

# $\gamma$ -Glutamyl Transpeptidase:

## Elevated Activities in Certain Neurologic Diseases

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### ABSTRACT

Ewen, Lilian M., and Griffiths, John:  $\gamma$ -glutamyl transpeptidase: Elevated activities in certain neurologic diseases. *Am. J. Clin. Pathol.* 59: 2-9, 1973.  $\gamma$ -glutamyl transpeptidase, an enzyme found by histochemical means to be located near the luminal border of endothelial cells, is shown to be elevated in the sera of patients with epilepsy. Elevations previously described have been confined to sera from patients with diseases of the liver and biliary system and from patients who have had myocardial infarctions. The increased activity in patients with epilepsy is apparently not the result of hepatotoxicity from drug administration. Elevated activity is not observed in cerebrospinal fluid of patients with epilepsy. Serum  $\gamma$ -glutamyl transpeptidase is also elevated following neurosurgery in patients with intracranial tumors. Similarities following neurosurgery in the patterns of this enzyme, 5'-nucleotidase, and alkaline phosphatase to those seen following myocardial infarction are described.

THE ENZYME  $\gamma$ -glutamyl transpeptidase has been used clinically as a diagnostic aid in diseases of the hepatobiliary system.<sup>11, 12, 16, 21, 26</sup> More recently, elevation of this enzyme has been described following myocardial infarction<sup>1, 8, 17, 20, 26</sup> at which time the enzyme usually remains in the normal range for 3 to 4 days, then increases to reach a peak about the eighth to the eleventh day after infarction.

This study describes elevations of serum  $\gamma$ -glutamyl transpeptidase in patients with epilepsy and in certain patients with intracranial tumors. The pattern of serum  $\gamma$ -glutamyl transpeptidase following neurosur-

gery is described and possible implications of elevations of this enzyme in serum are discussed.

### Method

The study included a total of 142 patients. There were 75 patients with epilepsy, of whom 25 were outpatients. In addition, there were 33 patients with multiple sclerosis, 11 patients with Parkinson's disease, and 23 patients with intracranial tumors. Specimens of blood were obtained from hospitalized patients with epilepsy and patients with tumors three times weekly during their hospital stays. Single blood specimens were obtained from outpatients with epilepsy, and from patients with multiple sclerosis and with Parkinson's disease. Serum was assayed for  $\gamma$ -glutamyl transpeptidase, alkaline phosphatase, 5'-nucleotidase, aspartate aminotransferase, lactate dehydrogenase, and creatine

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Table 1. Range of Values for Normal Persons

Test	E.C. No.	Abbreviation	Range of Normal Values
$\gamma$ -glutamyl transpeptidase	2.3.2.1	$\gamma$ -GTP	5-40 I.U./l.
Alkaline phosphatase	3.1.3.1	Alk P <sup>ase</sup>	12-42 I.U./l.
5'-nucleotidase	3.1.3.5	5'NTD	2-15 I.U./l.
Aspartate aminotransferase	2.6.1.1	AAT	5-35 I.U./l.
Bilirubin—total			To 1.0 mg./100 ml.
direct			To 0.4 mg./100 ml.
Creatine kinase	2.7.3.2	CPK	9-52 (women) 9-86 (men)
Lactate dehydrogenase	1.1.1.27	LDH	150-350 I.U./l.

kinase activities and for serum bilirubin by methods previously described.<sup>8</sup> Normal ranges for these parameters established using these technics are shown in Table 1.

Results

*Patients with Epilepsy*

$\gamma$ -glutamyl transpeptidase was elevated in the sera of 64 of 75 patients (85.4%) studied. The activity of this enzyme showed no consistent distinctive pattern changes when followed three times weekly in patients whose hospital stays ranged from 1 week to 2 months. Enzyme activity usually remained at a relatively constant level characteristic of the patient, although in some, peaks or depressions in activity did occur (Fig. 1). One patient admitted on two occasions during the period of study with an interval of 8 months between admissions was found to have similar mean serum  $\gamma$ -glutamyl transpeptidase activities of 200 and 225 I.U. per l. on the two occasions.

