# BIOCHEMISTRY OF SEIZURES

*By*

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Neuronal cells manifest the property of electrical activity and autorhythmicity. When this becomes hypersynchronous, there occurs dense volleys of axonal discharges capable of activating all the corresponding target tissues. This results in massive peripheral reactions, the most prominent of which are the "Convulsions".

"Epilepsy", in addition to the above disturbance is characterised by a tendency to recurrence and is accompanied by disorders of consciousness and behaviour disturbances Epilepsy has come to be regarded not as a disease entity but as a symptom of various pathological processes occurring in central nervous system gray matter. It is considered more appropriate to denote these disturbances under the broad term of "Seizures". A seizure is characterised by the paroxysmal firing of neurons which can be visualized by electroencephalographic recordings, or by clinical manifestations or both.

Many factors can produce acute and temporary convulsions or chronic epileptogenecity in brain. The important ones, according to Sir Russel Brain (1955) are intoxication, anoxia, disturbances of neuronal metabolism, endocrine disorders, allergy, increased intracranial pressure, inflammatory diseases, trauma, congenital abnormalities, degenerative and vascular diseases, etc. In most cases the exact modes of action of such factors are not known.

From histopathological point of view, "Ammon's horn sclerosis", characterised by diffused loss of laminar cells and gliosis of white matter and subpial layer of cortex has been observed in some epileptic brains (Malamud, 1957). Tower and Elliott (1952) compared the epileptogenic focus and adjacent non-epileptogenic tissue from human brain. They found no significant difference in neuron density, neuronal morphology, viability (Nissl stain), glial (astrocyte) reaction or lipid content of the two samples. All sections appeared to be within normal limits. From these studies Tower (1958) concluded that specific histopathologic changes need not and do not consistently occur in epileptogenic brain samples.

The abnormal reaction in seizures may probably be due to abnormality in functional control of synaptic transmission, both inhibitory and excitatory, leading to neuronal hyper-activity. Since it is assumed that neuronal activity viz. the reception, conduction and transmission of impulses—depends directly or indirectly on chemical and metabolic events at cellular level, various workers (Tower, Waelsch, Elliott, Nachmansohn) have hinted at the possibility of a biochemical lesion underlying the occurrence of seizures. A number of studies over the past twenty-five years have sought to demonstrate biochemical abnormalities associated with seizures.

## ENERGY METABOLISM AND SEIZURES

Brain is an organ with a very high metabolic rate. Though it accounts only for about 2 per cent of body weight in an adult, its oxygen consumption in basal state accounts for approximately 20 per cent of oxygen consumption of the whole body (Lassen, 1959). It was therefore, logical to suppose that a defect in energy supply or utilization or both might be at the root of seizure process. Convulsions are accompanied by an increased rate of cerebral circulation and oxygen uptake (Geiger, 1957). An increased rate of circulation has been demonstrated in epileptogenic regions of brain during experimental convulsions in animals (Gibbs, Lennox and Gibbs, 1934). Penfield *et al.*(1939) have observed similar changes in human epileptic seizures. Similarly increased rate of blood circulation and oxygen consumption of brain have been demonstrated in monkeys by Schmidt, Kety & Pennes (1945) and in cats by Geiger and Magnes (1947) during convulsions produced by drugs. Penfield *et al.* (1939) showed that the increase in circulation begins a little time after the onset of local neuronal discharge and is limited only to the parts involved in seizures. None of the above mentioned changes persist in between the attacks or precedes the occurrence of a seizure. In the interictal period there is no detectable difference between epileptogenic and nonepileptogenic areas of the brain tissue as regards the energy metabolism. Elliott (1955) has pointed out that changes in blood flow or oxygen consumption occur only after the onset of seizures. These changes may only be reflecting demands for energy concident with increased neuronal activity. He concludes from these studies that the primary derangement may not be located in the energy metabolism.

*Changes in ATP and labile phosphorylated compounds*:—Olsen and Klein (1946, 1947) observed that there occurs significant decrease in creatine phosphate together with a fall in ATP during stimulation of nervous system, caused by various agents like metrazol, picrotoxin, camphor, caffeine, and electric

shock. Similar results have been reported by Stone *et al.* (1945), Dawson and Richter (1950), Gerard and Tupikova (1939) and McIlwain (1951).

Shapot (1957) using camphor as the convulsant, has shown that there occurs a fall in ATP content of the brain of rats followed by a rise in inorganic phosphate and ADP. Naka (1957) has observed that the concentration of labile phosphate decreases considerably in brains of rabbits subjected to electrical shock, insulin hypoglycaemia and sulphonal administration. Sadasivudu and Talwar (1961) obtained similar results in rats during metrazol convulsions.

The depletion of high energy phosphate compounds would be in response to increased energy demands. The synthesis of these compounds during the heightened neuronal activity is not sufficient to maintain the steady state concentrations (Abood *et al.* 1952). According to these authors the rate of ATP regeneration is also lowered during convulsions.

## ROLE OF ACETYLCHOLINE

The presence in gray matter of the central nervous system of a system for synthesis, storage, liberation and destruction of acetylcholine which can produce neuronal hyperactivity and evoke epileptiform activity in brain, led Forster (1945) to suggest that an abnormality in acetylcholine metabolism might underlie seizures.

