STUDIES ON A PROPOSED COMMON MECHANISM FOR THE ACTION OF GENERAL AND LOCAL ANAESTHETICS IN THE CENTRAL NERVOUS SYSTEM

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by

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ABSTRACT

Procaine and several general anaesthetics have been reported to block action potential production in frog's skeletal muscle fibres and nerve fibres by a single mechanism of action suggesting a common basic mechanism of action on all excitable cells. It was proposed, therefore, that these agents produce their effects on the central nervous system by a similar common mechanism.

Procaine, dibucaine, lidocaine, cocaine and a convulsant barbiturate 5-ethyl-5-(1,3-dimethylbutyl) barbiturate (DMB) given alone to intact white mice produced 'excitement' and convulsions but when given 60 minutes after phenobarbital caused central nervous system depression. Large convulsant doses of these agents caused a loss of the righting reflex in mice pretreated with small subanaesthetic doses of phenobarbital. In contrast, pentylenetetrazol only antagonized the depression produced by phenobarbital. Pentylenetetrazol given after a combination of phenobarbital and procaine antagonized only the phenobarbital depression and added to the depression produced by procaine.

When applied topically to neuronally isolated slabs of cat's cerebral cortex, procaine or pentobarbital reduced the sizes of the surface negative response and surface positive burst response to direct stimulation to the cortex. Pentylenetetrazol had the opposite

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effect. DMB usually depressed both responses of the cortex.

When given systemically, procaine and high doses of DMB raised the threshold for the surface positive burst response, ether raised or did not change this threshold and pentylenetetrazol and low doses of DMB either lowered the threshold or left it unchanged. In addition, DMB in low doses lowered the threshold for the surface negative response.

Systemically administered procaine and pentylenetetrazol usually increased the primary component of the click evoked response in the auditory cortex of spinalized cats. In contrast, the amplitude of this response was decreased by subanaesthetic doses of thiopental. Pentylenetetrazol given after thiopental increased the amplitude of the evoked response over that produced by thiopental alone. Procaine, depending on the dose, potentiated or antagonized the thiopental response.

The results support the contention that local and general anaesthetics act by a single common basic mechanism in the central nervous system. It is suggested that the differences in the central nervous system effects observed after local and general anaesthetic administration is due to the greater degree of conduction block produced by local anaesthetics on small fibres in inhibitory pathways.

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I. INTRODUCTION

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A. GENERAL INTRODUCTION

In 1846, Morton demonstrated the general anaesthetic properties of ether and in 1884, Koller introduced cocaine as a local anaesthetic into clinical practice (Goodman and Gilman, 1955a). Many new drugs having these effects have been introduced since that time and various theories have been proposed to explain these effects on nervous tissue. Since both general and local anaesthetics act on excitable tissue, theories concerning the mode of action of one group might apply to the other as well. The present investigation is based on the concept that both groups of drugs have a common basic mechanism of action on nervous tissue at the cellular level.

B. CHEMISTRY

Those agents usually classified as general anaesthetics have a wide divergency in chemical structure. Until 1939, these agents included little more than various analogues of short chain hydrocarbons and a wide variety of urea derivatives which included the barbiturates. The discovery of the anaesthetic effects of argon (Behnke and Yarborough, 1939) added a completely new type of anaesthetic compound. Later, krypton and xenon were also shown to be anaesthetics (Lawrence et al., 1946). It becomes clear, then, that no specific structure is absolutely necessary for anaesthetic activity, and indeed, this lack of specificity can be regarded as an outstanding characteristic of anaesthetics (Butler, 1950).

Despite this lack of structural specificity there are nevertheless some structural features which appear to be incompatible with anaesthetic properties. These features have been summarized by Butler (1950), and are presented here. First, active anaesthetics have not been found among organic compounds that are ionized to a large extent at physiological hydrogen ion concentrations. Compounds that are highly soluble in water are also poor anaesthetics. The introduction of some group e.g., hydroxyl, which increases the water solubility of a compound by the formation of hydrogen bonds, reduces anaesthetic activity. Finally, metabolic reactions leading to strongly ionized or highly soluble products are important in terminating the effects of many anaesthetics. This is particularly true for the barbituric acid derivatives (Burger, 1960a). Predictions on the lack of anaesthetic activity based on structure alone can be made with considerably more confidence than can predictions of the presence of such activity (Butler, 1950).

In contrast to this lack of structural specificity in the general anaesthetics, the clinically used local anaesthetics constitute a relatively homogeneous chemical group. For the most part they are tertiary amino esters of aromatic acids although the ether and amino analogues may also show local anaesthetic activity. In addition to the para-amino benzoic acid derivatives, quinine and quinoline derivatives are also active, as are several primary and secondary aromatic alcohols. The structural features of the local anaesthetics have been reviewed by Hirschfelder and Bieter (1932).

All antihistaminic drugs are also capable of exerting a limited degree of local anaesthesia if applied topically. These agents are all chemically related to each other but bear only a very remote resemblance to the clinically used local anaesthetics. There appears to be no correlation between local anaesthetic effectiveness and antihistaminic activity (Goodman and Gilman, 1955b). A major difference between these two classes of drugs is their effects on the central nervous system. Systemic administration of the clinically used local anaesthetics will, in adequate doses, produce central convulsions. The antihistaminics given in this way, may produce sedation or convulsions (Goodman and Gilman, 1955b).

C. COMPARISON OF THE EFFECTS OF GENERAL AND LOCAL ANAESTHETICS AT THE CELLULAR LEVEL

The classification of general and local anaesthetics into separate categories may be artificial. Gros (1929) showed that cocaine, eucaine and procaine prevented the normal motion of paramecia. Conversely, cutaneous injections of chloroform, ethylurethane and several other general anaesthetics into the centre of a wheal in mammals, produced local anaesthesia. Alcohol and ether have been shown to produce conduction block when applied directly to peripheral nerve (Davis et al., 1925). Cocaine and amyl alcohol were shown to produce peripheral nerve block by stabilization of the cell membrane i.e., block without depolarization (Bishop, 1932) and similar results have been obtained for the barbiturates (Heinbecker and Bartley, 1940). Low concentrations of homologous series of urethanes were found to produce a small hyperpolarization of the cell membrane while depolarization occurred with higher concentrations (Cresticelli, 1948). Conduction block in the nerve, however, occurred both during depolarization and hyperpolarization.

Any similarity between the effects of ether and those of local anaesthetics has been obscured by observations indicating that ether prevents the action potential in nerve and muscle by depolarization of the membrane rather than by stabilization (Wright, 1947; Lorente de No, 1947; Heinbecker and Bartley, 1940; Alcock, 1906). The evidence, however, is equivocal. Gross and Cullen (1943), for example, observed that ether reduces the response of skeletal muscle to nerve stimulation and to close intra-arterial injection of acetylcholine. They also showed that ether enhances the effect of curare (which stabilizes the membrane) and could be antagonized by neostigmine, a cholinesterase inhibitor which blocks impulse transmission by depolarization.

Strong evidence for the stabilizing action of ether has been obtained recently by Yamaguchi (1961) and by Inoue and Frank (1962a). It was shown that ether blocks the production of action potentials in skeletal muscle fibres by inhibiting the specific increase in sodium conductivity of the membrane which normally follows an adequate stimulus. Procaine, a local anaesthetic which has consistently been shown to block by stabilization of the membrane (Bennet and Chinburg, 1946; Bishop, 1932), also blocks action potential production by an identical mechanism in skeletal muscle (Inoue and Frank, 1962b) and in nerve (Taylor, 1959; Shanes, et al., 1959).

There exists, therefore, a distinct possibility that both local and general anaesthetics act by some single basic mechanism of action at the cellular level. The concept is not new, but between the first unified field theory of Claude Bernard (1875) and the microcrystal hypothesis of Pauling (1961), many theories of narcosis (or anaesthesia) have been advanced.

D. HISTORICAL REVIEW

1. Theories of general anaesthesia and their relevance to local anaesthesia

One of the controversies which has plagued theories of general anaesthesia has been the use of the terms 'narcosis' and 'anaesthesia'. The multiplicity of meanings given to the former have made this term almost meaningless unless specifically defined. As used in discussion of the following theories of anaesthesia, narcosis will be taken to mean the general depressant effect produced by drugs. As such, this term will also include anaesthesia and may be substituted for it. The reverse substitution i.e., anaesthesia for narcosis, may not be made. Anaesthesia, as used below, will be considered to be a special case of narcosis implying a loss of consciousness in some animal, or, if used in the clinical sense, includes Stage I (analgesia), Stage II (excitement), Stage III (surgical anaesthesia).

None of the theories discussed below explains adequately the mechanism by which various agents produce anaesthesia. The purpose in reviewing these theories is to point out how unsatisfactory is our knowledge about anaesthesia and to show in what respects these theories failed to elucidate anaesthetic mechanisms.

a) The Overton-Meyer Theory

H.H. Meyer (1899) proposed a theory of narcosis which was almost immediately endorsed by Overton (1901). It is based on the fact that narcotics are soluble in lipoids and that the strength of their action is related to their distribution coefficient in oil and water. Meyer stated his theory in the following way:

1) All chemically indifferent substances which are fat solvents exert a narcotic action upon living protoplasm insofar as they can diffuse into it.

2) The effect shows itself first and most strongly in those cells where there is a preponderance of lipoid material viz., the nerve cells.

3) The relative efficiency of such narcotics is dependent on their physical affinity for lipoids on the one hand and for water on the other hand. It is dependent therefore, on the partition coefficient which determines their distribution in water and lipoid.

Both Meyer and Overton used an olive oil-water system in testing this hypothesis although they were aware that neutral fats probably did not exist as a normal component of the cell membrane. On the basis of the proposed theory, the depressant effect on the cell would parallel the molar concentration of narcotic in the cell lipoid independent of the chemical structure of the narcotic. K.H. Meyer and Gottlieb-Billroth (1920), however, tested a large number of anaesthetics and found that the narcotic concentrations, calculated by multiplying the concentration of the substance necessary to produce narcosis by the olive oil-water partition coefficient, varied between .001 for ethylurethane to .09 for phenylurethane - a factor of almost 100. Much better agreement was obtained for the volatile anaesthetics, the range being between .04 for ethylene and .10 for amylene (Meyer and Hopff, 1923).

Thus, from the outset, the Overton-Meyer theory could not account adequately for all anaesthetics. Meyer and Hemmi (1935) suggested that oleyl alcohol be substituted for olive oil as it appeared to give a much closer correlation with narcotic effectiveness in tadpoles than did the olive oil-water system. Lofgren (1948), however, decided that local and general anaesthetics could not be compared even with this improved model, since substances with widely differing activities appeared to have the same partition coefficients. He concluded that the minimum effective concentrations of local anaesthetics were not a function of the partition coefficients alone, but showed nevertheless

that these agents were most effective in the lipid phase i.e., local anaesthesia was greater in oil than in water.

The most serious blow to the Overton-Meyer theory was given by Winterstein (1926). He showed that narcotics are capable of exerting their effects on organisms which are completely free of lipoids e.g., acetone-extracted yeasts. It is obvious, therefore, that the Overton-Meyer theory of narcosis is incorrect, since a fat layer of some type is required to give the hypothesis meaning.

From this and other observations, Butler (1950) suggested that the oil-water partition coefficient is merely a measure of the barrier through which the drug must pass in order to exert its effect. This interpretation permits an explanation of the observation that two drugs with the same partition coefficient may produce effects ranging from depression to convulsions as occurs, for example, with several barbiturates (Albert, 1960a).

b) The Traube Hypothesis

A second physico-chemical theory of narcosis was advanced by Traube (1904). He observed that many narcotics were included among a large group of substances which lowered interfacial tension between water and some other phase. He suggested that a definite relationship existed between the surface activity and the narcotic strength of a drug. According to the Gibbs adsorption equation (Glasstone, 1946), the lowering of the surface tension of a solution by a substance is directly proportional to the degree that it accumulates at the surface. Traube regarded this theorem from a different point of view. He stated that the more a substance lowered the surface tension of its solution, the less it attached to the main body of the fluid and this could be

measured by changes in the capillary activity at an air-water interface. The implications of this theory are that narcosis is achieved when the interfacial tension at the cell membrane is lowered to a critical point which is independent of the chemical structure of the surface-active agent. Later, Traube (1912) suggested that since alkalisation increases the surface activity of alkaloids, the increased activity of local anaesthetics in alkaline solution could be attributed to this cause.

The adsorption theory is fraught with many difficulties. Ethylene, ethyl chloride, chloroform and carbon tetrachloride do not lower the interfacial tension between oil and water (Lazarew, 1930). Soaps and detergents, which lower the interfacial tension of an oleyl alcohol-water model do not possess narcotic properties (Meyer and Hemmi, 1935). Among these compounds are naphthalene sulphonates, cetyl sulphonate and salts of fatty acids with carbon chains of six units or more. The Traube theory is also incompatible with the observation that both lidocaine and chloroform increased the surface tension of a cholesterol film (Lofgren, 1948). More recently, Luduena and his co-workers (1955) tested 37 local anaesthetics for surface tension lowering ability. No correlation was obtained for local anaesthetic potency and surface activity although a positive correlation between irritancy and surface activity was found. Finally, Traube's experimental model can be criticized. Values obtained for capillary activity at an air-water interface are merely a measure of wettability and probably bear no resemblance to the events occurring on cellular surfaces (Hober, 1945; Albert, 1960b).

c) The Warburg Theory

Verworn (1912), suggested that narcotics act by interfering with cell oxidations, i.e., anaesthesia could be considered as a type of asphyxia. Some presumptive experimental evidence supporting this hypothesis was supplied by Warburg (1914) using a charcoal catalyst. He showed that narcotics inhibit the oxidation of amino acids by occupying the catalytic surface. He later showed that with larger narcotic molecules, fewer of them were required to coat a given surface area of the charcoal and to produce a given degree of catalyst inactivation. He claimed, therefore, that the anaesthetic activity of a drug is totally dependent on the degree to which it is adsorbed onto the cell surface (Warburg, 1921). In support of this theory, Lofgren (1948) suggested that in vivo the anaesthetic adheres strongly to the node surface of the nerve fibres which results in the formation of a highly concentrated layer of the drug. When this concentration reaches a critical value, anaesthetic effects result. Hober (1945), however, criticized this hypothesis on two counts. First, he pointed out that some narcotic substances are not adsorbable on any cell surface and secondly, some substances which are strongly adsorbed exert no anaesthetic effect.

Watson (1960) considers that adsorption can potentially influence two processes. It might decrease metabolism by blanketing the oxidative process or it might affect permeability by decreasing porosity. Both of these points are covered by other theories and it is unlikely that adsorption alone can explain anaesthesia.

d) Ferguson's Hypothesis

Ferguson (1939) and later Brink and Posternak (1948) attempted to resolve the discrepancies between the foregoing theories. They suggested that chemical potential is a more suitable index of anaesthetic activity than is concentration in oil or adsorption on charcoal surfaces. This concept is based on the assumption that all substances can exert a physiological effect by a physical process. If an equilibrium exists between the external phase and the biologically affected phase, the chemical potential must be the same in each phase. The thermodynamic potential in the external phase can be measured and therefore its value in the biologic phase is known. For gaseous substances, the activity is given by the ratio of the partial pressure of the gas mixture (p_t) required to produce anaesthesia, to the saturated vapour pressure of the substance (p_s) at the temperature of the experiment. If the anaesthetic agent is in solution and is of limited solubility, the activity of the anaesthetic concentration can be put approximately equal to S_t/S_o, where S_t is the molar concentration of the narcotic solution and S_o its solubility in moles per litre. Ferguson applied these calculations to a wide variety of volatile anaesthetics and showed that there was a narrow range of values into which many of these substances fell. He concluded that the principal cause of narcosis was the same for all drugs giving the same p_t/p_s values. He assumed that these agents had a non-specific action which was purely physical in nature. Those agents whose p_t/p_s values fell outside this range were assumed to be acting both by a physical and a chemical mechanism. Ferguson's calculations imply that the ability of the drug to reach the biological phase is more important than whether the agent is soluble in oil or whether it is

adsorbed at the surface of the cell. It seems likely, however, that the specific chemical action of the drug at the biologically affected phase is also of considerable importance.

There is no doubt that Ferguson's calculations are based on sound thermodynamic principles. A major criticism of this work must be that there is no evidence to support the contention that all substances can exert a physiological effect by a physical mechanism. No cognizance is taken of the possibility that when an agent reaches the biophase its physiological effect may result from chemical union with some enzyme system preventing it from carrying out its normal function. A further weakness in this work is that the calculations do not apply to all anaesthetics and two separate and distinct theories are required - one to explain the physical effects and one the chemical.

e) Metabolic Theories

All the metabolic theories are based on the premise that anaesthetics interfere with cell oxidations resulting in a type of asphyxia. The first proponent of such a theory was Verworn (1909). He suggested, on the basis of some rather meagre evidence, that during anaesthesia the ability of a cell to absorb oxygen was impaired. At about the same time, Heaton (1910) showed that a stimulated nerve became inexcitable before a non-stimulated one when both were placed in a narcotic solution. Verworn explained this by assuming that utilization of oxygen is greater when the cell works regardless of whether or not it is narcotized. He further postulated that the ability of the cell to take up oxygen is lost in the presence of anaesthetics. According to this theory, an increase in the duration of narcosis would cause a greater asphyxia.

