the more superficial layers, but rather give a network appearance, with axons and dendrites projecting in all directions (e.g. the lower 2 cm of Plate IIB). It was evident however that the layer VI had decreased in depth (between 1/3 and 2/3 in most preparations). Layer V, as already indicated, lost most large pyramidal cells. The remainder of the cortical shrinkage probably occurred because of slight cell losses in the other layers. Layer II appeared to be virtually intact in most chronic preparations, although some cells must have been lost since the chronic slabs shrunk in width as well as in depth.

The astrocytosis appeared to be heaviest in the deepest layers (Plate IIB). Most of these fibrous astrocytes were located in layer VI, and some in layer IV or V, and occasionally one or two showed up in the superficial layers. As has been indicated the areas of the acute slabs counted did not include layer VI (e.g. Plate IIA) however the acute slabs had only the occasional astrocyte show up in this layer (Golgi-Cox preparations).

(ii) Dendritic organization and survival. Although there was no difference in the density of neurones present in the acute

and chronic slabs, dendritic survival did not appear to be as great in the chronic slabs as in the acute. The chronic slabs were generally more disorganized in appearance: the dendrites were not as numerous, partly destroyed, or degenerated, and there was considerably more unclassifiable debris.

Apical dendrites projecting into Layer I were sparser in chronic slabs than in acute slabs or normal cortex in 11 of 13 animals examined. Dendrites in the other layers also appeared to be fewer in number in the chronic slabs, and to have sustained greater degeneration than in acute slabs, in 12 of the 13 animals examined. These estimates were highly subjective and in many preparations the differences were not great, and were doubtless influenced by the presence of debris in most of the chronic slab preparations. In the cases where survival of dendrites was rated equal, the general condition of the slabs was almost as good as normal cortex.

Some neurones were seen that had grossly distorted apical dendrites as described by Ward (1961), however these were seen in normal cortex as well as in the chronically isolated slabs. Furthermore such cells were a very small minority.

(iii) Axon survival. Only a few axons in any preparation could be followed more than 5 mu from the cell soma, since the Golgi-Cox method stains axons poorly. Even so, among the few which could be followed were examples of all the types classified by Sholl (1956), and these could all be found in both acute and chronic slabs (Fig. V - 2). The chances of finding P_4 neurones (pyramidal cells with only recurrent axons) with multiple axon collaterals seemed to be the same in the acute as in the chronic slabs.

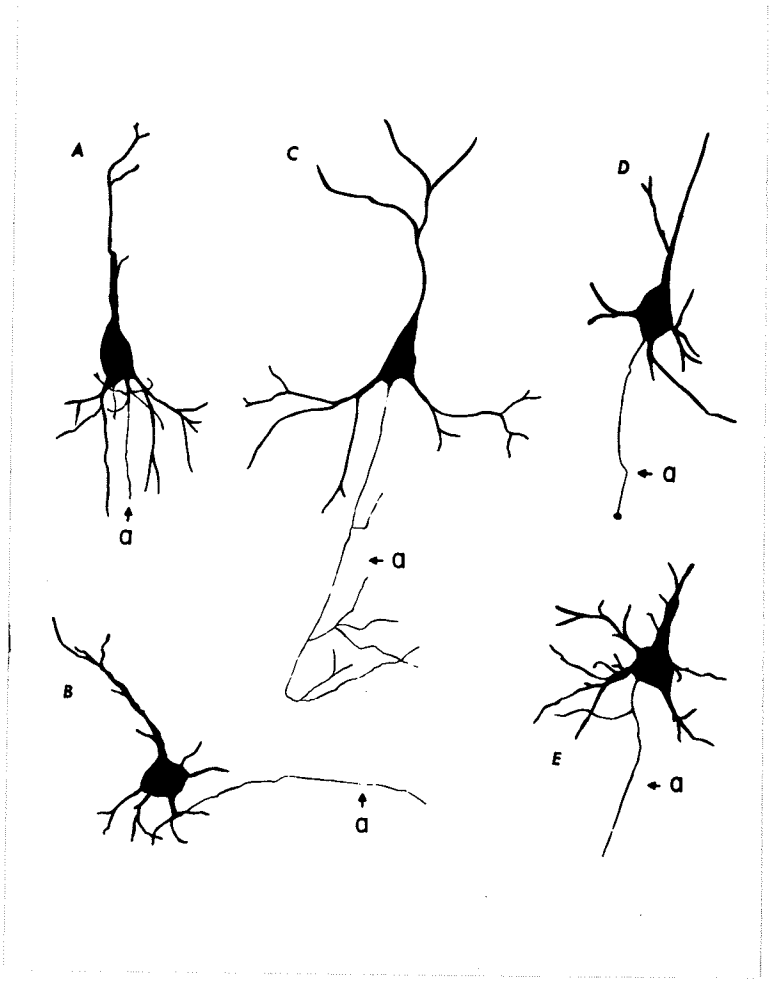

Fig. V - 2 Drawings of typical neurones

Typical cells as seen in Golgi-Cox preparations normal and chronically isolated cerebral cortex.
Neurone classification is that of Sholl (1956).
(A) P_3 neurone from intact visual cortex, gyrus adjacent to a chronic slab (no. 20 in tables V-1 and V-2). Pia is upward in all drawings.
(B) P_4 neurone from chronic slab (No. 16) layer VI.
(C) P_4 neurone from acute slab (No. 20) layer II.
(D) P_1 neurone from chronic slab (No. 20) layer IV. Note terminal free axonic ball.
(E) P_2 neurone from chronic slab (No. 19) layer V or VI.

Examples of some of the cells found are shown in the drawings of Fig. V - 2: "A" is a type P_3 neurone (branched axon to the white matter and recurrent collaterals). This example was seen in the intact marginal gyrus, adjacent to a suprasylvian gyrus with a chronic slab. Many more of these cells were noticed in the visual cortex than in the suprasylvian gyrus. "B" does not fit into the Sholl classification, but appears closest to the P_4 category of a "pyramidal cell with axon forming recurrent collaterals and branches only". This was found in a chronic slab. "C" is a P_4 neurone which was found in layer II of an acute slab. "D" is a P_1 neurone (pyramidal cell with unbranched axon to white matter) found in a chronic slab. Unsuccessful regrowth has occurred: the axon ends in a free axonic ball. Many of these axons, which showed no evidence of axon collaterals over the distance which the axon could be followed, were noticed. Similarly "E" is a typical P_2 neurone (branched axon to white matter) which in this case sent out one horizontal collateral during the distance it could be followed. The axon appeared to go out of the plane of the section. These latter two cell types appeared to be the most frequently encountered types in both chronic and acutely isolated slabs.

(e) Completeness of isolation of the slabs.

No neural connections between the slab and the cortex adjacent to the slab were ever seen in the cross-sections of chronic slabs. Three acute slabs were found to have layer I intact for a short distance on one side of the slab. All the samples were taken from the middle of the slabs however, so the ends of the slabs were never examined.

In the chronic slabs there was usually a heavy glial scar along the sides. In the Golgi-Cox preparations a region of 50-100 mu on either

side of the isolation lesion appeared devoid of all cellular elements. In the Nissl stains this region proved to be populated almost exclusively by microglial cells. Plate IV shows an example of such a microglial scar, near the pia; the scar appears to be continuous with the pia.

No such accumulation of microglial cells is seen in the acute slabs. In these the isolation cut was often filled with erythrocytes, and the Golgi-Cox preparations showed that the neurones in the immediate vicinity had been severely damaged. There was generally no cellular destruction beyond 30 mu on either side of the cut.

Plate Legends

Plate I. (page 72)

(A) Upper left: acute cortical slab #17, cresyl violet 173X. Slab numbers refer to numbers in Tables V-1 and V-2. Pia is upward in all photographs.
(B) Upper right: chronic slab, as for A. Note absence of large pyramidal neurones.
(C) Lower left: acute slab as in A, 865X.
(D) Lower right: chronic slab as in B, 865X.

Plate II. (page 73)

(A) Upper left: acute cortical slab #1. A cluster of large pyramidal cells is visible in the bottom right. Golgi-Cox, 865X.
(B) Upper right: chronic cortical slab, as in A. Note that 9 fibrous astrocytes are visible, but no large pyramidal neurones.
(C) Lower left: acute cortical slab #1, cresyl violet, 173X.
(D) Lower right: chronic slab, as in C.

Plate III. (page 74)

(A) Upper left: acute cortical slab #18, single large pyramidal neurone from layer V. Golgi-Cox, 865X.
(B) Upper right: chronic slab #18, largest cell in layer equivalent to layer V in the acute slab cortex. Golgi-Cox, 865X. Axons are visible at the bottom of cells in both A and B, but are not stained for more than a few microns.
(C) Lower left: acute slab, #18, several neurones visible. Cresyl violet, 865X.
(D) Lower right: chronic slab, #18, neurones lack dark staining Nissl substance. Cresyl violet, 865X.

Plate IV. (page 75)

Chronic cortical slab #1. Glial scar of chronic isolation cut at its junction with the pia. Nuclei of the scar cells are mainly of microglia. Cresyl violet, 173X.

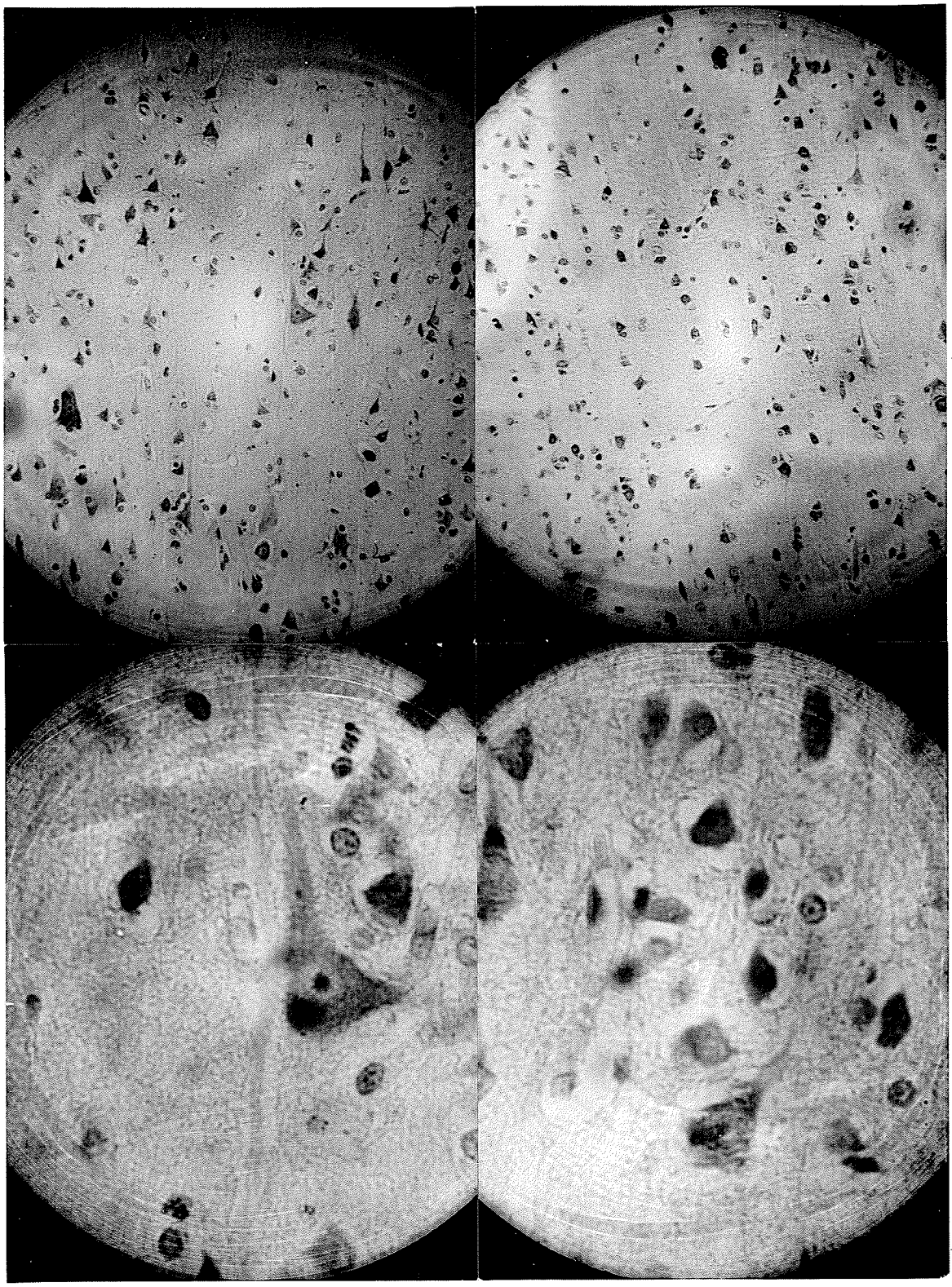

Plate I.

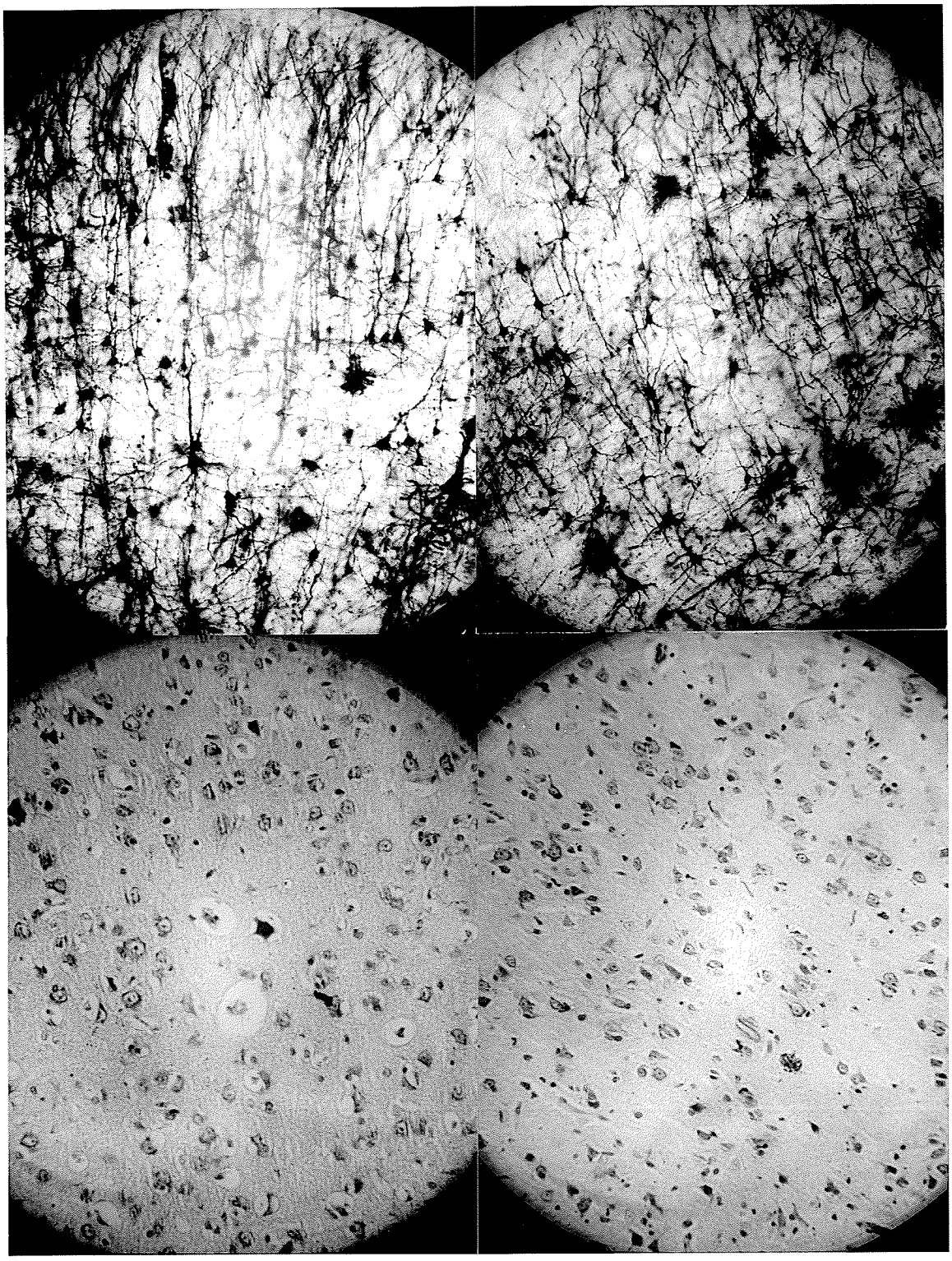

Plate II.

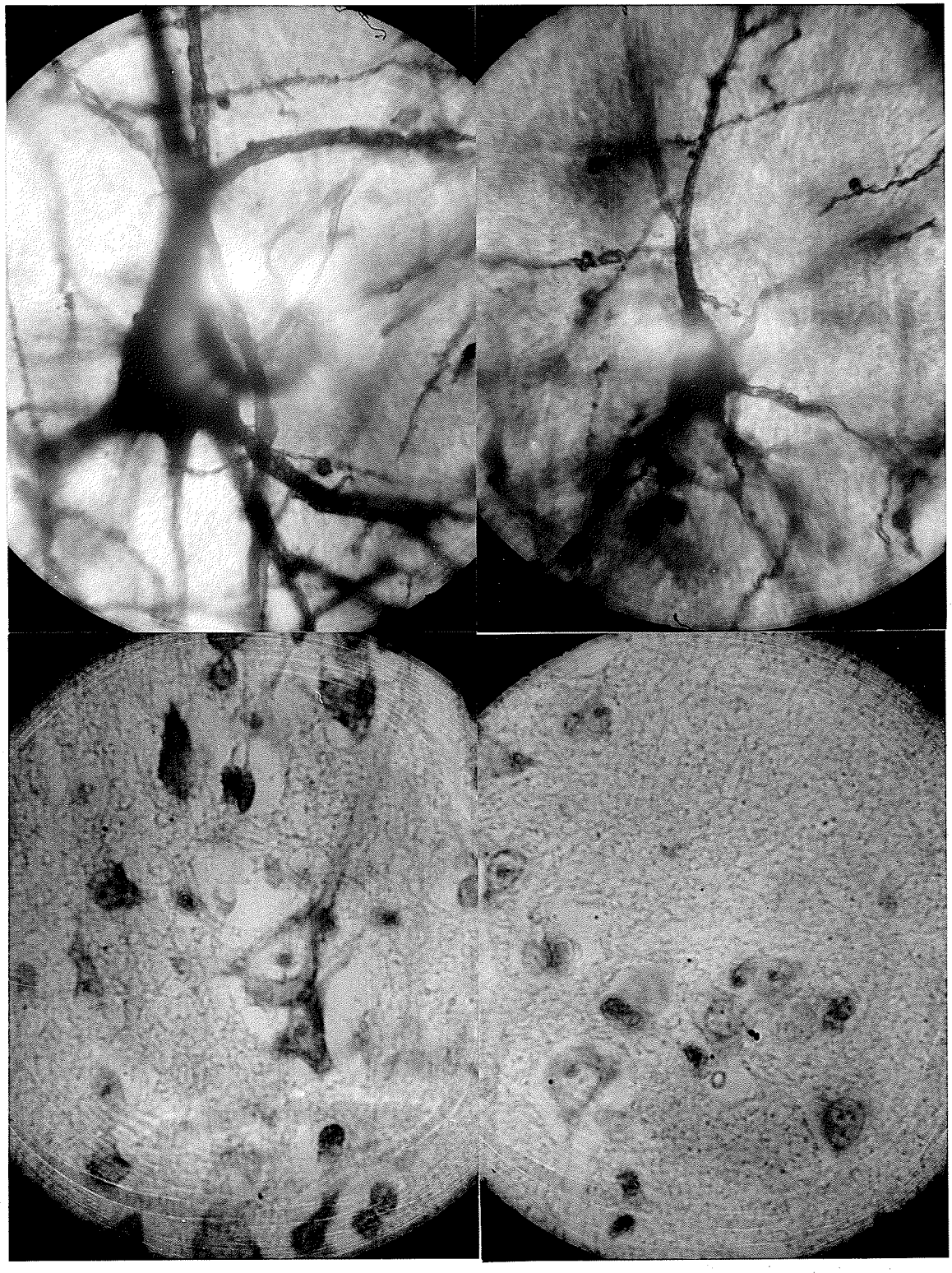

Plate III.

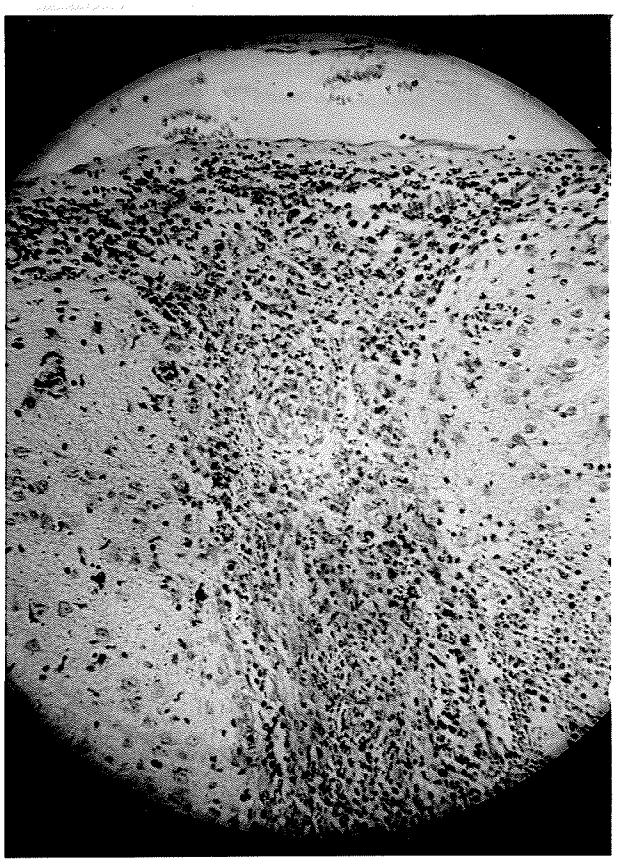

Plate IV.

D. DISCUSSION

The effects of isolation seen were not caused indirectly by an effect of the surgery on the blood supply to the slab. The destructive effects observed are probably entirely due to the severance of axons during undercutting and not to a decrease in blood supply resulting from damage to the pial blood vessels. Certainly acutely isolated slabs are adequately perfused and functional as measured by their ability to respond to stimuli (e.g. Burns, 1958).

In fact the blood supply to the chronic slabs may have been greater than normal. All the original blood vessels appeared to be intact, yet the volume of the slab and its containing gyrus was considerably less than when acutely isolated, so that blood flow per weight of tissue might be expected to be higher. Certainly the blood supply was at least equal to that of acute slabs. It is as unlikely that the prolonged afterdischarges of chronically isolated slabs could result from accumulation and persistance of any transmitter substance as it is that afterdischarges in acutely isolated or intact cortex could result from this type of mechanism (Pinsky, 1961).

Since all slabs were originally made close to the same width, the shrinkage of the chronic slabs, and the decrease in the depth of their cortex must be an indication that considerable destruction and degeneration of neurones must have occurred. The decrease in cortex depth without a decrease in neurone density at first suggested that the loss was entirely of cells at the sides of the slabs next to the isolation cut, and the loss of the entire deepest cortical layer where axons were most likely to have been severed close to their somata. However it is apparent that the loss of neurones was more widespread than that, and

that cell losses occurred from other cortical layers besides layer VI. The most obvious such loss occurred in layer V. The neurone density appears to have been maintained by the "collapse" of surrounding tissue into the space formerly occupied by the degenerated neurones. The average size of the remaining neurones must have been considerably smaller than before, for the same volume of cortex was then occupied by an increased number of glia as well as the same number of neurones. Indeed the Nissl stains showed that the neurones were, on the average, smaller than those of normal cortex; often the neurone nuclei had only a very thin layer of cytoplasm, sometimes darkly stained (Nissl substance) but more often quite pale.

The distribution of astrocytes in the Golgi-Cox preparations is additional evidence for a widespread loss of cells. The presence of astrocytes is common following stab wounds of the cerebral cortex, and it is possible that their function is phagocytotic (Glees, 1955). It is suggested that distribution of astrocytes is causally related to the presence of neurone destruction in the various layers of cortex, and that they are present to remove the debris of the dead cells.

Thus the destruction of neurones and the degree of disorganization in the various layers seems to be directly related to their depth in the cortex. In general, this is in agreement with Cajal's (1929) observation that the cell bodies far from an undercutting lesion were generally unaffected while layers closest to the lesion suffered greatest cell losses.

The shrinkage of the chronic slabs affected the cell counts by changing the layers in which they were done. For example, the counts in the Golgi-Cox preparations included some of layer VI in the chronic preparations, whereas in the acute samples layer VI, and the deeper part of

layer V were not counted at all. The change in the number of astrocytes counted was not, however, the result of this shrinkage, for fibrous astrocytes were infrequently found in any layer of normal cortex. While there may have been a bias toward astrocytes in the counting of Nissl preparations of chronic slabs, there was no difficulty in distinguishing astrocytes in the Golgi-Cox stains. There is of course no way of knowing whether a similar proportion of neurones are stained in chronic and acute slabs by these techniques, or if the distribution of staining among different neurone types is the same. However the relation between chronic and acute neurone density is about the same for the two techniques used, and it would seem unlikely that the same variation in staining properties should affect two such different staining methods. Previous experience has shown that the staining ability of the Golgi-Cox technique is unaffected by lesions or pathological conditions (Sholl, 1956).

Generally, nothing was found to confirm the contention of Purpura and Housepian (1961) and Purpura (1961) that extensive collateral sprouting of axons in the chronically isolated cortex is responsible for increased epileptogenesis of such cortex. Few neurones with multiple recurrent axon collaterals were found in either acute or chronic slabs. Of those which were found in the chronic slabs (e.g., Fig. V - 2B) many looked as if they could have been derived from P_3 or P_2 cells by degeneration of the main axon branch to white matter without further sprouting of axon collaterals. For example, neurone B of Fig. V - 2 might well have been derived from a cell such as A or E (Fig. V - 2) simply by degeneration of the main axon. Furthermore the isolated cortex prepared by Purpura and Housepian included part of the medial and ectosylvian gyri as well as the suprasylvian gyrus, and cells such as A (P_3 cells) are apparently

more plentiful in the visual cortex than in the suprasylvian gyrus (see also Sholl, 1956). The examples of cells with supposed newly sprouted axon collaterals shown by Purpura (1961) are from "near the edge of the slab" and it seems likely that they were indeed from the visual cortex.

It may be that the increase in collaterals over normal immature cortex observed by Purpura (1961) was only apparent and not real. Swelling of the axon and axon collaterals accompanies chromatolysis (Cajal, 1929). The "sprouting" observed by Purpura may have been mostly that normally occurring in the first 21 postnatal days, but the collaterals may have become more visible or prominent solely due to this swelling.

Even the identity of what are called axons may be in some doubt. Purpura also used a Golgi-Cox technique for staining; certainly some of the "axons" could well be fine branches of basilar dendrites (for example see his Fig. 25). The same may be said of the "axons" of Fig. V - 2A, B. Moreover it was observed that many fragmented apical dendrites of other cells often appeared to be axons of more superficial cells.