Acetylcholine has been shown to be liberated at synaptic junctions of cholinergic fibres (Birks and MacIntosh, 1957) and, therefore, is believed to be inherently concerned with nerve impulse transmission. Hebb and Waites (1956) have further observed that acetylcholine vesicles travel along the fibres and get concentrated at synaptic junctions. Nachmansohn (1959 a) is of the opinion that acetylcholine is the "specific operative substance" in conduction. Feldberg (1945) has found that acetylcholine has stimulating effect on central nervous system or may facilitate response to other stimuli. Spikes and bursts of high amplitude, resembling epileptic activity have been produced by local application of solutions of acetylcholine to the cortex of cats (Miller *et al.* 1940; Brenner *et al.* 1942; and Forster, 1945). Intracisternal injections of acetylcholine also produce epileptiform brain waves (Bornstein, 1946) and generalized convulsions. Freedman *et al.* (1949) have observed epileptiform brain waves in curarized rabbits following intracarotid injection of DFP, a cholinesterase inhibitor. Di-isopropyl fluorophosphate (DFP) or Tetraethyl pyrophosphate (TEPP) when administered give rise to an increased

concentration of free active acetylcholine which is responsible for convulsions (Elliott, 1953).

In epileptogenic cortical foci, cholinesterase activity has been seen to be elevated as compared to that found in nonepileptogenic cerebral cortex (Tower and Elliott, 1952). Tower and McEachern (1949) have noted an appreciable amount of free, active acetylcholine in cerebrospinal fluid of the patients with frequent seizures, whereas none is detectable in samples of the patients suffering from other neurological disorders.

It is known that acetylcholine is concentrated and active in cerebral cortex and subcortical nuclei (gray matter), which are generally the seat of seizure activity. Acetylcholine as well as cholinesterase activity is present in all cortical layers (Pope, 1952), and a relationship is seen to exist between preponderance of acetylcholine system and neuronal cell density in mammalian cerebral cortex (Tower, 1954).

Mann *et al.* ( 1938 ) demonstrated the existence of acetylcholine in bound form in the brain. Acetylcholine in this form is physiologically inactive. Nachmansohn (1959 b) believes that excitation of the membrane by an intrinsic process leads to a dissociation of the complex, and free acetylcholine is released. The free ester acts upon a receptor, presumably a protein leading to a change in membrane permeability. The receptor-acetylcholine combination is reversible, and the free acetylcholine is spontaneously hydrolysed by the action of cholinesterase. This reaction takes place wiihin a fraction of a millisecond and thus permits the rapid return of receptor to its resting state. The permeability barrier is reestablished. In the recovery stage, acetylcholine is resynthesised from acetyl CoA and choline through the agency of the enzyme choline acetylase.

Richter and Crossland (1949) studied the sequence of changes in acetylcholine content of rat brain during electric shock stimulation and seizures. A decrease of 56 per cent in the bound form occured during 3-second period of stimulation. In the same study it was noted that free acetylcholine content of rat brain increased by 40 per cent in anaesthesia caused by barbiturates. The amount of bound acetylcholine thus appears to vary inversely with the functional activity of brain. Tower and Elliott (1952, 1953) have conducted *in vitro* studies on tissue slices from human epileptogenic foci and normal cortex slices. After 60 min incubation, the amount of acetylcholine (bound form) was determined in the slices. It was observed that epileptogenic slices failed to show an increase in bound acetylcholine, whereas non-epileptogenic tissue showed regularly an increase under the same experimental conditions.

From these studies they have put forward a hypothesis that "acetylcholine binding capacity" is impaired in epileptogenic tissues. Recently Takahashi *et al.* (1961) have studied the relationship between acetylcholine content, the seizure threshhold and seizure intensity. They observed that acetylcholine levels in brain did not correlate with changes in seizures threshhold. The acetylcholine level, however, was related to the intensity of seizures.

### ROLE OF AMMONIA

Ammonia in the brain arises as a normal consequence of neuronal activity (Torda, 1953), chiefly from cellular deamination and transamination reactions which are particularly active in brain ( Meister, 1954 ). The possible toxicity of ammonium ions has been proposed as an important factor in seizures (Sapirstein, 1943 and Braganca *et al.* 1953) but the literature concerning this point is contradictory. It should be emphasized that the results of determination of ammonia in brain tissue have to be interpreted with caution because of difficulties in its determination and variations caused by different methods of killing the animals. Richter, Dawson and Less (1949) found the level of ammonia in normal rat brain to be 0·28 or 0·47 mg/100 g depending on the method of killing. A rise of ammonia content was observed in rat brains during picrotoxin, metrazol and electric shock seizures and a fall in its content in nembutal narcosis. The same authors in a subsequent study (Richter, Dawson and Less, 1949) were, however, unable to detect significant rise of ammonia in cerebrospinal fluid in patients after electroshock or grandmal epileptic seizures. Stone (1955) has reported a 7-fold increase of ammonia level in dog brain preceeding the onset of convulsions produced by fluoroacetate poisoning. In metrazol convulsions also an increase of 3-4 fold was observed. Tower (1958) has reported an increase in the ammonia content of the medium during incubation of human epileptogenic slices. Nauruse *et al.* (1960) have also seen a marked increase in brain ammonia content in an epileptogenic strain of mice during preconvulsive and convulsive stages.

