STRUCTURAL, FUNCTIONAL, AND CLINICAL ASPECTS OF γ-GLUTAMYLTRANSFERASE

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INTRODUCTION

The enzyme designated by the IUPAC Commission as EC.2.3.2.2 has been variously trivialized in the literature as γ-glutamyl transpeptidase, D-glutamyltransferase, and γ-glutamyltransferase. The latter (abbreviated as GGT) will be employed in this review.

Interest in GGT has taken many forms, and an attempt will be made to survey these diverse areas of interest in the present work. Following its "discovery" by Hanes and his colleagues in Toronto in 1950, it received modest attention from scientific investigators who, in general but with considerable overlap, pursued one or other of the following themes:

1. What is the function of GGT?
2. What is its structure?
3. How does it carry out its catalytic action?
4. What role can it play in laboratory diagnosis?

The first three questions have been probed by basic scientists at the fundamental level, and unequivocal answers are emerging which have provided a generally agreed consensus as to the structure of this membrane glycoprotein and its catalytic function, although very many details remain to be explored and expanded. The functional role of GGT in the ecology of the cell has led to some ingenious proposals, although some question their universality and others their very validity. It is in the field of laboratory diagnosis that the greatest confusion exists. While the greater part of this review will be devoted to this latter aspect, the opportunity must not be lost to bring the reader up to date with more fundamental aspects. These are the areas where the greatest amount of real knowledge of the enzyme has been accumulated. An understanding of the pathophysiology of GGT requires knowledge of its function and location, as well as its behavior in various experimental situations. Exploitation of the enzyme and its isoenzymes for diagnostic purposes requires knowledge of their structure and catalytic behavior. The diagnostician who attempts to proceed without this solid background will soon find himself in trouble, generating information which at best may be useful but empirical, and more often data that are without real utility and cannot be rationalized by the limited facts at his disposal.

In choosing a broad approach to the subject in hand, this reviewer has decided to focus attention primarily on advances in the areas defined over the last 5 years referring to earlier work only where this is necessary for an understanding of present endeavors. The material designated as "References" will amplify this background where the reader finds this necessary.

CATALYTIC ACTIONS

The catalytic activities carried out by GGT are basically of three types.

The Transfer Reaction

The first, and functionally the most important, involves transfer of the γ-glutamyl residue from glutathione and other γ-glutamyl peptides to amino acids or small peptides to form the γ-glutamyl amino acid and cysteinyl-glycine, thus:

γ-glutamyl-cysteinyl-glycine + amino acid (peptide)
↓ GGT
γ-glutamyl-amino acid (peptide) + cysteinyl-glycine

This is the fundamental transpeptidation reaction and although in most biological systems glutathione is indeed the γ-glutamyl donor, others can also serve this purpose, such as a γ-glutamyl residue attached to another peptide or amino acid, or even to an artificial chromogen such as napththylamide or *p*-nitroanilide.

Autotranspeptidation

Another interesting reaction is autotranspeptidation in which the γ glutamyl residue can be transferred from one substrate molecule to another molecule of the same substrate to form a γ-glutamyl-γ-glutamyl peptide:

$$\begin{array}{l}\gamma\text{-glutamyl-NH-R} + \gamma\text{-glutamyl-NH-R} \\ \qquad\qquad\quad \downarrow \text{GGT} \\ \gamma\text{-glutamyl-}\gamma\text{-glutamyl-NH-R} + NH_2R\end{array}$$

This reaction is blocked by the presence of an α methyl group attached to the γ-glutamyl residue of the substrate itself.[1]

Hydrolysis

A third possibility is transfer of the γ-glutamyl residue to water — simple hydrolysis — with formation of free glutamic acid and either the amino acid or peptide to which the γ-glutamyl residue was initially attached:

$$\gamma\text{-glutamyl-NH-R} + H_2O \longrightarrow \text{glutamic acid} + NH_2R$$

Enzyme Mechanism

The enzyme reaction follows a Ping Pong Bi Bi kinetic mechanism[1a] with a γ-glutamyl-donor site exhibiting low specificity for L- and D-γ-glutamyl donors, whereas the separate acceptor site exhibits absolute L-specificity.[2,3] Values for the kinetic constants of hog kidney GGT have been presented.[4] A detailed kinetic analysis has shown that GGT is prone to substrate inhibition by both γ-glutamyl-p-nitroanilide and glycylglycine.[5] Use of active site-directed inhibitors has demonstrated the importance of a serine residue in the catalytic mechanism of rat kidney GGT.[5a] Consistent with its Ping Pong Bi Bi kinetic mechanism, it has been reported that GGT shows product inhibition by free *p*-nitroanaline,[6,7] although this effect could not be reproduced by Rosalki.[8] Further work is needed to resolve this disagreement.

An extensive study of amino acid and peptide acceptors carried out with rat and human intestinal GGT preparations confirmed glycylglycine to be the most efficient, with glycylalanine as the next most active in this role.[9] Dipeptides were more suitable acceptors than tripeptides or individual amino acids. Interestingly, the pattern of activation of both rat and human enzymes by peptides was stated to be compatible with the currently known specificity of the peptide transport system.

It has been reported[10] that addition of bilirubin to serum causes inhibition of GGT activity. This has not been confirmed by Dickson and Beck[11] who, on adding either bile or bilirubin standards as a source of bilirubin, found that serum GGT activity increased as the bilirubin concentration rose. However at the lower concentration of bile corresponding to 50 μmol/l bilirubin concentration, all samples showed decreased GGT activity, although samples in which the bile was added to a concentration of 250 μmol/l bilirubin demonstrated 59% activation. The results of these experiments are not entirely clear or well explained. Further complexity has been added to this controversy by Stromme,[22,23] who considers the reported inhibition by bilirubin to be an artifact dependent upon departure from linearity of the absorbance recorded by spectropho-

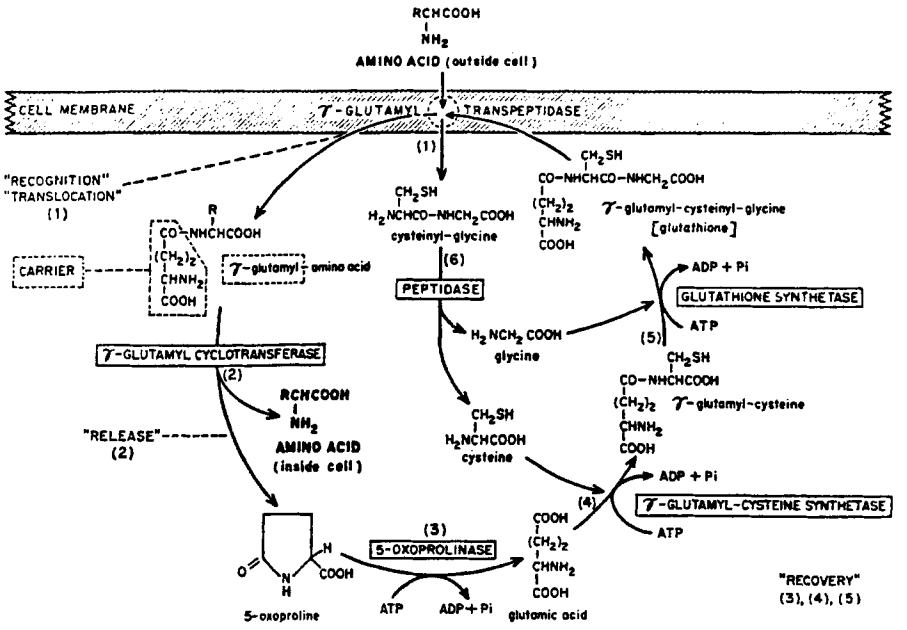

FIGURE 1. The gamma-glutamyl cycle for glutathione synthesis and amino acid translocation and release into cells. (From Meister, A., *Science*, 180, 33, 173. With permission. Copyright 1973 by the American Association for the Advancement of Science.)

tometers used for the assay procedure. This departure from linearity will generally be seen only at high absorbance values, but will vary with the instrument which should be carefully checked in this regard when setting up the assay.

FUNCTIONS OF GGT

The γ-Glutamyl Cycle

The possibility that GGT may play a crucial role in the absorption of amino acids from fluids such as the glomerular filtrate, and their uptake into cells from the extracellular fluid in general, is crystallized through the concept of the γ-glutamyl cycle, developed and promulgated by Meister[12,13] on the basis of his own observations, and the earlier work of Hanes and Connell,[14-17] and outlined in Figure 1. It has been shown to function in the renal tubule and is potentially responsible for reabsorption of all amino acids except proline from the glomerular filtrate. The transport of amino acids across the blood-brain barrier probably involves the operation of this cycle. Since GGT forms an integral part of the plasma membrane of mammalian cells, the cycle is likely to be operational in all cells capable of assimilating amino acids from body fluids. Most cancer cells fall into this category and membrane-associated GGT is especially rich in such cells.[18-20]

The crucial step in the cycle occurs at the cell membrane where GGT is located. The amino acid external to the cell becomes coupled in a ternary complex to the enzyme to which glutathione is also complexed. Translocation of the amino acid into the cell is postulated to occur through the mechanism described in Figure 2. Group X on the membrane-bound GGT interacts with the γ-glutamyl moiety of glutathione to yield the γ-glutamyl-enzyme and releasing cysteinyl-glycine. Attack by the amino acid nitrogen directed towards the γ-carbon of the γ-glutamyl-enzyme complex yields the γ-glutamyl-amino acid, and this linkage is formed in such a way that the amino acid is translocated

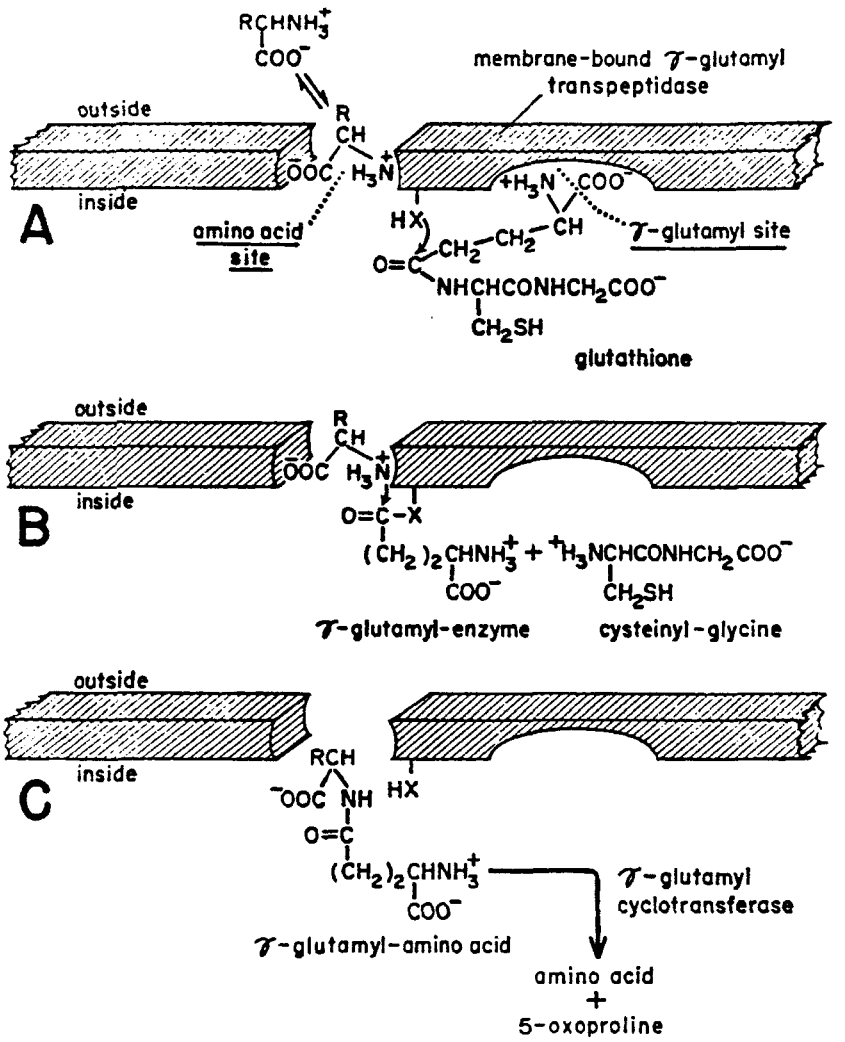

FIGURE 2. Detailed scheme for translocation of amino acids into cells by membrane-bound gamma-glutamyl transpeptidase. (From Meister, A., *Science*, 180, 33, 1973. With permission. Copyright 1973 by the American Association for the Advancement of Science.)

from the external to the internal surface of the membrane through a "pore" or by virtue of a conformational change in the membrane. The intracellularly located γ-glutamyl-amino acid-GGT complex is attacked by γ-glutamyl cyclotransferase, a soluble intracellular enzyme, releasing the amino acid and converting the γ-glutamyl residue to 5-oxoproline. The remaining steps involve "cycles within cycles" (Figure 1) in that the products of the original glutathione which was initially complexed to GGT (5-oxoproline and cysteinyl-glycine) go through a series of reactions leading to regeneration of the glutathione. 5-oxoproline is hydrolyzed by a remarkable reaction carried out by the enzyme 5-oxoprolinase but requiring energy input in the form of ATP, and the product is glutamic acid. Cysteinyl-glycine, the remaining two-thirds of the initial glutathione, is cleaved by a specific peptidase to yield cysteine and glycine which are reattached to glutamic acid in two independent synthetic transpeptidase reactions, each requiring a molecule of ATP. Cysteine is the first to be attached through the enzyme γ-glutamyl-cysteine synthetase, and addition of glycine by glutathione synthetase com-

pletes the cycle with restoration of glutathione. At the end of the cycle there is as much glutathione within the cell as at the start, but three molecules of ATP have been expended so that energetically speaking it is an expensive reaction. It has been estimated that 0.5 mol of amino acids are reabsorbed from the glomerular filtrate each day, which implies that 1.5 mol of ATP are utilized in this process. In comparison, 2 mol of ATP are required each day to operate the urea cycle. There is little difference, therefore, in the energy requirements of the γ-glutamyl cycle and the urea cycle, both of which are likely to be essential steps in the intermediary metabolism and transportation of amino acids.

Glutamine Formation and Amino Acid Transport

A number of investigators have sought to determine the universality of the GGT cycle in terms of how essential it is for amino acid transport in all mammalian cells capable of carrying out this process. It is too soon to bring in a firm verdict on this question. Recent work from Meister's laboratory supports the concept of a significant physiological connection between the metabolism of glutathione, transport of amino acids, and the γ-glutamyl cycle, since specific inhibitors of enzymes of the cycle when given to mice blocked the depletion of kidney glutathione otherwise occurring after intraperitoneal injection of amino acids which are substrates for cycle enzymes and therefore capable of cell translocation at the expense of intracellular glutathione.[20a] A note of caution was introduced in consideration of the fact that these changes could also be mimicked by agents that are neither substrates nor inhibitors of cycle enzymes. Uptake of glutamine was studied in human lymphoid cell lines whose GGT activities varied by two orders of magnitude. A direct correlation could not be shown between GGT activity and the transport and utilization of glutamine, which was extensively converted to glutamate in all cell lines.[21] A role for GGT in the metabolic response to acidosis in the rat which is characterized by increased ammonia production and uptake of glutamine is suggested by the fact that GGT activity in crude homogenates and purified brush border membranes of rat kidney increased during acidosis.[22] The increased GGT activity was not measurable until acidosis had persisted for 36 hr and seemed therefore a secondary rather than a primary response to acidosis. An enzyme, glutaminase-γ-glutamyltransferase, present in the cytosol of rat kidney appears to contribute 30% of ammonia produced from glutamine in mild acidosis.[22a] It is probably distinct from GGT, and the relative roles of both enzymes in ammonia production by the kidney need further elucidation. GGT activity of a rat ascites tumor cell line was shown to have its active site on the outer surface.[23] When enzyme activity was blocked with an affinity label, the rate of transport of amino acids into the cell was not affected, suggesting that the activity of GGT on the outer surface of the tumor cell membrane is not required in the transport process.

In the rat small intestine, the relative specificity of GGT with diglycine and eight essential amino acids as substrates correlated well with their rate of intestinal absorption.[24] GGT activity was 12-fold greater in the duodenum than in the ileum, with specific activity 10 times greater in the villus tip cells than in crypt cells; amino acid uptake differed by the same magnitude between the two cell types. Bromosulfophthalein, which inhibits GGT activity, also inhibited amino acid uptake. Other reports on the behavior and distribution of GGT in intestinal cells and brush border membranes provide similar indirect evidence for the involvement of GGT in amino acid absorption in the gut.[25,26]

Evidence against the participation of the γ-glutamyl cycle in amino acid transport by rabbit erythrocytes has been presented.[27] The situation with regard to human erythrocytes is controversial. One group of authors reported a marked derangement in the

free amino acids of erythrocytes from an adult patient with glutathione synthetase deficiency,[28] but similar studies performed in two children with the same condition failed to demonstrate any evidence of a general derangement of amino acid transport in the glutathione-deficient erythrocytes of these patients.[29] Data from experiments with normal human fibroblasts and those grown from a case of glutathionuria in which the fibroblasts manifest extreme reduction of GGT activity demonstrated normal amino acid uptake by the enzyme-deficient cells. The GGT cycle therefore does not appear to be essential for amino acid uptake in human fibroblasts.[30,31]

There is doubt that the γ-glutamyl cycle accounts for transport of all amino acids in all cell types. The weight of current evidence however, suggests that it could be the principle mechanism for amino acid uptake in the intestine and the renal tubule, and probably accounts for significant amino acid uptake by those cells in which it is present in a surface form, even though alternative and as yet unidentified mechanisms may coexist in some of the latter.

