

## Serum $\gamma$ -glutamyl transpeptidase activity in liver disease

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*From the Departments of Chemical Pathology and Medicine, Royal Postgraduate Medical School, London*

**SUMMARY** Serum  $\gamma$ -glutamyl transpeptidase (GGT) activity correlates closely with the activities of alkaline phosphatase (ALP) and 5'-nucleotidase (5NT) in various forms of liver disease. Maximum elevations of all three enzyme activities are observed in diseases which particularly affect the biliary tract. Compared with the other two enzymes GGT is generally increased to a greater extent and is thus the most sensitive indicator of biliary-tract disease, while estimations of serum GGT are more reproducible than those of 5NT. However, a group of patients who had been treated with phenytoin and barbiturates were found to have elevated serum GGT activities without any other evidence of liver disease. The apparent effect of certain drugs on serum GGT activity indicates the need for caution in interpreting the results of this test.

In the past few years a number of reports have appeared describing the conditions in which there is an elevation of circulating  $\gamma$ -glutamyl transpeptidase (GGT) (eg, Goldbarg, Pineda, Smith, Friedman, and Rutenburg, 1963; Orłowski, 1963; Zein and Discombe, 1970; Rosalki, Rau, Lehmann, and Prentice, 1970). The level of this enzyme has been shown to be raised mainly in patients with liver disease and to a lesser extent in a number of other conditions, not apparently involving the liver (Table I). It has been suggested that  $\gamma$ -glutamyl transpep-

an additional one is unlikely to gain widespread acceptance unless it offers advantages either clinically or technically over existing tests.

In this paper, we describe a critical study of the value of GGT in the diagnosis and assessment of a group of 160 unselected patients undergoing investigation at Hammersmith Hospital. In these patients we compare GGT with two well established markers of hepatic parenchymal cell damage (aspartate transaminase, AST, and isocitrate dehydrogenase, ICD) and two biliary tract enzymes (alkaline phosphatase, ALP, and 5'-nucleotidase, 5NT). We have also assessed the diagnostic sensitivity of GGT in 81 of the 160 patients who were shown to have a definite liver disorder. Serial studies of liver enzymes were undertaken in selected patients in an attempt to determine whether the relative value of GGT compared with that of other enzymes varies with the stage of the disorder as described previously for AP and 5NT (Phelan, Neale, and Moss, 1970). On the basis of the results of this investigation, we have tried to determine whether or not GGT should be selected as a routine test of liver function. During the course of the study, we have also demonstrated elevated levels of circulating GGT in a proportion of patients taking anti-epileptic drugs. This finding confirms a preliminary communication by Rosalki, Tarlow, and Rau (1971) and may prove to be the most serious limitation in the use of what appears otherwise to be a sensitive indicator of liver disease.

Disease	Series
Renal neoplasm	Orłowski (1963)
Nephrotic syndrome	Orłowski (1963)
Renal transplant rejection	Lehmann <i>et al</i> (1970)
Acute and chronic pancreatitis	Rutenburg <i>et al</i> (1963)
Diabetes mellitus	Goldbarg <i>et al</i> (1963)
Congestive cardiac failure	Rutenburg <i>et al</i> (1963)
Myocardial infarction	Agostini <i>et al</i> (1965)
Angina	Hedworth-Whitty <i>et al</i> (1967)

Table I Diseases in which serum GGT has been found to be raised

tidase is a useful enzyme to measure in serum in the assessment of liver disease, particularly when this is due to alcoholism or malignant infiltration (Zein and Discombe, 1970). In view of the considerable number of enzymic tests for liver disease already available,

**Patients and Methods**

Two hundred and seventy nine samples of serum for liver function tests were received from 160 unselected patients attending Hammersmith Hospital. In addition to undertaking the specific tests requested, GGT was measured together with as many serum enzymes known to be related to hepatic disease as possible with the amount of serum available. In 81 of the 160 patients a definite diagnosis of liver disease was established (Table II). The results of enzyme studies in these patients were studied in more detail. Some of the patients with liver disease were studied sequentially over a period of weeks or months.

