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Biochemical studies on the epileptic cerebral cortex

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Abstract

Within the range of our investigations the most important biochemical characteristics in the brain of idiopathic epileptic patients seem to be defect in the production of and the attendant decrease in free amino acids of the brain. On account of these phenomena there seem to occur the acceleration of the ChE activity and a poor utilization of glucose. Of the free amino acids in the brain the combined amount of glutamic acid, glutamine and γ-aminobutyric acid (GABA) will occupy the major portion of the total free amino acids found in brain, and thus diminution in the contents of glutamic acid and GABA in the brain of idopathic epileptic patients has quite an important meaning. At the present stage it is not yet poible to give any definitive answer to the question why such decrease occurs but it is believed that the most urgent problem facing us today is the amino acid metabolism that is aociated with glutamic acid and the comparative studies of the amino acid metabolism in the epileptic brain to that in the non-epileptic brain are required. The fact that γ-amino-β-hydroxybutyric acid (GABOB), the substance that supprees the central excitation, is decreased seems to indicate biochemically the existence of a defect in the procees of excitation in the brain of idiopathic epleptic patients.

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BIOCHEMICAL STUDIES ON THE EPILEPTIC CEREBRAL CORTEX

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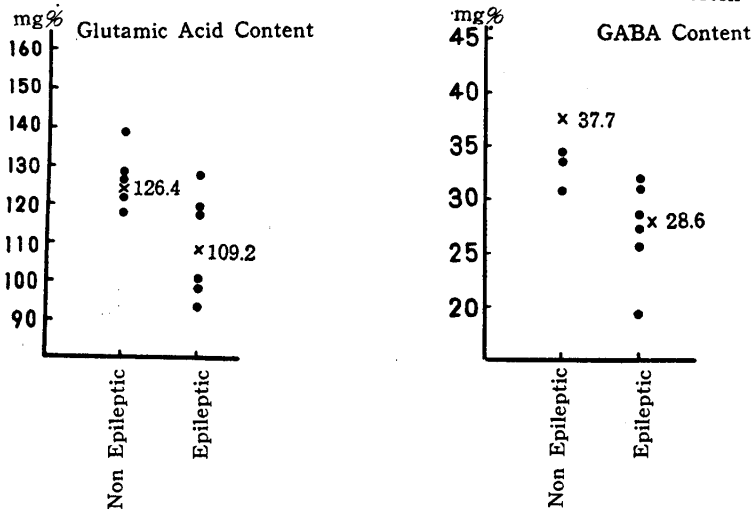
With advance in the technics of brain operation for therapeutic purpose, recently it has become possible to use the human brain tissue of epileptic patient for biochemical analysis. Since around 1950 Elliott and his colleagues published several papers of the biochemical studies on the epileptic human brain, and TOWER[1,2] has contributed an excellent review in this field. In our laboratory, too, we have studied the human brains of idiopathic epilepsy since 1952, a portion of which has been reviewed by JINNAI[3]. Present report describes a review of our recent biochemical research works on the cerebral cortex, area 6, of the patients with idiopathic epilepsy, about 100 cases and of those other than epilepsy, frontal lobe, 100 cases, with some correlation to other authors.

AMINO ACID METABOLISM

According to the work of INOUE[4] (1952) free amino acid nitrogen in the epileptic cerebral cortex is markedly decreased as compared with that in the non-epileptic, while no significant differences can be recognized in ammonia nitrogen and urea nitrogen. Further studies clarified that the nitrogen from amino acids, glutamic acid and γ-aminobutyric acid (GABA) are particularly decreased in the epileptic brain (KOKUDO[5], 1959) (Fig. 1). The results obtained by applying the recently advanced method of MOORE[7] (1959) with an ion exchange resine, Amberlite IR-120, also revealed the diminution of GABA and other amino acids in epileptic cerebral cortex (YAMAMOTO[8], 1960). Details of these results will be published elsewhere.

