File copy

CHOLINERGIC MECHANISMS IN CONVULSIVE THERAPY

Max Fink, M.D.

From the Department of Psychiatry at the Missouri Institute of Psychiatry, University of Missouri School of Medicine, 5400 Arsenal Street, St. Louis, MIssouri 63139.

Aided, in part, by USPHS grants MH-927, MH-2715, MH-07249, and MH-11380; and the Psychiatric Research Foundation of Missouri.

VII: 7-25-65

CHOLINERGIC MECHANISMS IN CONVULSIVE THERAPY

Despite extensive use and many efforts at understanding, the mode of action of the convulsive therapy process remains enigmatic. The neurophysiological, psychological, clinical, and social aspects, of many studies served to elucidate the present neurophysiological-adaptive view of the process (Fink, 1957, 1962). In this view, the early development and persistance of signs of altered cerebral functions were reported to be requisite to changes in behavior (Fink and Kahn, 1956), with electroencephalographic slow wave activity as the most significant index of altered brain function. Demonstrations that premedication with atropine inhibited this slow wave activity (Ulett and Johnson, 1957) and that anticholinergic compounds reversed the clinical as well as electrographic changes (Fink, 1958) suggests that the biochemical basis for the convulsive therapy process may be in cholinergic mechanisms of the central nervous system. This review discusses the available data regarding acetylcholine and the cholinesterases in the convulsive therapy process.

Acetylcholine has been extensively studied as an active agent in the transmission of nervous impulses since the first agent in the transmission of nervous impulses since the first descriptions of Dale (1914) and Loewi (1921). It is a constituent of nervous tissue, existing in a bound form which is liberated during the excitation process. It is rapidly hydrolyzed through the specific action of cholinesterase and is rapidly reconstituted by the choline-acetylase system (Richter and Crossland, 1949). In normal cerebrospinal fluid, free acetylcholine is not present despite the rapid breakdown of bound acetylcholine during periods of

activity and excitement (Tower and McEachern, 1949a). The cerebrospinal fluid does have measurable cholinesterase activity, however, principally of the "true" or mecholyl hydrolyzing type (Nachmanson and Rothenberg, 1945). In the absence of free acetylcholine and under the conditions described, electroencephalograms fail to show abnormality.

(a) Cholinergic Aspects of Craniocerebral Trauma: Free acetylcholine was found in the cerebrospinal fluid within a few minutes after experimental head trauma in cats and persisted for varying periods up to 48 hours (Bornstein, 1946). The quantity of free acetylcholine varied between 2.7 and 9.0 gamma percent, and the amount was related to the degree of induced trauma.

Concurrent electroencephalograms demonstrated patterned changes. The records were first filled with high voltage fast activity, interpreted as evidence of an intense neuronal discharge, soon to be succeeded by a short period of flattening of all recorded electrical activity. These phases were then followed by prolonged periods of high amplitude sharp waves in the delta frequencies.

The behavioral changes related both to the degree of trauma and the amount of measured free acetylcholine. With higher levels of acetylcholine, Bornstein reported greater degrees of EEG abnormality and greater changes in consciousness. Spontaneous post-traumatic seizures were also related to the amount of free acetylcholine appearing in the spinal fluid.

Bornstein applied acetylcholine to exposed cat cerebral cortex. When the concentration of acetylcholine was 1 gamma percent or less, high amplitude sharp waves of low frequency appeared in the electroencephalogram. When the concentration was increased to 2 gamma percent, the electroencephalogram flattened in a fashion parallel to the post-traumatic records.

Investigations in neurological patients by Tower and McEachern (1949a) demonstrated free acetylcholine in the cerebrospinal fluid only in patients with recent head trauma, recent grand-mal seizures or after electroconvulsive therapy. Free acetylcholine varied from 0.2 to 100 gamma percent. In assaying spinal fluid cholinesterase activity, they noted a sharp rise in the nonspecific cholinesterase fraction (benzoylcholine-splitting) and a drop in the specific cholinesterase fraction (mecholyl-splitting) in patients with head trauma and following convulsive therapy. The cerebrospinal fluid did not exhibit such inversion, although it contained free acetylcholine, after spontaneous seizures. They concluded that the level of free acetylcholine varied directly with the degree of cerebral damage and that reversal of the cholinesterase fractions was a more sensitive indicator of cerebral damage. Electroencephalograms, taken at varying intervals following trauma, also indicated a relation between the degree of EEG abnormality and the appearance of free acetylcholine in the cerebrospinal fluid.

These observations were recently confirmed by Kovach, et al. who recorded increased acetylcholine in rat brain after traumatic shock, and an inhibition of this activity by the administration of atropine to the muscle preparation.

Thus the amount of free acetylcholine may increase in the spinal fluid following craniocerebral trauma and the amount of free acetylcholine, the degree and type of electroencephalographic abnormality, and changes in clinical behavior appear as interrelated phenomena.

(b) Anticholinergic drugs and trauma: The electrographic, behavioral and neurologic signs of head trauma were blocked by the parenteral administration of 0.5-1.0 mg/kg atropine (Bornstein, 1946), as were similar clinical changes occurring after the intracisternal addition of acetylcholine. Ward (1950) applied these observations to the treatment of closed head injuries. In 20 patients with varying degress of trauma, he administered atropine subcutaneously in doses of 0.1 mg/kg, noting clinical improvement in some and a reversal of the electrographic effects in others. Similar alterations in the post-traumatic electroencephalogram were reported by Jenkner and Lechner (1955) in a study of diethazine; another anticholinergic drug. A single intravenous dose in forty patients resulted in normalizing the abnormal electroencephalogram in twenty-two and marked improvement in six others.

Similar observations have been reported with methylbenactyzine and transentin in animal experiments of post-traumatic shock and cerebral edema (Denisenko, 1965).

The effect of atropine was assessed in the convulsive therapy process by Ulett and Johnson (1957). With the administration of atropine up to mgm per day during the weeks the patients received electroshock therapy, the amount of slow wave activity was significantly less than in a control group who had not received the atropine administration. (In a later study these authors failed to replicate this study, suggesting that dosage factors or population changes may have contributed to different results [Johnson et al., 1960]).

Similar effects were observed with experimental anticholinergic compounds (Fink, 1958, 1960). The intravenous injection of diethazine, benactyzine, the piperidylbenzilates JB-318, JB-336 and JB-329 (Ditran), and WIN-2299 induced EEG desynchronization in psychiatric subjects. These EEG changes were associated with behavioral alerting, anxiety, tremors, illusions, and hallucinations. In patients who had recently received electroconvulsive therapy, there was a reduction in slow wave activity and a reversal of euphoria, denial and confusion. Atropine in low doses, was associated with EEG desynchronization accompanied by tachycardia, nervousness and tension. At higher dosages, hypersynchronous slow waves, followed by lower voltage, poorly organized delta activity with superimposed beta activity was associated with progressive confusion and disorientation.

Both in cerebral trauma and induced convulsions, the electrographic changes may be modified by the administration of anticholinergic drugs, suggesting that increased amounts of acetylcholine or increased cholinergic receptivity is associated with the high voltage slow wave activity.

(c) Brain acetylcholine and anticholinergic drugs:

Similar EEG changes and similar blocking by anticholinergic drugs has been observed following the direct application of acetylcholine to the central nervous system. The administration of a cholinesterase inhibitor DFP (di-isopropyl fluorophosphate) elicited high amplitude rapid frequency EEG patterns similar to status epilepticus, as well as changes similar to those of post-traumatic states (Freedman et al., 1949, Hampson et al., 1950, and Himwich et al., 1950, and Weseol et al., 1948). These EEG effects were blocked by small doses of parenteral atropine and scopolamine. The great increase in acetylcholine after tetraethyl pyrophosphate (TEPP) was measured and related to the toxic manisfestations and convulsions induced (Giarman and Pepeu, 1952; Stone, 1957).

Chatfield and Dempsey (1942) prepared exposed animal cortex with prostigmine and evoked eleetroencephalographic spike activity. The prior administration of atropine blocked this spiking, or if present, the abnormality could be eliminated by atropine.

In contrast to these findings, Brenner and Merritt (1942) applied topical acetylcholine in concentrations of 2-1/2 to 10% to the exposed cortex of cats, and noted no effect on the

electroencephalographic changes after intravenous atropine (1 mg/kg.) The concentrations of acetylcholine in these experiments, however, were higher than the topical applications (1-4 gamma percent) and the intracisternal (0.2-10 gamma percent) injections of Bornstein (1946). Brenner and Merritt also made note of electroencephalographic effects similar to acetylcholine from mecholyl (acetylbetamethylcholine) and doryl (carbamylcholine) in concentrations much lower than the acetylcholine concentrations. They ascribed the increased effectiveness of these cholinergic drugs to their lack of sensitivity to cerebral cholinesterases.

These data are conflicting and further study is necessary to qualify this issue.

(c) Cerebrospinal Fluid Acetylcholine and Seizures: One view of aeetylcholine metabolism indicates that it is found in nervous tissues in an inactive bound form. During periods of activity, acetylcholine is liberated at the cell membrane where it is rapidly deactivated by cholinesterase. The amount of bound acetylcholine is the resultant of the continuous processes of synthesis, liberation and breakdown. It has been postulated that the level rises during sleep and falls during activity. (Richter and Crossland, 1949; Elliott, Swank and Henderson, 1950; Giarman and Pepeu, 1962). Richter and Crossland measured the level of acetylcholine (micro-gamma per mg. brain tissue) during anesthesia and sleep in rat brain to be 300% higher than post-aeizure levels. The difference in tissue levels is transient, however, as the resynthesis rate for acetylcholine in rat brain is high (7 gamma/gm/minute). These observations were confirmed by

Elliot et al. (1950) and Crossland and Merrick (1954). Giarman and Pepeu (1962) found the increase in acetylcholine following various depressants to be roughly proportional to the degree of depression of the central nervous system and the reduction in motor activity. Maynert and Buck (1964), however, studying brain acetylcholine levels and sedation concluded that some sedating agents are associated with elevated brain acetylcholine, but that no rigorous relationships existed. In part, this may be related to the earlier observations of McLennan and Elliott (1951) that acetylcholine synthesis measured in rat brain slices, is accelerated by low dosages of narcotic drugs, but inhibited by high dosages.

Free acetylcholine was reported in the spinal fluid in patients with epilepsy (Cone, Tower and McEachern, 1948; Tower and McEachern, 1949b). Of 56 epileptic patients, 44 (77%) demonstrated free acetylcholine in quantities of 0.02 to 5.0 gamma percent with an average of 1.0 gamma percent. Acetylcholine levels were related to the frequency of seizures, the extent of electroencephalographic abnormality, and to the time since the last seizure, but bore no relation to medication, type of epilepsy or level of cholinesterase activity. Elliott et al. also noted that free acetylcholine was demonstrable in the spinal fluid in concentrations up to 3 gamma percent after metrazole convulsions.

Tower and McEachern (1949b) viewed the increased acetylcholine as a by=produce of the seizure, and not causal. Studying the hypothesis that the accumulation of acetylcholine induced seizures, Torda (1953) measured the level of acetylcholine in brain tissue after metrazole convulsions. She noted a rise in the acetylcholine content of brain, before a seizure and a fall during the convulsion. Below certain levels of acetylcholine, convulsions failed to occur. She suggested that the fall in tissue acetylcholine during a convulsion was due to inhibition of acetylcholine synthesis by increased concentrations of metabolites such as ammonium ions.

Giarman and Pepeu also measured changes in central nervous system acetylcholine following various stimulants. Only after mecholyl and 3, 5-dimethylbutylethyl-barbiturate was there a significant change in the acetylcholine level. They noted a decrease in association with induced convulsions. With other drugs which they classified as stimulants (LSD, iproniazid, iproniazid = hydroxytroyptophan and iproniazid = DOPA) there were no changes in acetylcholine level. They concluded that despite intense excitation produced by these compounds, there were no changes in acetylcholine levels unless these were accompanied by convulsions. (The differences in observations between these workers and Cone et al. and Tower and McEachern may be related to the differences in methods of biochemical measurements, for the latter measured changes in spinal fluid reflecting the free acetylcholine, while Giarmann and Pepeu

measured the total acetylcholine reflecting bound and free forms of acetylcholine. [McLennan and Elliott, 1951]).

These studies suggest that spontaneous or induced seizures are accompanied by an increase in intercellular free acetylcholine liberated from its bound form which may be reflected in the spinal fluid. Cerebral activity and seizures enhance acetylcholine destruction, lowering tissue levels of acetylcholine, while sleep and anesthesia may augment acetylcholine production increasing tissue levels.

(d) Central Nervous System Cholinesterases: Tower and McEachern (1949) also measured spinal fluid cholinesterase activity. Two types of cholinesterases are normally found in the spinal fluid: cholinesterase-I ("true," "specific," or mecholyl-hydrolyzing), which hasaa high specificity for acetylcholine; and cholinesterase-II ("pseudo," "non-specific," or benzoylcholine-hydrolyzing). Both compounds hydrolyze acetylcholine but have different rates of hydrolysis for mecholyl and benzoylcholine. This differential rate permits qualitative distinctions. By reporting the cholinesterase activity as a ratio of the activity with a mecholyl substrate and with a benzoylcholine substrate compared to a substrate of acetylcholine, two ratios are found: cholinesterase-I/acetylcholine and cholinesterase-II/acetylcholine. In such ratios normal cerebrospinal fluid contains esterases in the ratio of 33:17 for cholinesterase-I to cholinesterase-II.

In patients with head trauma, Tower and McEachern reported an inversion of the cholinesterases with an increase in the

cholinesterase-II fraction of the spinal fluid and a decrease in cholinesterase-I activity. The extent of the cholinesterase reversal was related to the severity of trauma and to the degree of the electroencephalographic abnormality.

In patients with elevated spinal fluid acetylcholine after spontaneous seizures, however, no change in the ratio of cholinesterases or total cholinesterase activity was found.

The change in cholinesterase activity may be understood in relation to changes in cell membrane permeability. Cholinesterase-I is found in highest concentration in the central nervous system while cholinesterase-II predominates in other tissues, especially blood serum. With an increase in acetylcholine levels in cerebral intercellular fluids, vasodilation and increased cellular permeability may be predicted, with a degree of transudation of vascular fluids into the intercellular spaces varying with the extent and duration of the vasodilation (Kabat et al., 1948). Spiegel, Spiegel-Adolf, and their co-workers (1941, 1942, 1944, 1948, 1953) demonstrated such permeability changes and increased conductivity of the tissues associated with the appearance of various ions (as potassium and phosphate) in the spinal fluid following electrically induced convulsions. Such non-electrolytes as nucleic-acid splitting enzymes also increased. Changes in cellular permeability may thus provide the basis for the high concentrations of acetylcholine and the increased concentrations of cholinesterase-II in induced seizures or head trauma (Tower and McEachern 1949c).

The persistence of acetylcholine in spinal fluid after head trauma and after seizures despite increased cholinesterase

activity may be related to the sensitivity of the acetylcholine-cholinesterase-I system to concentration relationships (Nachmanson and Rothenberg, 1945; Tower and McEachern, 1949c; Burgen and MacIntosh, 1955). At "physiologic" concentrations, hydrolysis of acetylcholine is rapid (3-4 microseconds) but at higher and lower concentrations, the activity falls off quickly. In contrast, the cholinesterase-II acetylcholine relationship is non-specific and the rate of hydrolysis increases with increased concentration.

One may relate these relationships to the induction of seizures. While the usual concentrations of acetylcholine at cell membranes are destroyed by the specific activity of cholinesterase-I in a few microseconds, an excessive concentration following excitation may exceed the rate of hydrolysis by cholinesterase-I. The seizure threshold is reached and a seizure induced, with the seizure itself adding to the amount of free acetylcholine. The increased acetylcholine diffuses rapidly, affecting vascular and cellular permeability and increasing the concentrations of various ions, including cholinesterase-II, in tissues and in the CSF. The activity of cholinesterase-II, though of low efficienty and depending on concentration kinetics reduces the acetylcholine in the tissues in hours to days to levels for the physiologic action of cholinesterase-I.

Cholinesterases appear in the spinal fluid as a reflection of their increase in intercellular fluids, resulting from changes in cell membrane permeability occasioned by increased acetylcholine. The increased cholinesterases are part of the homeostatic mechanisms,

controlling the levels of acetylcholine at cell membranes necessary for nervous system activity.

(e) Acetylcholine, EEG Hypersynchrony and Induced Convulsions: Alteration in the blood-brain permeability barrier by the continuing action of acetylcholine may be a biochemical substrate for the post-electroshock hypersynchrony of the electroencephalogram. Such a possibility is evident in the demonstration of an increase in the concentration of cocaine in brain tissues three days after a series of 12 induced convulsions (Aird et al., 1956). The change in concentration of this large molecule, ordinarily absent in brain tissue, was associated with the appearance of hypersynchrony delta bursts) in the electroencephalogram.

We have confirmed the many previous reports that convulsive therapy induces electrographic hypersynchrony (Fink and Kahn, 1956; Fink et al., 1961). Despite a constant application of treatments there is a great variability in the time of appearance, the duration and the extent of the electrographic slow wave activity as well as the sensitivity to modification by alerting, hyperventilation and barbiturates in psychiatric populations. The early appearance of high degree hypersynchrony and its persistence throughout a treatment course has been described as prerequisite to improvement following electroshock (Roth, 1951; Roth, et al., 1957; Fink and Kahn, 1956). It is possible that the differences in the degree of induced EEG hypersynchrony may be related to differences in central cholinergic activity. The failure of certain patients to develop hypersynchrony may be associated with the absence of free acetylcholine being related to minimal changes in cerebral function and thus precluding a

clinical response to induced convulsions. Tower and McEachern (1949a), in their study of craniocerebral trauma, included observations of six psychiatric patients undergoing convulsive therapy. Studying the patients after 3-7 treatments they reported free spinal fluid acetylcholine in two patients; and an increase in cholinesterase-II and a decrease in cholinesterase=I with a reversal of the ratio of cholinesterases in five of the six patients. From these observations they conduded that the spinal fluid changes in induced convulsions were more like those of craniocerebral trauma than those of spontaneous epilepsy.

They described the one patient in the series who failed to show either free acetylcholine or a cholinesterase ratio reversal in the spinal fluid as: "It is interesting that this patient was the only one of the six to show no response to treatment." If electrographic hypersynchrony is a reflection of increased free acetylcholine, subjects who maintain hypersynchrony and those in whom it disappears rapidly may be exhibiting differences in the kinetics of the cholinesterase-acetylcholine hydrolysis systems. Persistent hypersynchrony may result from a decreased rate of hydrolysis of acetylcholine, associated with low concentrations of either cholinesterase-I or cholinesterase-II. (Conversely, in patients with short-lived hypersynchrony, cholinesterase-I and -II in tissue and spinal fluid may be unusually high).

From these observations we would conclude that induced convulsions are associated with an increase in free acetylcholine in intercellular fluids, altering cerebral permeability and enhancing the appearance of cholinesterases. The level of free

acetylcholine is maintained by repeated induced seizures. EEG hypersynchrony is one reflection of altered levels of acetylcholine and altered permeability of other electrolytes. It is probable that these changes in intercellular electrolytes provide the biochemical substrate for the persistent behavioral changes following induced convulsions.

(f) Cholinesterases and the Classification of Psychoses:

These studies have application to the problem of autonomic reactivity and the classification of the psychoses. Funkenstein, et al. (1948, 1951, 1952) have demonstrated a relationship between the blood pressure response to injected methacholine (Mecholyl) and the clinical response of spychiatric patients to convulsive therapy. Methacholine is a potent cholinergic agent which induces vasodilation, tachycardia, sweating, and increased peristalsis. It is rapidly hydrolyaed by cholinesterase-I and slowly by cholinesterase-II. The blood pressure of subjects falls after injected mecholyl and returns to the baseline within five to more than 20 minutes. Patients whose blood pressure returns to the baseline within 5 minutes have been classified as Group I, II, or III reactors; those whose blood pressure takes 20 or more minutes to return to baseline, as Group VI and VII reactors. Group I and Group II-III reactors have a 9 and a 35% recovery rate, respectively, while Group VI and Group VII reactors 89% and 97% recovery rates (Funkenstein et al., 1952). Group I to III reactors may be looked upon as patients in whom the injected mecholyl is rapidly hydrolyzed; while Groups VI and VII have a slow hydrolysis rate. It is probable, therefore, that the blood and tissue cholinesterase activity levels of Groups I-III is high; while that of Groups

VI - VII is low. [For a recent review, see Rose, 1962.]

A similar analysis may be made regarding central nervous system levels of cholinesterase-I in the development of EEG hypersynchrony and spinal fluid levels of acetylcholine, providing the basis for a congruent hypothesis regarding central nervous system reactivity to induced convulsions and to peripheral cholinergic agents.

CONCLUSION:

~~This review permits the view that~~ central cholinergic mechanisms are significant in the convulsive therapy process. The published data indicates that induced convulsions are associated with an increase in intercellular acetylcholine to levels greater than can be destroyed by cholinesterase-I activity. Vasodilation and increased cellular permeability are followed by increased amounts of cholinesterase-II and other enzymes and electrolytes in inter-cellular fluids.

These changes are reflected in the increased electrical hypersynchrony which is recorded as EEG slow wave activity in scalp electrodes, and which can be modified by a wide variety of anticholinergic drugs.

In these regards, induced convulsions are more similar to cerebral trauma, than to spontaneous seizures.