Disorder	No. of Patients	No. of Samples
1 Hepatitis		
Viral	11	36
Active chronic hepatitis	8	15
Drug	1	1
2 Cirrhosis		
Alcoholic	11	17
Primary biliary cirrhosis	5	14
Cryptogenic	6	7
Haemochromatosis	1	1
3 Biliary tract disease (including carcinoma pancreas, cholangitis, biliary stricture)	7	23
4 Neoplasm		
Secondary carcinoma	7	15
Lymphoma	4	4
5 Abscess	2	6
6 Gilbert's disease	1	1
7 Systemic disorders (including colitis, infection, sarcoid, scleroderma, systemic lupus erythematosus, tuberculosis)	17	34
	81	174

Table II Diagnoses in 81 patients in whom liver disease was established

Serum GGT was also measured in two groups of patients who were apparently free from liver disease. The first group consisted of 10 patients with normal liver function but with an elevated serum ALP associated with bone disease. The second group consisted of 83 children aged from 10 to 16 years living in a residential hospital of whom 49 had been receiving barbiturates and/or phenytoin for the control of epilepsy for at least two years. The other 34 children in this group were of similar age and sex distribution to those with epilepsy but were receiving no drugs and acted as controls.

The enzymes estimated, with the methods used and the normal ranges, are listed in Table III. Enzyme activities are expressed in this paper as multiples of the upper limits of the corresponding normal ranges. Other biochemical estimations were carried out by standard Technicon AutoAnalyzer techniques or by the methods given by Wootton (1964) but the

Enzyme	Method	Normal Range
$\gamma$ -glutamyl transpeptidase (GGT)	Szasz (1969)	3-25 IU/l
Alkaline phosphatase (ALP)	AutoAnalyzer N6A	3-13 KAU/100 ml
5'-Nucleotidase (5NT)	Campbell (1962)	0-17 IU/l
Aspartate transaminase (AST)	AutoAnalyzer N25A	4-20 IU/l
Isocitrate dehydrogenase (ICD)	Bell and Baron (1960)	2-8 IU/l

Table III Methods used for enzyme estimations

results of these tests are not given in full except as part of individual case summaries.

**Results**

COMPARISON OF SERUM GGT WITH OTHER ENZYME TESTS IN ALL PATIENTS INVESTIGATED FOR SUSPECTED LIVER DISEASE

Figures 1-4 show the relationship between serum GGT activities and the serum levels of 5NT, ALP, ICD, and AST. In order to accommodate the wide range of results the figures have been drawn with logarithmic axes but all calculations were carried out on the raw data. It was not possible to carry out all five enzyme estimations on every specimen. Each point in Figs. 1-4 represents a specimen on which at least two enzyme assays were possible, so that the four Figures do not necessarily all contain the same number of experimental results. The calculated correlations between serum GGT and other enzyme activities are given in Table IV. For comparison the

X	Y	R	P	Regression Equation
GGT	ALP	0.659	$3.1 \times 10^{-9}$	$GGT = 2.00 ALP - 0.39$
GGT	5NT	0.760	$1.4 \times 10^{-8}$	$GGT = 1.50 5NT + 0.57$
GGT	AST	0.053	0.27	
GGT	ICD	0.079	0.11	
ALP	5NT	0.713	$3.0 \times 10^{-9}$	$5NT = 1.17 ALP - 0.37$
AST	ICD	0.500	$1.5 \times 10^{-8}$	$ICD = 0.17 AST + 1.09$

Table IV Correlations between enzyme results and regression equations

correlations between pairs of enzymes, not including GGT, which are accepted as giving similar information about the nature of pathological processes in the liver have also been calculated. The correlation coefficients between GGT and ALP or 5NT ( $r = 0.66$  and  $0.76$  respectively) are of the same magnitude as that which relates ALP and 5NT ( $r = 0.71$ ), two enzymes which respond similarly in intra- or extrahepatic biliary obstruction. On the other hand, the correlation between serum GGT and AST or ICD, enzymes which reflect hepatocellular damage,