In the 64 patients showing elevations of serum  $\gamma$ -glutamyl transpeptidase activity, 23 (36%) had a mean value between 40 and 100 I.U. per l., 33 (51.5%) had a mean value between 100 and 200 I.U. per l., and 7 (11%) had a mean value in excess of 300 I.U. per l. Only one patient (1.5%) had a mean  $\gamma$ -glutamyl transpeptidase value between 200 and 300 I.U. per l., at 212 I.U. per l. The distribution of values in the 75 patients studied is shown in Figure 2.

Bilirubin levels and activities of aspar-

tate aminotransferase, alkaline phosphatase, 5'-nucleotidase, lactate dehydrogenase, and creatine kinase were measured in all patients and are shown in Figure 3, grouped according to the  $\gamma$ -glutamyl transpeptidase activities of individual patients. Elevations in alkaline phosphatase, 5'-nucleotidase, and aspartate aminotransferase were observed in a small number of patients, but no significant correlation between these and  $\gamma$ -glutamyl transpeptidase activity was observed. Correlation coefficients derived from these parameters are shown in Table 2.

No relationship between serum  $\gamma$ -glutamyl transpeptidase and frequency of seizures, age of onset, length of time the patient had epilepsy, or type of epilepsy was observed. Patients with all types of epilepsy showed  $\gamma$ -glutamyl transpeptidase activities in each of the arbitrary subdivisions previously described.

Serum  $\gamma$ -glutamyl transpeptidase showed no apparent correlation with dosage or type of medication when either single or multiple drugs were being administered. All levels of activity, including normal, were seen in patients receiving either single or multiple medications. Activity of this enzyme in the sera of patients not receiving medication for periods of 7 days showed no immediate change when medications were administered, and cessation of drug administration for a similar period in patients normally receiving anticonvulsants did not result in a related change in  $\gamma$ -glutamyl transpeptidase activity. Drugs

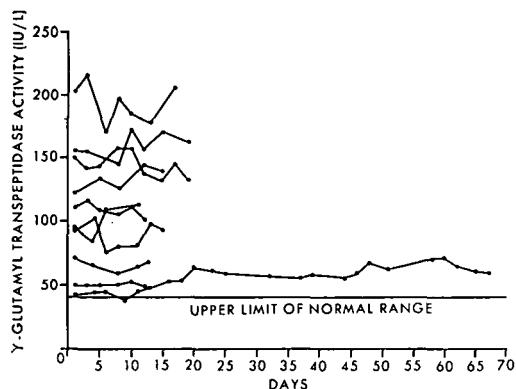


FIG. 1.  $\gamma$ -glutamyl transpeptidase activity in individual patients with epilepsy, related to time.

being taken by patients with elevated  $\gamma$ -glutamyl transpeptidase included anticonvulsants, tranquilizers, sedatives, both barbiturate and non-barbiturate, and analgesics.

Cerebrospinal fluid showed no detectable  $\gamma$ -glutamyl transpeptidase activity. Thirty patients were tested, 10 of whom had epilepsy with elevated serum  $\gamma$ -glutamyl transpeptidase activity, five of whom had other neurologic diseases, and 15 of whom had non-neurologic diseases.

#### *Patients with Other Neurologic Diseases*

Elevation of serum  $\gamma$ -glutamyl transpeptidase was not usually observed in patients who had certain demyelinating or neurodegenerative diseases.

**Multiple Sclerosis.** Of 33 patients under chronic hospital care for this disease, 27 had  $\gamma$ -glutamyl transpeptidase within the normal range. Six had values above the upper limit of normal of 40 I.U. per l., but only one had a value in excess of 51 I.U. per l.