Over the years, much experimental evidence has appeared to support this theory of anaesthesia. Mansfeld (1909) showed that polywogs are anaesthetized by lower concentrations of paraldehyde in the absence of oxygen and Hamburger (1912) observed that anaesthetics prevented the cell lipids from absorbing oxygen. Kisch (1913), although unable to confirm Hamburger's work, nevertheless held that many anaesthetics decrease the absorption of oxygen by cells. Green and Curry (1925) and Brown et al., (1927) presented evidence that nitrous oxide is a better anaesthetic at low oxygen concentrations but they felt that a considerable part of the anaesthesia was due to anoxia. There is little doubt that the earlier experiments can also be interpreted in this way.

More conclusive evidence can be cited to show the inadequacy of Verworn's hypothesis. Warburg (1910) observed that fission of sea urchin eggs was inhibited by urethane and phenylurethane but that oxidation was little affected. Much higher concentrations of various urethane derivatives were found to be necessary to inhibit oxidation of frog brain slices than to produce anaesthesia (Usui, 1912). Winterstein (1915) observed that the same concentration of ethanol required to produce anaesthesia in frogs resulted in an increased oxidation in nervous tissue. He also demonstrated that frogs anaesthetized with urethane, a long-acting anaesthetic, could be revived within two minutes by perfusion with oxygen-free saline. Pigeons anaesthetized continuously for 14 days, recovered with no ill effects (Ellis, 1923). Certainly the high metabolic rate in birds should be affected by anoxia within a short period of time. Finally, Warburg and Wiesel (1912) showed that the anaerobic growth of brewer's yeast could be halted by several urethanes and alcohols and obligate anaerobic bacteria were also shown to be capable of being

narcotized (Veszi, 1918). A theory of asphyxiation per se can therefore no longer be considered.

It has been postulated that anaesthetics disrupt cell metabolism by inhibition of oxidative enzyme systems essential for the function of the central nervous system. The chief advocates of this theory have been Quastel and his co-workers (Jowett, 1938; Jowett and Quastel, 1937a; Quastel, 1939; Jowett and Quastel, 1937b; Quastel and Wheatley, 1932; Quastel and Wheatley, 1934). They have shown that the oxygen consumption of brain tissue in vitro is inhibited by a number of barbiturates (Jowett, 1938; Jowett and Quastel, 1937; Quastel and Wheatley, 1932), ether (Jowett, 1938; Quastel and Wheatley, 1932), chloroform (Quastel and Wheatley, 1932), ethyl alcohol (Jowett, 1938), urethane (Quastel and Wheatley, 1932) and several other anaesthetics. Similar results for a large series of barbiturates were obtained by Fuhrman and Field (1943). Quastel demonstrated that these anaesthetics arrested the oxidation of glucose, pyruvate and lactate but not of succinate (Quastel, 1939). In contrast, Watts (1949) showed that succinate could be inhibted by many local anaesthetics.

Quastel concluded that anaesthesia is produced by local effects on some specific enzyme system rather than hypoxia in the whole brain. His basic concept has been supported by both Burger (1960b) and Barlow (1955a), the latter assuming that those anaesthetics which have a greater activity than is suggested by their physico-chemical constants act in this manner. Gerard (1947) also stated that functional anaesthesia is not always accompanied by a depressed tissue respiration. He felt that anaesthetics act by inhibition of oxidation but that his action was non-specific.

The evidence contradicting these theories is convincing. Quastel and Wheatley (1934) showed that the inhibitory effects of chlorbutanol and phenobarbital on oxidation are reversible - a property required of all clinical anaesthetics. The effects of ether (Jowett and Quastel, 1937b) and ethanol (Jowett, 1938), however, were irreversible. Jowett and Quastel conceded that the concentrations of these agents needed to inhibit oxidation were far in excess of the concentrations tolerated in the living mammal. Finally, several non-anaesthetic amines have been found to be potent inhibitors of oxidative processes in vitro (Quastel and Wheatley, 1933).

Sherif (1930) studied the effects of several agents on the isolated rabbit sciatic nerve. He found that the agent most effective in reducing the metabolism of nerve was eucupinotoxin, a drug with almost negligible effect on nerve conduction. He also showed that quinine, caffeine and procaine inhibited oxidation at similar concentrations although caffeine and quinine did not suppress nerve conduction.

Much of the work on metabolism has dealt with oxygen utilization but inhibition of other enzymatic reactions is also possible. Butler (1950) noted that many anaesthetics can inhibit the breakdown of adenosine triphosphate (ATP). Indirect evidence has shown that the cleavage of three high energy phosphate bonds is necessary for the extrusion of one sodium ion (Caldwell and Keynes, 1937). This evidence led Hodgkin (1958) to postulate that ATP is the energy source for sodium ion extrusion. Thus any agent interfering with the transfer of the energy rich phosphate from ATP, would prevent the cell from carrying out its normal function. Data in support of such a hypothesis are lacking at present. Moreover, if anaesthetics acted by inhibition of

the sodium transport mechanism, the nerve or muscle fibre membrane would necessarily be depolarized. That this does not occur has been shown by Yamaguchi (1961) and Inoue and Frank (1962a).

f) Coagulation Theories

In 1875, Claude Bernard stated that all agents which depress cells do so by producing the same modification in the cell. This statement arose out of several experiments in which muscle tissue was exposed to various anaesthetics. As a result of this treatment Bernard observed that the tissue became hard and more opaque but returned to its original state once the anaesthetic was removed. Experiments in lower forms revealed that these agents depressed many of the vital processes of the organisms. Regardless of the test organism used the narcotic increased tissue opacity. He concluded therefore, that the phenomenon of depression in all cells was the result of a single basic mechanism of action which he claimed was the reversible coagulation of colloids.

Moore and Roaf (1904; 1906) presented evidence in support of Bernard's hypothesis. They showed that the addition of 1% chloroform to blood serum produced a reversible opalescence. No precipitation was seen when the serum was observed under the microscope. Higher concentrations of chloroform resulted in frank preceipitation and this phenomenon was irreversible. Ether, benzol and xylol also caused precipitation but to a lesser degree than produced by chloroform. Moore and Roaf concluded that anaesthesia is produced by reversible reactions of the narcotics with cell proteins since they felt that only protein was present in sufficient quantities in the cell to account for flocculation.

The most ardent supporters of a coagulation theory of narcosis have been Bancroft and Richter (1931). They claimed that low concentrations of anaesthetic could cause coagulation of intracellular protein and lipid which they observed under the ultramicroscope. They classified all narcotics into two categories - direct narcotics which coagulate the cell colloids by direct action and indirect narcotics which interfere with some normal function of the cell resulting ultimately in coagulation. In addition, they held that all previous theories of anaesthesia could be explained by the coagulation hypothesis. They regarded the distribution coefficients of Meyer and Overton as a rough measure of the rate of transport of the various anaesthetics. The relative degree of adsorption determines the extent of coagulation, i.e., the narcosis. Traube's theory of the depression of the surface tension of water by various narcotics was interpreted in the same manner. They interpreted Verworn's theory of asphyxiation as a special case of the coagulation theory in that it deals only with indirect narcotics. Depression of oxidation alone does not cause narcosis; the accumulated waste products coagulate the colloids and are responsible for the anaesthetic effect.

Barlow (1955b) criticized this theory on three counts. First, he claims that coagulation is a toxic effect and is irreversible. This effect was observed by Moore and Roaf (1904; 1906) but this did not prevent them from supporting the coagulation theory. Secondly, drug concentrations which produce narcosis are much less than those required to flocculate colloids, the observations of Bancroft and Richter notwithstanding. This was earlier observed by Salkowski (1888) who showed that even long exposures to anaesthetic concentrations of chloroform did not precipitate the blood cell colloids. Thirdly, Barlow claims that

instances are known in which narcotics appear to decrease the dispersion of colloids. Similar criticisms of the coagulation theory have been advanced by Henderson (1930).

Butler (1950) has also commented on the coagulation theory. He stated that the simplicity of this concept tends to ignore the complex nature of biological systems. This criticism is not an unreasonable one in the light of what is known of biological phenomena today.

g) The Dehydration Theory

Dubois (1882) observed that plants which had been exposed to ether, chloroform, alcohol and cold, lost water. Consequently he suggested that narcosis resulted from the loss of water. Stephanowska (1902) extended these results to protozoa and observed that when the activity of the organism was decreased by the anaesthetics, the number of vacuoles increased. Removal of the anaesthetic led to a reversal of the phenomenon. Like Dubois, Stephanowska considered the loss of water to be the cause of narcosis.

Kochmann (1923) studied the effects of several anaesthetics on the frog gastrocnemius, observing in each case the concentration of drug necessary to make the muscle non-irritable and to produce a decrease in its weight. The decrease in muscle weight was found to be reversible in all cases. He concluded, therefore, that anaesthetics reversibly dehydrate the cell colloids thus reducing membrane permeability which results in metabolic inhibition and functional arrest.

The dehydration theory implies that any process which results in a loss of water from the tissues must lead to narcosis. Winterstein (1926) clearly showed that this is not the case. Muscles of curarized frogs which received subcutaneous injections of glycerine [illegible]

frogs which received subcutaneous injections of glycerine became highly irritable due to dehydration.

h) The Permeability Theory

It is well known that the cell membrane is essential in the propagation of the wave of excitation which normally follows an adequate stimulus to excitable tissue. Any agent, therefore, which interferes with this process might be acting at the membrane. Lillie (1923) proposed the theory that an anaesthetic could act in two ways: either by decreasing the permeability of the membrane to substances necessary in the excitability cycle, or by preventing the normal increase in permeability which gives rise to the action potential. The theory was based on several observations in various excitable tissues wherein the drugs used either decreased the permeability of the cell membrane or prevented the normal increase in permeability. For the most part, observations which have not supported Lillie's results have been in non-excitable tissues (Liebe, 1948; Jacobs and Parpart, 1937; Brooks, 1948; Davson and Danielli, 1943) and as such are not relevant to the discussion of this theory. Guttman (1939), however, showed that while low concentrations of isoamyl carbamate and other narcotics increased the resistance of the frog sartorius muscle membrane, higher concentrations decreased it. This finding is certainly incompatible with the hypothesis that anaesthetics decrease the permeability of the membrane in producing their effects but has no bearing on the concept that narcotics act by preventing the normal increase in permeability to some substance which produces the action potential. The latter hypothesis has been well supported. Eccles and his co-workers studied the blocking

action of pentobarbital on synaptic potentials in the spinal cord of cats (Eccles, 1946; Eccles and Malcolm, 1946; Brooks and Eccles, 1947). They concluded that this blocking action is due to the stabilization of the cell body membrane so that discharge of an impulse is not initiated by a synaptic potential that normally would be effective. Similar results have been reported for other barbiturates (Bremer and Bonnet, 1948; Bremer et al., 1942). Urethane and chloralose, however, produced a different effect (Bonnet and Bremer, 1948). The latter agents depressed the synaptic potentials without changing the voltage level at which the discharge was initiated.

Good agreement with Lillie's hypothesis has been also obtained in peripheral nerve fibres. It has been shown that transmission of an impulse over a nerve can be blocked by stabilization of polarization. This effect has been demonstrated for cocaine, procaine and a large series of other local anaesthetics (Bennet and Chinburg, 1946), amyl alcohol (Bishop, 1932) and urethane (Cresticelli, 1948). The apparently anomalous results obtained with ether have already been discussed (vide supra).

The membrane stabilization theory of anaesthesia has been supported by Shanes (1958). Like Bennet and Chinburg (1946), he defined anaesthetics as agents which block nerve or muscle impulses without any change in the resting potential. He attributed this effect to the ability of anaesthetics (specifically cocaine, procaine and calcium) to reduce the electrical effectiveness of sodium and potassium. It is his contention that the permeability of the membrane to these ions is decreased by a diminution in size for the membrane pores through which the ions normally pass. Shanes' concept is in good agreement with

Hodgkin's theory of nerve transmission wherein a selective increase in sodium conductance produces the action potential (Hodgkin, 1958).

Gerard (1947) criticized the permeability hypothesis on the grounds that many narcotics increase the permeability of the cell membrane. This criticism is untenable since a decrease in the permeability of one specific ion may be sufficient to prevent the action potential even in the presence of an overall increase in permeability to other substances.

i) Pauling's Microcrystal Hypothesis

The most recent theory of anaesthesia is that proposed by Pauling (1961). He found a good correlation between the partial pressure of anaesthetic gas required to produce narcosis and the partial pressure necessary to form the anaesthetic's hydrate crystals. He suggested, therefore, that anaesthesia was the result of the formation of such microcrystals in the brain, especially at synapses. Pauling was fully aware that the mechanism of narcosis could not be simply the formation of the hydrate crystals of the anaesthetic alone, because at body temperatures and pressures such structures are unstable. He assumed, therefore, that other substances in the extracellular fluid exert a stabilizing effect on hydrate formation. Such substances might include amino acids, alkyl ammonium side chains of lysyl residues and the alkyl carboxylate ion of aspartate and glutamate residues. Pauling stated that the end result of such microcrystal formation would be that these protein residues and ions would be entrapped so as to depress conduction in networks involved in the maintenance of consciousness.

The Pauling hypothesis emphasizes the water content of the brain rather than the lipid content. It resembles the Overton-Meyer theory, Traube hypothesis and Warburg hypothesis in that all of these anaesthetic theories are based on van der Waals attraction of anaesthetic molecules for other molecules, but differs from them in that water is absolutely essential for microcrystal formation. The concept that events occurring in the extracellular water may result in anaesthesia is attractive since if sodium ions, which are responsible for the production of the action potential, are trapped within the microcrystal, transmission of impulses becomes impossible. The possibility, however, that the microcrystals once formed act as a mechanical barrier for some transmitter substance cannot be ignored.

The Pauling hypothesis has several limitations. Firstly, it is restricted to those agents which are incapable of hydrogen bonding. Thus, although it provides a possible explanation for the mechanism of action of such anaesthetics as nitrous oxide, chloroform, ethylene and the rare gases, it fails to elucidate the mechanism of action of such anaesthetics as the alcohols and diethyl ether. In effect, then, its resemblance to the Overton-Meyer theory becomes even greater in that both theories are more specific than general.

The greatest weakness at present is the lack of adequate technology to either substantiate or disprove this hypothesis. Evidence gained from his own experiments is at best equivocal since many interpretations of his findings are possible. In addition to the similarities that this theory shares with the Overton-Meyer theory, it also bears a strong resemblance to Dubois' dehydration theory and Bernard's coagulation theory and in some respects shares their disadvantages.

2. Effects of anaesthetics on excitability and central nervous system function

None of the theories previously described, save for that of Lillie, have considered the effect of anaesthetics on the basic mechanisms involved in the transmission of impulses from one point to another. Lillie himself was not explicit as to the exact mechanisms that might be affected by anaesthetics. He envisaged the anaesthetic as so altering the membrane as to prevent the increase in permeability that accompanies depolarization. According to his theory, it is unimportant whether the anaesthetic increases or decreases the permeability of the membrane to several substances as long as the permeability to one specific substance responsible for excitation is decreased or halted. Some of the factors responsible for this excitation are reviewed below.

Bernstein (1902) observed that the concentration of potassium ions was much greater inside the nerve fibre than in the extracellular fluid. To explain this he considered that the membrane in its resting state was impermeable to all anions and sodium and therefore the resting potential was due to the difference in concentration of potassium ions in the intracellular and extracellular fluids. He suggested that during activity the selective potassium permeability is destroyed and the membrane potential approaches zero. In the same year, Overton (1902) showed the importance of the sodium ion in maintaining excitability and suggested that impulse conduction was accompanied by an exchange between extracellular sodium and intracellular potassium. Like Bernstein, he held that the membrane potential was effectively zero at the peak of the action potential.

The observations of Hodgkin and Huxley (1939; 1945) demonstrated the error of the latter hypothesis. They noted that the membrane potential of squid axons does not merely drop to zero at the peak of the action potential but is in fact reversed. Hodgkin and Katz (1949) suggested that during the rising phase of the action potential the permeability to sodium ions is increased and that this influx of sodium ions produces this rising phase. This hypothesis has been confirmed by several lines of evidence including measurement of ionic movements during nervous activity (Hodgkin, 1958; Shanes, 1958).

The foregoing theory implies that any agent interfering with the selective increase in sodium permeability would prevent the action potential. This provides a plausible explanation for the stabilization of the membrane by local anaesthetics (Bishop, 1932; Hirschfelder and Bieter, 1932) and several general anaesthetics (Eccles, 1946; Eccles and Malcolm, 1946; Brooks and Eccles, 1947; Bremer et al., 1942; Bremer and Bonnet, 1948; Bennet and Chinburg, 1946; Bishop, 1932; Cresticelli, 1948).