The changes in the cerebral biochemical milieu alter cellular activities sufficiently to be associated with altered behavior of subjects. Failure to induce high and persistent concentrations of acetylcholine and failiure to induce concomitant electrolyte changes results in a failure to produce behavioral change.

Differences in the rate of development of cerebral changes reflect differences in the dependence of subjects on cholinergic mechanisms or in their sensitivity to changes in acetylcholine levels. These differences provide the basis for the classification of the mentally ill by Funkenstein and by Fink and Kahn (1961).

The observations provide a rational biochemical basis for the mode of action of induced convulsions in altering the

behavior of psychotic subjects. These views are consistent with the more general neurophysiologic-adaptive theroy expressed earlier (Fink, 1957, 1962).

REFERENCES

Aird, R. B., Strait, L. A., Pace, J. W., Hrenoff, M. K. and Bowditch, S. C. Neurophysiologic effects of electrically induced convulsions. *Arch. Neurol. Psychiat. (Chic.)*, 1956, 75: 371-378.

Bornstein, M.D. Presence and action of acetylcholine in experimental brain trauma. *J. Neurophysiol.*, 1946, 9: 349-366.

Brenner, C. and Merritt, H. H. Effect of certain choline derivatives on electrical activity of the cortex. *Arch. Neurol. Psychiat. (Chic.)*, 1942, 48: 382-395.

Burgen, A. S. V. and MacIntosh, F. C. The physiological significance of acetylcholine. In K. A. C. Elliot, I. H. Page and J. H. Quastel (Editors), *Neurochemistry*. C. C. Thomas, Springfield, Ill., 1955: 374-375.

Chatfield, P. O. and Dempsey, E. W. Effects of prostigmine and acetylcholine on cortical potentials. *Amer. J. Physiol.*, 1942, 135: 633-640.

Cone, W. V., Tower, D. B. and McEachern, D. Acetylcholine and neuronal activity in epilepsy. *J.A.M.A.*, 1948, 73: 59-63.

Crossland, J. and Merrick, A. J. The effect of anaesthesia on the acetylcholine content of the brain. *J. Physiol.*, 1954, 125: 56-66.

Dale, H. H. The action of certain esters and ethers of choline, and their relation to muscarine, *J. Pharmacol. Exp. Ther.*, 1914, 6: 147.

Elliott, R. A. C., Swank, R. L. and Henderson, N. Effects of anesthetics and convulsants on acetylcholine content of brain. *Amer. J. Physiol.*, 1950, 162: 469-474.

Fink, M. A unified theory of the action of psychodynamic therapies. *J. Hillside Hosp.*, 1957, 6: 197-206.

Fink, M. Effect of anticholinergic agent, diethazine, on EEG and behavior: significance for theory of convulsive therapy. *Arch. Neurol. Psychiat. (Chic.)*, 1958, 80: 380-387.

Fink, M. Effect of anticholinergic compounds on post-convulsive electro-encephalogram and behavior of psychiatric patients. *Electroenceph. Clin. Neurophysiol.*, 1960, 12 (2): 359-369.

Fink, M. The Mode of action of convulsive therapy: the neurophysiologic-adaptive view. *J. Neuropsychiat.*, 3: 231-233.

Fink, M. and Kahn, R. L. Quantitative studies of slow wave activity following electroshock. *Electroenceph. Clin. Neurophysiol.*, 1956, 8: 158.

Fink, M., Kahn, R. L., Karp, E., Pollack, M., Green, M. A., Alan, B. and Lefkowits, H. J. Inhalant-induced convulsions. *Arch. Gen. Psychiat.*, 1961, 4: 259-266.

Freedman, A. M., Bales, P. D., Willis, A. and Himwich, H. E. Experimental production of electrical major convulsive patterns. *Amer. J. Physiol.*, 1949, 146: 117-124.

Funkenstein, D. H., Greenblatt, M. and Solomon, H. C. Autonomic nervous system changes following electric shock treatment. *J. Nerv. Ment. Dis.*, 1948, 108: 409-422.

Funkenstein, D. H., Greenblatt, M. and Solomon, H. C. Autonomic changes paralleling psychologic changes in mentally ill patients. *J. Nerv. Ment. Dis.*, 1951, 114: 1-18.

Funkenstein, D. H., Greenblatt, M. and Solomon, H. C. Autonomic nervous system test of prognostic significance in relation to electroshock treatment. *Psychosom. Med.*, 1952, 14: 347-362.

Giarman, N. J. and Pepeu, G. Drug-induced changes in brain acetylcholine. *Brit. J. Pharmacol.*, 1962, 19: 226-234.

Hampson, J., Essig, C. F., McCauley, A. and Himwich, H. E. Effects of di-isopropyl fluorophosphate (DFP) on electroencephalogram and cholinesterase activity. *Electroenceph. Clin. Neurophysiol.*, 1950, 2: 41-48.

Himwich, H. E., Essig, C. F., C. F. Hampson, J. L. Bales, P. D. and Freedman, A. M. Effect of trimethadione (Tridione) and other drugs on convulsions caused by di-isopropyl fluorophosphate (DFP). *Amer. J. Physiol.*, 1950, 106: 816-820.

Jenkner, F. L. and Lechner, H. The effect of Diparcol on the electroencephalogram in the normal subject and in those with cerebral trauma. *Electroenceph. Clin. Neurophysiol.*, 1955, 7: 303-305.

Johnson, L. C., Ulett, G. A., Johnson, M., Smith, K. and Sines, J. O. Electroconvulsive therapy (with and without atropine); effect on electronically analyzed electroencephalogram. *Arch. Gen. Psychiat.*, 1960, 2: 324-336.

Kabat, E. A., Glusman, M. and Knaub, V. Quantitative estimation of the albumin and gamma globulin in normal and pathologic cerebrospinal fluid by immunochemical methods. *Amer. J. Med.*, 1948, 4 (5): 653-662.

Kovach, A. G. B., Fonyo, A. and Halmagyi, M. Acetylcholine content of the brain in traumatic shock. *Acta Physiologica (Hung.)*, 13: 1-4.

Loewi, O. Uber humorale Ubertragbarkeit der Herznervenwirkung. *Arch. f. d. ges. Physiol.*, 1921, 189: 239-242.

Maynert, E. W. and Buck, E. G. Effects of C.N.S. Depressants on Brain Acetylcholine. *Pharmacologist*, 1964, 6: 191.

McLennan, H. and Elliott, K. A. C. Effects on convulsant and narcotic drug on acetylcholine syntheses. *J. Pharm. & Exper. Therap.*, 1951, 103: 35.

Nachmansohn, D. and Rothenberg, M. A. Studies on cholinesterase: on specificity of enzymes in nerve tissue. *J. Biol. Chem.*, 1945, 158: 653-666.

Richter, D. and Crossland, J. Variation in acetylcholine content of the brain with physiological state. *Amer. J. Physiol.*, 1949, 159: 247-255.

Rose, J. T. The Funkenstein Test - A review of the literature. *Acta Psych. Scand.*, 1962, 38: 124-153.

Roth, M. Changes in the EEG under barbiturate anaesthesia produced by electro-convulsive treatment and their significance for the theory of ECT action. *Electroenceph. Clin. Neurophysiol.*, 1951, 3: 261-280.

Roth, M., Kay, D. W. K., Shaw, J. and Green, J. Prognosis and pentothal induced electroencephalographic changes in electro-convulsive treatment. *Electroenceph. Clin. Neurophysiol.*, 1957, 9: 225-237.

Spiegel, E. A. and Spiegel-Adolf, M. Permeability changes in the brain induced by Metrazol and insulin convulsions. *J. Nerv. Ment. Dis.*, 1941, 93: 750-755.

Spiegel, E. A. and Spiegel-Adolf, M. Physiocochemical effects of electrically induced convulsions (cerebrospinal fluid studies). *Trans. Amer. Neurol. Ass.*, 1944, 70: 130-132.

Spiegel, E. A. and Spiegel-Adolf, M. Physiological and physicochemical mechanisms in electroshock treatment. *Confin. Neurol.*, 1953, 13: 38-63.

Spiegel, E. A., Spiegel-Adolf, M. and Henry, G. Physiocochemical changes in the brain accompanying electrically induced convulsive discharges. *Trans. Amer. Neurol. Ass.*, 1942, 68: 174.

Spiegel-Adolf, M., Wilcox, P. H. and Spiegel, E. A. Cerebrospinal fluid changes in electroshock treatment of psychoses. *Amer. J. Psychiat.*, 1948, 104: 697-706.

Stone, W. E. The role of acetylcholine in brain metabolism and function *Amer. J. Phys. Med.*, 1957, 36: 222-255.

Torda, C. Effect of convulsion inducing agents on the acetylcholine content of the brain. *Amer. J. Physiol.*, 1953, 173: 179-183.

Torda, C. Effects of single injection of corticotropin (ACTH) on ammonium ion and acetylcholine content of brain. *Amer. J. Physiol.*, 1953, 173: 176-178.

Tower, D. B. and McEachern, D. Acetylcholine and neuronal activity. I. Cholinesterase patterns and acetylcholine in the cerebrospinal fluids of patients with craniocerebral trauma. *Canad. J. Research*, 1949a, 27 (Sect. E): 105-119.

Tower, D. B. and McEachern, D. The content and characterization of cholinesterases in human cerebrospinal fluids. *Canad. J. Research*, 1949b, 27 (Sect. E): 132-145.

Tower, D. B. and McEachern, D. Acetylcholine and neuronal activity. II. Acetylcholine and cholinesterase activity in the cerebrospinal fluids of patients with epilepsy. *Canad. J. Research*, 1949c, 27 (Sect. E): 120-131.

Ulett, G. A. and Johnson, M. W. Effect of atropine and scopolamine upon electroencephalographic changes induced by electro-convulsive therapy. *Electroenceph. Clin. Neurophysiol.*, 1957, 9: 217-224.

Ward, A. A. Atropine in the treatment of closed head injury. *J. Neurosurg.*, 1950, 7: 398-402.

Wescoe, W. C., Green, R. E., McNamara, B. P., and Krop, S. The influence of atropine and scopolamine on the central effects of DFP. *J. Pharmacol. Exper. Therap.*, 1948, 92: 63-72.

DEPARTMENTAL CORRESPONDENCE

DATE July 29, 1965

SUBJECT Cholinergic Mechanisms in Convulsive Therapy

TO Max Fink, M.D. DEP'T

FROM George A. Ulett, M.D. DEP'T

A most interesting paper, well put together and documented and with which I am in the main in agreement.

There is one minor typographical error on page 12.

GAU:lz

CHOLINERGIC MECHANISMS IN CONVULSIVE THERAPY

Max Fink, M.D.

From the Department of Psychiatry at the Missouri Institute of Psychiatry, University of Missouri School of Medicine, 5400 Arsenal Street, St. Louis, Missouri 63139.

Aided, in part, by USPHS grants MH-927, MH-2715, MH-07249, and MH-11380; and the Psychiatric Research Foundation of Missouri.

CHOLINERGIC MECHANISMS IN CONVULSIVE THERAPY

Despite extensive application and study, the mode of action of the convulsive therapy process remains enigmatic. Much study has been devoted to neurophysiological ( ), psychological ( ), clinical ( ), and social ( ) aspects, elucidating the present neuro-physiological-adaptive view of the process ( ). The early development and persistance of signs of altered cerebral functions were reported to be requisite to changes in behavior (Fink and Kahn, 1956), with electroencephalographic slow wave activity as the most significant index of altered brain function. The demonstration that premedication with atropine inhibited this slow wave activity (Ulett and Johnson, 1957) and the report that anticholinergic compounds reversed these clinical as well as electrographic changes (Fink, 1958) suggests that the biochemical basis for the convulsive therapy process may be in the cholinergic mechanisms of the central nervous system. This review discusses the available data regarding acetylcholine and

and the cholinesterases in the convulsive therapy process.

Acetylcholine has been extensively studied as an active agent in the transmission of nervous impulses since the first descriptions of Dale (1914) and Loewi (1921). It is a constituent of nervous tissue, existing in a bound form which is liberated during the excitation process. It is rapidly hydrolyzed through the specific action of cholinesterase and is rapidly reconstituted by the choline-acetylase system (Richter and Crossland, 1949). In normal cerebrospinal fluid, free acetylcholine is not present despite the rapid breakdown of bound acetylcholine during periods of activity and excitement (Tower and McEachern, 1949a). The cerebrospinal fluid does have measureable cholinesterase activity, however, principally of the "true" or mecholyl hydrolyzing type (Nachmanson and Rothenberg, 1945). In the absence of free acetylcholine and under the conditions described, electroencephalograms fail to show abnormality.

(a) Cholinergic Aspects of Craniocerebral Trauma: Free acetylcholine was found in the cerebrospinal fluid within a few minutes after experimental head trauma in cats and persisted for varying periods up to 48 hours (Bornstein, 1946). The quantity of free acetylcholine varied between 2.7 and 9.0 gamma percent, and the amount was related to the degree of induced trauma.

Concurrent electroencephalograms demonstrated patterned changes. The records were first filled with high voltage fast activity, interpreted as evidence of an intense neuronal discharge, soon to be succeeded by a short period of flattening of all recorded electrical activity. These phases were then followed by prolonged periods of high amplitude sharp waves in the delta frequencies.

The behavioral changes were related both to the degree of trauma and to the amount of measured free acetylcholine. With higher levels of acetylcholine, Bornstein reported greater degrees of EEG abnormality and greater changes in consciousness.

Spontaneous post-traumatic seizures were also related to the amount of free acetylcholine appearing in the spinal fluid.

Bornstein applied acetylcholine to exposed cat cerebral cortex. When the concentration of acetylcholine was 1 gamma percent or less, high amplitude sharp waves of low frequency appeared in the electroencephalogram. When the concentration was increased to 2 gamma percent, the electroencephalogram flattened in a fashion parallel to the post-traumatic records.

Parallel investigations in neurological patients by Tower and McEachern (1949a) demonstrated free acetylcholine in the cerebrospinal fluid only in patients with recent head trauma, recent grand-mal seizures or after electroconvulsive therapy. Free acetylcholine varied from 0.2 to 100 gamma percent. In assaying spinal fluid cholinesterase activity, they noted a sharp rise in the nonspecific cholinesterase fraction (benzoylcholine-splitting) and a drop in the specific cholinesterase fraction (mecholyl-splitting) in patients with head trauma and following convulsive therapy. The cerebrospinal fluid did not

exhibit such inversion, although it contained free acetylcholine, after spontaneous seizures. They concluded that the level of free acetylcholine varied directly with the degree of cerebral damage and that reversal of the cholinesterase fractions was a more sensitive indicator of cerebral damage. Electroencephalograms, taken at varying intervals following trauma, also indicated a relation between the degree of EEG abnormality and the appearance of free acetylcholine in the cerebrospinal fluid.

[Add ¶ Kovach et al, 1957]

Thus the amount of free acetylcholine may increase in the spinal fluid following craniocerebral trauma and the amount of free acetylcholine, the degree and type of electroencephalographic abnormality, and changes in clinical behavior appear as interrelated phenomena.

(b) Anticholinergic drugs and trauma: The electrographic, behavioral and neurologic signs of trauma were blocked by the parenteral administration of 0.5-1.0 mg/kg atropine (Bornstein, 1946), as were similar clinical changes occurring after the intracisternal addition of acetylcholine. Ward (1950) applied these observations to the treatment of closed head injuries. In 20 patients with varying degrees of trauma, he administered atropine subcutaneously in doses of 0.1 mg/kg, noting clinical improvement in some and a reversal of the electroencephalographic effects in others. In a study of another anticholinergic drug, diethazine, Jenkner and Lechner (1955) reported alterations in the post-traumatic electroencephalogram. A single intravenous dose in forty patients with abnormal electroencephalograms resulted in normalizing in twenty-two and marked improvement in six others.

Similar observations have been reported by Denisenko (1965) using methylbenactyzine and trasentin in animal experiments of post-traumatic shock and cerebral edema.

These observations were assessed in the convulsive therapy process by Ulett and Johnson (1957). These workers administered dosages of atropine up to ___ mgm per day during the weeks the patients received electroshock therapy. They demonstrated that the amounts of slow wave activity produced in these patients was significantly less than the control group who had not received the atropine administration.

(In a later study these authors failed to replicate this study, suggesting that dosage factors or population changes may have contributed to different results [Johnson et al, 1960]).

These observations provided the basis for studies with other known anticholinergic compounds (Fink, 1958, 1960). The intravenous injection of experimentally active anticholinergic compounds as diethazine, benactyzine, the piperidylbenzilates JB-318, JB-336 and JB-329 (Ditran), and WIN-2299 induced EEG desynchronization in psychiatric subjects. These EEG changes were associated with behavioral alerting, anxiety, tremors, illusions and hallucinations. In patients who had recently

received electroconvulsive therapy, the administration of these compounds was associated with a reduction in slow wave activity and reversal of euphoria, denial and confusion. Atropine was also examined in low doses, and in these administrations, EEG desynchronization was observed accompanied by tachycardia, nervousness and tension. At higher dosages, hypersynchronous slow waves, followed by lower voltage, poorly organized delta activity with superimposed beta activity was accompanied by progressive confusion and disorientation.

Both in cerebral trauma and induced convulsions, the electrographic changes may be modified by the concurrent administration of anticholinergic drugs, thus suggesting that increased amounts of acetylcholine or increased cholinergic receptivity is associated with the high voltage slow wave activity.

(c) Brain acetylcholine and anticholinergic drugs:

Similar EEG changes and the blocking thereof has been observed following the direct application of acetylcholine to the central nervous system.

The administration of a cholinesterase inhibitor DFP (di-isopropyl fluorophosphate) elicited high amplitude rapid frequency EEG patterns similar to status epilepticus, as well as changes similar to those of post-traumatic states (Freedman et al, 1949, Hampson et al, 1950; and Himwich et al, 1950). These EEG effects were blocked by small doses of parenteral atropine. The great increase in acetylcholine after tetraethyl pyrophosphate (TEPP) was measured and related to the toxic manisfestations and convulsions induced (Giarman and Pepeu, 1952; Stone, 1957.)

Chatfield and Dempsey (1942) prepared exposed animal cortex with prostigmine and evoked electroencephalographic spike activity. The prior administration of atropine blocked this spiking, or if present, the abnormality could be eliminated by atropine.

In contrast to these findings, Brenner and Merritt (1942) applied topical acetylcholine in concentrations of 2-1/2 to 10% to the exposed cortex of cats, and noted no effect on the electroencephalographic changes after intravenous atropine (1 mg/kg.) The concentrations of acetylcholine in these experiments,

however, were higher than the topical applications (1-4 gamma percent) and the intracisternal (0.2-10 gamma percent) injections of Bornstein (1946). Brenner and Merritt also made note of electro-encephalographic effects similar to acetylcholine from mecholyl (acetylbetamethylcholine) and doryl (carbamylcholine)in concentrations much lower than the acetylcholine concentrations. They ascribed the increased effectiveness of these cholinergic drugs to their lack of sensitivity to cerebral cholinesterases.

These data are conflicting and further study is necessary to qualify this issue.

(c) Cerebrospinal Fluid Acetylcholine and Seizures: One view of acetylcholine metabolism indicates that it is found in nervous tissues in an inactive bound form. During periods of activity, acetylcholine is liberated at the cell membrane, where it is rapidly deactivated by cholinesterase. The amount of bound acetylcholine is the resultant of the continuous processes of synthesis, liberation and breakdown. It has been postulated that the level rises during sleep and falls during activity. (Richter and Crossland, 1949; Elliott, Swank and Henderson, 1950; Giarman

Pepeu, 1962). By using liquid air quick freezing methods, Richter and Crossland measured the level of acetylcholine (micro-gamma per mg. brain tissue) during anesthesia and sleep to be 300% higher than post-seizure levels. The difference in tissue levels is transient, however, as the resynthesis rate for acetylcholine in rat brain is high (7 gamma/gm/minute). Elliot et al (1950) confirmed these observations, also noting that after metrazole convulsions free acetylcholine was demonstrable in the spinal fluid in concentrations up to 3 gamma per cent. Giarman and Pepeu (1962) found an increase in acetylcholine caused by a group of central nervous system depressants to be roughly proportional to the degree of depression of the central nervous system and no reduction in motor activity.

Free acetylcholine was reported in the spinal fluid in patients with epilepsy (Cone, Tower and McEachern, 1948; Tower and McEachern, 1949 b). Of 56 epileptic patients, 44 (77%) demonstrated free acetylcholine in quantities of 0.02 to 5.0 gamma per cent with an average of 1.0 gamma per cent.

Acetylcholine levels were related to the frequency of seizures, the extent of electroencephalographic abnormality, and to the time since the last seizure, but bore no relation to medication, type of epilepsy or level of cholinesterase activity.

Tower and McEachern (1949b) viewed the increased acetylcholine a by-product of the seizure, and not causal.

Studying the hypothesis that the accumulation of acetylcholine induced seizures, Torda (1953) measured the level of acetylcholine in brain tissue metrazole convulsions. She noted a rise in the acetylcholine content of brain, before a seizure and a fall during the convulsion. Below certain levels of acetylcholine, convulsions failed to occur. She suggested that the fall in tissue acetylcholine during a convulsion was due to inhibition of acetylcholine synthesis by increased concentrations of metabolites such as ammonium ions.

