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Gamma Glutamyl Transferase

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ABSTRACT: Serum gamma-glutamyl transferase (GGT) has been widely used as an index of liver dysfunction and marker of alcohol intake. The last few years have seen improvements in these areas and advances in understanding of its physiological role in counteracting oxidative stress by breaking down extracellular glutathione and making its component amino acids available to the cells. Conditions that increase serum GGT, such as obstructive liver disease, high alcohol consumption, and use of enzyme-inducing drugs, lead to increased free radical production and the threat of glutathione depletion. However, the products of the GGT reaction may themselves lead to increased free radical production, particularly in the presence of iron.

There have also been important advances in the definition of the associations between serum GGT and risk of coronary heart disease, Type 2 diabetes, and stroke. People with high serum GGT have higher mortality, partly because of the association between GGT and other risk factors and partly because GGT is an independent predictor of risk.

This review aims to summarize the knowledge about GGT's clinical applications, to present information on its physiological roles, consider the results of epidemiological studies, and assess how far these separate areas can be combined into an integrated view.

I. INTRODUCTION

Activity of the enzyme gamma glutamyl transferase (GGT)* in serum or plasma is commonly measured in clinical laboratories as a sensitive but not very specific liver function test. The measurement of GGT using automated analyzers is quick, cheap, and precise. However, the application of the test is based mostly on empirical evidence and on evaluations of its clinical characteristics, rather than on any deep understanding of the pathophysiological basis of the abnormalities of GGT in liver disease or in other conditions.

GGT measurement was introduced into clinical laboratories some 35 years ago, and over that time a large amount of information on factors influencing its activity in serum has accumulated. Theories have been put forward about its normal function within the body and its role in numerous pathological conditions. Within the last few years there have been significant advances in understanding of GGT's physiological roles and their consequences at a cellular level. In addition, prospective epidemiological studies have shown that variation in GGT in the general population is associated with variation in mortality and morbidity.

General reviews from a clinical perspective were undertaken by Rosalki[3] in 1975 and by Goldberg[4] in 1980, and material from these are not repeated in detail. Nevertheless, some older work is mentioned where it is relevant to the main theme of this review. Reviews on selected aspects of GGT have been written by Nemesanszky and Lott[5] on isoenzymes, by Lieberman *et al*.[6] on gene expression, Wolf and Gassen[7] on GGT activity at the blood-brain barrier, Taniguchi and Ikeda[8] on the catalytic mechanism of GGT and gene expression, Hanigan[9] on GGT in carcinogenesis, and Ristoff and Larsson[10] on enzyme defects in the gamma-glutamyl cycle. Reviews in languages other than English have also appeared, but, unfortunately, they are less accessible to most readers.

The aim of the current review is to collect and connect experimental, epidemiological, and clinical facts and opinions on GGT. In particular, it attempts to assess and explain some of the epidemiological and clinical results in light of our knowledge of the enzyme's physiological actions and pathological changes. With this knowledge, the interpretation of results in either the clinical or epidemiological context can be improved, and some directions for further research can be identi-

* The name γ-glutamyltransferase was preferred by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology for this enzyme, E.C.2.3.2.2, (5-L-Glutamyl)-peptide:amino-acid 5-glutamyl transferase.[1] The Expert Panel on Enzymes of the International Federation of Clinical Chemistry, which developed the Recommended Method,[2] also used this name. Some authors have continued to use the older name gamma-glutamyl transpeptidase. In order to conform with clinical practice and to avoid use of the Greek character, the abbreviation GGT is used throughout this review.

fied. The topic has been divided into three main sections: first, the physiological and pathophysiological significance of GGT; second, the clinical uses of GGT; and third, the epidemiological evidence that increased serum GGT is a prospective risk factor for death or ill health in humans.

II. OCCURRENCE, EXPRESSION, AND FUNCTIONS OF GGT

A. Occurrence, Genes, and Gene Expression

Gamma glutamyl transferases are widely distributed, being found in bacteria,[11,12] and plants,[13,14] as well as in members of the animal kingdom, ranging from the nematode *Ascaris suum*[15] to humans.