An intravenous infusion of a solution containing ammonium chloride and sodium bicarbonate which brings rapid rise in cerebral ammonia level induced cortical seizures only in two animals while cortical depression was observed in three. Subdural injections of ammonium chloride also failed to induce localized cortical excitatory activity. Recently, Takahashi *et al.* (1961) have shown that chronic administration of ammonium salts decreases brain excitability as regards seizure threshhold and seizure intensity. It has been demons-

trated that ammonia levels in brain do not run parallel to changes in excitability.

From these findings it may be concluded that the increase of ammonia content in brain does not initiate convulsive activity, but is one of the metabolic consequences of functional excitation of nervous tissue.

## GLUTAMIC ACID AND SEIZURES

Glutamic acid is situated at metabolic crossroads in cerebral metabolism between tricarboxylic acid cycle on one hand and aminoacid metabolism on the other. Its importance is attested by the fact that its concentration in the free amino acid pool of brain is higher (35 precent) than any other amino acid (Weil-Malherbe, 1952). Furthermore, it is present in higher concentration in brain than in any other tissue of the body (Krebs *et al.* 1949 and Tallan, Moore and Stein, 1954). Of all the amino acids, it is the only one that can support the respiration effectively in the brain (Krebs, 1935). In addition, glutamic acid decarboxylase is present only in the brain in mammalian tissues (Albers and Brody, 1959 and Lowe *et al.* 1958). This enzyme converts glutamic acid to gamma-amino butyric acid, a compound which exerts a powerful inhibitory action on stretch receptor neurons (Bazemore, Elliott and Florey, 1956, 1957).

Relationship of glutamic acid to epilepsy dates back to the work of Price, Waelsch and Putnam (1943) who reported beneficial effects obtained by oral administration of this amino acid in patients suffering from petitmal type of seizures. The seizures were decreased in frequency. Waelsch (1949) has also observed that the use of glutamic acid anti-metabolites in animals produces convulsions and mental retardation, the states in which glutamic acid therapy is beneficial for human beings Studies in mental defectives, without convulsions, by Albert, Hoch and Waelsch (1946) and by Zimmerman *et al.* (1946, 1949) have also shown that I.Q. of these patients rose significantly during the period of glutamic acid administration and dropped to almost original level when glutamic acid therapy was discontinued.

Investigations in experimental animals have shown that in a strain of mice susceptible to audiogenic seizures, glutamic acid administration reduced the incidence of seizures (Ginsberg *et al.* 1951). Administration of methionine sulfoximine which blocks the synthesis of glutamine in brain and other tissues, also leads to violent convulsions (Speck, 1949). Using this convulsant, Peters and Tower (1959) have reported that the content of glutamic acid falls on in-

cubation of epileptogenic slices while that of control cerebral cortex slices increases significantly under similar experimental conditions. Narang (1961) observed that glutamic acid and glutamine content of brain falls significantly in the course of metrazol or electroshock convulsions in rats and in chronic epileptogenic foci in monkeys. Similar results were reported by Berl *et al.* (1959) in epileptogenic foci produced by ethylchloride spray on cerebral cortex of cats. Investigations with other convulsants such as strychnine (Haber and Saidel, 1948), fluoroacetate (Dawson, 1953) and magimide (Tower, 1957) have also exhibited significant decrease in cerebral glutamic acid content.

A fall in the glutamic acid content might be probably due to its increased oxidation or decreased synthesis in convulsive states. Normally carbohydrates form the main fuel for energy supply of brain. In convulsions, there is an increased rate of metabolism, which cannot be fully sustained by oxidation of glucose. It has been pointed out by Geiger (1958) that during seizures, there occurs also on exidation of alternative substrates to keep pace with the demand of energy. Glutamic acid is probably the most important substance in this connection because of its presence in large amounts in the free amino acid pool of the brain and is, therefore, metabolized at a high rate during convulsions to support the oxidative metabolism of hyperactive neurons.

As regards the route for glutamic acid oxidation, there can be three possible pathways, (i) conversion to alpha-ketoglutaric acid by glutamic dehydrogenase. Alpha-ketoglutaric acid being one of the intermediates in tricarboxylic acid cycle, is further metabolized in the usual manner, (ii) transamination of glutamic acid with oxaloacetate or pyruvate by GOT and GPT respectively, yielding alpha-ketoglutatic acid and corresponding amino acids, iii)decarboxylation of glutamic acid to GABA by glutamic acid decarboxylase (GAD). GABA can further transaminate with alpha-ketoglutaric acid to form glutamic acid and succinic semialdehyde which in turn is converted to succinic acid by a dehydrogenase. Succinic acid again, is an intermediate of Kreb's citric acid cycle and is further metabolized.