Glutathione Metabolism

The close involvement between GGT and glutathione implicit in the γ-glutamyl cycle points to an obvious role for the enzyme in regulating tissue levels of this highly important compound. Since the γ-glutamyl link of glutathione is resistant to the action of usual peptidases, its removal by GGT will enable the rest of the molecule to become available as a source of amino acids for protein synthesis. Moreover, GGT may initiate secondary transpeptidation reactions which may have the potential to bring about post-transcriptional changes in intracellular peptides. These aspects are described in the reviews by Rosalki[32] and Meister,[33] and more recently, further evidence in favor of a key role for GGT in glutathione catabolism has been elicited from a kinetic analysis of semipurified human liver enzyme.[33a] Indeed Griffith and Meister have produced data suggesting that glutathione synthesized by cells possessing GGT may be translocated to and used by the membrane-bound enzyme, whereas glutathione synthesized in cells lacking GGT may be transported via plasma to GGT located on the membranes of other cells.[33b]

GGT and Immunoglobulin Secretion

A function for GGT has been proposed by Binkley and colleagues in relation to the transport of proteins (as well as amino acids) into and out of cells.[34] This suggestion is based upon a parallelism between transport of IgA into mucosal secretions and colostral milk and increased GGT activity in these fluids; copurification of secretory IgA with GGT also occurred, prompting the speculation that GGT might serve as the secretory component of IgA.[35,36] A high content of GGT is found in cells which are relatively rich in secretory IgA.[37,38] This view no longer seems tenable in the light of two recent independent studies reporting purification of the secretory component and lack of GGT activity demonstrated by the pure preparation.[39,40]

PURIFICATION AND MECHANISM OF ACTION

Most such studies have been done with the rat kidney enzyme, since this has been brought to a high degree of purification. Not unexpectedly, studies on the mechanism of GGT action have proceeded *pari passu* with elucidation of the molecular structure of the purified enzyme itself. For that reason, it is necessary to consider both aspects in the following narrative.

D-γ-glutamyl-*p*-nitroanilide was hydrolyzed by rat kidney GGT with a K_m value for hydrolysis of 31 μM compared with 5 μM for the L-derivative; the V_{max} values for both compounds were identical. Autotranspeptidation could not be demonstrated with the

D-derivative, or when the L-derivative was used at concentrations lower than 10 μM. The γ-glutamyl donor site of the enzyme thus exhibits low stereospecificity, but the acceptor site appears to be absolutely specific for L-γ-glutamyl donors. The data were consistent with the existence of separate donor and acceptor enzyme sites.[41] The inability of D-γ-glutamyl-*p*-nitroanilide to undergo autotranspeptidation enabled it to be used as the γ-glutamyl donor in a study designed to examine the affinity of various amino acids and dipeptides for the acceptor site.[42] Highest affinity was shown by L-cystine and aminoacylglycines, lower values for glycyl amino acids and free amino acids, and very low values for peptides containing D-amino acids or more than two amino acid residues. The authors suggested that acceptor substrates bind to the region of the glutathione binding site vacated by cysteinylglycine during formation of the γ-glutamyl enzyme, and that free amino acids bind to the cysteinyl locus of this site. A close correlation was found between the specificities of the enzyme for various aminoacylglycines and for the corresponding free amino acids. The increased activity towards aminoacylglycines as compared to the corresponding free amino acids appears to reflect substantial assistance in binding by specific interactions between the enzyme and the COOH-terminal glycyl residue of the substrate. These interactions decrease when the latter residue is larger than alanine, indicating high specificity for glycine at the COOH-terminus of the dipeptide, but relatively low specificity for the NH_2-terminal residue which binds to the cysteinyl portion of the cysteinylglycine site. On the other hand, free amino acids also act as competitive inhibitors by binding to the γ-glutamyl site. With the purified enzyme from sheep kidney cortex, various metal ions (Ca^{2+}, Mg^{2+}, Na^+, and K^+) cause acceleration of the transfer reaction when glutathione is substrate, but are without effect upon the rate of hydrolysis of the γ-glutamyl bond.[43]

Early studies on rat kidney GGT led to the purification of two isoenzymes with similar amino acid composition, and similar specificities for various γ-glutamyl donors and acceptors. Each contained about 30% carbohydrate, but showed different mobilities on polyacrylamide gels.[44,45] It was shown that maleate inhibits transfer of the γ-glutamyl moiety to an acceptor, thereby increasing hydrolysis of the γ-glutamyl donor. Thus glutamine, a poor substrate for transpeptidase as compared to glutathione, is rapidly hydrolyzed by transpeptidase in the presence of maleate. This apparently explains the role of maleate in stimulating phosphate-independent glutaminase activity which can thus be assigned to a catalytic function of GGT. A heavy form of rat kidney GGT with a molecular weight of 200,000 was isolated by detergent extraction and affinity chromatography. Treatment of this heavy form with bromelain generated a light form of the enzyme with a molecular weight of 68,000 separable by electrophoresis and isoelectric focusing into 12 isozymes, also with a molecular weight of 68,000 and with similar catalytic behavior, amino acid, and carbohydrate content, but differing significantly with respect to sialic acid. Each isozyme was dissociated by SDS electrophoresis into two nonidentical glycopeptides with molecular weights of 46,000 and 22,000. The heavy form of the enzyme contained the dimeric light form as well as other membrane proteins.[46] The γ-glutamyl binding site of rat kidney GGT was subsequently shown to be located on the light subunit with a molecular weight of 22,000.[47] Other workers have confirmed the fact that rat kidney GGT is composed of two nonidentical polypeptide chains, one small and one large, with the active site predominantly located in the smaller subunit.[48] Further studies involving denaturation and renaturation experiments indicated that the large subunit possesses an active site which is masked when the enzyme is in its native state.[49] Recent investigations of the rat renal brush border membrane have shown that GGT faces the luminal side of the renal tubule, being anchored to the brush border membrane mainly via the large subunits of the enzyme. The evidence in favor of this conclusion rested upon the ability of a high molecular weight (215,000 daltons) derivative of glutathione, which is believed to

be impermeable to plasma membrane, to inhibit GGT activity. Moreover, the membrane-bound GGT was quantitatively released by treating the membranes with papain immobilized to Sephadex® G-10.[50]

Human kidney GGT purified by similar procedures to those used in the rat yielded a glycoprotein with a molecular weight of 84,000, composed of two nonidentical glycopeptides with molecular weights of 62,000 and 22,000. As with the rat kidney enzyme, izozymic forms separable by isoelectric focusing differed predominantly in sialic acid content. The light subunit of the human enzyme closely resembled that of rat kidney in molecular weight, amino acid composition, and in possessing the γ-glutamyl binding site. The heavy subunits of the two enzymes are however markedly different, accounting for differences in acceptor amino acid specificity and magnitude of the maleate effect which stimulates glutamine utilization only threefold with the human enzyme, compared with tenfold by the rat kidney enzyme.[51] A previous report that human kidney GGT comprises four subunits each of molecular weight 21,000[52] was attributed to the use by these authors of autopsy samples stored at 4°C for periods up to 15 days rather than fresh perfused kidneys. If this interpretation is correct, one would expect the 62,000 mol wt subunit to consist of three light subunits. Currently, there is no evidence that this is so and this discrepancy is in need of resolution.

Using the concept of "reactivity ratios" as a measure of the relative efficiencies with which equimolar concentrations of amino acids would serve as acceptors in the transpeptidation reaction, it was determined that at physiological concentrations of amino acids found in serum, 30 to 50 times as much glutamine would serve as substrate compared with branched chain or aromatic amino acids.[53] The significance of these observations is not altogether clear, since sheep kidney GGT and human serum were used in the experiments.

METHODOLOGY

Older Substrates

The assay of GGT activity is dependent upon the availability of synthetic substrates which are predominantly derivatives of glutamic acid in which a diazotisable radical or a chromogenic ion is coupled to the γ-carboxyl group. The first to be used was α-(N-γ-DL-glutamyl)-aminopropionitrile,[54] followed by *N*-(DL-γ-glutamyl) aniline.[55] Synthesis of γ-L-glutamyl α- and β-naphthylamides extended the range, the former being preferred because of its greater solubility, although higher activity was obtained with the latter.[56] In the above procedures, the reaction is stopped by protein precipitation and an aliquot of the protein-free filtrate taken for diazotisation employing the Bratton-Marshall reaction. Two molecules of substrate were utilized in each transpeptidation step, one acting as acceptor for the glutamyl group transferred from the other, thus:

2 γ-L-glutamyl-α-naphthylamide ⟶ γ-L-glutamyl-γ-L-glutamyl-α-naphthylamide + α-naphthylamine

An important development was the use of glycylglycine as acceptor for the γ-L-glutamyl residue[57,58] resulting in improved sensitivity of the assay. These authors also used fast blue B salt as the coupling agent instead of the more cumbersome Bratton-Marshall reaction, while they and a later worker[59] selected conditions that avoided the need for protein precipitation.

The commonest substrate used at the present time is γ-L-glutamyl-*p*-nitroanilide. Transfer of the γ-glutamyl residue to glycylglycine releases *p*-nitroaniline which, at the pH for optimal activity, absorbs strongly at 405 to 420 nm. This property may be used to monitor the reaction continuously,[60] or it may be used to measure the concentration

of free *p*-nitroaniline after stopping the reaction.[61-63] An alternative approach involves diazotisation of the *p*-nitroaniline and is said to overcome the problem of jaundiced sera and to yield a more sensitive assay.[64] Automated methods based on this substrate have been presented.[65,66] A later modification incorporates glutamate in a concentration of 1 mmol/ℓ in the reaction mixture, since this enhances activity by 20-100%.[67] An automated kinetic method in which the γ-L-glutamyl-*p*-nitroanilide is dissolved with greater ease than in previous methods by using dilute hydrochloric acid as the solvent and in which the reaction is initiated with γ-glutamyl *p*-nitroanilide instead of serum, has recently been described.[68] Polyoxyethylene nonylphenol has also been used to promote substrate solubility.[69] Kinetic methods using this substrate are preferable to fixed-point assays since the free *p*-nitroaniline is inhibitory, and therefore the reaction should be monitored over the shortest possible time.[7] It has been reported that glutathione, phenol tetrabromophthalein sulphonate and L-serine reduce activity with some of the above synthetic substrates.[70] A "Selected Method" for serum GGT assay has been published by Szasz[71] and favorably evaluated by four distinguished colleagues independently. The substrate is simply dissolved in the aqueous buffer at 50 to 60°C and the pH of the complete reaction mixture is adjusted to 8.0 at 25°C or 7.9 at 30°C. The final recommended concentrations were: 2-amino-2-methyl-1,3-propanediol, 100 m*M*; glycylglycine, 50 m*M*; L-glutamyl-4-nitroanilide, 4 m*M*. The latter compound is supersaturated under these conditions, but the solution is stable for at least 3 days.

New Substrates

Attempts have been made to introduce alternative substrates and to modify conditions from those recommended by Szasz.[71] It has been recommended that γ-glutamyl-*p*-nitroanalide be dissolved in an acidic aqueous organic solution.[72] Of six organic solvents tested, dimethylsulfoxide was preferred, since the rate of spontaneous decomposition of substrate was low in this reagent and activity of the enzyme was in no way inhibited; methylcellosolve, in which the γ-glutamyl-*p*-nitroanalide was relatively stable, inhibited enzyme activity by 5%. Solution of substrate in a mixture of hydrochloric acid and dimethylsulfoxide was therefore recommended, especially for continuous flow analyses. γ-Glutamyl-*p*-aminobenzoic acid has also been proposed as a substrate.[73] It is more soluble than the *p*-nitroanilide derivative, but the *p*-aminobenzoic acid liberated by enzyme action has to be measured in a subsequent chemical reaction and it is hard to see what advantages it has to offer. A benzfurozan derivative of glutathione has been described as a chromogenic substrate which yields the benzfurozan derivative of cysteinylglycine as a consequence of GGT action. Spontaneous molecular shifts and coupling with a sulfydryl reagent generate a product which absorbs intensely; the maximum difference in molar extinction between substrate and product is at 470 nm with a coefficient of 13,200.[73a] It has a higher affinity but lower maximal velocity with GGT than γ-glutamyl-*p*-nitroanilide. Another substrate *N*-γ-L-glutamyl-5-aminoisophthalic acid dimethyl ester hydrochloride has found use in a fluorometric determination.[74] Enzyme activity with this substrate is tenfold higher in terms of relative fluorescence response than γ-L-glutamyl-α-naphthylamide which has been previously used as a fluorochromatic substrate for GGT assay.[75,76] Fluorometric assays are, in general, technically more demanding and more prone to interference than spectrophotometric procedures; they are also difficult to standardize in molar terms. Their chief advantage is their remarkable sensitivity, but this is not a problem with conventional spectrophotometric GGT assays, and they are an inconvenient and unnecessary luxury in routine laboratories. They may have a place in the analysis of GGT isoenzymes should these ever become of genuine clinical value.

The 3-carboxy derivative of γ-glutamyl-*p*-nitroanilide has been introduced as a substrate.[77] Although it is much more soluble in water than the parent compound, it dis-

plays more pronounced substrate inhibition so that higher initial substrate concentrations are disadvantageous; it also suffers the disadvantage of a much higher absorbance in the range 400 to 420 nm used to monitor the product.[2] It was passed over in favor of γ-glutamyl-*p*-nitroanilide in the methods for GGT assay recommended by the Scandinavian Society for Clinical Chemistry.[78] This group advised incorporation of $MgCl_2$ in a concentration of 10 m*M* to enhance solubility of the substrate, and found higher concentrations to be slightly inhibitory. A comparative study between the two variant methods using γ-glutamyl-*p*-nitroanilide and two sources of the 3-carboxy derivative revealed that the latter gave lower values than the former and overall precision was poorer; moreover, results with the two sources of substrate were not in good agreement.[79] Other authors emphasized the superior solubility and lack of spontaneous hydrolysis shown by γ-glutamyl-3-carboxy-4-nitroanilide, and were unable to confirm its property of substrate inhibition.[80] The major difference between the two substrates reported by these authors was reduced auto-γ-glutamyl transferase activity with the 3-carboxy substrate. In the opinion of this reviewer, there is presently no justification in the search for alternatives to γ-glutamyl-*p*-nitroanilide whose use has been endorsed by all national societies whose recommended methods have been published.

GGT IN SELECTED TISSUES AND BODY FLUIDS

GGT and the Kidney

Renal GGT

In the light of the importance of GGT in the reabsorption of amino acids from the glomerular filtrate and its location in the brush border of the proximal renal tubular cell, it is hardly surprising that the earliest attempts to purify the enzyme utilized renal tissue: beef kidney,[81] hog kidney,[82] and human kidney.[83] The success of more recent efforts with these tissues has been described in a previous section of this review. Another source has been rat hepatoma.[84] It is therefore not surprising that raised serum activity of GGT may reflect disease processes within the kidney, such as renal tumors and chronic pyelonephritis.[85,86] Acute rejection of renal homotransplants is frequently accompanied by raised serum GGT activity.[87] Another feature of transplant rejection is that there occurs during the rejection process a dramatic increase in the clearance of GGT relative to that of creatinine.[88] In terms of specificity, however, this test has not become established as a biochemical marker for renal transplant rejection, although this is an area for future exploration.

Serum GGT activity was measured in 108 uremic patients, 110 patients on regular hemodialysis, and 71 successful renal transplant patients.[89] The frequency of abnormal results was 11, 14, and 28% respectively, indicating that these are uncommon in nondialyzed and dialyzed patients, and therefore in the uremic subject significant hepatic microsomal induction does not seem to occur per se. The higher frequency of raised values after renal transplantation might suggest additional pathology, such as hepatobiliary disease or the administration of microsomal enzyme-inducing drugs. The authors did not include the possibility of an impairment in renal excretion of the enzyme being a factor contributing to their results. A report in which standard biochemistry tests, but not GGT, were used revealed hepatic dysfunction in 38% of 82 renal transplant patients followed from 6 to 48 months.[90] Serum GGT activity may therefore not be a sensitive indicator of hepatic dysfuncion in transplant patients.

Urine GGT

Activity of this enzyme in urine is increased in acute renal disease and, in such circumstances, the properties of the enzyme are identical to those of renal tissue.[32] Inhib-

itors present in urine may be removed by a rapid ion-exchange method which is superior to gel chromatography, since the latter causes appreciable loss of activity;[91] the urinary enzyme resisted freezing and thawing at −5°C. Urinary excretion of GGT was significantly higher in pregnant women than in a nonpregnant control group.[92] The increase first became obvious before the end of the first trimester and continued thereafter. This was attributed to hypertrophy or hyperplasia of the nephron, and to be part of overall renal adaptation to normal pregnancy. Values outside this range were reported in three pregnant patients whose course was consistent with renal complications.

Patients receiving aminoglycoside antibiotics demonstrated increased urinary GGT excretion ranging from 1.6 to 14 times normal for tobramycin; 1.6 to 19 times normal for gentamicin; and 1.2 to 6 times normal for streptomycin.[93] In normal subjects and those with a variety of renal diseases, there was a direct relationship between the urinary GGT and the creatinine clearance. In patients receiving aminoglycoside antibiotics, there was an inverse relationship between GGT excretion (expressed as the GGT: creatinine clearance) and the initial creatinine clearance, although it is not clear why the authors chose to express the data in this way. The unrelated antibiotic flucloxacillin was also associated with increased urinary GGT excretion in a number of patients. The interpretation of these results is difficult, since all patients given aminoglycoside antibiotics demonstrated increased urinary GGT excretion, whereas only 3 of the 41 developed a fall in creatinine clearance. It is unlikely that clinically significant renal damage could have occurred in all, and the authors offer no decision point at which withdrawal of the drug should be considered. Urine collections were stored at 4°C until assay, but the duration of the storage period is not given; neither was the urine treated in any way to remove GGT inhibitors. The possibility that by preventing bacterial growth during storage the antibiotics prevented GGT degradation, although remote, was neither considered nor excluded.