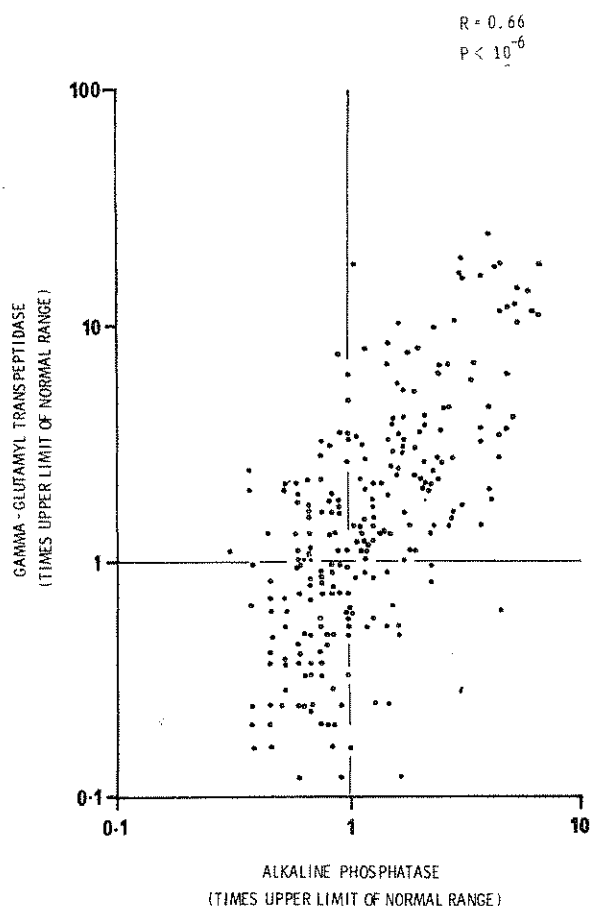


Fig. 1  $\gamma$ -Glutamyl transpeptidase and alkaline phosphatase activities in 245 serum specimens from patients in whom liver function tests were requested.

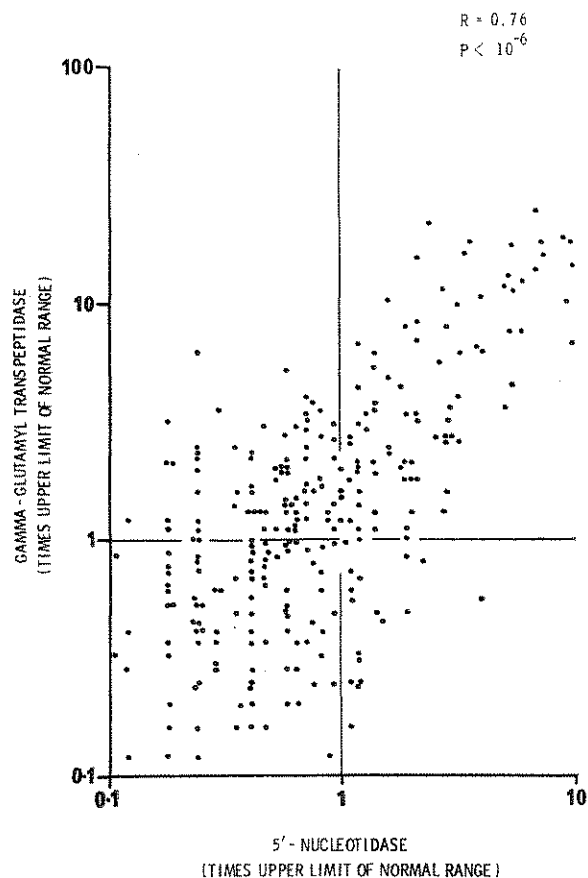


Fig. 2  $\gamma$ -Glutamyl transpeptidase and 5'-nucleotidase activities in 247 serum specimens from patients in whom liver function tests were requested.

is not significant. The correlation between AST and ICD is itself highly significant but with a lower correlation coefficient ( $r = 0.50$ ) than that between the pairs of 'biliary function' enzymes.

Serum GGT was raised in a higher proportion of the samples represented in Figs. 1 and 2 than were ALP or 5NT. Where an elevation of more than one enzyme was present, the degree of elevation of GGT tended to be greater with respect to the upper limit of normal serum activity than for either of the other two enzymes, as shown by the slopes of the regression lines. The greater potential sensitivity of serum GGT measurements in the detection of liver disease is emphasized further by the results of 174 estimations of GGT, ALP, and 5NT carried out on 81 unselected patients with proven liver disease. A single measurement of serum GGT would have suggested liver disease in 74% of these cases, compared with 57% for ALP and 43% for 5NT, so that on this basis GGT

appeared to be the best single biliary enzyme test. Combination of serum GGT with ALP or 5NT estimations produced only a marginal increase in the number of abnormalities detected, and even when all three of these enzymes were determined, the proportion of abnormal results was only increased to 80%.