In addition to oxidative metabolism, glutamic acid can also be converted to glutamine. As ammonia concentration in convulsions is high, glutamine synthesis should be favoured. Glutamine should thus rise during convulsions. In experiments reported by Berl *et al.* (1959) and Narang (1961), it has been observed that decrease in glutamine content occurs in convulsive tissues. Waelsch (1959) has reported that glutamine synthesis from glutamic acid and ammonia, a reaction catalyzed by glutamine synthetase takes place in endoplasmic reticulum of the neurons. This compartment is in continuity with the

extracellular compartment. The glutamine synthesised there, goes immediately out of the cells and does not mix with the pre-existing glutamine pool. Moreover, it is known that blood-brain barrier is permeable to glutamine, which can result in the excretion of this metabolite in jugular venous blood. Thus the synthesis of gluamine occuring from glutamic acid and ammonia does not lend material contribution to the cellular glutamine pool.

## GAMMA-AMINO BUTYRIC ACID (GABA)

Presence of this amino acid in brain was first noted by Roberts, Frankel and Hartman in 1950. Roberts and Frankel(1950) and later Tallan, Moore and Stein (1954) demonstrated that most, if not all, of GABA is present as free amino acid. Berl and Waelsch (1958) reported that GABA is predominently a constituent of gray matter rather than white matter of brain. Studies on subcellular fractions of cat cerebral cortex have revealed the distribution of this compound as follows : Mitochondria—45 per cent, Nucleus—26 per cent Supernatant—29 per cent and Microsomes—0 per cent (Tower and Albers, 1959). VanGelder and Elliott (1958) have shown that GABA does not cross blood brain barrier in normal animals.

Formation of GABA from glutamic acid is a reaction unique to central nervous system in mammalian tissues (Roberts and Frankel, 1950, 1951), The enzyme catalyzing this reaction, glutamic acid decarboxylase is found only in gray matter (neuronal) areas of central nervous system (Albers and Brody, 1959; Lowe *et al.*, 1958). Roberts and Frankel (1950) found that optimum pH for this enzyme is between 6.4-6 5. Pyridoxal phosphate is required as cofactor for the enzyme reaction.

Bessman *et al.* (1953) and Roberts and Bregoff (1953) showed the presence of an enzyme which can catalyze the transamination of GABA with alphaketoglutaric acid to give rise to glutamic acid and succinic semialdehyde. The latter is oxidised by a dehydrogenase to succinic acid which in turn is oxidized through the tricarboxylic acid cycle (Albers and Salvador, 1958; McKhann and Tower, 1959). The optimum pH for GABA—transaminase is 8.2. The pyridoxal phosphate, which acts as a coenzyme, is more firmly bound to this enzyme in contrast to glutamic acid decarboxylase (Killam, 1957; Killam and Bain, 1957).

It, therefore, appears that the route alphaketoglutaric acid via glutamic acid, GABA and succinic semialdehyde to succinic acid constitutes a shunt pathway by passing the classical alpha-ketoglutaric acid, succinyl CoA to succinate pathway via the tricarboxylic acid cycle This pathway accounts for

a considerable portion of normal cerebral metabolism both *in vivo* and *in vitro*. In isolated cortical slices, an average of 40 per cent of total metabolism could be accounted for by turnover of constituents of this pathway (McKhann, 1959).

*Florey's factor* I *and GABA*—In 1953, Florey found that extracts from mammalian central nervous tissue exert inhibitory effects on crustacean stretch receptor neuronal activity. Employing this preparation as an assay procedure, Bazemore *et al.* (1957) have purified Factor I from beef brain in a crystalline form, which has been found to be identical to GABA in its infra red spectrum and chromatographic Rf value. Florey and Florey (1958) further demonstrated the proportionality between the GABA content of the factor I preparations and their biological potencies. From these studies they concluded that GABA content could account for virtually all the factor I activity.

*Role of GABA in seizures*—The total tone at any time in the central nervous system is a resultant of numerous excitatory and inhibitory factors. Decrease in the content of inhibitory factors will lead to increased neuronal activity. As GABA constitutes one of the inhibitory factors in central nervous system gray matter, it was logical to postulate that depletion of this compound might be responsible for seizures.

The content of GABA in brain would depend on the relative activities of glutamic acid decarboxylase, which forms it, and GABA-alpha ketoglutaric acid transaminase which removes it. As pointed out already, both the enzymes are active at normal pH of 7.4-7.5, the optimum pH for decarboxylase is at 6.5 and that for transaminase is at 8.2. Roberts, Rothstein and Baxter (1958)suggested that the known tendency of acidosis to decrease the incidence of epileptic and experimental seizures and of alkalosis to increase them may be due to changes in the content of GABA which has been shown to increase in acidosis and decrease in alkalosis.

Acetzoleamide is a carbonic anhydrase inhibitor. It is known to have anti-convulsant properties. Koch and Woodbury (1958) have ascribed this action to an increase in intracellular carbonic acid. This would lower the pH and more of GABA would be produced by activation of decarboxylase.

Pyridoxine deficiency is known to cause convulsions in experimental animals (Chick *et. al.*, 1940; Daniel *et al.*, 1942 and Patton *et al.*, 1944) and in human infants (Hunt *et al.*, 1954 and Molony and Parmelee, 1954). Roberts, Younger and Frankel (1951) have demonstrated that glutamic acid

decarboxylase activity falls in pyridoxine deficiency. This leads to the fall in content of GABA. These findings suggest that in pyridoxine deficiency, convulsions may occur as a consequence of reduction in GABA content.