A study in rats injected with a single dose of mercuric chloride sufficient to cause acute nephropathy revealed a sharp increase in urine GGT output which was more specific than other biochemical tests performed, and as sensitive as cytology in detecting renal tubular lesions.[94]

Seminal Vesicles

Histochemical studies demonstrated a high concentration of GGT in the secretory epithelium of the seminal vesicles of the rat, disappearing when the animals were orchidectomized, and restored by administration of testosterone.[95] Subsequently it was shown that the epididymis contained fivefold higher activity[96] and GGT was present in epididymal fluid in a particulate form. The suggestion that determination of the activity of the enzyme in seminal fluid might assist in the investigation of infertility[97] has not been confirmed, since the enzyme activity in samples from normal males and those subjected to vasectomy show overlap.[98] Comparison of GGT assay with that of acid phosphatase in vaginal wash samples from normal females and those suspected of rape indicated the former to be too insensitive and to be subject to contamination of the wash fluid with blood or urine.[99] The higher GGT activities in normal male serum may be due to enzyme contributed by the male reproductive tract.[100]

Pancreatic Juice

Following stimulation with an analogue of cholecystokinin, GGT activity of duodenal aspirate increased in normal subjects, but even more so in patients with chronic pancreatitis, in contrast to the typical pancreatic enzymes trypsin, lipase, and amylase, which showed a lesser increase in the latter patients.[101] No increase following pancreatic stimulation occurred in cholecystectomized patients,[102] and the authors postu-

TABLE 1

GGT Activity of Ascitic Fluid in Various Disease States

Diagnosis	No. of patients	Enzyme activity (U/ℓ) Mean ± SE	Range
Primary hepatoma	5	33.50 ± 5.0	17—56
Inactive cirrhosis	5	0.86 ± 0.40	0.46—1.40
Cirrhosis and dysplasia	4	6.29 ± 2.40	3.50—8.50
Secondary carcinoma	5	2.44 ± 2.10	1.20—3.50
Serositis (SLE)	3	1.04 ± 1.10	0.66—1.48
Chronic hepatitis	5	3.20 ± 2.10	0.21—3.50
Miscellaneous	4	2.72 ± 1.10	0.57—4.80

From Peters, T. J., Seymour, C. A., Wells, G., Fakunle, F., and Neale, G., *Br. Med. J.*, 2, 1576, 1977. With permission.

late that an abnormality of the gall bladder in chronic pancreatitis leads to a high concentration of GGT in gall bladder bile. This seems a more satisfactory explanation than the earlier suggestion that it originates from the diseased pancreas itself.[101] No change in serum GGT activity occurred after endoscopic retrograde cholangiopancreatography.[103]

Ascitic Fluid

A study performed in 31 patients with ascites associated with liver disease revealed exceptionally high levels of GGT in 5 patients with primary hepatoma.[104] Values in 5 patients with inactive cirrhosis were lower than in 4 showing macronodular regeneration, there being no overlap between the groups (Table 1). There was a good correlation between the GGT activity in the ascitic fluid and that in biopsy samples of the liver. The numbers in the series were small, but the results appear promising, although in this series GGT did not appear to be superior to α-fetoprotein which was elevated in all the hepatoma patients. This procedure is worthy of more extensive evaluation.

REFERENCE VALUES FOR SERUM GGT ACTIVITY

Before considering the role of serum GGT in clinical medicine, it is necessary to address this important problem. Most studies demonstrate a striking sex difference for this enzyme activity in serum, values being lower in females. Pregnancy brings about a decrease in plasma GGT activity which is most marked in the third trimester.[32] Table 2 shows the widely differing normal ranges obtained by various authors even when the same substrate was used. According to one study entirely devoted to the problem, the normal range for children was somewhat lower than that for adult females, except in the newborn when very high levels were encountered.[105]

Szasz, in his now classic methodological paper,[60] defined normal ranges at 25°C, and gave conversion factors for various other temperatures. Unfortunately we have not found these applicable in population studies. What is not adequately appreciated is the importance of rigidly defining the nature of the population used to establish reference ranges. Insight into this problem is provided in Figure 3, which contrasts the ranges obtained on examining healthy laboratory staff with those from an age-matched population of blood donors.[7] As many as 5 females in the latter group exceeded the 95% confidence limit of 22 U/ℓ obtained among laboratory staff, and 40 males had values > 27 U/ℓ, the upper limit derived from our male laboratory staff. Seasonal, diurnal, and postabsorptive changes do not seem to affect serum GGT activity,[32] nor

TABLE 2

Normal Ranges[a] for Serum GGT Activity in U/l at 37°C (unless otherwise stated)

Males/females	Substrate	Ref.
0.425—2.18/0.375—1.485	α (*N*-γ-DL-glutamyl)-aminoproprionitrile	54
1.65—6.9/1.15—5.3	γ-L-glutamyl-α-naphthylamide	56
3—20; all subjects	γ-L-glutamyl-α-naphthylamide	58[b]
2.1—10.5; all subjects	γ-glutamyl-α-naphthylamide	59
Up to 2.0/up to 1.85 mean ± 3 SD	*N*-(DL-γ-glutamyl) aniline	55
9—16, all subjects	γ-glutamyl-*p*-nitroanilide	64
4.5—24.8/3.2—13.5	γ-glutamyl-*p*-nitroanilide	60[b,c]
Up to 45/up to 35 bimodal distribution	γ-glutamyl-*p*-nitroanilide	87[b]
4.5—22.0/4.0—13.0	γ-glutamyl-*p*-nitroanilide	200[b]
5—19/4—14	L-γ-glutamyl-*p*-nitroanilide	65[b]
Up to 50/up to 30 from probability plots	L-γ-glutamyl-*p*-nitroanilide	68[b]
Up to 22/up to 27 from probability plots	L-γ-glutamyl-*p*-nitroanilide	116
10—45/9—28.5	L-γ-glutamyl-*p*-nitroanilide	32[b]
5—30/3—20 Nonparametric calculation	L-γ-glutamyl-*p*-nitroanilide	71[b,c]

[a] Mean ± 2 SD unless otherwise stated.
[b] Normal population included or consisted of blood donors.
[c] Assayed at 25°C.

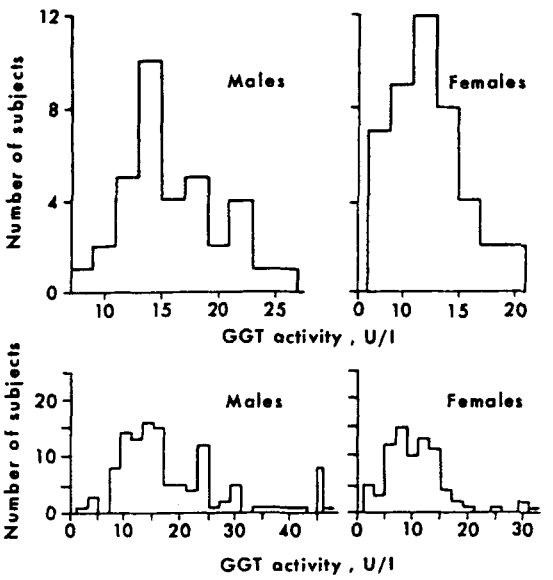

FIGURE 3. Distribution of serum GGT activities in healthy laboratory staff (upper panel) and in age-matched healthy blood donors (lower panel) using identical assay procedure. (From Martin, J. V., Gray, P. B., and Goldberg, D. M., *Clin. Chim. Acta*, 61, 99, 1975. With permission.)

does physical exercise.[106] The recent alcohol intake of the population, however, seems to be an important factor[107] and could account for the different ranges we observed in the two populations, since the samples from laboratory staff were collected toward the end of the working week, whereas the blood donor samples were from an industrial community and taken on a Monday — perhaps following an increased alcohol intake at the weekend. It is essential that each center determines its own normal range on a group of healthy subjects whose recent alcohol and drug intake are known.

A study was carried out on 6729 subjects attending for routine health examination.[108] The most important factors affecting GGT activity were drug intake, alcohol consumption, sex, age, and excessive weight. Reference intervals were derived. Table 3 presents these intervals on a "selected" subpopulation, excluding subjects who drank more than 1 *l* of beer or wine daily and women who took oral contraceptives. However, a high proportion of the original population was overweight and hypertensive, and it does not appear that such subjects were excluded from the subpopulation.

HEPATIC GGT AND ENZYME INDUCTION

It is mainly as a test for disease of the liver and biliary system that serum GGT has found a role in the diagnostic laboratory. Yet the liver ranks well behind many tissues such as kidney, brain, and testis in its GGT concentration. According to Wieme and Demeulenaire,[109] it is only sixth among human tissues rated for GGT concentration. One reason for its popularity in this regard is the acceptance by the laboratory community of the enthusiastic claims made by ardent advocates, which, as will subsequently be indicated, rarely stand up to critical analysis. The function of the enzyme in liver physiology is not clear, but presumably the γ-glutamyl cycle also operates in this organ. A special expression of its relation to glutathione metabolism in this tissue may have to do with the conjugation of drugs such as naphthalene and sulfobromophthalein which are coupled with glutathione in the liver prior to excretion. According to Hunter and Chasseaud[110] the glutathione-conjugating enzymes of human liver are soluble, whereas most of the enzymes involved in drug hydroxylation reactions are located in smooth endoplasmic reticulum (the main site of GGT in human liver). These enzymes are inducible by their substrates, the phenomenon being referred to as hepatic microsomal enzyme induction. For reasons that are not entirely clear, serum GGT activity is increased in many patients receiving enzyme-inducing drugs under circumstances where liver damage is highly improbable. Such increases are associated with alterations in other constituents indicative of hepatic microsomal enzyme induction.

The recent literature provides much evidence that enables us to be reasonably confident in making this statement. Data from a patient studied by Whitfield, Moss, Neale, et al.[111] are presented in Figure 4. Already receiving a standard dose of warfarin, a recognized enzyme-inducing drug, the serum GGT activity was elevated. No further increase occurred when barbiturate in a dose of 100 mg was given. When the dose was raised to 200 mg and 300 mg nocte, serum GGT rose proportionately, suggesting a dose-response relationship, and there was a simultaneous reduction in the serum warfarin concentration. Figure 5, from Bartels, Evert, and Hauck, et al.[112] describes the activities of serum enzymes frequently used to diagnose liver disease in 103 epileptic children on long-term anticonvulsant drug therapy. Some have increased alkaline phosphatase, but GGT manifests much the largest percentage of abnormal values in this patient population, and it is obviously responding much more effectively to hepatic microsomal enzyme induction than the other enzymes whose activities were measured. The situation among children receiving the anti-inflammatory drug aminopyrine is shown in Figure 6, again from the work of Bartels, Hauck, and Vogel.[113] Every patient had a serum GGT activity above the normal limit, whereas most of the other enzymes

TABLE 3

Serum GGT Activity (U/l at 37°C) as a Function of Sex and Age for a "Selected" Population

Percentiles age (years)	Males		Females	
	50	95	50	95
8—12	14.0	23.8	13.9	21.7
12—16	15.4	23.6	13.7	23.9
16—20	17.5	35.0	13.4	21.6
20—30	23.6	63.8	15.7	32.2
30—40	28.6	81.2	17.4	43.9
40—50	32.4	85.6	18.5	53.2
50—60	32.6	86.3	20.2	59.4
>60	28.6	72.5	22.5	48.5

From Schiele, F., Guilmin, A. M., Detienne, H., and Siest, G., *Clin. Chem. (Winston-Salem, N.C.)*, 23, 1023, 1977. With permission.

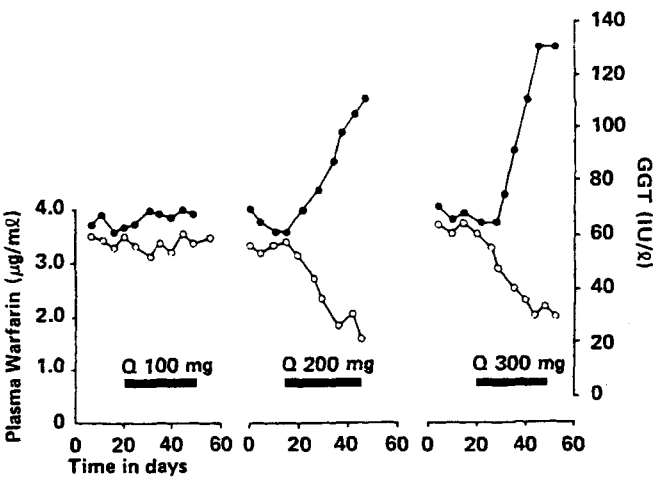

FIGURE 4. Changes in plasma GGT activity and plasma warfarin concentration in a patient on warfarin therapy given quinalbarbitone at the three dose levels indicated for 30 days at each level. • = plasma GGT activity; o = plasma warfarin concentration. (From Whitfield, J. B., Moss, D. W., Neale, G., Orme, M., and Breckenridge, A., *Br. Med. J.*, 1, 316, 173. With permission.)

remained normal. By examining the response pattern in one individual (Figure 7), it is apparent that serum GGT rose to almost 10 times the upper normal limit when the child was given aminopyrine and fell promptly on withdrawal. There was no change in the other enzymes measured, and no relationship with the other drugs (prednisone and penicillin) administered to the patient.

These same authors have provided an animal model for their clinical observations. When rats were fed phenytoin by stomach tube at 2-dose levels for 17 to 25 days, there were only minor changes in hepatic GGT and cytochrome P-450 content at the lower dose (100 mg/kg); at the higher dose (300 mg/kg) there was a 15-fold increase in hepatic content of GGT (partly due to increased liver weight), and a fourfold increase in enzyme specific activity in microsomes and cytosol associated with a moderate increase

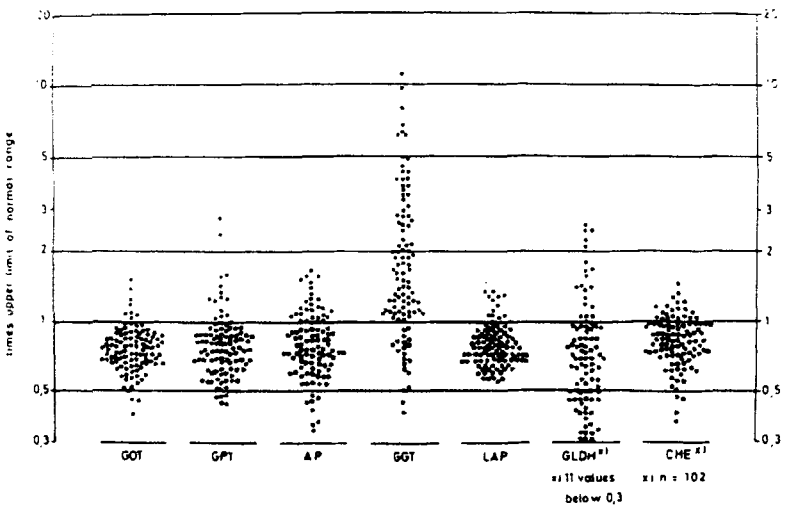

FIGURE 5. Serum activities of seven enzymes related to diagnosis of hepatobiliary disease in 103 epileptic children on long-term anticonvulsant therapy. Abbreviations as follows: GOT (aspartate aminotransferase), GPT (alanine aminotransferase), AP (alkaline phosphatase), GGT (gamma-glutamyl transpeptidase), LAP (leucine aminopeptidase), GLDH (glutamate dehydrogenase), CHE (pseudocholinesterase). Ordinate represents the activity of each case on a log scale in relation to the upper normal limit for each enzyme. (From Bartels, H., Evert, W., Hauck, W., Petersen, C., Putzki, H., and Schulze, W., *Neuropaediatrie*, 6, 77, 1975. With permission.)

in cytochrome P-450 (Table 4). Similar experience was reported among rats fed aminopyrine at a dose of 600 mg/kg daily for the same time period, except that the increased GGT content and specific activity of the two fractions were even more dramatic, despite a more modest increase in cytochrome P-450, emphasizing the important fact that indices of enzyme induction tend to show a loose, rather than a rigid correlation in many clinical and experimental situations (Table 5).

Bartels, Evert, and Hauck et al.[112] took the opportunity of demonstrating a good correlation between the urinary excretion of D-glucaric acid (an established index of microsomal enzyme induction) and serum GGT activity in their patients (Figure 8). An earlier study had shown a close association between serum GGT activity and D-glucaric acid excretion in patients with presumptive evidence of hepatic microsomal enzyme induction.[114] Although a poor correlation has been claimed in normal patients who have not been exposed to drugs,[115] this proves little except that basal levels of these constituents are independently regulated by genetic and environmental factors. It certainly does nothing to disprove the assertion that the similar increases that follow drug exposure are attributable to a common mechanism, namely, hepatic microsomal enzyme induction. Urinary excretion of 6β-hydroxycortisol is another index of microsomal enzyme induction. As shown in Figure 9, there occurred a parallel increase in serum GGT activity and urinary excretion of D-glucaric acid and 6β-hydroxycortisol in a patient given glutethimide, followed by a parallel decrease on withdrawal of the drug.[116] Even in newborns, who have higher GGT activities than normal adults, increased serum levels concomitant with increased urinary D-glucaric acid excretion accompanied phenobarbital administration.[117]

The usefulness of serum GGT activity as an index of hepatic microsomal enzyme induction appears secure on the basis of these and many related observations. Three points should be emphasized at this stage:

1. Whereas it takes a minimum of 24 hours to collect the urine necessary for assay

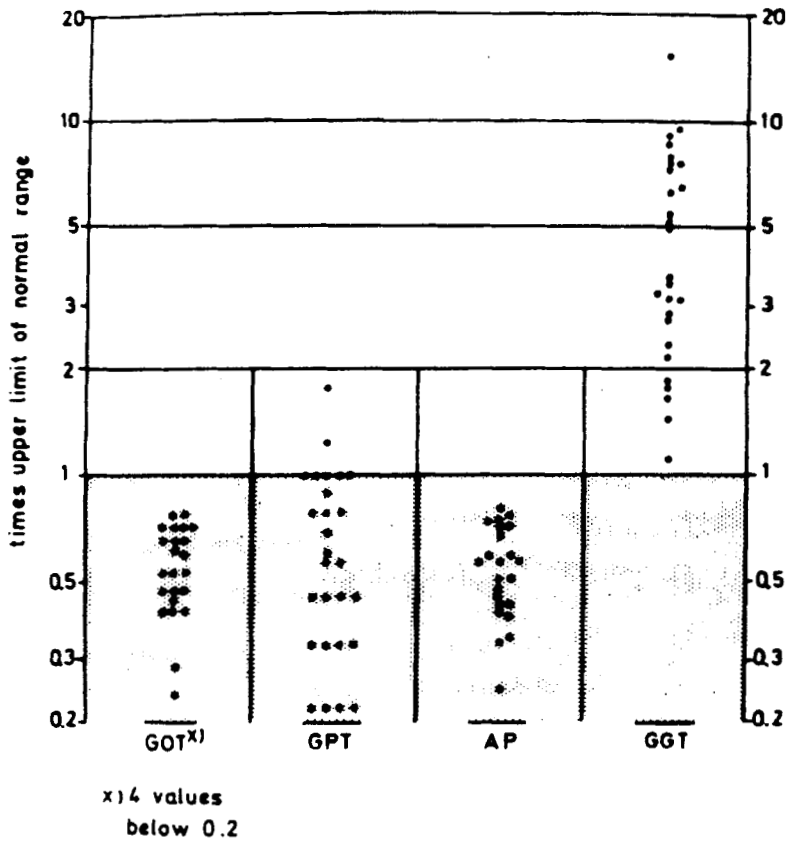

FIGURE 6. Serum activities of four enzymes in children on long-term treatment with animopyrine. Abbrevations and ordinate as described in legend to Figure 5. (From Bartels, H., Hauck, W., and Vogel, I., *J. Pediatrics*, 86, 298, 1975. With permission.)

of D-glucaric acid and 6β-hydroxycortisol, both of which are fraught with technical uncertainties, serum for GGT can be obtained with ease and the assay completed in a matter of minutes.