**Parkinson's Disease.** Of 11 patients, nine had serum  $\gamma$ -glutamyl transpeptidase in the normal range. The remaining two had values in excess of 200 I.U. per l. One of these had a markedly elevated aspartate aminotransferase (195 I.U. per l.), and the other had elevated alkaline phosphatase and 5'-

nucleotidase activities (83 I.U. per l. and 18 I.U. per l., respectively).

**Other Degenerative Diseases.**  $\gamma$ -glutamyl transpeptidase was normal in five patients suffering from other degenerative diseases of the nervous system, including cerebral and cerebellar degeneration and motor neuron disease.

#### *Patients with Brain Tumors*

**Preoperative.**  $\gamma$ -glutamyl transpeptidase was elevated preoperatively in 13 of 23 patients with brain tumors. Those showing elevations included seven of 13 patients with astrocytomas, Grades I to IV, four of six patients with metastatic brain tumors, and two of four patients with cerebral meningiomas. No correlation between elevated serum levels and type or vascularity of the tumor was apparent. Histochemical study of the activity of  $\gamma$ -glutamyl transpeptidase in tumor tissue and its relationship to the vascularity of the tumor itself would provide more meaningful data. No significant differences were seen in the other parameters studied between the group with normal  $\gamma$ -glutamyl transpeptidase and elevated  $\gamma$ -glutamyl transpeptidase activity, although as in some patients with epilepsy, alkaline phosphatase and 5'-nucleotidase activities were elevated in a

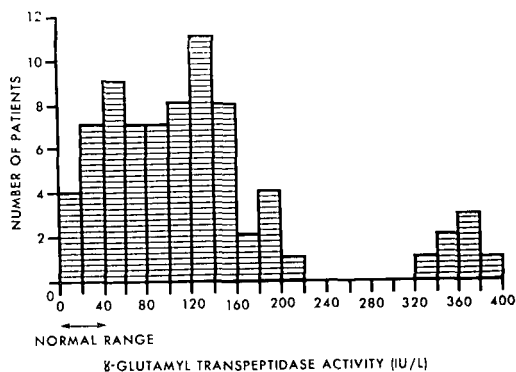


FIG. 2. Distribution of  $\gamma$ -glutamyl transpeptidase activities in 75 patients with epilepsy.