Giarman and Pepeu also measured changes in central nervous system acetylcholine following various stimulants. Only after

mecholyl and 3, 5-dimethylbutylethyl-barbiturate was there a significant change in the acetylcholine level. They noted a decrease in association with induced convulsions. With other drugs which they ~~quantified~~ classified as stimulants (LSD, iproniazid, iproniazid + hydroxytroyptophan and iproniazid + DOPA) there were no changes in acetylcholine level. They concluded that despite intense excitation produced by these compounds, there were no changes in acetylcholine level unless these were accompanied by convulsions. (The differences in observations between these workers and Cone et al and Tower and McEachern may be related to the differences in methods of biochemical measurements, for the latter measured changes in spinal fluid reflecting the free acetylcholine, while Giarmann and Pepeu measured the total acetylcholine ~~in tissues~~ reflecting bound and free forms of acetylcholine.)

These studies suggest that spontaneous or induced seizures are accompanied by an increase in intercellular free acetylcholine

liberated from its bound form which may be reflected in the spinal fluid. Cerebral activity and seizures enhance acetylcholine destruction, lowering tissue levels of acetylcholine, while sleep and anesthesia augment acetylcholine production increasing tissue levels.

(d) Central Nervous System Cholinesterases: Tower and McEachern (1949) also measured spinal fluid cholinesterase activity. Two types of cholinesterases are normally found in the spinal fluid: cholinesterase-I ("true," "specific," or mecholyl-hydrolyzing), which has a high specificity for acetylcholine; and cholinesterase-II ("pseudo," "non-specific," or benzoylcholine-hydrolyzing). Both compounds hydrolyze acetylcholine but have different rates of hydrolysis for mecholyl and benzoylcholine. This differential rate permits qualitative distinctions. By reporting the cholinesterase activity as a ratio of the activity with a mecholyl substrate and with a benzoylcholine substrate compared to a substrate of acetylcholine, two ratios are found: cholinesterase-I/acetylcholine

and cholinesterase-II/acetylcholine. In such ratios normal cerebrospinal fluid contains esterases in the ratio of 33:17 for cholinesterase-I to cholinesterase-II.

In patients with head trauma, Tower and McEachern reported an inversion of the cholinesterases with an increase in the cholinesterase-II fraction of the spinal fluid and a decrease in cholinesterase-I activity. The extent of the cholinesterase reversal was related to the severity of trauma and to the degree of the electroencephalographic abnormality.

In patients with elevated spinal fluid acetylcholine after spontaneous seizures, however, no change in the ratio of cholinesterases or total cholinesterase activity was found.

The change in cholinesterase activity may be understood in relation to changes in cell membrane permeability. Cholinesterase-I is found in highest concentration in the central nervous system while cholinesterase-II predominates in other tissues, especially blood serum. With an increase in acetylcholine levels in cerebral intercellular fluids,

vasodilation and increased cellular permeability may be predicted, with a degree of transudation of vascular fluids into the intercellular spaces varying with the extent and duration of the vasodilatation (Kabat et al, 1948). Spiegel, Spiegel-Adolf, and their co-workers (1941, 1942, 1944, 1948, 1953) demonstrated such permeability changes and increased conductivity of the tissues associated with the appearance of various ions (as potassium and phosphate) in the spinal fluid following electrically induced convulsions. Such non-electrolytes, as nucleic-acid splitting enzymes, also increased. Changes in cellular permeability may thus provide the basis for the high concentrations of acetylcholine and the increased concentrations of cholinesterase-II in induced seizures or head trauma (Tower and McEachern 1949c).

The persistence of acetylcholine in spinal fluid after head trauma and after seizures despite increased cholinesterase activity may be related to the sensitivity of the acetylcholine-cholinesterase-I system to concentration relationships (Nachmanson

and Rothenberg, 1945; Tower and McEachern, 1949c; Burgen and MacIntosh, 1955). At "physiologic" concentrations, hydrolysis of acetylcholine is rapid (3-4 microseconds) but at higher and lower concentrations, the activity falls off quickly. In contrast, the cholinesterase-II acetylcholine relationship is non-specific and the rate of hydrolysis increases with increased concentration.

One view of these relationships suggests that while the usual concentrations of acetylcholine at cell membranes are destroyed by the specific activity of cholinesterase-I in a few microseconds, an excessive concentration following excitation may exceed the rate of hydrolysis by cholinesterase-I. The seizure threshold may be reached and a seizure induced, with the seizure itself adding to the amount of free acetylcholine. The increased acetylcholine diffuses rapidly, affecting vascular and cellular permeability and increasing the concentrations of various ions and cholinesterase-II in the CSF. The activity of cholinesterase-II, though of low efficiency and depending on

the concentration kinetics, reduces the acetylcholine in the tissues in hours to days to levels for the physiologic action of cholinesterase-I..

Cholinesterases appear in the spinal fluid as a reflection of their increase in intercellular fluids, resulting from changes in cell membrane permeability occasioned by increased acetylcholine. The increased cholinesterases are part of the homeostatic mechanisms controlling the levels of acetylcholine at cell membranes necessary for nervous system activity.

(e) Acetylcholine, EEG Hypersynchrony and Induced Convulsions:

Alteration in the blood-brain permeability barrier by the continuing action of acetylcholine may be the biochemical substrate for the post-electroshock hypersynchrony of the electroencephalogram. Such a possibility is evident in the demonstration of an increase in the concentration of cocaine in brain tissues three days after a series of 12 induced convulsions (Aird et al, 1956). The change in concentration of this large molecule, ordinarily absent

in brain tissue, was associated with the appearance of hyper-synchrony (delta bursts) in the electroencephalogram.

We have confirmed the many previous reports that convulsive therapy induces electrographic hypersynchrony (Fink and Kahn, 1956; Fink et al, 1961). Despite a constant application of treatments there is a great variability in the time of appearance, the duration and the extent of the electrographic slow wave activity as well as the sensitivity to modification by alerting, hyperventilation and barbiturates in psychiatric populations. The early appearance of high degree hyper-synchrony and its persistence throughout a treatment course has been described as prerequisite to improvement following electroshock (Roth, 1951; Roth et al, 1957; Fink and Kahn, 1956). It is possible that the differences in the degree of induced EEG hypersynchrony may be related to differences in the activity of central cholinergic mechanisms. The failure of certain patients to develop hypersynchrony may thus be associated precluding a clinical response to induced convulsions. Tower

and McEachern (1949a), in their study of craniocerebral trauma, included observations of six psychiatric patients undergoing convulsive therapy. Studying the patients after 3-7 treatments they reported free spinal fluid acetylcholine in two patients; and an increase in cholinesterase-II and a decrease in cholinesterase-I with a reversal of the ratio of cholinesterases in five of the six patients. From these observations they concluded that the spinal fluid changes in induced convulsions were more like those of craniocerebral trauma than those of spontaneous epilepsy.

Regarding the one patient in the series who failed to show either free acetylcholine or a cholinesterase ratio reversal in the spinal fluid, they wrote: "It is interesting that this patient was the only one of the six to show no response to treatment." If electrographic hypersynchrony is a reflection of increased free acetylcholine, subjects who maintain hypersynchrony and those in whom it disappears rapidly may be exhibiting differences in the kinetics of the cholinesterase-

acetylcholine hydrolysis systems. Persistent hypersynchrony may result from a decreased rate of hydrolysis of acetylcholine, associated with low concentrations of either cholinesterase-I or cholinesterase-II. (Conversely, in patients with short-lived hypersynchrony, cholinesterase-I and -II in tissue and spinal fluid may be unusually high).

From these observations we would conclude that induced convulsions are associated with an increase in free acetylcholine in intercellular fluids, altering cerebral permeability and enhancing the appearance of cholinesterases. The level of free acetylcholine is maintained by repeated induced seizures. EEG hypersynchrony is one reflection of altered levels of acetylcholine and altered permeability of other electrolytes. It is probably that ~~it is~~ these changes in intercellular electrolytes ~~that~~ provide the biochemical substrate for the persistent behavioral changes following induced convulsions.

(6) Cholinesterases and the Classification of Psychoses:

These studies may also have application to the problem of autonomic reactivity and the classification of the psychoses. Funkenstein and others ( ) have demonstrated a relationship between the blood pressure response to injected mecholyl and the clinical response of psychiatric patients to convulsive therapy. Mecholyl is a potent cholinergic agent which induces vasodilation, tachycardia, sweating, and increased peristalsis. It is rapidly hydrolyzed by cholinesterase-I and slowly by cholinesterase-II. The blood pressure of subjects falls after injected mecholyl and returns to the baseline within five to more than 20 minutes. Patients whose blood pressure returns to the baseline within 5 minutes have been classified as Group I, II, or III reactors; those whose blood pressure takes 20 or more minutes to return to baseline, as Group VI and VII reactors. Group I and Group II-III reactors

have a 9 and a 35% recovery rate, respectively, while Group VI and Group VII reactors 89% and 97% recovery rates (Funkenstein et al, 1952). Group I to III reactors may be looked upon as patients in whom the injected mecholyl is rapidly hydrolyzed; while Groups VI and VII have a slow hydrolysis rate. We may predict, therefore, that the blood cholinesterase activity levels of Groups I-III would be high; while the activity of Groups VI - VII would be low.

A similar analysis may be made regarding central nervous system levels of cholinesterase-I in the development of EEG hypersynchrony and spinal fluid levels of acetylcholine, providing a basis for a congruent hypothesis regarding central nervous system reactivity to induced convulsions and to peripheral cholinergic agents.

CONCLUSION:

Central cholinergic mechanisms appear ~~to be~~ a significant ~~factor~~ in the convulsive therapy process. The published data ~~may be interpreted to~~ indicate that induced convulsions are associated with an increase in intercellular acetylcholine to levels greater than can be destroyed by cholinesterase-I activity. Vasodilation and increased cellular permeability are followed by increased amounts of cholinesterase-II and other enzymes and electrolytes in intercellular fluids.

These changes are reflected in the increased electrical hypersynchrony which is recorded in scalp electrodes, and which can be modified by anticholinergic drugs as atropine, benactyzine, and diethazine.

The changes in the cerebral biochemical milieu alter cellular activities sufficiently to be associated with altered behavior of subjects. Failure to induce high and persistent concentrations of acetylcholine and failure to induce concomitant electrolyte changes results in a failure to produce behavioral change.

Differences in the rates of development of cerebral changes reflect differences in the dependence of subjects on cholinergic mechanisms or in their sensitivity to changes in acetylcholine levels. These differences provide the basis for the classification of the mentally ill by Funkenstein and by Fink and Kahn (1961).

These observations provide a rational biochemical basis for the mode of action of induced nonvulsions in altering the behavior of psychotic subjects. These views are consistent with the more general neurophysiologic-adaptive theory expressed earlier (Fink, 1957).

REFERENCES

Aird, R. B., Strait, L. A., Pace, J. W., Hrenoff, M. K. and Bowditch, S. C. Neurophysiologic effects of electrically induced convulsions. *Arch. Neurol. Psychiat. (Chic.)*, 1956, 75: 371-378.

Bornstein, M.D. Presence and action of acetylcholine in experimental brain trauma. *J. Neurophysiol.*, 1946, 9: 349-366.

Brenner, C. and Merritt, H. H. Effect of certain choline derivatives on electrical activity of the cortex. *Arch. Neurol. Psychiat. (Chic.)*, 1942, 48: 382-395.

Burgen, A. S. V. and MacIntosh, F. C. The physiological significance of acetylcholine. In K. A. C. Elliot, I. H. Page and J. H. Quastel (Editors), *Neurochemistry*. C. C Thomas, Springfield, Ill., 1955: 374-375.

Chatfield, P. O. and Dempsey, E. W. Effects of prostigmine and acetylcholine on cortical potentials. *Amer. J. Physiol.*, 1942, 135: 633-640.

Cone, W. V., Tower, D. B. and McEachern, D. Acetylcholine and neuronal activity in epilepsy. *J.A.M.A.*, 1948, 73: 59-63.

Dale, H. H. The action of certain esters and ethers of choline, and their relation to muscarine. *J. Pharmacol. Exp. Ther.*, 1914, 6:147.

Elliott, R. A. C., Swank, R. L. and Henderson, N. Effects of anesthetics and convulsants on acetylcholine content of brain. *Amer. J. Physiol.*, 1950, 162: 469-474.

Fink, M. A unified theory of the action of psychodynamic therapies. *J. Hillside Hosp.*, 1957, 6: 197-206.

Fink, M. Effect of anticholinergic agent, diethazine, on EEG and behavior: significance for theory of convulsive therapy. *Arch. Neurol. Psychiat. (Chic.)*, 1958, 80: 380-387.

Fink, M. Effect of anticholinergic compounds on post-convulsive electroencephalogram and behavior of psychiatric patients. *Electroenceph. Clin. Neurophysiol.*, 1960, 12 (2): 359-369.

Fink, M. and Kahn, R. L. Quantitative studies of slow wave activity following electroshock. *Electroenceph. Clin. Neurophysiol.*, 1956, 8:158.

Fink, M., Kahn, R. L., Karp, E., Pollack, M., Green, M. A., Alan, B. and Lefkowits, H. J. Inhalant-induced convulsions. *Arch. Gen. Psychiat.*, 1961, 4:259-266.

Freedman, A. M., Bales, P. D., Willis, A. and Himwich, H. E. Experimental production of electrical major convulsive patterns. *Amer. J. Physiol.*, 1949, 146: 117-124.

Funkenstein, D. H., Greenblatt, M. and Solomon, H. C. Autonomic nervous system test of prognostic significance in relation to electroshock treatment. *Psychosom. Med.*, 1952, 14: 347-362.

Giarman, N. J. and Pepeu, G. Drug-induced changes in brain acetylcholine. *Brit. J. Pharmacol.*, 1962, 19: 226-234.

Hampson, J., Essig, C. F., McCauley, A. and Himwich, H. E. Effects of di-isopropyl fluorophosphate (DFP) on electroencephalogram and cholinesterase activity. *Electroenceph. Clin. Neurophysiol.*, 1950, 2: 41-48.

Himwich, H. E., Essig, C. F., Hampson, J. L., Bales, P. D. and Freedman, A. M. Effect of trimethadione (Tridione) and other drugs on convulsions caused by di-isopropyl fluorophosphate (DFP). *Amer. J. Physiol.*, 1950, 106: 816-820.

Jenkner, F. L. and Lechner, H. The effect of Diparcol on the electroencephalogram in the normal subject and in those with cerebral trauma. *Electroenceph. Clin. Neurophysiol.*, 1955, 7: 303-305.

Johnson, L. C., Ulett, G. A., Johnson, M., Smith, K. and Sines, J. O. Electroconvulsive therapy (with and without atropine); effect on electronically analyzed electroencephalogram. *Arch. Gen. Psychiat.*, 1960, 2: 324-336.

Kabat, E. A., Glusman, M. and Knaub, V. Quantitative estimation of the albumin and gamma globulin in normal and pathologic cerebrospinal fluid by immunochemical methods. *Amer. J. Med.*, 1948, 4 (5): 653-662.

Loewi, O. Über humorale Übertragbarkeit der Herznervenwirkung. *Arch. f. d. ges. Physiol.*, 1921, 189: 239-242.

Nachmansohn, D. and Rothenberg, M. A. Studies on cholinesterase: on specificity of enzymes in nerve tissue. *J. Biol. Chem.*, 1945, 158: 653-666.

Richter, D. and Crossland, J. Variation in acetylcholine content of the brain with physiological state. *Amer. J. Physiol.*, 1949, 159: 247-255.

Roth, M. Changes in the EEG under barbiturate anaesthesia produced by electro-convulsive treatment and their significance for the theory of ECT action. *Electroenceph. Clin. Neurophysiol.*, 1951, 3: 261-280.

Roth, M., Kay, D. W. K., Shaw, J. and Green, J. Prognosis and Pantothal induced electroencephalographic changes in electro-convulsive treatment. *Electroenceph. Clin. Neurophysiol.*, 1957, 9: 225-237.

Spiegel, E. A. and Spiegel-Adolf, M. Permeability changes in the brain induced by Metrazol and insulin convulsions. *J. Nerv. Ment. Dis.*, 1941, 93: 750-755.

Spiegel, E. A. and Spiegel-Adolf, M. Physicochemical effects of electrically induced convulsions (cerebrospinal fluid studies). *Trans. Amer. Neurol. Ass.*, 1944, 70: 130-132.

Spiegel, E. A. and Spiegel-Adolf, M. Physiological and physicochemical mechanisms in electroshock treatment. *Confin. Neurol.*, 1953, 13: 38-63.

Spiegel, E. A., Spiegel-Adolf, M. and Henny, G. Physiocochemical changes in the brain accompanying electrically induced convulsive discharges. *Trans. Amer. Neurol. Ass.*, 1942, 68: 174.

Spiegel-Adolf, M., Wilcox, P. H. and Spiegel, E. A. Cerebrospinal fluid changes in electroshock treatment of psychoses. *Amer. J. Psychiat.*, 1948, 104: 697-706.

Stone, W. E. The role of acetylcholine in brain metabolism and function. *Amer. J. Phys. Med.*, 1957, 36: 222-255.

Torda, C. Effect of convulsion inducing agents on the acetylcholine content of the brain. *Amer. J. Physiol.*, 1953, 173: 179-183.

Torda, C. Effects of single injection of corticotropin (ACTH) on ammonium ion and acetylcholine content of brain. *Amer. J. Physiol.*, 1953, 173: 176-178.

Tower, D. B. and McEachern, D. Acetylcholine and neuronal activity. I. Cholinesterase patterns and acetylcholine in the cerebrospinal fluids of patients with craniocerebral trauma. *Canad. J. Research*, 1949a, 27 (Sect. E): 105-119.

Tower, D. B. and McEachern, D. The content and characterisation of cholinesterases in human cerebrospinal fluids. *Canad. J. Research*, 1949b, 27 (Sect. E): 132-145.

Tower, D. B. and McEachern, D. Acetylcholine and neuronal activity. II. Acetylcholine and cholinesterase activity in the ~~cerebrospinal~~ cerebrospinal fluids of patients with epilepsy. *Canad. J. Research*, 1949c, 27 (Sect. E): 120-131.

Ulett, G. A. and Johnson, M. W. Effect of atropine and scopolamine upon electroencephalographic changes induced by electro-convulsive therapy. *Electroenceph. Clin. Neurophysiol.*, 1957, 9: 217-224.

Ward, A. A. Atropine in the treatment of closed head injury, *J. Neurosurg.*, 1950, 7: 398-402.

7
E. Domino

CHOLINERGIC MECHANISMS, CONVULSIVE THERAPY AND BEHAVIOR

Max Fink, M.D.

From the Department of Psychiatry at the Missouri Institute of Psychiatry, University of Missouri School of Medicine, 5400 Arsenal Street, St. Louis, Missouri 63139.

Aided, in part, by USPHS grants MH-00927, MH-2715, MH-07249, and MH-11380; and the Psychiatric Research Foundation of Missouri.

This report is part of the study program "Brain Function and Behavior" undertaken at Hillside Hospital in New York.

CHOLINERGIC MECHANISMS, CONVULSIVE THERAPY, AND BEHAVIOR

Studies of induced convulsions have shown that the early development and persistence of signs of altered cerebral function are prerequisite to changes in behavior (Fink and Kahn, 1956). Electroencephalographic slow wave activity was found to be a significant index of altered brain function, and the demonstration that premedication with atropine inhibited this slow wave activity (Ulett and Johnson, 1957) suggested a relation to cholinergic mechanisms. In the following review the role of acetylcholine and the cholinesterases in convulsive therapy is discussed.

Acetylcholine has been extensively studied as an active agent in the transmission of nervous impulses since the first descriptions of Dale (1914) and Loewi (1921). It is a constituent of nervous tissue, existing in a bound form which is liberated during the excitation process. It is rapidly hydrolyzed through the specific action of cholinesterase and is rapidly reconstituted by the choline acetylase system (Richter &

Crossland, 1949). In normal cerebrospinal fluid free acetylcholine is not present despite the rapid breakdown of bound acetylcholine during periods of activity and excitement (Tower and McEachern, 1949a). The cerebrospinal fluid does have measurable cholinesterase activity, however, principally of the "true" or mecholyl hydrolyzing type (Nachmanson and Rothenberg, 1945). In the absence of free acetylcholine and under the conditions described, electroencephalograms fail to show abnormality.

(a) Effect of Craniocerebral Trauma: Free acetylcholine was found in the cerebrospinal fluid within a few minutes after experimental head trauma in cats and persisted for varying periods up to 48 hours (Bornstein, 1946). The quantity of free acetylcholine varied between 2.7 and 9.0 gamma percent, and the amount was related to the degree of induced trauma.

Concurrent electroencephalograms demonstrated patterned changes. The records were first filled with high voltage fast activity, interpreted as evidence of an intense neuronal discharge, only to be followed by a short period of flattening of all recorded

electrical activity. These phases were then followed by prolonged periods of high amplitude sharp waves in the delta frequencies.

The behavioral changes were related both to the degree of trauma and to the amount of measured free acetylcholine. With higher levels of acetylcholine, Bornstein reported greater degrees of EEG abnormality and greater changes in consciousness. In addition, spontaneous post-traumatic seizures were also related to the amount of free acetylcholine appearing in the spinal fluid.

