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

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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

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.

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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

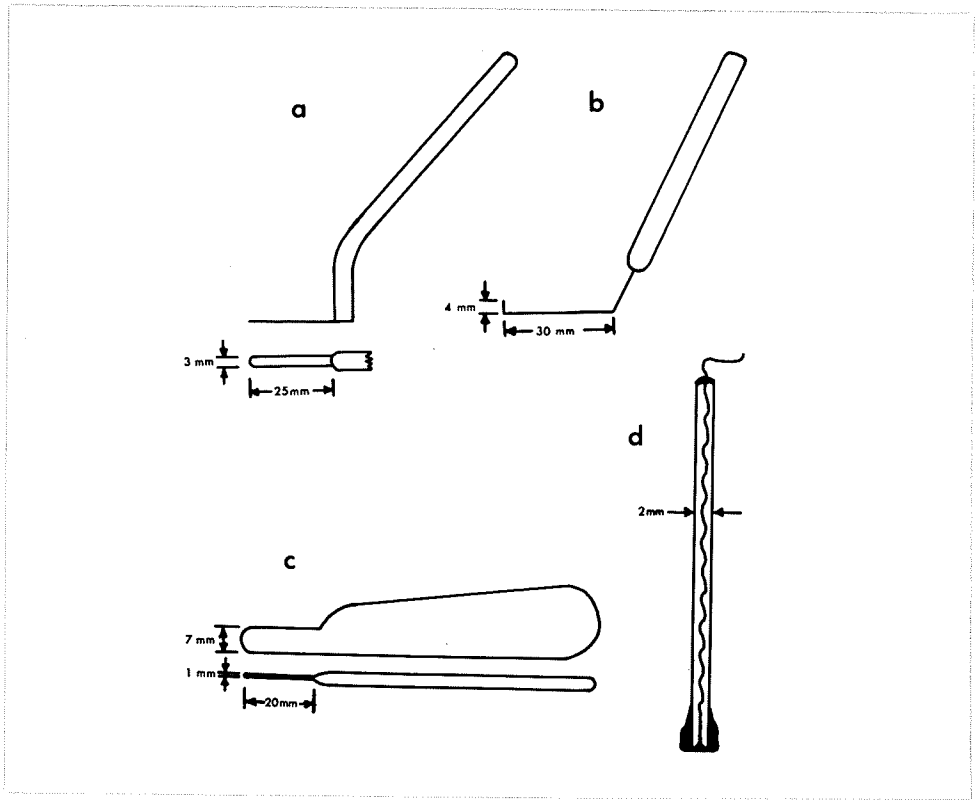