Even if there is axon collateral sprouting in isolated cortex, there is some doubt that many of such collaterals could form functional synapses, especially in mature cortex. It has been demonstrated that it is possible for nerves to innervate previously end-plate free regions of skeletal muscle, but only if the muscle has been denervated (Miledi, 1962) or has had nerve transmission chronically blocked by chemical means (Thesleff, personal communication). Thus it would be theoretically possible for newly sprouted axon collaterals to form functional synapses with the partially denervated neurones in the slabs. However since the terminals of regrowing axons in the cortex end up as free axonic balls, it is very likely that the collaterals also would be prevented by glia

from making contact with other cells, and would likewise end up as free axonic balls. This is less likely to be the case in immature neocortex, but does seem to be the probable course of events in mature chronically isolated cortex should collateral sprouting actually occur. Colonnier and Gray (1962) found that in mature isolated cortex there was only degeneration of terminals and no new end-plate formation during 21 days following isolation, the same period during which Purpura (1961) reported an increase in excitatory synapses because of collateral sprouting.

A major objection to the conclusions of Purpura and Housepian (1961) is that they never truly demonstrated any increase in epileptogenesis in their chronically isolated cortical slabs. Thus they are showing a cause for something that they have not demonstrated to exist! Their criteria for "increased epileptogenesis" seem to be the presence of elicited surface positive bursts, and spontaneous positive bursts (afterbursts, Burns, 1954) in the electrical activity recorded from the chronic slabs. However both these phenomena can be obtained from acutely isolated cortex, and therefore can have no connection with presence or absence of collateral sprouting, or the development of increased epileptogenesis, either of which takes more than a few hours to develop. Purpura and Housepian (1961) and Purpura (1961) seem to have made the same mistake as a number of others (Henry and Scoville, 1952; Echlin, Arnett and Zoll, 1952; Echlin, McDonald, Duke and Peck, 1953; Echlin, 1959) in confusing afterbursting with epileptic phenomena. There is also some doubt if it would be possible at all for immature cat neocortex to sustain an epileptiform seizure of the "grand mal" type (Grossman, 1955). There is a strong likelihood that immature cortex may be chemically supersensitive anyway; newborn rat diaphragm is supersensitive before functional

innervation is established (Diamond and Miledi, 1959).

With the general picture of degeneration and disorganization, together with lack of convincing evidence for functional axon collateral sprouting, it is logical to assume that there are many fewer functional synapses, rather than more, in chronically isolated cerebral cortex. The increased epileptogenesis in chronically isolated cortex is therefore much more likely to be the consequence of denervation supersensitivity in the remaining neurones, rather than a hyper-innervation due to collateral sprouting.

The chronic-depolarization-of-dendrites mechanism for epilepsy was proposed by Ward (1961) on the basis of results obtained from aluminium hydroxide lesions. The results do not agree with the results obtained in this study. No such pattern of dendritic deformation was seen in chronically isolated slabs. The closest that was seen was like cell A of Fig. V - 2, where the apical dendrite does appear bent. However this particular cell was seen in intact cortex. Depopulation of chronic aluminium hydroxide and other epileptic lesions has been a fairly consistent finding. Most studies (e.g., Goldring, Jerva, Holmes and O'Leary, 1960) indicate that epileptic discharges arise from the margins of such lesions, where there is partial destruction, and hence partial isolation of neurones. Thus it may be said that the chronic depolarization theory of Ward (1961) is not applicable to chronically isolated cortex, and that the theory of denervation supersensitivity may be applied to explain the increased epileptogenesis of aluminium hydroxide lesions.

VI. THRESHOLDS AND RESPONSES TO ELECTRICAL STIMULATION

A. INTRODUCTION

It is well established that repetitive electrical stimulation of the cerebral cortical surface can initiate epileptiform afterdischarges. The relationship between the various parameters of stimulation and certain features of the afterdischarge have been studied in intact cerebral cortex by Rosenblueth and Cannon (1942) and in acutely isolated cerebral cortex by Pinsky and Burns (1962). The latter authors found that the discharge bears an all-or-none relationship to the electrical stimulus when either the pulse width, stimulus strength (volts) or the total number of pulses in the stimulating train is varied. They found also that the threshold stimulus strength for initiation of afterdischarges is independent of the total area of cortex (over an 8-fold range) being stimulated. They concluded that a minimum density of cells at the focus has to be excited (driven) a minimum number of times before an afterdischarge will occur.

Because of its important relation to the experiments described in this section, the theory of Pinsky and Burns warrants detailed examination here. In contrast to the other parameters of stimulation, the frequency of stimulation appeared to be the only one which could alter the duration of the afterdischarges in acutely isolated cortex. Pinsky and Burns postulated that the factor which leads to the outbreak of the epileptiform activity is what they called a "state of exhaustion" induced by each stimulating pulse, and that the afterdischarge is initiated during the recovery from this state. The "exhaustion" was related to a prolonged depolarization of the somatic region of the cortical pyramidal neurones which was found experimentally by Pinsky (1961). It is apparent that they considered that the depolarizations produced during the afterdischarge did

not contribute significantly to the "exhaustion state" since they postulated that the afterdischarge was proportional to the degree of "exhaustion" produced during the period of stimulation only. They suggested that the cells recover from "exhaustion" during the period between each pulse, so that only a small total sum of "exhaustion" can be obtained when the cortex is stimulated at low frequencies. This would explain why afterdischarges produced by low frequency stimulation lasted only a relatively short time. Further, they postulated that at high stimulus frequencies some of the stimulating pulses occur during the refractory period of the neurones so that a proportion of the pulses are ineffective, and the effective frequency is lower. This would explain why the discharge duration decreased with higher stimulus frequencies. The observed maximally effective stimulus frequency was approximately 64/sec, which suggested an average refractory period of about 15 msec (1/64 sec) for the neurones involved. At present it is not clear what part of the cells is stimulated by the electrical shocks delivered to the cortical surface, nor what part of the cell might be refractory in the manner postulated by Pinsky and Burns. However Kandel, Spencer and Brinley (1961) found that, in response to antidromic stimulation, the B-spike of archicortical pyramidal cells did not occur when the interval between two antidromic stimuli was less than 15 msec. This suggests that the figure arrived at by Pinsky and Burns is a reasonable one for cortical neurones, and that the cell soma is involved in this functional refractory period that they described.

Pinsky and Burns presented a formula which expresses the effect of stimulus frequency and the total number of pulses upon the development of the "state of exhaustion". According to this formula, the effect on

the "exhaustion state" of increasing the number of pulses asymptotes very quickly to a maximum for a given frequency of stimulation. This is in agreement with the observed all-or-none relationship between the discharge duration and the total number of stimulating pulses.

The series of experiments to be described in this section were designed to test whether chronic isolation alters those relationships between stimulus and discharge which Pinsky and Burns described for acutely isolated cerebral cortex. If the tendency of chronically isolated cortex to produce prolonged epileptiform afterdischarges is due primarily to denervation supersensitivity, then there is no a priori reason to expect that the threshold values of the stimulus parameters in chronic slabs will differ from those found for acute slabs. A supersensitivity to chemical agents would not change the threshold to an electrical stimulus which excites directly the cells involved in the focus. On the other hand, if the electrical threshold for the initiation of afterdischarges is well above the true electrical threshold for the primary neurones directly stimulated, and an afterdischarge only occurs when a certain number of post-synaptic neurones are excited it is conceivable that the electrical threshold for afterdischarges would be lowered by denervation supersensitivity development. Thus any change, or lack of a change would be compatible with denervation supersensitivity. In contrast, the lack of a change in "electrical threshold" for afterdischarges can only mean that denervation supersensitivity, or some other process (e.g., sprouting) which results in a larger number of post-synaptic neurones being excited for each pre-synaptic discharge, can account for the occurrence of prolonged afterdischarges in chronic slabs. These alternates were examined in the following experiments.

B. METHODS

Frequency of stimulation, pulse width, strength (voltage) and total number of pulses in the stimulus train were varied independently of each other. Stimulus threshold values were found, and curves showing the relationship between each stimulus parameter and the duration of the afterdischarge were constructed for chronically and acutely isolated cortical slabs. The graph lines in each figure are fitted by eye to the cumulative data of all experiments. The points shown are data from single typical experiments. Since the ordinates are logarithmic, there is no zero. However, stimuli which caused an afterdischarge of 1 sec or less were defined as subthreshold. The logarithmic ordinates also have the effect of deemphasizing the large difference between the discharge durations of chronic and acute slabs.

Pulse width was kept constant at either 1 or 2 msec, unless the pulse width was the variable being examined. In experiments in which frequency was varied the total number of pulses was kept at 100. The voltages used were arbitrarily selected except for the requirement that for the voltage used the threshold number of pulses at a stimulating frequency of 20/sec be between 20 and 64 for the acute slab. Determination of the pulse-number thresholds were done at several stimulus frequencies (5, 10, 20, 40 and 100/sec) but all frequencies were not necessarily examined in each experiment. The 20/sec threshold usually was tested first, but the other frequencies were tested in a haphazard order. The order of testing was the same in each pair of acute and chronic slabs.

C. RESULTS

a) General observations

(i) Frequency-vs-discharge duration curves. It was found that in chronically isolated cortex the stimulus frequency at all suprathreshold values had no effect on the duration of the afterdischarge. This all-or-none relationship between stimulus frequency and discharge duration is shown by the solid line in Fig. VI-1. This relationship contrasted sharply with the dependency of discharge duration on stimulus frequency in the acute slabs, where there was an increase and then a decline in discharge duration as the stimulus frequency was increased (broken line in Fig. VI-1; cf. Pinsky and Burns, 1962).

The thresholds are reported separately below (part b).

(ii) Stimulus-response curves for the other stimulus parameters. In both acute and chronic preparations there was an all-or-none relationship between the afterdischarge duration and variation in the pulse width, in the stimulus voltage, or in the number of stimulating pulses (Fig. VI-2, Fig. VI-3 and Fig. VI-4 respectively). The stimulus frequencies used were between 20 and 100/sec. The pulse-width graph (Fig. VI-2) was constructed with data from 3 pairs (acute and chronic) of slabs, stimulus strength data (Fig. VI-3) was obtained from 7 pairs of slabs, and the number-of-pulse data (Fig. VI-4) from 13 pairs. Tests of stimuli more than 50% above threshold were made in only a few pulse-variation experiments.

When this group of experiments was begun, there appeared to be a tendency for extremely long-lasting discharges, some of several hours duration, to be set up at higher values of stimulation. It later became clear that there was no such relationship (just threshold stimuli caused the prolonged discharges in some animals and strong stimuli only occasionally

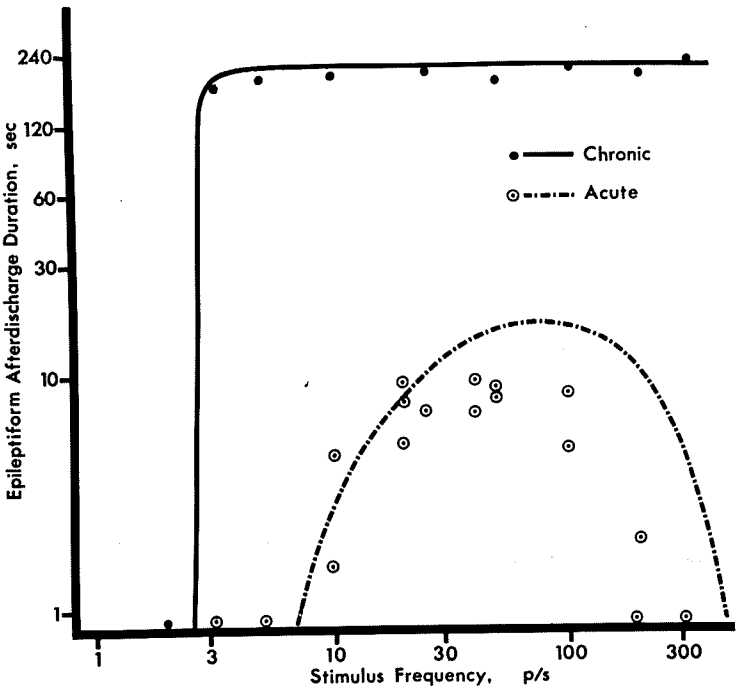

Fig. VI - 1 Stimulus frequency - response curve

Relationship between stimulus frequency and the duration of the epileptiform afterdischarge.
Lines are fitted by eye to data from all animals.
Points plotted are from single sample experiments.

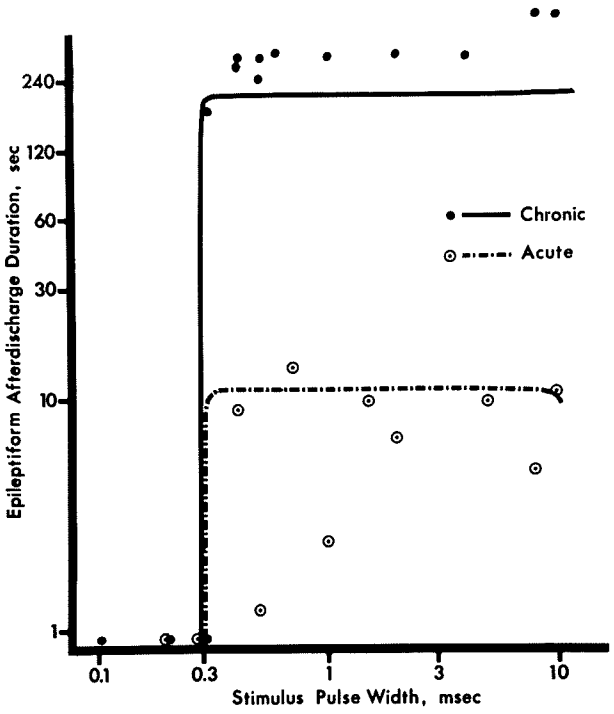

Fig. VI - 2 Stimulus pulse width - response curve

Details as for Fig. VI - 1.

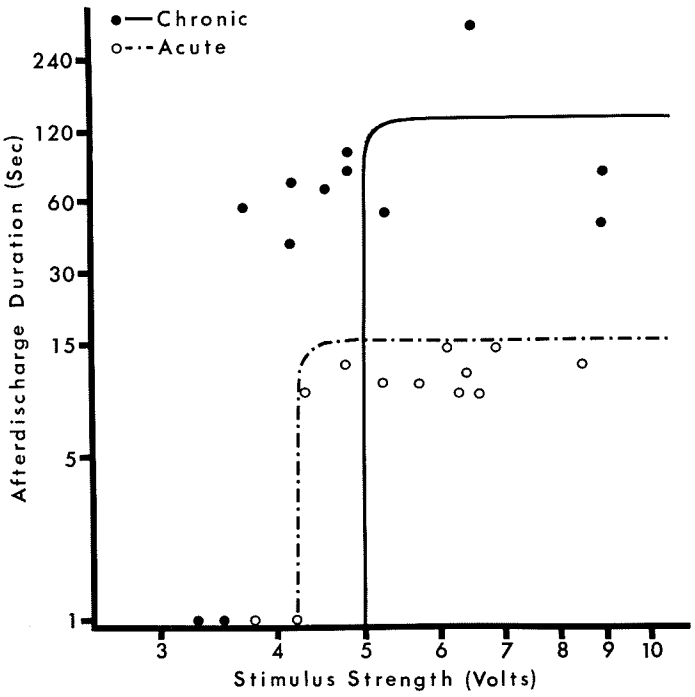

Fig. VI - 3 **Stimulus strength - response curve**

Details as for Fig. VI - 1.

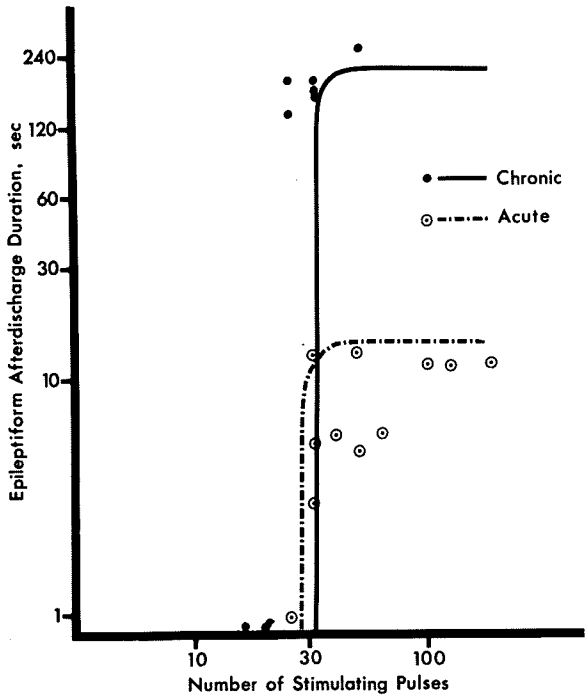

Fig. VI - 4 Number of stimulating pulses - response curve

Details as for Fig. VI - 1.

produced very prolonged discharges in some others), but until then it was decided to avoid risking the occurrence of these long-lasting discharges since when they occurred they usually prevented further testing. Thus tests were rarely carried out with stimuli more than 200% of threshold.

Most discharges in chronically isolated slabs lasted between 1 and 6 min (Fig. VI-5b); a few chronic slabs regularly gave discharges lasting between 30 and 60 sec, or between 8 and 15 min. Most discharges from acutely isolated cortex lasted under 20 sec, usually about 8 sec (Fig. VI-5a), but several acute preparations had occasional discharges which lasted up to 50 sec. The discharges became extremely short (5-20 sec) in some of the chronic preparations which were tested for longer than 24 hours. It seemed likely that this was due to a general deterioration of the preparations rather than to a "desensitization", as has been suggested by Sharpless and Halpern (1962). One chronic slab was tested repeatedly over 36 hours with no decrease in discharge duration.

Patterns of afterdischarge were remarkably similar in many preparations. The course of a discharge in a chronic slab (Fig. VI-5b) usually started with a very high frequency, low amplitude phase which generally lasted 30 to 60 sec. This was followed by intermittent bursts of high frequency activity superimposed on large surface-positive waves which waxed and waned during the discharge but which were usually largest shortly before the end of the seizure. The size of the waves was inversely related to the frequency at which they occurred. These two phases have been termed "tonic" and "clonic" because of their close association with the motor phases of a clinical seizure (Rosenblueth and Cannon, 1942). In some animals the seizures ended with a series of surface negative spikes as shown in the example in Fig. VI-5b. Within any one preparation the

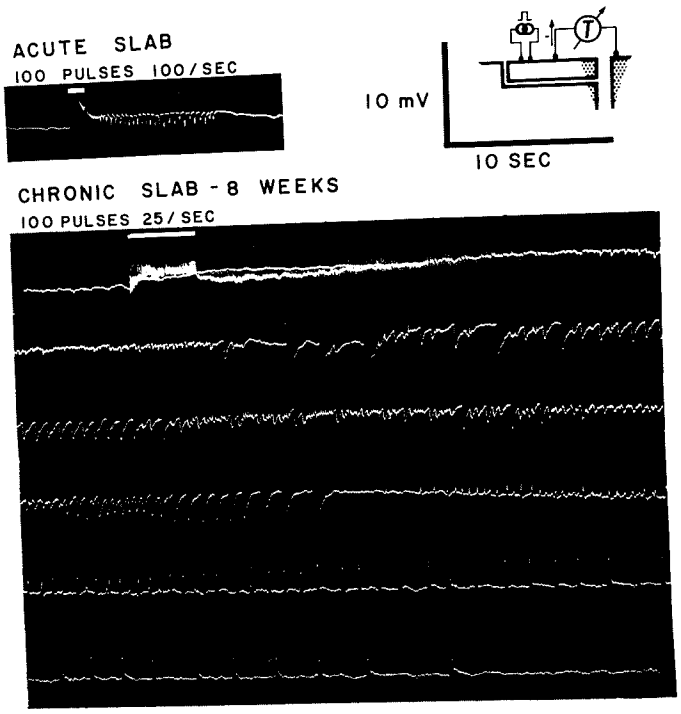

Fig. VI - 5 Typical epileptiform afterdischarges

Recording of tangential potentials during afterdischarges in acute and chronic slabs, "a" and "b" respectively. Horizontal white bars in records indicate stimulus. Negativity at the active electrode is upward in the record (see insert for arrangement of electrodes). Record of discharge of chronic slab is continuous.

sequence of waxing and waning of the potentials was closely stereotyped in the various afterdischarges. In the acutely isolated slabs the "tonic" phase was often absent. Its presence or absence often appeared to be related to the frequency of stimulation.

(iii) Prolonged afterdischarges of the chronic slabs. Afterdischarges which lasted over 30 min were observed in 12 chronic slabs. Such long-lasting discharges have been reported previously by Grafstein and Sastry (1957), by Echlin (1959) and by Sharpless and Halpern (1962). All of these authors related the occurrence of the prolonged discharge to the duration of the isolation. They had observed these very prolonged discharges in slabs which had been isolated 3 to 11 months prior to testing. In the present study very prolonged afterdischarges were observed throughout the range of isolation times studied (8 weeks to 15 months).

In the first 3 experiments in which such afterdischarges were encountered, they appeared to result from the adjustment of the various parameters of stimulation to values several times the threshold, and such stimuli were generally avoided thereafter. However, in subsequent experiments prolonged discharges were observed following the first or second successful test stimulus, close to threshold. There was no correlation between the value of the stimulation at which the prolonged discharges occurred and the duration of the isolation. In some animals prolonged discharges did not occur following stimuli 2 or 3 times threshold. Thus no clear relationship was found between the probability of occurrence of very prolonged paroxysmal afterdischarge and either the parameters of stimulation or the duration of the isolation.

The long-duration afterdischarges started off in the same manner as the discharges of other chronic slabs, that is with a period of

fast (tonic) discharge lasting 30-60 sec, followed by slow (clonic) bursting. This clonic bursting appeared to decrease gradually in amplitude, after reaching a maximum within the first 3 min, until the amplitude of the clonic bursts was about one half maximum. The bursting rate gradually declined throughout the discharge until it was between 1/sec and 0.3/sec (Fig. VI-7; Fig. VII-1).

This late, prolonged clonic phase somewhat resembled, in form and frequency, the spontaneous surface-positive "afterbursting" described by Burns (1954). Afterbursting has been suggested to be an epileptic phenomenon (Echlin, 1959) and elicited surface positive burst responses (SPBR) (Burns, 1951) have been suggested to be single paroxysmal events (Eccles, 1953). However certain characteristics of the prolonged clonic discharge clearly distinguished it from afterbursting. For example, in the former the frequency of the bursts was faster and their occurrence was more regular than is usually seen with afterbursting. In addition, elicited surface-positive burst responses (SPBR) (Burns, 1951) obtained from chronic slabs had only a small, variable positive component (Fig. VI-6). This was also true of the spontaneous positive bursts (afterbursting) observed in many of the chronic slabs (see Fig. VII-2, also Section VIII C (c)). In contrast, the clonic bursts of chronic slabs' afterdischarge had a large surface-positive component. Finally, strong epileptogenic stimulation (equal to or greater than that which caused the discharge initially) during the course of the prolonged afterdischarge did not inhibit the bursts (Fig. VI-7). Occasionally a very fast tonic activity was observed during the first 2 or 3 sec after such stimulus (Fig. VI-7c). More usually, however, the stimulation did not alter the form of the discharge in any way. That is, a new discharge with a long tonic

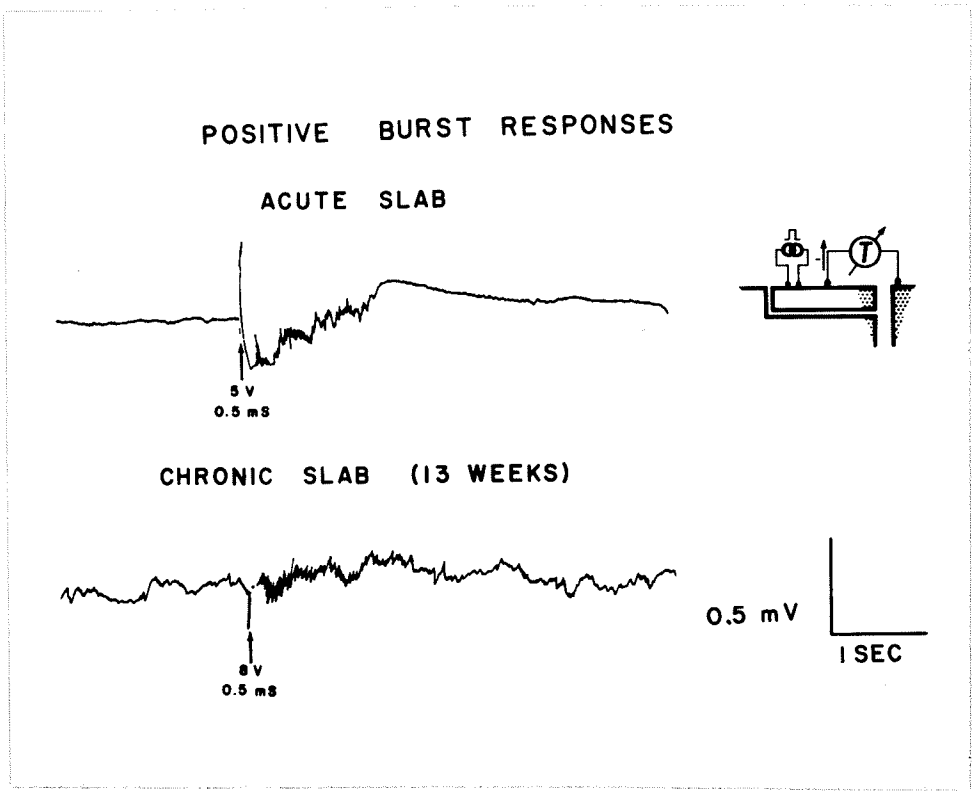

Fig. VI - 6 "Surface positive" burst responses

The burst response of the acute slab has a pronounced positive component in addition to the bursting of high-frequency activity.
The burst response of the chronic slab has only the high-frequency activity and not the slow positivity.