Bornstein also applied acetylcholine to exposed cat cerebral cortex. When the concentration of acetylcholine was 1 gamma ? percent or less, he reported high amplitude sharp waves of low frequency in the electroencephalogram. When the concentration was increased to 2 gamma percent, the electroencephalogram flattened in a fashion parallel to the post-traumatic records.

gamma ? gamma/100cc

Parallel studies were carried out in neurological patients by Tower and McEachern (1949a). Free acetylcholine was found in

the cerebrospinal fluid only in patients withrecent head traume recent grand-mal seizures or after convulsive therapy. Free acetylcholine varied from 0.2 to 100 gamma percent. Tower and McEachern also assayed spinal fluid cholinesterase activity, noting a sharp rise in the nonspecific cholinesterase fraction (benzoylcholine-splitting) and a drop in the specific cholinesterase fraction (mecholyl-splitting) in patients with head trauma and following convulsive therapy. Following spontaneous seizures the cerebrospinal fluid did not exhibit such inversion, although it contained free acetylcholine. They concluded that the level of free acetylcholine varied directly with the degree of cerebral damage and that reversal of the cholinesterase fraction was a more sensitive indicator of cerebral damage. Electroencephalograms, taken at varying intervals following trauma, also indicated a relation between the degree of EEG abnormality and the appearance of free acetylcholine in the cerebrospinal fluid.

Thus, the amount of free acetylcholine may increase in the spinal fluid following craniocerebral trauma and the amount of

free acetylcholine, the degree and type of electroencephalographic abnormality, and changes in clinical behavior may be interrelated phenomena.

(b) Atropine, anticholinergic drugs and trauma: The EEG behavioral and neurologic signs of trauma were blocked by the parenteral administration of 0.5-1.0 mg/kg atropine (Bornstein,), as were similar clinical changes occurring after the intracisternal addition of acetylcholine. Ward (1950) applied these observations to the treatment of closed head injuries. In 20 patients with varying degress of trauma, he administered atropine subcutaneously in doses of 0.1 mg/kg, noting clinical improvement in some and a reversal of the electroencephalographic effects in others. In a study of ~~diethazine~~ diethazine, another anticholinergic drug, Jenkner and Lechner (1955) reported alterations in the post-traumatic electroencephalogram. A single intravenous dose in forty patients with abnormal electroancephalograms resulted in normalizing in twenty-

two and marked improvement in six others.

In subjects following convulsive therapy the effect of atropine in blocking the appearance of slow wave activity was reported (Ulett and Johnson, 1956). (In a later study these authors failed to replicate this study, suggesting that dosage factors or population changes may have contributed to different rasults [Johnson et al, 1960]).

Should be pursued further

These observations provided the basis for studies with other known anticholinergic compounds (Fink, 1958, 1960). The intravenous injection of experimentally potent anticholinergic compounds as diethazine, benactyzine, the piperidylbenzilates JB-318, JB-336 and JB-329 (Ditran), WIN-2299 and procyclidine induced EEG desynchronization. The EEG changes were associated with behavioral alerting, anxiety, tremors, illusions and hallucinations in psychiatric subjects. In patients with recent induced convulsions these compounds resulted in a reduction in slow wave activity associated with a reversal of euphoria, denial and confusion.

Atropine was also examined in low doses, EEG desynchronization was observed, accompanied by tachycardia, nervousness and tension. At higher dosages, hypersynchronous slow waves, followed by lower voltage, poorly organized delta activity with superimposed beta activity was associated with progressive confusion and disorientation.

The administration of a cholinesterase inhibitor, DFP (di-isopropyl fluorophosphate) elicited high amplitude rapid frequency EEG patterns similar to status epilepticus, as well as changes similar to those of post-traumatic states (Freedman et al, 1949; Hampson et al, 1950; and Himwich et al, 1950). These EEG effects were blocked by small doses of parenteral atropine.

Chatfield and Dempsey (1942) prepared exposed animal cortex with prostigmine and evoked electroencephalographic spike activity. The prior administration of atropine blocked this spiking, or if present, the abnormality could be eliminated by atropine.

In contrast to these findings, Brenner and Merritt (1942), applied topical acetylcholine in concentrations of 2-1/2 to 10% to the exposed cortex of cats, and noted no effect on the

electroencephalographic changes after intravenous atropine (1 mg/kg). The concentrations of acetylcholine in these experiments, however, were higher than the topical applications (1-4 gamma percent) and the intracisternal (0.2-10 gamma percent) injections of Bornstein (1946). Brenner and Merritt, also made note of electro-encephalographic effects similar to acetylcholine from mecholyl (acetylbetamethylcholine) and doryl (carbamylcholine) in concentrations much lower than the acetylcholine concentrations. They ascribed the increased effectiveness of these cholinergic drugs to their lack of sensitivity to cerebral cholinesterases.

These experimental and clinical studies suggest that electro-encephalographic activity induced by acetylcholine either as a result of trauma, topical application, or interference with cholinesterase activity can be blocked or eliminated by anti-cholinergic agents as atropine, benactyzine, etc.

(c) Cerebrospinal Fluid Acetylcholine and Seizures One view of acetylcholine metabolism indicates that it is found in nervous tissues in an inactive bound form. During periods of activity, acetylcholine is liberated at the cell membrane, where it is

rapidly deactivated by cholinesterase. The amount of bound acetylcholine is the resultant of continuous processes of synthesis, liberation and breakdown. It has been postulated that the level rises during sleep and falls during activity. This hypothesis was supported in animal experiments by Richter and Crossland (1949) and Elliott, Swank and Henderson (1950). By using liquid air quick-freezing methods, Richter and Crossland observed the level of acetylcholine during anesthesia and sleep (measured as micro-gamma per mg. brain tissue) to be 300% higher than post-seizure levels. The difference in tissue levels is transient, however, as the resynthesis rate for acetylcholine in rat brain is high (7 gamma/gm/minute). Elliott et al, (1950) confirmed these observations. After metrazole convulsions they also noted that free acetylcholine was always demonstrable in the spinal fluid in concentrations up to 3 gamma per cent.

In spinal fluid studies in man, Cone, Tower and McEachern (1948) and Tower and McEachern (1949b) reported significant

quantities of free acetylcholine in patients with epilepsy.

Of 56 epileptic patients, 44 (77%) demonstrated free acetylcholine in quantities of 0.02 to 5.0 gamma per cent with an average of 1.0 gamma per cent. The acetylcholine level was related to the frequency of seizures, the extent of electroencephalographic abnormality, and to the time since the last seizure. It bore no relation to medication, type of epilepsy or level of cholinesterase activity.

Whether the acetylcholine appearing in the spinal fluid is a by-product of the convulsion or whether the increase in acetylcholine is a cause of the seizure is problematical. Tower and McEachern (1949b) suggested that increased acetylcholine liberation is not due to the seizure itself but to the process causing the seizure. In a study of the hypothesis that the accumulation of acetylcholine is causal for seizures, Torda (1953) induced convulsions in animals by metrazole and determined the level of acetylcholine in brain tissue before and during convulsions. She noted that convulsions are preceded by a rise in the acetylcholine content of

tissue, that the content falls during the convulsion, and that below certain levels of acetylcholine, convulsions failed to occur. She suggested that the fall in tissue acetylcholine during a convulsion was due to inhibition of acetylcholine synthesis by ~~the~~ increased concentration of metabolites, such as ammonium ions.

Seizures are accompanied by an increase in intercellular free acetylcholine liberated from its bound form, and ~~that~~ this increase may be reflected in the spinal fluid; that cerebral activity and seizures enhance acetylcholine destruction, lowering tissue levels of acetylcholine; while sleep and anesthesia augment acetylcholine production increasing tissue levels.

(d) Central Nervous System Cholinesterases: Concomitant with their observations of changes in acetylcholine, Tower and McEachern (1949) measured spinal fluid cholinesterase activity. Two types of cholinesterases are normally found in the spinal

fluid: cholinesterase-I ("true," "specific," or mecholyl-hydrolyzing), which has a high specificity for acetylcholine; and cholinesterase-II ("pseudo," "non-specific," or benzoylcholine-hydrolyzing). Both compounds hydrolyze acetylcholine but have different rates of hydrolysis for mecholyl and benzoylcholine. This differential rate permits qualitative distinctions. By reporting the cholinesterase activity as a ratio of the activity with a mecholyl substrate and with a benzoylcholine substrate compared to substrate of acetylcholine, two ratios are found: cholinesterase-I/acetylcholine and cholinesterase-II/acetylcholine (with Ach/Ach=100). In such ratios normal cerebrospinal fluid contains esterases in the ratio of 33:17 for cholinesterase-I to cholinesterase-II.

In patients with head traums Tower and McEachern reported an inversion of the cholinesterases, with an increase in the cholinesterase-II fraction of the spinal fluid and a decrease in cholinesterase-I activity. The extent of the cholinesterase reversal was related

to the severity of trauma and to the degree of the electro-encephalographic abnormality.

In patients with elevated spinal fluid acetylcholine after spontaneous seizures, however, no change in the ratio of cholinesterases or total cholinesterase activity was found.

The increase in cholinesterase activity may be understood in relation to changes in cell membrane permeability. Cholinesterase-I is found in highest concentration in the central nervous system while cholinesterase-II predominates in other tissues, especially blood serum. With an increase in acetylcholine levels in intercellular cerebral fluids following stimulation, convulsions or trauma, vasodilation and increased cellular permeability may be predicted, with a degree of transudation of vascular fluids into the inter-cellular spaces varying with the extent and duration of the vasodilation (Kabat et al, 1948). Spiegal and Spiegel-Adolf demonstrated such permeability changes in numerous reports (1941, 1942, 1944, 1948, 1953). They observed increased conductivity of the tissues

associated with the appearance of various ions (as potassium and phosphate) in the spinal fluid following electrically induced convulsions. There was also a significant increase in such non-electrolytes as nucleic-acid splitting enzymes. Changes in cellular permeability may thus provide the basis for the high concentrations of acetylcholine and the increased concentrations of cholinesterase-II in induced seizures or head trauma (Tower and McEachern 1949c).

The increase in cholinesterase activity should be associated with the rapid destruction of free acetylcholine. The persistence of acetylcholine in spinal fluid after head trauma and after seizures may be related to the sensitivity of the acetylcholine-cholinesterase-I system to concentration relationships (Nachmanson and Rothenberg, 1945; Tower and McEachern, 1949; Burgen and MacIntosh, 1955). At "physiologic" concentrations, hydrolysis of acetylcholine is rapid (3-4 microseconds) but at higher and lower concentrations, the activity falls off quickly. In contrast, the cholinesterase-II-

acetylcholine relationship is non-specific and the rate of hydrolysis increases with concentration.

One view of these relationships suggests that while the usual concentrations of acetylcholine at cell membranes are destroyed by the specific activity of cholinesterase-I in few microseconds, an excessive concentration following excitation may exceed the rate of hydrolysis by cholinesterase-I. The seizure threshold may be reached and a seizure induced, with the seizure itself adding to the amount of free acetylcholine, The increased acetylcholine diffuses rapidly, affecting vascular and cellular permeability and increasing the concentrations of various ions and cholinesterase-II in CSF. The activity of cholinesterase-II, though of low efficiency and depending on the concentraiton kinetics, reduces the acetylcholine in the tissues in hours to days to levels for the physiologic action of cholinesterase-I.

one should calculate this to see if more than a few seconds are involved

excess Ach may block rather than

Cholinesterases appear in the spinal fluid as a reflection of their increase in intercellular fluids, resulting from changes in cell membrane permeability occasioned by increased acetylcholine. The increased cholinesterases are part of the cybernetic mechanisms controlling the levels of acetylcholine at cell membranes necessary for nervous system functioning.

(e) Acetylcholine, EEG Hypersynchrony and Induced Convulsions:

Alteration in the blood-brain permeability barrier by the continuing action of acetylcholine may be the biochemical substrate for the post-electroshock hypersynchrony of the electroencephalogram. Such a possibility is evident in the report by Aird et al. (1956) demonstrating an increase in the concentration of cocaine in brain tissues three days after a series of 12 induced convulsions. His data shows the change in concentration of this large molecule, ordinarily absent in brain tissue to be associated with the appearance of hypersynchrony (delta bursts) in the electro-encephalogram.

In studies of induced convulsions, we have confirmed the many previous reports that convulsive therapy induces electrographic hypersynchrony (Fink and Kahn, 1956). Despite a constant application of treatments, the time of appearance, the duration and the extent of the electrographic slow wave activity as well as its sensitivity to modification by alerting, hyperventilation and barbiturates vary greatly in psychiatric populations. The early appearance of high degree hypersynchrony and its persistence throughout the treatment course has been described as prerequisite to improvement following electroshock (Fink and Kahn, 1956). The failure of certain patients to develop hypersynchrony may be related to differences in the activity of central acetylcholine and cholinesterases, with the absence of free acetylcholine being related to minimal changes in cerebral function and thus precluding a clinical response to induced convulsions. Tower and McEachern (1949a), in their study of craniocerebral trauma, included observations of six psychiatric patients undergoing convulsive therapy. Studying the patients after 3-7 treatments

they reported free acetylcholine activity in the spinal fluid in two patients; and an increase in cholinesterase-II and a decreass in cholinesterase-I with a reversal of the ratio of cholinesterases in five of the six patients. From these observations they concluded that the spinal fluid changes in induced convulsions were more like those of craniocerebral trauma than those found in epilepsy.

Regarding the one patient in the series who failed to show either free acetylcholine or a cholinesterase ratio reversal in the spinal fluid, they wrote: "It is interesting that this patient was the only one of the six to show no response to treatment."

It is probable that induced convulsions increase free acetylcholine in intercellular fluids, altering cerebral permeability and enhancing the appearance of cholinesterases. Free acetylcholine is maintained by repeated seizures. EEG hypersynchrony is one reflection of altered levels of acetylcholine and altered permeability of other electrolytes. It is these changes in intercellular electrolytes that provide the biochemical substrate for the persistent behavioral changes following induced convulsions.

Maybe is enhancing synthesis of Ach primarily, rather than uptake + liberating free Ach which may be in equilibrium with bound Ach

(f) Cholinesterases and the Classification of Psychoses:

These studies may also have application to the problem of autonomic reactivity and the classification of the psychoses. Funkenstein and others ( ) have demonstrated a relationship between the blood pressure response to injected ~~mecholyl~~ (methacholine) and the clinical response of psychiatric patients to convulsive therapy. Mecholyl is a potent cholinergic agent which induces vasodilation, tachycardia, sweating, and increased peristalsis. It is rapidly hydrolyzed by cholinesterase-I and slowly by cholinesterase-II. The blood pressure of subjects falls after injected mecholyl and returns to the baseline ~~in a variable length of time~~ within, i.e., five to more than 20 minutes. Patients whose blood pressure returns to the baseline within 5 minutes are classified as Groups I, II, or III reactions; those whose blood pressure takes 20 or more minutes to return to baseline, as Group VI and VII reactions. The Group I reactors have a 9% improvement rate with convulsive therapy, and the Group II-III reactors a 35% recovery rate. In contrast, the Group VI

reactors have a 89% and the Group VII a 97% recovery rate (Funkenstein et al, 1952).

Patients in Funkenstein Groups I to III may be looked upon as patients in whom the injected mecholyl is rapidly hydrolyzed; while the Groups VI and VII patients have a slow hydrolysis rate. We may predict, therefore, that the blood cholinesterase activity levels of Groups I-III would be high; while the activity of Groups VI - VII would be low.

Helkone believes (on the basis of cat data) that another explanation of hypothalamic reactivity is on the basis of

A similar analysis may be made regarding central nervous system levels of cholinesterase-I in the development of EEG hypersynchrony and spinal fluid levels of acetylcholine.

In studies of EEG changes following induced convulsions subjects were identified in whom a few seizures resulted in a great amount of slow wave activity; while other subjects exhibited few changes to many and to frequent seizures (Fink and Kahn, 1956). If electrographic hypersynchrony is a reflection of increased free acetylcholine, subjects who maintain hypersynchrony and those in whom it disappears rapidly may be exhibiting differences in the kinetics of the cholinesterase-acetylcholine hydrolysis systems. Persistent hypersynchrony may result from a decreased rate of hydrolysis of acetylcholine, associated with low concentrations of either cholinesterase-I or cholinesterase-II. (Conversely, in patients with short-lived hypersynchrony, cholinesterase-I and -II in tissue and spinal fluid may be unusually high). Thus these speculations provide

a basis for a congruent hypothesis regarding central nervous system reactivity to electroshock, and to peripheral cholinergic agents ~~indices~~.

CONCLUSION:

Central cholinergic mechanisms appear to be a significant factor in the convulsive therapy process. A review of the published data ~~may be interpreted to~~ indicates that induced convulsions are associated with an increase in intercellular acetylcholine to levels greater than can be destroyed by ~~cholinesterase I~~ acetylcholinesterase activity. Vasodilation and increased cellular permeability ~~is~~ are followed by the appearance of butyrylcholinesterase, increased amounts of ~~cholinesterase II~~, other enzymes and electrolytes in intercellular fluids.

biochemical
These changes are reflected in ~~the~~ increased electrical
as EEG slow wave activity
hypersynchrony ~~which is~~ recorded in scalp electrodes, and which

~~can be modified~~ by anticholinergic drugs, as atropine, benactyzine,

diethazine, procyclidine, ~~etc.~~ and piperidyl benzilates.

~~biochemical~~ in extracellular fluids
These changes provide the changes in biochemical milieu which
firing and recovery rates to a degree
alters cerebral cellular ~~activities~~ sufficient to alter the

Thus,
behavior of subjects. Failure to induce high and persistent

or
concentrations of acetylcholine, ~~and~~ failure to induce concomitant
does not alter cerebral cellular activities, and
electrolytes changes, results in a failure to produce behavioral

change.

Differences in the rates of development of cerebral changes to
the same number and frequency of induced convulsions may
reflect differences in the dependence of subjects on cholinergic

mechanisms, or in their sensitivity to changes in acetylcholine

levels. These differences provide the basis for the classifica-
based on neurophysiologic responsivity
tion of the mentally ill by Funkenstein and by Fink and Kahn ~~(1956)~~.
The data on cholinergic mechanisms ~~viewed~~
~~These observations~~ provide a rational biochemical ~~basis for~~ theory for
the mode of action of induced convulsions in altering the behavior
of psychotic subjects. These views are consistent with the more
general neurophysiologic-adaptive theory expressed earlier. ~~(Fink, 1957).~~

V: File copy

CHOLINERGIC MECHANISMS, CONVULSIVE THERAPY AND BEHAVIOR

Max Fink, M.D.

From the Department of Psychiatry at the Missouri Institute of Psychiatry, University of Missouri School of Medicine, 5400 Arsenal Street, St. Louis, Missouri 63139.

Aided, in part, by USPHS grants MH-00927, MH-2715, MH-07249, and MH-11380; and the Psychiatric Research Foundation of Missouri.

This report is part of the study program "Brain Function and Behavior" undertaken at Hillside Hospital in New York.

CHOLINERGIC MECHANISMS, CONVULSIVE THERAPY, AND BEHAVIOR

Studies of induced convulsions have shown that the early development and persistence of signs of altered cerebral function are prerequisite to changes in behavior (Fink and Kahn, 1956). Electroencephalographic slow wave activity was found to be a significant index of altered brain function, and the demonstration that premedication with atropine inhibited this slow wave activity (Ulett and Johnson, 1956) suggested a relation to cholinergic mechanisms. In the following review the role of acetylcholine and the cholinesterases in convulsive therapy is discussed.

Acetylcholine has been extensively studied as an active agent in the transmission of nervous impulses since the first descriptions of Dale (1914) and Loewi (1921). It is a constituent of nervous tissue, existing in a bound form which is liberated during the excitation process. It is rapidly hydrolyzed through the specific action of cholinesterase and is rapidly reconstituted by the choline-acetylase system (Richter &

Crossland, 1949). In normal cerebrospinal fluid free acetylcholine is not present despite the rapid breakdown of bound acetylcholine during periods of activity and excitement (Tower and McEachern, 1949a). The cerebrospinal fluid does have measurable cholinesterase activity, however, principally of the "true" or mecholyl hydrolyzing type (Nachmanson and Rothenberg, 1945). In the absence of free acetylcholine and under the conditions described, electroencephalograms fail to show abnormality.

(a) Effect of Craniocerebral Trauma: Free acetylcholine was found in the cerebrospinal fluid within a few minutes after experimental head trauma in cats and persisted for varying periods up to 48 hours (Bornstein, 1946). The quantity of free acetylcholine varied between 2.7 and 9.0 gamma percent, and the amount was related to the degree of induced trauma.

Concurrent electroencephalograms demonstrated patterned changes. The records were first filled with high voltage fast activity, interpreted as evidence of an intense neuronal discharge, only to be followed by a short period of flattening of all recorded

electrical activity. These phases were then followed by prolonged periods of high amplitude sharp waves in the delta frequencies.

The behavioral changes were related both to the degree of trauma and to the amount of measured free acetylcholine. With higher levels of acetylcholine, Bornstein reported greater degrees of EEG abnormality and greater changes in consciousness. In addition, spontaneous post-traumatic seizures were also related to the amount of free acetylcholine appearing in the spinal fluid.

Bornstein also applied acetylcholine to exposed cat cerebral cortex. When the concentration of acetylcholine was 1 gamma percent or less, he reported high amplitude sharp waves of low frequency in the electroencephalogram. When the concentration was increased to 2 gamma percent, the electroencephalogram flattened in a fashion parallel to the post-traumatic records.