Thesleff, (1956) studied the effects of several non-volatile general anaesthetics on the electrical activity of skeletal muscle fibres. He found that all of these agents blocked the production of action potentials by inhibiting the specific increase in membrane sodium conductivity which normally follows an adequate stimulus. An identical effect and mechanism of action has since been shown for chloroform (Yamaguchi, 1961) and ether (Yamaguchi, 1961; Inoue and Frank, 1962b). Thesleff also compared the ability of the anaesthetics to produce a hypnotic effect in intact frogs with their ability to inhibit electrical activity of muscle cells. The potencies of these

agents in producing these two distinct effects were well correlated and he suggested the possibility that these drugs produced narcosis by an action on the electrical excitability of cells in the central nervous system identical to that observed in skeletal muscle fibres.

More recently, Inoue and Frank (1962a) found that procaine also blocks the production of action potentials in skeletal muscle fibres by suppressing the increase in sodium conductivity. Since this effect is produced by both general and local anaesthetics, they suggested the possibility that local and general anaesthetics had a single basic mechanism of action on cells in the central nervous system.

E. STATEMENT OF THE PROBLEM

The aim of this work is to investigate the possibility that general and local anaesthetics exert the same effect upon cells of the central nervous system. If this concept is correct then some explanation for the seemingly different effects of these two groups of drugs on the central nervous system must be advanced. Most clinically used local anaesthetics can produce central nervous system excitation and convulsions after systemic administration. General anaesthetics, on the other hand, produce a stage of excitement (Stage II anaesthesia) which is followed by a reversible stage of depression of nervous activity known as surgical anaesthesia (Stage III anaesthesia). These differences might be quantitative rather than qualitative, i.e., convulsions due to local anaesthetics may be the result of the same mechanism responsible for the excitement stage of general anaesthesia. This excitement (Stage II anaesthesia) is held to be due to suppression of the activity or effects of inhibitory neurones (French et al., 1953). This is believed to be different than the mechanism of action of such drugs as pentylenetetrazol, the direct action of which is solely excitatory upon central nervous tissue (Ten Cate and Swijgman, 1945; Preston, 1955; Jolly and Steinhaus, 1956; Curtis, 1959; Jones and Lombroso, 1955; Okuma, 1960; Lewin and Esplin, 1961; Bircher et al., 1962; Desmedt and Monaco, 1960).

The experiments reported here were designed to determine whether the central effects of local anaesthetics resembled those of general anaesthetics more closely than the effects of solely excitatory drugs such as pentylenetetrazol. The experimental procedures employed included:

1) studies in intact animals

2) comparison of some of the aforementioned agents in isolated cortex

3) alterations of click-evoked auditory responses in intact cortex by drugs.

II. EXPERIMENTAL

A. EFFECTS OF DRUGS ON THE MOTOR ACTIVITY OF INTACT MICE

1. Method

Drugs dissolved in 0.1 to 0.2 ml of saline were injected intraperitoneally (i.p.) into female Swiss Albino mice (Lemberger Co.) weighing between 20 and 30 g. Effects on gross motor activity were noted. Loss of the righting reflex, i.e., loss of the ability of the animal to right himself when placed on its side or back, was used as the criterion of central nervous system depression. Animals losing the righting reflex without convulsions either before or after the loss of this reflex were considered to be anaesthetized. Absence of convulsions was considered to be necessary to distinguish righting reflex loss due to anaesthesia from that of postictal depression or a preconvulsive state - two situations where loss of righting reflex can occur. Eventual recovery of the righting reflex and the presence of spinal reflexes during the phase of depression were two additional criteria of the test system that had to be met. The withdrawal response to pinching of the foot was also taken as an index of the absence of neuromuscular blockade. Animals which met these criteria were in a state indistinguishable from 'general anaesthesia'. Increased motor activity in the absence of convulsions was taken to indicate central nervous system excitement.

The drugs used were procaine hydrochloride, dibucaine hydrochloride (Nupercaine^{R}- Ciba), cocaine hydrochloride, lidocaine hydrochloride (Xylocaine^{R}- Astra), sodium 5-(1,3-dimethylbutyl)-ethyl barbituric acid (DMB), sodium phenobarbital and pentylenetetrazol (Metrazol^{R}- Knoll). DMB is one of the convulsant barbiturates, i.e., a barbituric acid derivative which produces convulsive activity in

low doses, instead of the mild excitation generally observed with the clinically used barbiturates (Knoefel, 1936).

The animals were observed continuously for 15 minutes after the administration of procaine, cocaine, lidocaine or DMB. The observation period lasted 60 minutes when phenobarbital was administered. When drug interaction studies were done phenobarbital was administered 60 minutes before any of the local anaesthetics, DMB or pentylenetetrazol to be certain that the maximum response to phenobarbital had been obrained. This was ascertained by the fact that no additional animals lost the righting reflex after 45 minutes from the time of injection of the drug. The dose-response curves relating the percentage of animals losing the righting reflex to the dose of drugs used were analyzed and compared by the method of Litchfield and Wilcoxon (1949).

Several experiments were carried out using procaine, phenobarbital and pentylenetetrazol in the same animal. Procaine and pentylenetetrazol were always injected 1 minute apart and 60 minutes after the injection of phenobarbital. Loss of righting reflex, convulsions and deaths were noted.

2. Results

The purpose of these drug interaction studies was to determine if the local anaesthetics and the convulsant barbiturate would potentiate or antagonize the central depressant effect of a general anaesthetic. The effects of each drug given alone were determined. Phenobarbital given i.p. in a dose of 30 mg/kg or more generally produced within 15 minutes some degree of mild excitement which disappeared 10 to 20 minutes later. Animals losing the righting reflex did so within 45 minutes of drug injection. The dose-response curve for phenobarbital alone is presented in Figs. 1-5.

Procaine, dibucaine, cocaine, lidocaine or the convulsant barbiturate (DMB) when given alone produced only signs of central nervous system stimulation. This stimulation was indicated by overt excitement, increased motor activity, increased respiration and sometimes convulsions. Convulsions were always preceded by a period of excitement. The righting reflex was lost during or after a convulsion in some of the animals. The mice were observed for 15 minutes after injection of a local anaesthetic or DMB but in all cases excitement began within 10 minutes. This excitement seldom exceeded 10 minutes duration with dibucaine and lidocaine or 5 minutes with procaine. Cocaine, however, often produced excitement and convulsions which lasted for 20 minutes. In approximately 70% of the animals death occurred after a convulsion. A peculiar phenomenon was observed in some animals which died after a convulsion due to DMB. This was a very pronounced rigidity which persisted after death. These animals died in extensor spasm with the back arched convexly. In contrast, death resulting from convulsions produced by any of the other agents

always left the animals flaccid.

Convulsant doses of the local anaesthetics and DMB are shown in Table I. The CD_{50} and CD_{90} (doses causing convulsions in 50% and in 90% of the animals respectively) were calculated according to the method of Litchfield and Wilcoxon (1949).

a) Interaction of phenobarbital with local anaesthetics and DMB

A convulsive or subconvulsive dose of local anaesthetic or DMB was given 60 minutes after an anaesthetic or subanaesthetic dose of phenobarbital. The responses observed in this series were in marked contrast to those obtained after administration of these agents alone. The results are plotted in Figs. 1-5. Each point on the graph represents at least 10 mice.

b) Phenobarbital-procaine interaction

Doses of procaine up to 175 mg/kg given after phenobarbital did not cause excitement or convulsions. This treatment further depressed the central nervous system since the righting reflex was lost in many animals not previously losing this reflex after phenobarbital alone. These results are shown in Fig. 1.

Only the curves for procaine (125 and 150 mg/kg) could be compared statistically with the phenobarbital curve since the curve for procaine (175 mg/kg) deviated significantly from parallelism. A significantly lower pretreatment dose of phenobarbital produced loss of righting reflex with 150 mg/kg of procaine. A still smaller dose of phenobarbital produced loss of righting reflex in 50% of the mice with 175 mg/kg. The curve for this dose of procaine, however, deviated

TABLE I

Convulsant Doses of Drugs Given Intraperitoneally

Drug	CD_{50} mg/kg	CD_{90} mg/kg
Procaine HCl	180	200
Dibucaine HCl	18	28
Lidocaine HCl	68	90
Cocaine HCl	38	57
DMB Na	15	19

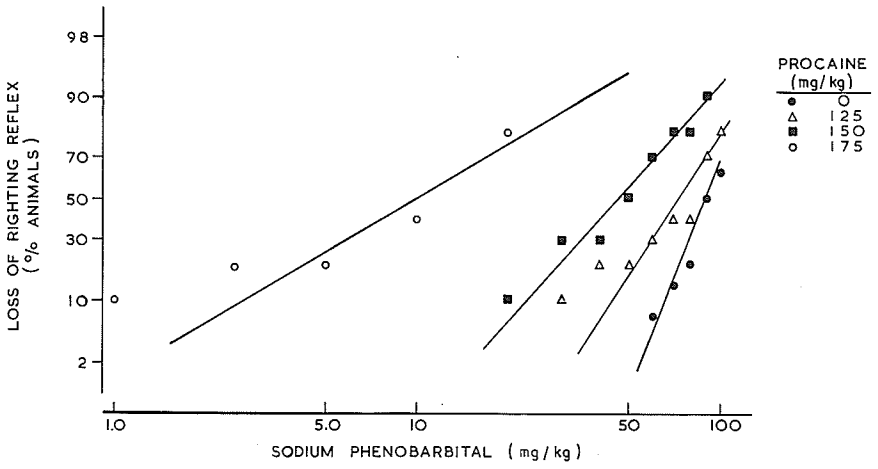

Fig. 1. Central nervous system depression produced by procaine given to white mice 60 minutes after phenobarbital. Logarithmic probability plots. 10-15 mice used to determine each point.

significantly from parallelism when compared with the lower doses of procaine. Statistical comparisons of the ED_{50}'s for these curves were therefore impossible. Similar drug interaction tests were carried out with procaine 200 mg/kg after varying doses of phenobarbital, but satisfactory dose-response curves could not be obtained because the results became very erratic with very small doses of phenobarbital; with higher doses of the barbiturate all animals lost the righting reflex (100% effect). Procaine (100 mg/kg) caused loss of the righting reflex in a few animals not previously affected by phenobarbital but the effectiveness of this dose was small.

c) Phenobarbital-dibucaine interaction

A similar series of tests was carried out with dibucaine as the local anaesthetic. The results are shown in Fig. 2. Dibucaine in doses up to 20 mg/kg produced results that were essentially the same as obtained with procaine, i.e., a loss of righting reflex without preceding or concomitant convulsions. In many cases, however, with phenobarbital pretreatment doses of less than 70 mg/kg the animals exhibited brief periods of active limb movement while lying on the side or back after losing the righting reflex. A distinction was made between this type of activity and convulsive activity. An animal was considered to be convulsing if it showed uncontrolled motor activity regardless of position. This activity, however, was not regarded as a convulsion if an animal could not right itself and exhibited, while on its side or back, some sort of overt activity which ceased when the animal was placed on its ventral surface. This activity was regarded merely as an attempt of the mouse to right itself. Several animals did indeed convulse before or after the loss of the righting reflex with

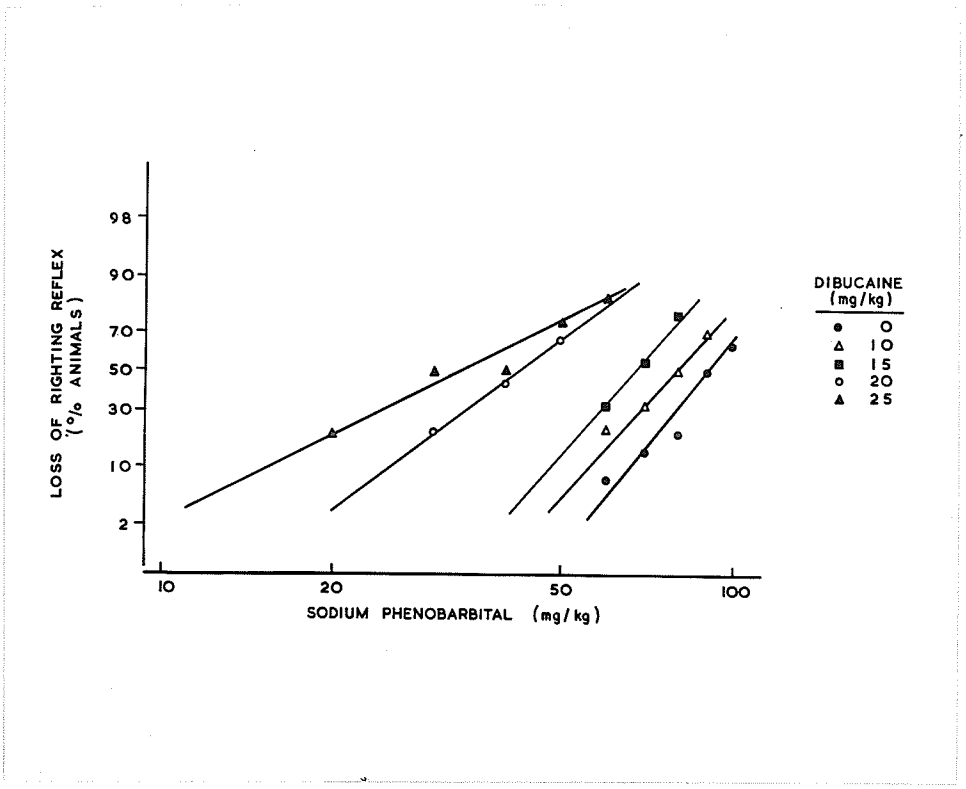

Fig. 2. Central nervous system depression produced by dibucaine given to white mice 60 minutes after phenobarbital. Logarithmic probability plots. 10-20 mice used to determine each point.

dibucaine (25 mg/kg) and a subanaesthetic dose of phenobarbital. These animals are not included in the results shown in Fig. 2.

Statistical comparison of the dose-response curves showed that only those for dibucaine (10 and 15 mg/kg) did not deviate significantly from parallelism. The ED_{50}'s were significantly different in these two cases. Deviation from parallelism did not permit statistical comparison of the results in the remaining curves but it is nevertheless readily apparent that the ED_{50} decreases as the dose of dibucaine increases.

d) Phenobarbital-lidocaine interaction

The curves obtained in the phenobarbital-lidocaine interaction studies are shown in Fig. 3. Statistical comparisons of the ED_{50}'s from the dose-response curves could not be carried out at any dose due to deviation from parallelism. A pattern of responses similar to the other local anaesthetic-phenobarbital interaction studies was nevertheless obtained, i.e., the ED_{50} decreases with increasing doses of lidocaine.

Despite this similarity, there is a difference which may be of importance. The shift of the dose-response curves to the left with other local anaesthetics and DMB is accompanied by a decreased or unchanged slope of the curve. In the case of phenobarbital-lidocaine, however, low doses of the local anaesthetic resulted in an increase of the slope and only at higher doses did the slope decrease.

e) Phenobarbital-cocaine interaction

Fig. 4 shows the results of the phenobarbital-cocaine interaction experiments. Comparison of the ED_{50}'s from the dose-response curves on a statistical basis is only possible with cocaine at doses

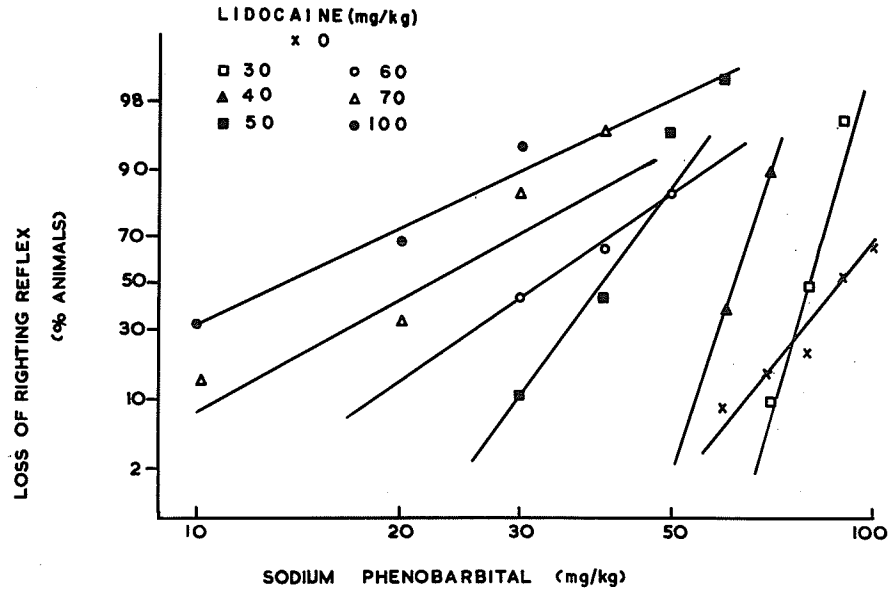

Fig. 3. Central nervous system depression produced by lidocaine given to white mice 60 minutes after phenobarbital. Logarithmic probability plots. 10-20 mice used to determine each point.