2. The phenomenon of hepatic microsomal enzyme induction shows a spectrum of effects in that some drugs switch on the synthesis of enzymes responsible for the metabolism of a wide range of other drugs, some switch on the hydroxylating enzymes for relatively few other drugs, and some actually inhibit the metabolism of a number of other drugs.[118]
3. Sequential studies in the same patient are much more valuable than single determinations at one point in time if one is seeking evidence of enzyme induction, since fourfold changes can occur in the chemical indices without the upper reference limits necessarily being exceeded.

By and large, serum GGT activity has a useful role in clinical studies on enzyme induction, and can be of special help in distinguishing the patient whose drug control is faulty due to rapid metabolism occasioned by induction with the necessity for much larger dosage from the patient whose lack of stabilization is due to poor compliance with drug therapy.[119]

Rosalki[120] has made a major contribution in this area. He observed increased plasma activity for GGT in 72% of males and 53% of females receiving anticonvulsant drugs. The isoenzyme pattern in epileptics resembled that of patients with chronic hepato-

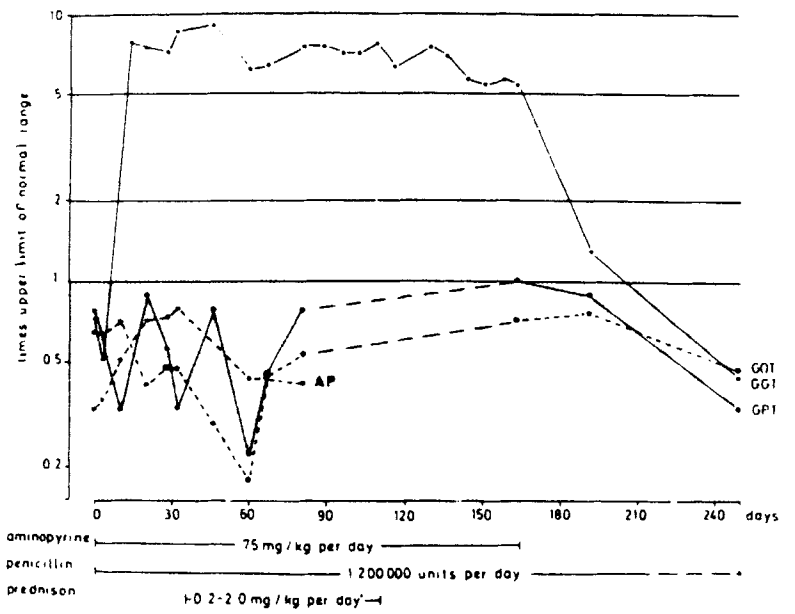

FIGURE 7. Alteration in serum activities of four enzymes in a girl during antirheumatic treatment to demonstrate influence of aminopyrine. Abbreviations and ordinate as described in legend to Figure 5. (From Bartels, H., Hauck, W., and Vogel, I., *J. Pediatrics*, 86, 298, 1975. With permission.)

TABLE 4

Gamma-glutamyltransferase Activity in Liver and Serum and Cytochrome P-450 Content in Liver Microsomes of Phenytoin-treated Rats

		Phenytoin	
	Controls	100 mg/kg	300 mg/kg
Liver			
Total GGT activity (U per liver)		7.07	74.00
Homogenate	4.54		
Supernatant 100,000 × g	0.20	0.38	0.77
Specific GGT activity (U/g protein)		8.85	47.40
Microsomes	8.65		
Supernatant 100,000 × g	0.21	0.33	0.83
Cytochrome P-450 ($\Delta E \times 10^{-1}$)	7.00	8.30	11.90
Serum GGT activity (U/*l*)	1.50	1.50	4.23

From Bartels, H., Evert, W., Hauck, W., Petersen, C., Putzki, H., and Schulze, W., *Neuropaediatrie*, 6, 77, 1975. With permission.

cellular disorders, and the increased activity was presumed to be of liver origin. There was no correlation between plasma GGT elevation, anticonvulsant drugs scores, and time since the last convulsion; however, serial studies in individual patients demonstrated a relationship between increasing drug dose and serum GGT levels. Tolbutamide, a weak enzyme inducer in man, was not associated with increased serum GGT activity, and only 6 of 59 female subjects receiving oral contraceptives had raised levels. The author speculated that female sex hormones may be inhibitory in vivo to GGT, citing the generally lower levels in females and the further decrease which occurs during pregnancy.

TABLE 5

Gamma Glutamyl Transpeptidase Activity in Liver and Serum and Cytochrome P-450 Content in Liver Microsomes of Aminopyrine-Treated Rats (Mean Values and SD of Four Animals)

	Controls		Aminopyrine, 600 mg/kg daily for 18—25 days	
	$\bar{x}$	S_D	$\bar{x}$	S_D
Liver				
Total GGT activity (U per liver)		1.41	147.90	42.80
Homogenate	4.24			
Supernatant 100,000 × g	0.18	0.04	1.11	0.10
Specific GGT activity (U/g protein)		3.00	75.30	10.40
Microsomes	10.4			
Supernatant 100,000 × g	0.23	0.03	1.23	0.46
Cytochrome P-450 ($\Delta E \times 10^{-2}$)	5.70	0.50	7.20	1.30
Serum GGT activity (U/l)	1.41	0.13	6.44	0.58

From Bartels, H., Hauck, W., and Vogel, I., *J. Pediatrics*, 86, 298, 1975. With permission.

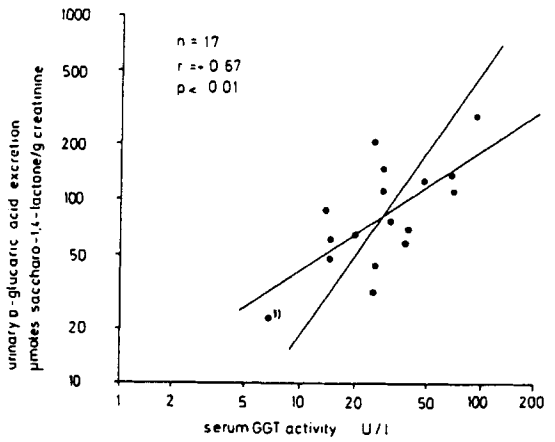

FIGURE 8. Relation between serum GGT activity and urinary D-glucaric acid excretion on log/log scale in 17 epileptic children treated with anticonvulsants demonstrated by regression lines of x on y and y on x. The point "1)" denotes a patient whose treatment differed somewhat from the remainder. (From Bartels, H., Evert, W., Hauck, W., Petersen, C., Putzki, H., and Schulze, W., *Neuropaediatrie*, 6, 77, 1975. With permission.)

In 49 epileptic patients taking anticonvulsant drugs, serum GGT was clearly elevated in 12 and borderline in 8. Total serum alkaline phosphatase was raised in 9 of the patients;[121] electrophoresis on polyacrylamide gels showed that the bone isoenzyme of alkaline phosphatase was responsible for the elevation. There was no correlation between total serum alkaline phosphatase and GGT, but increased serum GGT was often accompanied by increase in the proportion of the bone enzyme in the total serum alkaline phosphatase activity. The assocation between raised serum GGT and alkaline phosphatase activities in epileptic patients can clearly be due to divergent mechanisms: microsomal enzyme induction for the former, and osteomalacia due to deranged Vitamin D metabolism for the latter.[122] This report emphasizes the fallacy of making deductions about alkaline phosphatase isoenzymes on the basis of GGT assays.

Using a different protocol and a different oral contraceptive combination, Martin, Martin, and Goldberg[123] found a significant increase in serum GGT activity in a cohort

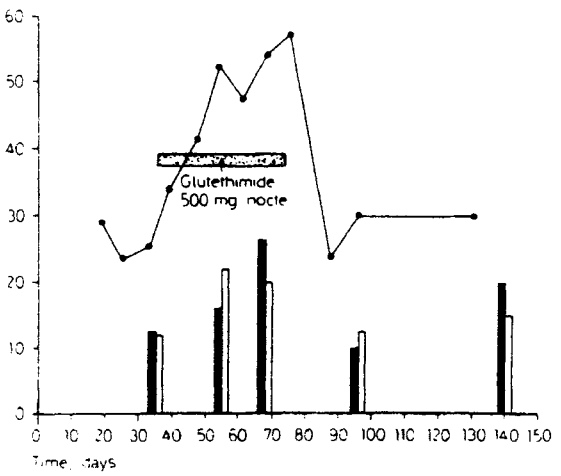

FIGURE 9. Changes in serum GGT activity (• = IU/ℓ), and urinary excretion of D-glucaric acid (■ = mg/24 hr) and 6β-hydroxycortisol (□ = μg/24 hr) in a patient during administration of glutethimide. (From Goldberg, D. M. and Martin, J. V., *Digestion*, 12, 232, 1975. With permission).

of females 6 months after being placed on the drug. A dissociation between D-glucaric acid and serum GGT activity in pregnancy was reported.[124] The former almost doubled between the first and third trimesters, whereas no change in GGT occurred. In fact, the mean value during the third trimester was a little lower than that during the first. This is in accord with a study indicating that females with viral hepatitis had lower mean values for serum GGT activity if they were receiving oral contraceptives or were in the last 20 weeks of pregnancy.[10] Attempts to discover an inhibitor of GGT activity in the serum of such patients were not fruitful, but produced results demonstrating interference by high bilirubin concentration in the GGT assay leading to lower activity. With regard to the first of these reports,[124] the authors accepted the possibility of a hemodilution effect. Moreover, it is possible that pregnancy brings about metabolic changes in carbohydrate metabolism leading to increased flux through the glucuronic pathway resulting in increased excretion of D-glucaric acid. A further critical point is that the authors related the excretion of the latter to urinary creatinine which is known to alter in pregnancy.

In contrast with the behavior of the enzyme in plasma and liver, the GGT content of leukocytes did not vary with sex and was unaffected by anticonvulsant drugs.[125]

GGT AND ALCOHOLISM

Animal Experiments

Alcohol is one of the most potent and most natural enzyme inducers in the world, as the work of Lieber and his associates has clearly shown.[126] Chronic alcohol consumption for 4 to 5 weeks was said to cause enhanced GGT activity in serum of rats, together with increase of hepatic GGT, slightly in the cytosol, and quite dramatically in the microsomes.[127] Deoxycholate added to the microsomes caused an increase of 20 to 30% in both control and alcohol-fed rats. Normal rat serum activity was around 1 U/ℓ and the method of Szasz[60] was used. It is difficult to accept the conclusions of this paper, since the value for the serum and cytosol recorded would barely be measurable. Moreover, the microsomal data are referred to gram of liver rather than to

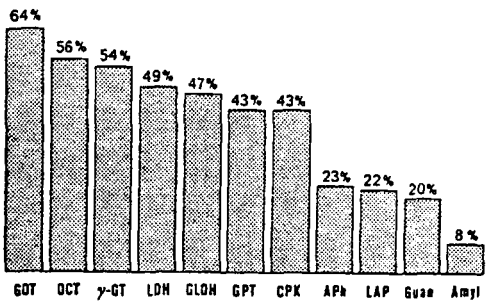

FIGURE 10. Percentage of 100 chronic alcoholic patients with abnormal activities for the following serum enzyms: GOT (aspartate aminotransferase), OCT (ornithine carbamoyltransferase), Y-GT (GGT), LDH (lactate dehydrogenase), GLDH (glutamate dehydrogenase), GPT (alanine aminotransferase), CPK (creatine phosphokinase), APH (alkaline phosphatase), LAP (leucine aminopeptidase), Guan (Guanase) and Amyl (Amylase). (From Konttinen, A., Hartel, G., and Louhija, A., *Acta Med. Scand.*, 188, 257, 1970. With permission.)

protein content and could merely reflect increased microsomes per liver cell. Six weeks of feeding ethanol as 36% of total calories to female rats doubled hepatic microsomal GGT activity whether expressed per gram of liver or per milligram of microsomal protein.[128] Intestinal GGT activity was also significantly enhanced by ethanol, whereas there was no change in the activity of the enzyme in kidney or pancreas. As in the previous report, a significant increase in plasma GGT activity was claimed, but once again, this is based on activities that seem to the present reviewer to be barely measurable, the mean for the control group being 1.92 U/*l*, rising to 2.76 U/*l* in the ethanol-fed animals. Exposure of adult rats to ethanol in air over a 13-day period increased GGT content in some, but not all, regions of the brain. The author speculated that this may be related to increased levels of γ-amino butyric acid noted in alcohol-treated animals.[128a]

Clinical Studies

One of the first systematic studies of serum GGT activities among chronic alcoholics revealed an incidence of 54% of abnormal values for this enzyme compared with 56% for ornithine carbamoyl transferase (OCT) and 64% for aspartate aminotransferase (GOT) as outlined in Figure 10. The authors postulated that the dominance of GOT was due to an associated myopathy known to accompany chronic alcoholism.[129] More recently, a frequency of 74% of abnormal GGT activities has been reported in 66 alcoholic patients with histological evidence of liver damage, and the levels did not change in these patients during abstinence from alcohol.[130] Between these publications, there appeared a number which sought to establish the utility of the serum GGT assay in the diagnosis and prognosis of chronic alcohol consumption. Values up to tenfold the upper normal limit were obtained in outpatients classified as alcoholics, but with no clinical evidence of liver disease, and up to fourfold in "heavy drinkers" who were not alcoholics.[131] Confusion has been added to this picture by the data presented in Table 6, taken from an analysis of GGT in relation to drinking habits graded as abstainers (teetotal), social drinkers, and heavier drinkers ranked 1 to 6 and > 6. From abstainers up to grade 6 drinkers there was no significant increase in the incidence of raised GGT activity.[132] Although these authors advocated the assay as a good index

TABLE 6

Relationship Between Abnormal Serum GGT Activity and Alcohol Consumption

Drinking group	No. in group	No. with abnormal GGT	% with abnormal GGT
Teetotal	37	8	21.6
Social	50	6	12.0
1—2	49	8	16.0
3—6	53	13	24.5
Over 6	49	23	46.9
Total	238	58	24.4

From Rollason, J. G., Pincherle, G., and Robinson, D., *Clin. Chim. Acta*, 39, 75, 1972. With permission.

of alcoholism or alcoholic consumption, it is unwise to adopt this recommendation without more critical analysis. A study of 2034 healthy men in the upper social classes revealed 14.9% with elevated GGT activity.[133] In a subgroup of 146 patients, whose alcohol consumption was carefully assessed, serum activities of GGT and aspartate aminotransferase appeared to reflect this consumption. Both enzymes seemed to be extremely sensitive markers of alcohol intake, with high levels commonly found among individuals whose drinking habits would be considered normal and acceptable (Figure 11). GGT was recommended as a marker of alcohol intake primarily in the sense that normal activity would be most unlikely in a patient developing physical illness due to alcohol abuse. An evaluation of the drinking habits in 55 adolescents aged 12 to 22 years was carried out.[134] Serum GGT activity was elevated in 12 of 15 who consumed 6 or more drinks a day, and none of the remaining subjects whose consumption was below this cutoff point.

Rosalki[120] reported higher activities of GGT and a greater incidence of abnormal values in alcoholics and heavy drinkers than for the aminotransferases and alkaline phosphatase. The isoenzyme pattern in alcoholics resembled that of patients with chronic hepatocellular disorders, and the increased activity was therefore presumed to be of hepatic origin as earlier described by this author for epileptic patients. In a later paper, he presented further data (Table 7) from which it is possible to argue that aspartate aminotransferase is a better test for alcoholism, since it provided a measure of discrimination between alcoholics and heavy drinkers, whereas GGT was raised in more than 80% of patients in both groups.[135] Rosalki more recently reviewed the role of GGT in the detection and monitoring of alcoholism and its associated liver damage.[136] The incidences in heavy drinkers and alcoholics were similar — around 85%. This underlines the fact that GGT is a test for excess alcohol consumption independent of liver damage. One gram of alcohol per kilogram of body weight produced only mild elevations of GGT in healthy volunteers and these had reverted to predrinking levels within 19 hr. Some 3 weeks of daily alcohol consumption at this dose level seemed to be required before pathological serum GGT activities were seen. When an alcoholic stopped drinking, GGT usually reverted to normal within days. The majority showed reduction of 50% within 2 weeks and near-normal values within 5 weeks. More persistent elevation was however seen in the presence of underlying cirrhosis. In many of these cases, the presence of an α-1 alkaline phosphatase isoenzyme associated with cholestasis and said to be elevated in a high proportion of patients who were alcoholics was less frequently detected than the raised GGT activity. Indeed the presence of bone alkaline phosphatase isoenzyme was noted in 5 of 20 subjects examined and may related to the enzyme-induced action of alcohol affecting Vitamin D metabolism with

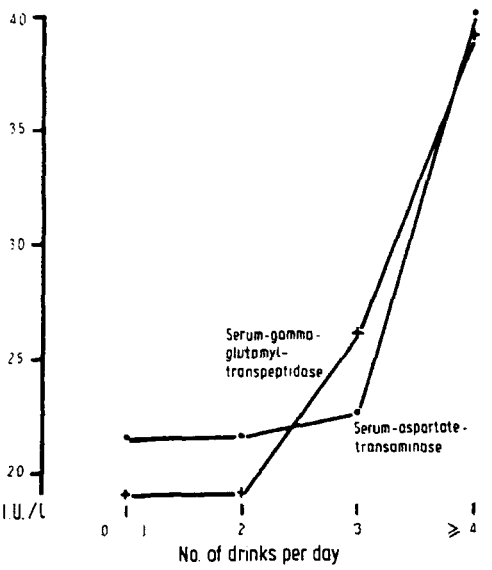

FIGURE 11. Serum GGT and aspartate aminotransferase activities related to daily alcohol intake in a subgroup of 146 subjects. (From Whitehead, T. P., Clarke, C. A., and Whitfield, A. G. W., Lancet, 1, 978, 1978. With permission.)