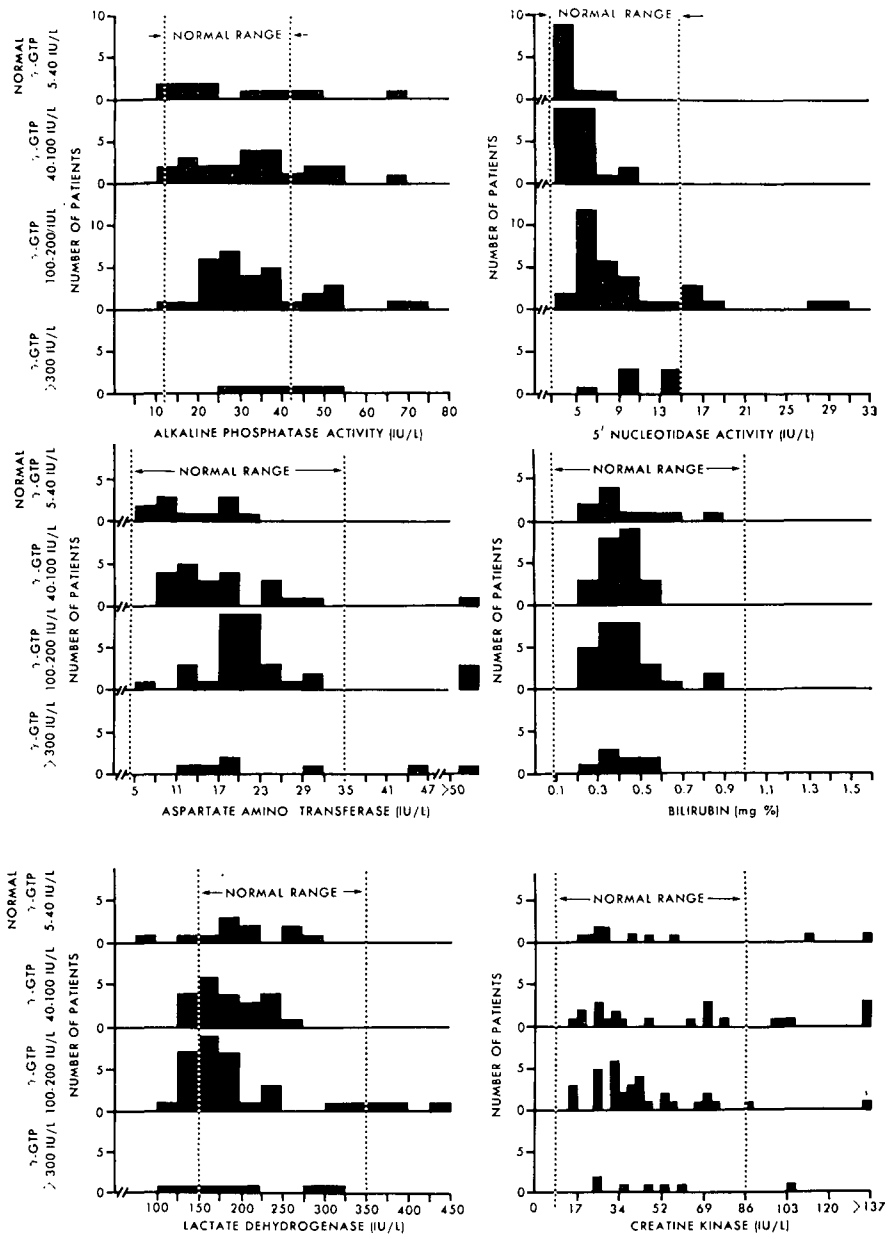


FIG. 3. Distribution of bilirubin levels and aspartate aminotransferase, alkaline phosphatase, 5'-nucleotidase, lactate dehydrogenase, and creatine kinase activities in patients with epilepsy, grouped according to  $\gamma$ -glutamyl transpeptidase activity.

small number of patients. Results are shown in Table 3.

**Postoperative.** Postoperatively  $\gamma$ -glutamyl transpeptidase activity became elevated. The pattern of activity, followed over a

period of 3 weeks, showed an increase in activity which reached a peak during the second week postoperatively. No significant change in bilirubin, lactate dehydrogenase, or creatine kinase was observed in this pe-

**Table 2. Correlation Coefficients Between  $\gamma$ -Glutamyl Transpeptidase Activity and Other Parameters Measured in 75 Patients with Epilepsy**

Test	r
Alkaline phosphatase	0.1374
5'-nucleotidase	0.4169
Aspartate aminotransferase	0.2961
Bilirubin	-0.0061
Lactate dehydrogenase	0.0888
Creatine kinase	-0.0139

riod of time. In those patients showing the most marked elevations of  $\gamma$ -glutamyl transpeptidase, alkaline phosphatase and 5'-nucleotidase also became elevated, reflecting the pattern of  $\gamma$ -glutamyl transpeptidase but with a less marked change in activity. Aspartate aminotransferase also increased slightly in a number of these patients. In patients showing elevated  $\gamma$ -glutamyl transpeptidase preoperatively, a secondary elevation in  $\gamma$ -glutamyl transpeptidase was observed, with a peak in the second week postoperatively, as in those patients with normal preoperative  $\gamma$ -glutamyl transpeptidase activities. Enzyme activities obtained over a 3-wk. period in patients representative of the two patterns of  $\gamma$ -glutamyl transpeptidase activity are shown in Figure 4.