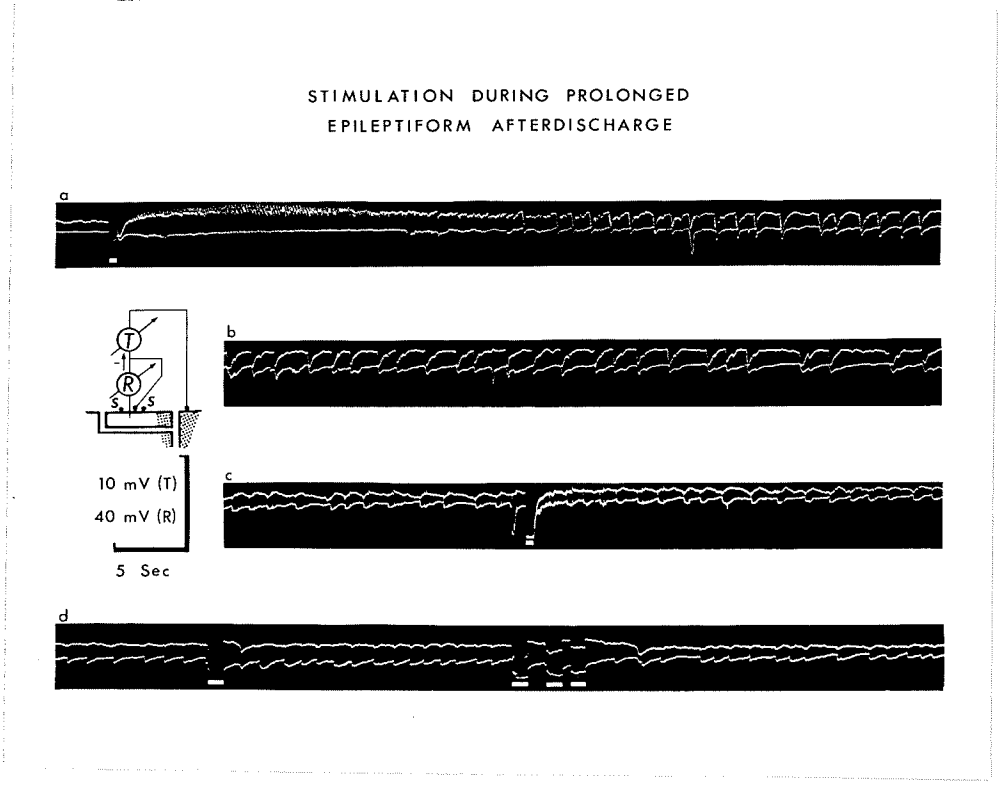

Fig. VI - 7 Stimulation during prolonged afterdischarge

DC Recordings from cortical slab isolated for 10 weeks. Upper channel is tangential record (T); lower channel is radial potential record (R) with the microelectrode 0.688 mm deep in cortex. Polarity of R: negativity at microelectrode tip - downward deflection.

(a) Beginning of epileptiform afterdischarge induced by 20 pulses (6.25 volts) at 40/sec.

(b) Continuation of "a"; 60 sec omitted.

(c) Same discharge 110 min after "b". Stimulus in middle of section is same as in "a". (The deflection just before the stimulus is a movement artifact).

(d) Same discharge 135 min after "c". The first stimulus is 40 pulses (6.25 volts) at 40/sec. 20 sec later the stimulus is repeated 3 times within 5 sec.

White bars under records indicate periods of stimulation. Spikes retouched.

phase could not be initiated during the prolonged slow clonic phase of the previous afterdischarge. This contrasts with the observation that afterbursting in acute or chronic slabs is inhibited for 15 to 60 seconds after an induced epileptiform seizure, or following a subthreshold epileptogenic stimulus. Moreover, the presence of afterbursting alone is no hinderance to the initiation of afterdischarges.

Various procedures were tried in attempts to stop the long-lasting discharges. Pentobarbital (15 mg/kg, i.v.) stopped the prolonged discharge and reduced both the amplitude and duration of subsequent epileptiform discharges. In two animals, ether, administered from a variable-by-pass bottle set at half the dial reading necessary for surgical anaesthesia, abolished the prolonged discharges within one minute. However when the anaesthetic was discontinued 2 min later, the discharge returned within the next couple of minutes. One animal was cooled rapidly by directing a fan on the skin of its trunk, which had been wet with water. Cooling down to 34-36°C abolished the discharge and inhibited further seizure initiation while the temperature was kept low. Long-lasting afterdischarges were obtained again when the animal was rewarmed. 1% procaine, applied topically, stopped the prolonged discharge in the one case in which it was tried.

Strong stimulation (1 or 2 stimulus parameters set at many times threshold, e.g., up to 2000 pulses delivered at rates up to 400/sec) was tried in an attempt to induce spreading depression (e.g., Fig. VI-7d). This could not be accomplished, and the stimulus had no effect on the course of the discharges. Application of 1% KCl to the cortex in one animal caused a typical wave of spreading depression, but discharge activity, although greatly reduced in amplitude, persisted throughout the spreading depression wave. Complete recovery of the activity did not occur over the

next hour despite saline washes of the cortex.

The slab was reisolated during prolonged afterdischarges in two animals (see Section VII). The reisolation presumably caused considerable trauma. Nevertheless the discharges were still recorded immediately after the surgery. Cerebral hypoxia had a variable effect. In one animal clamping the common carotid and the vertebral arteries for periods up to 20 sec did not stop the clonic discharges; this procedure was effective in stopping the discharge in 2 animals where clamping the common carotids alone was ineffective, and clamping only the common carotid arteries was effective in stopping the discharges in 2 others. In one of the latter, however, the discharges normally lasted only 15-20 min and were deliberately stopped at 10 min or sooner. The prolonged afterdischarges precluded further testing in 6 animals because of the difficulties in stopping the afterdischarges, or because of the general unresponsiveness caused by most of the effective methods.

b) Electrical thresholds for the induction of epileptiform afterdischarges.

(i) Stimulus frequency thresholds. There was approximately a 2-fold difference between the threshold frequency of stimulation of acute and chronic slabs. The results of 5 experiments are shown in Table VI-1. The average threshold frequency for acute slabs was about 7/sec while the average threshold for the chronic slabs was about 3/sec.

(ii) Pulse width threshold. No difference in pulse width threshold was seen for 3 acute and 3 chronic slabs (Table VI-2). In the one pair of slabs tested in the same animal the threshold pulse width was exactly the same.

(iii) Threshold number of stimulating pulses. The

Table VI-1

STIMULUS FREQUENCY THRESHOLDS

Exper.	Isolation Time (Weeks)	Stimulus Strength (Volts)	Frequency Threshold (Pulses per second) CHRONIC SLABS	ACUTE SLABS	Longest afterdischarge duration (seconds) CHRONIC SLABS	ACUTE SLABS
1	8	7.5	2-3	5-10	200	7
2	9	7.5	3-5	5-8	190	26
3	10	5	2-3	5-10	58	10
4	11	7.5	3-5	5-10	>3 hrs.	3
5	25	10	2-2.5	4-5	180	10
Average			3.05	6.70		

These results are from 10 animals, 5 with slabs isolated the indicated length of time and 5 with acutely isolated slabs.
All data was obtained with a train of 100 pulses
Thresholds are expressed as the range between the highest frequency that failed to cause an afterdischarge and the lowest successful frequency tried. $p<0.001$ for threshold means (Student's t-test).

Table VI-2

STIMULUS PULSE DURATION THRESHOLDS

Exper. No.	Isolation Time (Weeks)	Stimulus Strength (volts)	Stimulus frequency (p.p.s.)	Number of stimulating pulses	Pulse width threshold (msec) CHRONIC	ACUTE	AD duration (sec) CHRONIC	ACUTE
1*	8	6.25	20	160	0.1-0.2	0.1-0.2	235	4
2	14	8	20	32	0.65-0.75	0.6-0.7	130	5.5
3	26	10	50	13	0.1-0.2	0.3-0.4	312	8
Average					0.28-0.37	0.33-0.43		

Chronic and acute slabs done in different animals with the exception of (*).

Thresholds are expressed as range between the widest pulse that failed to cause an afterdischarge and the narrowest effective pulse width.

threshold number of stimulating pulses required to initiate an epileptiform afterdischarge was found to be the same in acutely and chronically isolated cortex. The data is presented in Table VI-3, subdivided according to the frequency of stimulation used. There was no significant difference between the threshold number of pulses of acute and chronic slabs at any of the frequencies studied. The mean thresholds for all tests at all frequencies are 29.2 pulses for the chronically isolated slabs and 27.3 pulses for the acutely isolated slabs.

Where threshold data from only one of a pair of acute and chronic slabs were obtained, thresholds found in that slab have been excluded from the averages given in the table. Thus the thresholds found with 100/sec stimulation in experiment 4 have been excluded from the averages since the chronic slab of this pair had been damaged by attempts at penetration of the pia with the microelectrode just before this threshold was obtained. The slab bled severely in several areas, and other areas were severely blanched when this test was made. Shortly thereafter no threshold could be obtained at either frequency tested. No thresholds could be obtained at the 5/sec stimulus frequency in the acute slabs of experiments 1 and 13, although the highest numbers of pulses tested were 2.5 times the thresholds of the chronic slabs in these experiments. These two acute slabs discharged during the stimulation but this activity ceased abruptly when the stimulation was stopped. The thresholds at 5/sec for the other 3 acute slabs were not very different from those of the chronic slabs.

The hypothesis presented by Pinsky and Burns (1962) implies an inverse relationship between the stimulus frequency and the threshold number of pulses, at least for frequencies up to about 100/sec. No such relationship could be found in either acute or chronic slabs. Comparison

Table VI-3

THRESHOLD NUMBERS OF PULSES											
	Isolation	Stimulus	Threshold Number of Pulses at Indicated Stimulus Frequencies									
			CHRONIC					ACUTE				
Exper. No.	Time (Weeks)	Strength (Volts)	100/s	40/s	20/s	10/s	5/s	100/s	40/s	20/s	10/s	5/s
1*	3	12.0	13 - 16	16 - 20	26 - 29	23 - 26	16 - 20	25 - 33	32 - 40	38 - 46	40 - 51	▣50 <
2	8	6.2	-	25 - 32	-	-	-	-	32 - 40	-	-	-
3*	8	8.0	18 - 19	-	26 - 32	-	29 - 32	13 - 16	-	26 - 32	-	20 - 22
4	10	8.0	▣100 - 130	-	56 - 63	-	-	17 - 18	-	42 - 43	-	-
5	13	8.0	73 - 76	-	56 - 57	-	-	19 - 20	-	35 - 39	-	-
6	14	4.0	-	-	50 - 63	-	-	-	-	50 - 63	-	-
		6.0	-	-	20 - 25	-	-	-	-	40 - 50	-	-
		8.0	-	-	20 - 26	-	-	-	-	26 - 32	-	-
7	15	10.0	-	▲20 - 25	40 - 50	-	-	-	▲16 - 18	29 - 32	-	-
		16.0	-	▲16 - 20	26 - 32	-	-	-	▲13 - 15	25 - 29	-	-
8	22	12.0	20 - 22	23 - 26	32 - 36	32 - 34	35 - 37	9 - 11	14 - 16	22 - 27	24 - 26	22 - 24
9	25	10.0	◉13 - 16	▲10 - 13	-	-	-	◉16 - 20	▲20 - 25	-	-	-
10*▽	28	4.2	-	25 - 32	-	-	-	-	25 - 32	-	-	-
11	60	8.0	20 - 25	16 - 20	26 - 27	20 - 27	20 - 26	10 - 13	8 - 10	16 - 20	20 - 26	34 - 36
12*	60	10.0	-	52 - 64	40 - 50	25 - 32	-	-	16 - 20	32 - 40	32 - 40	-
		20.0	16 - 20	25 - 32	26 - 32	-	-	8 - 10	10 - 13	16 - 20	-	-
13*	71	8.0	22 - 22	-	24 - 28	-	32 - 40	12 - 15	-	31 - 32	▣58 - 63	▣120 <
14*	77	8.0	-	-	26 - 26	-	-	-	-	26 - 26	-	-
Mean of all data in column			25.6	28.3	37.0	27.4	28.7	15.8	20.4	32.5	32.4	26.3

* Control acute experiment done in same animal; all other slab pairs in different animals.
◉ Data at 200/sec rather than 100/sec.
▲ Data at 50/sec rather than 40/sec.
▽ Animal moribund (distemper) at the time of experiment.
Results reported as the range between highest unsuccessful stimulus and lowest successful stimulus.
▣ Data not included in averages; see text.

of thresholds at different frequencies in those chronic slabs where paired data were available (i.e., where testing was done at 2 or more frequencies in the one slab) showed that there was no significant difference between any thresholds. In the acute slabs there was a significant difference between the thresholds at 20/sec and 40/sec, but not between any other adjacent columns of Table VI-3.

(iv) Stimulus strength (voltage) thresholds. There was no difference between thresholds voltages necessary for the production of afterdischarges in acute and in chronic slabs (Table VI-4). These data were derived from tests on 4 pairs of slabs in which bipolar stimulating electrodes were used, and 3 pairs in which stimulation was delivered via monopolar electrodes having different contact areas. In 2 of the former, tests were made at several frequencies or total numbers of pulses (experiments 1 and 2). Where testing was conducted with monopolar stimulation (experiments 5, 6 and 7) the thresholds given in Table VI-4 are means of tests done with electrode areas larger than 0.35 mm^2 (see Table VI-5). Since, of necessity, these tests were conducted under a limited number of conditions, the absolute values of the means have little direct significance. They merely serve as an indication of the approximate voltages necessary to produce an effective stimulus under these experimental conditions. The mean thresholds of 7 pairs of slabs were 5.0 volts for the chronic and 4.2 volts for the acute slabs.

(v) Effects of stimulating electrode area on stimulus strength threshold. Monopolar electrodes (Fig. IV-1d) were mounted in a micromanipulator so that the centers of all electrodes were returned to the identical location on the cortical surface. Contact of the electrodes with the cortex was monitored visually with a binocular operating

Table VI-4

STIMULUS STRENGTH THRESHOLDS

Exper. No.	Isolation Time (Weeks)	Stimulus frequency (pps)	No. of stimulus Pulses	Threshold stimulus (volts) CHRONIC	ACUTE
1B	8	40	20	5.5 - 5.8	5.4 - 5.8
		40	32	5.0 - 6.3	4.4 - 4.8
		MEAN		5.7	5.1
2B	10	10	50	7.0 - 8.0	5.6 - 7.2
		20	50	7.0 - 8.0	4.0 - 4.8
		20	32	12.0 - 13.0	5.6 - 6.4
		40	32	12.0 - 13.0	4.0 - 4.8
		MEAN		10.0	5.3
3*B	13	40	32	6.0 - 6.5	6.0 - 6.5
4B	14	20	32	5.0 - 6.0	4.4 - 4.8
5*M	7	20	40	1.9 - 2.1	2.2 - 2.4
6M	9	20	50	2.9 - 3.1	3.2 - 3.3
7M	65	20	40	2.5 - 2.8	2.4 - 2.6
MEAN				5.0	4.2

Thresholds are given as range between highest ineffective stimulus and lowest effective stimulus.

* = Control acute slabs in same animal as the chronic slabs.
B = Bipolar stimulating electrode.
M = Monopolar stimulating electrode.

microscope, and electrically by monitoring the current passed by the electrodes. Very weak pulses were applied to the electrodes during placement on the cortex. After initial electrical contact was made the current passed by the electrode increased rapidly to a maximal value as the electrode was lowered an additional few tenths of a millimeter. The minimum pressure on the cortex necessary to obtain this maximal current was maintained during each experiment, and was checked during the test run. Such pressure did not appear to interfere with the blood supply to the area under the electrode. At times the voltage threshold appeared to vary quite widely during the course of several hours of testing and in these cases there may have been some vascular occlusion. This was especially the case whenever there was cortical swelling. In general, swollen preparations did appear to deteriorate more rapidly than most.

The effect of variation in the area of cortex stimulated on the voltage threshold for epileptiform afterdischarge was studied in 3 acute and 3 chronic slabs (Table VI-5). When the electrode areas were 0.35 mm^2 or greater there was no appreciable variation in the threshold voltage in either the acute or chronic slabs. There was, however, a slight tendency for the threshold to be inversely related to the stimulated area. In two experiments (1 and 3) the thresholds obtained using the 0.17 mm^2 electrode were about twice the values obtained with the larger electrodes.

These electrodes can be regarded as a number of parallel conducting pathways, and ideally the total conductance of the electrode-cortex contact should be directly related to the area of the electrode. However, edge effects and conductance through surrounding fluid on the cortical surface can give a small electrode a much larger conductance than

Table VI-5

EFFECT OF STIMULATION AREA ON STRENGTH THRESHOLD

Exper. No.	Isolation Time (Weeks)	Stimulus (Pulses & Frequency)	Electrode Area (mm^2)	CHRONIC THRESHOLDS: Electrode Conductance (mMho)	CHRONIC THRESHOLDS: Strength (volts) or Current Density (mA/mMho)	CHRONIC THRESHOLDS: Current Density (mA/mm^2)	ACUTE THRESHOLDS: Electrode Conductance (mMho)	ACUTE THRESHOLDS: Strength (volts) or Current Density (mA/mMho)	ACUTE THRESHOLDS: Current Density (mA/mm^2)
1*	7	{40 p / 20 pps}	0.17	0.25 - 0.26	3.3 - 3.3	4.88 - 5.00	0.34 - 0.33	6.3 - 6.6	☐12.41 - 12.00
			0.35	0.39 - 0.41	2.3 - 2.9	3.13 - 3.60	0.32 - 0.33	4.1 - 4.5	3.65 - 4.12
			1.36	0.87 - 0.92	2.3 - 2.4	1.43 - 1.58	0.94 - 0.98	2.6 - 2.7	1.77 - 1.93
			4.90	0.84 - 0.88	2.0 - 2.2	0.33 - 0.39	0.72 - 0.70	1.9 - 2.2	0.27 - 0.31
			16.75	1.26 - 1.19	1.6 - 1.8	0.12 - 0.13	1.10 - 1.06	2.2 - 2.4	0.13 - 0.15
			△Mean:	0.99 - 1.00	1.9 - 2.1		0.92 - 0.91	2.2 - 2.4	
2	9	{50 p / 20 pps}	0.17	-----	-----	-----	0.27 - 0.27	4.2 - 4.3	6.76 - 6.76
			0.35	0.36 - 0.40	2.1 - 2.5	2.14 - 2.85	-----	-----	-----
			1.36	0.94 - 0.98	2.9 - 3.3	2.01 - 2.35	0.48 - 0.50	3.2 - 3.4	1.14 - 1.25
			4.90	0.88 - 0.92	2.8 - 3.0	0.50 - 0.57	0.59 - 0.65	3.1 - 3.2	0.39 - 0.41
			16.75	1.11 - 1.16	2.5 - 2.8	0.17 - 0.19	-----	-----	-----
			△Mean:	0.91 - 0.95	2.9 - 3.1		0.54 - 0.58	3.2 - 3.3	
3	65	{40 p / 20 pps}	0.17	0.11 - 0.11	8.1 - 8.8	5.00 - 5.52	0.22 - 0.26	5.0 - 5.3	6.32 - 7.94
			0.35	0.14 - 0.15	3.5 - 4.2	1.43 - 1.79	0.40 - 0.38	2.8 - 3.4	2.21 - 3.64
			1.36	-----	2.8 - 3.3	-----	0.41 - 0.43	2.8 - 3.1	0.86 - 1.02
			4.90	0.69 - 0.71	2.0 - 2.3	0.28 - 0.33	0.69 - 0.71	2.0 - 2.3	0.28 - 0.33
			16.75	1.14 - 1.16	2.8 - 2.9	0.19 - 0.23	1.03 - 1.04	2.4 - 2.6	0.15 - 0.16
			△Mean:	0.92 - 0.93	2.5 - 2.8		0.86 - 0.88	2.4 - 2.6	
Average of the 3 expts.			0.17	0.18 - 0.19	5.7 - 6.1		0.28 - 0.27	5.2 - 5.4	
			0.35	0.30 - 0.32	2.6 - 3.2		0.36 - 0.35	2.3 - 2.9	
			1.36	0.91 - 0.95	2.7 - 3.0		0.61 - 0.64	2.9 - 3.1	
			4.90	0.80 - 0.84	2.3 - 2.5		0.63 - 0.67	2.3 - 2.6	
			16.75	1.17 - 1.17	2.3 - 2.5		1.06 - 1.05	2.3 - 2.5	

* Acute slab in same animal as chronic.
☐ Average of two trials; cortex was swelling during this test series.
△ Means are for data from electrode areas greater than 0.35 mm^2 where data from both slabs available.
Data is expressed as the range between the highest unsuccessful and the lowest effective stimuli.

predicted from its dry surface area. Therefore the conductance of each electrode is quoted in Table VI-5 as an index of the "effective" (rather than measured) electrode area. Since the conductance was observed to increase as the stimulating voltage was increased, the conductance at threshold is given in the table. The voltage applied across the electrode system is equivalent to current density per conductive pathway (amps/mho = volts), i.e., the current density per "effective" electrode contact area. The threshold current density per measured area of electrode is also given in the table ("current density"). Where more than one measurement with an electrode was made in a slab, the average for that electrode is given.

Comparison of conductances of the electrode-cortex contact (Table VI-6) shows little difference between acute and chronic preparations. The bipolar electrodes had a higher resistance than the monopolar electrodes since the area of contact was less in the former; also there were in effect 2 such electrodes in series in the bipolar system. Therefore the ratio of the conductance in the chronic slabs to the conductance of the acute slabs is given in the last column of the table (C/A). The average of this ratio is not significantly different from 1.00. The distribution of current through the cortex is probably much more restricted to a local area when bipolar electrodes are used than when the stimulus is monopolar (Penfield and Erickson, 1941).

Table VI-6

CONDUCTANCE OF CORTEX-ELECTRODE CONTACT

Exper. No.	Stimulating Electrode	Isolation Time (Weeks)	Chronic (mMhos)	Acute (mMhos)	C/A
1*	B	10	0.250	0.242+	1.03
2*	B	10	0.210@	0.314	0.67
3*	B	8	0.108	0.167	0.65
4*	M	7	0.995	0.925	1.08
5	M	9	0.930	0.560	1.66
6	M	65	0.925	0.870	1.06
Average			0.569	0.513	1.025

* Acute control in same animal as chronic
B Bipolar stimulating electrode.
M Monopolar stimulating electrode, averages from electrodes areas greater than 0.35 mm^2 where data for both slabs available (see Table VI-5).
@ Slab was unresponsive at the time the measurement was made.
\+ Intact contralateral suprasylvian gyrus.

D. DISCUSSION

Any attempt to explain the results of electrical stimulation of the cortex must include a consideration of which structures may be excited by a burst of stimulating pulses applied to the cortical surface. Notwithstanding the opinions of some authors (e.g., Purpura and Grundfest, 1956b; Grundfest, 1957, 1958, 1961) the somata of cortical neurones are electrically excitable and capable of action-spike propagation. Indeed, axon-hillock or axon initial-segment initiation of some spikes (Fuortes, Frank, and Becker, 1957; Coombs, Curtis and Eccles, 1957) has been shown for neurones of the motor cortex (Phillips, 1956b) and for pyramidal cells of the hippocampus (Kandel, Spencer and Brinley, 1961; Kandel and Spencer, 1961c; Spencer and Kandel, 1961a). Thus the voltage threshold for spike initiation is lower in the axon and axon hillock than in the soma. In terms of current this difference will be much greater because of the much greater area of somatic membrane which must be depolarized. Weak stimulus strengths are probably insufficient to excite the neurones directly (Phillips, 1956b).

There is some controversy as to whether the dendrites of motoneurones and of cortical neurones are electrically excitable (Eccles, 1960). The dendrites probably can maintain local responses at least (Bishop, 1956, 1958; Lorente de Nó, 1947, 1960). Spinal motoneurones can, under conditions of chromatolysis, develop all-or-none spikes (Eccles, Libet and Young, 1958). Dendrites of hippocampal pyramidal cells can also propagate all-or-none spikes (Cragg and Hamlyn, 1955; Spencer and Kandel, 1960, 1961b; Kandel and Spencer, 1961b).

a) Minimum electrical excitability of slab neurones.

Since neither the voltage thresholds for epileptiform afterdischarge nor the conductance of the electrode-cortex contact of the chronic

slabs are significantly different from those of acutely isolated cortex, the currents passed at threshold must also be identical in the acute and chronic slabs. It is not known, however, if the distribution of this current in the cortex is similar in the two preparations, and therefore this information by itself does not define the thresholds of the individual cells. However it was also shown that the threshold pulse widths (equivalent to the minimum utilization times) in the two types of preparations were the same. Thus it may be concluded that the electrical excitabilities of the stimulated cells in chronically isolated slabs are not different from those of acutely isolated cortical neurones. While this does not differentiate between the various possible alterations of post-synaptic events, it does eliminate the possibility that chronic denervation increases epileptogenesis in the cortex by altering the electrical excitabilities of the primary neurones stimulated.

The threshold pulse widths found in the present study for both acute and chronic slabs are in close agreement with the values reported by Gerin (1960) for intact cortex and by Pinsky and Burns (1962) for acutely isolated cortex.

The part of the cell excited by the stimulus, and whether the pre- or post-synaptic cell is the site of the focus may be determined by comparison of the threshold pulse-width requirements of the epileptiform afterdischarge with those of a known post-synaptic response, the DCR (Eccles, 1951; Clare and Bishop, 1955a,b; Frank and Pinsky, 1964). The minimum pulse width for epileptiform afterdischarge as found in this study is more than 20 times the minimum pulse width required for the DCR in either acutely or chronically isolated cortex (Goldring, O'Leary, Holmes and Jerva, 1961) for approximately equal pulse amplitudes. On

the basis that the axonal and somatic membranes must have quite different current thresholds (as outlined above) it seems reasonable to conclude that direct stimulation of the somata, rather than the axons, of the neurones of the focus is necessary to initiate afterdischarges, as compared with the DCR which results from activation of a pre-synaptic site (presumably axons or axon-terminals). A corollary is that synaptic activation alone (at least that produced by repetitive strong pulses of less than 0.2 msec duration) is unable to start afterdischarges.

The voltage thresholds found for both acute and chronic slabs are similar to those previously obtained for normal cortex (Gerin, 1960), for acute cortical slabs (Pinsky and Burns, 1962) or for chronic slabs (Grafstein and Sastry, 1957). However the finding that acute and chronic slabs have identical voltage and current thresholds contrasts with the results of previous investigators (Grafstein and Sastry, 1957; Sharpless and Halpern, 1962) who found that chronic isolation of the cortex lowered these thresholds. The difference between the results of Grafstein and Sastry (1957) and those of the present study may have two explanations. It is possible that in their study the slabs may have been depressed by the recent surgery, whereas in the present investigation acute slabs that were especially prone to spreading depression were rejected (<u>vide supra</u>, p. 51). Such slabs appeared to have an unusually high threshold for epileptiform afterdischarges and an unusually low threshold for spreading depression. The alternate explanation may involve the fact that Grafstein and Sastry, in determining the voltage threshold, used a stimulus frequency (8/sec) which was submaximal, and indeed very close to the threshold stimulus frequency for acute slabs (see Table VI-1). Ideally a maximal stimulus frequency should have been used. In the present investigation parameters

other than the one being studied were well above their respective thresholds. Goldring, O'Leary, Holmes and Jerva (1961) also obtained results which differ from those reported by Grafstein and Sastry (1957). The latter authors found that the voltage threshold for the DCR was higher in chronic slabs than in acute slabs, whereas Goldring et al. (1961) found no difference in the thresholds.

Sharpless and Halpern (1962) reported that chronic slabs developed a lower current threshold than intact cortex, at least over the first 3 weeks of isolation. Beyond this time they found it impossible to compare thresholds because of the great variation in the resistivity of the implanted stimulating electrodes. It seems likely that the difference in thresholds of the chronically isolated and intact cortex may have been due to differences in current distribution resulting from changes in histological structure or connective tissue above the slab during the first 3 weeks of isolation. The proliferation of connective tissue was probably more rapid over the chronic slabs than over the intact cortex because of the additional trauma received by the slabs. This latter difficulty was partly avoided in the present study by the removal, prior to testing, of all connective tissue above the pia-arachnoid, and the conductivities of the electrode-cortex contact were the same in acute and chronic slabs. Clearly there are differences in techniques used by the various workers in this field which could account for the different results obtained, but it is not apparent which differences in the techniques are the critical ones.

b) The minimum size of the focus of epileptiform afterdischarge.