Fig. IV - 1

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

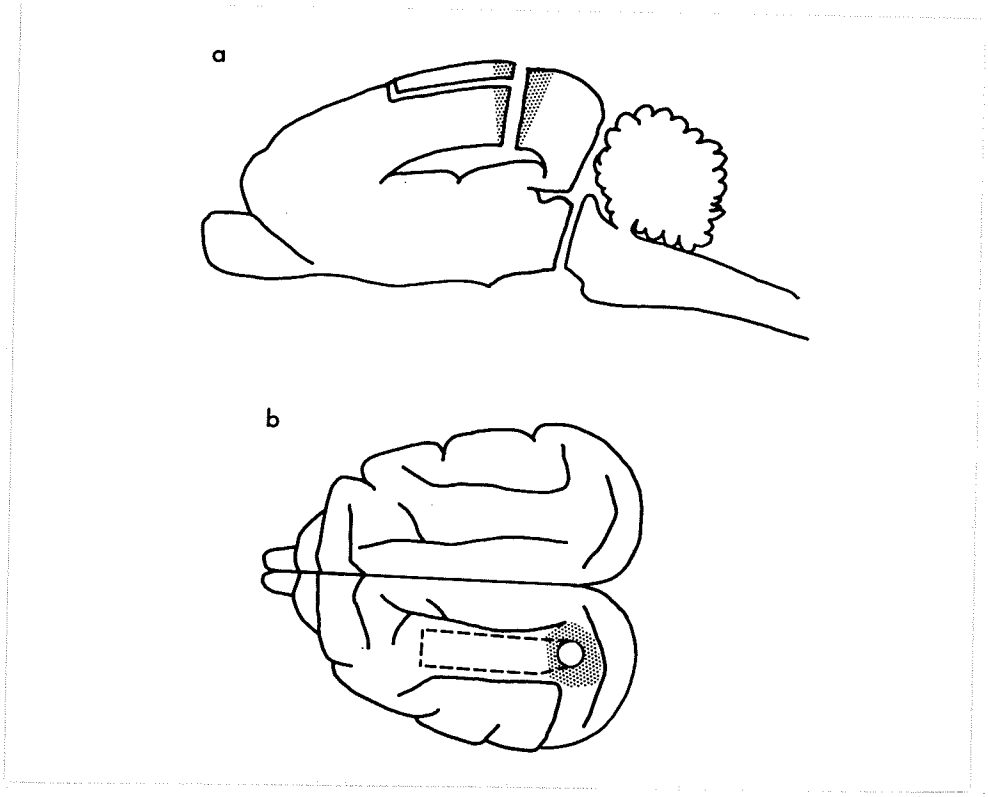

Fig. IV - 2 Diagrams of the preparation

(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.

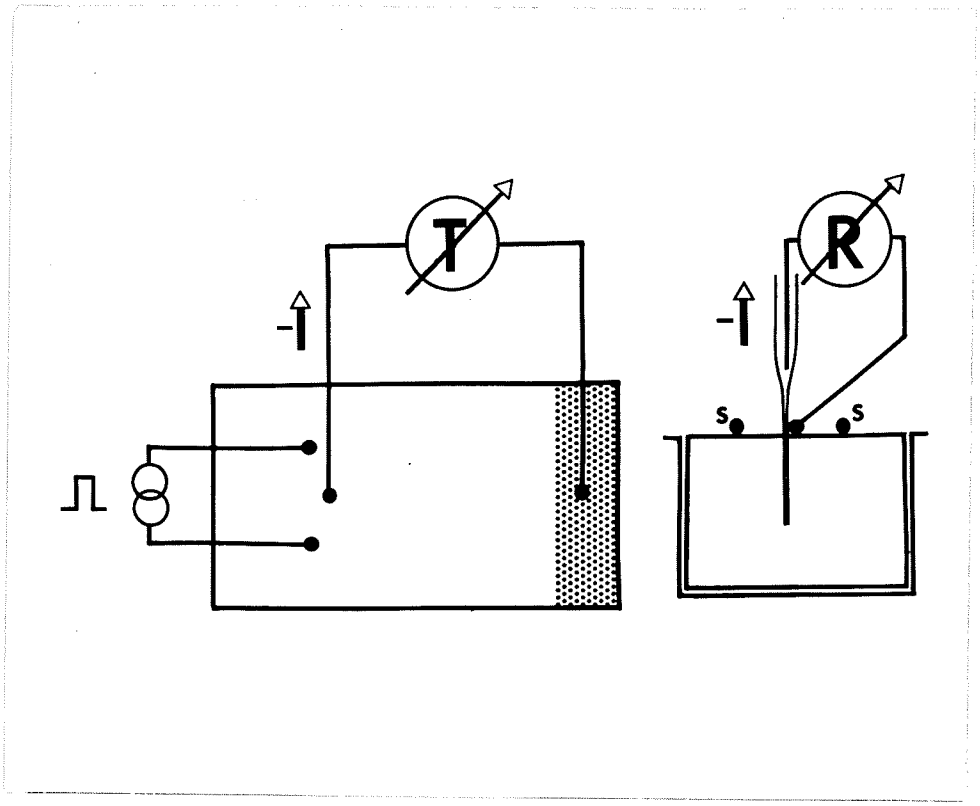

Fig. IV - 3 Stimulating and recording arrangements

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.