### Discussion

$\gamma$ -glutamyl transpeptidase has been shown by histochemical means to be located in endothelial cells of a variety of tissues, including kidney, biliary tract, spleen, intestinal wall, heart, and brain.<sup>3, 4, 17</sup> It is an enzyme of large molecular weight, in excess of 200,000 as shown by gel filtration chromatography.<sup>9, 18</sup> The  $\gamma$ -glutamyl transpeptidase found normally in serum is believed to originate in the hepatobiliary system,<sup>14</sup> and it is logical that damage to this area, as may occur in obstructive hepatic disease, would release the enzyme into the blood stream.

It is not in dispute that  $\gamma$ -glutamyl transpeptidase is a sensitive indicator of

hepatobiliary disturbance, but there is now considerable evidence to show that the elevation of this enzyme in serum does not occur exclusively in hepatic disease. Previous publications<sup>8, 20</sup> have postulated that elevations of  $\gamma$ -glutamyl transpeptidase which occur following myocardial infarction may be related to the metabolic processes that occur during tissue repair. The pattern seen after neurosurgery with elevation of serum  $\gamma$ -glutamyl transpeptidase occurring over a time period similar to that following myocardial infarction is consistent with this suggestion. Although the molecular size of the enzyme might suggest that it would be unlikely to be found in serum except after destruction of tissues, the permeability characteristics of regenerating vascular membranes have not been completely investigated and may not be inconsistent with this postulate.

Because of the established relationship of elevated  $\gamma$ -glutamyl transpeptidase to hepatobiliary disease, increases of serum  $\gamma$ -glutamyl transpeptidase immediately suggest that the elevation is the result of changes in hepatic metabolism, either through hepatic damage following neurosurgery, or through drug-mediated hepatotoxicity, particularly in patients with epilepsy. Evaluation by several parameters of liver function in the patients studied did not confirm this. While slight elevations of aspartate aminotransferase were noted in some patients following neurosurgery, the elevation was not consistent nor sufficiently great to confirm parenchymal damage of the liver. This enzyme is widespread throughout the body, and the small elevation noted in some patients could have originated in the cerebral tissue subjected to surgical procedures. In patients with epilepsy this enzyme remained in the normal range and no other signs of hepatic dysfunction, either clinical or biochemical, were apparent, although  $\gamma$ -glutamyl transpeptidase was elevated.

Table 3. Preoperative Biochemical Data for Patients with Brain Tumors\*

	Type of Tumor	$\gamma$ -GTP	Alk P'ase	5'NTD	AAT	Bilirubin		LDH	CPK
						Total	Direct		
Elevated $\gamma$ -GTP	Astrocytoma, Grades I-IV	52	27	8	14	0.3	0.2	190	12
		55	23	8	23	0.7	0.3	255	42
		91	42	21	20	—	—	270	99
		105	36	11	11	1.1	0.9	270	34
		196	108	15	15	0.4	0.3	190	36
		323	56	53	20	0.5	0.4	200	—
	Metastatic brain tumor	69	30	14	23	0.4	0.2	150	9
		84	21	6	24	0.6	0.5	280	38
		152	40	10	46	0.4	0.3	239	7
	Cerebral meningioma	122	21	10	25	0.4	0.3	175	96
		136	20	8	18	0.3	0.3	200	22
Normal $\gamma$ -GTP	Astrocytoma, Grades I-IV	16	52	5	29	0.9	0.4	185	114
		18	15	6	11	0.7	0.4	140	22
		20	28	11	21	0.6	0.3	225	—
		32	36	9	23	0.5	0.3	260	9
		36	32	2	20	0.5	0.2	255	—
		35	39	9	14	—	—	140	16
	Metastatic brain tumor	13	25	5	18	0.4	0.2	245	26
		41	35	7	24	—	—	355	9
	Cerebral meningioma	18	25	9	14	0.7	0.3	215	21
		34	24	9	14	0.3	0.2	180	30

\* $\gamma$ -GTP =  $\gamma$ -glutamyl transpeptidase; Alk. P'ase = alkaline phosphatase; 5'NTD = 5'-nucleotidase; AAT = aspartate amino-transferase; LDH = lactate dehydrogenase; CPK = creatine kinase. Values given in I.U. per liter.