#### COMPARISON OF SERUM GGT WITH OTHER ENZYME TESTS IN DIFFERENT DISEASES OF THE LIVER

When patients with altered liver function were classified retrospectively into categories of disease the data further demonstrated the association of raised serum GGT activity with disease of the biliary tract. The diseases were grouped into two broad categories, those in which the lesion can be regarded as affecting principally the biliary system and its function and those in which the disease

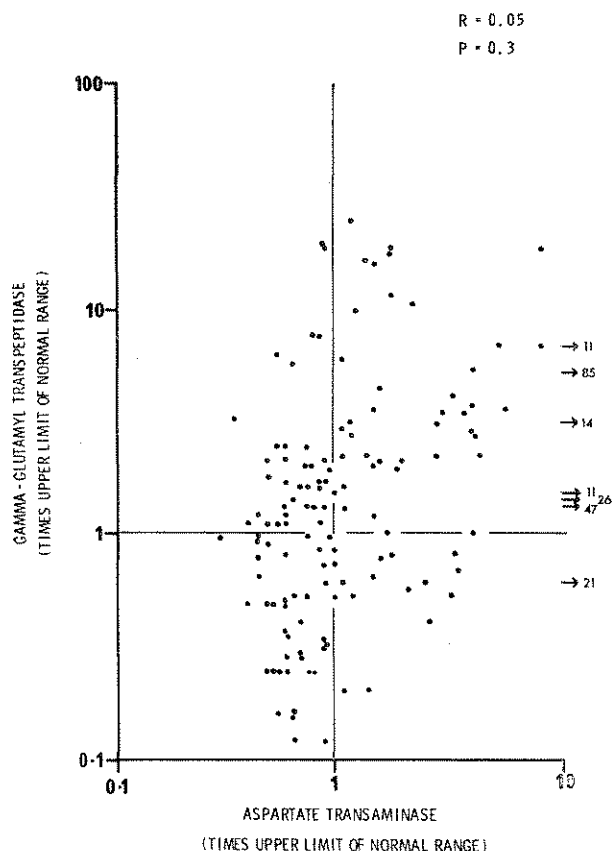


Fig. 3  $\gamma$ -Glutamyl transpeptidase and aspartate transaminase activities in 137 serum specimens from patients in whom liver function tests were requested.

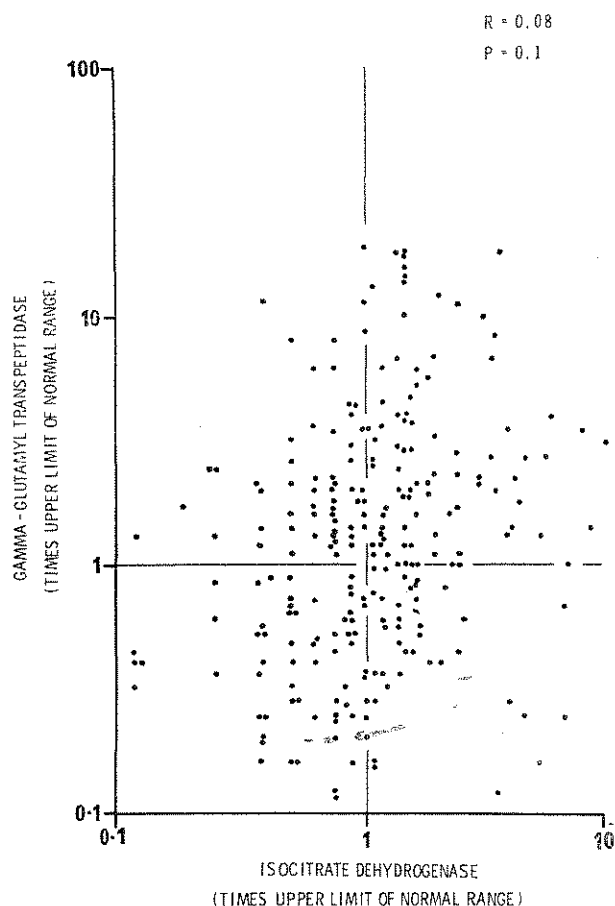


Fig. 4  $\gamma$ -Glutamyl transpeptidase and isocitrate dehydrogenase activities in 243 serum specimens from patients in whom liver function tests were requested.

causes mainly parenchymal cell damage. Average serum activities of GGT, 5NT, and ALP were each three to six times greater in biliary tract disease than in the parenchymal disease category, while in both categories the ratios of mean GGT activity to activity of 5NT or ALP were approximately the same (Table V). Thus, as far as the pattern of these enzyme changes is concerned, the distinction between biliary-

tract and parenchymal disease is quantitative rather than qualitative.