Since the current spread and hence the number of neurones that will be stimulated depends on the voltage, the all-or-none relationship of

stimulus voltage to the duration of the afterdischarge indicated that either a critical total number or a critical density of excited (or "exhausted") cells is required to start an afterdischarge. The results of variation (over a 100-fold range) of the area of cortex stimulated allows further examination of this question. If the necessary factor is the total number of cells excited, then there should be an inverse relationship between the threshold current density (i.e., volts) and the area stimulated. Instead the stimulus voltage threshold was constant for electrode areas between 0.35 mm^2 and 16.75 mm^2, which implies that the critical factor, when large areas are stimulated, is a minimum density of stimulated cells.

The all-or-none curves of the discharge duration vs. the number of stimulating pulses or pulse width also confirms the concept of a minimum density of stimulated cells required for an epileptiform afterdischarge. In these cases the voltage, and therefore the spread of the current, was held constant. Therefore there must have been an increasing density of excited cells as either of these stimulus parameters was increased. Since the discharge duration is independent of the stimulus pulse width or number of pulses, the discharge duration must also be independent of the density of neurones, above this minimum, excited by the stimulus. Possibly this is because, in both acute and chronic slabs, synaptic spread of the discharge involves more neurones more rapidly or more effectively than does the increase in the number of stimulating pulses.

In contrast to the independence of the voltage threshold from the area of cortex stimulated for large electrode sizes, the voltage threshold was much higher when the stimulating electrode was reduced to

0.17 mm^2. This implies that the current spread must extend beyond the small electrode area, or that a greater proportion of the cells in this small focus must be excited before an afterdischarge will occur. Quite possibly both these conditions must be met to elicit an afterdischarge with the small-area electrode. In any case there appears to be a minimum volume of cortex (i.e., that under 0.17 - 0.35 mm^2 of cortical surface) which must be excited to set up an epileptiform afterdischarge. Such a volume must contain many thousands of neurones. This minimum number of neurones must be stimulated a minimum number of times, in chronic slabs as well as in acute, for an afterdischarge to occur. These results also give a negative answer to the question posed by Pinsky and Burns (1962) of whether a single neurone can be driven to epileptiform afterdischarge.

The volume stimulated by bipolar electrodes placed 1.5 - 2 mm apart on the cortical surface would be well above this minimum volume, and thus the stimulated area also conforms to the requirement that stimulus parameters, other than that being tested, be greatly suprathreshold.

c) Stimulus frequency and the afterdischarge.

The major differences between chronically and acutely isolated cortex, apart from the difference in discharge durations, were in the relationships between frequency of stimulation and the initiation and maintenance of the epileptiform afterdischarge. Two alternate hypotheses might be advanced to explain the lower stimulus frequency threshold after chronic isolation: (1) the "exhaustion" state of each neurone in the chronic slabs might last longer between stimulus pulses (i.e., repolarization might be slower or the depolarizing afterpotential be greater or more prolonged than normal) or (2) the self re-excitation of the

neurones during the interval between stimulus pulses might be more effective, as would be the case if the cells were supersensitive to impinging synaptic excitation or if inhibition became less efficient. The first hypothesis seems unlikely, since it would require depolarizing afterpotentials lasting over 400 msec to explain the development of seizures at stimulus frequencies of 2.5 to 3 pulses per sec. Such afterpotentials would be expected to decrease the electrical threshold (current x pulse-width), which did not happen. Even in normal cortex the afterpotentials (Kandel and Spencer, 1961c) are not of sufficient duration to explain the frequency threshold on this basis. Moreover, previous studies have shown that denervated structures have normal action-potential configurations. For example, Nicholls (1956) and Levine (1961) found that the shape of action potentials in denervated frog muscle was normal. Kandel and Spencer (1961a,b) made intracellular recordings of spike potentials of hippocampal pyramidal cells chronically deprived of their afferent supply from the fornix. Spikes of these cells were of normal amplitude and configuration. During seizures these partially denervated neurones exhibited smaller depolarizing afterpotentials than those with their afferent supply intact. Records presented by these authors suggest that these denervated preparations gave much longer seizures than intact hippocampus, indicating that in this respect the deafferented hippocampus preparations are comparable to chronic slabs of cerebral cortex. Other types of experimental epileptic foci also have lower than normal stimulus frequency thresholds (Matsumoto and Ajmone-Marsan, 1964b), and the configurations of action potentials of such epileptic cells were not different from those of normal neurones (Atkinson, Macs and Ward, 1961). Results obtained below (Section VIII) also suggest that depolarizations of chronic slab cells are

of normal duration. It must therefore be concluded that the second hypothesis is the more likely one.

One is then left with the alternates that either decreased synaptic inhibition or increased synaptic excitation is the cause of the lowered stimulus frequency threshold of chronic slabs. IPSP's in cortical cells have longer time courses and lower amplitudes than do EPSP's (Kandel et al., 1961) and therefore with low frequency stimulation of synaptic pathways, summation of IPSP's predominates, while with high frequency stimulation, afterpotentials and EPSP's summate and overwhelm the IPSP hyperpolarization. Thus following destruction of pyramidal cells, which are part of the pathway for recurrent inhibition in the cortex, a lower frequency might be capable of initiating a discharge. Sawa et al., (1963) suggest that the termination of seizures occurs when the discharge frequency decreases enough for the IPSP's to predominate. On the other hand, it is the pyramidal cells themselves which are inhibited by this pathway, and the chronic isolation could be said to provide the ultimate inhibition of these cells: namely their death.

In contrast to initiation of afterdischarges, which is quite similar in acute and chronic slabs, the process which maintains the discharges in chronic slabs must be very different from that of acute slabs. Discharges of both acute and chronic slabs are independent of stimulus pulse width, strength, and the number of pulses. However in acute slabs the discharge is independent on the stimulus frequency whereas the paroxysmal afterdischarge of chronic slabs is independent of stimulus frequency as well as the other parameters, i.e., it is completely independent of the exciting stimulus, the maximal discharge characteristic of the particular slab resulting from a minimal effective stimulus. The duration of the

discharges in chronic slabs must therefore depend on processes initiated by the discharge itself, some form of self maintenance or auto-excitation. Obviously this process is more effective in chronically isolated than in acutely isolated cortex. The same alternates may be suggested for this process as those discussed above for the difference in frequency threshold. In addition to those arguments already advanced against a hypothesis of decreased inhibition, it may be questioned whether the presence or absence of pyramidal cell inhibition would affect the duration of the seizures, for Kandel and Spencer (1961a,b) showed that the deafferented hippocampal pyramidal cells can be strongly inhibited without shortening the seizure. The most plausible explanation is that the seizures of chronic slabs are prolonged by recurrent excitatory synaptic pathways which are made supersensitive by denervation, so that minimal synaptic activity is capable of maintaining the seizure.

Some other evidence is available to suggest such a concept. Prolonged discharges can be maintained in acute slabs by augmented synaptic activity. Rosenblueth, Bond and Cannon (1942) described how epileptiform activity could be prolonged by administration of low-frequency shocks. (the "epileptiform sustaining response", ESR). Sanders and Pinsky (personal communication) have succeeded in producing the ESR in acutely isolated slabs by starting weak stimulation, at about the natural frequency of the clonus, just before the expected end of an afterdischarge. The stimulus pulse width used could be as brief as that used to initiate the DCR, i.e., a pulse which excites axons or axon-terminals directly but not the cell body. These stimuli could be applied anywhere in the slab, not necessarily at the original focus. Thus the effects of this stimulation are likely to be synaptically mediated. Discharges could often be

sustained for several minutes in this manner. In at least one animal the discharge was prolonged over 45 minutes, or in the same range as the very prolonged afterdischarges observed in some chronic slabs. It is suggested that the ESR stimuli were reinforcing synaptic activation produced by the discharge itself so that the synaptic activation was sufficient to maintain the discharge. This may be equivalent to reinforcement of synaptic activity by development of denervation supersensitivity.

Whereas Pinsky and Burns (1962) were able to deduce the refractory period of the excited cortical neurones of acute slabs from the stimulus frequency which produced seizures of maximum duration, this approach cannot be used in chronic slabs because of the all-or-none relationship between the stimulus and the afterdischarge. According to the formula developed by Pinsky and Burns the threshold number of pulses should vary inversely with the stimulus frequency, and it was expected that deviations from this relationship might indicate the frequency at which the refractory period might begin to interfere with the effectiveness of some of the stimulus pulses. The results did not conform to this relationship for either chronic or acute slabs. The threshold number of pulses was the same for all frequencies. The higher threshold at 20/sec is probably the result of the experimental procedure whereby the 20/sec threshold was the first tested, and some of the slabs survived only for the one threshold determination. It might be deduced that the "refractory" periods of cells in chronic slabs are normal, since the thresholds of chronic and acute slabs are the same at each stimulus frequency. On the other hand the proposed relationship between stimulus and discharge is possibly incorrect. It was assumed that the refractory period was an absolute value, whereas it may be dependent on interactions between cells which might be frequency

dependent, such as occlusion between synaptic and electrical activation. In any event, the chronic slabs were not different from the acute.

In summary, the concept of increased and prolonged excitatory synaptic effectiveness due to denervation supersensitivity would appear to be the most satisfactory explanation for the decreased stimulus frequency threshold, and the independence of the discharge from the stimulus in chronically isolated cortical slabs. If supersensitivity to chemical transmitters does in fact develop with chronic isolation of cortical slabs, then the effects of interactions of neurones in such slabs should be expected to lower the threshold for any stimulus parameter, provided that the rate of stimulation is sufficiently slow that recurrent synaptic activation builds up the required "epileptic state" faster than does the direct electrical activation. This proviso is necessary to explain why, in this study, no differences were observed for stimulus strength, pulse width, or number of pulses. These parameters were observed at frequencies of 20/sec or more, which was quite a bit above the threshold frequency. This may be the basis of the seeming discrepancy between the results of this study and those of Grafstein and Sastry (1957) with regard to the voltage threshold, as discussed above (p. 111). This may also explain why Halpern (1962) failed to detect any difference between the pentylenetetrazol thresholds of chronically isolated and intact cortex, while Echlin et al., (1953) found that pentylenetetrazol selectively activated chronically isolated cortex. However, it may be that in the experiments of Echlin et al. the latency of onset of seizures was shorter in the chronic slabs, while the threshold dose level of pentylenetetrazol was the same in the chronic slabs and in the intact cortex.

VII. REISOLATION OF THE CHRONIC SLABS

A. INTRODUCTION

Spontaneous activity can be recorded from the surface of an unstimulated chronically isolated slab of cerebral cortex (Grafstein and Sastry, 1957). This contrasts with slabs acutely isolated by the same technique which are electrically silent if completely isolated (Burns, 1958). An almost normal electrocorticogram is retained with an incomplete acute isolation. This suggested that the continual activity in the chronically isolated slabs might be due to regeneration of axons carrying impulses across the cut, particularly if some fibres had been only crushed rather than severed by the isolation surgery. Functional regeneration of neurones in other areas of the central nervous system has been observed by previous workers (Windle, 1955; Liu and Chambers, 1958; McCouch, Austin, Liu and Liu, 1958). Functional regeneration might also explain the prolonged afterdischarges which occur in the chronically isolated slabs. There might be a spread of the seizures outside the slab and back via regenerated axons. Thus both the spontaneous continual activity and the longer afterdischarges of the chronically isolated slabs might be maintained by activity entering the slabs from the surrounding cortex. On the other hand the spontaneous activity might arise solely within the chronic slab as a consequence of denervation. Chronically denervated mammalian skeletal muscle is known ti fibrillate, a phenomenon which is associated with an oscillation of the resting membrane potential (RMP) and random spikes in individual fibres (Li, Shy and Wells, 1957). The oscillations in the RMP may be due to a reappearance of EPSP's without reinnervation (Birks, Katz and Miledi, 1960; Miledi, 1960) rather than to an "instability" of the membrane.

In an attempt to resolve this point 6 chronically isolated slabs were reisolated to sever any remaining or regenerated connections between the slab and the rest of the cortex.

B. METHODS

The isolation technique was much like that employed originally to isolate the slab. However there were several additional technical difficulties due largely to the formation of a tough glial scar in the original cut and in the original drainage hole. It proved almost impossible to make a new hole (to provide access for the re-undercutting of the slab) in the original location. Moreover the area usually was highly vascular and bleeding was difficult to control. The scar tissue in the old isolation cut was very resistant to the blunt knives used for isolation. The first 2 reisolations attempted were cut approximately in the original line of section, a practice which was abandoned when it was found that this procedure led to extensive damage in the second reisolation attempted. In subsequent experiments an attempt was made to reisolate the slabs by sectionning about 1 mm outside the original cut. Even with this type of peripheral reisolation the scar tissue interferred with the smooth manipulation of the knives, and caused distortion of the slab and surrounding cortex. Some damage to the pial circulation was impossible to avoid. Sharper knives could not be used because of the danger of severing the pial blood vessels. The reisolation undoubtedly caused much more trauma than the original (or acute) isolation.

C. RESULTS

Since there was considerable trauma involved in the reisolation procedure, it was necessary to obtain physiological evidence concerning the condition of the reisolated slabs. On the basis of such evidence it can be assumed that the physiological state of 4 of the slabs was not modified greatly by the reisolation. In 2 of the animals the slabs were reisolated during the course of afterdischarges which had been initiated more than 3 hours previously. In both the discharge apparently survived the reisolation procedure. In both cases the discharge could not be altered by suprathreshold stimulation (as determined prior to the reisolation) after the reisolation. In one of these cases the discharge stopped after a half hour after the reisolation and then a just suprathreshold stimulus started another long discharge. The other slab was still discharging 3 hours after reisolation and further tests could not be made.

In the preparation which stopped discharging 1/2 hr after reisolation, and in 2 of the other preparations (Table VII-1) it was observed that the duration of the discharges produced by pre-reisolation suprathreshold stimulation were unchanged from those obtained prior to reisolation, and the discharge pattern peculiar to each animal was retained. The threshold was carefully redetermined in one of these reisolated slabs and was found to be unchanged (Table VII-1, animal 1). Also in the preparation which discharged for 1/2 hr after reisolation (Table VII-1, animal 3) the threshold could not have been significantly raised since the weakest stimulus tried after reisolation was only slightly above the original pre-reisolation threshold, and this stimulus caused a discharge of over 1 hr. In the three animals just discussed it

Table VII-1

REISOLATION OF CHRONICALLY ISOLATED SLABS

Exper. No.	Isolation (weeks)	Stimulus			Afterdischarge duration (sec)	
		freq. (pps)	no. of p. pulses	width (mSec)	Initially	after reisolation
1	9	2	100	2	0	0
		3			175	185
		5			190	185
		10			195	195
		25			205	35 + Sp. dpr.
		50			185	47 + Sp. dpr.
		100			210	47 + Sp. dpr.
		200			200	Sp. dpr.
2	18	50	100	2	116	114; 102
			200		48	88
		100	100		>1800	59 + Sp. dpr.
3	8	20/s	160	0.1	0	
				0.2	240; 230	▼No change
				0.3	>3 hrs.	>300
				1.0	▼No change	65 + Sp. dpr.
				5.0	▼No change	65 + Sp. dpr.

Sp. dpr. = Spreading depression.
▼ = Still discharging after 0.3 ms stimulation before reisolation.

also was observed that strong stimulation (several times greater than the threshold) resulted in afterdischarges which were superimposed on a wave of spreading depression (Grafstein, 1956) which reduced the duration of the afterdischarges (Fig. VII-1 and Table VII-1).

In the remaining 2 of the 6 reisolated preparations, the reisolations were exceptionally difficult, and the slabs were extensively damaged. In these the threshold was raised and the duration of the afterdischarges greatly reduced. One slab which had given afterdischarges of 3 to 4 minutes before reisolation gave a 40 sec afterdischarge upon the first trial after reisolation. The duration of the discharges progressively decreased to 10 sec by the third trial and spontaneous spreading depression occurred between trials. This slab became totally unresponsive before further trials could be made. The other slab gave discharges of 40-75 sec before reisolation, while after reisolation discharges of 5-30 sec with much reduced amplitude were observed. This slab was the most difficult reisolation, and one which resulted in over half the slab being destroyed outright and most of the remainder being severely blanched. Testing had to be confined to a small (2x3 mm) area.

Several animals at the start of this investigation were examined for the presence of afterdischarge in cortex adjacent to the chronic slab during afterdischarges in the slab (e.g., Fig. VIII-11). In one case, afterdischarge spread out of an incompletely isolated acute slab, and in one case afterdischarge spread out of a chronic slab.

Chronic slabs generally showed considerable spontaneous activity. This was of two kinds. The first was a recurring pattern which somewhat resembled the afterbursts in acutely isolated cortex as described by Burns (1954). The bursts did not, however, have the pronounced positive

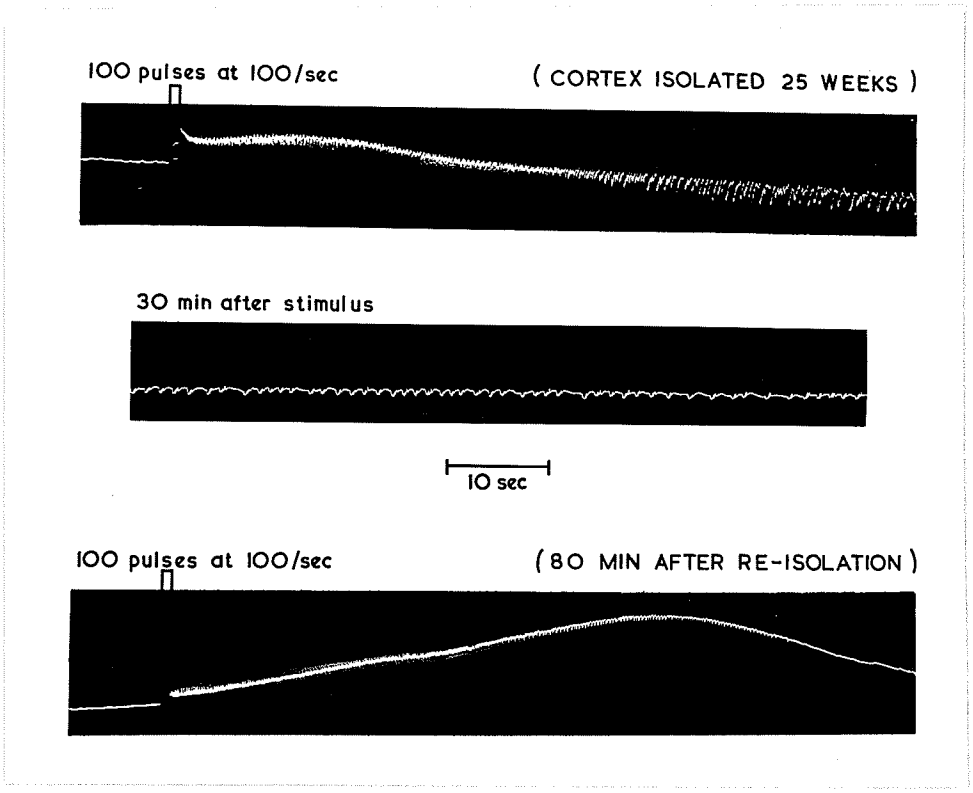

Fig. VII - 1 Effect of reisolation on afterdischarges

Upper record is tangential potential at the start of a prolonged epileptiform afterdischarge (Table VII - 1, Exper. 2).
Middle record is the same afterdischarge a half hour later.
Bottom record is the response to the same stimulus as in the upper record, 80 min after reisolation of the slab. Only the fast low amplitude activity characteristic of the start of afterdischarges in chronic slabs is present. This is followed by a large (10 mV) swing of spreading depression.

D-C component described by Burns. The elicited burst responses of Fig. VI-6 are almost identical in form to the afterbursts, and illustrate the difference between the bursts of the two types of slabs. The second type of spontaneous activity in the chronic slabs consisted of an attenuated ECG similar to that of normal intact cortex. Both kinds of spontaneous activity were almost completely eliminated in all slabs by the reisolation, in the most viable slab (Fig. VII-2) as well as in the most severely damaged ones. Some afterbursting, (bursts of 0.5-1.0 sec duration occurring irregularly at intervals of not less than 15 sec) were observed in one animal about an hour after reisolation. In contrast to the afterbursting activity before reisolation, this afterbursting had a definite positive D-C component and more closely resembled the afterbursting of acutely isolated cortex.

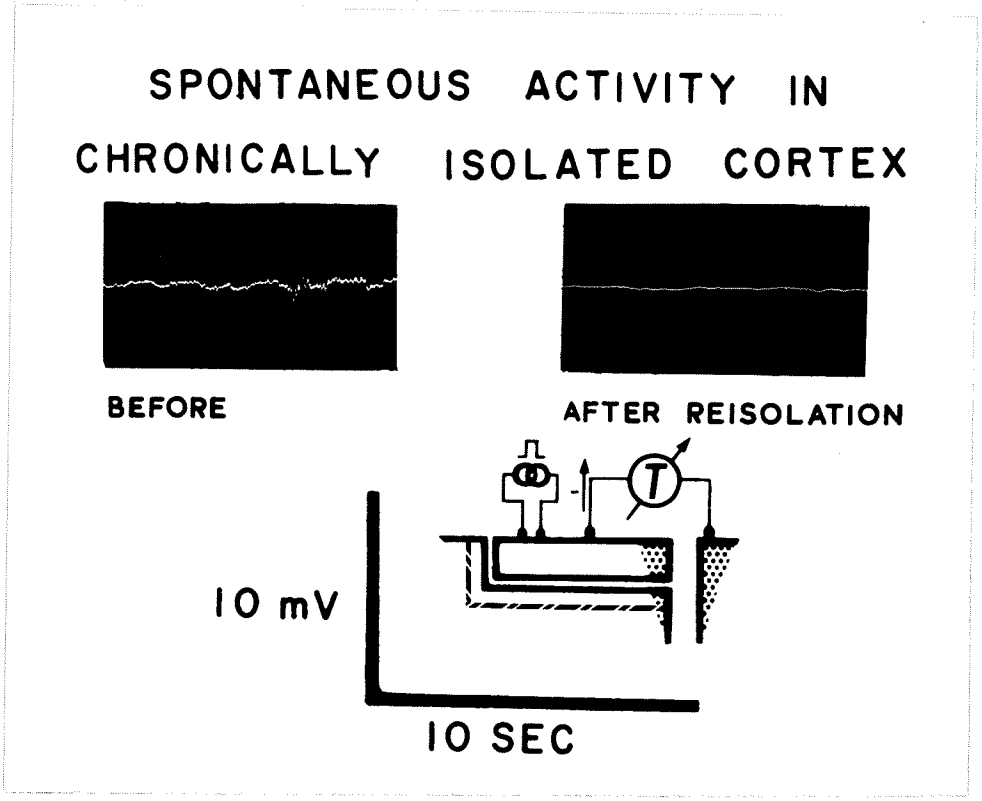

Fig. VII - 2 Effect of reisolation on spontaneous activity of chronic slabs

Left: Spontaneous activity in slab isolated 9 weeks. (Table VII - 1, Exper. 1).

Right: Reisolation of the slab eliminated all spontaneous activity, but did not alter excitability.

D. DISCUSSION

The principal conclusion that can be drawn from the results obtained using reisolated chronic slabs is that the maintenance of prolonged afterdischarges in chronically isolated cortex must be independent of any cortico-cortical pathways and must depend entirely upon the altered properties of the cell population within the slab. The ability of the chronic slabs to maintain long afterdischarges is unchanged by the reisolation except that strong stimulation is then prone to cause spreading depression.

There is other published evidence to suggest that a reverberating circuit with the surrounding cortex could not be responsible for the prolonged seizure maintenance in chronically isolated cortex. While Sharpless and Halpern (1962) found that seizures initiated in intact cortex contralateral to the chronic isolation had an increased tendency to spread and hence to last longer, minimal effective stimulation still produced the same short localized seizures in the contralateral cortex, whereas the chronically isolated cortex yielded maximal seizures in response to minimal stimulation. Livingston, French, Richland and Konigsmark (1956) reported that seizures could not spread out of cortex isolated along the sides but not undercut (although a generalized seizure could spread inwards) presumably because the bombardment of surrounding cortex by cortico-cortical paths via the white matter was not intense enough to start up a seizure. Similar results have been reported for both acutely and chronically partially isolated slabs (undercut and isolated along 3 sides) by Echlin, Arnett and Zoll (1952), Echlin (1954, 1956, 1959) and Echlin and Battista (1961). They found that seizures initiated in partially isolated cortex by direct electrical stimulation, by topical

application of ACh, or by peripheral nerve stimulation only occasionally spread out of the partially isolated area. Thus it is unlikely that the chronically isolated cortex, with a few connections with the surrounding cortex, could drive the rest of the cortex sufficiently to re-excite the slab.

The "continual" spontaneous activity seen in chronic slabs is probably due to some viable connections with the cortex surrounding the slabs, even though histological evidence for this was never seen (vide supra, Section V). The "continual" portion of the spontaneous activity observed in the chronic slabs is quite different from the spontaneous activity that other investigators have observed in acutely isolated cortex (Kristiansen and Courtois, 1949; Echlin, Arnett and Zoll, 1950, 1952; Echlin, Arnett, Zoll and Peck, 1952; Henry and Scoville, 1952; Preston, 1955; Ingvar, 1955a, b; Domino, 1957). The latter type of activity almost uniformly appears to be the afterbursting described by Burns (1954). Although Domino (1957) has taken the presence of this activity as a necessary index of the viability of isolated cortex, it is evident that perfectly viable cortical slabs may be electrically silent (Burns, 1958; Sanders, 1963).

Echlin (1959) and his co-workers (op. cit.) have postulated that this spontaneous activity (afterbursting) seen on acute isolation is the basis of focal epilepsy. However, the susceptibility to prolonged epileptic seizures develops gradually over several weeks following isolation (Sharpless and Halpern, 1962) rather than immediately. In the present experiments, afterbursting was observed after reisolation in only one of the 4 viable reisolated slabs, which is about the same incidence as the occurrence of afterbursting in unstimulated acute slabs. The

afterbursting seen in this one animal may have developed the more pronounced positive D-C component after reisolation because of the absence of other activity, as in acute slabs. Thus the presence or absence of afterbursting appears independent of epileptic phenomena such as the long lasting afterdischarge in the chronic slab, nor is afterbursting an epileptic phenomenon itself. It is known that brief anoxia will stop such spontaneous afterbursting in acute slabs, so it is possible that brief hypoxia during the reisolation surgery was responsible for eliminating the afterbursting in the chronically isolated slabs. Whereas afterbursting can occur in completely isolated cortex independent of connections with the rest of the cortex, it appears that the "continuous" part of the spontaneous activity of chronic slabs was dependent upon such connections (possibly fibres close to the pia mater which escaped complete destruction by the initial isolation) since this activity was completely eliminated by the reisolation.