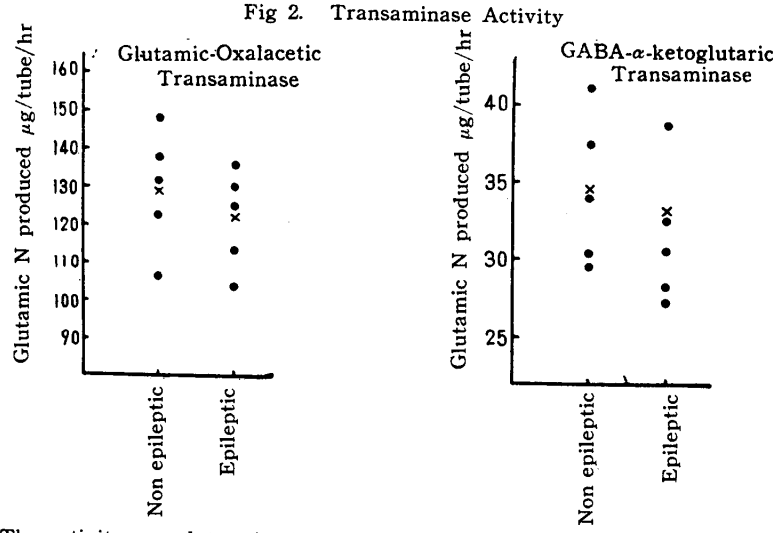
For the elucidation of the mechanism of diminution of free amino acid contents in the epileptic brain the transaminase activity of the brain was examined. Contrary to our expectation, the result showed no abnormalities in glutamic-oxalacetic transaminase and γ-aminobutyric-α-ketoglutaric transaminase (KOKUDO[5], 1959) (Fig. 2). As is well known in the deficiency

Fig 1. Contents of Glutamic Acid and GABA in the Cerebral Cortex



After separating alcohol extract of brain on paperchromatogram, spots are picked by Stein and Moore's method[6].
Comparative quantitative analysis by their ninhydrin coloration.

Fig 2. Transaminase Activity



The activity was determined by Awapara's technic[9].

of vitamin B_6, a certain kind of convulsion occurs. But chemical analysis of the brain of vitamin B_6 deficient mice gave data inconsistent with those of the epileptic brain suggesting that the mechanism of convulsion occurring in these mice is different from that in the idiopathic epileptic patient as will be touched upon later in detail.

It is generally accepted that the blood-brain barrier can hardly per-

meate amino acids and most of amino acids in the brain tissue should be produced *in situ*. Our observation proved the amino acids are produced in the brain in support of this view, and incorporation of radioactive carbon of ^{14}C-labeled glucose into amino acids could be demonstrated after incubating brain homogenate with ^{14}C-labeled glucose in Simon's solution for 24 hours at 37°C and developing the extract of this reaction mixture on the two dimension paperchromatography, proving that most of amino acids in brain tissues are produced from glucose *in situ*. Subsequently by means of autoradiographic technics radioactivities of amino acids were detected with X-ray films, and measured counts per minute of spots on paperchromatography using G-M counter on the other hand. The radioactivity of ^{14}C was detected on the spots of glutamine, asparagine, glutamic acid, aspartic acid and GABA. Identical experiments were conducted with the idiopathic epileptic and non-epileptic brains and obtained the results as shown in Table 1.

Table 1. ^{14}C-Glucose Conversion into the Brain Free Amino Acids (Av. of 8 cases)

cpm of the spot	non-epileptic	epileptic
Glutamic acid	84 ± 4	76 ± 4
Aspartic acid	63 ± 4	39 ± 3
Glutamine	32 ± 3	34 ± 3
Asparagine	30 ± 3	28 ± 3
γ-Aminobutyric acid	7 ± 3	0 ± 2

Conversion experiments were done according to Rafelson's method [11.10]. The reaction solution was separated on paperchromatogram, and count/min. of spots was measured with a low energy G-M counter.

The same observation on the material from the focus and the non-focus areas in one case of focal epilepsy proved the suppressed synthesis of aspartic acid in the focus area as illustrated in Table 2 (Kuroda[12], 1959).