In 22 serum samples from 13 patients an elevated serum GGT activity was the only enzyme abnormality. The most common diagnosis in this group was alcoholic liver disease (five patients), followed by resolving viral hepatitis (four), and active chronic hepatitis (two). Diagnoses of cardiac failure and gallstones, respectively, were made in the two remaining cases.

Disease	Mean Serum Activities as Multiples of Upper Limit of Normal		
	GGT	5NT	ALP
Biliary tract disease including primary biliary cirrhosis (12 patients; 37 samples)	11.9	6.2	4.0
Other liver disease (69 patients; 137 samples)	2.3	1.1	1.5

Table V Relative sensitivity of biliary tract enzymes

SEQUENTIAL CHANGES IN SERUM GGT AND OTHER ENZYME LEVELS IN LIVER DISEASE  
Three cases are illustrated in Figures 5-7.

Case 1 (J.M., Fig. 5)

A 42-year-old publican was admitted to hospital following a period of several months during which he had been drinking two bottles of brandy a day. On admission he was jaundiced and had marked oedema

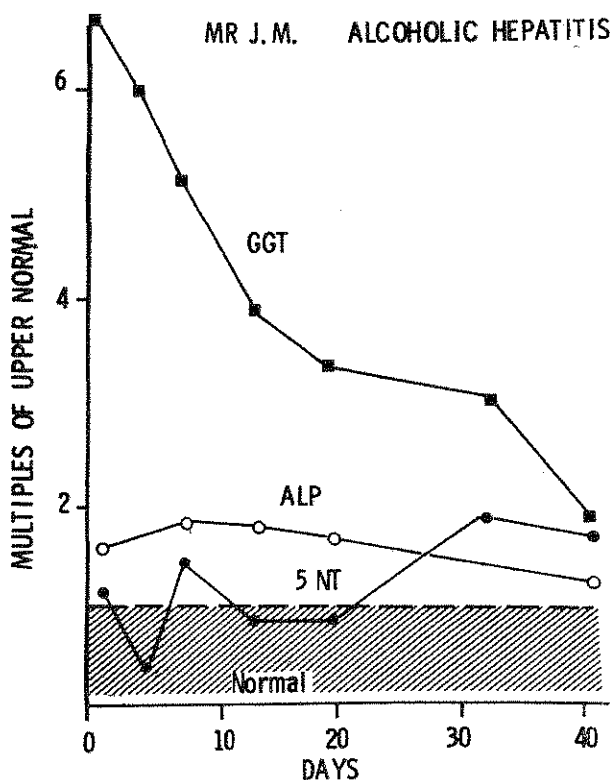


Fig. 5 Changes in serum GGT, ALP, and 5NT activities in a case of alcoholic hepatitis.

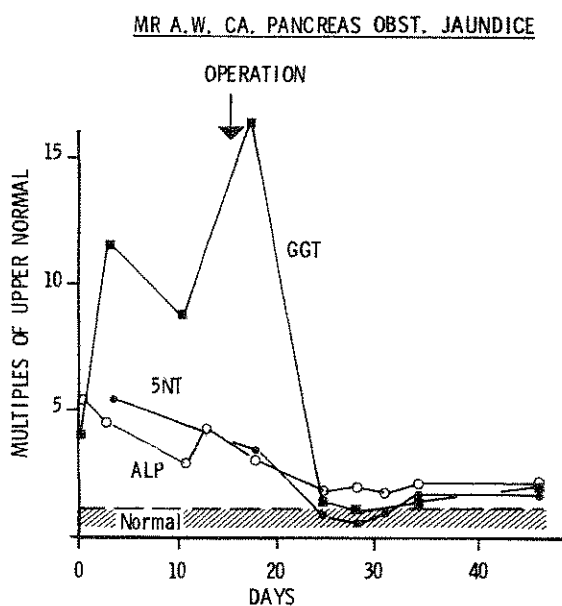


Fig. 6 Changes in serum GGT, ALP, and 5NT activities in a case of extrahepatic obstructive jaundice.

and ascites. He was given a low salt diet, thiazide diuretics, and spironolactone. His condition improved rapidly over the next month with clearance of the jaundice and fluid retention. Initially GGT was more markedly raised than ALP or 5NT. After four weeks' abstention from alcohol, GGT had fallen progressively to about the same level as the other two enzymes. Liver biopsy showed evidence of florid alcoholic cirrhosis. Serial studies in this patient demonstrated the disproportionately marked elevation of GGT found in many patients with alcoholic liver disease who have recently been taking alcohol.