Parallel studies were carried out in neurological patients by Tower and McEachern (1949a). Free acetylcholine was found in

the cerebrospinal fluid only in patients withrecent head traume recent grand-mal seizures or after convulsive therapy. Free acetylcholine varied from 0.2 to 100 gamma percent. Tower and McEachern also assayed spinal fluid cholinesterase activity, noting a sharp rise in the nonspecific cholinesterase fraction (benzoylcholine-splitting) and a drop in the specific cholinesterase fraction (mecholyl-splitting) in patients with head trauma and following convulsive therapy. Following spontaneous seizures the cerebrospinal fluid did not exhibit such inversion, although it contained free acetylcholine. They concluded that the level of free acetylcholine varied directly with the degree of cerebral damage and that reversal of the cholinesterase fraction was a more sensitive indicator of cerebral damage. Electroencephalograms, taken at varying intervals following trauma, also indicated a relation between the degree of EEG abnormality and the appearance of free acetylcholine in the cerebrospinal fluid.

Thus, the amount of free acetylcholine may increase in the spinal fluid following craniocerebral trauma and the amount of

free acetylcholine, the degree and type of electroencephalographic abnormality, and changes in clinical behavior may be interrelated phenomena.

(b) Atropine, anticholinergic drugs and trauma: The EEG behavioral and neurologic signs of trauma were blocked by the parenteral administration of 0.5-1.0 mg/kg atropine (Bornstein,), as were similar clinical changes occurring after the intracisternal addition of acetylcholine. Ward (1950) applied these observations to the treatment of closed head injuries. In 20 patients with varying degress of trauma, he administered atropine subcutaneously in doses of 0.1 mg/kg, noting clinical improvement in some and a reversal of the electroencephalographic effects in others. In a study of ~~diethazine~~ diethazine, another anticholinergic drug, Jenkner and Lechner (1955) reported alterations in the post-traumatic electroencephalogram. A single intravenous dose in forty patients with abnormal electroencephalograms resulted in normalizing in twenty-

two and marked improvement in six others.

In subjects following convulsive therapy the effect of atropine in blocking the appearance of slow wave activity was reported (Ulett and Johnson, 1956). (In a later study these authors failed to replicate this study, suggesting that dosage factors or population changes may have contributed to different results [Johnson et al, 1960]).

These observations provided the basis for studies with other known anticholinergic compounds (Fink, 1958, 1960). The intravenous injection of experimentally potent anticholinergic compounds as diethazine, benactyzine, the piperidylbenzilates JB-318, JB-336 and JB-329 (Ditran), WIN-2299 and procyclidine induced EEG desynchronization. The EEG changes were associated with behavioral alerting, anxiety, tremors, illusions and hallucinations in psychiatric subjects. In patients with recent induced convulsions these compounds resulted in a reduction in slow wave activity associated with a reversal of euphoria, denial and confusion.

Atropine was also examined in low doses, EEG desynchronization was observed, accompanied by tachycardia, nervousness and tension. At higher dosages, hypersynchronous slow waves, followed by lower voltage, poorly organized delta activity with superimposed beta activity was associated with progressive confusion and disorientation.

The administration of a cholinesterase inhibitor, DFP (di-isopropyl fluorophosphate) elicited high amplitude rapid frequency EEG patterns similar to status epilepticus, as well as changes similar to those of post-traumatic states (Freedman et al, 1949; Hampson et al, 1950; and Himwich et al, 1950). These EEG effects were blocked by small doses of parenteral atropine.

Chatfield and Dempsey (1942) prepared exposed animal cortex with prostigmine and evoked electroencephalographic spike activity. The prior administration of atropine bbcked this spiking, or if present, the abnormality could be eliminated by atropine.

In contrast to these findings, Brenner and Merritt (1942), applied topical acetylcholine in concentrations of 2-1/2 to 10% to the exposed cortex of cats, and noted no effect on the

electroencephalographic changes after intravenous atropine (1 mg/kg). The concentrations of acetylcholine in these experiments, however, were higher than the topical applications (1-4 gamma percent) and the intracisternal (0.2-10 gamma percent) injections of Bornstein (1946). Brenner and Merritt, also made note of electro-encephalographic effects similar to acetylcholine from mecholyl (acetylbetamethylcholine) and doryl (carbamylcholine) in concentrations much lower than the acetylcholine concentrations. They ascribed the increased effectiveness of these cholinergic drugs to their lack of sensitivity to cerebral cholinesterases.

These experimental and clinical studies suggest that electro-encephalographic activity induced by acetylcholine either as a result of trauma, topical application, or interference with cholinesterase activity can be blocked or eliminated by anti-cholinergic agents as atropine, benactyzine, etc.

(c) Cerebrospinal Fluid Acetylcholine and Seizures One view of acetylcholine metabolism indicates that it is found in nervous tissues in an inactive bound form. During periods of activity, acetylcholine is liberated at the cell membrane, where it is

rapidly deactivated by cholinesterase. The amount of bound acetylcholine is the resultant of continuous processes of synthesis, liberation and breakdown. It has been postulated that the level rises during sleep and falls during activity. This hypothesis was supported in animal experiments by Richter and Crossland (1949) and Elliott, Swank and Henderson (1950). By using liquid air quick-freezing methods, Richter and Crossland observed the level of acetylcholine during anesthesia and sleep (measured as micro-gamma per mg. brain tissue) to be 300% higher than post-seizure levels. The difference in tissue levels is transient, however, as the resynthesis rate for acetylcholine in rat brain is high (7 gamma/gm/minute). Elliott et al, (1950) confirmed these observations. After metrazole convulsions they also noted that free acetylcholine was always demonstrable in the spinal fluid in concentrations up to 3 gamma per cent.

In spinal fluid studies in man, Cone, Tower and McEachern (1948) and Tower and McEachern (1949b) reported significant

quantities of free acetylcholine in patients with epilepsy. Of 56 epileptic patients, 44 (77%) demonstrated free acetylcholine in quantities of 0.02 to 5.0 gamma per cent with an average of 1.0 gamma per cent. The acetylcholine level was related to the frequency of seizures, the extent of electroencephalographic abnormality, and to the time since the last seizure. It bore no relation to medication, type of epilepsy or level of cholinesterase activity.

Whether the acetylcholine appearing in the spinal fluid is a by-product of the convulsion or whether the increase in acetylcholine is a cause of the seizure is problematical. Tower and McEachern (1949b) suggested that increased acetylcholine liberation is not due to the seizure itself but to the process causing the seizure. In a study of the hypothesis that the accumulation of acetylcholine is causal for seizures, Torda (1953) induced convulsions in animals by metrazole and determined the level of acetylcholine in brain tissue before and during convulsions. She noted that convulsions are preceded by a rise in the acetylcholine content of

tissue, that the content falls during the convulsion, and that below certain levels of acetylcholine, convulsions failed to occur. She suggested that the fall in tissue acetylcholine during a convulsion was due to inhibition of acetylcholine synthesis by ~~the~~ increased concentration of metabolites, such as ammonium ions.

Seizures are accompanied by an increase in intercellular free acetylcholine liberated from its bound form, and ~~that~~ this increase may be reflected in the spinal fluid; that cerebral activity and seizures enhance acetylcholine destruction, lowering tissue levels of acetylcholine; while sleep and anesthesia augment acetylcholine production increasing tissue levels.

(d) Central Nervous System Cholinesterases: Concomitant with their observations of changes in acetylcholine, Tower and McEachern (1949) measured spinal fluid cholinesterase activity. Two types of cholinesterases are normally found in the spinal

fluid: cholinesterase-I ("true," "specific," or mecholyl-hydrolyzing), which has a high specificity for acetylcholine; and cholinesterase-II ("pseudo," "non-specific," or benzoylcholine-hydrolyzing). Both compounds hydrolyze acetylcholine but have different rates of hydrolysis for mecholyl and benzoylcholine. This differential rate permits qualitative distinctions. By reporting the cholinesterase activity as a ratio of the activity with a mecholyl substrate and with a benzoylcholine substrate compared to substrate of acetylcholine, two ratios are found: cholinesterase-I/acetylcholine and cholinesterase-II/acetylcholine (with Ach/Ach=100). In such ratios normal cerebrospinal fluid contains esterases in the ratio of 33:17 for cholinesterase-I to cholinesterase-II.

In patients with head traums Tower and McEachern reported an inversion of the cholinesterases, with an increase in the cholinesterase-II fraction of the spinal fluid and a decrease in cholinesterase-I activity. The extent of the cholinesterase reversal was related

to the severity of trauma and to the degree of the electro-encephalographic abnormality.

In patients with elevated spinal fluid acetylcholine after spontaneous seizures, however, no change in the ratio of cholinesterases or total cholinesterase activity was found.

The increase in cholinesterase activity may be understood in relation to changes in cell membrane permeability. Cholinesterase-I is found in highest concentration in the central nervous system while cholinesterase-II predominates in other tissues, especially blood serum. With an increase in acetylcholine levels in intercellular cerebral fluids following stimulation, convulsions or trauma, vasodilation and increased cellular permeability may be predicted, with a degree of transudation of vascular fluids into the inter-cellular spaces varying with the extent and duration of the vasodilation (Kabat et al, 1948). Spiegal and Spiegel-Adolf demonstrated such permeability changes in numerous reports (1941, 1942, 1944, 1948, 1953). They observed increased conductivity of the tissues

associated with the appearance of various ions (as potassium and phosphate) in the spinal fluid following electrically induced convulsions. There was also a significant increase in such non-electrolytes as nucleic-acid splitting enzymes. Changes in cellular permeability may thus provide the basis for the high concentrations of acetylcholine and the increased concentrations of cholinesterase-II in induced seizures or head trauma (Tower and McEachern 1949c).

The increase in cholinesterase activity should be associated with the rapid destruction of free acetylcholine. The persistence of acetylcholine in spinal fluid after head trauma and after seizures may be related to the sensitivity of the acetylcholine-cholinesterase-I system to concentration relationships (Nachmanson and Rothenberg, 1945; Tower and McEachern, 1949; Burgen and MacIntosh, 1955). At "physiologic" concentrations, hydrolysis of acetylcholine is rapid (3-4 microseconds) but at higher and lower concentrations, the activity falls off quickly. In contrast, the cholinesterase-II-

acetylcholine relationship is non-specific and the rate of hydrolysis increases with concentration.

One view of these relationships suggests that while the usual concentrations of acetylcholine at cell membranes are destroyed by the specific activity of cholinesterase-I in few microseconds, an excessive concentration following excitation may exceed the rate of hydrolysis by cholinesterase-I. The seizure threshold may be reached and a seizure induced, with the seizure itself adding to the amount of free acetylcholine, The increased acetylcholine diffuses rapidly, affecting vascular and cellular permeability and increasing the concentrations of various ions and cholinesterase-II in CSF. The activity of cholinesterase-II, though of low efficiency and depending on the concentration kinetics, reduces the acetylcholine in the tissues in hours to days to levels for the physiologic action of cholinesterase-I.

Cholinesterases appear in the spinal fluid as a reflection of their increase in intercellular fluids, resulting from changes in cell membrane permeability occasioned by increased acetylcholine. The increased cholinesterases are part of the cybernetic mechanisms controlling the levels of acetylcholine at cell membranes necessary for nervous system functioning.

(e) Acetylcholine, EEG Hypersynchrony and Induced Convulsions:

Alteration in the blood-brain permeability barrier by the continuing action of acetylcholine may be the biochemical substrate for the post-electroshock hypersynchrony of the electroencephalogram. Such a possibility is evident in the report by Aird et al. (1956) demonstrating an increase in the concentration of cocaine in brain tissues three days after a series of 12 induced convulsions. His data shows the change in concentration of this large molecule, ordinarily absent in brain tissue to be associated with the appearance of hypersynchrony (delta bursts) in the electro-encephalogram.

In studies of induced convulsions, we have confirmed the many previous reports that convulsive therapy induces electrographic hypersynchrony (Fink and Kahn, 1956). Despite a constant application of treatments, the time of appearance, the duration and the extent of the electrographic slow wave activity as well as its sensitivity to modification by alerting, hyperventilation and barbiturates vary greatly in psychiatric populations. The early appearance of high degree hypersynchrony and its persistence throughout the treatment course has been described as prerequisite to improvement following electroshock (Fink and Kahn, 1956). The failure of certain patients to develop hypersynchrony may be related to differences in the activity of central acetylcholine and cholinesterases, with the absence of free acetylcholine being related to minimal changes in cerebral function and thus precluding a clinical response to induced convulsions. Tower and McEachern (1949a), in their study of craniocerebral trauma, included observations of six psychiatric patients undergoing convulsive therapy. Studying the patients after 3-7 treatments

they reported free acetylcholine activity in the spinal fluid in two patients; and an increase in cholinesterase-II and a decreass in cholinesterase-I with a reversal of the ratio of cholinesterases in five of the six patients. From these observations they concluded that the spinal fluid changes in induced convulsions were more like those of craniocerebral trauma than those found in epilepsy.

Regarding the one patient in the series who failed to show either free acetylcholine or a cholinesterase ratio reversal in the spinal fluid, they wrote: "It is interesting that this patient was the only one of the six to show no response to treatment."

It is probable that induced convulsions increase free acetylcholine in intercellular fluids, altering cerebral permeability and enhancing the appearance of cholinesterases. Free acetylcholine is maintained by repeated seizures. EEG hypersynchrony is one reflection of altered levels of acetylcholine and altered permeability of other electrolytes. It is these changes in intercellular electrolytes that provide the biochemical substrate for the persistent behavioral changes following induced convulsions.

(f) Cholinesterases and the Classification of Psychoses:

These studies may also have application to the problem of autonomic reactivity and the classification of the psychoses. Funkenstein and others ( ) have demonstrated a relationship between the blood pressure response to injected mecholyl and the clinical response of psychiatric patients to convulsive therapy. Mecholyl is a potent cholinergic agent which induces vasodilation, tachycardia, sweating, and increased peristalsis. It is rapidly hydrolyzed by cholinesterase-I and slowly by cholinesterase-II. The blood pressure of subjects falls after injected mecholyl and returns to the baseline ~~in a variable length of time, i.e.,~~ within five to more than 20 minutes. Patients whose blood pressure returns to the baseline within 5 minutes are classified as Groups I, II, or III reactions; those whose blood pressure takes 20 or more minutes to return to baseline, as Group VI and VII reactions. The Group I reactors have a 9% improvement rate with convulsive therapy, and the Group II-III reactors a 35% recovery rate. In contrast, the Group VI

reactors have a 89% and the Group VII a 97% recovery rate (Funkenstein et al, 1952).

Patients in Funkenstein Groups I to III may be looked upon as patients in whom the injected mecholyl is rapidly hydrolyzed; while the Groups VI and VII patients have a slow hydrolysis rate. We may predict, therefore, that the blood cholinesterase activity levels of Groups I-III would be high; while the activity of Groups VI - VII would be low.

A similar analysis may be made regarding central nervous system levels of cholinesterase-I in the development of EEG hypersynchrony and spinal fluid levels of acetylcholine.

In studies of EEG changes following induced convulsions subjects were identified in whom a few seizures resulted in a great amount of slow wave activity; while other subjects exhibited few changes to many and to frequent seizures (Fink and Kahn, 1956). If electrographic hypersynchrony is a reflection of increased free acetylcholine, subjects who maintain hypersynchrony and those in whom it disappears rapidly may be exhibiting differences in the kinetics of the cholinesterase-acetylcholine hydrolysis systems. Persistent hypersynchrony may result from a decreased rate of hydrolysis of acetylcholine, associated with low concentrations of either cholinesterase-I or cholinesterase-II. (Conversely, in patients with short-lived hypersynchrony, cholinesterase-I and -II in tissue and spinal fluid may be unusually high). Thus these speculations provide

a basis for a congruent hypothesis regarding central nervous system reactivity to electroshock, and to peripheral cholinergic agents ~~indices~~.

CONCLUSION:

Central cholinergic mechanisms appear to be a significant factor in the convulsive therapy process. The published data may be interpreted to indicate that induced convulsions are associated with an increase in intercellular acetylcholine to levels greater than can be destroyed by cholinesterase-I activity. Vasodilation and increased cellular permeability is followed by increased amounts of cholinesterase-II, other enzymes and electrolytes in intercellular fluids.

These changes are reflected in the increased electrical hypersynchrony which is recorded in scalp electrodes, and which can be modified by anticholinergic drugs, as atropine, benactyzine, diethazine, procyclidine, etc.

These changes provide the change in biochemical milieu which alters cerebral cellular activities sufficient to alter the behavior of subjects. Failure to induce high and persistent concentrations of acetylcholine, and failure to induce concomitant electrolyte changes, results in a failure to produce behavioral change.

Differences in the rates of development of cerebral changes reflect differences in the dependence of subjects on cholinergic mechanisms, or in their sensitivity to changes in acetylcholine levels. These differences provide the basis for the classification of the mentally ill by Funkenstein and by Fink and Kahn (1960).

These observations provide a rational biochemical basis for the mode of action of induced convulsions in altering the behavior of psychotic subjects. These views are consistent with the more general neurophysiologic-adaptive theory expressed earlier (Fink, 1957).

IV a: 6/21/65

~~IV: 6-6-65~~

CHOLINERGIC MECHANISMS, CONVULSIVE THERAPY, AND BEHAVIOR

Studies of cerebral changes associated with convulsive therapy have indicated that the development of early and persistent signs of altered cerebral function are prerequisite to changes in behavior (Fink and Kahn, 1956). In these studies, electroencephalographic slowing was the most significant index of altered brain function. The demonstration that premedication with high doses of atropine prevented the appearance of slow wave activity (Ulett and Johnson, 1956) suggested a relation to cholinergic mechanisms. The role of acetylcholine and the cholinesterases in convulsive therapy is amplified in the following review which summarizes the published data.

The role of acetylcholine in the transmission of nervous impulses has been studied extensively since the first descriptions of Dale (1914) and Loewi (1921). Acetylcholine is a normal constituent of nervous tissue existing in a bound form which is

liberated during the excitation process. It is rapidly hydrolyzed through the specific action of cholinesterase and as rapidly reconstituted by the choline acetylase system (Richter & Crossland, 1949). No free acetylcholine has been reported in normal cerebrospinal fluid despite the rapid breakdown of bound acetylcholine during periods of activity and excitement (Tower and McEachern, 1949a). The cerebrospinal fluid normally has measurable cholinesterase activity, however, principally of the "true" or mecholyl hydrolyzing type (Nachmanson and Rothenberg, 1945).

In the absence of free acetylcholine and under the conditions described, electroencephalograms fail to show any consistent abnormality.

(a) Effect of Craniocerebral Trauma: Free acetylcholine was found in the cerebrospinal fluid within a few minutes after experimental head trauma in cats and persisted for varying periods up to 48 hours (Bornstein 1946). The quantity of free acetylcholine varied between 2.7 and 9.0 gamma percent, and the amount was related to degree of induced trauma.

~~Concomitant~~ Concurrent electroencephalograms demonstrated patterned changes. The records were first filled with high voltage fast activity, interpreted as evidence of an intense neuronal discharge, only to be followed by a short period of flattening of all recorded electrical activity. These phases were then followed by prolonged periods of high amplitude sharp waves in the delta frequencies.

The behavioral changes were both related to the degree of trauma and to the amount of measured free acetylcholine. With higher levels of acetylcholine, Bornstein reported greater degrees of EEG abnormality and greater changes in consciousness. In addition, spontaneous post-traumatic seizures were also related to the amount of free acetylcholine appearing in the spinal fluid.

Bornstein ~~later~~ also applied acetylcholine to exposed cat cerebral cortex. When the concentration of acetylcholine was 1 gamma percent or less, he observed high amplitude sharp waves of low frequency in the electroencephalogram. When the concentration was increased to

2 gamma percent, the electroencephalogram flattened in a fashion parallel to the post-traumatic records.

Parallel ~~These~~ studies were carried out ~~repeated~~ in neurological patients by Tower and McEachern (1949a). Free acetylcholine was found in the cerebrospinal fluid only in ~~the~~ patients with recent head trauma, recent grand-mal seizures or after convulsive therapy. The free acetylcholine varied from 0.2 to 100 gamma percent. Tower and McEachern also assayed the cholinesterase activity of the spinal fluid. They noted a sharp rise in the nonspecific cholinesterase fraction (benzoylcholine-splitting) and a drop in the specific cholinesterase fraction (mecholyl-splitting) both in patients with head trauma and in those following convulsive therapy. The cerebrospinal fluid following spontaneous seizures containing free acetylcholine did not exhibit such inversion. They ~~These authors~~ concluded that the level of free acetylcholine varies directly with the degree of cerebral damage

and that the reversal of the cholinesterase fraction was a more sensitive indicator of cerebral damage.

Electroencephalograms, taken at varying intervals following trauma in most of these subjects, indicated a relation between the degree of EEG abnormality and the appearance of free acetylcholine in the cerebrospinal fluid.

These studies indicate that the amount of free acetylcholine may increase in the spinal fluid following craniocerebral trauma and that a direct relation may exist between the amount of free acetylcholine, the degree and type of electroencephalographic abnormality, and changes in clinical behavior.

(b) Effect of Atropine on post-traumatic EEG and Behavior:

Bornstein administered 0.5-1.0 mg/kg atropine parentually after head trauma and demonstrated blocking of the EEG, behavioral and neurologic signs of trauma. ~~XXXXXXXXXXXXXXXXXX~~ Similar EEG and clinical changes occurring after the experimental addition of intracisternal acetylcholine were also blocked by atropine.