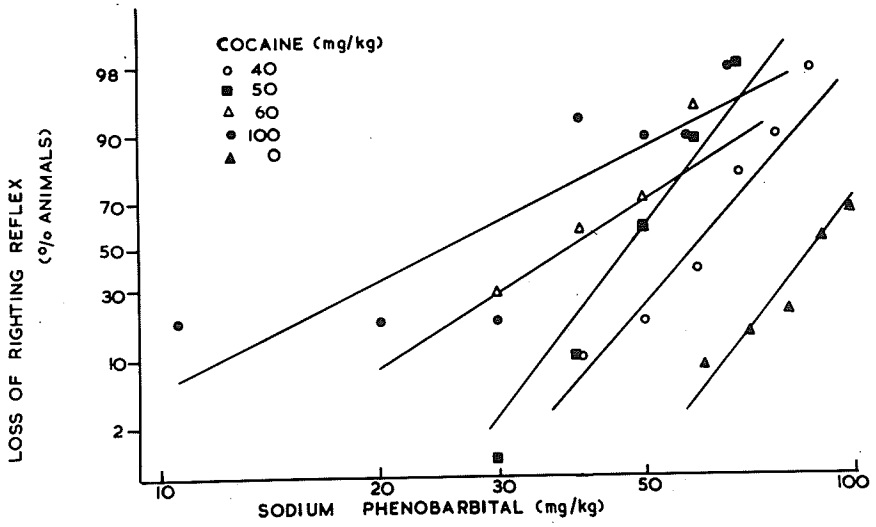

Fig. 4. Central nervous system depression produced by cocaine given to white mice 60 minutes after phenobarbital. Logarithmic probability plots. 10-15 mice used to determine each point.

of 40 and 50 mg/kg. The ED_{50} was significantly lower at 50 mg/kg. At higher doses, there is an obvious decrease in the ED_{50} even though statistical comparison was not possible.

f) Phenobarbital-DMB interaction

The same pattern of responses occurred also in the phenobarbital-DMB study shown in Fig. 5. Only the dose-response curve for DMB at a dose of 14 mg/kg lends itself to statistical comparison with the phenobarbital control. In this case the decrease in the ED_{50} was found to be significant. Higher doses of DMB resulted in a further decrease in both the slope of the line and the ED_{50}.

g) Phenobarbital-procaine-pentylenetetrazol interaction studies

The results are presented in Table II. Pentylenetetrazol, in a dose of 25 mg/kg given to mice pretreated with phenobarbital (70 mg/kg) and procaine (150 mg/kg) resulted in loss of righting reflex in 8 of 10 mice. In contrast, only 4 of 10 mice, similarly pretreated with phenobarbital and procaine, lost the righting reflex when the dose of pentylenetetrazol was raised to 75 mg/kg. In addition, 4 of 10 animals convulsed and subsequently died, whereas at the lower dose of pentylenetetrazol there were no convulsions and no deaths. This same dose of pentylenetetrazol (75 mg/kg) given to mice pretreated with phenobarbital (70 mg/kg) and procaine (125 mg/kg) resulted in only 1 of 10 animals losing the righting reflex. At this dose there were no convulsions although 8 of 10 mice exhibited marked excitement. None of the animals in this test group died.

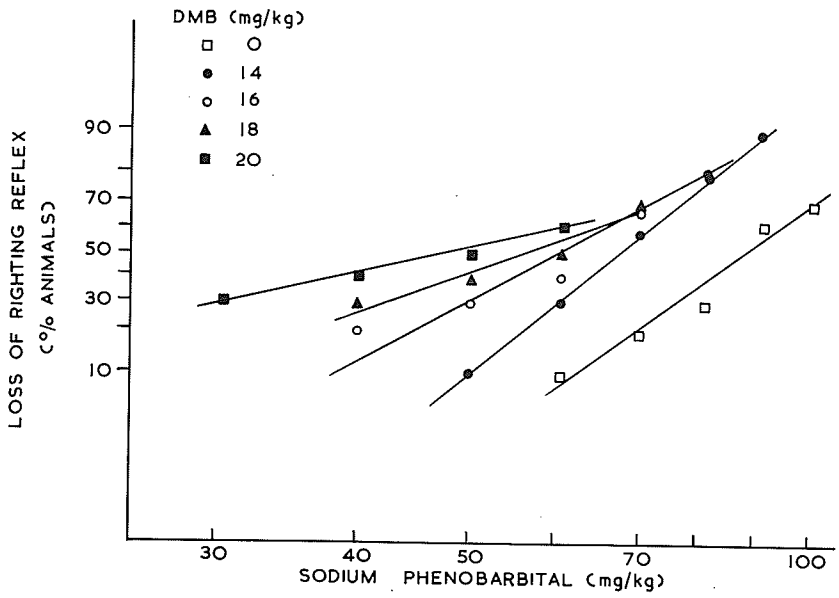

Fig. 5. Central nervous system depression produced by DMB given to white mice 60 minutes after phenobarbital. Logarithmic probability plots. 10-20 mice used to determine each point.

TABLE II

Phenobarbital - Procaine - Pentylenetetrazol Interaction

Dose mg/kg			Righting reflex lost	Convulsions	Deaths	Remarks
Phenobarbital	Procaine	Pentylenetetrazol				
-	-	25	0/10	0/10	0/10	
-	-	50	0/5	1/5	0/5	4/5 excited
-	-	75	0/5	5/5	1/5	
-	125	-	0/10	1/10	0/10	
-	150	-	0/10	3/10	0/10	
-	100	25	0/10	0/10	0/10	
-	100	50	0/10	4/10	0/10	6/10 excited
70	-	75	0/10	0/10	0/10	
70	125	-	4/10	0/10	0/10	
70	150	-	9/10	0/10	0/10	
70	125	75	1/10	0/10	0/10	8/10 excited
70	150	25	8/10	0/10	1/10	
70	150	50	7/10	0/10	0/10	
70	150	75	4/10	4/10	4/10	All deaths in convulsed animals

3. Discussion

The results support the contention that local anaesthetics and general anaesthetics produce qualitatively similar effects on the central nervous system. All the local anaesthetics showed marked convulsive activity in mice when administered alone but resulted in an increased depression in these animals when given in combination with phenobarbital. This depression, reflected by a shift of the dose-response curve to the left, is in contrast to the results obtained when another convulsant, pentylenetetrazol, is combined with barbiturates in mice (Loewe, 1955), rabbits (Pickrell and Richards, 1945) and humans (Pickrell and Richards, 1945; Booker et al., 1950). These results confirm those of Maykut and Kalow (1955) who showed that guinea pigs receiving subanaesthetic doses of pentobarbital lost the righting reflex when a suitable dose of procaine was given.

Convulsant doses of DMB in phenobarbital pretreated mice also caused a shift of the dose-response curve to the left. These results are in good agreement with those of Swanson and Chen (1939) who found that the convulsant barbiturate enchanced the depressant action of amobarbital although amobarbital antagonized the stimulant effects of DMB. Leonard and Harrison (1953) also noted that addition of DMB to pentobarbital or hexobarbital pretreated mice prolonged the sleeping time. The mortality of the mice was also increased. In contrast, Knoefel (1945) observed that the fatal dose of DMB in rats and rabbits was raised by a factor of three by the previous administration of a depressant barbiturate, but the reverse antagonism did not occur. These seeming discrepancies were resolved by Domino and his co-workers (1955) who compared the central nervous system effects of pentobarbital

and DMB in cats and found that with careful selection of the dose both DMB and pentobarbital increased the mean convulsive dose of pentylenetetrazol. The depressant effects were furthermore real and not secondary to the stimulant effects. Several thiobarbiturates also show mixed convulsant and hypnotic properites in mice (Richards, 1951).

The loss of the righting reflex was taken to indicate anaesthesia. This test was used in preference to prolongation of sleeping time which appears to be less selective in the type of agent that can produce it. Barbiturate sleeping times i.e., the length of time an animal remains asleep after barbiturate administration, are prolonged by glucose (Lamson et al., 1949), alpha-tocopherol (Giarman et al., 1954), dimercaprol (Kahn, 1953), beta-diethylaminoethyldiphenylpropyl acetate (SKF-525A) (Cook et al., 1954), thiamine (de Boer, 1948), sorbital and several iodides (Krantz and Fassell, 1951), histamine and antihistamines (Ambrus et al., 1952), nitrates (Wooster and Sunderman, 1949) and ascorbic acid (Giarman and Flick, 1951) to mention but a few. Chloral hydrate sleeping times are prolonged by adrenaline, noradrenaline, serotonin, atropine, histamine and ergotamine (Fastier et al., 1957). On the other hand, the ability of an agent to potentiate subanaesthetic doses of barbiturate seems to be restricted to analgesics (Glassman and Seifter, 1955), other anaesthetics (Aston and Cullumbine, 1959) and local anaesthetics (Maykut and Kalow, 1955), all of which have pronounced central nervous system activity. Indeed, Sanders (1961), showed that beta-hydroxythujaplicin, a potent sedative (Sanders Halliday, 1962) did not potentiate subanaesthetic doses of hexobarbital and thiopental although the sleeping times were markedly prolonged.

Pentylenetetrazol, however, can arouse animals made comatose with barbiturates (Loewe, 1955; Pickrell and Richards, 1945; Booker et al., 1950; Zipf, 1936). None of the drugs used except lidocaine in low doses roused animals already anaesthetized with phenobarbital. This antagonism is anomalous since potentiation of the anaesthetic effect of phenobarbital occurred with higher doses of lidocaine where the convulsant effect is most marked. A true antagonism between phenobarbital and lidocaine may indeed occur at low doses of licocaine, but the possibility that the observed results may be due to variations between individuals or colonies cannot be ignored.

Parallel log dose-response curves have been taken to mean that two agents act at the same site (Gaddum, 1937). Conclusions based on the shape of dose-response curves alone, however, may be erroneous (Nickerson, 1956; Gaddum, 1939). For example, the response in the whole animal may be the result of combinations in many tissues where binding is not the same in all of them (Chen and Russell, 1955). Parallel dose-response curves are also obtainable with acid-base neutralization data (Gaddum, 1939). It has also been suggested that the response of a tissue is the sum of the responses of a large number of receptors with different thresholds which have a normal distribution (Gaddum, 1957). It is therefore not possible to state whether the local anaesthetics and DMB are acting at the same site as phenobarbital. It is possible that all the aforementioned drugs act at the same site, while the local anaesthetics and DMB have an additional action. Such a concept is most reasonable for DMB since it is a close structural analogue of pentobarbital (Domino et al., 1955) and it is not unlikely that all barbiturates act, at least in part, on the same cells in the central

nervous system. The similarity between the dose-response curve for the phenobarbital-DMB interaction and the local anaesthetic-phenobarbital interaction is striking and it is tempting to assume a similar site of action for all of the agents. The foregoing considerations, however, make this conclusion highly speculative.

Several experiments were done to obtain further information about sites of action. If procaine and phenobarbital were acting on the same site, then the depression produced by a combination of procaine and phenobarbital should be antagonized by a proper dose of pentylenetetrazol. Cursory examination of the results in Table II might lead to the conclusion that this is in fact the case since fewer animals lost the righting reflex in the presence of pentylenetetrazol than in its absence. More careful analysis, however, reveals that as the dose of pentylenetetrazol was increased, more and more animals convulsed, an effect which appears to be a function of the procaine dosage rather than that of pentylenetetrazol. This is seen in comparing the results of the interaction of the three drugs with procaine in a dose of 125 and 150 mg/kg, the doses of the other agents remaining the same. Not only is the incidence of convulsions greater at the higher dose of procaine, but there also is an increased mortality. The data indicate no antagonism between procaine and pentylenetetrazol, a result also reported by Frey (1962). It appears, therefore, that when pentylenetetrazol is administered to animals pretreated with phenobarbital and procaine, antagonism between pentylenetetrazol and phenobarbital occurs allowing the convulsant activity of procaine to become manifest. Procaine, then, seems to have a dual action, i.e., excitation and depression, but excitation occurring with high doses must be masked to allow the depressant action of the

drug to be observed. The increased mortality at the higher dose of procaine is probably due to the addition of the postictal depressive phase after pentylenetetrazol convulsions to the depressant action of procaine. This conclusion is borne out by the fact that this dose of procaine is not lethal whether given alone or after phenobarbital (70 mg/kg).

These results are inconsistent with the concept that procaine and phenobarbital act on the same site to produce anaesthesia since pentylenetetrazol seems to be selective in antagonizing the effects of the barbiturates. Maykut and Kalow (1955) assume that the hypnotic and convulsive effects of procaine represent two independent actions i.e., they are not related as in the general anaesthetics. The foregoing results seem to support their concept, but a more complete understanding of the actions of procaine within the central nervous system is necessary before final conclusions can be drawn.

B. EFFECTS OF DRUGS ON ISOLATED CEREBRAL CORTEX

1. Methods

a) General

Slabs of neuronally isolated cortex were prepared according to the method of Burns (1950) and Burns and Grafstein (1952) in the suprasylvian gyrus of cats of either sex weighing between 1.9 and 3.4 kg. The animals were anaesthetized in a closed box measuring 38 cm on each edge and containing a piece of cotton soaked in ether. The anaesthetized cat was removed from the box, the trachea was exposed and anaesthesia continued by tracheal cannula. Ether was delivered to the animal through an ether bottle with a variable by-pass which permitted control of the concentration of anaesthetic administered. Both carotid arteries were exposed and a loose tie, extending about 20 cm outside the animal, was placed around each in order to facilitate rapid clamping of the arteries when required. The head of the cat was then fixed in a modified Czermak holder which allowed both vertical and rotary adjustment. The scalp was incised in the midline to expose the temporal muscles. The left temporal muscle was reflected from its origin, clamped off as close as possible to its insertion, tied and finally removed above the tie. One, two or three trephine holes, each about 12 mm in diameter, were made in the left skull. The cortex was exposed by nibbling away the bone with a rongeur, from the bony tentorium to the anterior border of the ectosylvian gyrus, and from the middle of the marginal gyrus to the inferior border of the ectosylvian gyrus. Bleeding from the bone was controlled with bone wax or plasticine. Clamping of one or both carotid arteries and raising the cat's head also helped control bone bleeding in a few cases. The dura mater over the exposed

cortex was removed and bleeding from small surface vessels was controlled by placing Gelfoam^{R}-(Upjohn) or a small piece of crushed temporal muscle directly on the bleeding vessel. The brain was kept moist by applying saline (0.9%) throughout the course of the surgery. Most animals were made decerebrate by section at the midcollicular level as described by Burns (1950). The details are as follows: the brain was reflected slightly at the bony tentorium with a small, curved spatula to permit access to the superior petrosal sinus. This vessel was sealed and burnt through by the passage of an electric spark delivered from a high frequency cauterizer (Model X-712, Birtcher Corp.). The brain stem was cut across with a blunt plastic knife, 25 mm long, 7 mm wide and 1 mm thick. The edges of the tentorium cerebelli were used as a guide for the knife. The plastic knife had smooth round edges in order to avoid damage to the vertebral arteries; for the same reason it was not dragged across the base of the skull, but was pressed down carefully upon the bone in a number of places so as to cut all nervous connections, while producing only a temporary compression of the vertebral arteries. The animals were taken off ether after decerebration. Successful preparations usually resulted in the appearance of a characteristic decerebrate rigidity when sufficient ether had blown off. A drawing of a sagittal section of the preparation is shown in Fig. 6.