Human GGT genes are located on chromosome 22q11,[16,17] with related sequences that may be pseudogenes on chromosomes 18, 19, and 20.[16] There are seven or more GGT genes in humans,[6,18] but of these only one gives rise to a complete and functional protein. Rat and mouse GGTs seem to be single-copy genes that show considerable sequence divergence from the human genes,[19] although the exonic structure of human and rodent GGT genes, and the existence of multiple forms of mRNA, show similarities.[20] GGT genes have multiple promoters and multiple RNA transcripts that lead to the same protein and are thought to be involved in tissue-specific and developmental GGT expression.[6,18,20] There is a GGT-related enzyme (GGT-rel) with approximately 40% amino acid homology that catalyzes many of the presumed physiological reactions of GGT but that is not active against the artificial substrates used for GGT measurement. The similarities and differences in GGT genes, and in their expression, across species were reviewed by Chikhi *et al*.[18]

The GGT protein is produced as a single polypeptide that is cleaved by a protease to produce a heavy and a light chain.[8] The heavy chain has an aminoterminal sequence that is intracellular, a single transmembrane domain, and an extracellular component that binds the light chain. The active site is extracellular and in the light chain. The light chain on its own has protease activity and can digest the heavy chain *in vitro;* presumably, *in vivo* the heavy chain not only secures the light chain to the cell membrane but also modifies its catalytic activity. There are up to eight potential sites for glycosylation, and the protein is in fact heavily glycosylated with considerable heterogeneity.

GGT does not have isoenzymes in the sense of proteins of different amino acid sequence but with the same catalytic activity, or is there any known allelic variation in human GGT. However, there is a considerable variety of isoforms that differ in their carbohydrate content or carbohydrate structure. These may be separated by electrophoresis or isoelectric focusing[5,21,22] or by lectin affinity.[23-25] Much of the variation in electrophoretic mobility is due to the isoforms' association with lipoproteins[26] or with IgA.[27] A hydrophilic form of GGT, possibly

equivalent to native GGT with the transmembrane domain cleaved by protease treatment, has been reported, and this is cleared from the circulation more quickly than the lipoprotein-associated forms.[28]

The activity of GGT varies considerably between normal tissues and over the stages of embryonic development. Activity also varies between normal and neoplastic tissue and between normal and transformed cells in culture. The distribution of immunoreactive GGT in normal human tissues was studied by Hanigan and Frierson.[29] They used immunohistochemistry, which has the advantage of localising activity within tissues but the disadvantage of nonquantitative results. The immunohistochemical results were cross-checked in some tissues by measuring enzyme activity. As a generalization, most cells staining for GGT were epithelial. Like previous authors, they found the highest activity in the kidneys, where GGT was localized to the luminal surface of the proximal tubule cells; the distal tubules and glomeruli gave negative results. Activity in homogenates of liver was approximately one-fifth that in kidney, and staining was most intense in biliary epithelial cells and bile canaliculi. Pancreatic acinar cells, but not islets, stained strongly. There was strong staining of endothelial cells lining the capillaries in the brain and spinal cord, and of many cell types within the male reproductive system. Seminal fluid, but not sperm, showed strong GGT activity. These results are consistent with a functional view of GGT in which it is involved in transport at cell membranes, some aspect of the "blood-brain barrier", and the protection of the environment in which sperm are produced and stored against oxidative damage. These authors also studied the distribution of GGT in tumors, and these results are discussed below.

Examination of fetal tissue showed similar relativities between tissues as in adults, although other authors have shown major developmental changes in hepatic GGT mRNA and protein.[30] GGT mRNA was approximately twice as abundant in 12-week fetal liver as in adult liver, and by 40 weeks gestation this had risen to approximately seven times adult levels. On the other hand, human amniotic fluid GGT decreased across the second trimester of pregnancy.[31] Species differences in fetal GGT may be significant, and these differences combined with the gene variation between rodents and humans mean that caution should be exercised in extrapolating animal results.