Thus, either  $\gamma$ -glutamyl transpeptidase is an exquisitely sensitive indicator of changes in hepatic metabolism, or some other explanation must be sought. In patients with epilepsy two other possibilities exist: (1) that the elevation is drug-mediated but not the result of hepatotoxicity, or (2) the elevation is unrelated to drug administration. While our results show no apparent relationship to drug administration, the majority of patients with epilepsy studied were on continuous drug therapy, and medications such as barbiturates and some anticonvulsants have been shown to stimulate microsomal enzymes *in vivo* and to give abnormal liver function tests,<sup>15</sup> presumably due to normal turnover of cells which contain increased concentrations of the induced enzymes.  $\gamma$ -glutamyl transpeptidase in the hepatobiliary system is confined to endothelial cells of the biliary tract,<sup>17</sup> however, and is not found in

parenchymal hepatic cells as are the amino-transferases. Study of  $\gamma$ -glutamyl transpeptidase in patients with epilepsy receiving no medication will be necessary to clarify this question or rule out entirely a drug-mediated elevation. In this study no changes in drug dosage, type, or frequency of administration resulted in related changes in  $\gamma$ -glutamyl transpeptidase activity, and cessation of drug administration for periods of as long as 7 days did not result in related changes in  $\gamma$ -glutamyl transpeptidase activity. Eleven (15%) of 75 patients receiving similar medications for periods of several years showed no elevation of  $\gamma$ -glutamyl transpeptidase activity. An apparent drug-mediated response of serum  $\gamma$ -glutamyl transpeptidase to administration of lidoflazine\* to patients follow-

\* Lidoflazine, 1-[4, 4-di(4-fluoro-phenyl)-butyl]-4-[(2, 6-dimethyl-anilo-carbonyl)-methyl]-piperazine (McNeil Laboratories, Inc.): a long-acting vasodilator.