VIII. MICROELECTRODE STUDIES OF THE RADIAL POTENTIALS ASSOCIATED WITH EPILEPTIFORM AFTERDISCHARGE IN ACUTELY AND CHRONICALLY ISOLATED CORTEX

A. INTRODUCTION

The amplitude of the brief negativity, deep in the cortex, which follows subthreshold epileptogenic stimuli in acutely isolated cortex (the negative after-deflection, "n.a.d.") is closely related to the intensity of the stimulation (Pinsky, 1961, 1963). The time course and depth of occurrence of this potential suggests that it is caused by differing rates of repolarization in the superficial and deep ends of radially oriented neurones in the cortex after they have been completely depolarized by the stimulus. The deep ends of these neurones apparently repolarize slower than the superficial ends. Pinsky (1961, 1963) and Pinsky and Burns (1962) suggest that this potential gradient along radially oriented cortical cells is responsible for the outbreak of epileptiform afterdischarge, and that the amplitude of the "n.a.d." is therefore critically related to the threshold for epileptiform afterdischarge.

Similarly Gloor et al. (1961, 1962) and Gloor (1962) have suggested that the maintenance of a potential gradient between the somatic and superficial-dendritic ends of the neurones is essential for the maintenance of epileptiform discharge. Since these observations imply that radial potential gradients play a causative role in the generation of epileptiform afterdischarges these gradients have been examined in acutely and chronically isolated slabs of cerebral cortex following sub- and suprathreshold epileptogenic electrical stimulation.

B. METHODS

a) General methods.

The radial cortical potentials which occur following epileptogenic electrical stimulation were examined using extracellular microelectrodes in 15 chronic and 12 acute slabs of cerebral cortex. In all cases the surface reference electrode was as close as possible to the point of insertion of the glass microelectrode through the pia. Shafts of the microelectrodes used were usually between 50 and 70 mu in diameter, because smaller electrode shafts tended to bend and break in attempts to puncture the pia of chronic slabs. Electrodes having shafts of 40 mu could be used without such trouble in acute slabs because the pia of acute slabs was not as tough. The tip diameters of the microelectrodes (between 2 and 7 mu) had no influence on the ease with which the electrodes penetrated the pia. In a couple of experiments the pia of chronic slabs was cut (using a razor blade held in a micromanipulator) to facilitate penetration with the microelectrode.

b) Measurement of depth of microelectrode tip penetration.

Measurement of the depth of the microelectrode tip in the cortex was difficult because of several factors. The most difficult problem was the distortion of the cortex by the microelectrode. Because of the plasticity of the cortex and the resistance of the pia to puncture, the microelectrode tended to drag the pia both on insertion and withdrawal. Since the distortion appeared to be less on withdrawal, all electrode placements and measurement of depth were made during withdrawal of the microelectrode after it had been thrust through the cortex.

Even localization of the surface proved difficult. The electrode could make electrical contact before making physical contact with

the pia because of a layer of leucocytes which gradually formed over the surface of the cortex. For this reason visible dimpling of the pia was used to confirm physical contact with the pia, even though the process of dimpling implies distortion of the cortical surface. Moreover, the tip of the electrode (but not the shaft) may have punctured the pia when the dimpling occurred. The last point of contact on withdrawal was even more difficult to assess because a long thread of tissue frequently adhered to the electrode, providing electrical contact even when the electrode tip was more than 0.5 mm above the pia. Another criterion for locating the surface was the appearance of noise and blood pressure artifacts as the electrode tip neared the surface (see Fig. VIII-1). This noise disappeared when the electrode tip re-entered the cortex. Similar artifacts also were seen when the electrode tip was deeper than the cortex.

In practice the electrode was lowered with the Prior mechanical micromanipulator until the pia was just visibly dimpled by the electrode tip. This point was used as the zero reference for depth ("dial" depth). Further movement of the electrode was controlled by the hydraulic micromanipulator system. Both the dimpling and the possibility that the tip had already punctured the pia tended to make the point at which contact was lost during withdrawal above the original arbitrary "dial" zero. As will be described below, a correction factor based on the recorded potentials was added to the "dial" depth.

C. RESULTS

a) The negative after-deflection ("n.a.d.").

Radially oriented potential changes at the focus of stimulation following subthreshold epileptogenic electrical stimulation were observed in chronically and in acutely isolated slabs of cerebral cortex. These potential changes were similar in form and amplitude to those observed by Pinsky (1961, 1963) in acutely isolated cortex. The deeper layers of the cortex became negative with respect to the surface for several seconds after stimulation.(e.g., Fig. VIII-1).

(i) Distribution of the amplitude of the "n.a.d." with depth in cortex. The magnitude of the "n.a.d." at various depths was examined in 11 acute slabs and in 6 chronic slabs. A standard stimulus, previously determined to be just subthreshold, was given at each stopping point (0.07 to 0.14 mm apart) during the withdrawal of the microelectrode from the depths of the cortex. The results obtained in typical experiments are shown in Fig. VIII-1 and Fig. VIII-2.

It was found that a slight "n.a.d." could often still be recorded when the electrode tip was completely withdrawn from the cortex according to the "dial" reading on the micromanipulator. This was especially the case when threads of tissue remained adhering to the electrode (e.g., Fig. VIII-2). To compensate for this, and also for the possibility that the electrode tip was deeper than the "dial" reading, the following adjustment was made. It was assumed that the curve of "n.a.d." vs. depth was linear as zero depth was approached, and that the amplitude of the "n.a.d." at zero depth should be zero (since both electrodes would then be at the same point). These assumptions rarely held true in practice because the orientation of the electrodes, while radial when far apart,

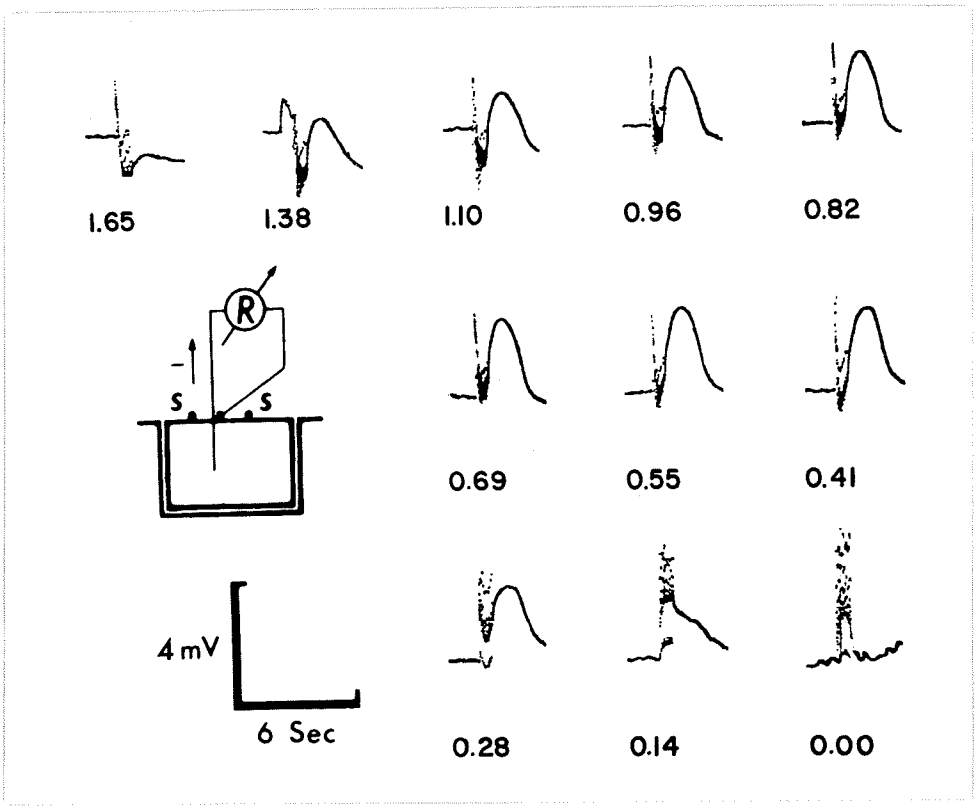

Fig. VIII - 1 "n.a.d."s at various depths in acutely isolated cortex

Numbers under each record are the depth, in mm, of the microelectrode tip.
Records were made during the withdrawal of the electrode.

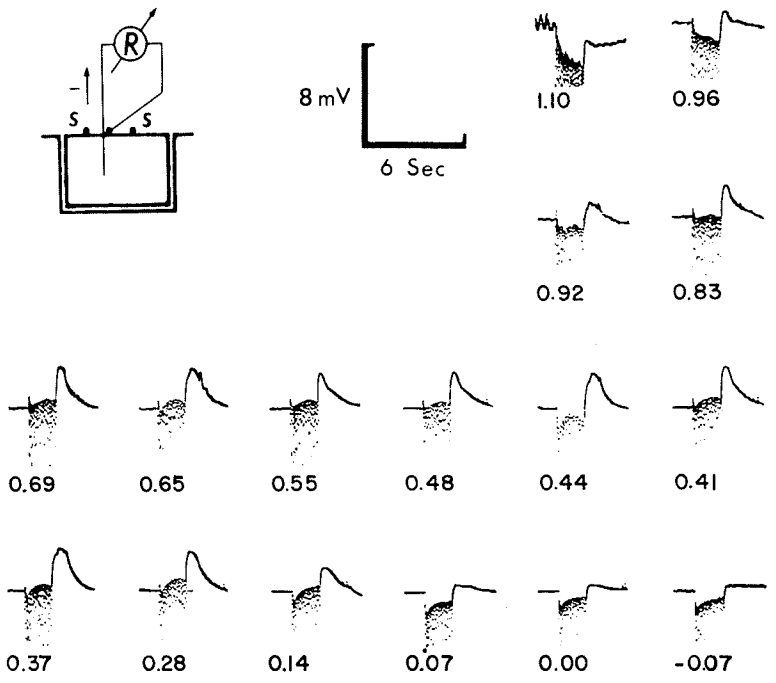

Fig. VIII - 2 "n.a.d."s at various depths in chronically isolated cortex

As for Fig. VIII - 1. Cortex isolated 22 weeks.

became more nearly tangential as the microelectrode tip approached the surface. The amplitude of the "n.a.d." was plotted against the "dial" depth. The curve thus obtained was extrapolated to zero amplitude, ignoring any departure from linearity at low amplitudes of the "n.a.d.", and the intercept on the "depth" axis assumed to be the true zero. The "dial" depths were then adjusted according to this new estimate of the "true" zero depth. The usual corrections obtained in this manner were between 0.1 and 0.2 mm. This adjustment has been made to the depths listed in all figures.

The usual distribution of "n.a.d." amplitude is similar to those shown in Fig. VIII-3; this is a curve with a single peak occurring between 0.35 and 0.85 mm deep in the cortex. Several distribution curves, however, showed two peaks both of which occurred between 0.35 and 0.85 mm (Fig. VIII-4). Of the 11 acute slabs, a double peak in "n.a.d." distribution was obtained in 2; one of these gave only a single-peaked curve in a later test while the other gave only a double-peaked curve in 4 tests over a period of 20 hours and during both insertion and withdrawal of the electrode. All 6 chronic slabs showed a double peak in the "n.a.d." distribution curve in at least one test; however curves with single maxima were obtained in earlier tests in 3 of these slabs, and in later tests in another. The data making up the double-peaked curve in one chronic slab is shown in Fig. VIII-2, while the single-peaked "n.a.d." distribution from the same chronic slab is graphed in Fig. VIII-3. The remaining 2 chronic slabs were tested only once. Thus the incidence of occurrence of at least one double-peaked distribution of "n.a.d." vs. depth is as follows:

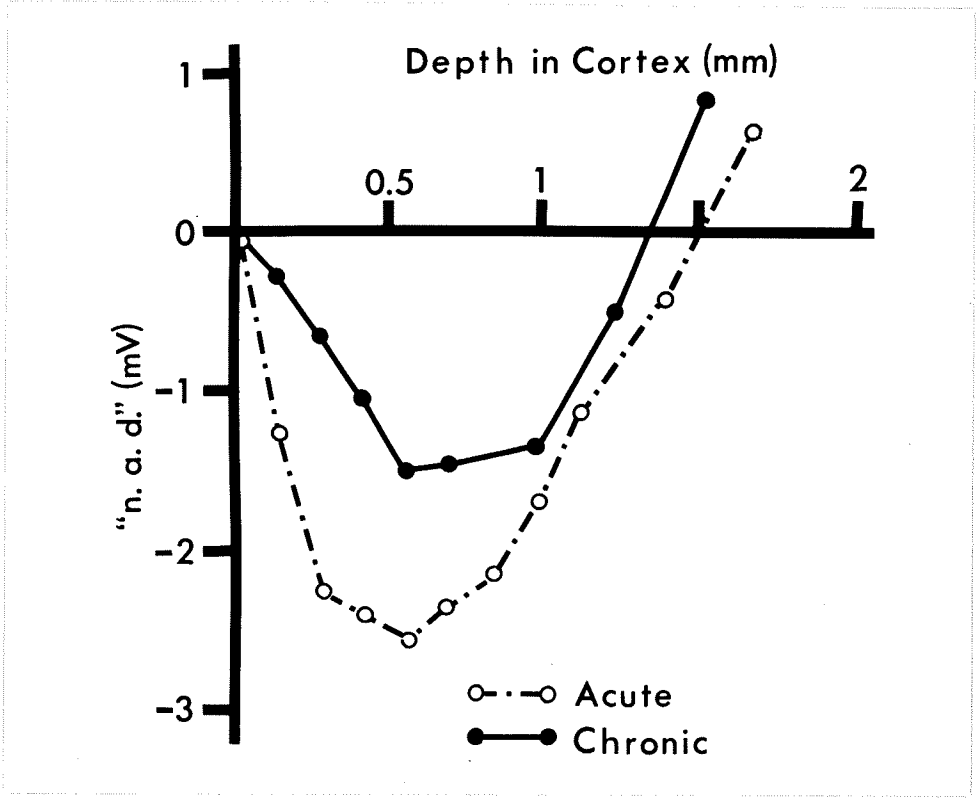

Fig. VIII - 3 "n.a.d." vs. depth (single-peaked curves).

These are the results of typical experiments.
The chronic slab was isolated 22 weeks.
Each curve was obtained from a single electrode penetration.

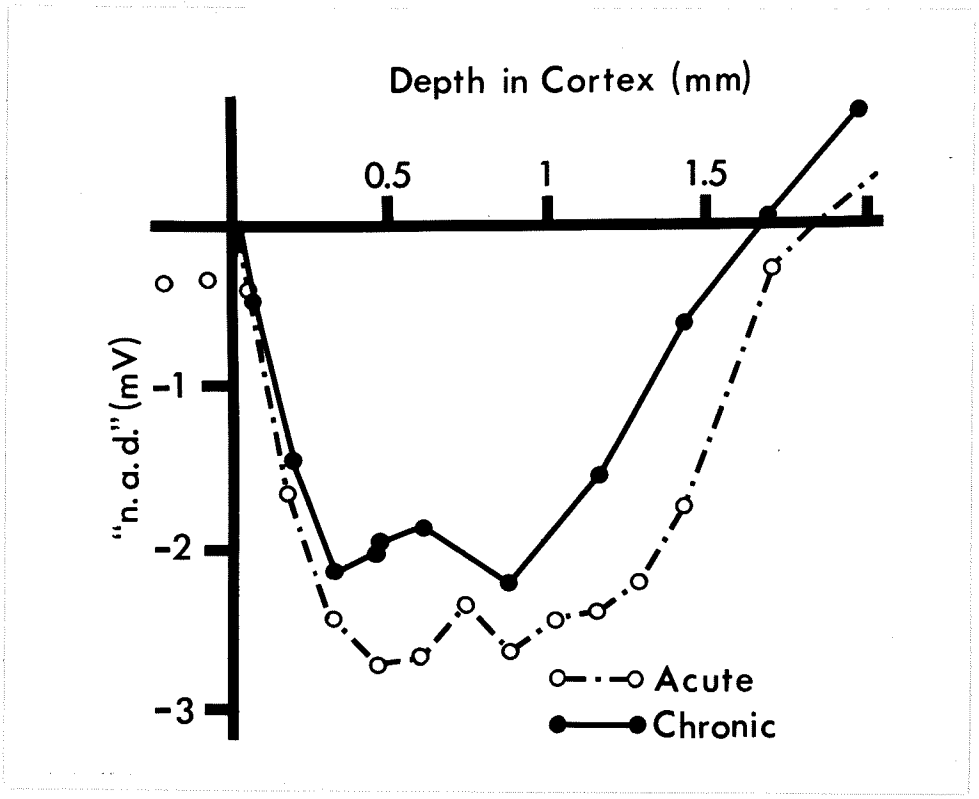

Fig. VIII - 4 "n.a.d." vs. depth (double-peaked curves).

Results of single typical experiments.
The chronic slab was isolated 8 weeks.
Each curve was obtained from a single electrode penetration.

	Acute	Chronic	Total
Only single-peaked:	9	0	9
At least one double-peaked:	2	6	8
Total:	11	6	17

The exact probability (single-tailed) for a 2-by-2 table with this distribution is 0.0023. Thus the difference in incidence of double-peaked distributions of "n.a.d." vs. depth is statistically significant.

A comparison of depths of maximal "n.a.d."s is shown in Table VIII-1. Where only double-peaked curves were obtained the depth of the largest peak is given in the table; otherwise the quoted data is from single-peaked curves. For various reasons complete curves could not be obtained in the chronic slabs of experiments 3, 4 and 5. The other point necessary to describe the average curves is the depth at which the polarity of the "n.a.d." becomes positive rather than negative relative to the original base line. The mean and standard error of this point was 1.45 ± 0.15 mm for the 6 chronic slabs, and 1.38 ± 0.11 mm for the 11 acute slabs. The difference is clearly not significant.

(ii) The decay time constant of the "n.a.d.". The time constants for the decay of the "n.a.d." are presented in Table VIII-2. These were estimated graphically from the slope of a line fitted by eye to a logarithmic plot of the amplitude of the "n.a.d." vs. the time elapsed from the peak of the "n.a.d." (Fig. VIII-5). Each value in the table is the average of the time constants of 3 haphazardly selected "n.a.d."s from each slab. There is no significant difference between the rates of decay of "n.a.d."s in chronic and acute slabs.

(iii) Relationship of the magnitude of the "n.a.d." to the threshold for epileptiform afterdischarge. The magnitude of the

Table VIII-1

DEPTH OF MAXIMUM "n.a.d."

Exper. No.	Weeks Isolation	Depth (mm) CHRONIC	ACUTE
1	0	-	0.78
2	0	-	0.75
3*	77	-	0.36
4*	71	-	0.35
5	60	-	0.50
6	22	0.41	0.65
7	15	0.50	0.50
8*	3	0.50	0.50
9*	8	0.35	0.35
10*	10	0.75	0.73
11	13	0.86	0.78
MEANS		$\overline{0.56}$	$\overline{0.57}$

* Indicates acute and chronic slabs in same animal

Exper. 1 and 2 were initial test expts. on acute preparations.

Table VIII-2

DECAY TIME CONSTANTS OF "n.a.d."

Exper. No.	Weeks Isolation	Time Constants (ms) CHRONIC	ACUTE
1	0	-	1095
2	0	-	740
3*	77	-	1350
4*	71	1680	840
5	60	660	1140
6*	13	650	455
7	22	965	950
8	15	395	795
9*	3	360	470
10*	8	2100	1980
11*	10	1260	1545
12	13	1200	600
MEANS		1030	997

* Indicates acute and chronic slabs in same animal.
Exper. 1 and 2 were initial test Expts. using only acute slabs.
"n.a.d.'s" not recorded from chronic slab of Exper. 3 because the electrode was too deep.

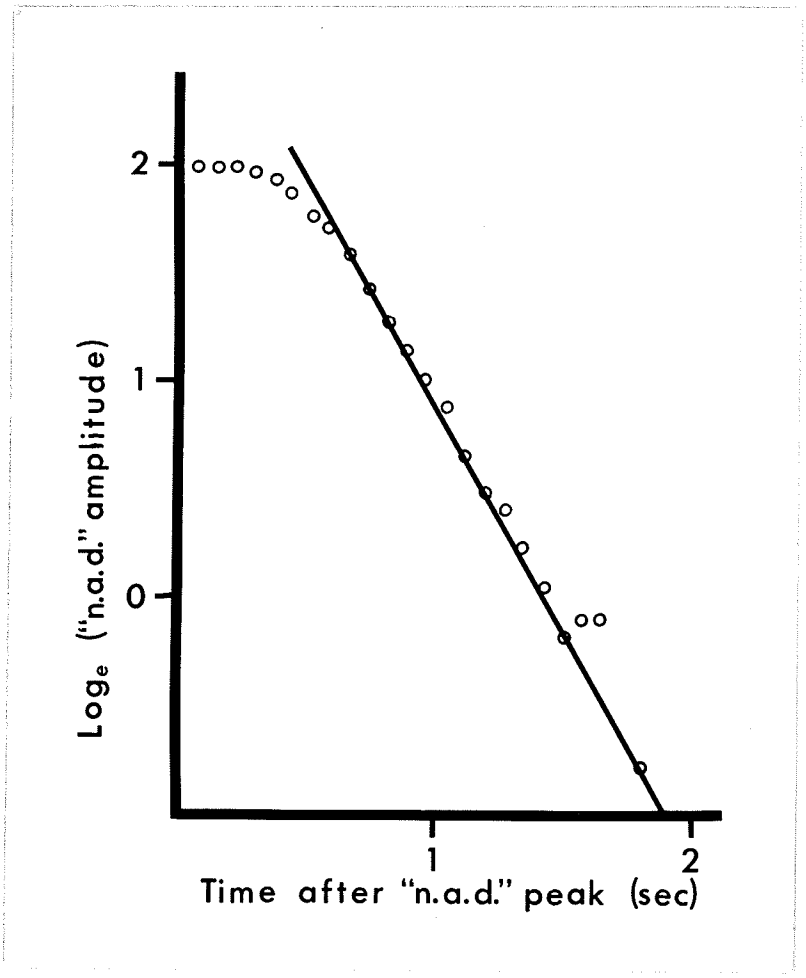

Fig. VIII - 5 Decay time course of a single "n.a.d."

The amplitude of a single "n.a.d." is plotted on a logarithmic scale. The time constant of decay, as derived from the slope of the line, is 461 mSec.
This example is taken from experiment 2 of Table VIII - 2. The stimulus was 8 volts at 40/sec for 64 pulses. The maximum amplitude of the "n.a.d." was 4.20 mV.

"n.a.d." was approximately linearly related to the logarithm of the number of stimulating pulses, at least near the threshold number of pulses, both in acutely and chronically isolated slabs. The results from a typical chronic slab are shown in Fig. VIII-6 and Fig. VIII-7, and from an acute slab in Fig. VIII-8 (a and b). The "n.a.d." increased with increasing stimulus until, at the threshold stimulus, an afterdischarge started from the peak of the "n.a.d.". The "n.a.d." was not always apparent following suprathreshold stimulation. Sometimes the first firing of the epileptiform discharge started at a greater radial potential than would have been predicted for that stimulus value from the subthreshold "n.a.d." amplitudes (e.g., Fig. VIII-7), with the radial potential often increasing to this point very much faster than the usual rate of rise of the "n.a.d.". In other cases the epileptiform firing began at a lower radial potential than would have been predicted (e.g., Fig. VIII-6 and Fig. VIII-8) or before the development of any "n.a.d." at all. This was particularly noticeable with stimuli which were slightly above the lowest effective stimulus: the radial potential at the start of the afterdischarge decreased with increases in the stimulus (e.g., the 10/sec stimulation of the chronic slab, Fig. VIII-9).

It was often difficult to determine exactly at what potential level epileptiform firing first occurred. This was especially true when the initial rate of rise of the radial potential was very rapid and the firing started sometime during the rising phase. In other cases, as in Fig. VIII-8c, there was a positive-going burst of spikes near the peak of the "n.a.d." when the stimulus was just subthreshold. This spiking was not considered epileptiform since it did not spread away from the point of stimulus, and because the polarity of the spikes was opposite to those in

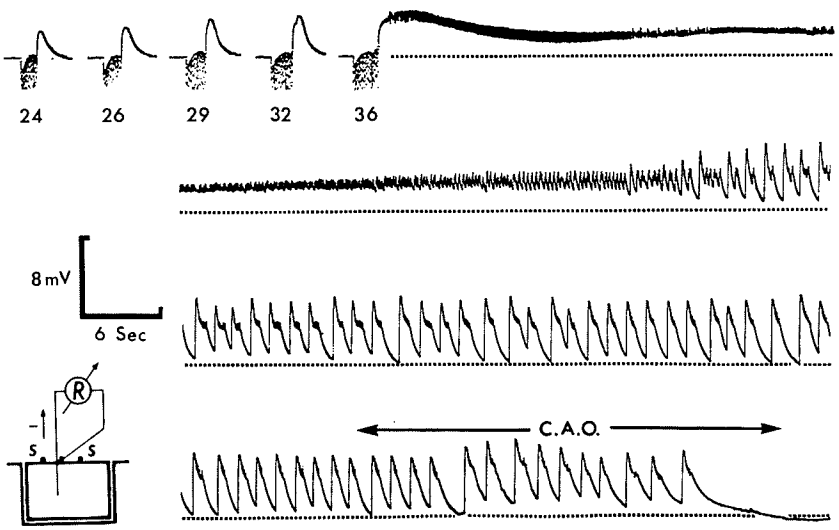

Fig. VIII - 6 Effects of sub- and suprathreshold epileptogenic stimuli on the radial potential of the chronic cortical slab.

The numbers under the stimulus artifacts indicate the number of stimulus pulses (12 volts, 20/sec).
The microelectrode depth was 0.33 mm.
The slab had been isolated 15 weeks.
"C.A.O." indicates carotid artery occlusion to stop the discharge, which in this animal normally lasted over 15 minutes.
Dotted line in the original base-line (zero) potential.

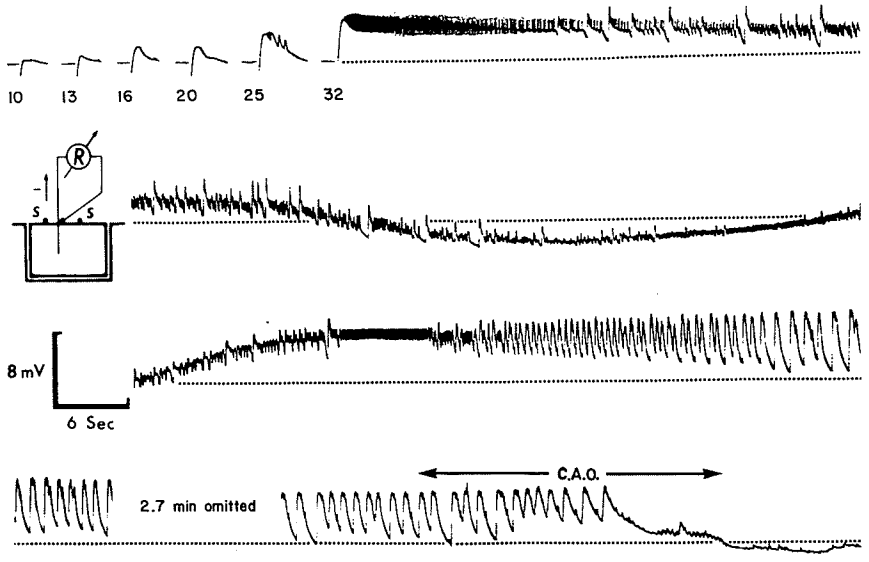

Fig. VIII - 7 Sub- and suprathreshold stimuli and the radial potential of the chronic slab.