A number of cell types show significant GGT activity but do not conform to the generalization that it is particularly associated with membrane transport. In humans, GGT is expressed in astrocytes around the blood vessels in the brain[32,33] and is believed to play a role in the blood-brain barrier, either by conjugating potentially toxic xenobiotics or by metabolizing vasoactive leukotrienes. Secondly, GGT is found in white blood cells, and the enzyme activity or protein content varies with cell type and with the stage of differentiation.[34-38] The function of GGT on these cells is uncertain, but two theories have been put forward: first, that expression of GGT protects cells against free radical injury, particularly in regions of inflammation; and second, that GGT participates in events that modify receptor-ligand interactions at the cell membrane.

B. Measurement of GGT Activity

The enzymatic function and specificity of GGT, as well as its probable physiological role or roles, have been discussed in many previous reviews.[3,4,6,8] The reaction catalyzed is the transfer of a glutamyl residue, linked through glutamate's gamma carboxylic acid to an amine or to another amino acid, to an acceptor. The GGT reaction takes the general form:

$$\text{Gamma-glutamyl-X} + \text{acceptor} \longrightarrow \text{Gamma-glutamyl-acceptor} + \text{X}$$

and a wide range of compounds can participate as the gamma-glutamyl donor or as the acceptor. Among the gamma-glutamyl donors the most significant natural substrate is believed to be glutathione (gamma-glutamyl cysteinyl glycine), but a range of artificial substrates are also acted on by GGT. The acceptors are amino acids or peptides, with glycylglycine the most active and commonly used example. In the absence of these preferred acceptors water can be a substitute, leading to formation of glutamate.

A number of artificial substrates have been developed for convenient measurement of GGT activity. These include gamma-glutamyl-β-naphthylamide, gamma-glutamyl-*p*-nitroanilide, and gamma-glutamyl-3-carboxy-4-nitroanilide. The nitroanilides have the advantage of being chromogenic substrates, and the progress of the reaction can be monitored continuously. The carboxylic acid avoids some solubility limitations that occur with gamma-glutamyl-*p*-nitroanilide. GGT activity is usually measured by kinetic spectrophotometric methods based on the work of Szasz,[39] which are precise and inexpensive. The IFCC recommended method[2] is based on this.

GGT is a comparatively stable enzyme *in vitro*.[40] Serum GGT assays have at various times been carried out at 25, 30, or 37°C. Activity values are reported in this review as the numerical values reported in the original papers: to convert values measured at 25°C to 37 °C, multiply by 1.78, and to convert from 30°C to 37°C multiply by 1.31.[41] There may be some variation in these temperature conversion factors with variation in the GGT isoforms present,[25] but probably no more than 10%.

Immunoassays, and high-sensitivity methods based on fluorescence, have also been described for specialized uses.[42,43] The study of isoenzymes has led to the conclusion that changes in the carbohydrate composition of GGT affect association with lipoproteins and produce variation in electrophoretic patterns, but isoenzyme analysis has not been widely used because of technical inconvenience and lack of evidence for diagnostic usefulness.

GGT can be inhibited by acivicin (AT-125, α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid) or by serine-borate complex; both have been used to establish the physiological functions of GGT.

C. Normal Function

As mentioned previously, the most abundant substrate for GGT is glutathione, and its physiological roles relating to glutathione have been investigated extensively. Some other compounds found *in vivo* have the necessary gamma-glutamyl structure, including glutathione conjugates of xenobiotics such as the antitumor agent cisplatin, and leukotrienes, and GGT participates in their metabolism.

One of the early observations about GGT was that its activity was greatest in tissues with a transport function, such as the kidneys and in the bilary system. This led to the suggestion that GGT played an important role in the transport of amino acids, through a sequence of reactions forming a "gamma-glutamyl cycle". Amino acid transport is probably not a significant function of GGT, because humans or animals with GGT deficiency do not show generalized disturbances of amino acid transport, but it is clear that GGT is important for the availability of the amino acid cysteine. However, GGT does form a key part of a cyclic process whereby glutathione in extracellular fluids can be broken down at the cell membrane to its constituent amino acids that can readily be taken up by cells possessing GGT (because the amino acids are released in close proximity) and used for synthesis of glutathione. The sequence of reactions is shown in Figure 1.