In some animals spinal section between the second and third cervical vertebrae was made instead of decerebration. The animal's head was held in the left hand and deflected sharply downward allowing easy access to the vertebrae which were then exposed. The bone overlying the spinal cord between the second and third cervical vertebrae

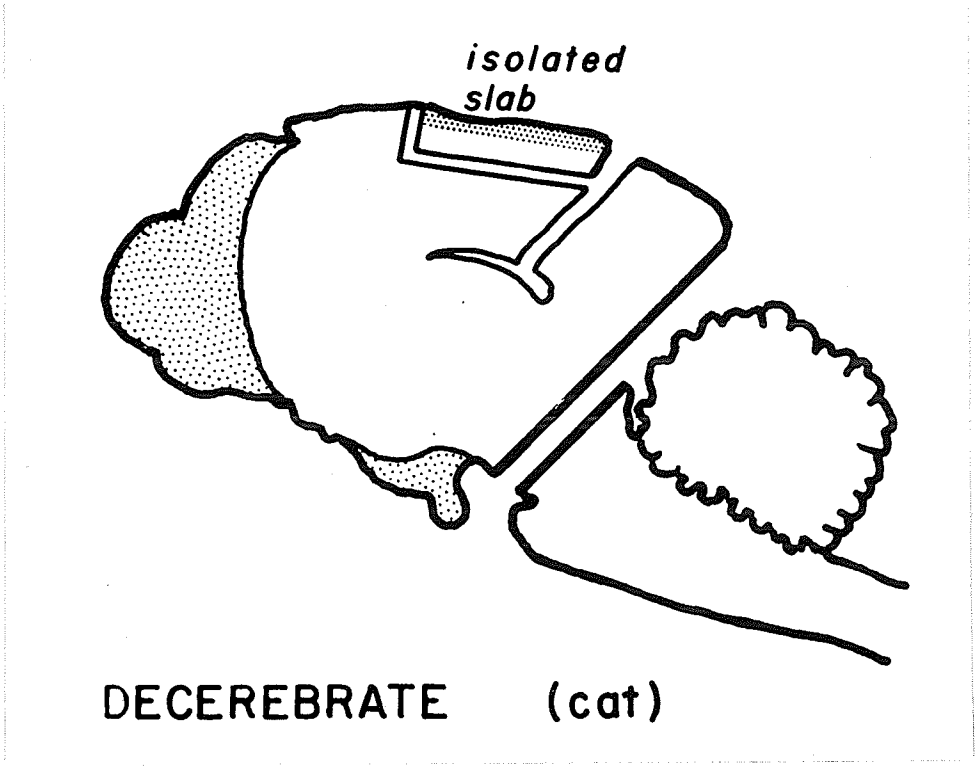

Fig. 6. Schematic drawing of a sagittal section of cat's brain showing cortical slab and transection of the brain stem at the mid-collicular level.

was removed and the cord cut with a pair of blunted iris scissors. The animal was maintained on artificial respiration during the whole of this procedure. There was little bleeding from the area in successful preparations and the mean arterial blood pressure was generally 70 mm Hg or more. The blood pressure usually bore a distinct relation to the speed of the surgery; the more rapidly the operation was performed, the higher the blood pressure afterwards.

After either of these surgical techniques, a small area of cortex was made bloodless by electrocautery of the surface of the posterior part of the suprasylvian gyrus. A small hole, extending from the outside of the brain into the lateral ventricle was made in this area by suction. The purpose of this opening was to permit drainage of cerebrospinal fluid which might otherwise accumulate in the lateral ventricle and cause swelling of the brain. After preparation of the drainage hole, the cortical slab was prepared in the suprasylvian gyrus by passing a tool made of a piece of razor blade 20 mm long and 3 mm wide into the hole to a depth of 4 mm and moving it anteriorly at that depth parallel to the surface of the gyrus. The final step, that of neurological isolation around the circumference of the chosen area, was performed with a piece of straight steel wire of about 1 mm diameter bent to a right angle 4 mm from its rounded tip. The bent wire was inserted in the plane of the cut made with the razor blade so as to pass beneath the cortical surface. By rotation of the shaft, the tip was then brought up so that it appeared just beneath the pia mater. The tip of the wire was then worked carefully around the border of the area of cortex to be isolated; during this process, the tip was watched closely and could always be made to slide harmlessly beneath

the pial vessels. The finished preparation thus consisted of a neuronally isolated piece of cortex with an intact blood supply which entered the slab from the pia. The whole of the exposed bone and cortex was covered by a pool of warm mineral oil; skin flaps were used to form the walls of the pool. The rectal temperature was kept at 35-37^{o}C by a warming plate placed under the cat or a heat lamp above the cat. Femoral arterial blood pressure was measured with a mercury manometer.

The foregoing procedure provided viable slaps which were electrically silent until driven by a suitable stimulus. At least two hours were allowed to elapse to allow for the ether to be blown off and the slab was tested for viability before any recordings were made. The criterion for viability was the appearance of the surface negative response with a single stimulus of 0.03-0.2 msec duration and a surface positive burst response when the voltage was increased (Burns, 1958). Any slabs which were not silent after isolation were made so by clamping both carotid arteries for 20 secs. This procedure had no effect on the viability of the slab. Only silent viable slabs were used in these experiments.

Stimulating and Recording

Rectangular pulse stimuli were generated by a combination of Tektronix pulse and waveform generators (Types 161 and 162). The stimuli were delivered via a 4:1 step-down isolation transformer (Type 578-B, General Radio Co.) through bipolar platinum electrodes having ball tips which rested about 1.5-2.0 mm apart on the surface of the exposed cortical slab. The recording electrodes consisted of saline-agar filled glass tubing 4 mm in diameter with a silk thread embedded in the agar and protruding from one end. The moist silk

thread contacted the cortex and a chlorided silver wire inserted into the agar made the connection with the recording equipment. Essentially monopolar recordings were obtained by placing one electrode on the cortical surface 1-5 mm from the stimulating electrodes and the other on the killed reference area at the end of the slab (Fig. 7). The responses were amplified by a differential D.C. amplifier (Model TA-2, Princeton Science Associates) and displayed on a Model 502 Tektronix oscilloscope. Measurements were made from photographic records.

b) Direct application of drugs to the isolated cortex

A strip of filter paper 5 mm long and 1 mm wide was moistened with 0.9% saline and placed across the width of the slab. The recording electrode was placed either on top of the strip or on the cortex at one edge of the strip. A stimulus intensity was chosen so that one stimulus pulse produced a surface negative response followed by a surface positive burst response (Burns, 1950), as illustrated in Fig. 8. The cortex was stimulated every 30 secs throughout the test. One stimulus, however, was usually missed when applying or removing the drug. Control responses were recorded after which approximately 0.015 ml of drug solution was applied to the filter paper strip at the appropriate concentration either in saline or distilled water. The filter paper was removed after about 5 minutes and stimulation continued for at least an additional 5 minutes. Changes in the amplitude of the negative and positive responses and in the duration of the positive burst response were plotted as a percentage of the mean control values.

The drugs tested in this manner were pentylenetetrazol, sodium pentobarbital, procaine hydrochloride and sodium DMB.

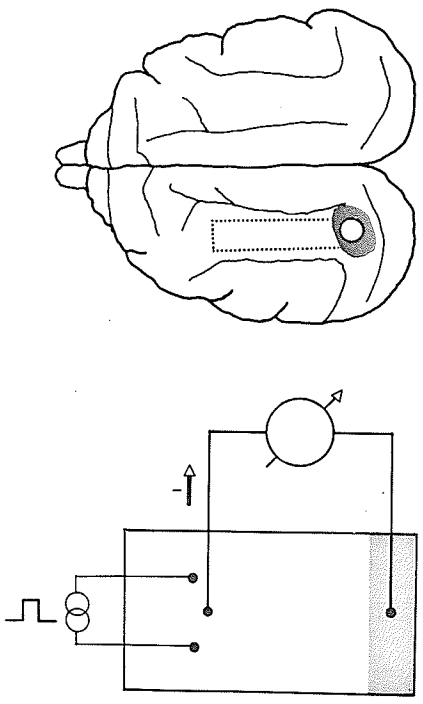

Fig. 7. Recording arrangement for responses of the isolated cortical slab.

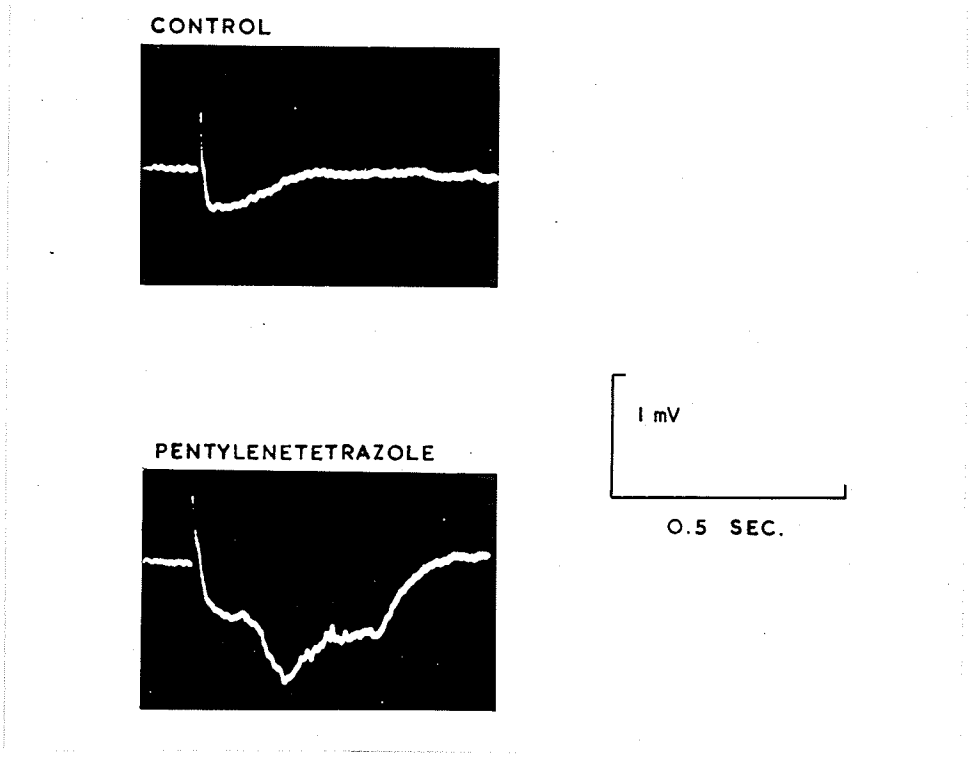

Fig. 8. Effect of local application of pentylenetetrazol on the response to direct stimulation of the cat's isolated cerebral cortex. Surface negative followed by surface positive burst response. Stimulus artifact not visible at this sweep speed.

c) Responses of the isolated cortex after systemic administration of drugs

The threshold stimulation voltages needed to produce the surface negative response and surface positive burst response were determined. The stimulus voltages were increased in 2.5 volt increments until the threshold was reached. Procaine, pentylenetetrazol or DMB were then given intravenously or in the case of ether by inhalation. The threshold was then redetermined and a minimum of one hour allowed to elapse before testing another drug. A return to the control threshold was always required to confirm that there was no persistent drug effect. The result of a test was discarded if the threshold did not return to the control value after one hour. The results are reported as the ratio of the stimulus voltage required to produce a surface positive burst response in the presence of drug to the stimulus voltage required for the same response with saline. All voltages were from dial readings. Absolute threshold voltages were not determined.

In the initial experiments it was observed that there was a fall in blood pressure after the intravenous administration of procaine. This fall in pressure was prevented in later experiments by intravenous dextran (Intradex^{R}-Glaxo). Usually 10 ml/kg of dextran were given after completion of the surgical procedures, and additional volumes were administered as required during the experiment. The total dose never exceeded 25 ml/kg. In some experiments the administration of convulsant drugs resulted in movement of the limbs which lasted for one to two minutes. This increased activity was treated by intravenous administration of d-tubocurarine (1 mg/kg) and was found to be adequate in preventing motor movements due to subsequent injections of convulsants. Animals so treated were respired artificially by a

respiratory pump (Model E106, C.F. Palmer Ltd.). Neither the administration of dextran nor the injection of d-tubocurarine affected the responses of the slab.

2. Results

a) Effects of the direct application of drugs to the isolated slab on the surface negative and surface positive burst responses

The purpose of this series of tests was to compare the effects of the drugs under investigation on two relatively uncomplicated electrical responses of cerebral cortical neurones. A total of 22 cats were used. At the start of each test a stimulus strength was chosen to produce a large surface negative response followed by a surface positive burst (Fig. 8 control). The drug concentrations used were: procaine hydrochloride 1.5%; pentylenetetrazol, 0.75%; sodium pentobarbital, 3.5% and DMB sodium, 0.3% and 0.5%. Two cats were also tested with DMB 1.0%.

Direct application of procaine and pentobarbital to the slab decreased the maximum amplitudes of the surface positive and surface negative responses and decreased the duration of the surface positive burst. In contrast, pentylenetetrazol increased the amplitude of these responses and increased the duration of the burst. A typical response of the isolated slab to direct stimulation following topical application of pentylenetetrazol is shown in Fig. 8.

Measurements of all responses from an experiment of this type are plotted in Fig. 9. This animal is one of thirteen tested in this manner with procaine, pentylenetetrazol and pentobarbital. Although the detailed form of the plotted curves varied in different animals, the general pattern was the same throughout, i.e., procaine and pentobarbital decreased these responses whereas pentylenetetrazol increased them.

The changes in these responses after DMB application depended on the concentration (see Table III). At a concentration of 0.3%, the

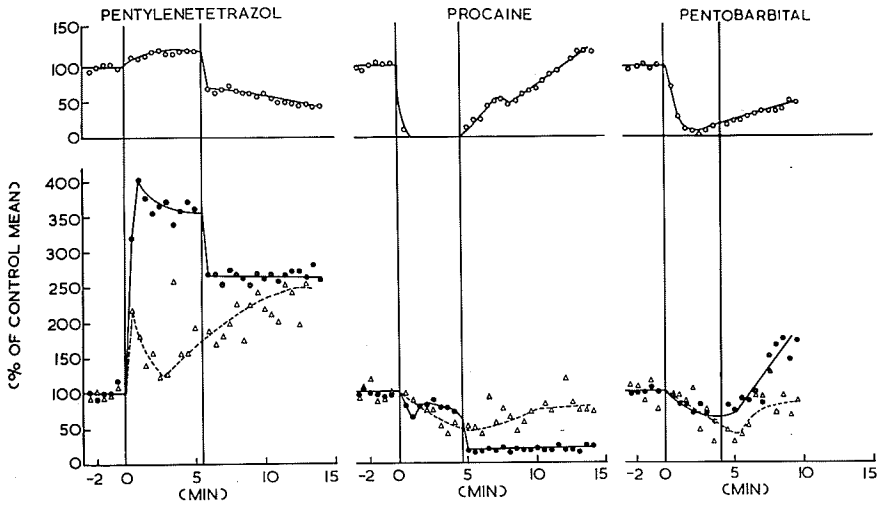

Fig. 9. Effects of local application of pentylenetetrazol, procaine and pentobarbital on the responses to direct stimulation of the cat's isolated cerebral cortex. o, amplitude of surface negative response; ●, amplitude of the surface positive burst, solid line; Δ, duration of surface positive burst response, dashed line.

TABLE III

Effects of Topical Application of DMB on Responses of Isolated Cortex

Cat	Concentration	Negative Response	Positive Burst Response
	0.3%		
28;37		=	↓
29;32		↓	↓
34		↓	=↑
	0.5%		
27;32;33;34;37		↓	↓
29;31		↓	=
36		↓	↑↓
	1.0%		
31;37		↓	↓

↑ Increase

↓ Decrease

= No Change

negative response was decreased in 3 of 5 animals. In the other two cats the response was unaffected. The positive burst response was depressed in 4 of 5 cases at this concentration, the remaining preparation being initially unaffected. The positive burst response in this latter animal was unaffected during the first few minutes after DMB application, but then showed an increase in the amplitude of the response in excess of 20%. Lower concentrations of DMB had no effect. When the concentration of DMB was raised to 0.5%, the negative response was always depressed. The positive burst response was also depressed in 5 of 8 animals tested at this concentration. Two out of 3 of the remaining animals showed no change in the response while the other cat showed an increase of almost 100% for about three minutes. This was followed by a marked decrease. DMB(1.0%)was tried in two animals and in these both the negative and positive responses were depressed for periods exceeding 30 minutes. DMB, unlike pentylenetetrazol, procaine and pentobarbital, did not produce consistent changes in the duration of the positive burst response; an increase in the amplitude of the response was often accompanied by a decrease in the duration of the response and vice versa. The results in 1 of 8 animals tested with 0.5% DMB are shown in Fig. 10.