#### Case 2 (A.W., Fig. 6)

An 84-year-old retired man was admitted to hospital with a short history of jaundice following a period of epigastric pain over the previous six months. The biochemical studies favoured a diagnosis of obstructive jaundice and two weeks later laparotomy was performed. The patient was found to have a carcinoma at the head of the pancreas and the obstructed common bile duct was bypassed by performing cholecystoduodenostomy. Serial enzyme studies showed disproportionately high GGT compared with ALP and 5NT, and a rapid fall to normal values immediately after the operation. This indicates the sensitivity of GGT and also demonstrates that the normal half life of the enzyme in the circulation is of the order of hours rather than of days.

#### Case 3 (J.V., Fig. 7)

A 24-year-old housewife suffered an acute febrile illness consisting of hepatitis, renal failure, and a period of marrow hypoplasia. She presented initially with pyrexia and symptoms suggestive of a urinary tract infection which was treated with short courses of ampicillin, tetracycline, and finally trimethoprim-sulphamethoxazole. She remained pyrexial and developed mild jaundice with raised levels of circulating hepatic enzymes. Two weeks after the start of her illness, she became uraemic and oliguric, and one week later was found to be anaemic (haemoglobin 6.8 g/100 ml) with pancytopenia. The bone marrow was shown to be profoundly hypoplastic. The patient made a complete recovery without further treatment.

In these three cases serum GGT responds in a way that is broadly similar to ALP and 5NT, but with a greater degree of abnormality than either of these enzymes. These observations again suggest that the greater sensitivity of serum GGT as a liver function test, compared with ALP and 5NT, reflects a quantitative, rather than a qualitative, difference in behaviour in liver disease.

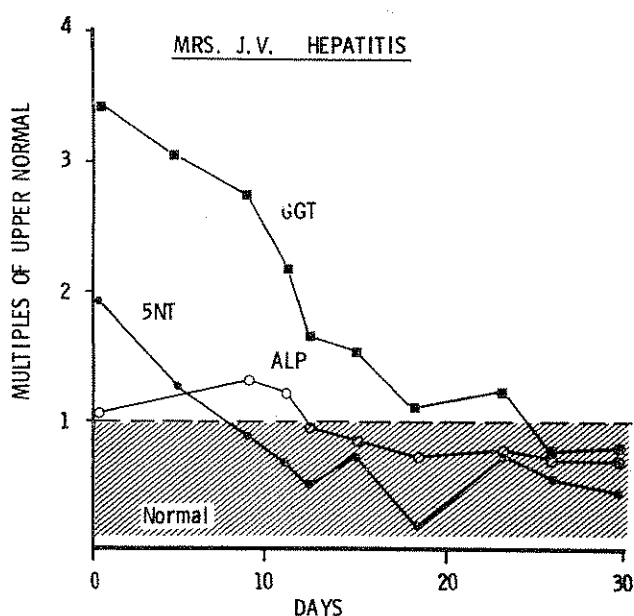


Fig. 7 Changes in serum GGT, ALP, and 5NT activities in a case of hepatitis.

SERUM GGT IN NON-HEPATOBIILIARY CONDITIONS

The 10 patients with osteoblastic bone disease but with no evidence of liver disease all had normal GGT and 5NT values, whereas serum ALP was elevated on each occasion on which enzyme measurements were made. However, the group of 49 patients who were receiving phenobarbitone and/or phenytoin for the treatment of epilepsy had significantly higher serum GGT levels than a control group of 34 patients who were not receiving these drugs (Fig. 8). The mean value and range of GGT activity for the treated group was 31 (0-125) iu/l, compared with 7 (0-25) iu/l for the controls. The difference between the means was significant at the 0.001 level.

Discussion

The strong correlation between serum GGT and ALP or 5NT in liver disease confirms that changes in the activity of GGT reflect principally alterations in biliary function, rather than damage to the parenchymal cells. The possible diagnostic advantages of serum GGT estimations are therefore to be considered in relation to measurements of ALP and 5NT.