Table 2. Aspartic acid formation from ^{14}C-glucose in the cerebral cortex

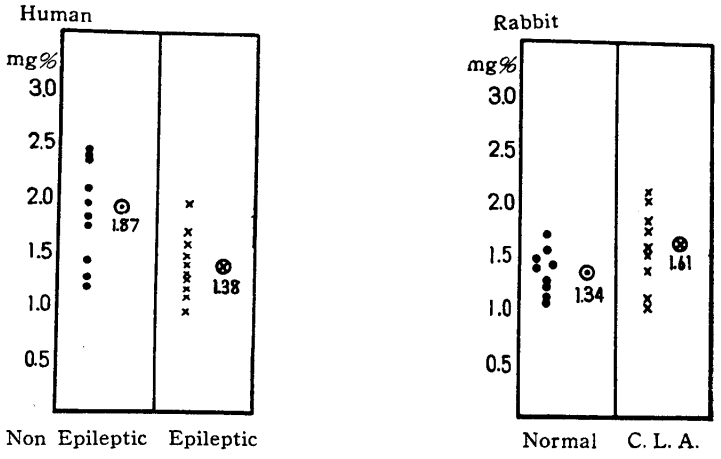
	cpm
focus	42 ± 3
non-focus	63 ± 4

Conversion experiments were done according to Rafelson's method. [10 11] The reaction solution was separated on paperchromatogram, and count/min. of spots was measured with a low energy G-M counter.

The synthesis of aspartic acid and GABA is markedly inhibited in the idiopathic epileptic brain with a slight inhibition of glutamic acid synthesis. On the basis of these findings we have carried further investigations on several enzyme system associated with the metabolism of GABA, aspartic acid and glutamic acid.

Recently INOUE[13] has proved that a portion of GABA undergoes β-oxidation in the brain tissue and is converted into γ-amino-β-hydroxybutyric acid (GABOB). This is the substance that suppresses the excitation of the central nervous system and now is a focus of the general attention. In his study on the contents of this substance in the cerebral cortex INOUE[15] also observed that this substance is slightly decreased in the idiopathic epileptic brain when compared with that in the non-epileptic brain (Fig. 3). This fact seems to indicate some disturbances in the excitation-suppressing processes in the idiopathic epileptic cerebral cortex.

Fig 3. Content of GABOB in the Cerebral Cortex



Quantative analysis by Inoue's method[15]

ACETYLCHOLINE METABOLISM

Since Loewi's[16,17] discovery (1921) of the so-called "Vagusstoff", associated with the stimulus transport of the vagus nerve terminals, leading to the modern theory of chemical transmission of nerve impulses, the biological significance of acetylcholine, cholinesterase (Ch E), and cholineacetylase (Ch A) has been clarified. At present it is well known that acetylcholine (ACh) plays an important role in the excitatory processes

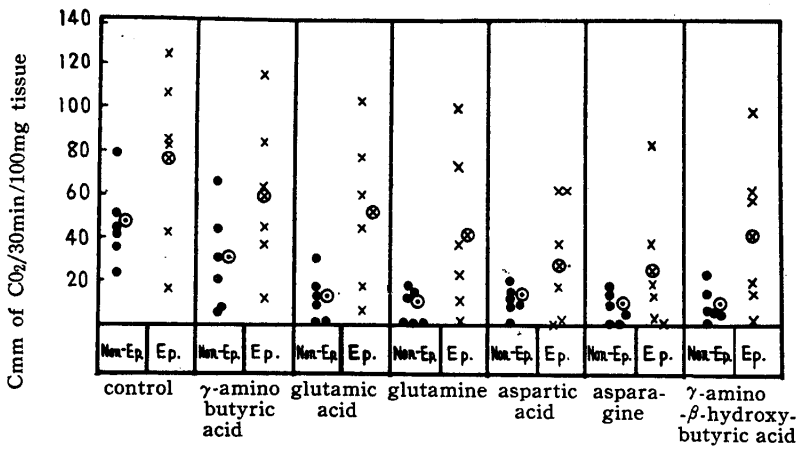
not only in the peripheral but also in the central nervous system.

Studies on the role of ACh on the function of the central nervous system have developed in conjunction with the observation on the changes in the local stimulation of the brain or with changes in the electroencephalograms after the application of this substance. However, with the discovery of two facts, namely, that ACh appears in the cerebro-spinal fluid after convulsive attack (TOWER and EACHERN[18]) and that convulsion can be induced by making ACh act on the surface of brain, has opened a way to the investigation of the ACh metabolism in epilepsy. Electroencephalographically, SJÖSTAND and MILLER[19,20,21] found the way to increase electrical activity by applying a high concetration of ACh on the surface of the brain of the animals pretreated with physostigmin. In addition, BRENNER and MERRITT[22] proved that similar changes can be brought about by applying a high concentration of ACh to the brain surface of animals without pretreatment of physostigmin and in encephalographical observation these changes are responsible for *grand mal*. Following upon these, there appeared reports by NAKAZIMA[23] that free ACh increases markedly immediately before metrazol convulsion and by ITO[24] that an epilepsy-like attack can be induced in the dog by applying a higher concentration of ACh solution on the cerebral motor cortex.