Same experiment as in Fig. VIII - 6.
Simulus at 100/sec.
Depth of microelectrode: 0.55 mm.
Other details as in Fig. VIII - 6.

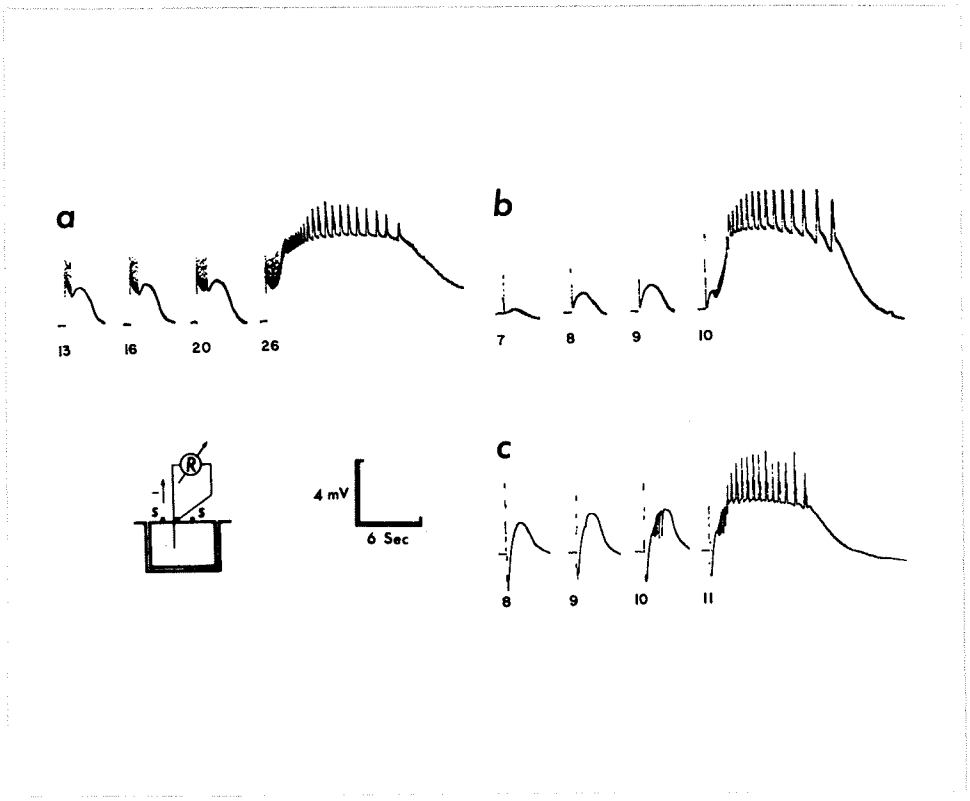

Fig. VIII - 8 Sub- and suprathreshold stimuli and the radial potential of the acute slab.

Numbers under the stimulus artifacts indicate the number of 12 volt stimulus pulses.

(a) Stimulus at 20/sec; microelectrode 0.50 mm deep.

(b) Stimulus at 100/sec; microelectrode 0.50 mm deep.

(c) Stimulus at 100/sec; microelectrode 0.65 mm deep.

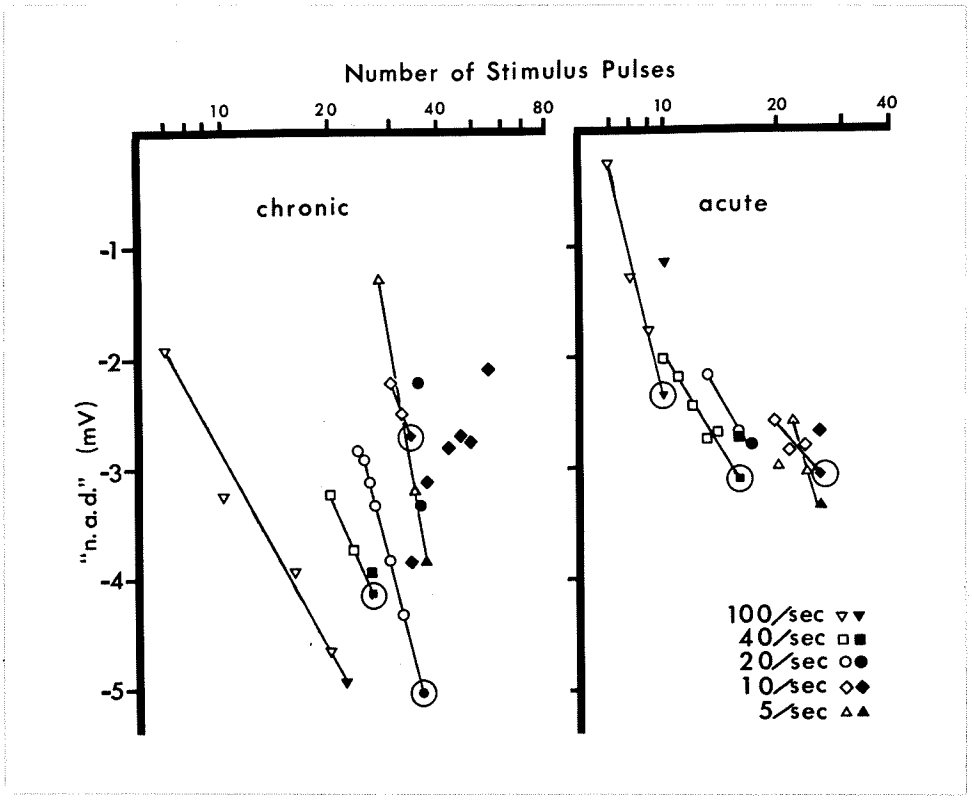

Fig. VIII - 9 Graph of "n.a.d." vs. stimulus.

Typical experiment where the "n.a.d." was studied as a function of the number of stimulating pulses at different stimulus frequencies.
The open symbols represent the amplitude of the "n.a.d."s following subthreshold stimuli, the filled symbols represent the amplitude of the "n.a.d."s following suprathreshold stimuli. The smaller filled symbols enclosed in circles are graphically obtained "expected threshold" values in cases where the "n.a.d." recorded was greater or less than these expected thresholds.
Lines are fitted to the subthreshold data by eye.
Data obtained during a single electrode penetration in each slab.

the first propagated phase (tonic phase) of the epileptiform seizure. Obviously, however, this type of discharge was related in some manner. In one acute slab it was noticed that these spikes reversed polarity when the electrode was withdrawn from 0.35 to 0.21 mm deep (Fig. VIII-10).

Comparison of the threshold amplitudes of "n.a.d."s in acute and chronic slabs showed no definite differences. Because the exact amplitude of the "n.a.d." was difficult to determine when the stimulus was suprathreshold, the "n.a.d." for the lowest suprathreshold stimulus was estimated graphically (the circled points in Fig. VIII-9) from the subthreshold "n.a.d."s assuming a logarithmic relationship between the stimulus and the amplitude of the "n.a.d.". For purposes of comparison, the values of the "n.a.d."s were adjusted to those for a recording depth of 0.55 mm by reference to the curve of "n.a.d." vs. depth for each slab. In 7 chronic and 11 acute slabs, 70 out of 100 observed threshold "n.a.d."s lay within the range of 1 - 3 mV.

While roughly the same level of radial potential was associated with threshold in both acute and chronic slabs, a definite relationship between radial potential and threshold did not exist in individual slabs, either acute or chronic. Where thresholds at several frequencies of stimulation were found during a single puncture, the threshold "n.a.d." amplitudes were compared. Two thresholds were considered equal if the ranges between the largest subthreshold "n.a.d." and the "n.a.d." at the lowest suprathreshold stimulus tried overlapped (the latter "n.a.d." was a graphically derived value, as outlined above). Among 11 acute slabs and 7 chronic slabs the threshold values of the "n.a.d." were the same for all frequencies of stimulation tested in only 3 slabs, all acute. If only those slabs where 3 or more frequencies of stimulation were tested are considered, then only 2 out of 15 slabs had equal values of the "n.a.d." at all

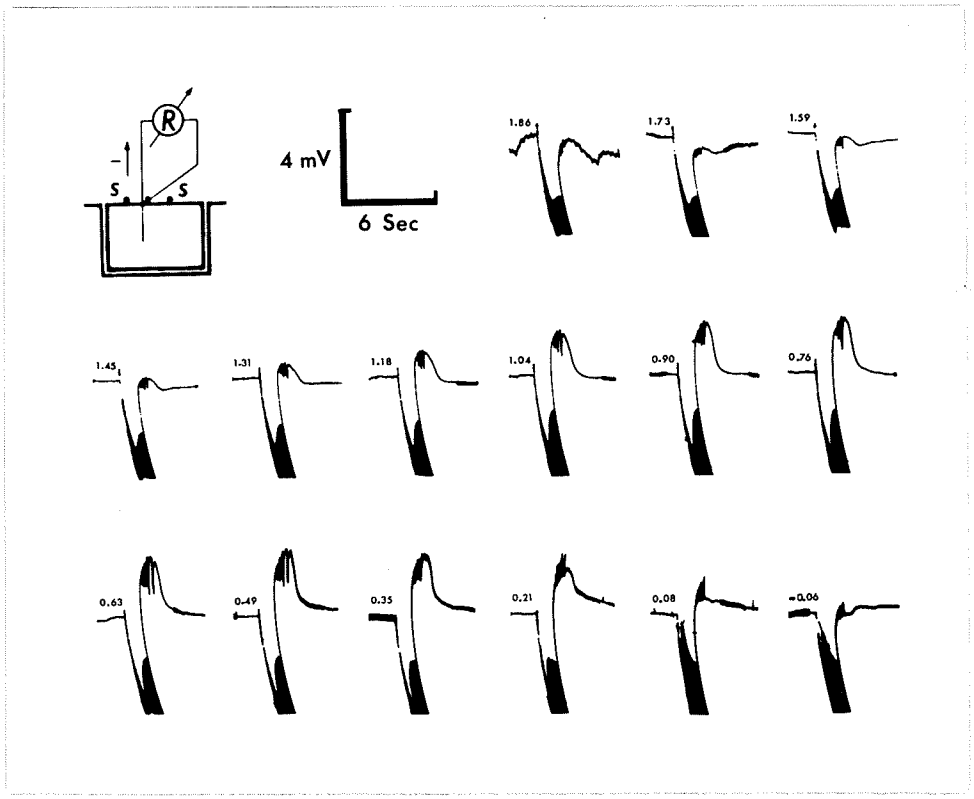

Fig. VIII - 10 Spiking during the "n.a.d."

Variation of the "n.a.d." with depth in an acute slab.
Small figures before each stimulus artifact indicate the depth, in mm, of the microelectrode tip.
Note that the positive-going spiking which occurs near the crest of each "n.a.d." reverses polarity between 0.35 and 0.21 mm.
Stimulus is 6 volts at 40/sec for 41 pulses, which is just below threshold.
Recording is curvilinear.

tested frequencies. A tabulation was made of all instances in 19 slabs (8 chronic and 11 acute) in which the stimulus threshold, or the value of the "n.a.d." at threshold, or both changed during the course of the experiment (24 cases in all). In 6 cases the change in the "n.a.d." was in the same direction as the change in stimulus threshold; in 7 cases the change in the "n.a.d." was in the opposite direction to the change in stimulus threshold; in 7 cases there was a change in the stimulus threshold without a change in the size of the "n.a.d." at threshold; and in 4 cases the threshold value of the "n.a.d." changed without a change in the stimulus threshold. This would be compatible with a random distribution of events in these 4 categories. In addition there was no definite ordering of the threshold values of the "n.a.d." with increasing stimulus frequency; while in several slabs the "n.a.d." at threshold was lowest with stimuli at 100/sec and highest at 5/sec stimuli, there were as many cases where the ordering was scattered. Neither was there any relationship between the order of testing and the threshold "n.a.d.".

b) Radial potentials during the epileptiform afterdischarge.

During an afterdischarge there is a displacement of the radially recorded potential level from the pre-stimulus base line. Superimposed on this displacement of steady potential is the bursting activity of the discharge. Burst amplitude, as indicated by "B" in the examples of epileptiform discharge shown in Fig. VIII-11, is measured from the lowest potential to the highest potential in the burst complex. The steady potential, as indicated by "E" in Fig. VIII-11, was taken to be the potential between the bursts of the afterdischarge and was measured from the base line to the potential level just before each discharge burst.

(i) Distribution of epileptiform burst amplitude ("B") with depth in the cortex. The burst amplitude at different depths was

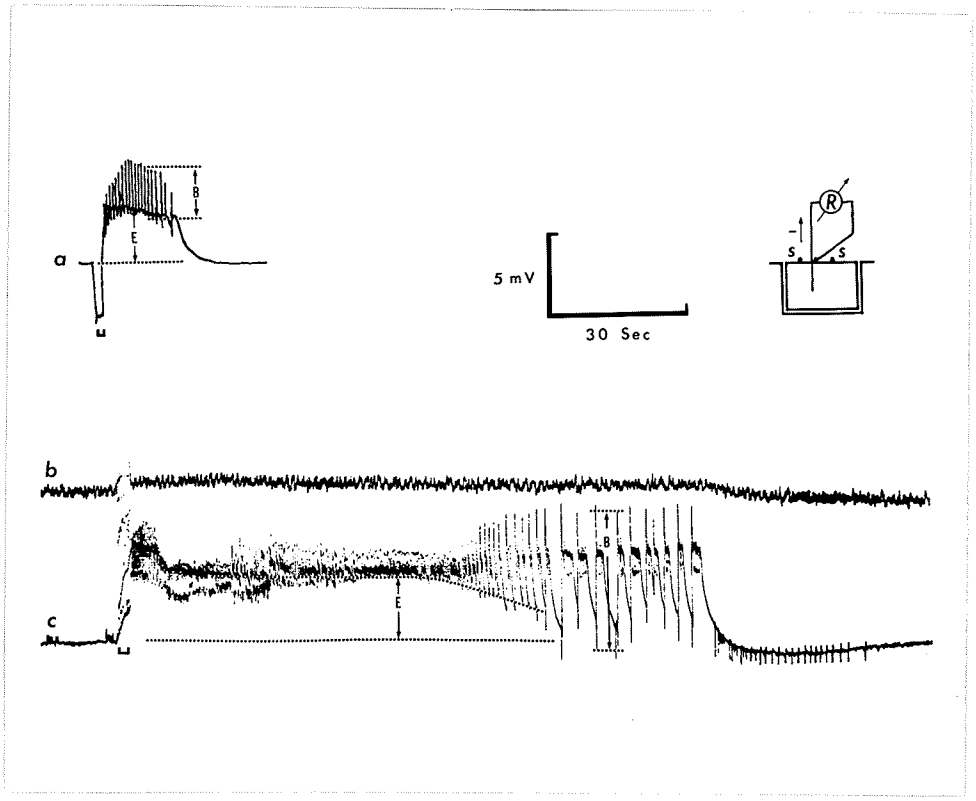

Fig. VIII - 11 Radial potentials during epileptiform afterdischarge.

(a) Afterdischarge in an acute slab following stimulus of 8 volts at 20/sec for 28 pulses.
Microelectrode depth: 0.50 mm.
"E" is maximum discharge steady potential;
"B" is maximum burst amplitude.

(b) Tangential potential of intact cortex immediately adjacent to a chronic slab during an epileptiform afterdischarge in the chronic slab (shown in c).
Amplification is double that shown for a and c.
The slight positive shift at the same time as the end of the afterdischarge in the chronic slab is a recording artifact.

(c) Afterdischarge of a chronic slab; stimulus was 8 volts at 20/sec for 57 pulses.
Microelectrode depth: 1.18 mm.
"E" is envelope potential, "B" is maximum burst amplitude.

Slab isolated 13 weeks.

Spikes retouched in (a) and (c).

examined in 3 acute slabs and in 8 chronic slabs. Typical results are shown in Fig. VIII-12. The burst amplitude has roughly the same distribution in acute and chronic slabs, with peaks at 0.60 mm (range: 0.40 - 0.83 mm) in acute slabs and 1.09 mm (range: 0.35 - 2.00 mm) in chronic slabs.

(ii) Distribution of the between-burst steady potential ("E") with depth in the cortex. The steady potential at various depths was studied in 5 chronic and 3 acute slabs. Examples of the distributions found are shown in Fig. VIII-13. If the maximum steady potentials of several discharges, or the steady potential during the second and third minutes of a single discharge in a chronic slab were plotted against depth of recording, the curve had a peak at about 1 mm deep (range: 0.55 - 1.40 mm; 3 slabs) and was roughly the same shape as the distribution of the maximum steady potential of acute slab discharges (peak: 0.54 mm; range: 0.40 - 0.83 mm; 3 slabs). If, on the other hand, the depth of the microelectrode was varied during the course of a prolonged afterdischarge (one of over 30 min duration) when the steady potential and burst amplitude had stabilized, then the steady potential remained within $\pm$ 1 mV throughout the cortex, and increased rapidly below depths of about 2 mm.

The zero reference potential of the 2 examples from chronic slabs in Fig. VIII-13 may not have been equivalent, since no compensation was made for the variation of potential with depth in resting cortex. However, in one experiment both types of curves were obtained in one chronic slab.

(iii) Relationship of the steady potential to maintenance of, and end of epileptiform afterdischarges.

The steady potential of acute slabs was usually negative with

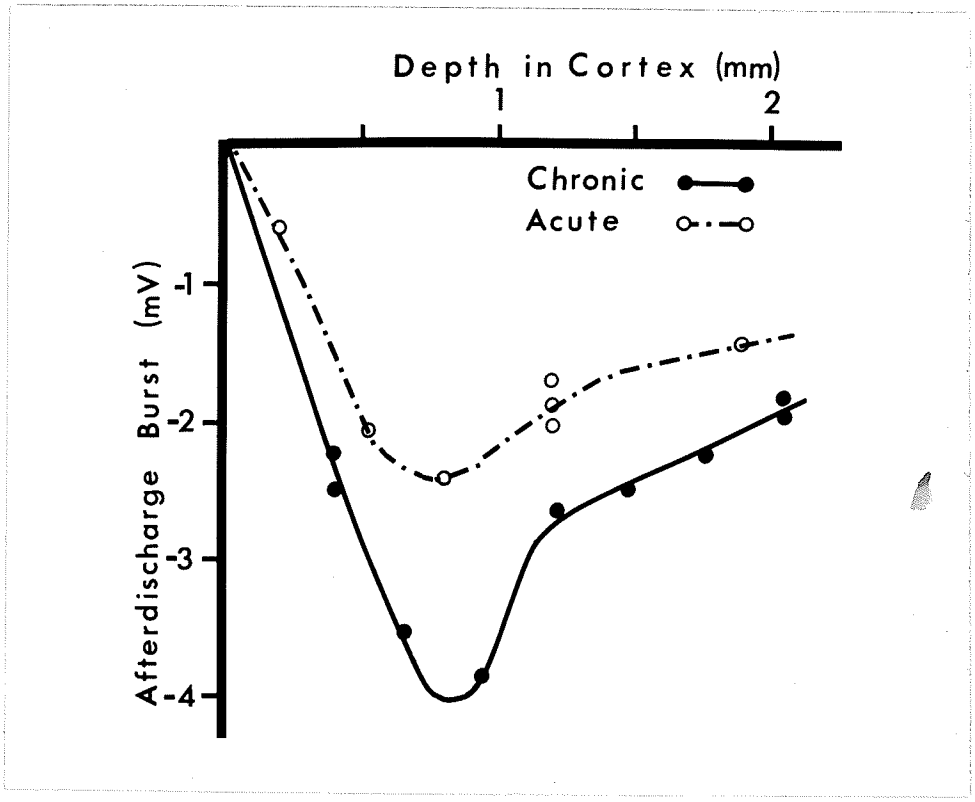

Fig. VIII - 12 Variation of epileptiform afterdischarge burst amplitude with depth in the cortex.

Chronic slab data obtained during one prolonged afterdischarge, while the amplitude of the tangentially recorded burst had a stable amplitude.
Chronic slab was isolated 13 weeks.
Acute slab data is maximum burst amplitude of discharges initiated while the microelectrode was at the indicated depth.

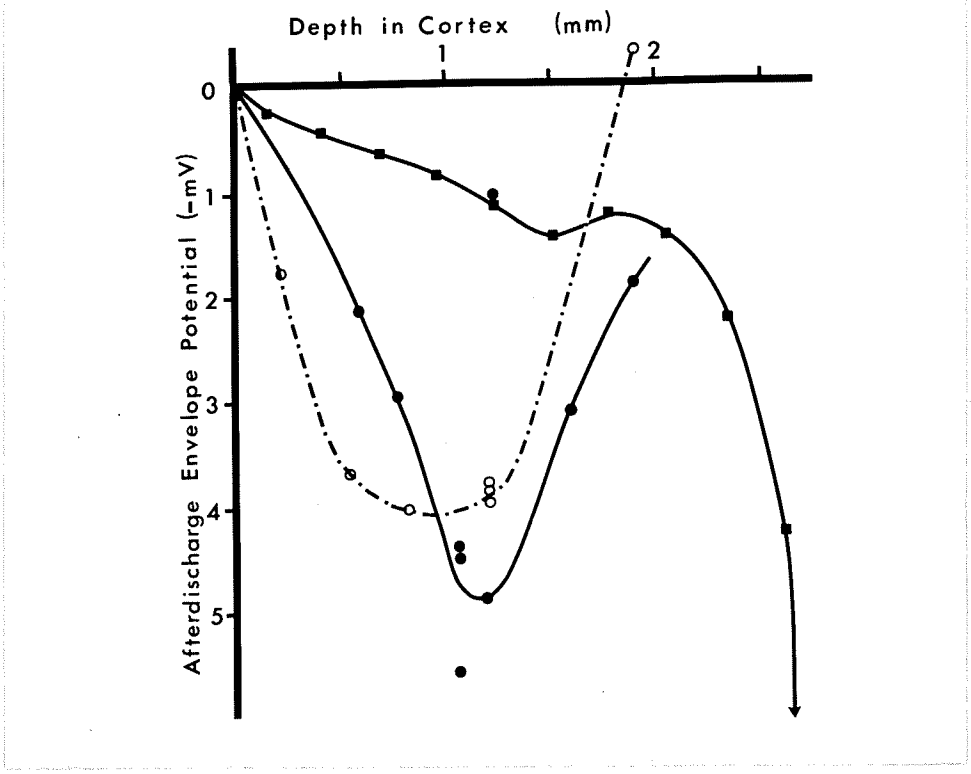

Fig. VIII - 13 Variation of afterdischarge steady potential with depth in the cortex.

Open circles and broken line are data from an acute slab. Solid circles and solid line are data from a chronic slab (isolated 13 weeks). Each point is the maximum steady potential of different afterdischarges which lasted about 120 sec. The prestimulus base-line at each depth is the zero reference level.
Solid squares and solid line are data taken during a single prolonged afterdischarge from a chronic slab (isolated 77 weeks). Acquisition of data was begun after the steady potential had declined from its maximum and stabilized. Zero reference was the potential level with the microelectrode at the surface.

respect to the pre-stimulus base line throughout the bursting activity of the afterdischarge (when recorded at depths above 1.5 mm). The end of the afterdischarge was always associated with a decline in the steady potential towards the base line. There was no clear relationship between the radial potential level and cessation of the bursting. Sometimes the burst amplitude and steady potential grew until the bursts ceased abruptly, and the steady potential then began to fall off. Sometimes the decline in steady potential preceded the last burst, the burst amplitude declining slightly as the steady potential declined (Fig. VIII-11a). In some cases an abortive burst occurred just before the steady potential reached the base line. For purposes of comparison the potential just before the start of the last burst was regarded as the steady potential at the end of the discharge. This had values all the way from the maximum steady potential to just positive to the pre-stimulus base line. While in most cases the steady potential at the end of the seizure was slightly lower than the potential level at which the discharge firing began (the threshold "n.a.d."), there were many cases where the discharge ended while the steady potential was very much above the "n.a.d.", and also many others where the discharge ended after the steady potential had fallen much below the "n.a.d." for the discharge. No account was taken of differences, if any, between the distributions of steady potential and "n.a.d." with depth.

The same general pattern also held for chronic slabs, except that the steady potential at the end of the seizure was, with one or two isolated exceptions, always less than the "n.a.d." when the discharge started. Moreover the steady potential frequently went below this level, or even reversed polarity, for much of the discharge. Some, like the

discharge in Fig. VIII-7, went positive in the middle of the discharge (although the bursting amplitude did not always become smaller at this time), or else the steady potential gradually declined to the base line potential (as in the discharge in Fig. VIII-6) or below (positive) after a minute or two of discharge. At this time the frequency of the bursting was usually lowest in the discharge, and the burst amplitude larger than at the beginning of the discharge.

c) Radial potentials of surface positive burst responses (SPBR) and spontaneous positive bursts (afterbursting).

Depth-recording of afterbursting and SPBR's was done in one chronic slab. This experiment had been going on for over 24 hours at this time, and the "continuous" background activity in the slab was so low that the high-frequency repetitive potentials of the bursts were easily distinguished from background activity. Two things were measured: the amplitude of the high-frequency repetitive activity, and the mean displacement of the steady potential from the base line ("envelope") on which the repetitive activity is superimposed.

The envelope of the afterbursting and SPBR's, and their repetitive activity, had the same form and distribution with depth. Only the results for the SPBR's are shown in Fig. VIII-14, but the results for afterbursting were exactly the same.

The envelope of the SPBR's and afterbursting recorded tangentially (lower trace in each panel of Fig. VIII-14) did not have a steady positive component as occurs in acute slabs (cf., Fig. VI-6). At all depths there was a negative peak immediately after the start of the burst, corresponding to the brief positivity at the beginning of the tangential record. Its amplitude was directly related to depth, as is

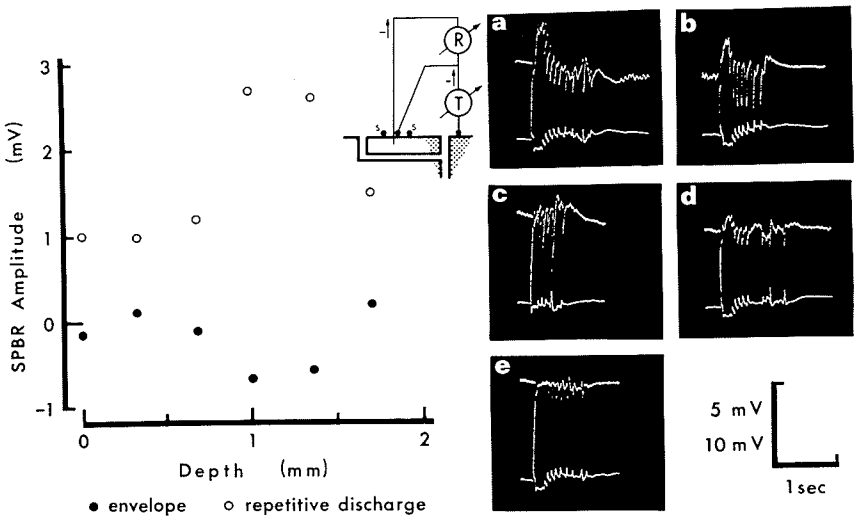

Fig. VIII - 14 Radial potentials of surface positive burst responses (SPBR) in chronic slab.