An immediately obvious advantage of GGT is its greater sensitivity than either of the other two enzymes, of which 5NT is probably the next in order of sensitivity (Phelan *et al*, 1971). On these grounds, therefore, GGT would be the enzyme of choice if

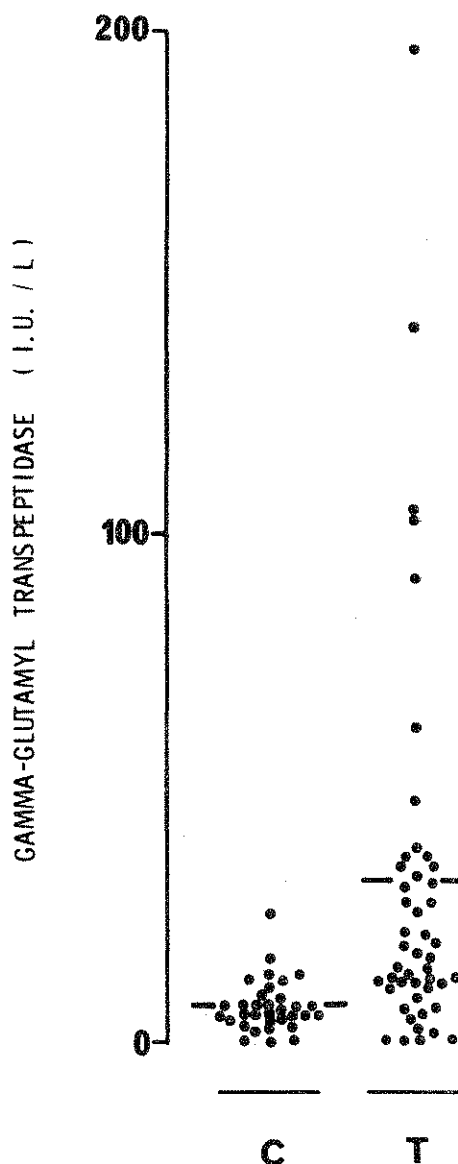


Fig. 8 Serum  $\gamma$ -glutamyl transpeptidase activities observed in 49 epileptics receiving barbiturates and/or phenytoin (T), compared with those in 34 controls of similar age and sex who were not receiving these drugs (C). ( $P = 0.001$ )

only one estimation were to be relied upon for the detection of latent or chronic liver disease. Furthermore, the procedure for estimation of GGT activity is considerably simpler than that for 5NT, since in the case of 5NT the need to correct for the action of non-specific alkaline phosphatase increases the complexity and reduces the precision of the assay. A between-batch standard deviation of  $\pm 2.6$  at the level of 49.5 iu/l ( $n = 18$ ) was found for the GGT method in use in our laboratory, compared with

$\pm 9.2$  at a level of 68 iu/l ( $n = 28$ ) for the measurement of 5NT.

However, two considerations caution against the use of GGT alone for the detection of liver disease. The first of these is the lack of specificity for hepatobiliary disease: although some of the changes in serum GGT listed in Table I may be attributable to secondary liver disease, this cannot be assumed in every case. Changes in 5NT, on the other hand, seem to be confined exclusively to hepatobiliary disease and, while bone, intestine, and placenta can contribute to an increased serum ALP, the organ specificity of this enzyme also can be increased by isoenzyme characterization. The second consideration concerns the effect of certain drugs in raising the serum GGT, possibly as a result of induction of the enzyme in the liver. The range and nature of those drugs which can produce this effect are not well defined at present, nor whether an increased serum GGT is an invariable consequence of their administration, and a raised serum GGT unsupported by other chemical or clinical abnormalities in a patient who has been receiving medication should therefore be interpreted with caution. This limitation erodes some of the clinical value of the sensitivity of GGT assays. Enzyme induction may also account for the marked elevations of serum GGT activity in patients with alcoholic liver disease which was noted in the present study as well as by earlier workers (Zein and Discombe, 1970). In hepatobiliary disease due to other causes, some factor associated with biliary retention may stimulate production of hepatic GGT, which is normally present in small amounts only (Orlowski, 1963), in the way that biliary obstruction is now believed to result in synthesis of hepatic alkaline phosphatase *de novo* (Kaplan and Righetti, 1970).

Our conclusions are that serum GGT is a valuable diagnostic aid when used in conjunction with ALP assays for the investigation of latent or chronic liver disease. The use of serum GGT measurements in this way can largely replace 5NT, except when the

possibility of renal disease, recent myocardial infarction or, most importantly, a history of drug administration, are present to make the interpretation of serum GGT levels doubtful.

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