TABLE 7

Incidence of Serum Enzyme Elevation* in Alcoholics and Heavy Drinkers

	GGTP (%)	GOT (%)	GPT (%)	AP (%)
Outpatients				
Heavy drinkers	85	0	9	0
Alcoholics	88	45	25	6
Inpatients				
For "drying-out"	85	42	21	11
For complications	81	65	—	38
Total	85	48	21	20
	(137/162)	(67/140)	(19/89)	(24/123)

* Above the 97.5th percentile, or more than 2 standard deviations above the normal mean. GOT, aspartate transaminase. GPT, alanine transaminase. AP, alkaline phosphatase.

From Rosalki, S. B., *Rev. Epidém. Santé Publ.*, 25, 147, 1977. With permission.

consequent metabolic bone disease. As emphasized previously, this potentially jeopardizes use of serum GGT assays to draw conclusions about alkaline phosphatase isoenzymes.

A study of 202 patients with alcoholic liver disease whose investigations included a liver biopsy revealed that 87.7% had abnormal GGT activity in serum, compared with 68.8% who had elevation of serum aspartate aminotransferase activity.[137] Since neither the frequency of raised enzyme activities in patients suspected of alcoholic liver disease, but in whom the biopsy did not support the diagnosis, nor the prevalence of alcoholic liver disease in patients presenting to the authors over the 7-year examination period were given, a proper evaluation of this report is not possible. The upper reference level for serum GGT was exceeded by 63% of a group of 135 chronic alcoholics admitted

for detoxification.[138] The same authors reported that ingestion of 70 g ethanol caused a 20% elevation in mean serum GGT activity 12 hr later. This effect of ethanol has been confirmed on healthy subjects[139] although the increase was reported to be 25% at 60 hr after ethanol ingestion. In a further study,[140] the same authors exposed eight healthy volunteers to four consecutive identical ethanol challenges. Increases in the group mean activities were 12, 11, 22, and 18% following these four challenges. It is not clear whether this reflects increasing sensitivity to alcohol with repeated exposure, since the changes were not analyzed statistically. Peak values ranged from 1% in 1 individual to 57% in another, and large variations were noted within the same individual during the four challenge periods, 1 subject showing a slight decrease below baseline during the second exposure and a 25% increase following the third exposure.

Much depends on the question one asks of a test. Lamy and his colleagues, in a series of papers, have emphasized the dynamics of serum GGT activity following alcohol withdrawal and have drawn attention to the value of sequential determinations in charting the progress of such patients.[107,141-143] Their claim that values in alcoholics with proven cirrhosis may return to normal after 1 year of abstinence is contrary to the experience of other authors already cited,[130] but their contention that serum GGT activity falls and rises on withdrawal and reexposure to alcohol in the chronic alcoholic has been confirmed.[144] However, withdrawal of alcohol led to a slight reduction in GGT activity of heavy drinkers which was not statistically significant, and a much more dramatic reduction in chronic alcoholics which was significant.[145] In summary, there is controversy as to the utility of serum GGT assays in detecting covert alcoholics, in monitoring abstinence in known alcoholics, and in distinguishing functional alcoholism from alcoholic liver disease. There is a real need for a multicenter study employing standardized criteria to settle these issues, since this does appear to be a diagnostic area in which GGT could potentially make a major contribution.

GGT AND CANCER

Experimental Studies

Dramatic increases follow exposure of animals to chemical carcinogens.[146] Whereas a slight increase in GGT activity took place in homogenates prepared from regenerating rat liver, hepatomas showed increases around 150-fold suggesting that these changes are characteristic of hepatocyte transformation and not of rapid cell proliferation as such.[147] GGT activity in the tumor and body fluids of experimental animals does not necessarily correlate with the growth rate of the tumor, since a rapidly growing variant of the Morris hepatoma 5123D had much lower GGT activity than the parent tumor and actually inhibited GGT activity in the tumor and body fluids of recipient animals bearing both the original and variant tumor lines.[148] Evidence was presented that this was due to an inhibitor of GGT synthesis rather than to an inhibitor of enzyme activity. Studies with the carcinogens 2-acetylaminofluorine and diethylnitrosamine have shown a 20- to 30-fold increase in GGT activity in preneoplastic nodules of rat liver.[149,150] Increased activity was even seen in very early putative preneoplastic hepatocytes 7 days after a single dose of carcinogen. Histochemical techniques confirmed these biochemical observations and demonstrated most intense staining in bile canaliculi. The authors are of the opinion that GGT can now be utilized as an effective phenotypic marker to follow the progression to malignancy of at least one early population of carcinogen-altered hepatocytes. However, these observations will have to be reconciled with an earlier report that kinetically, immunologically, and structurally, the GGT of azo dye-induced hepatomas was similar to the enzyme of fetal rat liver.[151]

The activity of GGT in whole-skin of the mouse was elevated ninefold during periods

of hair growth, rising and falling abruptly with progression through the growing phase and return to the resting phase of the hair growth cycle.[152] Further studies were performed by these authors on chemically induced skin tumors. No GGT could be demonstrated histochemically in eight benign papillomas; however, seven of eight malignant squamous carcinomas demonstrated pockets with intense GGT staining associated with single cells, islands of epithelial cells, and follicle-like structures. Among the suggestions they raise as a result of this work is the possibility of a follicular origin for the GGT-positive carcinomas.

Isoelectric focusing has been used to study the GGT isoenzymes of normal and cancerous rat mammary tissue.[153] At least 12 enzymatically active species were detected, tumor tissue having a greater proportion of negatively charged species. This difference was greatly reduced by treatment with neuraminidase, but not with papain. A 13-fold increase in sialyltransferase activity in the tumors compared with normal mammary tissue suggested that increased sialylation of the tumor enzyme was responsible for the preponderance of negatively charged species.

A novel GGT found in renal carcinoma moved on electrophoresis more quickly than the enzyme of normal kidney, but more slowly than normal liver enzyme. The fast mobility of the carcinoma enzyme was not abolished by neuraminidase. Its molecular weight was 130,000 compared with 90,000 for normal kidney, but the catalytic properties of the 2 enzymes were very similar.[154] This enzyme was found in 5 of 10 patients with renal carcinoma. It appears to differ from the normal renal enzyme in chemical constituents other than sialic acid.

Clinical Studies

The main interest of GGT in human cancer diagnosis lies in its role as a marker of hepatic secondaries from a primary tumor. The early clinical literature cites much experience of raised serum GGT activities in patients with liver secondaries, often as the only chemical abnormality detectable. A histochemical study in human biopsy material defined the source of GGT as the surrounding normal liver tissue exposed to cholestasis and vascular proliferation, in addition to the tumor itself.[155] Aronsen and co-workers published an important study in which cancer patients were classified by surgical diagnosis into those with and without hepatic metastases.[156] Some pertinent findings are indicated in Figure 12. Better discrimination between patients with and without metastases was provided by GGT than by alkaline phosphatase or alanine aminotransferase (GPT), and it did not seem to matter greatly whether the former patients were jaundiced or not. Using a cutoff point of 30 units for GGT, the true positive rate was 90% and the false positive rate 9%. However, the authors excluded all patients with evidence of extrahepatic biliary obstruction, so that the true false positive rate could be expected to be much higher. Later authors who examined a series of 262 patients with colo-rectal cancer and found operative evidence of hepatic secondaries in 45 reported that approximately 25% of the patients without and 50% of those with metastases demonstrated raised serum GGT activity.[157] Such poor discrimination did not encourage the use of GGT as a screening procedure for liver secondaries.

In a study of 30 patients with histologically verified liver secondaries, serum GGT activity was raised in all 30, whereas liver scanning was positive in only 20 of the cases.[158] A similar 100% frequency of abnormal values was recorded for 5′-nucleotidase in this same series. GGT activity was measured in 79 patients, of whom 49 had malignant tumors and 18 benign diseases of the hepatobiliary system.[159] Enzyme activity was increased in all but two patients in whom liver metastases were found, and in only one of the cancer patients without liver metastases. The authors conclude that elevated GGT activity in the absence of jaundice suggests the presence of liver metastases, but these conclusions are unwarranted on the basis of the small numbers they

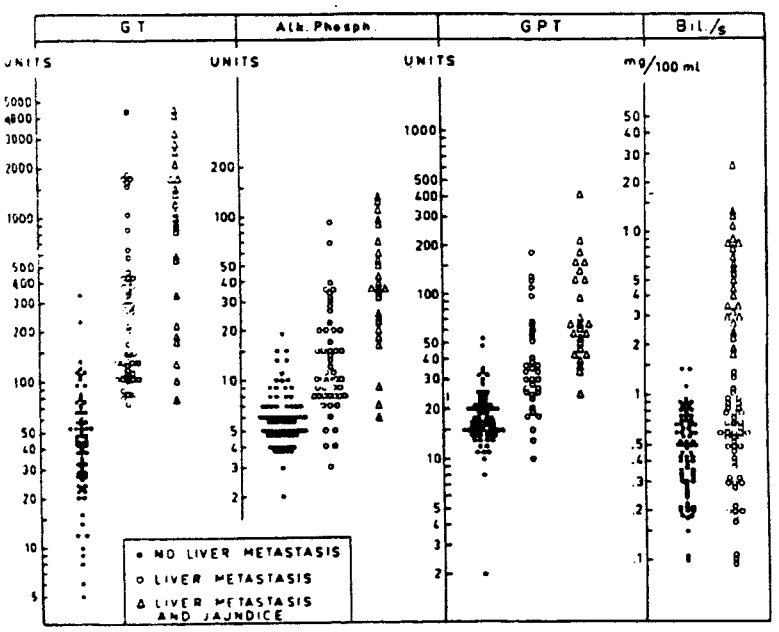

FIGURE 12. Activities of serum gamma-glutamyl transpeptidase (GT), alkaline phosphatase and alanine aminotransferase (GPT) and serum bilirubin concentration in patients with cancer classified according to presence or absence of liver metastases and jaundice. (From Aronsen, K. F., Nosslin, B., and Pihl, B., *Acta Chir. Scand.*, 136, 17, 1970. With permission.)

have examined. French authors compared GGT, aminotransferases, alkaline phosphatase and 5′-nucleotidase in 25 patients who were controls, 25 cancer patients without metastases, and 25 with hepatic metastases.[160] The numbers with increased GGT activity were 2, 3, and 24 in the 3 groups, respectively — a higher frequency than for the other enzymes tested in hepatic metastases, but it is remarkable that aspartate and alanine aminotransferase were reported to be abnormal in 5 and 6 of the patients, respectively, in the control group. This study was subsequently criticized with special reference to the exclusion of patients who consumed alcohol in the control group.[161] These authors found only 85% positivity for serum GGT activity in a larger series of cases with hepatic metastases and a much higher proportion of elevations in cases who did not have hepatic metastases, emphasizing the lack of specificity of the test and the bias introduced by selection of inappropriate reference populations.

Use of GGT in prognosis does not seem to be established on a secure foundation. Figure 13 describes the activities of GGT, 5′-nucleotidase and alkaline phosphatase in patients with Hodgkin's disease treated with nitrogen mustard therapy between the times indicated by the arrows.[162] Despite the dramatic elevation of GGT, no metastases were found in the liver at autopsy when the patients deceased at the end of the period of observation described in the figure. Suggestions have been made that by combining serum GGT assays with determination of carcino-embryonic antigen (CEA) titres, the prognostic value of both tests in cancer can be improved.[163-165] In a survey of 500 patients with colo-rectal cancer following surgery, 29 patients were confirmed to have liver metastases. Excluding 19 exposed to subsequent chemotherapy, the GGT values of the remaining 10 are charted in Figure 14 in relation to the time of detection of metastases, and abnormalities of the associated enzymes alkaline phosphatase and leucine aminopeptidase.[164] It will be intriguing and important to learn what happens to 39 patients with rising CEA and/or GGT levels over a period not less than 3 months

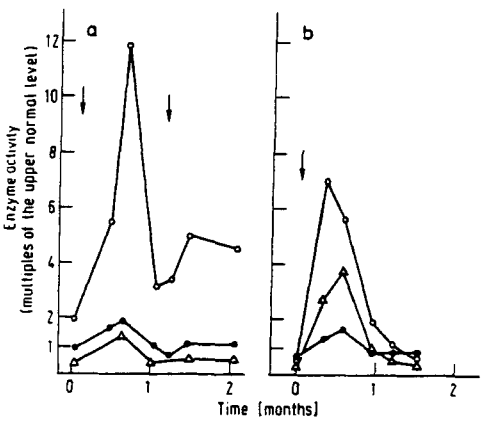

FIGURE 13. Variation with time of GGT (0), 5′-nucleotidase (•) and alanine aminotransferase (△) activities of serum in two patients with lymphoma who had no autopsy evidence of hepatic spread. (From Korsten, C. B., Persijn, J.-P., and van der Slik, W., *Z. Klin. Chem. Klin. Biochem.*, 12, 116, 1974. With permission.)

identified by these authors, who have not yet had metastases confirmed.

In a comparison of 4 enzyme tests for diagnosing metastases to the liver[166] 5′-nucleotidase showed the greatest diagnostic value, having the lowest proportion of false-positive results (7.4%) with the highest predictive value of a positive test (85.7%). GGT showed the lowest proportion of false-negative results (2.8%), but was the least specific (35% false-positive results). As with alcoholism, serum GGT assays may be of greater utility in excluding hepatic metastases. Again, disparate results from the many authors who have published on this topic emphasize the differences in criteria and patient material which probably underly this disagreement, and the need for authoritative multicenter trials to settle this question.

GGT AND LIVER DISEASE

Experimental Observations

In the rat, intrahepatic cholestasis induced by norethandrolone was characterized by the appearance of GGT activity at the cannalicular membranes of periportal parenchymal cells, staining intensity being dose-dependent and rapidly reversible. Extrahepatic cholestasis was characterized by early increase of GGT activity in ductular cells, with positive staining on lateral cell membranes of biliary lining cells. The parenchymal cells stained positively only after several days. This was interpreted as reflecting superimposed intrahepatic cholestasis produced by accumulation of biliary constituents in the parenchymal cells.[167]

The activity of GGT increased slowly in the liver and more rapidly in the serum of rats with ligated bile ducts.[168] These observations have been followed up in more sophisticated experiments by the same group of authors who studied the distribution of GGT in liver cell types, together with analysis of the changes seen after bile duct ligation.[169,169a] In control rat livers, highest enzyme concentration for GGT was found in Kupffer cell preparations. Fractions enriched with biliary tract cells had 2 to 4 times the enzyme activity found in isolated parenchymal cells. GGT concentrations tended to decrease 24 hr after bile duct ligation, most markedly in Kupffer cells. However, after 7 days the overall activity of GGT in the liver had increased fourfold, primarily

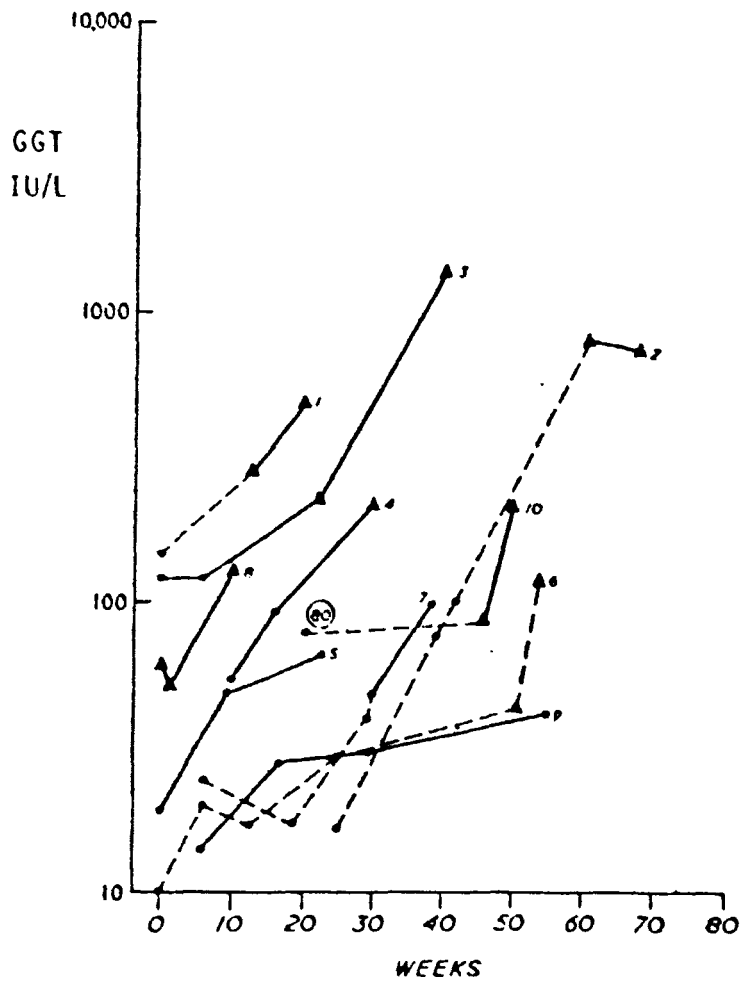

FIGURE 14. Serum GGT activities in 10 patients (numbered accordingly). Broken line indicates absence, and solid line the presence of clinically apparent metastases. Triangles indicate subjects with raised activity of serum alkaline phosphatase or leucineaminopeptidase. (From Cooper, E. H., Turner, R., Steele, L., Neville, A. M., and Mackay, A. M., *Br. J. Cancer*, 31, 111, 1975. With permission.)