Left: Distribution of SPBR envelope and repetitive potential amplitudes with depth in the cortex. Depths are approximate. Envelope data are averages of midpoints of 5 SPBRs at each depth.

Right: Sample SPBRs at (a) 1.72 mm, (b) 1.38, (c) 1.03, (d) 0.69, (e) 0.34 mm deep in cortex. Upper trace in each panel is radial potential (R), lower trace is the surface tangential record (T).
Spikes in records retouched.
Chronic slab isolated 13 weeks.

apparent from the sample SPBR's in Fig. VIII-14. The envelope potential at the mid-points of the SPBR's did not have any particular polarity (relative to the pre-burst base line); at any depth both positive and negative envelope potentials occurred at the mid-points of the bursts. The potential at the end of the bursts generally was slightly positive.

The repetitive activity amplitude of the SPBR's and afterbursting had a distribution with depth that was identical to that described for the SPBR in acute slabs by Burns and Grafstein (1952).

The amplitudes of the envelope and repetitive activity of afterbursting in two acute slabs studied had distributions with depth similar to the distributions of envelope and repetitive potential of acute slab SPBR's as described by Burns and Grafstein (1952).

D. DISCUSSION

a) The negative after-deflection.

There appears to be no difference between acutely and chronically isolated slabs in the processes responsible for the production of the "n.a.d.". Their time constants for decay of "n.a.d."s and their relationships between stimulus and "n.a.d." amplitude are the same. The distribution of "n.a.d." amplitude with depth is also similar since the depths at which the maximum and minimum amplitudes occur are the same.

The occurrence of doube-peaked "n.a.d." vs. depth distribution curves in chronic slabs may represent two separate (in depth) populations of cells responsible for the "n.a.d.". However this would not appear to be a stable condition, since in the chronic slabs when this was retested, single-peaked curves could be obtained. Since the double-peaked curves could also be obtained in acute slabs the occurrence of double-peaked distribution curves does not seem to be a factor (or at least an important one) in explaining the differences in epileptiform discharges of the acute and chronic slabs.

It seems most likely that the occurrence of the doube-peaked curves was an artifact caused by compression of the cortex by the electrode dragging the pia on insertion. It is suggested that the deeper peak results from compression of the cortex in the immediate area of the electrode, and that the more superficial peak is recorded from the surrounding tissue, or vice versa if the distortion is produced by dragging of the cortex on withdrawal. Thus there would be two parallel cell systems, one close to the electrode and compressed by it, the other a bit farther away and not compressed. The relative size of the two peaks would depend on the size of the compressed region. Thus the significance

of the double-peaked curves is one of differences in toughness of the pia in acute and chronic slabs rather than of differences in properties or distributions of the cells involved in the "n.a.d.".

The basic premise that there is a critical relationship between the radial potential gradient (and in particular the "n.a.d.") and the outbreak of epileptiform afterdischarge does not appear to have been upheld. While the "n.a.d." at threshold is approximately the same, on the average, in acute and chronic slabs, the size of the "n.a.d." does not appear to be critically related to the threshold for epileptiform discharge in individual slabs, either acute or chronic. If such a critical relationship existed, then the threshold "n.a.d." should be the same for every test during a single electrode placement, regardless of the stimulus frequency used. This was not the case in either acute or chronic slabs, nor was there any particular relationship between stimulus frequency and the amplitude of the "n.a.d." at threshold. Moreover, the value of the radial potential at which afterdischarge started was often inversely related to the stimulus when suprathreshold stimuli were used. This suggests that the processes responsible for the generation of afterdischarge have nothing directly to do with a potential gradient between the dendrites and the soma. Sometimes the discharge began at the base line potential, immediately after the stimulus and before a "n.a.d." had developed (although "n.a.d."s were recorded after subthreshold stimulation). Here the critical depolarization of the cell soma itself, rather than a potential gradient along the cells, was sufficient to start the discharge.

Therefore it is suggested that the "n.a.d." is not associated in a direct cause and effect relationship with the initiation of the afterdischarge, and that a critical value of the "n.a.d." is neither a

sufficient nor a necessary requirement for an afterdischarge to start. It seems more likely that the association between the two is fortuitous, and that both are due to a common cause (depolarization of the soma of the cells) with no direct interaction. It seems unlikely that a current sink in the apical dendrites would have much positive effect on firing of the axon hillock by the depolarized cell soma.

It is possible that the "n.a.d." is a poor gauge of the size of the potential gradient along individual cells, for the technique of recording in a volume detects current flow between the two points, so that the sum of currents from many parallel cells in the region of the electrode is recorded. Moreover, the recording of a radial potential tells little about the activity of the part of the cell population which is not radially oriented. The fact that no equivalent potential can be recorded in a tangential direction is probably an indication of the fact that there is no system of parallel cells in that direction equivalent to that in the radial direction.

The magnitude of the "n.a.d." does therefore depend in part on the number of excited, radially oriented cells around the electrode (i.e., the density of excited cells in the discharge focus), as well as the potential of each cell dipole. However, since the discharge has already been shown to be critically dependent on the density of excited cells, and since voltage and thus current spread was kept constant, it is likely that the variation of the threshold "n.a.d." is due to variations in the magnitude of the cell dipole rather than variations in the density of excited cells. The "n.a.d." may offer some information about the density of the excited cells at the focus. The increase of "n.a.d." with stimulus occurs only quite close to the threshold in most cases (cf., Fig.

VIII-9), which suggests that very few cells are depolarized until the stimulation is just subthreshold.

b) Radial potentials during afterdischarges.

The maintenance of a radial potential gradient in the cortex does not appear to be required to maintain epileptiform afterdischarges in chronically isolated cortical slabs. This is probably true in acutely isolated slabs as well, for discharges in acute slabs often continued after the envelope potential had declined below that at which the discharge began. A radial potential might, however, play an "ephaptic" role in the discharge by lowering the threshold of non-excited cells, but certainly could not be directly stimulatory to other cells. It may be that the decline in the steady potential represents a wandering of the focus away from the electrodes, since in acutely isolated slabs the displacement of the steady potential can only be recorded within a couple of mm of the focus (Pinsky, 1961). However, this aspect was not studied in the chronic slabs.

There does not seem to be any particular radial gradient required for the occurrence of single bursts of the afterdischarge, either in acute or chronic slabs. The radial steady potential may be a millivolt or more different at the start of two successive bursts.

Since the distribution of maximum steady potential displacement, and of burst amplitude are similar in acute and chronic slabs, it is probable that the same population of cells is involved. It would seem possible that the radial potential changes at the focus of an afterdischarge are the result of, rather than the cause of the intense activity at the focus of discharge. The occurrence of bursts, and the maintenance of the long discharges in chronically isolated cortex can be best explained by

synaptic activation rather than by standing potential gradients in the cells of the focus. The possibility that the focus may wander in the chronic slabs only serves to support the hypothesis that synaptic activation plays the more important role, and that the basic defect of cells in chronically isolated cortical slabs is denervation supersensitivity.

c) Surface positive burst responses.

The SPBR has been shown to be a synaptically propagated response of the cortex (Burns, 1958). In chronic slabs the surface positivity (and deep negativity) of the SPBR is absent. However the absence of the envelope in the SPBR of chronic slabs does not interfere with its initiation or propagation, just as the maintenance of epileptiform afterdischarge, also proposed to be synaptically maintained, is not dependent on an "envelope" potential.

These results also further differentiate afterbursting and SPBR's from epileptiform discharges.

IX. ROLE OF ACETYLCHOLINE IN EPILEPTIC SEIZURES

A. SENSITIVITY TO ACETYLCHOLINE

Acetylcholine (ACh) is the only example of a known neural transmitter substance which can cause epileptiform activity (Miller, Stavraky and Woonton, 1940; Brenner and Merritt, 1942; Chusid and de Gutierrez-Mahoney, 1953; Infantellina, 1955). ACh is released from brain and spinal cord during spontaneous and induced epileptic seizures and may then be recovered from the cerebrospinal fluid (Tower and McEachern, 1949a,b). Cholinoceptive cells have been identified in the cortex (Krnjević and Phillis, 1963b). Hence the role of ACh is of particular interest in any hypothesis which postulates denervation supersensitivity as a possible mechanism in focal epilepsy. In many parts of the central nervous system, chronic denervation has been shown to produce a sensitization to the convulsant actions of ACh (Stavraky, 1961). Chronically isolated cerebral cortex becomes particularly supersensitive to the epileptogenic effects of ACh (Echlin, 1954, 1956, 1959).

The effect of topically applied solutions containing ACh or eserine was tested in 4 acute slabs and in 1 chronic slab of cerebral cortex. Application of 1% ACh produced no effect in any of the 4 acute slabs (Fig. IX -1b). Eserine (0.1-1.0%) alone was found to slightly raise the threshold number of pulses required to start an afterdischarge in 2 of the acute slabs (not investigated in the other two) (Fig. IX - 1c). ACh (1%) applied after eserine had been placed on the filter paper caused spontaneous epileptiform discharges in all 4 animals (Fig. IX - 1d, IX - 2) after a latent period of up to 5 minutes. The filter paper was then removed within one minute and the cortex washed with saline, however,

Fig. IX - 1 (p. 168) Effects of Acetylcholine

Left: acute slab.
a-c: seizure responses to just subthreshold and just suprathreshold repetitive electrical stimuli; topical pretreatment with ACh in b, with eserine in c.
d: "high frequency" and "clonic" discharge (5 min apart) of a prolonged seizure induced by topical treatment of the acute slab with ACh and eserine.
e: long burst induced by single electrical stimulus pulse within 10 min after end of seizure in d.

Right: Chronic slab (14 weeks)
f: electrically induced seizure.
g: ACh induced seizure, 5 min after application of ACh. No eserine and no electrical stimulation.
Surface negativity is upward.
Records are retouched.

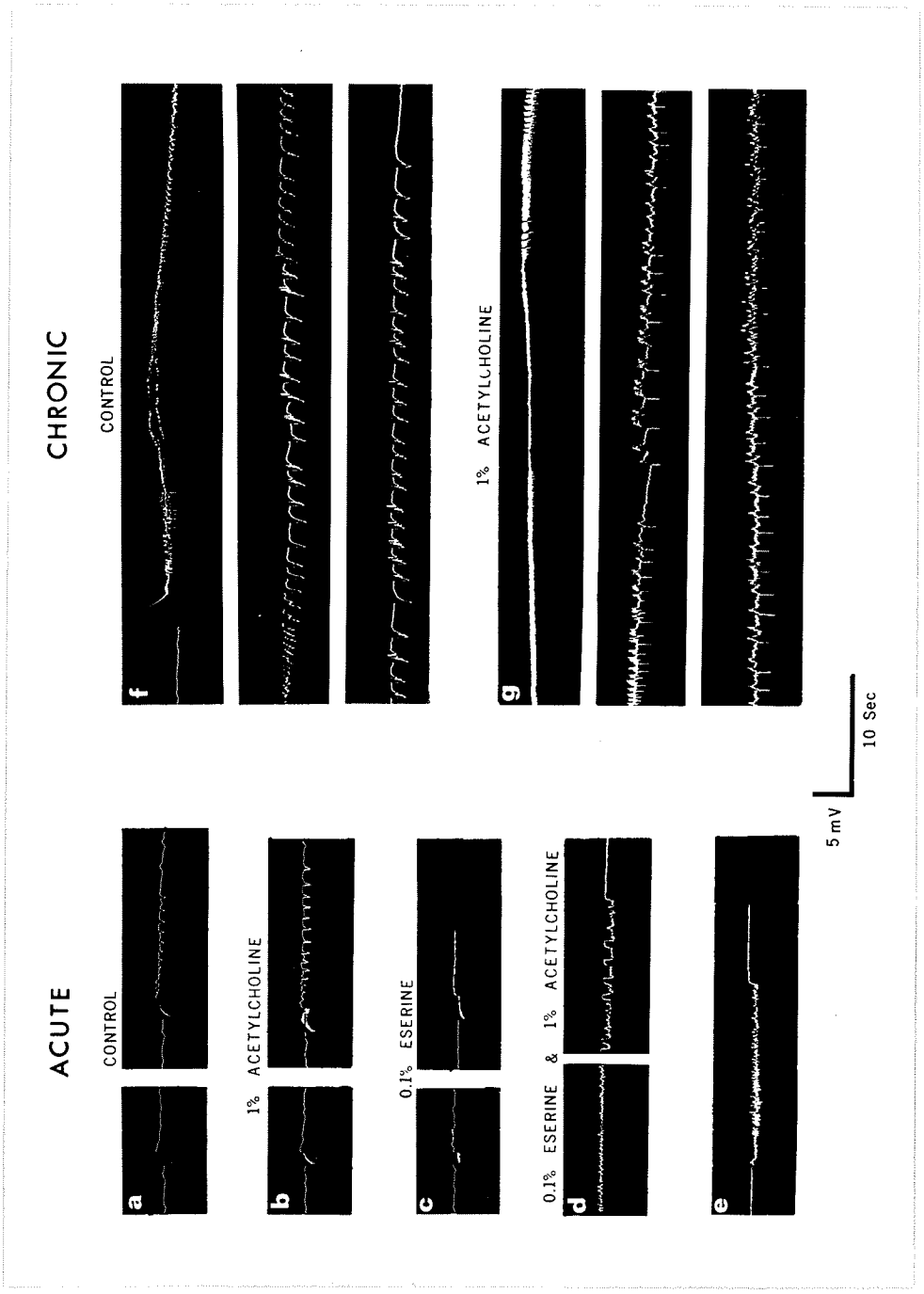
ACUTE
CONTROL
a
1% ACETYLCHOLINE
b
0.1% ESERINE
c
0.1% ESERINE & 1% ACETYLCHOLINE
d
e
CHRONIC
CONTROL
f
1% ACETYLCHOLINE
g
5 mV
10 Sec

Fig. IX - 1

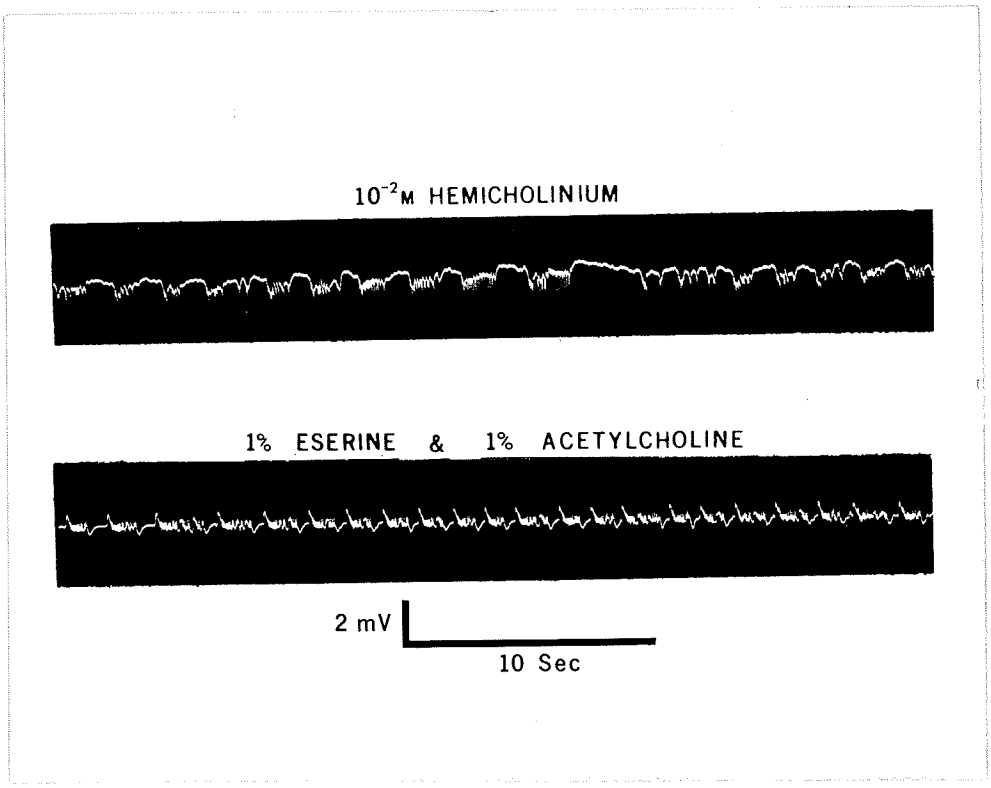

Fig. IX - 2 Effect of HC-3 on acute cortical slab

Upper record is discharge occurring spontaneously after topical treatment with Hemicholinium No. 3 (HC-3). Lower record is discharge produced by topical ACh and eserine. Acute slabs. Surface negativity up.

the discharges continued, sporadically towards the end, for at least 10 minutes. When the discharges stopped single pulses could initiate long bursts similar to the bursts of the intermittent phase of the seizure activity (Fig. IX - 1e). In one animal 0.1% atropine sulphate was applied at this juncture. It abolished these responses to single stimuli and prevented an epileptiform response to electrical stimulation. A prolonged discharge was produced in the chronic slab by the application of 1% ACh without prior treatment with an anticholinesterase and without application of electrical stimulation (Fig. IX - 1g).

Because of the possibility that the topical atropine blocked the electrical induction of epileptiform discharges by a local anaesthetic action rather than its specific anti-ACh action, atropine (1-5 mg) was administered intra-arterially to 1 acute and 1 chronic slab via the ipsilateral common carotid artery (1 mg/kg, i.v., will block cortical cholinoceptive neurones, Krnjević and Phillis, 1963c). The first injection of atropine (1 mg) into the acute slab preparation produced immediate motor convulsions (spinal level) and both animals were immobilized with gallamine for all other injections. Atropine, intra-arterially, in doses up to 5 mg, did not affect threshold or duration of electrically induced epileptiform afterdischarges in either acute or chronic slabs. 2 mg of atropine was administered to the chronic slab <u>during</u> a prolonged afterdischarge without altering the discharge.

B. THE EFFECT OF HEMICHOLINIUM NO. 3 (HC-3)

If acetylcholine is necessary for the maintenance of epileptiform activity in the cerebral cortex then decreases in the production of ACh should decrease the ability of the cortex to maintain electrically induced seizures. The drug HC-3 provides one possible means for interfering with ACh synthesis and thereby output. According to MacIntosh (1961) HC-3 competes with choline for uptake into cholinergic nerve terminals and thus prevents ACh synthesis. Nerve transmission fails only if nerve activity is maintained at levels sufficiently high to deplete preformed ACh stores.

Two acutely isolated slabs were treated topically with a 10^{-2} molar HC-3 solution. The filter paper used covered 75% of the slab. Stimulating electrodes were placed on the uncovered portion of the slab. Epileptiform afterdischarges were induced electrically every 10-15 min and in one of these slabs surface positive burst responses (Burns, 1951) were elicited every 10 seconds. (A third slab was treated with 10^{-5}M HC-3 and only surface positive bursts were elicited).

In one case 10^{-2}M HC-3 caused the elimination of the burst responses, an increase in the electrical threshold and a decrease in the duration of the afterdischarges (from 20 to 8 seconds). No equivalent changes were observed in the second slab. In both slabs the HC-3 eventually caused a spontaneous discharge which resembled the response to ACh. This occurred in one animal after the drug had been in contact with the cortex for 75 minutes and after 45 minutes in the other, and in both cases the discharges continued for over 10 minutes (Fig. IX - 2). (In the third animal 10^{-5}M HC-3 reduced the duration and amplitude of the positive burst response after 2 hours of application).

C. DISCUSSION OF THE ROLE OF ACh IN EPILEPSY

It is unclear from these results whether ACh has a necessary role in cortical seizures. It is by no means certain that the ACh-initiated discharge is truly epileptiform. Certainly the electrographic picture is very similar to electrically induced seizures, especially in chronically denervated cortex: i.e., a long high-frequency phase followed by a protracted "interrupted" or clonic phase. However, in the acutely isolated cortex the bursts of the clonic phase of the ACh-seizures bear a stronger similarity to the positive bursts of afterbursting or single positive burst responses of acutely isolated cortex than to the clonic bursts of electrically induced afterdischarge. Moreover, the bursts of activity (appearing identical to the ACh-seizure bursts) which could be elicited by single pulses when the chronic activity had died away had many of the properties of the positive burst response. Infantellina (1955) also noted this phenomenon and commented on the similarity of ACh-induced discharge to the positive burst response.

The present experiments confirm the previous observations of a difference in sensitivity to ACh between chronic slabs and normal or acutely isolated cortex. In intact cortex or acute slabs, a 5% concentration of ACh is required to start epileptiform activity, but only 0.1% is required if the cortex is pretreated with eserine (Brenner and Merritt, 1942; Infantellina, 1955) whereas chronic slabs respond to 0.1-0.2% ACh without eserine pretreatment (Echlin, 1954, 1956, 1959). However, it is still not possible to say if this difference is a Cannon-type supersensitivity (alteration of the receptor sensitivity), or due to a decrease in cortical cholinesterase (ChE) concentration. Chronic denervation or undercutting of the cortex has been shown to cause a decrease in ChE

(Echlin and Battista, 1962a,b; Hebb, Krnjević and Silver, 1963), a decrease in choline acetylase (Hebb et al., 1963) and a decrease in ACh content (Sastry, 1956). These results are similar to the effects of denervation in peripheral tissues innervated by cholinergic nerves (Brooks and Meyers, 1952; Hebb, 1957; Holmstedt, Lundgren and Sjöquist, 1963). Moreover the increase in sensitivity of the cortex to ACh brought about by chronic denervation appears to be the same as brought about by pretreatment of the cortex with an anticholinesterase or by the use of a cholinergic drug which is not split by ChE (Brenner and Merritt, 1942). However, a decreased ChE content is not a necessary feature of an epileptic focus, for Pope, Morris, Jasper, Elliott and Penfield (1946) found increased ChE contents in aluminium hydroxide foci in monkeys and in epileptogenic human tissue. "Mirror image" foci may also have slightly increased ChE activity (Krech, Rosenzweig and Bennett, 1960). There are many other conflicting reports regarding acetylcholinesterase and epileptic activity. Giachetti and Piva (1956, 1957, 1958a,b) found that intravenous administration of ChE could inhibit epileptiform seizures induced by strychnine or by direct electrical stimulation. Eserine or neostigmine (applied iontophoretically or systemically) enhances the action of ACh on individual cells in the cortex (Spehlmann, 1963; Krnjević and Phillis, 1963c) but it does not increase the tendency to electrically induced convulsions (cf., Infantellina, 1955). While convulsions are a known result of diisopropylfluorophosphate (DFP) poisoning, DFP does not enhance the development of epileptic activity in aluminium hydroxide lesions (Chusid, Kopeloff and Kopeloff, 1952).

Present results suggest that atropine only has a local anaesthetic action in antagonizing electrically induced seizures, since

massive intra-arterial doses failed to alter epileptiform afterdischarges of acute or chronic slabs. This is in agreement with Funderburk and Case (1951) who found that 1 mg/kg of atropine (intravenously) only abolished the epileptiform activity due to topical ACh, but not that of penicillin lesions (indeed atropine increased the spiking of penicillin foci). This suggests that ACh is not essential to the development and maintenance of seizures in either chronic or acute slabs. Atropine and other anticholinergic agents, applied topically, systemically or iontophoretically are quite effective and specific in blocking the excitatory effects of applied ACh, either at the cellular level (Spehlmann, 1963; and Krnjević and Phillis, 1963c) or at the gross electrographic level (Miller et al., 1940; Brenner and Merritt, 1942; Funderburk and Case, 1951; Infantellina, 1955).

It is possible that the action of ACh is an indirect one: that the high concentrations of ACh required to produce convulsions actually blocks (by depolarization) cholinoceptive cells involved in inhibitory pathways in the cortex. Such a system has been postulated by Chatfield and Lord (1955), however it is only in the spinal cord that inhibitory cholinergic pathways have been demonstrated. Chusid and de Guiterrez-Mahoney (1953) found that strychninization, known to block inhibitory synapses, or previous treatment with ACh reduces the latency of onset of ACh convulsions. Low doses of curare also facilitate development of strychnine seizures (Purpura and Grundfest, 1956a,b, 1957). High doses of curare cause convulsions, but these are undoubtedly the result of histamine release and consequent hypotension. This hypothesis of acetylcholine acting only on inhibitory pathways must be considered unlikely in view of the fact that iontophoretic application of ACh is known to directly excite many cells in the cortex (Krnjevic and Phillis, 1962,

1963a,b; Spehlmann, 1963) a significant proportion of which were identified as large pyramidal tract neurones, rather than interneurones. However, these authors demonstrated that iontophoresis of large quantities of ACh onto single cholinoceptive cells initially increased their rate of firing but did eventually block them (Krnjević and Phillis, 1963b). Non-cholinoceptive cells were not blocked by ACh (Krnjević and Phillis, 1963a).

HC-3 was no real aid in elucidating the problem, for it was found to initiate afterdischarges. It may be that this action was direct, since many other ACh-analogues are known to stimulate cholinoceptive cells (Krnjević and Phillis, 1963c). It certainly did not prevent epileptiform activity. There is other evidence to suggest that prevention of ACh release in the cortex does not interfere with seizure development. Chloralose blocks release of ACh from the cortex (MacIntosh and Oborin, 1953; Mitchell, 1963), blocks the antidromic activation of the Renshaw cell (Haase and van der Meulen, 1961) and blocks synaptic activation of cholinoceptive cells in the cortex (Krnjević and Phillis, 1963c) without affecting the effects of applied ACh. Krnjević and Phillis (1963c) suggested that a possible action of chloralose was to prevent the release of ACh from nerve terminals. However, afterdischarges can be initiated in the isolated cortex of the chloralosed cat (Burns, 1950).

ACh, despite the evidence available, still appears to be the most likely candidate for involvement in increased epileptogenesis produced by chronic denervation, if only because it seems to be the only candidate for such a role. This problem is likely only to be resolved by comparison of ACh sensitivities of chronically denervated and normal cholinoceptive cells by direct iontophoretic application of ACh, so that other variables such as the blood-brain barrier and the presence of ChE

are bypassed. Although ChE plays a relatively insignificant role in removal of ACh from the immediate synaptic region of cortical cholinoceptive cells (Krnjević and Phillis, 1963c), it does affect the amount of topically applied ACh reaching the cells.

X. INDUCTION OF SPREADING DEPRESSION IN CHRONICALLY ISOLATED CORTICAL SLABS

A. INTRODUCTION

Spreading cortical depression, first described by Leão (1944) can be induced easily in acutely isolated cortex (Grafstein, 1956). It can be produced by application of potassium chloride, mechanical deformation, or strong repetitive electrical stimulation. Increasing the strength of epileptogenic stimulation is likely to cause spreading depression (Pinsky and Burns, 1962). Spreading depression is characterized by a large (up to 10 mV) surface-negative wave which lasts over 30 seconds and is followed by a much longer surface-positive wave of lower amplitude. During the positive phase, which may last as long as 20 minutes, spontaneous activity is reduced and the cortex is inexcitable. It has been postulated that spreading depression results from a massive release of potassium from cortical neurones (Grafstein, 1956). This released potassium presumably diffuses to, and depolarizes, neighbouring cells which then release more potassium and thereby spread the response. Such a massive release of potassium has, in fact, been shown to occur during spreading depression (Brinley, Kandel and Marshall, 1960).