Thiosemicarbazide and other hydrazides can also produce convulsions (Parks *et al.*, 1952 and Reilly *et al.*, 1953). Killam and Bain (1957) found that in such seizures in rats the content of GABA and activity of decarboxylase are lowered. Killam (1957) also demonstrated that progress of electrical changes in semicarbazide induced convulsive animals was parallel to the progressive inhibition of glutamic acid decarboxylase activity. The convulsive activity is temporarily prevented by direct application of GABA to cortex or by its intraventricular administration. Addition of the coenzyme viz. vitamin $B_6$ prevents the seizures completely. These findings suggest that semicarbazide induced seizures also result from decrease in GABA content of brain.

In convulsions caused by insulin hypoglycemia in rats, Cravioto *et al.*, (1951) found that GABA content of the brain is reduced.

Hawkins and Sarret (1957) reported that oral administration of large doses of GABA or its lactam, 2–pyrrolidinone, a few hours before the convulsant, could protect the mice against metrazol and magimide seizures.

Tower (1960) incubated the cerebral cortex slices from human epileptogenic foci and methionine sulfoximine convulsive cat brains. He found a decrease in glutamate content of these slices during incubation. Presence in the medium of GABA or of its lactam, 2-pyrrolidinone, could prevent the loss of glutamic acid.

Tower (1960) has also reported clinical trials with GABA in patients with different types of epilepsy. Four out of fourteen patients experienced some improvement whereas in the rest of them there was no benefit from therapeutic employment of GABA.

Narang, Talwar and Singh (1960) studied the problem in acute convulsive brains as well as in chronic epileptogenic foci. They did not find any significant difference between the GABA content of these tissues and normal brain samples. Berl *et al.* (1959) have also reported similar results in cortical tissues made epileptogenic by ethylchloride spray. Baxter and Roberts 1960) have demonstrated the occurrence of seizures in presence of normal or elevated levels of GABA in brain. Seizures have been produced in mice and

cats with l-2, 4-diaminobutyric acid (Kessel, 1959). In these studies, seizures occured while GABA levels in brain were 2-3 times higher than normal. Kamrin *et al.* (1959) reported the therapeutic value of GABA in seizures produced by $MOB_6$ (4-methoxy-methyl pyridoxine), a specific pyridoxine anta gonist. However, Purpura *et al.* (1960) could not confirm this finding. They were unable to ameliorate the seizures by GABA or glutamic acid administration.

In view of these observations, the proposed role of GABA as an underlying factor in seizures needs reconsideration. Though the total concentration of GABA in brain may not be related to neuronal activity, yet it is possible that only a small fraction of its content may be functional and the changes occuring in this small amount may not be measurable by the currently available methods. Another function of GABA may lie in regulating an additional pathway for energy supply. Ammonia which increases during seizures has been shown to inhibit the classical alpha-ketoglutaric acid, succinyl CoA and succinate pathway (McKhann and Tower, 1961). Under such circumstances, therefore, the maintainance of metabolism through 'GABA-Shunt' pathway becomes more important during seizures.

The conversion of GABA to γ-guanidinobutyric acid has been demonstrated by Pisano *et al.* (1957). This pathway in the brain may serve as an additional mechanism for scavenging the ammonium ions, whose importance in seizures has already been discussed.

## ELECTROLYTES

The distribution of electrolytes within the cell and in the extracellular compartment is not uniform, $Na^+$ being the chief extracellular cation and $K^+$, the chief intracellular cation. The conducting cell makes use of the ionic concentration gradients resulting from unequal distribution of electrolytes for its special function i.e. for generation of small electric currents which conduct the impulse (Nachmansohn, 1959). The maintainance of appropriate ionic environment both within and outside the cell is necessary for the manifestation of the resting potential of cell membrane Spike potential in motor neurones arises because of a transitory increased permeability to $Na^+$ ions followed by an extrusion of $K^+$ ions (Hodgkin and Katz, 1949; Hodgkin, 1951). According to this hypothesis, the rising phase of the spike is caused by the inward movement of $Na^+$ ions along the electrochemical gradient, while falling phase is caused by the subseqnent outward movement of $K^+$ ions. In case the specific permeability to $Na^+$ ions increases, there occurs the generation of higher voltage with a concomitant increased rate of propagation, while

the subsequent increase in $K^+$ ions permeability accelerates the recovery of membrane potential so that it becomes ready to propagate another impulse.

*Role of potassium in neuronal metabolism* :—Potassium has been the subject of much speculation because of the well established association of this cation with neuronal activity. Presence of potassium ions in the medium stimulates the oxygen uptake and glucose utilization by the brain cortex slices (Dickens and Greville, 1953 and Ashfold and Dixon, 1935). The potassium stimulated brain respiration is inhibited by malonate (Braganca and Quastel,1952). Quastel (1959) has reported that malonate inhibition of the potassium stimulated brain respi ration may be reversed by the addition of oxaloacetate. This indicates the important role of citric acid cycle in stimulated brain respiration. Kini and Quastel (1959) have reported that the presence of potassium ions increases the rate of oxidation in brain cortex of both pyruvate-1-$C^{14}$ and pyruvate-2-$C^{14}$ to $CO_2$. This shows that the stimulating effect of potassium ions is probably due to its stimulating action on two reactions : conversion of pyruvic acid to acetyl CoA, and on another step in the citric acid cycle or a pace limiting reaction closely associated with the latter.