in parenchymal and biliary tract cells as shown in Table 8. One of the striking findings in this paper is the low activity of the enzyme in parenchymal cells and the very high activity in Kupffer cells. More than 90% of the total GGT activity of the normal rat liver was calculated to be present in nonparenchymal cells. Proliferation of biliary epithelium begins 2 days after obstruction of the bile ducts.[170] These changes in cell distribution have not been fully taken into account in computing the contribution of the different cell types to the increase occurring after biliary occlusion. The results do not make allowance for possible increase in size of the liver, and the enzymes are only given as specific activities and not per unit wet weight or per whole liver. These concerns have been partially met since Moss has previously assessed the contribution of each cell type in normal rat liver to total hepatic GGT content, finding 14% of the total in parenchymal cells, 50% in Kupffer cells and 36% in biliary tract cells.[170a] He has also assessed the changes in these contributions after bile duct ligation. By the seventh day, GGT content of whole liver had risen almost fivefold but the Kupffer cell contribution had declined. The modest increases in parenchymal (fourfold) and

TABLE 8

Specific Activities (U/g protein) of GGT in Perfused Unfractionated Liver and in Enriched Cell Fractions for Control and Bile Duct — Ligated Rats

	Control	1 day after ligation	7 days after ligation
Whole liver	1.1 ± 0.2	0.9 ± 0.2	4.5 ± 0.6
Parenchymal cells	0.4 ± 0.1	0.5 ± 0.1	1.7 ± 0.3
Kupffer cells	23.3 ± 6.9	3.2 ± 1.2	17.6 ± 5.6
Biliary tract	12.4 ± 3.0	7.4 ± 1.5	21.4 ± 2.7

From Wootton, A. M., Neale, G., and Moss, D. W., *Clin. Sci. Mol. Med.*, 52, 585, 1977. With permission.

biliary (twofold) contribution did not seem adequate to account for the changes in whole liver.[170b] A comprehensive account of these and further studies to resolve these discrepancies by this eminent group would be timely and welcome.

Somewhat different results were obtained by Grant and Billing[171] who separated rat liver cells on gradients of Ficoll and metrizamide. Their experiments were chiefly concerned with the distribution of the enzyme between parenchymal and nonparenchymal cells. In normal rat liver, GGT activitiy was preferentially located in a fraction enriched with bile ductule cells. After bile duct ligation, specific activity increased twofold in the nonparenchymal cells and fourfold in the parenchymal cells. Since they only used one time interval (12 days) after ligation, and did not separate ductular cells from Kupffer cells, it is difficult to reconcile their studies with those of Wootton, Neale, and Moss.[169] Interestingly enough, both groups reported almost identical activity for GGT in parenchymal cells (approximately 0.5 U/mg protein).

Ligation of the bile-duct in the guinea pig generated a prompt increase in serum GGT activity reaching 10 to 20 times control values by 3 hr but falling slightly over the next 70 hr.[171a] Hepatic GGT declined over this period, and administration of cycloheximide, an inhibitor of protein synthesis, did not block the rise in serum GGT activity. Changes in serum bile salt levels paralleled those in GGT and the authors suggested that solubilization of membrane-bound enzyme by bile salts accounts for the increased serum activity. The authors convincingly spell out the advantages of the guinea pig over the rat as an experimental model to simulate conditions affecting GGT, although they do not explain why the increased serum activity reaches a plateau so abruptly — a phenomenon which does not seem to conform with the behavior of GGT in human cholestatic disease where the activity tends to progressively increase with prolongation of cholestasis, at least over a time-frame of days if not weeks.

Studies in the rabbit point to cholestasis and enzyme induction as potent factors leading to increased serum GGT activity in this species, but a renal role in modulating the extent of GGT synthesis occurring under these circumstances was also suggested.[172,173] Further experimental work to clarify the mechanism of raised GGT activity in hepatobiliary disease is much needed. Marked species variation in the activity of GGT in liver and serum exists (Table 9), and highlights the need for cautious interpretation of animal data until it has been established which species provides the closest approximation to the situation existing in human liver.

Diagnostic Value of Serum GGT Activity in Liver Disease — General Remarks

Contrary opinions on the general utility of GGT in the diagnosis of hepatobiliary disease will be found in previous reviews already cited.[32,116] In a further review,[174] the

TABLE 9

Specific Activity of GGT (U/mg protein) in Liver and Blood Serum (U/ℓ) of Three Animal Species*

	Rat (12)	Guinea pig (8)	Rabbit (9)
Whole homogenate	1.64 ± 0.76	21.67 ± 3.78	3.43 ± 0.64
Microsomes	2.85 ± 1.21	43.60 ± 7.05	11.14 ± 3.05
Blood serum	Undetectable	11.75 ± 2.01	4.44 ± 1.52

* Number of observations in parentheses. Data as mean ± SD.

From Goldberg, D. M. and Roomi, M. W., unpublished, 1979.

usefulness of serum GGT assays was compared with that of other enzyme determinations employed for the diagnosis of liver disease. It was found to be of little value in acute hepatitis. An elevation of the ratio of GGT to alanine aminotransferase was a much better index for intrahepatic or extrahepatic cholestasis than GGT alone, but cirrhosis combined with alcoholism also yielded high values for this quotient. It was of dubious value in chronic active liver disease. The authors concluded that the determination of GGT has value in the classification and characterization of liver diseases only when combined with other enzymatic tests. They state that in cholestatic disease there is no association between GGT activity and the increment in bilirubin concentration; however, this disparity is not borne out by data they present relating the mean increase in serum GGT activity to serum bilirubin concentration stratified according to the severity of the icterus.

Attention is drawn to the data of Table 10, compiled from two fairly large patient series which sought to make a case for the diagnostic value of this enzyme[175,176] as well as data collected by the present reviewer during a collaborative study. Consideration of the percentage of the cases with each condition manifesting abnormal serum GGT activity leads to the conclusion that irrespective of the kind of liver disease with which the patient is afflicted, or whether this is primary or secondary (as in cardiac failure), the percentage lies between 70 and 100% and provides little in the way of diagnostic guidance. Examination of individual patient values or the mean values for the different disease categories in these two publications and in our own unpublished series reveals such overlap as to offer no reliable discrimination. It is surprising that Lum and Gambino[175] in particular should have waxed so enthusiastic about the utility of GGT. Application of the admirable criteria proposed by Galen and Gambino[177] to judge the sensitivity, specificity, and predictive value of laboratory tests would reveal serious shortcomings with this enzyme assay. Proposals for the application of GGT assays in screening for hepatobiliary disease would add a 13th dimension to 12-channel disease. A study was performed to evaluate the usefulness of GGT in the screening of hospitalized patients.[178] Of 1040 unselected adult inpatients, 139 (13.4%) had elevated serum GGT activity; 32.4% of the elevations occurred in patients with primary hepatobiliary disease. This hardly bears out the assertion of the authors that serum GGT is a strong indicator of hepatobiliary dysfunction, since this was not the primary abnormality in the majority of the 139 cases.

Serum GGT as an Adjunct to Alkaline Phosphatase

Suggestions that GGT may complement or replace enzyme electrophoresis in patients with raised alkaline phosphatase activities of uncertain origin appear to have some merit,[179-181] although there is still an inherent risk in interpreting GGT elevations as pointing unequivocally to a hepatic origin for alkaline phosphatase or even hepatic disease, in view of its lack of specificity. The tendency of enzyme-inducing agents to

TABLE 10

Percentage of Cases with Various Diseases Manifesting Raised Serum GGT Activity

Disease	Percentage total cases with raised GGT		
	Lum and Gambino[a]	Boone, Routh, and Schrantz[b]	Goldberg, Martin, and Martin[c]
Hepatitis	94	100	100
Cholelithiasis	100	88	100
Liver metastases	100	88	94
Alcoholism	80	85	100
Cirrhosis	83	85	97
Acute pancreatitis	100	71	100
Heart failure	100	89	94

[a] From Lum, G. and Gambino, S. R., *Clin. Chem. (Winston-Salem, N.C.)*, 18, 358, 1972. With permission.
[b] From Boone, D. J., Routh, J. I., and Schrantz, R., *Am. J. Clin. Pathol.*, 61, 321, 1974. With permission.
[c] From Goldberg, D. M., Martin, J. V., and Martin, P. J., in *Clinical Enzymology Symposia*, Vol. 2, Burlina, A. and Galzigna, L., Eds., Piccin Medical Books, Padua, Italy, 1979, in press.

increase bone alkaline phosphatase, as well as hepatic GGT, is another pitfall which has already been alluded to. The advantage of electrophoresis in revealing multiple enzyme zones and abnormal molecular variants is sacrificed in the use of such indirect interpretation.

Some recent studies emphasize these pitfalls of using serum GGT activity to elucidate the source of increased alkaline phosphatase. In 31 unselected patients with high serum alkaline phosphatase activity, the source of the alkaline phosphatase was correctly identified by use of urea denaturation in 64%, by serum GGT activity in 64%, and by urinary hydroxyproline in 69%. Simultaneous performance of all three tests correctly identified the source of alkaline phosphatase in 88% of the cases.[182] Electrophoresis for alkaline phosphatase isoenzymes was not carried out in any of these patients, and the data seem to suggest the need for this approach rather than the indirect approach used. Another study found a high degree of correlation between GGT and the bile (α_1) and liver (α_2) fractions of serum alkaline phosphatase separated on agar gel in 282 cases with increased total serum alkaline phosphatase activity.[182a] The presence of α_1-alkaline phosphatase and increased GGT were more sensitive indicators of ethanol-induced liver involvement than other commonly used tests, including the LDH_5/LDH_4 ratio. However, the authors stated that fractionation of alkaline phosphatase sometimes yielded clinical information that could not be obtained by determination of GGT and total alkaline phosphatase activities alone.

Samples from 269 nonjaundiced patients with raised alkaline phosphatase activity were analyzed for GGT and 5′-nucleotidase and also alkaline phosphatase isoenzymes.[183] GGT was found to be a more sensitive index than 5′-nucleotidase in confirming the presence of a liver component of elevated plasma alkaline phosphatase. The authors state that if GGT is normal, it is probable that increased alkaline phosphatase is of bone origin. However, elevated GGT does not exclude a bone component, and in this situation isoenzyme analyses should be performed, a suggestion that the present reviewer considers to be reasonable and sensible, since 18 of 33 patients with bone isoenzyme had increased GGT, and in 11 this was quite marked. Twenty-one cases were listed with marked increases of one or all three enzymes presumed due to drug administration; this happened much more frequently with GGT than with the other two. In another report, fractionation of alkaline phosphatase yielded clinical information which could not be obtained by determining the activity of this enzyme and

GGT alone.[184] Although there was good correlation between activity of total GGT and appearance of the biliary-specific band of alkaline phosphatase, the appearance of other bands in acute and chronic hepatitis, and in some histological types of cirrhosis (presumed due to fibroblastic activity) was not reflected in GGT activity.

Serum GGT Compared with Other Hepatobiliary Tests

Does GGT then have any useful role in the identification and elucidation of patients with hepatobiliary disease? This question would have received an altogether different answer in 1956 than in 1979 because today the question must be reformulated: can GGT replace an established diagnostic procedure or add information of real value not currently available through existing tests? This question has been addressed in a number of studies comparing serum GGT activity with other tests conventionally employed or recently advocated in the diagnosis of hepatobiliary disease. Some of these studies employed computer-assisted techniques to quantitate the measure of discrimination introduced by GGT into a diagnostic profile.

Solberg and colleagues included GGT along with the enzymes aspartate and alanine aminotransferase and alkaline phosphatase in a profile of almost 30 tests with which they sought by means of discriminant function analysis to define the best combination of tests for certain diagnostic decisions.[185] Only in the discrimination of active chronic hepatitis from chronic persistent hepatitis was GGT activity included in the list of four most discriminatory tests. The other diseases to which the profile was applied included acute viral hepatitis, alcoholic cirrhosis, primary biliary cirrhosis, fatty degeneration of the liver, and hepatic metastases. A somewhat similar study by Plomteux and colleagues utilized 32 laboratory tests, and the disease processes studied included intra- and extra-hepatic obstruction, as well as liver cancer.[186] Serum GGT activity was not among the 10 most discriminatory tests identified by their computer-assisted analysis. Detailed analyses were carried out by Skrede and colleagues on a number of enzyme tests in eight parenchymal diseases of the liver.[187] Prominent increases in GGT relative to the other four enzyme tests evaluated occurred only in acute alcoholic hepatitis, alcoholic cirrhosis, and alcoholic steatosis as shown in Figure 15. An evaluation of the ratio between GGT and alkaline phosphatase in the same disease groups was also carried out, high values for this ratio only being detected in the three conditions already mentioned (Figure 16).

Serum GGT activity had a higher mean value in 35 patients with histological evidence of cholestasis than in 45 who did not show cholestasis. All but one of the former had lipoprotein-X present in serum, whereas this was found in only two of the latter group. As a test for cholestasis, lipoprotein-X was far superior, since the GGT values showed unacceptable overlap between the two groups.[188] In another report, the LP-X test was positive in 93% of 59 cases showing histological evidence of cholestasis and negative in 95% of 98 cases in whom histology was negative.[189] GGT was raised in 97% of the patients with a positive LP-X test, but it was also raised in 47% of the cases who were LP-X negative. The authors cite recent evidence that a considerable amount of GGT is bound to LP-X in the plasma of cholestatic patients. In summary, no clear advantage for GGT over existing tests for hepatic and biliary integrity has emerged, and its capacity to discriminate the various categories of hepatobiliary diseases is unimpressive.

SERUM GGT ACTIVITY IN OTHER DISEASES

GGT and Cardiac Disease

Ischemic heart disease is an area to which GGT has contributed much confusion.

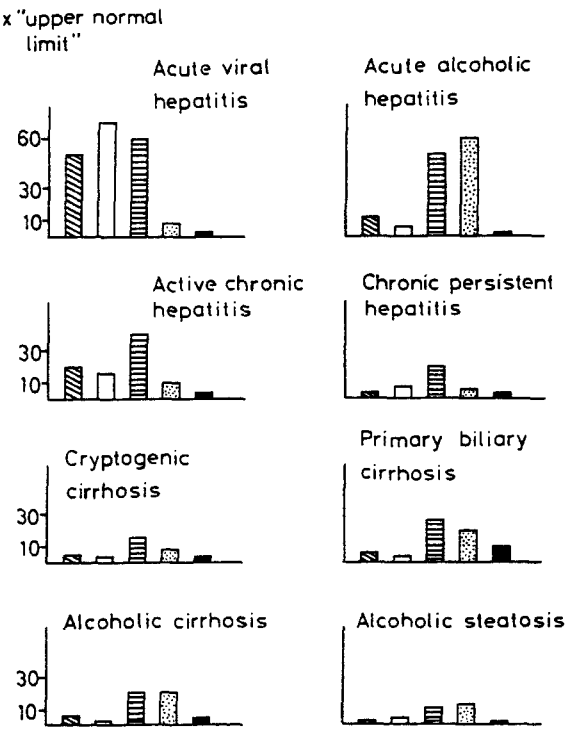

FIGURE 15. Mean values for various patient groups as related to upper-normal limits to show enzyme profiles in liver diseases. Symbols as follows ▨, aspartate aminotransferase; □, alanine aminotransferase; ▤, ornithine transcarbamoylase; ▩, GGT; ■, alkaline phosphatase. (From Skrede, S., Blomhoff, J. P., and Gjone, E., *Ann. Clin. Res.*, 8, 182, 1976. With permission.)