Grafstein and Sastry (1957) first observed that spreading depression was almost impossible to induce by electrical stimulation in chronically isolated cortex. However, they also observed that application of potassium chloride or mechanical stimulation could initiate spreading depression although the amplitude of the negative wave was lower than in normal cortex.

B. RESULTS

In the present experiments, spreading depression rarely occurred in chronically isolated cortex. Deliberate but unsuccessful attempts were made to stop prolonged afterdischarges by inducing spreading depression with hundreds of pulses delivered at rates over 100 per second (vide supra; Section VI-c). Such stimulation is invariably effective in causing spreading depression in acute slabs. In chronic slabs this strong stimulation not only failed to induce spreading depression but it also failed to alter the discharge. Similar strong stimulation delivered to quiescent chronic slabs caused only afterdischarges. Spreading depression was observed to occur spontaneously once in one animal and only once in 1 of 9 other animals in response to strong electrical stimulation. In both of these cases the experiments had been going on for over 8 hours prior to the occurrence of the spreading depression.

Reisolation of the chronic slab radically changed the ease with which strong electrical stimulation could produce spreading depression in the 6 chronic slabs which had been reisolated. Spreading depression was readily induced by strong stimulation after reisolation (Fig. VII-2). This was usually preceded by an epileptiform afterdischarge which was terminated by the spreading depression. In contrast, the mechanical trauma of the reisolation procedure did not by itself produce spreading depression, nor did it terminate an afterdischarge already in progress. For example, in 2 of the animals the slabs were reisolated during prolonged afterdischarge activity and this activity continued long after surgery (vide supra; Section VII).

In all 4 experiments in which isotonic KCL was applied topically to chronically isolated cortex it caused electrical waves, typical

of spreading depression several mm from the point of application. Twice the spreading depression spontaneously recurred after removal of the KCL soaked filter paper. In 8 acute cortical slabs, 10^{-4} and 10^{-3} veratradine or veratrine topically either lowered the electrical threshold for spreading depression or caused spreading depression. In 3 chronic slabs, 10^{-4} and 10^{-3} veratradine was applied topically. The lower concentration produced no effect, while 10^{-3} veratradine allowed induction of spreading depression in all animals with stimulation which previously caused only afterdischarge. By itself 10^{-3} veratradine twice caused a negative potential shift characteristic of spreading depression, but the spontaneous activity of the slab continued throughout and following this negative wave. This was also true in one case where the wave was induced by KCl. In all cases, as observed by Grafstein and Sastry (1957) the negative wave of spreading depression in chronic slabs was about half that in acute slabs. In contrast the negative wave amplitude in reisolated slabs was of the same order of magnitude as in acute slabs.

C. DISCUSSION

There are several possibilities which might explain the difference between the susceptibilities of chronically and acutely isolated cortex to spreading depression. Grafstein (1956) reported that the spread of the depression could be prevented if the cortex was kept activated by electrical stimulation. Thus, it might be that the spontaneous activity in chronically isolated slabs prevents the development of spreading depression. Reduction in this activity by reisolation would then permit the development of spreading depression. However, the records from some chronic slabs showed no greater activity than those from acute slabs. These quiet chronic slabs were not sensitive to initiation of spreading depression. Furthermore, the normal spontaneous activity in intact cortex does not prevent the development of spreading depression.

Van Harreveld, Terres and Dernburg (1956) found that spreading depression could not cross a glial scar. Thus the absence of spreading depression might be explained on the basis of the gliosis observed in chronically isolated slabs (Section V). However, reisolation could scarcely be expected to alter the gliosis.

Since the spreading depression is thought to be mediated by K^+ release from neurones, two possibilities involving cellular K^+ suggest themselves: either the neurones are relatively K^+ deficient, or have a decreased ability to release their internal K^+. In chronically denervated frog skeletal muscle the second alternate actually does occur. Denervated frog muscle has a decreased K^+ permeability but a normal internal K^+ content (Nicholls, 1956; Harris and Nicholls, 1956; Hubbard, 1963). In denervated cortex too, the second possibility seems to be the most likely. The observation, both in the present study and by Grafstein and Sastry

(1957), that the depression, once initiated, can spread and the fact that veratrum alkaloids, which increase K^+ permeability in frog muscle (Frank, 1958), permit electrical initiation of spreading depression suggest that the resistance of chronic slabs to initiation of spreading depression results from decreased K^+ permeability and the ineffectiveness of electrical stimulation to cause the release of sufficient K^+ to initiate spreading depression.

In relation to the first possibility concerning K^+ (that the cells may be K^+ deficient), Grafstein (1956) found that repeated induction of spreading depression depleted the neurones K^+ and reduced the amplitude of the spreading depression waves. Topical treatment of the slabs with KCl then restored the amplitude of the negative wave of spreading depression. Thus the fact that the negative wave of the spreading depression is consistently smaller in chronic than in acute slabs and the possibility that the trauma of reisolation of the slabs may supply K^+ to deficient cells with K^+ from cells destroyed or temporarily made anoxic by the surgery, still permit the interpretation that K^+ deficiency also plays a role, although an alternate explanation for the reduced negative wave is possible. It may be that fewer neurones are involved in spreading depression in the isolated slab. The occasional persistence of activity even during a spreading depression wave in chronic slabs suggests that not all neurones in the slab necessarily participate in the spreading depression. The lack of spontaneous activity in the reisolated slabs may permit the spreading depression to involve all neurones. The possibility of K^+ deficiency could perhaps be settled by testing for long term changes in electrical-stimulation threshold for spreading depression after brief topical treatment of the chronic slab with KCl. If

this treatment lowered the threshold it is probably that a K^+ deficiency existed previously.

The ease of induction of spreading depression is related to the amount of recent trauma sustained by the cortex. There is some controversy concerning this point (Marshall, 1959). Thus, Ochs, Hunt and Booker (1961) were able to produce spreading depression with chronically implanted electrodes in "normal" untraumatized cortex. On the other hand, a wide variety of damaging procedures such as dehydration (Marshall, Hanna and Barnard, 1950), exposure to air (Marshall and Essig, 1951; Van Harreveld and Bogen, 1956) and asphyxiation (Van Harreveld and Stamm, 1953, 1954) have been shown to predispose cerebral cortex to the induction of spreading depression. The insensitivity of chronic slabs could therefore be a consequence of the relative absence of recent damage to the cortex. It appears to be likely in the present study that the trauma of the surgical manipulation necessary to reisolate the slab is the factor which predisposed the reisolated chronic slab to spreading depression whether this may be by increasing K^+ permeability or by direct release of K^+. It was because of this possible relationship between non-specific damage and susceptibility to spreading depression that it was decided to reject, as damaged and probably anoxic, those acutely isolated slabs which had a lower threshold for spreading depression than for epileptiform activity.

XI. GENERAL DISCUSSION

The conclusion drawn from the results of this investigation and other available evidence is that the basic defect of chronically isolated slabs of cerebral cortex results neither from changes in the electrical properties of, nor from anatomical rearrangements of cortical cells, but rather from an alteration in the processes of synaptic activation due to the development of denervation supersensitivity. Further, it is suggested that the functional difference between focal epileptic tissue and normal cortex is a difference in the maintenance and propagation (and hence in the duration and intensity) of the seizure discharge rather than a difference in the threshold conditions required for the initiation of a discharge. Very localized, brief seizures can be elicited by minimal stimulation in normal cortex, whereas in epileptic tissue all seizures are more effectively self-maintained and spread by self re-exciting neurone chains made supersensitive by chronic partial denervation. A minimal number of cells must be recruited into the discharge before it becomes self-sustaining.

In this study it was shown that the minimal electrical stimulation required to start an epileptiform afterdischarge is very similar in acute and chronic slabs. These cells apparently have the same electrical excitability. This suggests that the electrical properties of the cortical neurones' membranes have not been significantly altered by chronic denervation, although the resistance of the chronic slabs to spreading cortical depression suggests that the K^+ permeability may be decreased.

The analysis of radial potential gradients in the cortex during epileptiform afterdischarges and following subthreshold electrical stimulation led to the conclusion that they were not related in a cause-and-effect manner to the initiation of or maintenance of the seizure. This

disassociation of the "n.a.d." and the outbreak of seizures was recently confirmed by Sanders and Pinsky (1964) who observed that the seizure threshold and the amplitude of the "n.a.d." at threshold could be independently altered. This is not to say that somatic depolarization of the cortical neurones is not an essential prerequisite for a seizure, but only that the development of a positive-to-negative gradient between the dendrites and soma is not a necessary factor in the discharge of either acutely or chronically denervated cortex. On the contrary, "paroxysmal depolarizing shifts" (PDS) of neurone somatic membranes have been observed during seizures in both normal (Sawa et al., 1963) and focal epileptic (Matsumoto, 1964) cortex. These depolarizations appear to result from massive synaptic bombardment (Matsumoto, 1964; Matsumoto and Ajmone-Marsan, 1964a,b). Matsumoto (1964) suggested that the process of seizure genesis involved an explosive recruitment of neurones by synaptic activity. These neurones then maintain their depolarization and act as a "pacemaker" for the seizure by their synaptic interactions. There appears to be little difference between the PDS of seizures induced in normal cortex and the spontaneous PDS of epileptic cortex, except that the latter are a bit larger, lasting longer and depolarizing below the soma firing level more often.

The major change caused by the chronic isolation of the cortex is the greatly prolonged discharge durations in chronic slabs as compared to those in the acute slabs. It is suggested that this increase in discharge duration is due to a sensitization of the synaptic interactions between the cells of the focus so that the electrically induced depolarization is sustained after the end of the stimulus by a synaptically induced depolarization sufficient to maintain axon firing and hence further

synaptic activation. Although, from the radial potential measurements, it is possible that the focus may wander away from the original point of stimulation during the long discharges of the chronic slabs, the "epileptic state" at that original point of stimulation must persist throughout the discharge at that point since further stimulation at that point does not induce a new seizure pattern nor alter the existing seizure. Toman, Swinyard and Goodman (1946) also noted that once a seizure was begun it could not be modified by further stimulation. Because the means of seizure maintenance involves intra-slab self re-exciting neurone chains it is probable that the large pyramidal neurones, whose axons terminate primarily outside the cortex, must play a rather minor role in seizure maintenance. The major role must be played by neurones with a local, intracortical distribution of their axons.

The results of many investigation listed above (p. 27) suggest that cells in epileptic cortex fire more rapidly than those of normal cortex. Rather than any electrical instability of the cell membrane, it would appear that afferent impulses impinging on supersensitive cells would be sufficient to maintain this higher rate of firing.

It has previously been established that the duration and intensity of a seizure in intact brain is dependent on the total area of cortex which becomes involved (i.e., the extent to which the seizure is propagated) which in turn is dependent on the strength of the stimulus (Cannon and Rosenblueth, 1942; Konigsmark, Abdullah and French, 1958; Sharpless and Halpern, 1962; Tedeschi, Swinyard and Goodman, 1956). It would appear that as more cells become involved, the secondary activation (re-excitation) of the cells in the seizure focus becomes greater, thus increasing the intensity and duration of the seizure. This process would

be intensified by denervation supersensitivity.

Some observations of Sharpless and Halpern (1962) provide an example of this in "mirror image" foci. The cortex in the homotopic area contralateral to a chronic slab might be expected to become slightly supersensitive, since it would also be partially denervated. Indeed, Franken and Desmedt (1957) did observe increased spread of responses under such conditions. Sharpless and Halpern (1962), in testing the sensitivity of intact cortex, wished to avoid the post-ictal depression of the animal which follows generalized seizures, and hence they attempted to keep the seizures of the intact cortex localized to the homotopic cortex contralateral to the chronic slab by using low stimulus strengths. This objective became harder to realize as the experiment progressed. After several months, stimuli which were only slightly suprathreshold at the time of testing produced generalized seizures. (At the same time they observed no change in the threshold of the intact cortex, just as could be expected from the results of the present study.) Although they suggested that the increased epileptogenesis of the intact cortex developed as a consequence of the repeated stimulation of the several months of testing (cf., Morrell's (1959, 1960b) "bombardment" theory for the origin of "mirror image" foci), a more satisfactory explanation would be the gradual development of denervation supersensitivity which would allow a more rapid recruitment of cells in the contiguous cortex into the seizure discharge pattern, and hence cause more intense seizures at the focus and produce greater seizure propagation. The development of supersensitivity in cortex contralateral to the chronic slabs was probably not very great compared to the chronic slabs themselves, since in the present study no difference was seen between the discharges of acute slabs cut

from otherwise intact cerebrum and the discharges of those slabs made in homotopic cortex contralateral to chronic slabs. Indeed the degree of chronic denervation of this cortex would be only a very small proportion of the total afferent input.

Metabolic theories of epilepsy have been discussed above (Section II). Nothing directly related to these theories was investigated in this study, except for the observation that severe anoxia (carotid occlusion) would usually stop existing discharges, or reduce the duration of discharges initiated immediately after the occlusion. It was assumed for the purposes of this study that no change in general oxidative metabolism is primarily involved in the epileptogenesis of chronic slabs, although biochemical changes doubtless occur secondarily to the seizures. The intermittent ischaemia theory of Penfield and Jarper (1954) may be assimilated into the theory of denervation supersensitivity insofar as local vascular insufficiencies could cause focal areas of necrosis which would leave the surrounding cells partially denervated. The concept that ischaemic episodes are responsible for initiating individual fits seems unlikely since under such conditions the cortex would be depressed by anoxia. Although anoxia causes convulsions, the caudad part of the reticular system is probably responsible for anoxic convulsions (Gastaut, Naquet and Fischer-Williams, 1958). Because GABA has been suggested as the or a central inhibitory transmitter, abnormal GABA metabolism has often been considered to be the basis of some epileptogenic foci (e.g., Purpura et al., 1958b; Symonds, 1959). However, there is a strong possibility that GABA is a nonspecific depressant in the mammalian central nervous system, depressing both IPSP's and EPSP's (Curtis, Phillis and Watkins, 1959), and not a specific inhibitory transmitter. Similarly,

GABA has been postulated to be involved in avitaminosis-B_6 seizures because of the known role of B_6 in GABA-glutamic acid metabolism (Tower, 1960), however some other metabolic pathway may well be involved in these seizures.

The role of ACh in the genesis of seizures, and in particular of seizures in chronically denervated cortex, remains uncertain. However, since atropine seems to block only ACh-induced seizures and not seizures of other origins, it is improbable that ACh has a necessary role in epileptiform seizures. Possibly the cholinergic pathway is only one of several alternates, any one of which may be sufficient but not requisite.

Isolation of the cortex in the manner described would only partially denervate a majority of cells in the slab (although all afferent activity would be eliminated). However, there is good evidence that partial denervation of a cell results in an increased sensitivity over the whole cell, not only to the normal transmitter, but to other "transmitter" substances as well. Frank (1959; 1961) has shown this in frog's sartorius muscle, which has at least two end-plates per fibre. Denervation of one end-plate region leads to increased sensitivity to ACh at another end-plate region on the same cells. Moreover, the muscle became sensitive to the muscarinic drug methacholine, which does not normally stimulate skeletal muscle. Similar results with respect to the spread of sensitivity over the whole cell following partial denervation of skeletal muscle have been obtained by Miledi (1960). Emmelin and Strömblad (1957), Emmelin and Engstrom (1960) and Burgen and Emmelin (1961) reported that denervation of the parasympathetic supply to the salivary glands causes increased responses to sympathetic stimulation and vice versa. The salivary glands' cells are normally innervated by both sympathetic and parasympathetic

systems.

There does not appear to be any general agreement as to the cellular basis of denervation supersensitivity, but the changes in the ACh-metabolic system in denervated cortex are typical of changes associated with denervation supersensitivity in a variety of places in the peripheral nervous system, namely decreases in the concentrations of the transmitter and of its synthetizing and inactivating enzymes. However there does not seem to be a causal relationship between denervation supersensitivity in all situations. The difference in sensitivity between the denervated and the innervated end-plates in partially denervated frog skeletal muscle fibres may be largely due to the reduction of ChE at the denervated end-plates (Frank, 1959), and the difference between completely denervated and normal frog muscle may be partly due to the reduction of ChE in the denervated muscle (Frank, 1959). The observation by Brooks and Myers (1952) that there was no correlation between ChE concentration and supersensitivity to ACh in denervated guinea pig skeletal muscle may be incorrect because they did not take into account the presence of large quantities of true ChE at the musculo-tendenous junction (Giacobini and Holmstedt, 1960). However, Brzin and Zajicek (1958) suggest that denervated mammalian skeletal muscle end-plates still retain appreciable true ChE activity. In the autonomic system there are many reports correlating the concentration of the inactivating enzyme with denervation supersensitivity (e.g., Burn and Robinson, 1953). However denervation of the sympathetic supply to the salivary glands causes an increased sensitivity to parasympathetic stimulation and parasympathomimetic agents without altering the concentration of ChE (Emmelin and Engstrom, 1960; Burgen and Emmelin, 1961). Denervation supersensitivity

is probably also independent of the store of transmitter since sympathetic decentralization (pre-ganglionic denervation) does not deplete noradrenaline stores, yet sensitizes the nictitating membrane to noradrenaline (Kirpekar, Cervoni and Furshgott, 1962). The only feature common to all these situations of denervation supersensitivity is the interruption of the "nerve traffic" and reduction in transmitter release. This is also true of the isolated cortex, since all the afferent stimulation has been eliminated. The decreased "nerve traffic" may well produce the supersensitization by different mechanisms in skeletal muscle, smooth muscle and the cortical cell. Even though ACh itself may not be involved, there is a strong possibility that the cortical cells which have become supersensitive to ACh will also be supersensitive to any other transmitter which normally excites those cells.

The correlation of the chronic isolation lesion with other types of experimental and clinical focal epileptogenic lesions is difficult to assess because the isolated slab cannot project its activity on, nor be influenced by, the rest of the brain. One of the obvious features of a clinical focus is that the seizures may occur spontaneously or be precipitated by various specific afferent stimuli. Both have been observed in chronic slabs. In the present study, apparently spontaneous seizures were observed at least once in each of 4 chronic slabs. Echlin and Battista (1961) found it difficult but not impossible to induce seizures in partially isolated chronic slabs by afferent stimulation. The difficulty probably arose because the afferent activity would only enter the slab secondarily, via cortico-cortical pathways. It is also difficult to induce self-sustained seizures in penicillin foci by afferent stimulation (Ralston, 1962); most often only a driven spiking can be obtained.

This is also true in many clinical cases, where the specific afferent stimulation may only produce pre-convulsive EEG activation, or perhaps driven spiking or myoclonic jerks, but not self-sustained seizures unless ajuvants such as hypocapnia or pentylenetetrazol are used (Penfield and Jasper, 1954; Jasper and Daly, 1955).

Clinical opinion seems to be that human epileptogenic foci have lower electrical (Walker, 1949) and pentylenetetrazol (Kaufman, Marshall and Walker, 1947; Cure, Rasmussen and Jasper, 1948; Walker, 1949) seizure thresholds than does the normal cortex. This is not in accord with the experimental results obtained in this study, or in the study of Halpern (1962), in chronically isolated slabs. There are some clinical reports, however, which are in agreement with the present study in suggesting that the electrical thresholds of epileptic foci do not differ from the normal (e.g., Garciadiega, Chávez and Alcalde, 1944; Kalinowsky and Kennedy, 1943). Moreover, Penfield and Erickson (1941) pointed out that the threshold of human foci to direct electrical stimulation can be highly variable, and that in some cases the thresholds of the epileptogenic foci may be much higher than the normal tissue. There may be several explanations for this difference between the results in the present study and in previous clinical reports such as that of Walker (1949). It is well known that the electrical threshold varies widely between different regions of the cortex, both in human (Penfield and Jasper, 1954) and in the monkey (French, Gernandt and Livingston, 1956), and most clinical comparisons appear to have been made between the foci and the surrounding normal cortex rather than the contralateral cortex. It is possible that the region containing the focus may have had a lower threshold to start with, since clinical foci seem to predominate in regions that are normally the most susceptible,

such as the motor cortex and hippocampus (cf., Gloor et al., 1961). Also, if because of denervation supersensitivity the electrically initiated seizures are propagated more easily from the clinical focus, these seizures are more likely to be detected in an electrocorticogram, or to produce motor effects, than would a seizure in normal cortex, which would remain very localized.

The concept that human epileptogenic foci have a lower pentylenetetrazol seizure threshold than normal cortex also warrants examination. It is asserted that both human foci and experimental foci (aluminium hydroxide lesions) in monkeys are "selectively activated" by pentylenetetrazol (Kaufman et al., 1947; Cure et al., 1948; Walker, 1949). In these experiments the pentylenetetrazol was administered slowly, the end-point being a pre-seizure EEG activation. Since the seizures which occur under such conditions generally resemble the spontaneous seizures of the foci, this production of pre-seizure activity was suggested as a technique for pre-operative localization of focal epileptogenic cortical tissue. This has turned out to be a useful technique when such localized activity actually is produced, but a re-examination of these reports makes it evident that the EEG activation produced may be neither localized nor particularly selective. While a majority of epileptic patients (about 85%) develop typical pre-seizure activation, probably in less than half of these was the activation localized. The patients with known focal cortical lesions were the least sensitive to pentylenetetrazol. However if focal cortical EEG abnormalities were present at the time of pentylenetetrazol administration these were aggravated at lower doses than affected normal cortex. Moreover, besides the large number of epileptic patients who developed EEG changes (activation) in normal cortex, a number of control

patients exhibited EEG abnormalities (but which were not estimated to be pre-seizure states) at dosages similar to those which caused the pre-seizure EEG activation in the epileptic patients. Of the post-traumatic patients (presumably with focal lesions) who developed seizures under this procedure, half had non-localized seizures. In the post-traumatic patients who developed only generalized EEG activation, retesting sometimes produced localized activation. Penfield and Erickson (1941) reported that pentylenetetrazol caused seizures in a high proportion of non-epileptic patients; and Penfield and Jasper (1954) commented that some epileptic patients do not develop even pre-seizure EEG activation as doses that would convulse normal individuals, and that even in the more usual cases the margin between activation of a patient's own localized seizure pattern and initiation of a generalized pentylenetetrazol seizure was very narrow. The observation that pentylenetetrazol aggravated existing EEG abnormalities may be the most significant observation. It may be suggested that pentylenetetrazol merely accentuates or amplifies previously existing subclinical EEG abnormalities or pre-seizure activity, but that the threshold dose level would be the same in quiescent epileptic foci as the threshold dose for EEG alteration in normal cortex. In the clinical focus, the augmented, but pre-pentylenetetrazol in origin, abnormal discharge would just superimpose its pattern on the normal EEG changes that would occur with that dose of the drug. Cure et al., (1948) did suggest that pentylenetetrazol might aid in "self-facilitation" of a seizure once it had begun.

From results of experimental investigations of anti-epileptic agents Toman et al., (1946) and Tedeschi et al., (1956) concluded that the efficacy of an antiepileptic is best estimated by the reduction in

duration and intensity of maximal seizures, rather than by alterations in the electrical or chemical thresholds. Thus the ultimate in antiepilepsis is the reduction of the duration of the seizure to zero, and not the raising of the threshold. This supports the concept that the difference between focal epileptic and non-epileptic cortex is the increase in the ability of the epileptic cortex to propagate the seizure to contiguous cortex due to the development of denervation supersensitivity and the consequent increase in "self-facilitation" in the focus.

Since virtually any damage to the cortex which would produce a local area of destruction of nerve cells, and thus an area of partial denervation around it or in the opposite hemisphere, might be expected to cause denervation supersensitivity and increased epileptogenesis in those regions, the rationale of surgical removal of foci may be in some doubt. Nevertheless Penfield and Jasper (1954) report considerable success with surgical removal of foci. They do however note that there were some cases where after several months the focus returned to the margin of the area removed. Echlin (1959) reported that the cortex surrounding the chronically partially isolated slabs was normally sensitive to electrical or chemical stimuli, but in the light of the present study, changes in threshold would be unexpected. The answer to this apparent dichotomy probably is that the cells around such an excision are only slightly denervated when compared to the total afferent input that such cells receive, and the remaining stimulation of these cells may be enough to keep them sufficiently "desensitized" that clinical seizures do not recur.

XII. SUMMARY

1. Chronically and acutely isolated cortical slabs were examined histologically. There was considerable neurones loss, seen as a shrinking of the slabs, and a mild astrocytosis. The most evident cell loss was of the large pyramidal cells of Layer V. No evidence of "deformed" dendrites, nor of increased numbers of axon collaterals was found. It was estimated that there was a decreased number of axon-dendrite synapses.

2. Of all the parameters of epileptogenic electrical stimulation studied, the only threshold affected by chronic isolation was the frequency threshold. This is ascribed to a more effective recurrent synaptic excitation due to the development of denervation supersensitivity. Since the voltage and pulse-width thresholds, as well as the cortico-electrode junction conductances of chronic and acute slabs were the same, it was concluded that the electrical excitability of cortical neurones was unchanged by chronic isolation.

3. The shape of the stimulus-response curves for all stimulus parameters except frequency were all-or-nothing for both acute and chronic slabs, however the afterdischarges of chronic slabs were always much longer than in acute slabs. The stimulus frequency-response curve was all-or-nothing only after chronic isolation. It was concluded that the development of the maximal response in response to minimal stimulation in the chronic slabs could best be explained by increased synaptic maintenance of the epileptic state induced by the electrical stimulus.

4. Reisolation of the chronic slabs (when conducted with a minimum of trauma) did not alter the stimulus threshold nor the duration of the afterdischarge. Thus surviving or regrown connections with the surrounding cortex could not be responsible for the increased duration of epileptiform afterdischarge of chronic slabs.

5. Examination of the voltage threshold, the effect of the area stimulated on the voltage threshold, the threshold number of pulses, and the threshold values of the "n.a.d." led to the conclusion that the minimum number and density of excited cells required for discharging focus is the same in chronically and acutely isolated cortex.

6. The positive burst response and spontaneous afterbursting were shown to have no relation to epileptic processes, since they can occur immediately after isolation, and are affected differently by chronic isolation than is the epileptiform afterdischarge.

7. Chronic isolation caused no significant changes in the radial potentials developed by the cortex during epileptiform afterdischarge. Further, there was no consistent relationship between radial potentials and the initiation and maintenance of afterdischarges. It was concluded that a potential gradient between the dendrites and the soma of radially oriented cells was not, per se, required for afterdischarges in either acutely or chronically isolated slabs.

8. It was confirmed that the cortex becomes supersensitive to ACh after chronic denervation. However a necessary role of ACh in cortical pathways mediating the epileptiform afterdischarge is doubtful because atropine has no effect on discharges other than those due to ACh.

9. Spreading depression can be induced in chronic slabs of cortex only after reisolation. It is suggested that reduced release of K^+ by neurones may be the reason that chronic slabs are not normally susceptible to spreading depression.

10. The data obtained in this investigation are compatible with the hypothesis that focal cortical epilepsy may be due to denervation supersensitivity in cortical neurones. It is suggested that this supersensitivity causes greater auto-excitation via recurrent pathways and greater recruitment of more distant cells into an established discharge, so that the discharge is maintained for longer and propagated to a greater degree than normally.

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