Some data is also available on the effect of $K^+$ ion administration *in vivo*. Bonnet and Bremer (1937) demonstrated the stimulating effect of potassium ions on cerebral activity of cat by intracarotid injection. Effects of direct application of potassium ions to cerebral cortex of cat were similar to those following intracarotid injection (Dubner and Gerard, 1939). On the other hand, Rubin *et al.* (1943) could not show any action when potassium was given intravenously.

*Potassium concentrating mechanisms.* Not much is yet known about the metabolic reactions which help in the concentration of potassium ions within the cell. Whittam (1958), has shown that ATP is utilized in the active transport of $K^+$ ions in the human red blood cells. It has been demonstrated by Krebs *et al.* (1951) that accumulation of potassium ions by the incubated cerebral cortex slices against the concentration gradient depends not only on the source of energy but also on the presence of glutamic acid. Pappius and Elliott (1956) and Ames (1956) have confirmed the above evidence by showing that the addition of glutamic acid to the incubation medium of cerebral cortex slices results in an increase in the content of intracellular potassium.

*Sodium pump.* Studies on a variety of preparations like invertebrase axons (Hodgkin and Keyne, 1954, 1955), the muscle fibre membrane (Botyle and Conway, 1941) and the frog skin (Ussing and Zerahn, 1951, 1952) have shown that sodium ions are actively transported across the membrane against

the electrochemical gradient. The sodium transport in these and other tissues is an energy requiring process. By injecting ATP into giant axons which had been poisoned with cyanide, Caldwell and Keynes (1957) obtained direct evidence that ATP can be utilized as an energy source for sodium pump. It has been suggested by a number of workers that sodium ions may be transported by a carrier situated in the cell membrane.

Schmidt-Nielsen *et al.* (1958) have shown that salt glands of marine bird albatross, respond to excessive intake of NaCl by secreting a hypertonic solution of NaCl. The secretory activity of these glands can be stimulated by injection of acetylcholine or acetyl β-methylcholine (Fange *et al.*, 1958) indicating that the secretory activity of these glands is regulated by a cholinergic nerve. Using this preparation, Hokin and Hokin (1960) have shown that addition of eserine either alone or together with low concentration of acetylcholine to the incubation medium results in an increase in turnover of phosphatidic acid and phosphoinositide. A similar effect has been demonstrated by the same authors in synaptic tissue incubated in contact with acetylcholine. From these series of observations, Hokin and Hokin have put forward the following scheme for active transport of $Na^+$ ions across the membrane :—

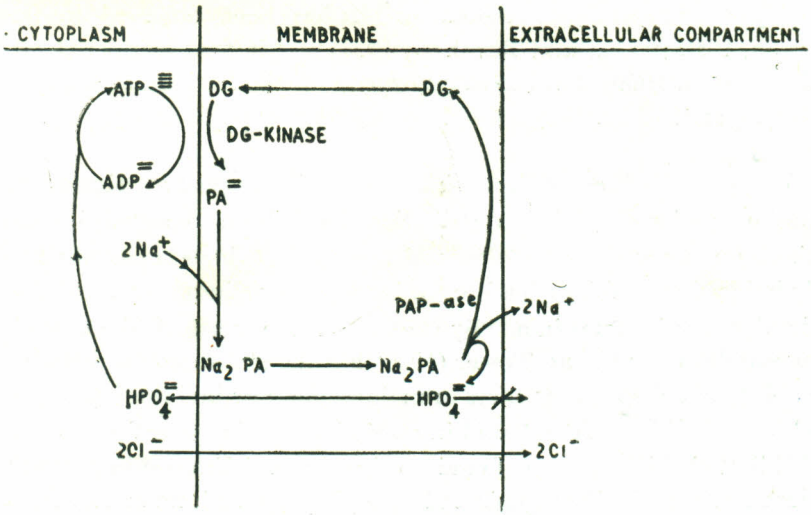


Fig. 1. Schematic representation of 'Sodium pump' adapted from *H*okin and *H*okin (1960)

At the inner surface of the membrane, diglyceride kinase (DG kinase) catalyzes the reaction between ATP and diglyceride (DG) to form phosphatidic acid. This combines with sodium ions, and sodium phosphatidate

crosses the membrane. On the other side of the membrane, sodium phosphatidate is hydrolyzed by phosphatidic acid phosphatase (PAP-ase) forming diglyceride and sodium phosphate. Digtyceride returns to inner surface of membrane, where the cycle is repeated. $Na^+$ ions are extruded out of the cell but a barrier to phosphate prevents it from accompanying sodium Phosphate is carried back to the cytoplasm where it is eventually incorporated into ATP through oxidative phosphorylation reactions in mitochondria. Membrane being selectively permeable to chloride ions, they are dragged across the membrane by electrical potential gradient established by the sodium pump. In addition to phosphatidic acid, phosphoinositide also plays an integral part in overall sodium transport.