As indicated in Figure 17 which summarizes data from 20 patients with proven myocardial infarction,[190] values for this enzyme climb slowly to a peak around 250% of the upper normal limit at the end of the 1st week and remain elevated for up to 30 days, and certainly long after aspartate aminotransferase and lactate dehydrogenase have returned to normal. Unfortunately, as shown in Figure 18 taken from the same report, abnormalities also occur in > 50% of patients with severe angina pectoris. Although some authors have confirmed the high frequency of serum GGT abnormalities in infarct patients with a peak incidence around the 10th day of illness,[191,192] others have found the test more often positive in patients subsequently proven not to have sustained infarction than in those who did.[193] The mechanism of the postinfarct elevation is also in doubt, since heart muscle is lacking in GGT. It has been suggested that the origin of the serum GGT may lie in reparative processes occurring within the myocardium, in particular, regeneration of vascular endothelium which is rich in GGT.[191] Support for this work comes from an important study by Ewen and Griffiths[194] who dissected the varying patterns of serum enzyme activities in 50 patients with a presumptive diagnosis of myocardial infarction and identified a profile combining elevations of GGT, alkaline phosphatase, and 5′-nucleotidase, all of which are rich in vascular endothelium. Other authors have found the postinfarct increase in serum GGT activity predominantly related to onset of congestive cardiac failure,[195] or related in many cases to increased LD_5, suggesting concomitant liver damage.[196,197] Rosalki, in his review[32] cites similar findings in his laboratory, but stresses the different isoen-

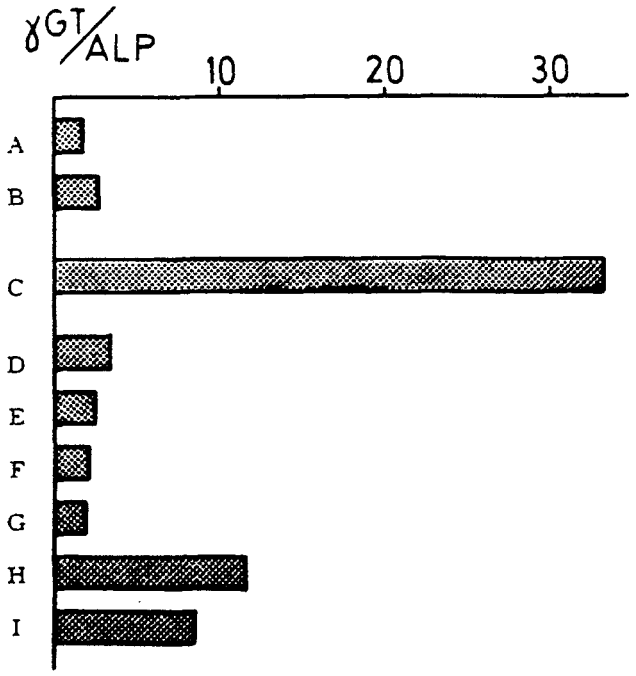

FIGURE 16. Mean ratio of GGT to alkaline phosphatase (ALP) in the following patient groups: A, reference group; B, acute viral hepatitis; C, acute alcoholic hepatitis; D, active chronic hepatitis; E, chronic persistent hepatitis; F, cryptogenic cirrhosis; G, primary biliary cirrhosis; H, alcoholic cirrhosis; I, alcoholic steatosis. GGT values were transformed to allow for sex-related reference values. (From Skrede, S., Blomhoff, J. P., and Gjone, E., *Ann. Clin. Res.*, 8, 182, 1976. With permission.)

zyme pattern for GGT in such cases compared with that reported in frank liver disease.[87,192] A sufficiently large number of patients following infarction have had documented increase in serum GGT unaccompanied by either cardiac failure or increase in other liver-specific enzymes to make it inherently improbable that release from a subclinically damaged liver can be the invariable cause of this increase. The possible contribution of microsomal enzyme induction occasioned by drugs given to treat these patients, such as anticoagulants and sedatives, does not appear to have been seriously entertained.

Other Diseases

Serum GGT increased in burns patients reaching a peak around 10 to 14 days and correlating with the degree of severity of the burns as shown in Figure 19. From parallel studies with serum urea and ribonuclease, it appears that this increase in GGT may be a consequence of increased protein catabolism and renal dysfunction.[198]

Prolonged elevation of serum GGT activity persisted for 24 months in 3 patients after infectious mononucleosis.[199] Among the reasons advanced for this phenomenon were an unusual degree of hepatic damage during acute infection, excessive induction of microsomal enzyme system activity by drugs, or EB virus persisting in a carrier state contributing to ongoing hepatic structural damage. Other enzymes commonly raised in hepatocellular disease were normal in late specimens from these three patients. A difficulty in interpreting these results is that the authors did not perform liver biopsies in their patients.

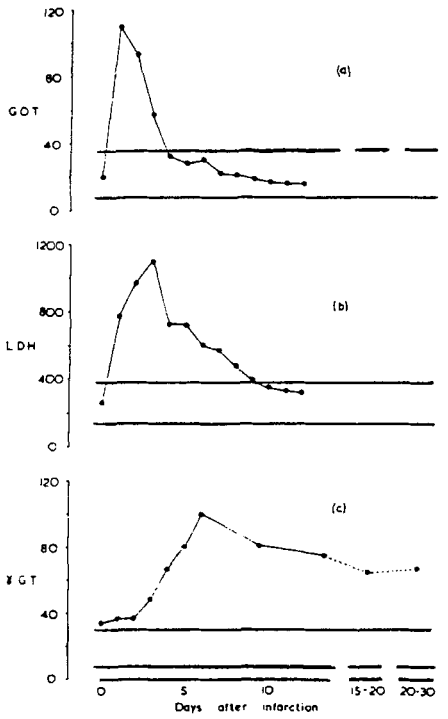

FIGURE 17. Mean value of serum activities of aspartate aminotransferase (GOT), (a); lactate dehydrogenase (LDH) (b); and GGT (c) with time after onset in 20 patients with proven myocardial infarction. Activities as U/*l*, with upper normal limit for each defined by horizontal line. (From Hedworth-Whitty, R. B., Whitfield, J. B., and Richardson, R. W., *Br. Heart J.*, 2, 432, 1967. With permission.)

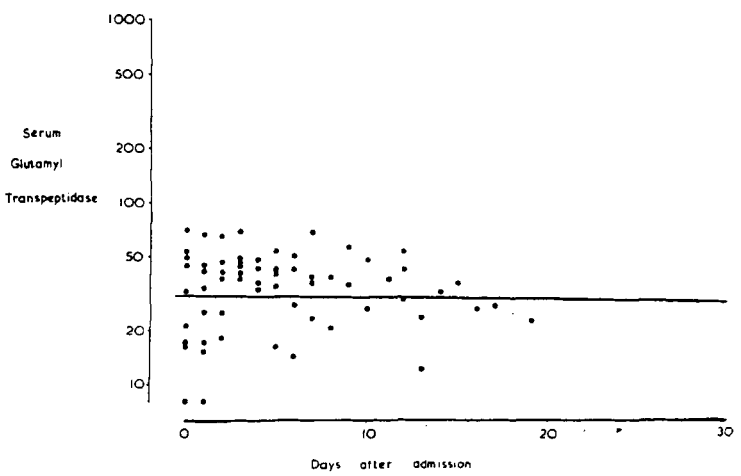

FIGURE 18. Serum GGT activities (IU/*l*) in relation to time after onset of symptoms in patients with severe angina pectoris. Horizontal line indicates upper limit of normal range. (From Hedworth-Whitty, R. B., Whitfield, J. B., and Richardson, R. W., *Br. Heart, J.*, 29, 432, 1967. With permission.)

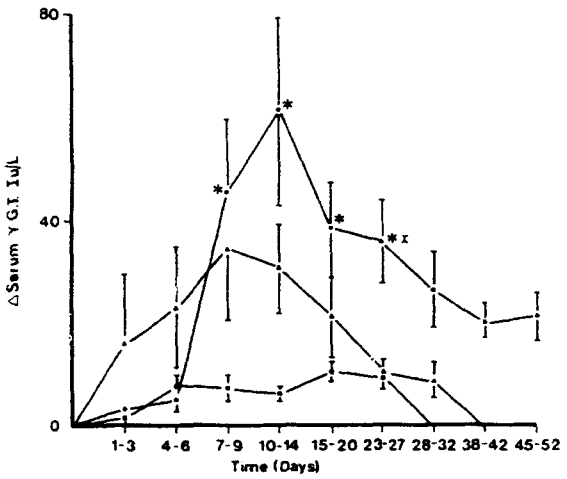

FIGURE 19. Mean change (± SE) in serum GGT activity with time in patients sustaining severe (•), moderate (▲), and mild (■) burns. (From Coombes, E. J., Shakespeare, P. G., and Batstone, G. F., *Clin. Chim. Acta*, 86, 279, 1978. With permission.)

Other diseases to be associated with increased serum GGT activity with high frequency are diabetes,[200,201] hypertension accompanied by type IV hyperlipoproteinemia,[202,203] and various neurological diseases other than epilepsy, such as vascular and neoplastic lesions of the brain.[204]

In the light of the many conditions other than hepatobiliary disorders which can lead to increased serum GGT activity, Beck[205] has attempted to develop a more specific test. After extraction with butanol, residual GGT activity was more than 75% in normal serum and less than 68% in normal urine. The residual serum GGT activity of patients with known hepatobiliary disease was reduced below this 75% cutoff point by butanol extraction more frequently than that of patients with raised serum GGT who did not have biochemical or clinical evidence of hepatobiliary disease, the figures being 81% and 34% respectively. However, the degree of overlap between the groups does not justify the author's contention that this is a valuable technique for distinguishing GGT elevations due to hepatobiliary and non-hepatobiliary diseases. Moreover, a meticulous examination of the effects of various detergents and organic solvents on human serum and urine GGT revealed major differences attributed to the different environments of the two enzymes rather than to intrinsic molecular factors, although the possiblity was raised that a lipid fraction associated with the enzyme protein modulated these responses.[205a]

ISOENZYMES OF GGT

Chemical Basis of Heterogeneity

The problem in utilizing GGT isoenzyme analyses for diagnostic purposes is that their genetic basis is poorly understood, and tissue-specific forms, or even tissue-specific patterns, have hardly been defined. The microheterogeneity seems to be posttranscriptional, and most likely related to changes in carbohydrate composition, since the enzyme is a glycoprotein with a carbohydrate content which can be as high as 35%. This property has been used to separate different forms by chromatography on Concanavalin A and Con A-Sepharose columns, with interesting results. A micro-adaptation of Con A-Sepharose chromatography has been published which the authors sug-

gest should enable the rapid separation of GGT isoenzymes for use in diagnosis of liver disease.[206] Rat liver and small intestinal mucosa contain a sialic acid-rich fetal enzyme and a sialic acid-poor adult form.[207] The kidney enzyme of several species was rich in sialic acid, and the pancreatic enzyme intermediate between this and the sialic acid-poor adult liver enzyme.[208] Human adult liver GGT like that of other animal species is poor in sialic acid, but it has been stated that in alcoholic hepatitis the enzyme has a much increased neuraminic acid content.[209] As mentioned earlier, the rat kidney enzyme has now been shown to comprise a dimer of two nonidentical glycopeptides with molecular weights of 46,000 and 22,000. This dimer is in turn associated with other as yet unidentified membrane proteins lacking GGT activity, since it is released on bromelain treatment of an aggregate with a molecular weight > 200,000.[46]

Clinical Utilization of GGT Isoenzyme

One of the first reported studies on GGT isoenzymes by Rutenburg, Smith, and Fischbein[211] used paper electrophoresis. They described two fractions in normal subjects which they called GT1 and GT2 (Figure 20). GT1 was less than GT2 and ran close to the albumin band. Figure 21 shows the pattern from a patient with portal cirrhosis; not only is the total GGT activity increased to 492 units, but there is a reversal of the normal pattern with GT1 predominating (the color density scale on the ordinate is only relative and does not correlate with total GGT activity of the sample). In extrahepatic obstruction, a third isoenzyme named GT3 and of β-globulin mobility made its appearance (Figure 22). The electrophoretic scan from a patient with adenocarcinoma metastatic to liver (Figure 23) shows a pronounced GT3 band, so that at this point one might conclude that the presence of this band is a good test for cancer or extrahepatic biliary obstruction. However, Figure 24 demonstrates this same band in a patient with drug hepatitis, so that the test appears to lack discriminatory capacity. Agarose gel electrophoresis was used in a more recent study,[212] and the authors identified fractions which seemed to approximate the GT1 and GT2 components of Rutenberg, Smith, and Fischbein.[211] Table 11 shows some of their data, and it may be seen that in chronic pancreatitis, carcinoma of the pancreas, and obstructive jaundice, the ratio of Fraction 1 to Fraction 2 was approximately unity. However, in chronic hepatitis and in intrahepatic cholestasis, this ratio was > 2.0, representing a significant increase. The mechanism of this change is hard to define, because few pathological features link intrahepatic cholestasis with chronic hepatitis (the former histological feature is generally absent from biopsy samples of patients with the latter disease), so this finding is rather puzzling.

Since these reports, many more have been published and have largely served to confuse the issue, because they have proliferated techniques without any commensurate increase in knowledge. One would be hard put to find two papers describing the authors' findings with the same methodology; consequently, claims for the diagnostic advantages of any particular method are for the most part unconfirmed. The clinical significance of the various isoenzymes is a matter of debate; their number, a matter for speculation; and their nomenclature a state of chaos. Seven zones of GGT activity were described following electrophoresis on cellogel;[213] one zone, in the α_2-β region, was stated to be especially prominent in patients with obstructive jaundice or drug-induced jaundice. However, later authors could only identify four bands on cellulose acetate membrane, and the patterns did not help to distinguish intra- from extrahepatic cholestasis.[214] Acrylamide gel electrophoresis revealed, in the hands of two groups, five zones of activity in serum from normal subjects or pregnant females.[215,216] Cord blood occasionally contained a sixth band, and patients with hepatobiliary disease additional slowly migrating bands, but this feature was of little value in diagnosing such diseases or in the detection of liver metastases.[215] Electrophoresis on cellogel revealed

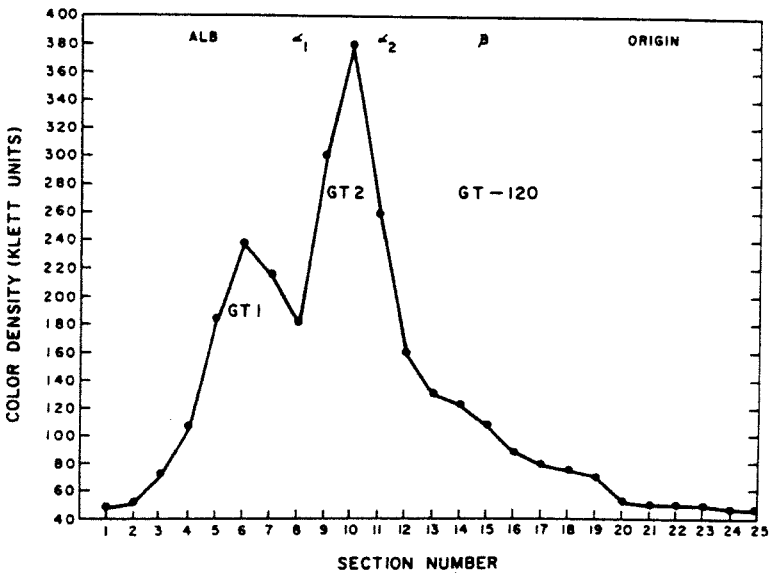

FIGURE 20. Electrophoretic pattern of GGT in normal serum with activity of 120 units, showing dominance of GT2. (From Rutenberg, A. M., Smith, E. E., and Fischbein, J. W., *J. Lab. Clin. Med.*, 69, 504, 1967. With permission.)

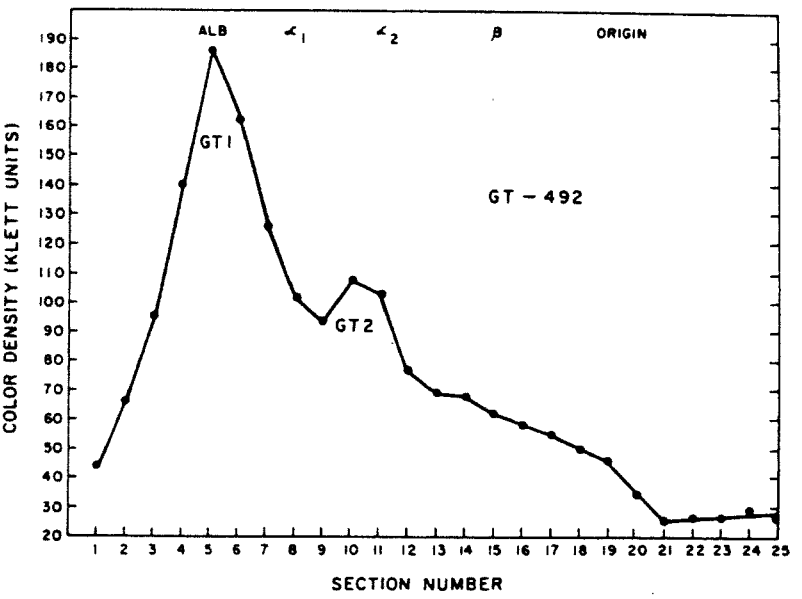

FIGURE 21. Electrophoretic pattern of GGT in serum from patient with portal cirrhosis and activity of 492 units, showing dominance of GT1. (From Rutenburg, A. M., Smith, E. E., and Fischbein, J. W., *J. Lab. Clin. Med.*, 69, 504, 1967. With permission.)

an α_2-β band which correlated with the presence of lipoprotein X and was said to be helpful in distinguishing extrahepatic from intrahepatic cholestasis.[217] A total of 14 of 19 patients with the former condition (74%) had a value > 30 U/ℓ for this isoenzyme, whereas only 7 of 22 patients with intrahepatic cholestasis (32%) exceeded this cutoff point for the same isoenzyme. In the opinion of this reviewer, such a degree of overlap is unacceptable.

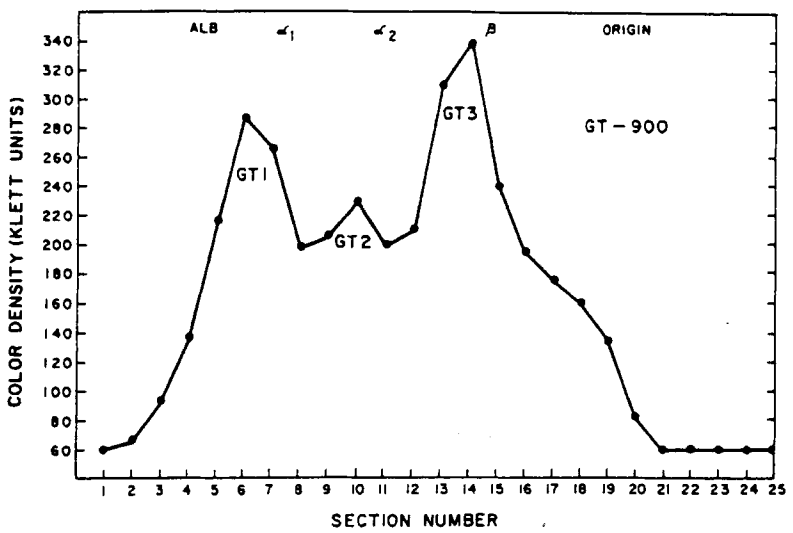

FIGURE 22. Electrophoretic pattern of GGT in serum of patient with extra-hepatic obstruction and activity of 900 units, showing appearance of GT3. (From Rutenburg, A. M., Smith, E. E., and Fischbein, J. W., *J. Lab. Clin. Med.*, 69, 504, 1967. With permission.)