With these experimental studies, some observations on the brain of epileptic patients have been done by several authors. POPE[25] et al. (1946) estimated the cholinesterase (ChE) activity in the cerebral cortex of focal epilepsy, and found that the ChE activity of the focus is greatly increased when compared with that of areas other than the focus and suggested that the ACh metabolism is accelerated in the epileptic brain. OKI[26] (1952) of our department found that the ACh metabolism in the cerebral cortex of idiopathic epilepsy is accelerated and also the more advanced is the disease the higher is the acceleration of the ChE activity, after comparing the ChE activity on the cerebral cortex of idiopathic epilepsy and non-epileptic cerebral cortex. TOWER[1,2] presented a very interesting fact in that he found the rate of the increase within a given time of the bound ACh in the epileptogenic cerebral cortex is extremely low when compared with that in non-epileptogenic cerebral cortex in his experiments where he incubated the brain tissue slice in a given medium. He is of the opinion that the ChE activity is accelerated in epileptogenic cerebral cortex and that this acceleration is a compensatory measure for these changes. In these experiments TOWER also found the addition of glutamine or asparagine to the experimental materials will eliminate the impediment in the increasing processes of the bound ACh, and this is an extremely interesting finding.

On the other hand, to the exactly identical experimental system as employed by OKI[26], YAMAGUCHI[28] of our department added 0.1 ml of aspartic acid, glutamic acid, glutamine, asparagine and GABA solution at the concentration of M/10 respectively and estimated the ChE activity of the cerebral cortex. The results are illustrated in Fig. 4. In other words, it

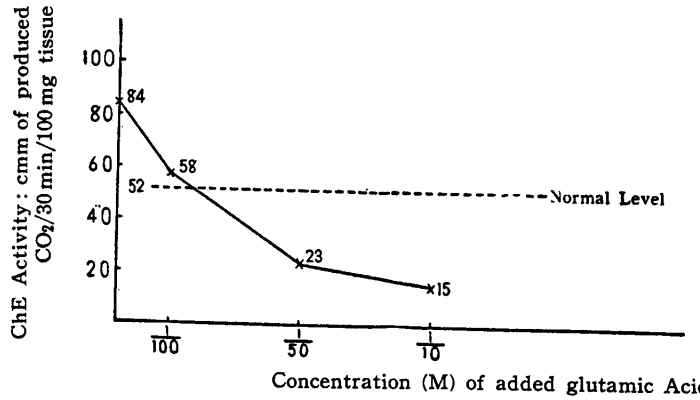
Fig 4. Influences of Amino Acids on ChE Activity of the Cerebral Cortex



Quantative analysis by Ammon's technics[27] with Warburg's manometer.

is obvious that every one of these amino acids inhibits the ChE activity more or less. In this instance, the ChE inhibitory effects of these amino acids can be recognized both in epileptic and non-epileptic brains, but by the addition of an equal volume of amino acid solution their inhibitory action in the epileptic brain is not so marked as in the non-epileptic. Therefore, it is assumed that the rise in the ChE activity of the idiopathic epileptic brain is due to the decrease in free amino acids in the epileptic brain as already mentioned. Concerning this problem, we further performed experiments by adding amino acids at various concentrations, and obtained the results as shown in Fig. 5. These are the results of our experiments in which glutamic acid solution of various concentrations was added to the experimental system, and it has been found that when 0.1 ml of M/100 glutamic acid is added to the experimental system (total volume of 2.2 ml), the ChE activity is restored to the normal level. This amount approximately coincides with the decreased amount of free amino acids observable in the idiopathic epileptic brain.

Fig 5. Influence of Glutamic Acid on ChE Activity of Epileptic Cerebral Cortex



Quantitative analysis done according to Ammon's technics[27] with Warburg's manometer.