An important feature of this mechanism is that it can explain in terms of chemical reactions how the energy of ATP can be utilized for active transport of sodium ions. This scheme conforms to the observed ratios of $Na^+$ ions transported to moles of oxygen consumed ($Na^+/O_2$). One phosphatidate carries two $Na^+$ ions. Therefore, one molecule of ATP, required for synthesis of one molecule of phosphatidic acid would lead to transfer of two $Na^+$ ions. Assuming the generally accepted overall P/O ratios of three, 12 $Na^+$ ions could be transported per molecule of oxygen. No figures are available for $Na^+/O_2$ ratio in salt gland of albatross. It has been observed in frog skin (Zerahn, 1956 ; Leaf and Renshaw, 1957) and in toad bladder (Leaf, Page and Andersen, 1959) that increased oxygen uptake for sodium transport gives a $Na^+/O_2$ ratio of 12.

*Changes in sodium and potassium ions during seizures :* Abnormalities in potassium and sodium ions distribution have been reported in convulsed brains by many workers. Colfer and Essex (1947) have reported that electrically induced convulsions lead to an increase in sodium content of the cortex and a decrease in potassium. By studies on the cortical slices under the same standard conditions, Tower (1960) has demonstrated an impairment of the ability of epileptogenic slices in maintainance or building up of potassium levels comparable to those found in nonepileptogenic cortex slices. Pappius and Elliott (1954), however, found no significant difference in potassium and sodium content of epileptogenic and nonepileptogenic human cortical tissues.

## CELLULAR STRUCTURAL COMPONENTS IN SEIZURES

In several types of experimental convulsive stresses, changes in the structural components of the brain such as nucleic acids, proteins and lipids have been repeatedly observed.

*Changes in nucleic acids*: Nucleic acids occupy a central position in cellular metabolism. Abood and Geiger (1955) have demonstrated that brain function can be sustained in cats on a glucose free perfusion fluid containing uridine and cytidine. Hyden (1943) showed that RNA content in anterior horn cells of guineapig decreases considerably when animals were exhausted in a running track. Geiger *et al.* (1956) observed that on stimulation of cerebral cortex directly by electrodes there occured an increase in non-protein nitrogen and a decrease in nucleic acid nitrogen and lipid nitrogen. All changes were reversible at rest for five minutes and were approximately proportional to the number of stimuli. Geiger-Ruth (1956) demonstrated histologically in neuron culture that there occurs an active extrusion of nucleolar material into the cytoplasm when they are stimulated by electric shock or metrazol. A fall in nucleolar PNA of ganglion cells of the supraoptic nucleus after stimulation was found by Edstrom and Eichner (1958). Sadasivudu and Talwar (1961) observed a decrease in PNA content of brain consistently in convulsive states. They further precised the nature of the change and showed that the major fractions undergoing depletion were the nuclear soluble and nucleolar PNA of brain (Talwar, Sadasivudu and Chitre, 1961). Vraa-Jensen (1957) from cytochemical studies has observed an increase in cytoplasmic RNA in intiial stages of neuron activity followed by a decrease on continued stimulation. Recently Hyden and Pigeon (1960) from a study on Deiter's cells observed increase in RNA and proteins in nerve cells after vestibular stimulation for 25 minutes per day for 7 days. A decrease occured in RNA and proteins of oligodendroglia surrounding the nerve cells.

In addition to the changes in the content of nucleic acids, there occurs a modification of the composition of cortex PNA on stimulation (Geiger, 1957). The total amounts of cytidine and adenine increase, whereas that of uridine and guanine remains constant. It has been suggested that these changes indicate an alteration in the type and molecular composition of the ribonucleic acids present in the brain, which in turn would induce the synthesis of new protein molecules associated with these nucleic acids. According to Holger Hyden (1958) such ribonucleoprotein molecules would be the structural components of memory.

*Changes in proteins*: Dawson and Richter (1948) were the first to show that ammonia was liberated during the functional activity of the nervous tissue. They suggested that the ammonia production was due to the utilization of nitrogeneous constituents by the nervous tissue. The liberation of ammonia was also noted in the *in vitro* incubation of brain slices (Weil-Malherbe and Drysdale, 1957 ; Vrba (1958). Vrba (1956) has implicated

brain proteins in ammonia formation and he has postulated a cyclic process during which amide-bound nitrogen of proteins is released on activity and combined with glutamic acid to form glutamine. The protein amide bonds are then resynthesised during rest. Changes in the brain proteins during stimulation have also been reported by Ungar *et al.* (1957, 1958). The changes were reversible and confined to the configuration of the protein molecules. Abood *et al.* (1958) showed a fall in aminoacid content of the bullfrog sciatic nerve when tetanised indicating a probable utilization of proteins during functional activity.

Findlay *et al.* (1954) have observed that radioactive phosphate is rapidly incorporated into phosphoproteins of the brain tissue. Heald (1958) has shown that the phosphoprotein fraction has the highest turnover following electrical stimulation of brain slices. The phosphoproteins have been suggested to be the probable intermediates in metabolic reactions involving phosphate transfer.