The pH of the solutions used were: procaine hydrochloride, 6.0; pentylenetetrazol, 7.2; sodium pentobarbital, 9.2 and DMB 9.2. In order to exclude the possibility that the results obtained were due to the hydrogen ion concentrations of the various solutions, controls were made using procaine and pentylenetetrazol solutions brought to pH 7.4 with sodium hydroxide. These solutions were applied to the slab after controls with saline had been done (see Methods). There was no

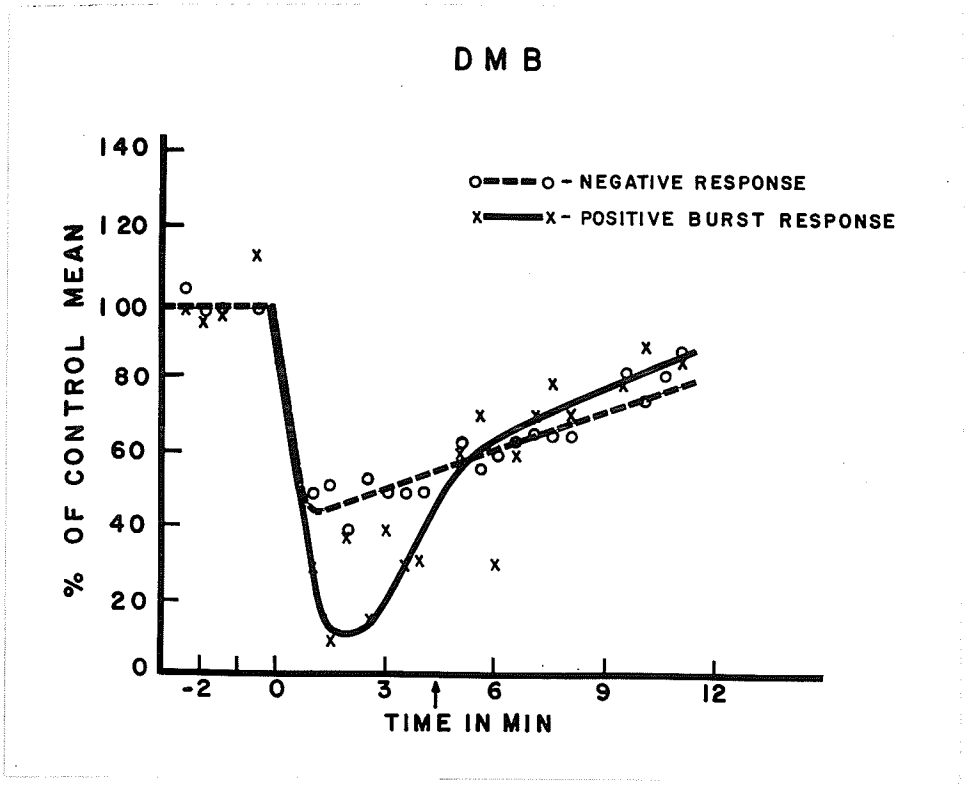

Fig. 10. Effects of local application of DMB on the responses to direct stimulation of the cat's isolated cerebral cortex. o, amplitude of surface negative response, dashed line; x, amplitude of the surface positive burst response, solid line.

difference between the results with the buffered and unbuffered solutions. The barbiturate solutions could not be adjusted to pH 7.4, since precipitation of barbituric acid occurred at pH 8.6. Therefore, a bicarbonate buffer, having the same molarity as the barbiturate solution, was prepared at pH 9.2 and applied to the slab as a control. The bicarbonate solution had no effect upon the responses of the slab.

b) Effects of systemic administration on the surface negative and surface positive burst responses

Procaine, pentylenetetrazol, DMB and ether were given systemically after determination of thresholds and then the thresholds were redetermined. Control threshold values never varied more than ± 2.5 volts during any 8 hour test period. In two animals maintained in good condition with liberal volumes of 0.9% saline and 5% glucose, the threshold for the positive burst response did not change for 36 hours. The range of control threshold voltages for all animals was 12.5-20.0 volts. Only those tests in which the control threshold voltages were the same before and one hour after giving of a drug are included in the results shown in Table IV.

The doses of pentylenetetrazol, procaine and DMB selected caused convulsions in normal, intact animals when given intravenously. Some increased motor activity was also observed in decerebrate animals given these drugs in convulsant doses. These movements, when caused by pentylenetetrazol or procaine, were seldom of sufficient magnitude to interfere with the recording since the head of the cat was firmly fixed in the head holder. In some animals the stimulating electrodes were nevertheless removed from the brain during this period and replaced immediately after the most active movements had ceased. The time that

TABLE IV

Positive Burst Response Ratio of Threshold Voltages after Systemic Drug Administration

Cat No.	Pent. (15 mg/kg)	(30 mg/kg)	Ether	DMB (2 mg/kg)	DMB (10 mg/kg)
2	-	1.4	-	-	-
3	-	> 2	2.0	-	-
5	0.13	16	1.0	-	-
7	1.0	-	1.0	-	-
8	1.0	2.0	2.0	-	-
10	1.0	1.6	1.3	-	-
17*	0.8	1.3	-	-	-
18*	0.8	2.2	> 2	-	-
19*	1.0	1.4	-	-	-
20*	0.8	1.4	-	-	-
21*	0.8	1.4	1.0	-	-
34*c	0.8	-	-	0.8	-
36*c	0.8	-	-	1.0	-
39*cs	0.8	-	-	0.75	-
40*cs	1.0	-	-	0.7	-
41*cs	-	-	-	0.7	-
42*cs	-	-	-	-	1.3
43*c	-	-	-	0.8	1.2
44*c	-	-	-	0.8	1.2

* Blood pressure stabilized by intravenous dextran

c Pre-treated with intravenous d-tubocurarine (1.0 mg/kg)

s Spinal cat

Pent. - Pentylenetetrazol

Proc. - Procaine

All ratios expressed as test/control

the electrodes were not in contact with the brain never exceeded 60 seconds. Administration of DMB, however, always resulted in the appearance of convulsions and it was always necessary to lift the electrodes to prevent damage to the brain when this drug was tested. Spinal cats were prepared in an attempt to eliminate this difficulty. Convulsant doses of pentylenetetrazol (15 mg/kg) and procaine (30 mg/kg) no longer produced any movement in the animal after spinal section, but DMB administration still caused convulsions. An intravenous dose of d-tubocurarine (1.0 mg/kg) at least 15 minutes before the injection of DMB in spinal or decerebrate cats prevented the convulsions although some minor twitching was sometimes observed in the hind limbs. None of the procedures designed to reduce motor activity had any observable effect on the threshold voltages or on the amplitudes of the responses. Movements were not observed when ether was tested for its effect on the cortical responses. It was given by inhalation at the concentration required to maintain surgical anaesthesia in the intact animal. During the period of ether administration, the positive burst response was monitored continuously in order to determine if threshold or amplitude changes occurred during the excitement stage of anaesthesia.

The threshold for the negative response was usually unaltered by pentylenetetrazol, procaine and ether although in two cats, one with ether and one after pentylenetetrazol, a slight decrease in the threshold voltage was noted. In contrast, DMB (2.0 mg/kg) produced a decrease in the threshold voltage of the negative response in 6 of 7 animals.

The threshold for the positive burst response was noticeably altered by the drugs. It was always increased by procaine, decreased or unchanged by ether, decreased or unchanged by pentylenetetrazol,

decreased by low doses of DMB and increased by high doses of DMB. The results are shown in Table IV. A value greater than 1.0 indicates an increase in the threshold and a value less than 1.0, a decrease (see Methods). These results represent values obtained 2-3 minutes after the intravenously administered drugs were given and 10 minutes after the start of the ether administration.

A pronounced increase in rate and depth of respiration always occurred after the administration of pentylenetetrazol. The possibility therefore existed that the decrease in threshold for the positive burst response that often occurred after this drug was given might be due to the blowing off of CO_2, which in turn could result in changes in cerebral circulation. This possibility was tested in five experiments in spinal and decerebrate cats with and without d-tubocurarine. These animals were respired artificially. This procedure did not modify the results (see Table IV). In one decerebrate animal, the threshold for the positive burst response was lowered even more after giving curare.

In addition to the changes in threshold caused by the drugs, the amplitudes of the responses were also affected. In about half the animals, procaine and ether caused a slight decrease and pentylenetetrazol a slight increase in amplitude of the negative response. DMB, in doses of 2.0 mg/kg consistently increased the amplitude of this response. In doses of 10.0 mg/kg, however, DMB always reduced the amplitude of the negative responses whereas the threshold was unaltered. Procaine and high doses of DMB always decreased and pentylenetetrazol and low doses of DMB increased the positive burst amplitude. This increase occurred whether the threshold was altered or not.

No depression in the responses of the slab was observed after systemically administered pentylenetetrazol. The decreased thresholds always returned to control values within 30 minutes after administration of the drug and there was no increase in the threshold for the positive burst response at any time during the recovery phase.

3. Discussion

It is generally agreed that as the connections of the cerebral cortex are progressively severed from the rest of the brain, there is a concomitant decrease in its electrical activity (Bremer, 1935a; 1935b; 1938; Dusser de Barenne, 1941; Obrador, 1943). It would then be expected that the relatively small slab of tissue prepared according to the method of Burns (1950) and Burns and Grafstein (1952) and used in the present experiments, would show very little activity until driven by a suitable stimulus. Such was indeed the case and only very small potential changes could be recorded in the absence of external stimuli. Bursts of spontaneous activity were noted in some of these preparations. Almost without exception, however, the activity disappeared within the first hour or two after isolation. This preparation is therefore suitable for the study of general anaesthetics because it provides a relatively isolated group of living cells within the central nervous system.

There is considerable disagreement among workers using this preparation as to what constitutes a viable cortical slab. Some are of the opinion that spontaneous burst activity in the slab is the measure of the health of the isolated cortex (Domino, 1957; Preston, 1955; Kristiansen and Curtois, 1949; Echlin et al., 1952). Others believe that this activity is due to an irritant focus (Burns, 1954) or injury potentials (Henry and Scoville, 1952). It has also been suggested that these bursts are due to humoral factors or changes in blood flow (Ingvar, 1955a; 1955b). This concept is based on the observation that animals with intact brain stems tend to show a greater incidence of spontaneous activity than those in which the brain stem

has been transected. The present experiments did not confirm this observation.

The only systematic investigation of this burst phenomenon has been done by Burns (1954). He was able to localize a focus of bursting in the slab. Occlusion of the blood supply to the brain for a brief period did not result in permanent changes in the excitability of the slab but the afterbursting stopped. Burns suggested that the initiation of this bursting is probably due to differential repolarization rates between deeper and superficial parts of neurones. It may therefore be initiated by electrical or mechanical stimulation. An injury potential could arise as a result of the isolation technique to establish the irritant focus. Domino's claim that spontaneous burst activity in the isolated slab is innate (Domino, 1957) must therefore be treated with considerable reserve. The results of the present experiments agree with Burns in that absence of spontaneous activity is no indication of lack of viability. Repetitive stimulation of the slab gives rise to bursts of electrical activity which may persist for many hours after removal of the primary stimulus (Burns, 1954). The isolated slab is then capable of 'spontaneous' activity although it may not exhibit it any one time. The appearance of a long-lasting positive burst response after direct stimulation, indicating that many neurones are being excited, is an adequate measure of the viability of the slab.

One of the major advantages of the isolated cortical slab preparation is that only two types of response are seen when single stimuli are applied to its surface. A stimulus of 0.03-0.2 msec duration produces the surface negative response. As the strength of

the stimulus is increased, there is a progressive increase in the amplitude of this response until a stimulus intensity is reached at which a completely different type of response is observed. This is the surface positive burst response which follows the surface negative response.

The surface negative response is also seen in intact cortex (Adrian, 1936). In the isolated cortical slab this response spreads out equally in all directions for a distance of about 10 mm but its amplitude decreases very quickly with the distance travelled and no response can be recorded at points further than 10 mm from the point of stimulation. The structures that conduct this response lie very close to the surface of the brain since a cut of 0.1 mm depth is sufficient to prevent spread of the response beyond the cut (Burns, 1958). It is still a disputed point whether this response is generated in pre-synaptic or post-synaptic neurones. Pinsky and Frank (1963) have shown that occlusion occurs when two stimuli, each of sufficient magnitude to produce a surface negative response, are applied at points more than 10 mm apart on the slab and the response is recorded at a point midway between them. This strongly suggests that the surface negative response is generated in post-synaptic neurones. On the other hand, the surface negative response is very resistant to general anaesthetics (Burns, 1958), an observation that has been confirmed in the present experiments. These observations would tend to indicate that the surface negative response is generated at a pre-synaptic site since synapses are generally considered to be the component in the transmission path of an impulse most sensitive to anaesthetics (Wright, 1954). It must be borne in mind, however, if the neurones conducting

the surface negative response are monosynaptic, they too would be expected to be resistant to anaesthesia. In the absence of evidence to the contrary, therefore, the negative response must be considered as post-synaptic.

The surface positive burst response consists of high frequency oscillations of potential suggesting that many neurones firing repetitively are involved. The amplitude and duration of the burst is independent of stimulus once the threshold has been reached. Moreover, the response spreads without attenuation over the whole of the slab. It may therefore be assumed that synapses are involved in its generation and transmission. The burst response, unlike the surface negative response, is not seen in intact cortex. It is conceivable that the normal afferent input to the intact cortex would prevent this response by the maintenance of a high degree of activity in the neurones involved in the burst pathway. The positive burst response was also found to be more sensitive to the effects of anaesthetics than was the surface negative response (Burns, 1951; Frank and Sanders, 1963), which is further evidence for the multisynaptic character of the burst pathway.

The systemic administration of procaine, ether and DMB (10 mg/kg) raised the threshold for the positive burst response and decreased its amplitude and duration. These results contrast with those obtained after intravenous pentylenetetrazol and DMB (2.0 mg/kg). It is therefore suggested that pentylenetetrazol and DMB in this low dose are acting in some manner different from that of procaine and ether. Ether, given in doses insufficient to produce surgical anaesthesia, did not decrease the threshold or increase the amplitude or

duration of the positive burst response. The convulsant activity of DMB, therefore, does not appear to be solely exaggerated Stage II excitement. A stage of excitement comparable to Stage II anaesthesia occurred, however, with higher doses of DMB. There was a marked increase in the motor activity of the cat after the intravenous administration of DMB (10 mg/kg), but the amplitude of the positive burst response decreased and the threshold was raised as well. At this higher dose, then, DMB produces the same effects as seen with ether and procaine. DMB, therefore, appears to act on more than one neuronal system within the central nervous system.

The results of the direct application of drugs of the isolated cortex must be treated with caution. These results were usually in the same direction as those obtained after systemic administration, but the concentrations used were completely arbitrary and no quantitative correlation between dose and effect is possible. Despite this drawback, the results obtained by this method qualitatively support those obtained after systemic administration, namely, pentylenetetrazol increased all responses, while procaine decreased them. Pentobarbital, like ether, also diminished these responses.

Topical application of DMB usually resulted in diminution of both the negative and positive burst response at all concentrations (see Table III). In only two cases was there facilitation of the positive burst response. Concentrations of DMB lower than 0.3% failed to produce any change in the responses of the slab. These results, therefore, emphasize the depressant effects of DMB and resemble those obtained with systemic doses of 10.0 mg/kg. It is not clear why a concentration of DMB which would facilitate the responses of the slab

could not be found. It may well be that the problem involves a diffusion barrier to the topically applied drug and that concentrations which produced no effect on the responses of the slab (0.2% and 0.1%) require a longer contact period with the cortex in order to produce an effect. The effect of DMB (0.3%), however, occurred within 30 seconds after application, which indicates that the drug moleucles diffuse rapidly to the sites where they act.

Of all the agents used, only DMB (2.0 mg/kg) lowered the threshold for the surface negative response. Higher doses of DMB did not raise the threshold. Lowering of the threshold can occur in several ways. Firstly, the drug may cause a partial depolarization of the cell membrane such that less current is required to produce the action potential. Another possibility is that more neurones are capable of contributing to the negative response i.e., spatial summation could occur. A third alternative, applying specifically to the post-synaptic membrane is that the drug causes an increased amount of transmitter to be released at the synapse. Evidence in support of any of these possible mechanisms is at present lacking.

The surface negative response was always affected before the surface positive burst response, further supporting the contention that the elements responsible for the negative response lie closer to the surface than those responsible for the burst.

Domino (1957) found that low doses of DMB or pentobarbital given intravenously caused an increase in the amplitude and a decrease in the frequency of the spontaneous activity of partially isolated cortex. Although these slabs differed from those used in the present experiments by showing spontaneous activity, the results are in good

agreement with those obtained with DMB on the responses of the directly stimulated slab. The effects of pentylenetetrazol on the spontaneous activity of the totally isolated slab was studied by Preston (1955). He showed that the slow intravenous infusion of pentylenetetrazol in cats produced the same pattern of effects on both the intact and isolated cortex. He further demonstrated that the slab went through a phase of postictal depression, a result never observed in the present experiments. It is difficult to resolve the differences in results. The 'spontaneous activity' described by Preston appears to be the same as the afterbursting studied by Burns (1954). Burns has described this afterbursting as being in every way similar to the positive burst response obtained on direct stimulation of the slab. It would be expected, therefore, that the results in the two cases would be similar. The only explanation offered for this discrepancy is that the doses of pentylenetetrazol used were markedly different. Preston infused the drug at a rate of 8 mg per minute for 8-16 minutes giving a total dose of 64-128 mg. This represents a much higher dose than was used in the experiments reported here and may account for the postictal depression observed.

C. EFFECT OF SYSTEMICALLY ADMINISTERED DRUGS ON RESPONSES EVOKED FROM THE AUDITORY CORTEX IN CATS

1. Method

The left cerebral cortex of cats of either sex weighing between 2.0 and 3.4 kg was exposed under ether anaesthesia (see II - B, Methods). The 'encephale isole' preparation of Bremer (1943) was made by cutting the spinal cord between the second and third cervical vertebrae either before or after exposure of the cortex. The dura mater was removed and the exposed brain covered with mineral oil. Rectal temperature was measured with a mercury thermometer, and maintained between 35° and 37°C by a heating lamp or warming plate. Blood pressure, recorded from a femoral artery by a mercury manometer, ranged from 70-110 mm Hg after the ether had blown off and before the administration of drugs. Dextran (6% in saline) was given intravenously as required up to 25 ml/kg to maintain the blood pressure. Most animals received only 10 ml/kg of dextran.