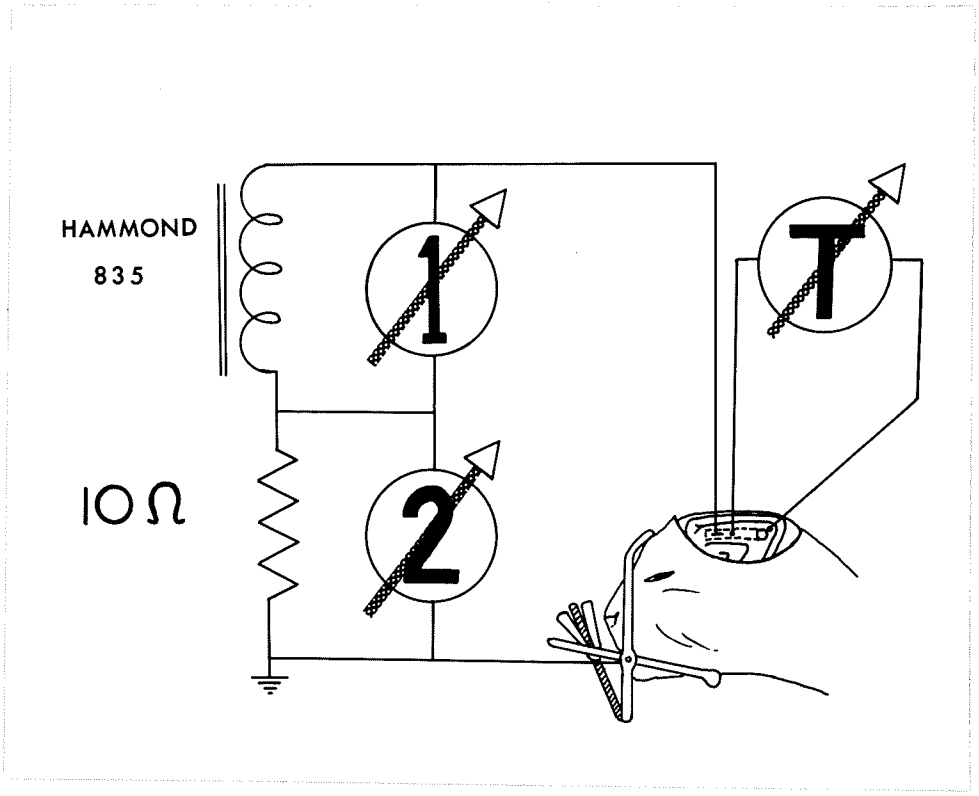

Fig. IV - 4 Arrangement for recording the effect of variations in stimulated area.

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.