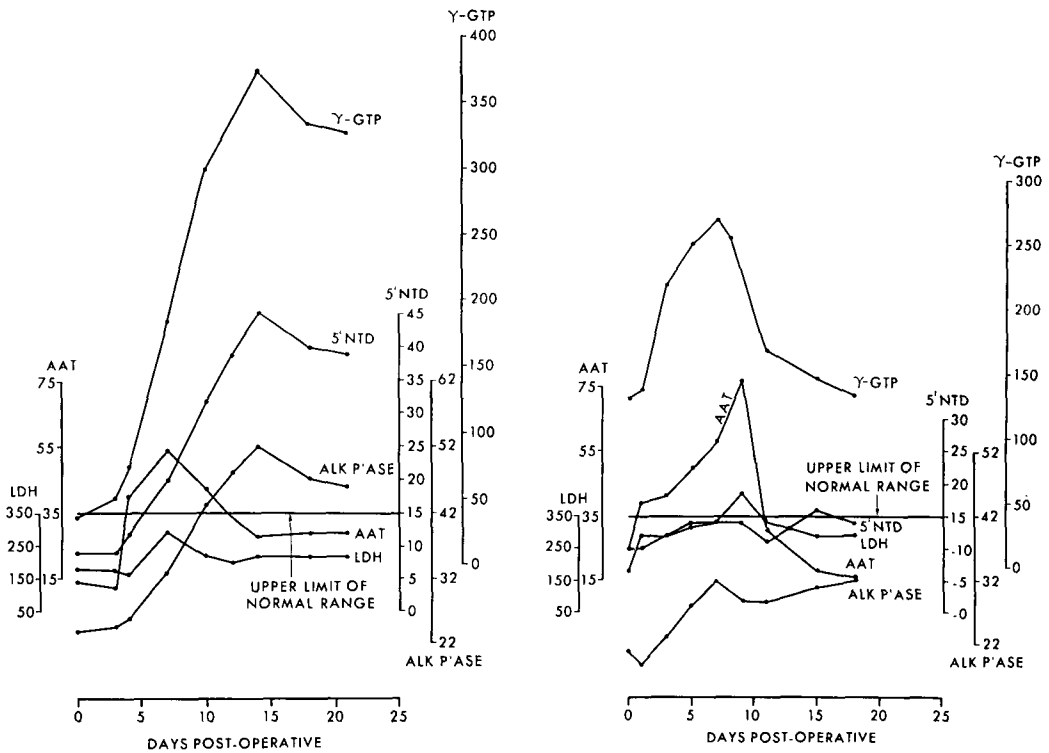


FIG. 4. Patterns of enzyme activity in individual patients following neurosurgery. Values expressed as I.U. per liter. *A* (left),  $\gamma$ -glutamyl transpeptidase in normal range preoperatively. *B* (right),  $\gamma$ -glutamyl transpeptidase elevated preoperatively.

ing myocardial infarction or with arteriosclerotic cardiac disease has been noted,<sup>6</sup> but the relationship of the enzyme to administration of and clinical response to lidoflazine described is not inconsistent with the previously postulated relationship of  $\gamma$ -glutamyl transpeptidase to vascularization, since this drug has been shown to stimulate development of collateral circulatory vessels in dogs<sup>23</sup> and elevation of the enzyme may be related to the vascularizing or vasodilatory effects of the drug. In this laboratory, serum  $\gamma$ -glutamyl transpeptidase has also been observed to be elevated in a number of patients with burns at a time when healing is occurring.<sup>9</sup> Therefore, elevation of serum  $\gamma$ -glutamyl transpeptidase in the conditions discussed may reflect changes in vasculature, because of regeneration, through a drug-mediated effect on the circulatory vessels, or through

some other metabolic action. The effect may reflect changes in capillary permeability or increased synthesis of the enzyme. The postulated function of  $\gamma$ -glutamyl transpeptidase in the transfer of amino acids across cellular membranes<sup>7, 19</sup> and the importance of glutamate in cerebral metabolism lead to speculation about a function of the enzyme in provision of glutamate to tissues. The concentration of adenosine triphosphate is known to be decreased before the onset of generalized convulsions.<sup>22</sup> Energy-yielding metabolism is necessary for the transfer of glutamic acid across bacterial cell membranes.<sup>10</sup> Convulsive agents are known to decrease nicotinamide adenine dinucleotide,<sup>24</sup> and to inhibit glutamate decarboxylase<sup>2</sup> and  $\gamma$ -amino butyrate transaminase.<sup>5</sup> A transpeptidation reaction has been postulated as part of the mechanism of transfer of glutamic acid



across cell membrane.<sup>10</sup> Isolated observations such as these may perhaps be combined to provide a key to an understanding of the metabolic processes involved in the findings reported here.

Conclusions which may be drawn from this study are as follows. Serum  $\gamma$ -glutamyl transpeptidase is elevated in patients with epilepsy, and the elevation apparently is not the result of hepatotoxicity from drug administration.  $\gamma$ -glutamyl transpeptidase may be elevated preoperatively in the sera of some patients with brain tumors. Post-operatively this enzyme may follow a pattern similar to that seen following myocardial infarction, with peak activity being reached in the second week after tissue insult.  $\gamma$ -glutamyl transpeptidase, while being a highly sensitive indicator of diseases of the hepatobiliary system, cannot be considered as specific as was at first thought, and care should be taken in interpretation of elevations of this enzyme to ensure that no other factors contribute to the elevated activity.

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