Ward (1950) applied these observations to the treatment of human cases of closed head injury. In 20 patients with varying degress of trauma, he administered atropine subcutaneously in doses of 0.1 mg/kg. In some cases he noted clinical improvement and in others a reversal of the electroencephalographic effects of the trauma. In a study of diethazine, another anticholinergic drug, Jenkner and Lechner (1955) reported significant alterations in the post-traumatic electroencephalogram. A single intravenous dose in 40 patients with abnormal electroencephalograms resulted in normalizing in 22 subjects and marked improvement in six others.

The same effect of atropine in blocking the appearance of slow wave activity was reported in subjects following convulsive therapy (Ulett and Johnson, 1956). (In a later study these authors failed to replicate this study, suggesting that dosage factors or population changes may have contributed to the different results [Johnson et al, 1960]).

6A

These observations provided the basis for a series of studies with other known anticholinergic compounds (Fink, 1958, 1960). The intravenous injection of experimental patent anticholinergic compounds as diethazine, benactyzine, the piperidylbenzilates, JB318, JB336, and JB329 (Ditran), WIN 2299 and procyclidine were shown to induce EEG desynchronization and behavioral alerting, anxiety, _____ illusions and hallucinations in non-electroshock subjects; and a reduction in slow wave activity associated with a reversal of the accompanying euphoria, denial and confusion in these with prior recent induced convulsions.

In these studies, atropine was also examined. In low doses, EEG desynchronization was observed, accompanied by considerable peripheral tachycordia, nervousness and tension. At higher dosages, confusion and disorientation accompanied a reduction of ~~hypersynchronization~~ hypersynchronous slow waves and their replacement by lower voltage, poorly organized delta activity with superimposed beta activity..

A variety of experiments with a potent cholinesterase inhibitor, DFP (di-isopropyl fluorophosphate) demonstrated high amplitude rapid frequency EEG patterns similar to status epilepticus, as well as lesser degress of abnormality similar to that noted in post-traumatic states (Himwich et al, 1950; Freedman et al, 1949; and Hampson et al, 1950). These electroencephalographic effects were blocked by small doses of parenteral atropine. Chatfield and Dempsey (1942) prepared exposed animal cortex with prostigmine and evoked electroencephalographic spike activity. The prior administration of atropine blocked this spiking, or if present, the abnormality could be eliminated by atropine.

In contrast to these findings, Brenner and Merritt (1942), applied topical acetylcholine in concentrations of 2-1/2 to 10% to the exposed cortex of cats, and noted no effect after intravenous atropine (1 mg/kg) on the electroencephalographic changes. The concentrations of acetylcholine in these experiments, however, was significantly higher than the topical applications (1-4 gamma percent)

and the intracisternal (0.2-10 gamma percent) injections of Bornstein (1946). Brenner and Merritt, also made note of electroencephalographic effects similar to acetylcholine from mecholyl (acetylbetamethulcholine) and doryl (carbamylcholine) in concentrations much lower than the acetylcholine concentrations. They ascribed the increased effectiveness of these cholinergic drugs to their lack of sensitivity to cerebral cholinesterases.

From a variety of experimental and clinical studies we may conclude that electroencephalographic activity induced by acetylcholine, either as a result of trauma, topical application of interference with cholinesterase activity can be blocked or eliminated by anticholinergic agents as atropine, benactyzine, etc.

(c) Cerebrospinal Fluid Acetylcholine and Seizures:

Acetylcholine is normally present in nervous tissues in an inactive bound form. During periods of activity, free acetylcholine is

liberated at the cell membrane, where it is rapidly deactivated by cholinesterase. The level of bound acetylcholine is thus the resultant of the processes of synthesis, liberation and breakdown. It may be postulated, therefore, that the level will rise during sleep and fall during activity. This hypothesis was supported in animal experiments by Richter and Crossland (1949) and Elliott, Swank and Henderson (1950). By using liquid air quick-freezing methods, Richter and Crossland observed the anesthesia and sleep level of acetylcholine (measured as micro-gamma per mg. brain tissue) to be 300% higher than post seizure levels. The difference in tissue levels is transient, however, as the resynthesis rate for acetylcholine in rat brain is high (7 gamma/gm/minute). Elliott et al, (1950) confirmed these observations. After metrazole convulsions, they also noted that free acetylcholine was always demonstrable in the spinal fluid in concentrations up to 3 gamma per cent.

In spinal fluid studies in man, Cone, Tower and McEachern (1948) and Tower and McEachern (1949b) reported significant quantities of free acetylcholine in patients with epilepsy. Of 56 epileptic patients, 44 (77%) demonstrated measurable free acetylcholine in quantities of 0.02 to 5.0 gamma per cent with an average of 1.0 gamma per cent. The acetylcholine level was directly related to the frequency of seizures, the extent of electroencephalographic abnormality, and the relation of the time of sampling to the last seizure. It bore no relation to medication, type of epilepsy or level of cholinesterase activity.

Whether the acetylcholine appearing in the spinal fluid is a by-product of the convulsion or whether the increase in acetylcholine is a cause of the seizure is problematical. Tower and McEachern (1949b) suggested that the increased acetylcholine liberation is not due to the seizure itself but is related to the process causing the seizure. In a study of the hypothesis that

the accumulation of acetylcholine is basic to the seizure process, Torda (1953) induced convulsions in animals by metrazole and determined the level of acetylcholine in brain tissue before and during convulsions. She noted that convulsions are preceded by a rise in the acetylcholine content of tissue; and that the content gradually falls during the convulsion; and that below certain levels, convulsions failed to occur. She suggested that the fall in tissue acetylcholine during a convulsion was due to inhibition of acetylcholine synthesis by increased concentrations of metabolites such as ammonium ions.

It is likely that seizures are accompanied by an increase in intercellular free acetylcholine liberated from its bound fame, and that this increase may be reflected in the spinal fluid; that cerebral activity and seizures enhance acetylcholine destruction, lowering tissue levels of acetylcholine; while sleep and anesthesia augment acetylcholine production increasing tissue levels.

(d) Central Nervous System Cholinesterases: Concomitant with their observations of changes in acetylcholine, Tower and McEachern (1949) measured spinal fluid cholinesterase activity. Two types of cholinesterases are normally found in the spinal fluid: cholinesterase-I ("true," "specific," or mecholyl hydrolyzing), which has a high specificity for acetylcholine; and cholinesterase-II ("pseudo,""non-specific," or benzoylcholine-hydrolyzing). Both compounds hydrolyze acetylcholine but have different rates of hydrolysis for mecholyl and benzoycholine. This differential rate permits qualitative distinctions. By reporting the cholinesterase activity as a ratio of the activity with mechdyl and with benzoylcholine substrates compared to an acetylcholine substrate two ratios are found: cholinesterase-I/acetylcholine and cholinesterase-II/acetylcholine (with Ach/Ach= 100). In such ratios ~~xxxxfxxxx~~ normal CSF contains esterases in the ratio of 33.17 for cholinesterase-I to cholinesterase-II. Thus, normal CSF consists

mainly of "specific" esterases with a small non-specific esterase component.

In patients with head trauma Tower and McEachern report and inversion of the cholinesterases with a significant increase in the cholinesterase-II fraction of the spinal fluid and a decrease in cholinesterase-I activity. They also observed a relation between the extent of the cholinesterase reversal with the severity of trauma and with the degree of the electroencephalograpic abnormality.

In patients with increased spinal fluid acetylcholine as a result of spontaneous seizures, however, no change in the ratio of cholinesterases or total cholinesterase activity was found.

Certain assumptions may be made regarding changes in cell membrane permeability as explaining the increase in cholinesterase activity. Cholinesterase-I is found in highest concentration in the central nervous system while cholinesterase-II predominates in other tissues, especially blood serum. With an increase in

acetylcholine levels in intercellular cerebral fluids following stimulation, convulsions or trauma, vasodilation and increased cellular permeability may be predicted, with a degree of transudation of vascular fluids into the inter cellular spaces dependent on the extent and duration of the vasodilation (Kabat et al, 1948). Spiegal and Spiegel-Adolf demonstrated such permeability changes in numerous reports (1941, 1942, 1944, 1948, 1953). They reported increased conductivity of the tissues associated with the appearance of various ions as potassium and phosphate in the spinal fluid following electrically induced convulsions. There was also a significant increase in such non-electrolytes as nucleic-acid splitting enzymes. Changes in cellular permeability may thus provide the basis for the high concentrations of acetylcholine and the increased concentrations of cholinesterase-II in induced seizures or head trauma (Tower and McEachern 1949c).

The increase in cholinesterase activity should be associated with the rapid destruction of free acetylcholine. Its persistence in spinal fluid after trauma and seizure may be related to the sensitivity of the acetylcholine-cholinesterase-I system to concentration relationships (Nachmanson and Rothenberg, 1945; Tower and McEachern, 1949; Burgen and MacIntosh, 1955). At "physiologic" concentrations, hydrolysis of acetylcholine is rapid (3-4 microseconds) but at higher and lower concentrations, the activity falls off quickly. In contrast, the cholinesterase-II-acetylcholine relationship is non-specific and the rate of hydrolysis increases with concentration. Thus, the usual levels of acetylcholine at cell membranes is destroyed by the specific activity of cholinesterase-I in few milliseconds. Where excitation leads to an excessive concentration of acetylcholine in nervous tissue, the rate of hydrolysis by cholinesterase-I is exceeded. The seizure threshold may be reached and a seizure occurs. The dissociation in acetylcholine-cholinesterase-I relationship results in a persistence of acetylcholine. The seizure may

itself ~~xx~~ add to the amount of free acetylcholine. The increased acetylcholine diffuses rapidly, affecting vascular and cellular permeability and increasing the concentrations of various ions and cholinesterase-II in CSF. The activity of cholinesterase-II, though of low efficiency and dependent on the concentration kinetics, reduces the acetylcholine in the tissues in hours to days to ~~xxxx~~ levels for the physiologic action of cholinesterase-I.

Cholinesterases
~~xCholinesx~~ appear in the spinal fluid as a reflection of their increase in intercellular fluids, resulting from changes in cell membrane permeability occasioned by increased acetylcholine. The increased cholinesterases are part of the cybernetic (?) mechanisms controlling the levels of acetylcholine at cell membranes necessary for nervous system functioning.

(e) Acetylcholine, EEG Hypersynchrony and Induced Convulsions:

Alteration in the blood brain permeability barrier by the continuing action of acetylcholine may be the biochemical substrate for the post-electroshock hypersynchrony of the electroencephalogram. Such a possibility is evident in the report by Aird et al, 1956, ~~demonstrt~~ demonstrating an increase in the concentration of cocaine in brain tissues three days after a series of 12 induced convulsions. His data shows the change in

concentration of this large molecule, ordinarily absent in brain tissue to be associated with the appearance of hypersynchrony (delta bursts) in the electroencephalogram.

In studies of induced convulsions, we have confirmed the many previous reports that convulsive therapy induces electrographic hypersynchrony (Fink and Kahn, 1956). Despite a constant application of treatments, however, the time of appearance, duration and extent of the electrographic slow wave activity; its sensitivity to modification by alerting, hyperventilation and barbiturates - all vary greatly in psychiatric populations. The early appearance of high degree hypersynchrony, and its persistence throughout the treatment course, has been described as prerequisite to improvement following electroshock (Fink and Kahn, 1956). The failure of certain patients to develop hypersynchrony may be relatedto differences in activity of central acetylcholine and cholinesterases. The degree of hypersynchrony may reflect the level of free acetylcholine and should follow a decay rate equal to the rate of acetylcholine destruction.

Since previous studies demonstrated that EEG hypersynchrony was prerequisite to the clinical response in convulsive therapy, it may be suggested that the absence of free acetylcholine suggests minimal changes in cerebral function and thus precludes a clinical response to induced convulsions. Tower and McEachern (1949a), in their study of craniocerebral trauma, included observations of six psychiatric patients undergoing convulsive therapy. Studying the patients after 3-7 treatments, they reported free acetylcholine activity in the spinal fluid in two patients; and an increase in cholinesterase-II and a decrease in cholinesterase-I with a reversal of the ratio of cholinesterases in five of the six patients. From these observations they concluded that the spinal fluid changes in induced convulsions were more like those of craniocerebral trauma than those found in epilepsy.

Regarding the one patient in the series who failed to show either free acetylcholine or a cholinesterase ratio reversal in the spinal fluid, they wrote: "It is interesting that this patient was the only one of the six to show no

response to treatment."

It is probably that induced convulsions increase free acetylcholine in intercellular fluids, altering cerebral permiability and enhancing the appearance of cholinesterases. Free acetylcholine is maintained by repeated seizures. EEG hypersynchrony is one reflection of altered levels of acetylcholine and altered permiability of other electrolytes. It is these changes in intercellulor electrolytes that provide the biochemical substrate for the persistent behavioral changes following induced convulsions.

(f) Cholinesterases and the Classification of Psychoses:

These studies may also have application to the problem of autonomic reactivity and the classification of the psychoses. Funkenstein and others ( ) have demonstrated a relationship between the blood pressure response of patients

to injected mecholyl and their clinical response to convulsive therapy. Mecholyl is a potent cholinergic agent which induces vasodilation, tachycardia, sweating, and increased peristalsis. It is rapidly hydrolyzed by cholinesterase-I and slowly by cholinesterase-II. The blood pressure of subjects falls after injected mecholyl and returns to the baseline in a variable length of time, i.e., five to more than 20 minutes. Patients whose blood pressure returns to the baseline in 5 minutes are classified as Groups I, II, or III reactions; those whose blood pressure takes 20 or more minutes to return to baseline, as Group VI and VIII reactions. The Group I reactors have a 9% improvement rate with convulsive therapy, and the Group II-III reactors a 35% recovery rate. In contrast, the Group VI reactors have a 89% and the Group VII a 97% recovery rate (Funkenstein et al, 1952).

Patients in Funkenstein Groups I to III may be looked upon as patients in whom the injected mecholyl is rapidly

hydrolyzed; while the Groups VI and VII patients have a slow hydrolysis rate. We may predict, therefore, that the blood cholinesterase activity levels of Groups I-III would be high; while the activity of Groups VI-VII would be low.

A similar analysis may be made regarding central nervous system levels of cholinesterase-I in the development of EEG hypersynchrony and spinal fluid levels of acetylcholine.

In studies of EEG changes following induced convulsions subjects were identified in whom a few seizures resulted in a great amount of slow wave activity; while other subjects exhibited few changes to many and to frequent seizures (Fink and Kahn, 1956). If electrographic hypersynchrony is a reflection of increased free acetylcholine, it is probable that subjects who maintain hypersynchrony and those in whom it rapidly (few hours) disappears may be exhibiting differences in the kinetics of the cholinesterase-acetylcholine hydrolysis systems. Persistent hypersynchrony may be seen to result from a decreased rate of hydrolysis of acetylcholine, associated with low concemtrations of either cholinesterase-I or

cholinesterase-II. Conversely, in patients with short-lived hypersynchrony, cholinesterase-I and -II in tissue and spinal fluid may be unusually high). Thus these speculations provide a basis for a congruent hypothesis regarding central nervous system reactivity to electroshock, and perepheral cholinergic indices.

CONCLUSION:

Central cholinergic mechanisms appear to be a significant factor in the convulsive therapy process. The published data may be interpreted to indicate that induced convulsions are associated with an increase in intercellular acetylcholine to levels greater than can be destroyed by cholinesterase-I activity. Vasodilation and increased cellular permiability is followed by increased amounts of cholinesterase-II, other and electrolytes in inter-cellulor fluids.

It is these changes that are reflected in the increased electrical hypersynchrony which is recorded in scalp electrodes, and which can be modified by anticholinergic drugs, as atropine, benactyzine, diethozine, procyclidine, etc.

It is these changes, also, that provide the change in biochemical milieu which alters cellular activities suffi cient to alter the behavior of subjects. Failure to induce high and persistent concentrations of acetylcholine, and failure

to induce concomitant electrolyte changes, results in a failure to produce behavioral change.

Differences in the rate of development of cerebral changes may reflect differences in subjects in their reliance on cholinergic mechanisms, or in their sensitivity to changes in acetylcholine levels. These differences provide the basis for the classification of the mentally ill by Funkenstein and by Fink and Kahn (1960).

CHOLINERGIC MECHANISMS, CONVULSIVE THERAPY, AND BEHAVIOR

Studies of cerebral changes associated with convulsive therapy have indicated that the development of early and persistent signs of altered cerebral function are prerequisite to changes in behavior (Fink and Kahn, 1956). In these studies, electroencephalographic slowing was the most significant index of altered brain function. The demonstration that premedication with high doses of atropine prevented the appearance of slow wave activity (Ulett and Johnson, 1956) suggested a relation to cholinergic mechnisms. The role of acetylcholine and the cholinesterases in convulsive therapy is amplified in the following review which summarizes the published data.

The role of acetylcholine in the transmission of nervous impulses has been studies extensively since the first descriptions of Dale (1914) and Loewi (1921). Acetylcholine is a normal constituent of nervous tissue existing in a bound form which is

liberated during the excitation process. It is rapidly hydrolyzed through the specific action of cholinesterase and as rapidly reconstituted by the cholineacetylase system (Richter & Crossland, 1949). No free ac etylcholine has been reported in normal cerebrospinal fluid despite the rapid breakdown of bound acetyl-choline during periods of activity and excitement (Tower and McEachern, 1949a). The cerebrospinal fluid normally has measurable cholinesterase activity, however, principally of the "true" or mecholyl hydrolyzing type (Nachmanson and Rothenberg, 1945).

In the absence of free acetylcholine and under the conditions described, electroencephalograms fail to show any consistent abnormality.

(a) Effect of Craniocerebral Trauma: Free acetylcholine was found in the cerebrospinal fluid within a few minutes after experimental head trauma in cats and persisted for varying periods up to 48 hours (Bornstein 1946). The quantity of free acetylcholine varied between 2.7 and 9.0 gamma percent, and the amount was related to degree of induced trauma.

Concomitant electroencephalograms demonstrated patterned changes. The records were first filled with high voltage fast activity, interpreted as evidence of an intense neuronal discharge, only to be followed by a short period of flattening of all recorded electrical activity. These phases were then followed by prolonged periods of high amplitude sharp waves in the delta frequencies.

The behavioral changes were related to degree of trauma and to the amount of measured free acetylcholine. With higher levels of acetylcholine, Bornstein reported greater degrees of EEG abnormality and greater changes in consciousness. In addition, spontaneous post-traumatic seizures were also related to the amount of free acetylcholine appearing in the spinal fluid.

Bornstein later applied acetylcholine to exposed cat cerebral cortex. When the concentration of acetylcholine was 1 gamma percent or less, he observed high amplitude sharp waves of low frequency in the electroencephalogram. When the concentration was increased to

2 gamma percent, the electroencephalogram flattened in a fashion parallel to the post-traumatic records.

These studies were repeated in neurological patients by Tower and McEachern (1949a). Free acetylcholine was found in the cerebrospinal fluid only in the patients with recent head trauma, recent grand-mal seizures or after convulsive therapy. The free acetylcholine varied from 0.2 to 100 gamma percent. Tower and McEachern also assayed the cholinesterase activity of the spinal fluid. They noted a sharp rise in the nonspecific cholinesterase fraction (benzoylcholine-splitting) and a drop in the specific cholinesterase fraction (mecholyl-splitting) both in patients with head trauma and in those following convulsive therapy. The cerebrospinal fluid following spontaneous seizures containing free acetylcholine did not exhibit such inversion. These authors concluded that the level of free acetylcholine varies directly with the degree of cerebral damage

and that the reversal of the cholinesterase fraction was a more sensitive indicator of cerebral damage.

Electroencephalograms were taken at varying intervals following trauma in most of these subjects. The authors reported the relation between the degree of EEG abnormality and the appearance of free acetylcholine in the cerebrospinal fluid.

These studies indicate that the amount of free acetylcholine may increase in the spinal fluid following craniocerebral trauma and that a direct relation may exist between the amount of free acetylcholine, the degree and type of electroencephalographic abnormality, and changes in clinical behavior.

(b) Effect of Atropine on post-traumatic EEG and Behavior:

Bornstein administered 0.5-1.0 mg/kg atropine parentually after head traume and demonstrated blocking of the EEG, behavioral and neurologic signs of trauma. Atropine also blocked the EEG and clinical changes similar to head trauma seen following the experimental addition of intracisternal acetylcholine.

Ward (1950) applied these observations to the treatment of human cases of closed head injury. In 20 patients with varying degress of trauma, he administered atropine subcutaneously in doses of 0.1 mg/kg. In some cases he noted clinical improvement and in others a reversal of the electroencephalographic effects of the trauma. In a study of diethozint, another anticholinergic drug, Jenkner and Lechner (1955) reported significant alterations in the post-traumatic electroencephalogram. A single intravenous dose in 40 patients with abnormal electroencephalograms resulted in normalizing in 22 subjects and marked improvement in six others.

The same effect of atropine in blocking the appearance of slow wave activity was reported in subjects following convulsive therapy (Ulett and Johnson, 1956). (In a later study then authors failed to replicate this study, suggesting that dosage factors or population changes may have contributed to the different results [Johnson et al, 1960]).

A variety of experiments with a potent cholinesterase inhibitor, DFP (di-isopropyl fluorophosphate) demonstrated high amplitude rapid frequency EEG patterns similar to status epilepticus, as well as lesser degress of abnormality similar to that noted in post-traumatic states (Himwich et al, 1950; Freedman et al, 1949; and Hampson et al, 1950). These electroencephalographic effects were blocked by small doses of parenteral atropine. Chatfield and Dempsey (1942) prepared exposed animal cortex with prostigmine and evoked electroencephalographic spike activity. The prior administration of atropine blocked this spiking, or if present, the abnormality could be eliminated by atropine.