Click stimuli were delivered via a 2.5 inch speaker placed 12-14 inches from the left ear of the cat. Click frequency was 12 per minute and the duration of each click was 0.2 msecs. The loudness of the click was adjusted with a pulse generator (Tektronix Model 161) to a level slightly above threshold for the evoked response in each cat. (The term evoked response as used here, refers to the response recorded from the auditory cortex as evoked by a click stimulus.) A monopolar silk wick electrode (see II - B, Methods) was placed on an active area of the ectosylvian gyrus with an indifferent electrode on a killed portion of cortex. A diagram of the cortical areas and the relative positions of the electrodes are shown in Fig. 11-A. The evoked responses were amplified with a Princeton Science Associates Model TA-2 amplifier

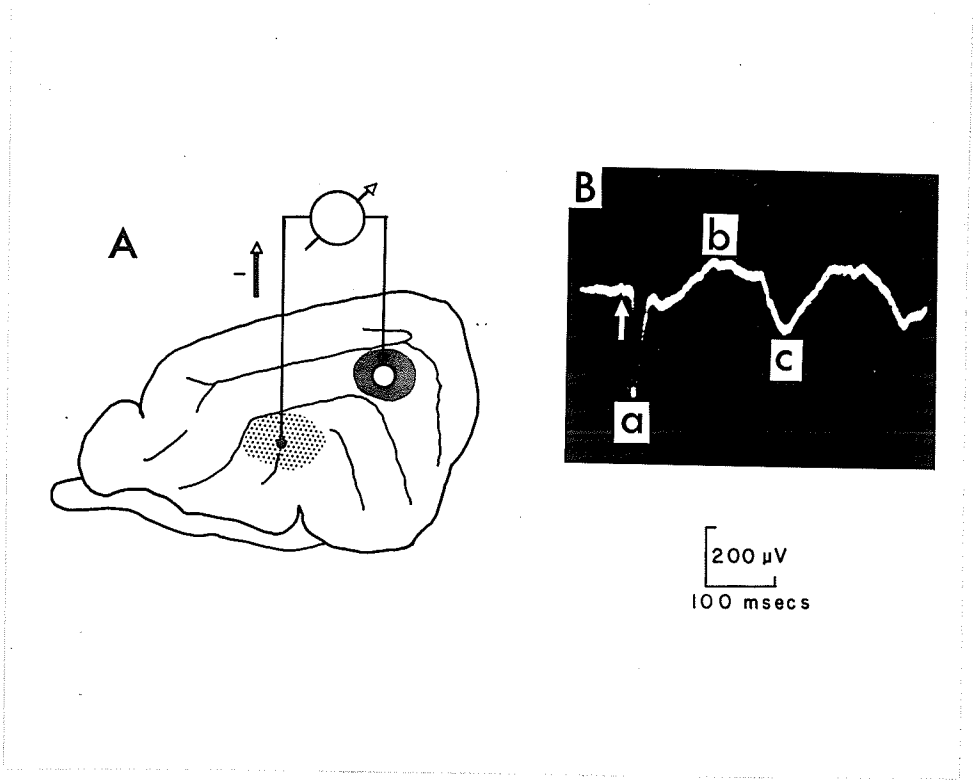

Fig. 11. (A) Diagram of the cat's cerebral cortex showing positioning of the recording electrodes. Shaded area in the ectosylvian gyrus is the acoustic cortex.

(B) Components of the evoked response recorded from the area shown in (A). 'a' primary positive component; 'b' negative component; 'c' secondary positive component.

adjusted to give a time constant of 100 msecs, and recorded photographically from a Tektronix Model 502 oscilloscope. Most measurements of the evoked responses were made from photographs of 10-15 superimposed traces. In four consecutive experiments, the amplitudes of 15 control traces recorded individually were averaged. These were compared statistically by a Student's 't' test with the average of an equal number of individually recorded responses obtained after administration of a drug. The amplitude of the primary positive component of the evoked response was measured from the middle of the baseline to the positive peak ('a' in Fig. 11-B). Component 'b' of the evoked response is called the negative component and 'c' the secondary positive component.

Pentylenetetrazol (15 mg/kg), procaine hydrochloride (15, 30 and 40 mg/kg) and thiopental (10 mg/kg) were given intravenously in a total volume of up to 3.5 ml over one minute via an indwelling catheter in a femoral vein. Control injections of 0.9% saline were made approximately 5 minutes before and 60 minutes after the administration of any drug. Recordings were made starting 2, 5, 10 and 60 minutes after completing the injection of any agent. A minimum of one hour was allowed to elapse between drug administrations (except where drug interaction was being studied) so that the effects of one drug would wear off before the next drug was given. Drug interaction studies were carried out using two combinations of drugs. In the first a convulsant or subconvulsant dose of procaine was given 2 minutes after a subanaesthetic dose of thiopental, and in the second, a convulsant dose of pentylenetetrazol was given 2 minutes after thiopental.

2. Results

The effect of these agents upon the primary component of the evoked response is summarized in Table V. Typical responses of the acoustic cortex after administration of the drugs tested are shown in Figs. 12-14. Procaine, in a dose of 30 mg/kg, produced an increase in the amplitude of component 'a' of the evoked response in 10 of 13 animals. A similar increase was noted in 5 of 5 animals when the dose of procaine was raised to 40 mg/kg. In a subconvulsive dose (15 mg/kg), however, only 3 out of 6 animals showed this increase in the amplitude and 2 of the 6 showed a decrease. Pentylenetetrazol increased the amplitude of this component in 11 of 12 animals, whereas thiopental, given in a subanaesthetic dose (10 mg/kg), decreased the amplitude in 16 out of 17 experiments.

The effects of procaine given 2 minutes after thiopental appear to be dose dependent. Procaine, at doses of 15 mg/kg and 40 mg/kg, and given in this manner, produced a further decrease in the amplitude of the response in 3 of 4 animals at each dose level. In contrast, procaine at a dose of 30 mg/kg, appeared to antagonize the effects of thiopental since the amplitude of the response was greater in 3 out of 4 animals tested in this fashion than with thiopental alone. Pentylenetetrazol given after thiopental increased the amplitude over that produced by thiopental alone in 4 out of 4 animals.

In those experiments where the average of individual traces was calculated, the differences between the test and control means was significant at the 0.01% level.

TABLE V

Effect of Drugs on the Primary Component of the Evoked Response

Cat	Dose in mg/kg								
	Proc 15	Proc 30	Proc 40	Thio 10	Thio + Proc 10 15	Thio + Proc 10 30	Thio + Proc 10 40	Pent 15	Thio + Pent 10 15
ER6		+16		-41				+27	
ER7		- 7						0	
ER8		+ 9		-48				+ 5	
E 2		+64						+ 9	
E 7		+31							
E 9		+40						+36	
E10		+ 9		-40				+200	
E11		+16						+155	
ED2	-13			-39	-53				
ED3		+25		-38		+13			
ED5		+23							
ED6		+50		-13		0			
ED7		-14		-58		-40			
ED8		- 5		-33		-66			
ED9			+33						
ED100			+31	-30			-54		
ED11			+70	-35			+108		
ED12			+54	- 9			-31		
ED13	+12			-38	-43				
ED15	+25			0				+85	+38
ED16	-18			-25				+50	-12
ED17								+75	
ED18	+ 6			-27	-27				
ED19	0			-39	-45			+30	+25
ED20			+25	-12			-20	+45	+17

Proc --- Procaine, Thio --- Thiopental, Pent --- Pentylenetetrazol
Values represent percentage increase or decrease in amplitude of primary component when compared to control.

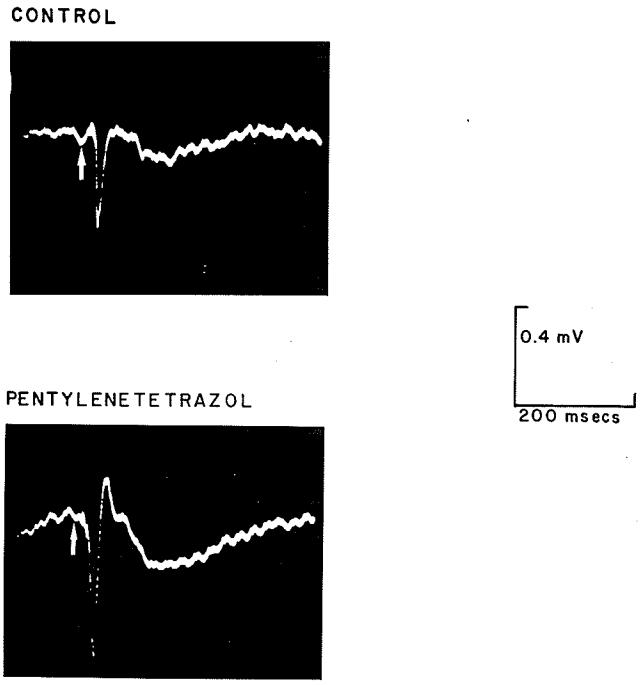

Fig. 12. Response of the acoustic cortex of the cat to a click stimulus 2 minutes after intravenous pentylenetetrazol (15 mg/kg).

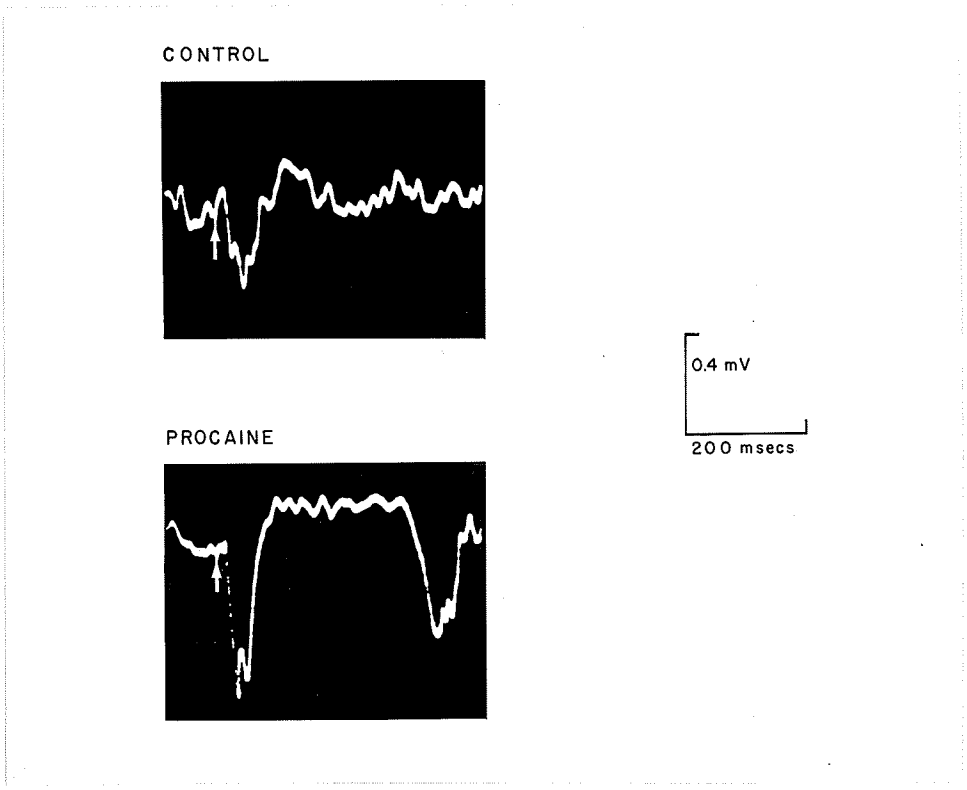

Fig. 13. Response of the acoustic cortex of the cat to a click stimulus 2 minutes after intravenous procaine (30 mg/kg).

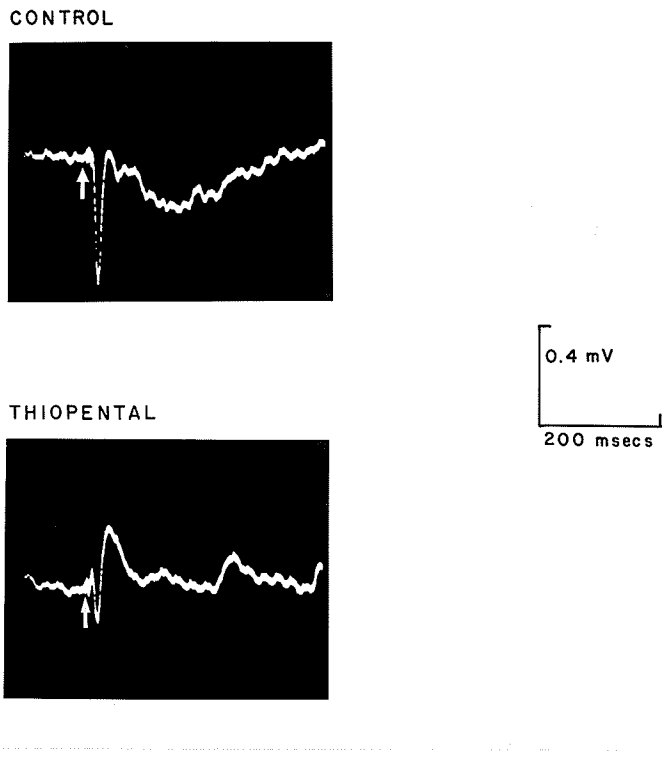

Fig. 14. Response of the acoustic cortex of the cat to a click stimulus 5 minutes after intravenous thiopental (10 mg/kg).

The negative component ('b' in Fig. 11-B) and the secondary positive component ('c' in Fig. 11-B) were also altered by the drugs. It is difficult to quantitate the effects of drugs upon these components of the evoked response since their form and amplitude varied considerably in individual responses. There were nevertheless certain obvious changes produced by the drugs. The negative component was increased by all doses of procaine, pentylenetetrazol and thiopental. On the other hand, only procaine and pentylenetetrazol increased the amplitude of the secondary positive component while thiopental decreased it. In 10 of 16 cats treated with thiopental in a dose of 10 mg/kg, the secondary positive component was not discernible (see Fig. 14).

Procaine, given after thiopental, invariably produced an increase in the secondary positive component whether the primary component was antagonized or enhanced. Pentylenetetrazol, however, caused only a very slight increase in the secondary component when given after thiopental in 2 of 4 cats. The remaining animals showed no change.

There was sometimes a fall in blood pressure (10-20 mm Hg) after the administration of procaine followed by a rise of as much as 60 mm Hg over control levels. A similar, although generally smaller response was often seen after thiopental administration. Pentylenetetrazol produced a rise in blood pressure which was never greater than 60 mm Hg above control levels.

It has been reported that decreases in blood pressure can produce electroencephalographic changes indistinguishable from those resulting from ether or pentobarbital anaesthesia (Beecher et al., 1938), and conversely that raising the blood pressure produces effects similar to those resulting from the administration of central

stimulants (Baust et al., 1963). Two control experiments were carried out in an attempt to determine whether alterations in the blood pressure affect the evoked response. In these experiments, the blood pressure was raised artificially with ephedrine, saline and dextran, or lowered by controlled haemorrhage. Lowering of the blood pressure had no effect on the evoked response as long as the pressure was not decreased to below 40 mm Hg. Below 40 mm Hg the components of the evoked response were markedly depressed. Pressures up to 160 mm Hg also had no noticeable effect on the evoked response although the background electrocortigram showed what is usually taken to be increased synchronization (Moruzzi and Magoun, 1949) when the pressure reached 180 mm Hg.

Despite the stability of the evoked response through wide variations in blood pressure, it was felt that pressures of 70-110 mm Hg would represent more reasonably physiological levels and were therefore chosen as the range of pressures to be attained before drug injection.

3. Discussion

The auditory area of the cat's brain represents a suitable surface from which evoked responses may be studied. First of all, the acoustic pathway lies wholly within structures above the spinal cord, thus permitting study of its responses without anaesthesia. Secondly, the acoustic area is very large and readily accessible. Finally, its responses to suitable stimuli applied to the periphery can be easily measured.

The components of the click-evoked response (see Fig. 11) have been analyzed by Bremer (1953), Bremer and Bonnet (1949) and Adrian (1941). The primary positive and succeeding negative components are both expressions of the reaction of cortical elements to afferent impulses arriving from the thalamus. The primary positive component is the sign of activity travelling from deeper parts to the surface of the cortex, while the negative response represents activity starting near the surface of the cortex and travelling away from it.

The secondary positive component of the evoked response was at one time considered to occur only in deep bsrbiturate narcosis (Derbyshire et al., 1936; Forbes and Morrison, 1939). The newer electronic averaging techniques (Brazier, 1961; Kiang et al., 1961) have indicated, however, that this slow secondary component occurs in the unanaesthetized preparation as well as in animals anaesthetized with other anaesthetics (Brazier, 1961). The source of this potential is still obscure but there is a suggestion that it may be due to a transitory intensification of autohythmic subcortical neurones (Bremer and Bonnet, 1949). This response was observed only with great difficulty in the present experiments in the absence of drugs since

separation of the response from the background electrical activity of the cortex was often impossible. It was even more difficult to discern after subanaesthetic doses of thiopental. It was sharply defined, however, in most animals after the administration of procaine or pentylenetetrazol.