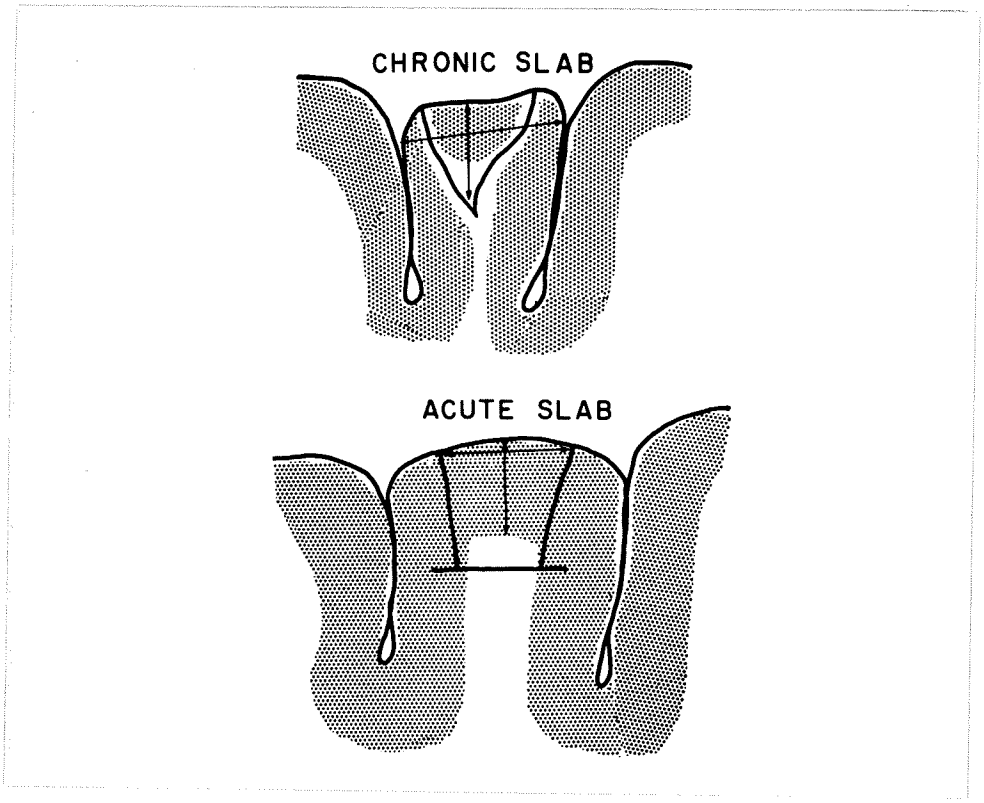

Fig. V - 1 "Camera lucida" drawings of slabs

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.

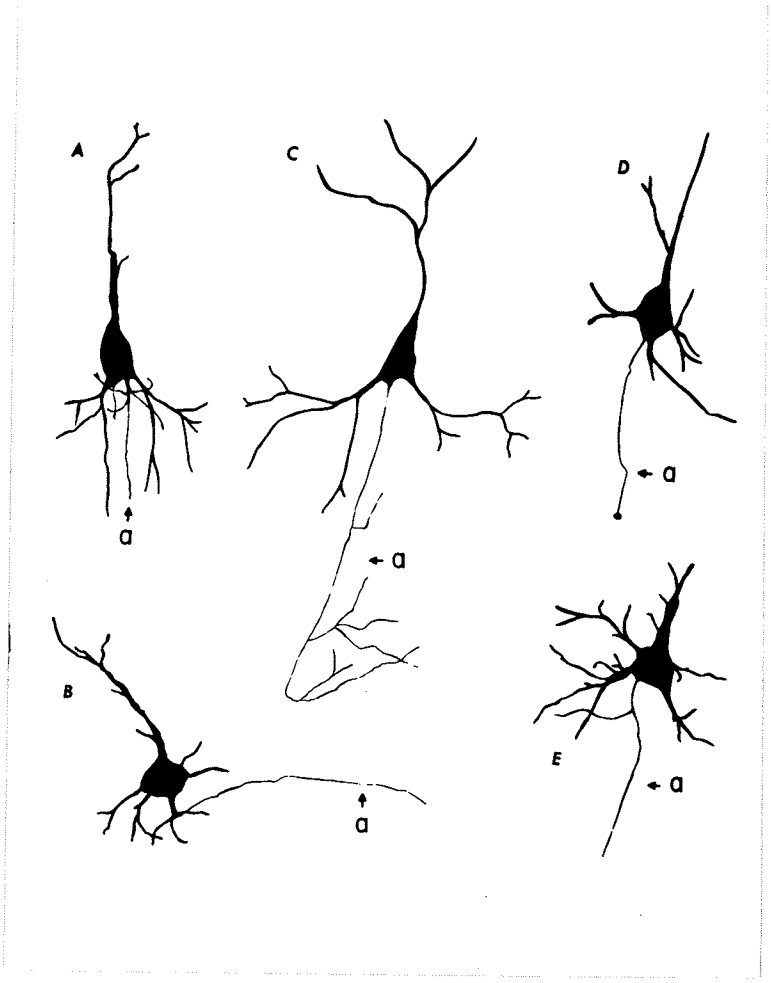

Fig. V - 2 Drawings of typical neurones

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.