GLUCOSE METABOLISM

In the brain tissues, glucose is the main source of energy supply, and significance of the glucose metabolism in the brain is great. The study on the glucose metabolism in the epileptic brain has its beginning with the study by ELLIOTT[29] in 1948. He observed the respiration and the aerobic as well as anaerobic glycolysis of human brain and found no differences between normal and epileptogenic brain tissues. Likewise KANEMATSU[30] (1953) of our department found no significant differences in the idiopathic epileptic brain and the non-epileptic brain as far as the respiration, aerobic- and anaerobic glycolysis, in his studies using Warburg's manometer. On the other hand, we performed the followig experiments, i. e. we estimated the glucose consumption before and after the incubation of brain homogenate by the direct determination and observed that the glucose utilization is impeded in the epileptic brain.

Furthermore, in this series of experiments similar estimation was conducted by adding aspartic acid, asparagine, GABA, and glutamine to the reaction system, and it was found that these amino acids accelerate the glucose consumption in the brain, and restore the decreased activity of the glucose consumption in the epileptic brains to the normal or higher level (YAMAMOTO[31]) (Fig. 6).

The same thing can be said of the hexokinase activity in the brain tissue as of the glucose metabolism. Namely, it has been proven that the hexokinase activity in the idiopathic epileptic cerebral cortex is lower than

Fig. 6. Using of Glucose in the Cerebral Cortex (μM/100mg tissue)

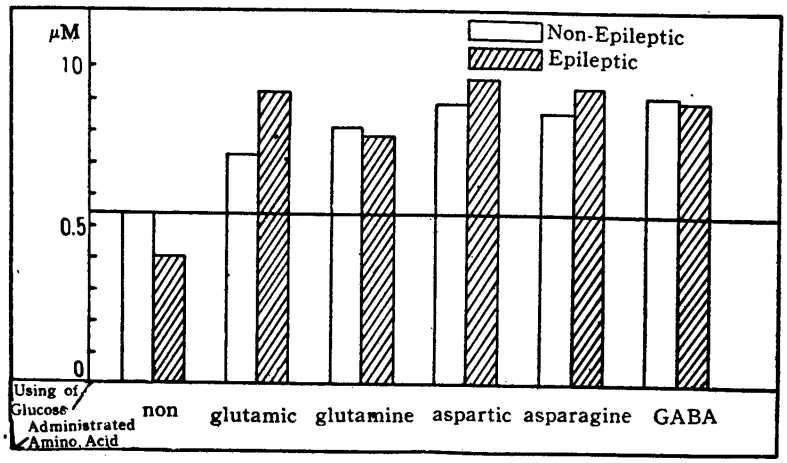
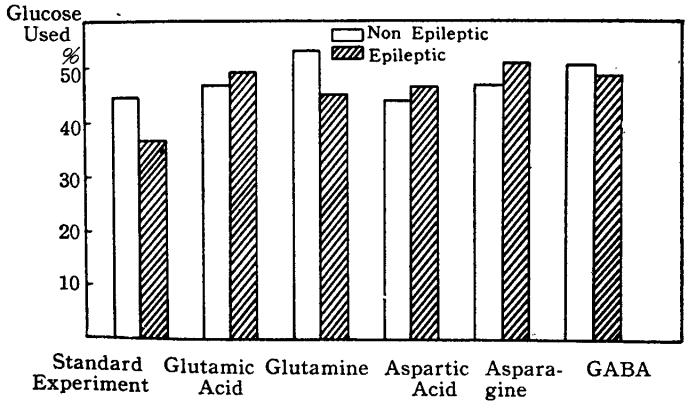


Fig. 6. Reaction system is composed of 0.5 ml phosphate buffer (pH 7.17), 0.5 ml 0.01M glucose, 1.0 ml 10% brain homogenate, 0.2 ml 0.1M amino acid, and enough distilled water is added to make the total vol. to 3.0 ml. Incubated 40 min. at 37.5°C. Glucose analysis is done by Somogyi's method[32,33].

that in the non-epileptic brain, and this decreased activity can be recovered or raised by the addition of such amino acids as asparagine, glutamic acid, GABA, glutamine and aspartic acid to the reaction system (YAMADA[34]) (Fig. 7).

Fig. 7. Influences of Amino Acids on the Cerebral Cortex Hexokinase (Av. of 8 cases)



The hexokinase actvity was estimated by Long's method[35].

Further, the oxidation of glucose-6-phosphate, 6-phosphogluconate, and ribose-5-phosphate, which are lying in the shunt pathway of glucose metabolism by Warburg-Dickens scheme, is somewhat inhibited in the idiopathic epileptic brain (ONODA[36]) (Fig. 8).