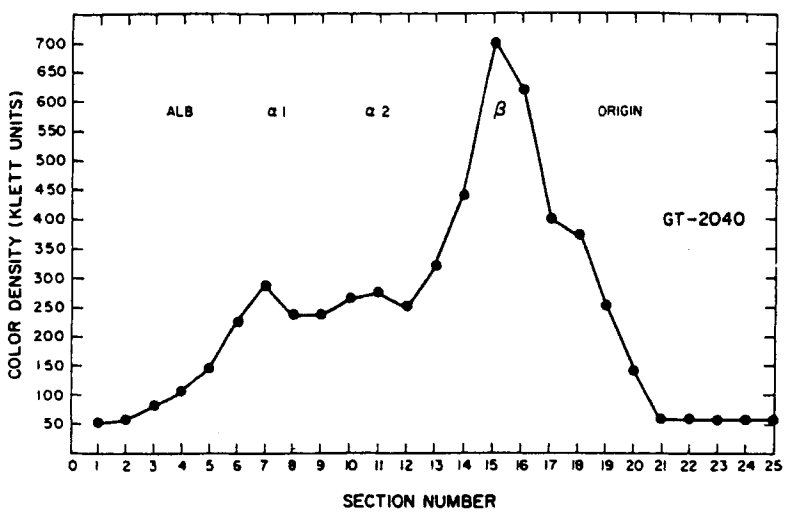

FIGURE 23. Electrophoretic pattern of GGT in serum of patient with hepatic metastases and activity of 2040 units showing dominance of GT3. (From Rutenburg, A. M., Smith, E. E., and Fischbein, J. W., *J. Lab. Clin. Med.*, 69, 504, 1967. With permission.)

Cellulose acetate has been recommended as a medium for separating GGT isoenzymes.[218] Normal sera had activity in the α_1-α_2-globulin regions. Four bands were usually present in patients with hepatic disease. Intra- and extrahepatic obstruction were characterized by an intense band of activity in the β-globulin zone, whereas patients with alcoholic liver disease had a marked increase in GGT activity in the α_2-region. The precise frequency of these abnormalities in the described diseases is not stated in this rather brief report. During a comparative evaluation of several methods from a purely technical standpoint, the localization procedure for identifying GGT isoen-

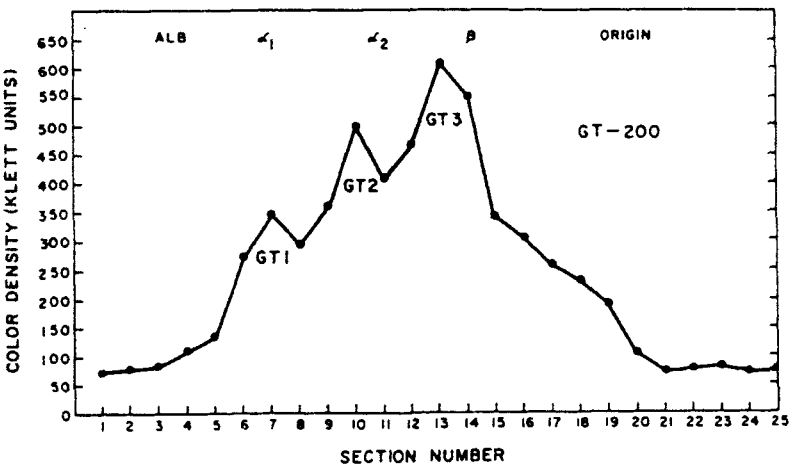

FIGURE 24. Electrophoretic pattern of GGT in serum of patient with iproniazid hepatitis and activity of 200 units showing dominance of GT3. (From Rutenburg, A. M., Smith, E. E., and Fischbein, J. W., *J. Lab. Clin. Med.*, 69, 504, 1967. With permission.)

TABLE 11

Ratio of Serum GGT Isoenzyme Fractions in Various Diseases

Disease	No. of patients	Total GGT*	Fr-1 (%)	Fr-2 (%)	Ratio 1:2
Chronic pancreatitis	22	20	54	46	1.1
Obstructive jaundice	9	108	47	53	1.0
Carcinoma of pancreas	16	505	49	51	1.0
Chronic hepatitis	11	286	71	29	2.4
Intrahepatic cholestasis†	17	186	69	31	2.2

* Upper normal limit 28 U/*l*.

† Defined as cholestatic hepatitis or liver metastases with high lipoprotein-X.

From Bornschein, W., *Clin. Chim. Acta*, 61, 325, 1975. With permission.

zymes was optimized using the substrate α-L-γ-glutamylnaphthylamide.[219] This was however achieved on the basis of experiments performed in cuvets rather than on the support media. The presence of cellulose acetate, agar gel, and polyacrylamide gel during staining reduced GGT activity to 89, 51, and 30%, respectively, of the control values. Further losses, more marked with polyacrylamide gel, occurred after electrophoresis on these media. This paper presents useful data, but the authors are not clear as to which medium they recommend for general use, since the extent of resolution of the different isoenzymes is a factor of paramount consideration.

Serum GGT was separated into two fractions by column chromatography on DEAE-Sephadex®.[210] These did not correlate with the underlying disease in the 53 patients studied and were believed not to be true isoenzymes, but a result of differences in the distribution of cholesterol-containing lipoproteins in the sera. Evidence for this assertion included the following observations: (1) a second peak appears in sera of high GGT activity but normal cholesterol concentration, when admixed with sera possessing low GGT activity but high cholesterol concentration; (2) the cholesterol-dependent peak, eluting at a conductivity of 0.2 mS, is abolished by Triton X-100, and moves to the lipid-rich supernatant when lipemic sera are clarified by adding heparin and $MgCl_2$.

This suggestion is consistent with later work on GGT isoenzymes in liver, bile, and serum[220] in which amphiphilic and hydrophilic forms were separated by gel filtration. Resolution was also performed with polyacrylamide gel and agarose electrophoresis revealing heterogeneous patterns in the three source materials. Detergent-treatment produced results in accord with solubilization of large aggregates explained by formation of complexes between GGT, lipids, and other amphiphilic proteins. The authors therefore counseled against the use of the term "isoenzymes" to describe these complexes.

A number of investigations have been made into the GGT isoenzyme composition of human bile. Using electrophoresis on agarose gel, liver homogenates yielded most of their GGT activity in the α_1- and α_2-globulin zones, whereas GGT activity of bile was localized with the β-globulins.[221] A later study on GGT isoenzymes in bile showed these to be highly method-dependent.[222] Gel filtration and ultracentrifugation revealed 19S and 4S fractions. Three zones of activity were detected on agar gel electrophoresis, the predominant fractions being of α_1 and α_2 mobility. With polyacrylamide gels, however, only two zones were visualized; the predominant band was at the origin, and the second component had 77% of the mobility of albumin. As the authors point out, it remains for these observations to be related to the isoenzyme patterns seen in the serum of patients with biliary obstruction.

In summary, the chemical and genetic basis for GGT isoenzymes are unknown. Their clinical utility has yet to be established. A consensus appears to be emerging, despite technical heterogeneity, that diseases of the biliary tree predominantly increase isoenzymes of β-mobility, whereas primary liver diseases occasion increased α_1- and α_2-components. The incidence and predictive value of these abnormalities have not been defined, and there is no real substance to the assertion that GGT isoenzyme fractionation can help in distinguishing between intra- and extrahepatic obstruction.

CONCLUSIONS

GGT catalyzes the transfer of γ-glutamyl residues to amino acids or small peptides. Autotranspeptidation and hydrolysis of γ-glutamyl peptides can also be performed by the enzyme which, under certain circumstances, can produce ammonia from glutamine. The reaction follows a Ping Pong Bi Bi kinetic mechanism with the γ-glutamyl-donor site exhibiting low stereo specificity, whereas the acceptor site displays absolute L-specificity.

A number of publications report the purification of GGT, the rat kidney enzyme being the best characterized. It is a membrane glycoprotein which apparently faces the luminal side of the renal tubule. It exists in a heavy form of molecular weight of 200,000 from which bromelain treatment liberates an active enzymic form, with a molecular weight of 68,000, separable by SDS electrophoresis into two nonidentical glycopeptides with molecular weights of 46,000 and 22,000; the latter contains the γ-glutamyl binding site. The human kidney enzyme appears to have a similar composition, although the molecular weights of the two nonidentical glycopeptides are somewhat higher. GGT is intimately concerned in the synthesis and metabolism of glutathione through the γ-glutamyl cycle. There is good evidence that this plays a role in the absorption of amino acids from the glomerular filtrate and from the intestinal lumen through a translocation mechanism. However, a number of observations are not consistent with its having a universal role in amino acid uptake by body cells, and a stumbling block to acceptance of its primacy in the gut and kidney is the fact that it is inconsistent with diseases presumed due to deficiency of specific amino acid transport proteins, such as cystinuria and Hartnup disease.

Many studies indicate that the GGT content of liver, especially that of smooth endoplasmic reticulum, is increased by enzyme-inducing drugs, and that this increase is usually reflected in elevated activity of the enzyme in blood serum. In many, but not in all, instances, good correlation has been shown between serum GGT activity and accepted parameters of hepatic microsomal enzyme induction. The serum assay has great potential value in monitoring drug compliance, but this role has yet to be convincingly demonstrated. The increased serum GGT activity encountered in chronic alcoholics seems to be partly due to microsomal enzyme induction, since high values occur among habitual drinkers who fall short of being alcoholics, or alcoholics who have no demonstrable liver injury. Even healthy volunteers demonstrate increased serum enzyme activity after exposure to ethanol. Utility of the assay in detecting alcoholism is controversial, but with few exceptions, it is a useful index to compliance with therapy in alcoholics who seek treatment.

Dramatic increases in activity are found in many chemically induced animal tumors. Indeed such increases can be recognized in premalignant cells long before any morphological changes become evident and could serve as a sensitive test for screening potentially carcinogenic drugs and environmental agents. In human cancer, its value is more debatable. It has largely been used as a test for hepatic metastases, but its predictive value in this situation has shown a wide range in the hands of many authors, some of whom recommend the test for this purpose, while others state that it is frankly misleading. Improvement in diagnostic accuracy may arise by combining serum GGT assay with that of other established cancer markers. A similar controversy applies to its role in monitoring cancer therapy. While increasing values may indicate spread of the tumor, especially to liver, these may also arise as a consequence of therapy itself, even though regression of the tumor can be clearly demonstrated. Again, combination with other cancer-specific tests in a prognostic profile may prove beneficial in the management of such patients.

Many synthetic substrates have been used to measure serum GGT activity. Currently, L-γ-glutamyl-*p*-nitroanilide is the most popular. Although its limited water-solubility can be a problem, many strategems have been devised to overcome this difficulty, and there seems little reason to pursue the quest for alternate substrates. A bigger difficulty lies in variability of reference ranges, even with the same techniques in the hands of different workers, or as applied to different populations. Males have higher values than females; activity is very high in the neonate, and rather low in pregnancy. Some publications indicate that the distribution of activity in a reference population is Gaussian, whereas other authors have found a non-Gaussian distribution. This controversy is in part due to inadequate selection of the reference population, which requires special emphasis on excluding apparently healthy subjects who are nonetheless taking tranquilizers, oral contraceptives, alcohol (in all but moderate amounts), and other enzyme-inducing drugs.

The most universal application of serum GGT assay is in diagnosis of liver and biliary tract disease. It is widely believed that higher values occur in biliary obstruction than in parenchymal disease. Experimental observations in the rat are supportive, since bile-duct ligation is accompanied by much more dramatic increases in GGT activity of Kupffer cells and bile duct epithelium than in parenchymal cells. However, the overlap of values in different disease categories is so great that the enzyme cannot be recommended for this purpose. Critical analyses, often with the aid of the computer, have confirmed that GGT has poor discriminatory capabilities compared with many other available laboratory tests used for the differential diagnosis of hepatobiliary diseases. Its employment as an alternative to electrophoresis of alkaline phosphatase isoenymes rests on a rather insecure foundation. Although it is widely regarded as a sensitive test for liver disease, its specificity is poor, and unselected hospital patients found to have

abnormal serum GGT activity on the basis of indiscriminate screening were more often than not proved to have an alternative reason for the abnormality. In part, this reflects its sensitivity to drug administration, alcohol, and poorly defined reference values. Further reasons include the wide range of other diseases in which increased serum GGT activity is encountered, which include diabetes mellitus, hypertriglyceridemia, cardiac disease, neurologic illness, and severe burns.

Isoenzyme analyses have been performed in an attempt to improve the diagnostic specificity of the serum GGT assay. Every conceivable support medium has been used, and a column technique with Con A-Sepharose has also been described. Different techniques yield different results. The genetic basis of the multiple forms of GGT has not been established and elucidation of their chemical nature is at an early stage. Tissue-specific patterns have not been described, and disease-specific patterns cannot be reproduced with confidence. There is a growing consensus, however, that primary liver diseases are associated with increased zones of GGT activity in the α-globulin region, whereas biliary tract disease causes elevation of isoenzymes with β-globulin mobility. The latter pattern does not distinguish intra- from extrahepatic obstruction, and the predictive values for the respective patterns do not warrant application of this procedure on a routine scale at the present time.

In summary, whereas exciting advances are being made in understanding the molecular structure, mechanism, and functions of the enzyme by basic biochemists, GGT presents clinical chemists with a genuine dilemma. Almost 2 decades after the first reports of its clinical use, and after many years of neglect, it has been introduced into the diagnostic armamentarium of many hospital laboratories. In this sense, it has found its "place in the sun", but a critical appraisal of the present literature leads the present reviewer to the conclusion that it has yet to find a genuinely useful diagnostic role substantiated by a convincing body of scientific data. It is to be hoped that these comments will stimulate the necessary experimental work or precipitate diminished utilization of the test, even to the point of its disappearance from the diagnostic laboratory scene.

ADDENDUM

During the interval between submission of this manuscript and receipt of the proofs, a number of important papers have appeared which expand our basic knowledge of GGT and illustrate its applications. This supplement seeks to provide a brief critical synopsis which supplements or qualifies some of the information and comments in the main body of the text.

Rat kidney GGT co-purifies with glutathione oxidase activity, and the evidence strongly suggests that conversion of glutathione to its disulfide is a function of GGT.[224] Between pH 8.5 and 6.0, the rate of transpeptidation carried out by rat kidney GGT decreased 29-fold whereas the rate of hydrolysis of glutathione remained constant at double the rate of transpeptidation under physiological conditions of glutathione and amino acid concentrations.[225] Glutathione hydrolysis rather than transpeptidation therefore appears to be the major reaction catalyzed by GGT in vivo. A similar conclusion was reached regarding the human liver enzyme.[226] The paradox that maleate stimulates hydrolysis of γ-glutamyl compounds by GGT at the expense of transpeptidation but increases both transpeptidation and hydrolysis of glutamine seems to be explained by the finding that the rate-limiting step in the reaction with glutamine is formation of the γ-glutamyl enzyme, whereas the rate-limiting step with other γ-glutamyl donors is the reaction of γ-glutamyl enzyme with water or acceptor.[227] A further affinity label, 6-diazo-5-oxo-D-norleucine, has been developed and compared with the corresponding

L-enantiomer and the results were consistent with the donor site of GGT being poorly stereospecific whereas the acceptor site exhibits strict L-specificity.[228] Griffith and Meister have sought to establish that mouse kidney GGT is capable of translocating intracellular glutathione and utilizing it at the outer surface of the cell membrane by demonstrating increased excretion of glutathione in the urine when inhibitors of GGT are administered in vivo.[229] The reasoning behind this proposal is not immediately obvious, and a more attractive schema has been suggested according to which GGT cooperates with other renal brush-border peptidases to hydrolyze glutathione and secure its conversion to a cysteine S-conjugate which is then reabsorbed from the glomerular filtrate, transferred to the liver, and utilized in the formation of mercapturic acids required for the biotransformation of certain foreign compounds.[230,231]

Two novel procedures have been employed in the purification of renal GGT. The first involved the separation of renal brush-border microvilli as the source material for the preparation.[232] The second utilized an antiserum to GGT which was covalently bonded to Sepharose® and permitted isolation of the enzyme from human urine.[233] Detailed analyses of liver cell fractions have shown that hepatic GGT is primarily a plasma membrane enzyme in the rat,[234] the guinea pig,[235] and the rabbit.[236] It is also present in high activity in the milk fat-globules of mouse mammary gland which are formed by budding of the plasma membrane.[237]

The role of serum GGT activity as a correlate of drinking habit and alcohol consumption has been further explored in a number of publications,[238-244] and its lack of value in the differential diagnosis of hepatobiliary disease has been emphasized.[245] Methodological developments have included definition of optimal substrate concentration for automated activity measurements,[246] and the synthesis of a new substrate which on hydrolysis liberates an aromatic amine capable of underoing a chemical reaction generating a chromogen measurable at 700 nm without the necessity of performing a serum blank.[247] Attempts to probe the origin of GGT isoenzymes in human serum have shown that when serum and liver tissue were incubated together, the electrophoretic mobility of the liver enzyme which leaches into the serum undergoes progressive modification due to a process which is not autolytic, but may be due to binding of the native enzyme (but not the purified enzyme) to serum components.[248]

Evidence for the participation of the γ-glutamyl cycle in amino acid transport has come from study of yeast cells, by demonstrating a high dependence of glutathione turnover upon the presence of external amino acids in the medium.[249] Further indirect evidence is the steady increase in rat mammary GGT during pregnancy with a dramatic further increase on lactation, ascribed to the enhanced amino acid transport into the gland required for milk-protein synthesis;[250] this is hormonally dependent but is not influenced by the intracellular concentration of cyclic AMP which seems to be a determinant of hepatic GGT activity.[251] Naturally occuring membrane phospholipids and their biological degradation products influence rat kidney GGT activity in vitro and may regulate its activity in vivo.[252]

Two reports on GGT in cancer are of interest in establishing that carcinoma of the breast in mouse and man[253] and rat hepatoma[254] are associated with a form of the enzyme much richer in sialic acid than that present in normal tissues, the speculation being that this represents an oncofetal alteration of the enzyme.

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