The significance of these observations is, for the present at least, not readily apparent. Understanding of the nature of this secondary positive response has been complicated even further by the report that although present in the unanaesthetized preparation, it is absent in physiological sleep (Gumnit, 1961; Huttenlocher, 1960) and in animals lightly anaesthetized with thiopental (Gumnit, 1961). As mentioned previously, this secondary component was not readily observed in the present experiments after subanaesthetic doses of thiopental (10 mg/kg). Subsequent administration of procaine, however, resulted in the appearance of this component and was often of approximately the same amplitude as the initial positive response. It would be tempting to assume that this secondary component of the evoked response is a measure of the anaesthetic state, but the fact that this portion of the response is observed with procaine alone, which does not result in anaesthesia in the absence of barbiturates, rules this out. On the other hand, it might represent depression of some central mechanism since it occurred after pentylenetetrazol convulsions and might indicate postictal depression. This suggestion is supported by the observation that the response can be evoked immediately after convulsive activity has ceased. Moreover, it is greatly diminished when convulsions are prevented by pretreatment of the cat with subanaesthetic doses of thiopental. The possibility exists, however, that this

component may be produced by more than one action on the central nervous system. If both inhibitory and facilitatory neurones exist in the system responsible for this portion of the response, then the same response might be observed if the former were inhibited or the latter excited. Inhibition of an inhibitory pathway by pentylenetetrazol, however, seems unlikely since the centrifugal inhibitory pathway in the acoustic system of cats was unaffected by pentylenetetrazol whereas strychnine inhibited it (Desmedt and Monaco, 1960, 1962).

Modification of the primary positive component of the evoked response by the various agents used in this study is also not readily explicable. Convulsant doses of procaine and pentylenetetrazol increased the amplitude of this component, while thiopental always decreased it. Nonconvulsant doses of procaine (15 mg/kg), either increased, decreased or did not change the amplitude of this component. Such effects could be explained by assuming that both inhibitory and facilitatory neurones are present in the pathway responsible for this component of the evoked response. Courville et al., (1962) injected procaine into the medial region of the rostral pontine reticular formation and found that the evoked photic response was increased. Injection of procaine into the rostral mesencephalic tegmentum, however, decreased the amplitude of the evoked response. These workers interpreted their results as being due to (1) a release of inhibition of the reticular activating system so that the amplitude of the evoked response increased and (2) a direct blocking effect of the reticular formation itself to cause a fall in the amplitude of the response. This interpretation seems entirely reasonable. In all localized systems where the effects of procaine on electrical activity have been measured, only depression of

such activity has been reported (Sanders and Frank, 1963; Inoue and Frank, 1962a; Nathan and Sears, 1961; Taylor, 1959; Shanes et al., 1959).

In addition to the effects procaine can exert on reticular pathways, it could perhaps alter the response of the cortex by effects on the primary acoustic pathway (Ades and Brookhart, 1950). Stimulation of the crossed olivo-cochlear bundles reduces the response to a click stimulus along the complete afferent auditory pathway (Galambos, 1956; Desmedt, 1960; Desmedt and Monaco, 1961; 1962; Desmedt, 1962a; 1962b). Therefore, procaine could cause an increase in the amplitude of the evoked response by inhibition of this inhibitory pathway also.

Another pathway that might be depressed by procaine is one postulated by Galambos et al., (1961). They have shown that transection of the classical auditory pathway at the level of the brachium of the inferior colliculus does not in any significant way alter the pattern of the evoked response as recorded from the acoustic cortex. These workers assume, therefore, that a more medial pathway exists which parallels the classical auditory projection. They have furthermore shown that conduction in this extralemniscal system is completely blocked by deep pentobarbital anaesthesia.

The seemingly anomalous results obtained when procaine was given to animals pretreated with thiopental might possibly be explained on the basis of a differential sensitivity of all of the aforementioned pathways to the actions of these two drugs. Procaine, in doses of 15 and 40 mg/kg, given after thiopental potentiated the depressant effects of the barbiturate. At a dose of 30 mg/kg, however, procaine antagonized the effects of thiopental. If it is assumed that procaine blocks both excitatory and inhibitory pathways, then

subconvulsive doses of procaine could be blocking excitatory neurones, while other excitatory neurones in the auditory pathway are being blocked by thiopental. It is postulated that procaine and thiopental are not acting on the same neurones since pentylenetetrazol antagonizes the barbiturate but not the local anaesthetic (see II-A, Results). This subconvulsive dose of procaine (15 mg/kg) appears to be just below threshold for the effects of the drug on some inhibitory neurones, since the amplitude of the evoked response was increased, decreased or unchanged when this dose of procaine was given alone. When the dose of procaine is increased (30 mg/kg) and given after thiopental, more and more inhibitory neurones become inhibited by the drug, with the result that an antagonism between the local anaesthetic and the barbiturate is observed. If this effect on the inhibitory neurones is almost maximum, then further increase of the dose of procaine could now inhibit one of the acoustic pathways that has a higher threshold for the drug. This latter pathway could be the extralemniscal pathway of Galambos, since only excitatory function has been described for it (Galambos et al., 1961).

No release from inhibition has been shown to occur with pentylenetetrazol (Jolly and Steinhaus, 1956; Lewin and Esplin, 1961; Desmedt and Monaco, 1962; Jones and Lombroso, 1955). It appears, therefore, that the increase in the amplitude of the primary component after pentylenetetrazol is due to direct excitation of a facilitatory pathway. Pretreatment of the cat with thiopental always reduced the amplitude of the pentylenetetrazol response, an effect that would be expected to occur if both drugs acted on the same neurones with pentylenetetrazol exciting these neurones and thiopental inhibiting them.

The negative componet of the evoked response is subject to the same analysis since it too is dependent on the afferent input to the cortex (Bremer, 1953). Modifications occurring anywhere in the many pathways that appear to be involved in the click evoked response could so alter this response as to make excitation and release phenomena indistinguishable.

It appears, therefore, that the effects of drugs on the evoked response cannot be used as a criterion of anaesthesia. Possibly a method to obtain more definitive evidence as to the mechanism of action of the various agents could be the measurement of responses evoked by light in the optic cortex in decerebrate preparations where the brain stem could be removed from the field of influence. This is possible since the whole of the optic radiation could be included above the level of the transection.

III. GENERAL DISCUSSION

GENERAL DISCUSSION

It would be ideal if drugs could be tested on a localized group of neurones specifically responsible for the maintenance of consciousness. Unfortunately no such group of neurones is known to exist. The most generally accepted theory assumes that the maintenance of consciousness depends on the overall activity in the reticular formation of the brain (Moruzzi and Magoun, 1949; Brazier, 1954; Coben and O'Leary, 1958). A brief review of the evidence for this concept is given below.

Bremer (1935) was the first to show that transection of the brain stem at the collicular level maintained an animal in a constant state of unconsciousness. Moruzzi and Magoun (1949) found that excitation of the reticular formation in the central core of the brain stem resulted in electroencephalogram arousal i.e., low voltage and fast activity waves appeared on the EEG in place of the high voltage slow waves occurring in the drowsing animal. This low voltage fast activity has also been called the alert pattern, the active pattern or desynchronization. Moruzzi and Magoun also found that when the reticular formation was stimulated under the influence of barbiturate anaesthesia, it was more difficult to obtain this alert pattern. Electrolytic lesions restricted to the reticular formation caused coma while interruption of the classical sensory pathways (auditory and tactile) in the presence of an intact reticular formation had no effect on the arousal mechanism (French and Magoun, 1952). Arduini and Arduini (1954) also showed that the arousal mechanism is much more sensitive to the effects of anaesthetics than is the primary sensory pathway. King et al., (1957) demonstrated that low doses of barbiturates affected

the brain stem reticular formation and higher doses were required to alter the thalamic responses.

Attractive as this hypothesis may be in explaining anaesthesia due to barbiturates, it does not explain chloralose anaesthesia, since anaesthetic doses of chloralose failed to block the reticular formation (Moruzzi and Magoun, 1949). Moreover, the reticular formation is depressed by chlorpromazine, a non-anaesthetic agent (Killam and Killam, 1957).

Attempts to use electrical responses evoked elsewhere in the central nervous system as criteria of anaesthesia have been equally unsuccessful. The secondary response of Forbes (see II-C, Discussion) which is enhanced by barbiturates is not altered by chloralose and ether (Brazier, 1961). Furthermore, the present experiments have shown this response to be enhanced by procaine and pentylenetetrazol. More recently, Pradhan and Galambos (1963) showed that click evoked responses in the cat's cortex were similar for ether, pentobarbital, ethyl chloride and paraldehyde but different for chloralose and chloroform. The effects of pentobarbital on the spontaneous EEG activity are in some respects strikingly different from those produced by inhalation anaesthetics (Domino and Ueki, 1959). It would appear, therefore, that the state known as anaesthesia can be produced by more than one action on the central nervous system or that the site at which all anaesthetics act to produce their ultimate effects has not been found.

Although the neurones responsible for the maintenance of consciousness have as yet not been found, the present experiments support the concept that the local and general anaesthetics act by a similar mechanism in the central nervous system at the cellular level.

In isolated cortical slabs, procaine, pentobarbital and ether produced only depression of the responses of the slab. The results support the hypothesis that these agents act by inhibition of the neurones responsible for the responses of the directly stimulated isolated cortex. Cortical responses evoked by click stimuli, however, showed an increase after convulsive doses of procaine. Jolly and Steinhaus (1956) injected cocaine into limited portions of the cerebral circulation and observed that cortical stimulation was produced. Similar results were later obtained with procaine (Jolly, 1956). If the action of a drug on the cerebral cortex is to be studied then the ideal test object is one whose responses are wholly due to cortical mechanisms. The isolated cortical slab meets these requirements and the effects of procaine on this preparation are only depressant. Therefore, stimulatory effects recorded from non-isolated cortex must be due to extracortical influences. Implicit in this conclusion is the assumption that all areas of the cortex when isolated would react similarly to the effects of procaine. Further experimentation is therefore necessary to substantiate this concept.

The concept that procaine produces its central effects by inhibition of inhibitory and excitatory pathways permits an explanation of the results of the phenobarbital-local anaesthetic studies. There can be no doubt that local anaesthetics depress the central nervous system in intact animals pretreated with phenobarbital. This central depression has been observed in a number of studies where local anaesthetics have been used as anticonvulsants (Bernhard and Bohm, 1955; Bernhard et al., 1956a; 1956b; Berry et al., 1961), as sedatives (Creech et al., 1950; Godman and Adriani, 1949), and as a supplement to

nitrous oxide-thiopental anaesthesia (Steinhaus and Howland, 1958). Suppression of the EEG after intravenous administration of local anaesthetics has also been reported, even in the presence of convulsions (Foldes et al., 1960). The anticonvulsant activity of the local anaesthetics, however, is unlike that of the barbiturates in that the former are more effective in preventing electroshock seizures than drug-induced seizures (Frey, 1962; Tanaka, 1955) while the reverse order obtains for the barbiturates (Goodman et al., 1953). This evidence further supports the concept that the barbiturates and procaine depress different groups of neurones in the central nervous system.

Although the depressant effects of general and local anaesthetics upon the central nervous system appear to be exerted on different groups of neurones, the possibility still exists that both groups of agents cause excitation by an effect on a common neuronal pathway. An inhibitory pathway in the ascending reticular formation has been postulated by Brazier (1961). Injection of procaine into this area enhanced the photically evoked response while injection of procaine into rostral mesencephalic areas reduced the response (Courville et al., 1962). Randt and his colleagues (Randt et al., 1958) have shown that nitrous oxide, cyclopropane and ethylene act differently on four somatic afferent systems of different fibre size. This suggests that anaesthetic effects, in addition to the effects on synaptic junctions, may be due to selective attack on small inhibitory fibres. Although no evidence exists for the presence of such fibres in the inhibitory pathway, such an assumption is not unreasonable. Thus, since both general and local anaesthetics probably depress both inhibitory and excitatory neurones, the excitation stage of general anaesthesia (Stage II) and the

excitement and convulsions produced by local anaesthetics could arise as a consequence of the inhibition of such small fibres by these agents. A greater degree of conduction block would be expected to occur with local than with general anaesthetics (Gros, 1929) thus permitting an explanation of the more marked central excitation produced by the local anaesthetics. On the basis of such a fibre system, it would be expected that local anaesthetics could produce anaesthesia when injected alone by inhibition of the excitatory fibres. This effect was not observed. It is possible that the fibres in the excitatory pathway are much larger than those in the inhibitory pathway and are not effectively blocked by local anaesthetics except with very large doses where death results due to depression of respiratory and cardiovascular functions (Steinhaus, 1957).

Some explanation is demanded to explain why general anaesthetics should preferentially attack the excitatory pathway of the postulated system. This may be explained by assuming that the excitatory pathway involves many more synapses than its inhibitory counterpart. Multisynaptic pathwyas are known to be more sensitive to the effects of general anaesthetics than are monosynaptic pathways (Wright, 1954). If such a system indeed exists, then transmission of impulses through the multisynaptic pathway could be blocked by the general anaesthetics resulting ultimately in anaesthesia. There is also some evidence that general anaesthetics are more effective at synapses than are local anaesthetics. Goldring et al., (1961) found that the effects of intravenous pentobarbital on the direct cortical response differed from that of intravenous procaine. Pentobarbital depressed the primary potential (surface negative response) which is believed to be post-synaptic in

origin (see II-B, Discussion) while this potential was unaffected by procaine. In contrast, procaine depressed the slow negativity while it was augmented by pentobarbital. The slow negativity is probably due to differential repolarization of neurones, i.e., one end of the neurone repolarizing before the other end and no synapses are involved (Pinsky, 1961).

The hypothesis also permits an explanation of the mechanism by which convulsions resulting from local anaesthetic administration are prevented by barbiturates. The net increase in excitatory activity resulting from depression of inhibitory pathways would not become manifest if synaptic inhibition of the excitatory pathway prevents impulse transmission. It explains also the results of the interaction studies with pentylenetetrazol and procaine. In this case both drugs produce the same effect since procaine inhibits the inhibitory fibres and pentylenetetrazol excites the excitatory pathway. Similarly, barbiturates are effective in antagonizing the excitatory effects of pentylenetetrazol, since excitation and depression are being produced in the same neurones.

Finally, the hypothesis is consistent with the concept that both general and local anaesthetics prevent the excitation that leads to propagated action potentials in cells within the central nervous system. Intracellular recording in isolated preparations could aid in the solution of these problems.

IV. SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

1. The local anaesthetics procaine, dibucaine, lidocaine, cocaine and the convulsant barbiturate 5-ethyl-5-(1,3-dimethylbutyl) barbiturate (DMB) increased the incidence of loss of righting reflex in mice pretreated with phenobarbital when compared to those given phenobarbital alone. Neither the local anaesthetics nor DMB caused loss of righting reflex when administered alone. These results are in contrast to those obtained when phenobarbital pretreated mice are given pentylenetetrazol.

2. Pentylenetetrazol administered to mice pretreated with both procaine and phenobarbital antagonized the depressant effects of the barbiturate only but deepened the depression produced by procaine. This suggests that procaine and phenobarbital act on neurones in different sites in producing central nervous system depression.

3. Procaine and pentobarbital decreased the amplitude of surface negative and surface positive responses when applied topically to isolated slabs of cerebral cortex in cats. Pentylenetetrazol increased the amplitude of these responses. DMB usually decreased the amplitude of these responses.

4. Systemic administration of procaine always raised the threshold for positive burst response of isolated cortex. Inhalation of ether either raised this threshold or left it unchanged, while pentylenetetrazol either lowered the threshold or left in unchanged. Low doses of DMB lowered the threshold for both the negative and

positive burst responses but higher doses raised the threshold for the positive burst response.

5. Procaine and pentylenetetrazol, administered to unanaesthetized spinal cats, usually increased the amplitude of the click-evoked response in the auditory cortex. This response was decreased by subanaesthetic doses of thiopental. Procaine, depending on the dose, either potentiated or antagonized the thiopental response while pentylenetetrazol always antagonized it.

6. It is suggested that local and general anaesthetics produce their excitatory effects on the central nervous system by inhibition of inhibitory pathways. It is further suggested that the fibres in the inhibitory pathway are very small and thus would be blocked to a greater degree by local than by general anaesthetics which may account for the greater excitation which occurs after systemic administration of local anaesthetics. In contrast, it is proposed that the excitatory pathway contains large fibres but many synapses which could account for the greater effectiveness of the general anaesthetics in blocking the excitatory pathway.

7. The results generally support the premise that local and general anaesthetics act by a common mechanism on neurones in the central nervous system.

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