Fig. 8. Oxidation of Intermediates in the Warburg-Dicken's Pathway (Av. of 8 cases)

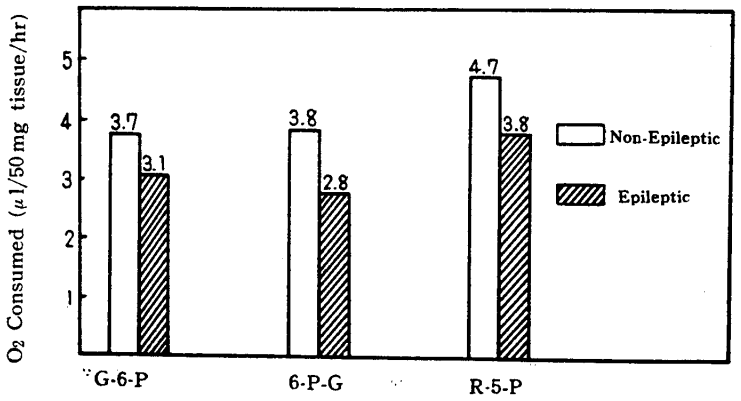
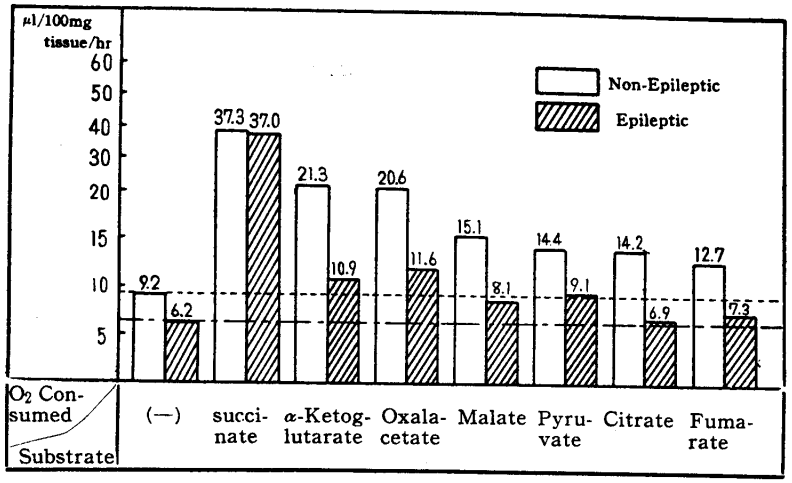


Fig. 8. Oxidation was estimated with Warburg's manometer[37]. Reaction system contained: 0.1 ml 1M NaF, 0.1 ml 0.1 M $MgCl_2$, 0.5 ml 0.06 M Tris.-buffer (pH 7.4), 0.2 ml of 1.5×10^{-3}M TPN, 0.5 ml M/10 substrate and 0.5 ml 10 % homogenate.

We have also studied the oxidation of members of TCA cycle, by using the mitochondrial fraction of brain separated by SUDA and HARA's method[14]. The oxygen consumption of the mitochondrial fraction was estimated with or without addition of these members as substrate in Warburg's apparatus. In this instance, first all, it has been found that the oxygen consumption of the brain mitochondria of the idiopathic epileptic brain prior to the addition of the substrate is lowered to two thirds that of the non-epileptic brain, and then the respiration is measured after the addition of various members of the TCA cycle as the substrate. The respiration in the idiopathic epileptic brain is decreased to about two thirds of the non-epileptic brain after the addition of pyruvate, in the case of addition of citrate, the oxidation hardly seems to take place in the idiopathic epileptic brain; in the case of adding α-keto-glutarate the respiration in idiopathic epileptic brain is inhibited, down to about one half. When succinate is added, the respiration between the two different brains shows hardly any significant difference. In the case of adding fumarate the respiration in the idiopathic epileptic brain is decreased to about one third that of the

non-epileptic brain, and in the case of oxalacetate the respiration is inhibited about one half of the non-epileptic brain (HIGUCHI[38]) (Fig. 9).

Fig. 9. Oxidation of TCA Cycle Members by the Cerebral Cortex Mitochondria (Av. of 8 cases)



Oxidation was estimated by means of Warburg's manometer[37]. Isolation of mitochondria was carried out by Suda and Hara's technics[39]. Experimental system was composed of 0.6 ml 0.1 M phosphate buffer (pH 7.3), 0.3 ml 0.01 M ATP-Na, 0.2 ml 0.02 M $MgCl_2$, 0.5 ml 10^{-5} M cytochrome C, 0.2 ml 0.1 M substrate and 1.0 ml mitochondria suspension.