In contrast to these findings, Brenner and Merritt (1942), applied topical acetylcholine in concentrations of 2-1/2 to 10% to the exposed cortex of cats, and noted no effect after intravenous atropine (1 mg/kg) on the electroencephalographic changes. The concentrations of acetylcholine in these experiments, however, was significantly higher than the topical applications (1-4 gamma percent)

and the intracisternal (0.2-10 gamma percent) injections of Bornstein (1946). Brenner and Merritt, also made note of electroencephalographic effects similar to acetycholine from mecholyl (acetylbetamethylcholine) and doryl (carbamylcholine) in concentrations much lower than the acetylcholine concentrations. They ascribed the increased effectiveness of these cholinergic drugs to their lack of sensitivity to cerebral cholinesterases.

From a variety of experimental and clinical studies we may conclude that electroencephalographic activity induced by acetylcholine, either as a result of trauma, topical application or interference with cholinesterase activity can be blocked or eliminated by atropine.

(c) Role of Cerebrospinal Fluid Acetylcholine in Seizures:

Acetylcholine is normally present in nervous tissue in an inactive bound form. During periods of activity, free acetylcholine is

liberated at the cell membrane, where it is rapidly deactivated by cholinesterase. The level of bound acetylcholine is thus the resultant of the processes of synthesis, liberation and breakdown. It may be postulated, therefore, that the level will rise during sleep and fall during activity. This hypothesis was supported in animal experiments by Richter and Crossland (1949) and Elliott, Swank and Henderson (1950). By using liquid air quick-freezing methods, Richter and Crossland observed the anesthesia and sleep level of acetylcholine (measured as micro-gamma per mg. brain tissue) to be 300% higher than post seizure levels. The difference in tissue levels is transient, however, as the resynthesis rate for acetylcholine in rat brain is high (7 gamma/gm/minute). Elliott et al, (1950) confirmed these observations. After metrazole convulsions, they also noted that free acetylcholine was always demonstrable in the spinal fluid in concentrations up to 3 gamma per cent.

In spinal fluid studies in man, Cone, Tower and McEachern (1948) and Tower and McEachern (1949b) reported significant quantities of free acetylcholine in patients with epilepsy. Of 56 epileptic patients, 44 (77%) demonstrated measurable free acetylcholine in quantities of 0.02 to 5.0 gamma per cent with an average of 1.0 gamma per cent. The acetylcholine level was directly related to the frequency of seizures, the extent of electroencephalographic abnormality, and the relation of the time of sampling to the last seizure. It bore no relation to medication, type of epilepsy or level of cholinesterase activity.

Whether the acetylcholine appearing in the spinal fluid is a by-product of the convulsion or whether the increase in acetylcholine is a cause of the seizure is problematical. Tower and McEachern (1949b) suggested that the increased acetylcholine liberation is not due to the seizure itself but is related to the process causing the seizure. In a study of the hypothesis that

the accumulation of acetylcholine is basic to the seizure process, Torda (1953) induced convulsions in animals by metrazole and determined the level of acetylcholine in brain tissue before and during convulsions. She noted that convulsions are preceded by a rise in the acetylcholine content of tissue; that the content gradually falls during the convulsion; and that below certain levels, convulsions failed to occur. She suggested that the fall in tissue acetylcholine during a convulsion was due to inhibition of acetylcholine synthesis by increased concentrations of metabolites such as ammoniumions.

The passage of electric current through brain substances induces a change in cellular activity with an increase in free acetylcholine to levels sufficient to induce a grand mal seizure. The presence of free acetylcholine in the intercellular fluids is associated with electrical hypersynchrony, reflected the EEG as delta slowing.

(d) Effect of Electroshock on Acetylcholine and Cholinesterases: Tower and McEachern (1949a), in their study of craniocerebral trauma, included observations of six psychiatric

patients undergoing convulsive therapy. Studying the patients after 3-7 treatments, they reported free acetylcholine activity in the spinal fluid in two patients; and an increase in cholinesterase-II and a decrease in cholinesterase-I with a reversal of the ratio of cholinesterases in five of the six patients. From these observations they concluded that the spinal fluid changes in induced convulsions were more like those of craniocerebral trauma than those found in epilepsy.

Regarding the one patient in the series who failed to show either free acetylcholine or a cholinesterase ratio reversal in the spinal fluid, they wrote: "It is interesting that this patient was the only one of the six to show no response to treatment."

While there is considerable argument about the role of acetylcholine in seizures, it is probable that free acetylcholine is increased during seizures and appears in the spinal fluid; that cerebral activity and seizures enhance acetylcholine destruction,

lowering tissue levels of acetylcholine; while sleep and anesthesia augment acetylcholine production increasing tissue levels.

(e) Central Nervous System Cholinesterases: Concomitant with their observations of changes in acetycholine, Tower and McEachern (1949) measured spinal fluid cholinesterase activity. Two types of cholinesterases are normally found in the spinal fluid: cholinesterase-I ("true," "specific," or mecholyl hydrolyzing), which has a high specificity for acetylcholine; and cholinesterase-II ("pseudo," "non-specific," or benzoylcholine-hydrolyzing). Both compounds hydrolyze acetylcholine but have different rates of hydrolysis for mecholyl and benzoylcholine. This differential rate permits qualitative distinctions. By reporting the cholinesterase activity as a ratio of the activity with mecholyl and with benzoylcholine substrates compared to an acetylcholine substrate two ratios are found: cholinesterase-I/acetylcholine and cholinesterase-II/acetylcholine (with Ach/Ach = 100). In such ratios normal CSF contains esterases in the ratio of 33:17 for cholinesterase-I to cholinesterase-II. Thus, normal CSF consists

mainly of "specific" esterases with a small non-specific esterase component.

In patients with head trauma Tower and McEachern report and inversion of the cholinesterases with a significant increase in the cholinesterase-II fraction of the spinal fluid and a decrease in cholinesterase-I activity. They also observed a relation between the extent of the cholinesterase reversal with the severity of trauma and with the degree of the electroencephalograpic abnormality.

In patients with increased spinal fluid acetylcholine as a result of spontaneous seizures, however, no change in the ratio of cholinesterases or total cholinesterase activity was found.

Certain assumptions may be made regarding changes in cell membrane permeability as explaining the increase in cholinesterase activity. Cholinesterase-I is found in highest concentration in the central nervous system while cholinesterase=II predominates in other tissues, especially blood serum. With an increase in

acetylcholine levels in intercellular cerebral fluids following stimulation, convulsions or trauma, vasodilation and increased cellular permeability may be predicted, with a degree of transudation of vascular fluids into the inter cellular spaces dependent on the extent and duration of the vasodilation (Kabat et al, 1948). Spiegal and Spiegel-Adolf demonstrated such permeability changes in numerous reports (1941, 1942, 1944, 1948, 1953). They reported increased conductivity of the tissues associated with the appearance of various ions as potassium and phosphate in the spinal fluid following electrically induced convulsions. There was also a significant increase in such non-electrolytes as nucleic-acid splitting enzymes. Changes in cellular permeability may thus provide the basis for the high concentrations of acetylcholine and the increased concentrations of cholinesterase-II (Tower and McEachern 1949c).

The increase in cholinesterase activity should be associated with the rapid destruction of free acetylcholine. Its persistence in spinal fluid after trauma and seizure may be related to the sensitivity of the acetylcholine-cholinesterase-I system to concentration relationships (Nachmanson and Rothenberg, 1945; Tower and McEachern, 1949; Burgen and MacIntosh, 1955). At "physiologic" concentrations, hydrolysis of acetylcholine is rapid (3-4 microseconds) but at higher and lower concentrations, the activity falls off quickly. In contrast, the cholinesterase-II-acetylcholine relationship is non-specific and the rate of hydrolysis increases with concentration. Thus, the usual levels of acetylcholine at cell membranes is destroyed by the specific activity of cholinesterase-I in few milliseconds. Where excitation leads to an excessive concentration of acetylcholine in nervous tissue, the rate of hydrolysis by cholinesterase-I is exceeded. The seizure threshold may be reached and a seizure occurs. The dissociation in acetylcholine-cholinesterase-I relationship results in a persistence of acetylcholine. The seizure may

itself add to the amount of free acetylcholine. The increased acetylcholine diffuses rapidly, affecting vascular and cellular permeability and increasing the concentrations of various ions and cholinesterase-II in CSF. The activity of cholinesterase-II, though of low efficiency and dependent on the concentration kinetics, reduces the acetylcholine in the tissues in hours to days to levels for the physiologic action of cholinesterase-I.

(f) Acetylcholine, EEG Hypersynchrony and Classification of Psychoses: Alteration in the blood brain permeability barrier by the continuing action of acetylcholine may be the biochemical substrate for the post-electroshock hypersynchrony of the electroencephalogram. Such a possibility is evident in the report by Aird et al, 1956 demonstrating an increase in the concentration of cocaine in brain tissues three days after a series of 12 induced convulsions. His data shows the change in concentration of this large molecule, ordinarily absent in brain tissue to be

associated with the appearance of hypersynchrony (delta bursts) in the electroencephalogram.

In studies of induced convulsions, we have confirmed the many previous reports that convulsive therapy induces electrographic hypersynchrony (Fink and Kahn, 1956). Despite a constant application of treatments, however, the time of appearance, duration and extent of the electrographic slow wave activity; its sensitivity to modification by alerting, hyperventilation and barbiturates - all vary greatly in psychiatric populations. The early appearance of high degree hypersynchrony, and its persistence throughout the treatment course, has been described as prerequisite to improvement following electroshock (Fink and Kahn, 1956). The failure of certain patients to develop hypersynchrony may be related to differences in activity of central acetylcholine and cholinesterases.

(Let us assume a grand mal seizure to be indicative of tissue levels of free acetylcholine in excess of the hydrolysis rate by cholinesterase-I, and that the electrographic hypersynchrony is a reflection of this abnormal concentration of acetylcholine. Patients who maintain hypersynchrony and those in whom it rapidly (few hours) disappears

may be exhibiting differences in the kinetics of the cholinesterase-acetylcholine hydrolysis systems. Persistent hypersynchrony may be seen to result from a decreased rate of hydrolysis of acetylcholine, associated with low concentrations of either cholinesterase-I or Cholinesterase-II. Conversely, in patients with short-lived hypersynchrony, cholinesterase-I and -II in tissue and spinal fluid may be unusually high).

These studies may also have application to the problem of autonomic reactivity. Funkenstein and others ( ) have demonstrated a relationship between the blood pressure response of patients to injected mecholyl and their clinical response to convulsive therapy. Mecholyl is a potent cholinergic agent which induces vasodilation, tachycardia, sweating, and increased peristalsis. It is rapidly hydrolyzed by cholinesterase-I and slowly by cholinesterase-II. The blood pressure of subjects falls after injected mecholyl and returns to the baseline in a variable length of time,

i.e., five to more than 20 minutes. Patients whose blood pressure returns to the baseline in 5 minutes are classified as Groups I, II, or III reactions; those whose blood pressure takes 20 or more minutes to return to baseline, as Group VI and VIII reactions. The Group I reactors have a 9% improvement rate with convulsive therapy, and the Group II-III reactors a 35% recovery rate. In contrast, the Group VI reactors have a 89% and the Groups VII a 97% recovery rate (Funkenstein et al, 1952).

Patients in Funkenstein Groups I to III may be looked upon as patients in whom the injected mecholyl is rapidly hydrolyzed; while the Group VIand VII patients have a slow hydrolysis rate. We may predict, therefore, that the blood cholinesterase activity levels of Groups I-III would be high; while the activity of Groups VI-Vii would be low. We recall here a similar prediction regarding the data for central nervous system levels of cholin-esterase-I, in which the development of early and sustained EEG hypersynchrony and elevated spinal fluid levels of acetylcholine was related to a low level of cholinesterase activity. Thus,

the data of peripheral stimulation by cholinergic agents is congruent to the hypothesis regarding central nervous system reactivity to electroshock.

VI : File Copy

CHOLINERGIC MECHANISMS IN CONVULSIVE THERAPY

Max Fink, M.D.

From the Department of Psychiatry at the Missouri Institute of Psychiatry, University of Missouri School of Medicine, 5400 Arsenal Street, St. Louis, Missouri 63139.

Aided, in part, by USPHS grants MH-927, MH-2715, MH-07249, and MH-11380; and the Psychiatric Research Foundation of Missouri.

CHOLINERGIC MECHANISMS IN CONVULSIVE THERAPY

Despite extensive application and study, the mode of action of the convulsive therapy process remains enigmatic. Much study has been devoted to neurophysiological ( ), psychological ( ), clinical ( ), and social ( ) aspects, elucidating the present neuro-physiological-adaptive view of the process ( ). The early development and persistance of signs of altered cerebral functions were reported to be requisite to changes in behavior (Fink and Kahn, 1956), with electroencephalographic slow wave activity as the most significant index of altered brain function. The demonstration that premedication with atropine inhibited this slow wave activity (Ulett and Johnson, 1957) and the report that anticholinergic compounds reversed these clinical as well as electrographic changes (Fink, 1958) suggests that the biochemical basis for the convulsive therapy process may be in the cholinergic mechanisms of the central nervous system. This review discusses the available data regarding acetylcholine and

and the cholinesterases in the convulsive therapy process.

Acetylcholine has been extensively studied as an active agent in the transmission of nervous impulses since the first descriptions of Dale (1914) and Loewi (1921). It is a constituent of nervous tissue, existing in a bound form which is liberated during the excitation process. It is rapidly hydrolyzed through the specific action of cholinesterase and is rapidly reconstituted by the choline-acetylase system (Richter and Crossland, 1949). In normal cerebrospinal fluid, free acetylcholine is not present despite the rapid breakdown of bound acetylcholine during periods of activity and excitement (Tower and McEachern, 1949a). The cerebrospinal fluid does have measurable cholinesterase activity, however, principally of the "true" or mecholyl hydrolyzing type (Nachmanson and Rothenberg, 1945). In the absence of free acetylcholine and under the conditions described, electroencephalograms fail to show abnormality.

(a) Cholinergic Aspects of Craniocerebral Trauma: Free acetylcholine was found in the cerebrospinal fluid within a few minutes after experimental head trauma in cats and persisted for varying periods up to 48 hours (Bornstein, 1946). The quantity of free acetylcholine varied between 2.7 and 9.0 gamma percent, and the amount was related to the degree of induced trauma.

Concurrent electroencephalograms demonstrated patterned changes. The records were first filled with high voltage fast activity, interpreted as evidence of an intense neuronal discharge, soon to be succeeded by a short period of flattening of all recorded electrical activity. These phases were then followed by prolonged periods of high amplitude sharp waves in the delta frequencies.

The behavioral changes were related both to the degree of traume and to the amount of measured free acetylcholine. With higher levels of acetylcholine, Bornstein reported greater degrees of EEG abnormality and greater changes in consciousness.

Spontaneous post-traumatic seizures were also related to the amount of free acetylcholine appearing in the spinal fluid.

Bornstein applied acetylcholine to exposed cat cerebral cortex. When the concentration of acetylcholine was 1 gamma percent or less, high amplitude sharp waves of low frequency appeared in the electroencephalogram. When the concentration was increased to 2 gamma percent, the electroencephalogram flattened in a fashion parallel to the post-traumatic records.

Parallel investigations in neurological patients by Tower and McEachern (1949a) demonstrated free acetylcholine in the cerebrospinal fluid only in patients with recent head trauma, recent grand-mal seizures or after electroconvulsive therapy. Free acetylcholine varied from 0.2 to 100 gamma percent. In assaying spinal fluid cholinesterase activity, they noted a sharp rise in the nonspecific cholinesterase fraction (benzoylcholine-splitting) and a drop in the specific cholinesterase fraction (mecholyl-splitting) in patients with head trauma and following convulsive therapy. The cerebrospinal fluid did not

exhibit such inversion, although it contained free acetylcholine, after spontaneous seizures. They concluded that the level of free acetylcholine varied directly with the degree of cerebral damage and that reversal of the cholinesterase fractions was a more sensitive indicator of cerebral damage. Electroencephalograms, taken at varying intervals following trauma, also indicated a relation between the degree of EEG abnormality and the appearance of free acetylcholine in the cerebrospinal fluid.

[Add ¶ Kovach et al, 1957]

Thus the amount of free acetylcholine may increase in the spinal fluid following craniocerebral trauma and the amount of free acetylcholine, the degree and type of electroencephalographic abnormality, and changes in clinical behavior appear as interrelated phenomena.

(b) Anticholinergic drugs and trauma: The electrographic, behavioral and neurologic signs of trauma were blocked by the parenteral administration of 0.5-1.0 mg/kg atropine (Bornstein, 1946), as were similar clinical changes occurring after the intracisternal addition of acetylcholine. Ward (1950) applied these observations to the treatment of closed head injuries. In 20 patients with varying degrees of trauma, he administered atropine subcutaneously in doses of 0.1 mg/kg, noting clinical improvement in some and a reversal of the electroencephalographic effects in others. In a study of another anticholinergic drug, diethazine, Jenkner and Lechner (1955) reported alterations in the post-traumatic electroencephalogram. A single intravenous dose in forty patients with abnormal electroencephalograms resulted in normalizing in twenty-two and marked improvement in six others.

Similar observations have been reported by Denisenko (1965) using methylbenactyzine and trasentin in animal experiments of post-traumatic shock and cerebral edema.

These observations were assessed in the convulsive therapy process by Ulett and Johnson (1957). These workers administered dosages of atropine up to mgm per day during the weeks the patients received electroshock therapy. They demonstrated that the amounts of slow wave activity produced in these patients was significantly less than the control group who had not received the atropine administration.

(In a later study these authors failed to replicate this study, suggesting that dosage factors or population changes may have contributed to different results [Johnson et al, 1960]).

These observations provided the basis for studies with other known anticholinergic compounds (Fink, 1958, 1960). The intravenous injection of experimentally active anticholinergic compounds as diethazine, benactyzine, the piperidylbenzilates JB-318, JB-336 and JB-329 (Ditran), and WIN-2299 induced EEG desynchronization in psychiatric subjects. These EEG changes were associated with behavioral alerting, anxiety, tremors, illusions and hallucinations. In patients who had recently

received electroconvulsive therapy, the administration of these compounds was associated with a reduction in slow wave activity and reversal of euphoria, denial and confusion. Atropine was also examined in low doses, and in these administrations, EEG desynchronization was observed accompanied by tachycardia, nervousness and tension. At higher dosages, hypersynchronous slow waves, followed by lower voltage, poorly organized delta activity with superimposed beta activity was accompanied by progressive confusion and disorientation.

Both in cerebral trauma and induced convulsions, the electrographic changes may be modified by the concurrent administration of anticholinergic drugs, thus suggesting that increased amounts of acetylcholine or increased cholinergic receptivity is associated with the high voltage slow wave activity.

(c) <u>Brain acetylcholine and anticholinergic drugs:</u>

Similar EEG changes and the blocking thereof has been observed following the direct application of acetylcholine to the central nervous system.

The administration of a cholinesterase inhibitor DFP (di-isopropyl fluorophosphate) elicited high amplitude rapid frequency EEG patterns similar to status epilepticus, as well as changes similar to those of post-traumatic states (Freedman et al, 1949, Hampson et al, 1950; and Himwich et al, 1950). These EEG effects were blocked by small doses of parenteral atropine. The great increase in acetylcholine after tetrarthyl pyrophosphate (TEPP) was measured and related to the toxic manisfestations and convulsions induced (Giarman and Pepeu, 1952; Stone, 1957.)

Chatfield and Dempsey (1942) prepared exposed animal cortex with prostigmine and evoked electroencephalographic spike activity. The prior administration of atropine blocked this spiking, or if present, the abnormality could be eliminated by atropine.

In contrast to these findings, Brenner and Merritt (1942) applied topical acetylcholine in concentrations of 2-1/2 to 10% to the exposed cortex of cats, and noted no effect on the electroencephalographic changes after intravenous atropine (1 mg/kg.) The concentrations of acetylcholine in these experiments,

however, were higher than the topical applications (1-4 gamma percent) and the intracisternal (0.2-10 gamma percent) injections of Bornstein (1946). Brenner and Merritt also made note of electro-encephalographic effects similar to acetylcholine from mecholyl (acetylbetamethylcholine) and doryl (carbamylcholine)in concentrations much lower than the acetylcholine concentrations. They ascribed the increased effectiveness of these cholinergic drugs to their lack of sensitivity to cerebral cholinesterases.

These data are conflicting and further study is necessary to qualify this issue.

(c) Cerebrospinal Fluid Acetylcholine and Seizures: One view of acetylcholine metabolism indicates that it is found in nervous tissues in an inactive bound form. During periods of activity, acetylcholine is liberated at the cell membrane, where it is rapidly deactivated by cholinesterase. The amount of bound acetylcholine is the resultant of the continuous processes of synthesis, liberation and breakdown. It has been postulated that the level rises during sleep and falls during activity. (Richter and Crossland, 1949; Elliott, Swank and Henderson, 1950; Giarman

Pepeu, 1962). By using liquid air quick freezing methods, Richter and Crossland measured the level of acetylcholine (micro-gamma per mg. brain tissue) during anesthesia and sleep to be 300% higher than post-seizure levels. The difference in tissue levels is transient, however, as the resynthesis rate for acetylcholine in rat brain is high (7 gamma/gm/minute). Elliot et al (1950) confirmed these observations, also noting that after metrazole convulsions free acetylcholine was demonstrable in the spinal fluid in concentrations up to 3 gamma per cent. Giarman and Pepeu (1962) found an increase in acetylcholine caused by a group of central nervous system depressants to be roughly proportional to the degree of depression of the central nervous system and no reduction in motor activity.