*Changes in phospholipids* :– Induction of convulsions by electric shock in rates, leads to a fall in the incorporation of $P^{32}$ into the phospholipids of brain (Dawson and Richter, 1950). Similar results where obtained by Torda (1954) in cerebral cortex slices.

## CONCLUSION

The evidence which has been reviewed does not as yet permit a precise definition of the mechanism responsible for seizure activity. Probably a number of factors are involved. Any one of them or a combination, lowers the threshhold of excitation so that impulse generation is facilitated and hyperactivity results.

In Fig. 2 metabolic reactions underlying some of the factors known to cause seizures states have been assembled to present an integrated picture. The integrity of these pathways must be maintained to prevent epileptogenecity.

Many of the essential processes of the cell require a regulated supply of energy in the form of ATP and other high energy compounds. Factors interfering in its production or utilization may cause convulsions (anoxia, hypoglycaemia, asphyxia etc,).

Maintenance of an appropriate ionic environment within and outside the cell is of primary importance for determining the polarization of the mem-

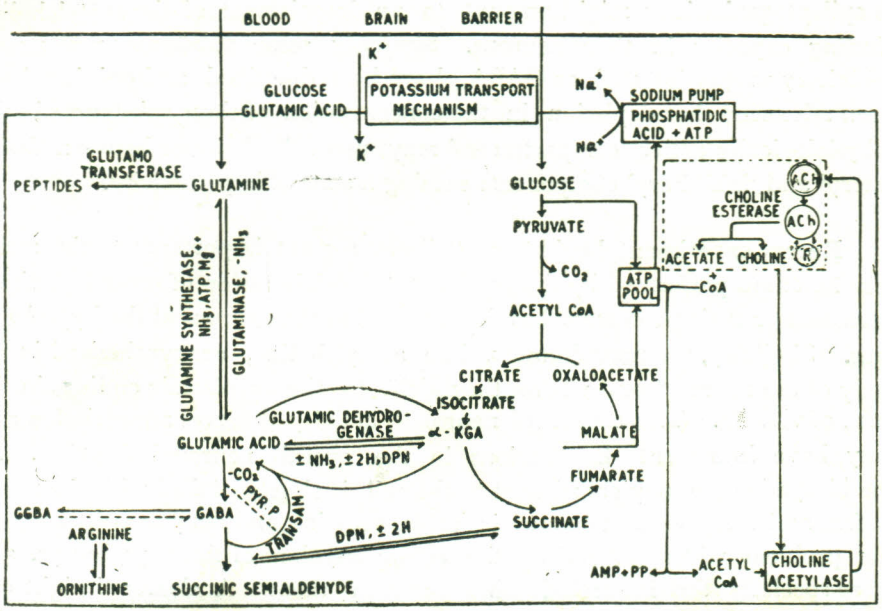


Fig. 2. Integrated representation of the metabolic reactions affecting the activity of neuronal cells. Mechanisms linked with the maintenance of appropriatiate K and Na concentration have been indicated.

brane, and in the processes connected with the transmission of the impulse. Only a hazy knowledge is as yet available to delineate the processes responsible for concentration of $K^+$ in the cell. Its transport is reported to be associated with the uptake of glucose and glutamic acid in the neuronal tissue. The series of papers published by Hokin and Hokin have given rise to a concept of "Sodium pump", a mechanism in which structural components of the membrane, mainly the phosphatidic acid and phosphoinositides play an active role in the extrusion of $Na^+$ through an alternate process of phosphorylation and dephosphorylation. Though this concept is well supported by their experimental results,its ultimate validity and its universal application should be accepted with reserve. According to McIlwain, such a mechanism falls short of the requirements on kinetic grounds. There is little need to point out that all mechanisms affecting the ionic distribution directly or through the via media of intracellular metabolic reactions, would be of extreme importance in the manifestation of convulsive phenomenon.

In the central nervous system, presence of various neurohumors, such as acetylcholine, has been recognised, which play a role in the synaptic transmission. Tower and his colleagues have proposed that the basic lesion

in epileptogenic tissue might amount to an impairment of the acetylcholine binding capacity in these neurons. Similarly some substances with strong inhibitory properties, such as GABA, have been described and opinions have been advanced that depletion in the concentration of these substances in the 'physiologically active compartments' may upset the balance between 'facilitatory' and 'inhibitory' components causing a state of hyperactivity.

Recent experimental evidence attributes a great importance to the secretory functions performed by the neurons. The elaboration of several pituitary hormones or their precursors has been traced to the neurons of the hypothalamus. Similarly, the neurohumors, like acetylcholine, are synthesized in the body of the neuron from where they drift to the axoplasmic endings in the form of vesicles. This aspect of neuronal activity deserves appreciation and may have important implications in the overall neuronal function. As the biosynthesis of any constituent in the cell is closely linked with the metabolism and turnover of ribonucleic acids, proteins, and enzymes, the scope of the problem widens to include the comprehension of these pathways, and more specially the interrelationship between them. A clearer insight can only emerge from a better understanding of the factors responsible for regulation and control of these reactions. The physicochemical barriers and the membrane phenomenon do have pertinence and the internal cytoskeleton of the cell requires further definition. The problem of hyperexcitability of the neuron, in its ultimate analysis, is not divorcable from the problems connected with the understanding of the normal neuronal function.

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