COMPARATIVE STUDY WITH EXPERIMENTAL EPILEPTIC ANIMALS

In 1954 IMAIZUMI[40] discovered "ep-mice" which have spontaneous convulsion by simply given postural stimulation such as gently throwing up and down about 10-15 cm high on a board for several times, and such a convulsion can be induced in 100 per cent of adult mice under a certain given stimulation. In this instance, no drugs or any other physiological stimulations are required. Therefore, these mice are very useful pure strain for the study on the onset of convulsion. With these "ep-mice" AKIMOTO[41] et al. are conducting comparative studies using gpc and dd strains as the control. Namely, they have measured the contents of ACh, NH_3, glutamic acid, glutamine, GABA and the ChE activity of the brain.

Observations clarified that the decrease in glutamic acid and glutamine in "ep-mice" brain the same as in human epileptic brain, but differing from the latter, an increase in GABA and a decrease in the ChE activity have been found quite contrary as compared with human epilepsy. From this fact it can be assumed that the mechanism that induces seizure

in "ep-mice" is different from the mechanism inducing *grand mal* in human epilepsy.

As is known vitamine B_6 deficient mice are in a preconvulsion state. We conducted biochemical studies on the brains of the mice in a chronic vitamine B_6 deficient state kept on the vitamine B_6 deficient diet for about one month. As the results, the ChE activity is markedly diminished in the brain of these mice, and an increase in the water content (MATANO[42]). In addition, a marked decrease in the transaminase activity can be recognized in vitamine B_6 deficiency, and this can easily be understood from the fact pyridoxal phosphate is the co-factor of transaminase. In the human epileptic brain, as has already been mentioned, the ChE activity accelerates[26] and the transaminase activity is normal[5], and also the water content in the brain is normal[4] differing from the vitamine B_6 deficient brain. These facts seem to suggest that epilepsy is not directly associated with vitamine B_6 deficiency.

In our laboratory in the latent cerebral anaphylaxis animals induced by repeated injection of dilute antigen into the brain, it has been found that biochemical changes in the brain resemble closely to those changes in human epileptic brain, and the results obtained after various chemical analyses amply substantiate these similarities (JINNAI[3]). However, recently we have discovered that the content of GABOB in these animal brains is conversely increased beyond the normal level[14] (Fig. 3).

Such a comparative study between the experimental epilepsy and human epilepsy will often yield different points, which seem to be the most important characteristics for human idiopathic epilepsy. Therefore, such a method of study is believed to be very useful in the studies of the epileptic brain (Table 3).

Table 3. Comparison between the human epileptic and the experimental animal's cerebral cortex

	Human epileptic	L. C. L. A. rabbit	B_6 deficient mice	Ep-mice
ChE activity	Increased	Increased	Decreased	Decreased
transaminase activity	Normal	Normal	Decreased	—
Free amino acid content	Decreased	Decreased	Decreased	Normal
GABOB content	Decreased	Increased	Increased	—
Water content	Normal	Increased	Increased	—
K content	Normal	Increased	Normal	—

SUMMARY

Within the range of our investigations the most important biochemical characteristics in the brain of idiopathic epileptic patients seem to be defect in the production of and the attendant decrease in free amino acids of the brain. On account of these phenomena there seem to occur the acceleration of the ChE activity and a poor utilization of glucose. Of the free amino acids in the brain the combined amount of glutamic acid, glutamine and γ-aminobutyric acid (GABA) will occupy the major portion of the total free amino acids found in brain, and thus diminution in the contents of glutamic acid and GABA in the brain of idopathic epileptic patients has quite an important meaning. At the present stage it is not yet possible to give any definitive answer to the question why such decrease occurs but it is believed that the most urgent problem facing us today is the amino acid metabolism that is associated with glutamic acid and the comparative studies of the amino acid metabolism in the epileptic brain to that in the non-epileptic brain are required.

The fact that γ-amino-β-hydroxybutyric acid (GABOB), the substance that suppresses the central excitation, is decreased seems to indicate biochemically the existence of a defect in the processes of excitation in the brain of idiopathic epleptic patients.

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