Free acetylcholine was reported in the spinal fluid in patients with epilepsy (Cone, Tower and McEachern, 1948; Tower and McEachern, 1949 b). Of 56 epileptic patients, 44 (77%) demonstrated free acetylcholine in quantities of 0.02 to 5.0 gamma per cent with an average of 1.0 gamma per cent.

Acetylcholine levels were related to the frequency of seizures, the extent of electroencephalographic abnormality, and to the time since the last seizure, but bore no relation to medication, type of epilepsy or level of cholinesterase activity.

Tower and McEachern (1949b) viewed the increased acetylcholine a by-product of the seizure, and not causal. Studying the hypothesis that the accumulation of acetylcholine induced seizures, Torda (1953) measured the level of acetylcholine in brain tissue metrazole convulsions. She noted a rise in the acetylcholine content of brain, before a seizure and a fall during the convulsion. Below certain levels of acetylcholine, convulsions failed to occur. She suggested that the fall in tissue acetylcholine during a convulsion was due to inhibition of acetylcholine synthesis by increased concentrations of metabolites such as ammonium ions.

Giarmann and Pepeu also measured changes in central nervous system acetylcholine following various stimulants. Only after

mecholyl and 3, 5-dimethylbutylethyl-barbiturate was there a significant change in the acetylcholine level. They noted a decrease in association with induced convulsions. With other drugs which they quantified as stimulants (LSD, improniazid, improniazid + hydroxytroyptophan and improniazid + DOPA) there were no changes in acetylcholine level. They concluded that despite intense excitation produced by these compounds, there were no changes in acetylcholine level unless these were accompanied by convulsions. (The differences in observations between these workers and Cone et al and Tower and McEachern may be related to the differences in methods of biochemical measurements, for the latter measured changes in spinal fluid reflecting the free acetylcholine, while Giarmann and Pepeu measured total acetylcholine in tissues reflecting bound and free forms of acetylcholine.)

These studies suggest that spontaneous or induced seizures are accompanied by an increase in intercellular free acetylcholine

liberated from its bound form which may be reflected in the spinal fluid. Cerebral activity and seizures enhance acetylcholine destruction, lowering tissue levels of acetylcholine, while sleep and anesthesia augment acetylcholine production increasing tissue levels.

(d) Central Nervous System Cholinesterases: Tower and McEachern (1949) also measured spinal fluid cholinesterase activity. Two types of cholinesterases are normally found in the spinal fluid: cholinesterase-I ("true," "specific," or mecholyl-hydrolyzing), which has a high specificity for acetylcholine; and cholinesterase-II ("pseudo," "non-specific," or benzoylcholine-hydrolyzing). Both compounds hydrolyze acetylcholine but have different rates of hydrolysis for mecholyl and benzoylcholine. This differential rate permits qualitative distinctions. By reporting the cholinesterase activity as a ratio of the activity with a mecholyl substrate and with a benzoylcholine substrate compared to a substrate of acetylcholine, two ratios are found: cholinesterase-I/acetylcholine

and cholinesterase-II/acetylcholine. In such ratios normal cerebrospinal fluid contains esterases in the ratio of 33:17 for cholinesterase-I to cholinesterase-II.

In patients with head trauma, Tower and McEachern reported an inversion of the cholinesterases with an increase in the cholinesterase-II fraction of the spinal fluid and a decrease in cholinesterase-I activity. The extent of the cholinesterase reversal was related to the severity of trauma and to the degree of the electroencephalographic abnormality.

In patients with elevated spinal fluid acetylcholine after spontaneous seizures, however, no change in the ratio of cholinesterases or total cholinesterase activity was found.

The change in cholinesterase activity may be understood in relation to changes in cell membrane permeability. Cholinesterase-I is found in highest concentration in the central nervous system while cholinesterase-II predominates in other tissues, especially blood serum. With an increase in acetylcholine levels in cerebral intercellular fluids,

vasodilation and increased cellular permeability may be predicted, with a degree of transudation of vascular fluids into the intercellular spaces varying with the extent and duration of the vasodilatation (Kabat et al, 1948). Spiegel, Spiegel-Adolf, and their co-workers (1941, 1942, 1944, 1948, 1953) demonstrated such permeability changes and increased conductivity of the tissues associated with the appearance of various ions (as potassium and phosphate) in the spinal fluid following electrically induced convulsions. Such non-electrolytes, as nucleic-acid splitting enzymes, also increased. Changes in cellular permeability may thus provide the basis for the high concentrations of acetylcholine and the increased concentrations of cholinesterase-II in induced seizures or head trauma (Tower and McEachern 1949c).

The persistence of acetylcholine in spinal fluid after head trauma and after seizures despite increased cholinesterase activity may be related to the sensitivity of the acetylcholine-cholinesterase-I system to concentration relationships (Nachmanson

and Rothenberg, 1945; Tower and McEachern, 1949c; Burgen and MacIntosh, 1955). At "physiologic" concentrations, hydrolysis of acetylcholine is rapid (3-4 microseconds) but at higher and lower concentrations, the activity falls off quickly. In contrast, the cholinesterase-II acetylcholine relationship is non-specific and the rate of hydrolysis increases with increased concentration.

One view of these relationships suggests that while the usual concentrations of acetylcholine at cell membranes are destroyed by the specific activity of cholinesterase-I in a few microseconds, an excessive concentration following excitation may exceed the rate of hydrolysis by cholinesterase-I. The seizure threshold may be reached and a seizure induced, with the seizure itself adding to the amount of free acetylcholine. The increased acetylcholine diffuses rapidly, affecting vascular and cellular permeability and increasing the concentrations of various ions and cholinesterase-II in the CSF. The activity of cholinesterase-II, though of low efficiency and depending on

the concentration kinetics, reduces the acetylcholine in the tissues in hours to days to levels for the physiologic action of cholinesterase-I..

Cholinesterases appear in the spinal fluid as a reflection of their increase in intercellular fluids, resulting from changes in cell membrane permeability occasioned by increased acetylcholine. The increased cholinesterases are part of the homeostatic mechanisms controlling the levels of acetylcholine at cell membranes necessary for nervous system activity.

(e) Acetylcholine, EEG Hypersynchrony and Induced Convulsions:

Alteration in the blood-brain permeability barrier by the continuing action of acetylcholine may be the biochemical substrate for the post-electroshock hypersynchrony of the electroencephalogram. Such a possibility is evident in the demonstration of an increase in the concentration of cocaine in brain tissues three days after a series of 12 induced convulsions (Aird et al, 1956). The change in concentration of this large molecule, ordinarily absent

in brain tissue, was associated with the appearance of hyper-synchrony (delta bursts) in the electroencephalogram.

We have confirmed the many previous reports that convulsive therapy induces electrographic hypersynchrony (Fink and Kahn, 1956; Fink et al, 1961). Despite a constant application of treatments there is a great variability in the time of appearance, the duration and the extent of the electrographic slow wave activity as well as the sensitivity to modification by alerting, hyperventilation and barbiturates in psychiatric populations. The early appearance of high degree hyper-synchrony and its persistence throughout a treatment course has been described as prerequisite to improvement following electroshock (Roth, 1951; Roth et al, 1957; Fink and Kahn, 1956). It is possible that the differences in the degree of induced EEG hypersynchrony may be related to differences in the activity of central cholinergic mechanisms. The failure of certain patients to develop hypersynchrony may thus be associated precluding a clinical response to induced convulsions. Tower

and McEachern (1949a), in their study of craniocerebral trauma, included observations of six psychiatric patients undergoing convulsive therapy. Studying the patients after 3-7 treatments they reported free spinal fluid acetylcholine in two patients; and an increase in cholinesterase-II and a decrease in cholinesterase-I with a reversal of the ratio of cholinesterases in five of the six patients. From these observations they concluded that the spinal fluid changes in induced convulsions were more like those of craniocerebral trauma than those of spontaneous epilepsy.

Regarding the one p-tient in the series who failed to show either free acetylcholine or a cholinesterase ratio reversal in the spinal fluid, they wrote: "It is interesting that this patient was the only one of the six to show no response to treatment." If electrographic hypersynchrony is a reflection of increased free acetylcholine, subjects who maintain hypersynchrony and those in whom it disappears rapidly may be exhibiting differences in the kinetics of the cholinesterase-

(6) Cholinesterases and the Classification of Psychoses:

These studies may also have application to the problem of autonomic reactivity and the classification of the psychoses. Funkenstein and others ( ) have demonstrated a relationship between the blood pressure response to injected mecholyl and the clinical response of psychiatric patients to convulsive therapy. Mecholyl is a potent cholinergic agent which induces vasodilation, tachycardia, sweating, and increased peristalsis. It is rapidly hydrolyzed by cholinesterase-I and slowly by cholinesterase-II. The blood pressure of subjects falls after injected mecholyl and returns to the baseline within five to more than 20 minutes. Patients whose blood pressure returns to the baseline within 5 minutes have been classified as Group I, II, or III reactors; those whose blood pressure takes 20 or more minutes to return to baseline, as Group VI and VII reactors. Group I and Group II-III reactors

have a 9 and a 35% recovery rate, respectively, while Group VI and Group VII reactors 89% and 97% recovery rates (Funkenstein et al, 1952). Group I to III reactors may be looked upon as patients in whom the injected mecholyl is rapidly hydrolyzed; while Groups VI and VII have a slow hydrolysis rate. We may predict, therefore, that the blood cholinesterase activity levels of Groups I-III would be high; while the activity of Groups VI - VII would be low.

A similar analysis may be made regarding central nervous system levels of cholinesterase-I in the development of EEG hypersynchrony and spinal fluid levels of acetylcholine, providing a basis for a congruent hypothesis regarding central nervous system reactivity to induced convulsions and to peripheral cholinergic agents.

CONCLUSION:

Central cholinergic mechanisms appear to be a significant factor in the convulsive therapy process. The published data may be interpreted to indicate that induced convulsions are associated with an increased in intercellular acetylcholine to levels greater than can be destroyed by cholinesterase-I activity. Vasodilation and increased cellular permeability are followed by increased amounts of cholinesterase-II and other enzymes and electrolytes in intercellular fluids.

These changes are reflected in the increased electrical hypersynchrony which is recorded in scalp electrodes, and which can be modified by anticholinergic drugs as atropine, benactyzine, and diethazine.

The changes in the cerebral biochemical milieu alter cellular activities sufficiently to be associated with altered behavior of subjects. Failure to induce high and persistent concentrations of acetylcholine and failure to induce concomitant electrolyte changes results in a failure to produce behavioral change.

Differences in the rates of development of cerebral changes reflect differences in their dependence of subjects on cholinergic mechanisms or in their sensitivity to changes in acetylcholine levels. These differences provide the basis for the classification of the mentally ill by Funkenstein and by Fink and Kahn (1961).

These observations provide a rational biochemical basis for the mode of action of induced convulsions in altering the behavior of psychotic subjects. These views are consistent with the more general neurophysiologic-adaptive theory expressed earlier (Fink, 1957).

REFERENCES

Aird, R. B., Strait, L. A., Pace, J. W., Hrenoff, M. K. and Bowditch, S. C. Neurophysiologic effects of electrically induced convulsions. *Arch. Neurol. Psychiat. (Chic.)*, 1956, 75: 371-378.

Bornstein, M.D. Presence and action of acetylcholine in experimental brain trauma. *J. Neurophysiol.*, 1946, 9: 349-366.

Brenner, C. and Merritt, H. H. Effect of certain choline derivatives on electrical activity of the cortex. *Arch. Neurol. Psychiat. (Chic.)*, 1942, 48: 382-395.

Burgen, A. S. V. and MacIntosh, F. C. The physiological significance of acetylcholine. In K. A. C. Elliot, I. H. Page and J. H. Quastel (Editors), *Neurochemistry*. C. C Thomas, Springfield, Ill., 1955: 374-375.

Chatfield, P. O. and Dempsey, E. W. Effects of prostigmine and acetylcholine on cortical potentials. *Amer. J. Physiol.*, 1942, 135: 633-640.

Cone, W. V., Tower, D. B. and McEachern, D. Acetylcholine and neuronal activity in epilepsy. *J.A.M.A.*, 1948, 73: 59-63.

Dale, H. H. The action of certain esters and ethers of choline, and their relation to muscarine. *J. Pharmacol. Exp. Ther.*, 1914, 6:147.

Elliott, R. A. C., Swank, R. L. and Henderson, N. Effects of anesthetics and convulsants on acetylcholine content of brain. *Amer. J. Physiol.*, 1950, 162: 469-474.

Fink, M. A unified theory of the action of psychodynamic therapies. *J. Hillside Hosp.*, 1957, 6: 197-206.

Fink, M. Effect of anticholinergic agent, diethazine, on EEG and behavior: significance for theory of convulsive therapy. *Arch. Neurol. Psychiat. (Chic.)*, 1958, 80: 380-387.

Fink, M. Effect of anticholinergic compounds on post-convulsive electroencephalogram and behavior of psychiatric patients. *Electroenceph. Clin. Neurophysiol.*, 1960, 12 (2): 359-369.

Fink, M. and Kahn, R. L. Quantitative studies of slow wave activity following electroshock. *Electroenceph. Clin. Neurophysiol.*, 1956, 8:158.

Fink, M., Kahn, R. L., Karp, E., Pollack, M., Green, M. A., Alan, B. and Lefkowits, H. J. Inhalant-induced convulsions. *Arch. Gen. Psychiat.*, 1961, 4:259-266.

Freedman, A. M., Bales, P. D., Willis, A. and Himwich, H. E. Experimental production of electrical major convulsive patterns. *Amer. J. Physiol.*, 1949, 146: 117-124.

Funkenstein, D. H., Greenblatt, M. and Solomon, H. C. Autonomic nervous system test of prognostic significance in relation to electroshock treatment. *Psychosom. Med.*, 1952, 14: 347-362.

Giarman, N. J. and Pepeu, G. Drug-induced changes in brain acetylcholine. *Brit. J. Pharmacol.*, 1962, 19: 226-234.

Hampson, J., Essig, C. F., McCauley, A. and Himwich, H. E. Effects of di-isopropyl fluorophosphate (DFP) on electroencephalogram and cholinesterase activity. *Electroenceph. Clin. Neurophysiol.*, 1950, 2: 41-48.

Himwich, H. E., Essig, C. F., Hampson, J. L., Bales, P. D. and Freedman, A. M. Effect of trimethadione (Tridione) and other drugs on convulsions caused by di-isopropyl fluorophosphate (DFP). *Amer. J. Physiol.*, 1950, 106: 816-820.

Jenkner, F. L. and Lechner, H. The effect of Diparcol on the electroencephalogram in the normal subject and in those with cerebral trauma. *Electroenceph. Clin. Neurophysiol.*, 1955, 7: 303-305.

Johnson, L. C., Ulett, G. A., Johnson, M., Smith, K. and Sines, J. O. Electroconvulsive therapy (with and without atropine); effect on electronically analyzed electroencephalogram. *Arch. Gen. Psychiat.*, 1960, 2: 324-336.

Kabat, E. A., Glusman, M. and Knaub, V. Quantitative estimation of the albumin and gamma globulin in normal and pathologic cerebrospinal fluid by immunochemical methods. *Amer. J. Med.*, 1948, 4 (5): 653-662.

Loewi, O. Über humorale Übertragbarkeit der Herznervenwirkung. *Arch. f. d. ges. Physiol.*, 1921, 189: 239-242.

Nachmansohn, D. and Rothenberg, M. A. Studies on cholinesterase: on specificity of enzymes in nerve tissue. *J. Biol. Chem.*, 1945, 158: 653-666.

Richter, D. and Crossland, J. Variation in acetylcholine content of the brain with physiological state. *Amer. J. Physiol.*, 1949, 159: 247-255.

Roth, M. Changes in the EEG under barbiturate anaesthesia produced by electro-convulsive treatment and their significance for the theory of ECT action. *Electroenceph. Clin. Neurophysiol.*, 1951, 3: 261-280.

Roth, M., Kay, D. W. K., Shaw, J. and Green, J. Prognosis and Pantothal induced electroencephalographic changes in electro-convulsive treatment. *Electroenceph. Clin. Neurophysiol.*, 1957, 9: 225-237.

Spiegel, E. A. and Spiegel-Adolf, M. Permeability changes in the brain induced by Metrazol and insulin convulsions. *J. Nerv. Ment. Dis.*, 1941, 93: 750-755.

Spiegel, E. A. and Spiegel-Adolf, M. Physicochemical effects of electrically induced convulsions (cerebrospinal fluid studies). *Trans. Amer. Neurol. Ass.*, 1944, 70: 130-132.

Spiegel, E. A. and Spiegel-Adolf, M. Physiological and physicochemical mechanisms in electroshock treatment. *Confin. Neurol.*, 1953, 13: 38-63.

Spiegel, E. A., Spiegel-Adolf, M. and Henny, G. Physiocochemical changes in the brain accompanying electrically induced convulsive discharges. *Trans. Amer. Neurol. Ass.*, 1942, 68: 174.

Spiegel-Adolf, M., Wilcox, P. H. and Spiegel, E. A. Cerebrospinal fluid changes in electroshock treatment of psychoses. *Amer. J. Psychiat.*, 1948, 104: 697-706.

Stone, W. E. The role of acetylcholine in brain metabolism and function. *Amer. J. Phys. Med.*, 1957, 36: 222-255.

Torda, C. Effect of convulsion inducing agents on the acetylcholine content of the brain. *Amer. J. Physiol.*, 1953, 173: 179-183.

Torda, C. Effects of single injection of corticotropin (ACTH) on ammonium ion and acetylcholine content of brain. *Amer. J. Physiol.*, 1953, 173: 176-178.

Tower, D. B. and McEachern, D. Acetylcholine and neuronal activity. I. Cholinesterase patterns and acetylcholine in the cerebrospinal fluids of patients with craniocerebral trauma. *Canad. J. Research*, 1949a, 27 (Sect. E): 105-119.

Tower, D. B. and McEachern, D. The content and characterisation of cholinesterases in human cerebrospinal fluids. *Canad. J. Research*, 1949b, 27 (Sect. E): 132-145.

Tower, D. B. and McEachern, D. Acetylcholine and neuronal activity. II. Acetylcholine and cholinesterase activity in the ~~xxxxxx~~ cerebrospinal fluids of patients with epilepsy. *Canad. J. Research*, 1949c, 27 (Sect. E): 120-131.

Ulett, G. A. and Johnson, M. W. Effect of atropine and scopolamine upon electroencephalographic changes induced by electro-convulsive therapy. *Electroenceph. Clin. Neurophysiol.*, 1957, 9: 217-224.

Ward, A. A. Atropine in the treatment of closed head injury, *J. Neurosurg.*, 1950, 7: 398-402.

(July 2, 1965)
Jan. 3, 1958

MODE OF ACTION OF BIOCHEMICAL AGENTS IN BEHAVIOR

The CNS is dependent upon a number of enzyme systems for proper metabolism and function, including cholinesterase-acetylcholine, glucose-phosphatase, etc.

The variety of agents that affect CNS function and, thereby, behavior is large, since processes in equilibrium may be shifted in either direction by increasing or decreasing the available quantity of a metabolite.

A. For drug action, therefore, the following are considerations in potency:

(a) Does a drug affect an enzyme system in a reliable way?

(b) Can it get to CNS to affect the system?

(c) What defenses does organism process to block or limit drug's action once in CNS?

B. Secondly, while an agent may directly affect the metabolism of a specific system, to what extent is the CNS dependent upon the system? Is the defect thus induced significant for the metabolism of CNS underlying behavior? What defenses can body call into play when system is affected to substitute other energy systems?

Thus, the variation in drug effects in behavior depend upon:

(a) Behavior at onset; and predisposition (personality) to response;

(b) Drug dosage - availability to CNS and the effect it has on an enzyme system;

(c) Dependence of organism on specific enzyme system - and organismic defenses (i.e., substitution for affected system).

(d) Whether effect was gradual (allowing for defenses, i.e., alternate metabolic system) or acute (not allowing defense).

Individual differences in response may be due, thus, to differences in:

(a) Dosage, ratio & route of administration

(b) dependence on the affected system

(c) adaptive ability to biochemical changes.

To these changes, EEG is a gross approximation and indicator, reflecting the homeostatic balance in various enzyme systems of the CNS.

Cholinergic Mechanisms and Behavior:

Coleton theory

Krech

intracerebral (cannula) drugs -

? Effect on memory

neurophysiol theory — Bradley & Elkes

Winkler.

acetylcholine hydrolysis systems. Persistent hypersynchrony may result from a decreased rate of hydrolysis of acetylcholine, associated with low concentrations of either cholinesterase-I or cholinesterase-II. (Conversely, in patients with short-lived hypersynchrony, cholinesterase-I and -II in tissue and spinal fluid may be unusually high).

From these observations we would conclude that induced convulsions are associated with an increase in free acetylcholine in intercellular fluids, altering cerebral permeability and enhancing the appearance of cholinesterases. The level of free acetylcholine is maintained by repeated induced seizures. EEG hypersynchrony is one reflection of altered levels of acetylcholine and altered permeability of other electrolytes. It is probably that it is these changes in intercellular electrolytes that provide the biochemical substrate for the persistent behavioral changes following induced convulsions.