Evidence of the normal function of GGT has come from multiple sources, some circumstantial and some direct. These include the location of the enzyme at membrane transport sites, where it has been detected by histochemical or immunohistochemical methods; experiments using GGT inhibitors such as acivicin and serine-borate, cell transfection, knockout animals and human subjects with GGT deficiency; and study of the response of GGT enzyme, protein, or mRNA concentrations to the manipulation of glutathione status.

The relationship between GGT and glutathione has been investigated extensively at the cellular level, and the results and implications of such studies are considered below. Specific interactions between alcohol and glutathione and their implications for the response of GGT to excessive alcohol consumption are considered in the section on GGT as a marker of alcohol use or abuse. Three general papers illustrate aspects of the interactions between GGT and its natural substrate — glutathione.

One of the three amino acids in glutathione is cysteine, and it is cysteine that is most likely to be undersupplied. When diets low in protein, and particularly in sulfur-containing amino acids, were fed to rats, there were significant changes in hepatic glutathione and GGT activity.[44] The effects of dietary composition on GGT and glutathione can be seen in Figure 2; there were three dietary conditions and each was given with 6% and 13% protein content. Compared with the control group with casein as a source of protein, animals on a rice and bean diet (which was low in sulfur-containing amino acids) had lower glutathione and higher GGT values. When methionine was added to the low-sulfur diet, glutathione increased to slightly more than control levels and GGT fell to almost the control value. This

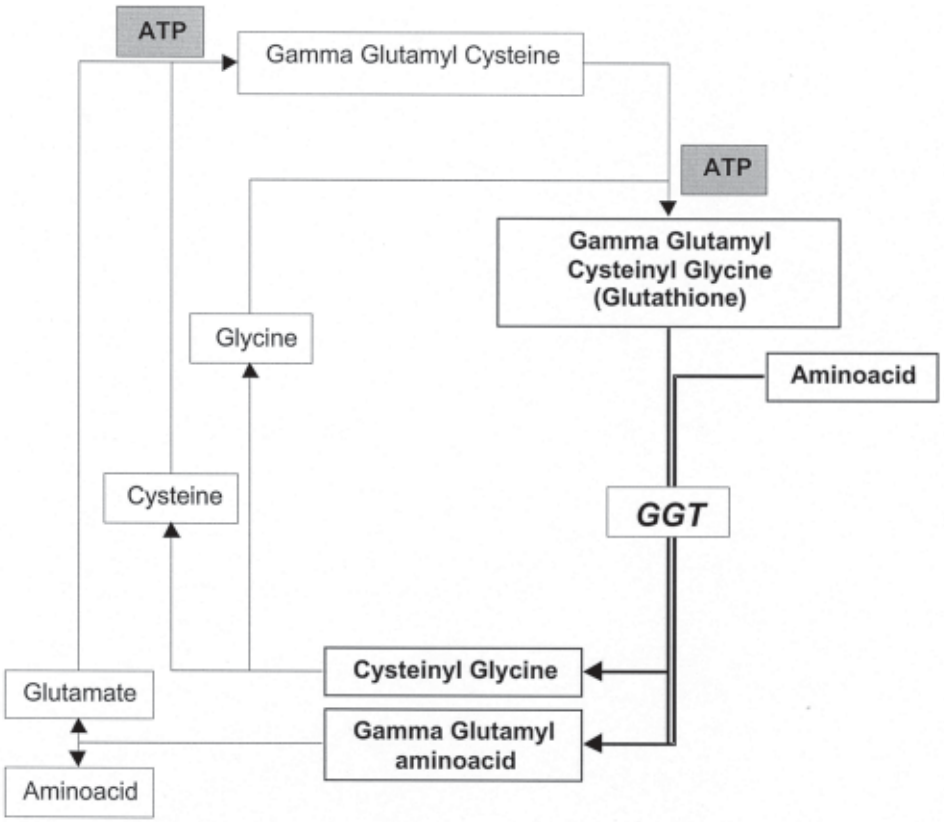

FIGURE 1. Reactions involved in the breakdown and synthesis of glutathione: the gamma-glutamyl cycle. GGT catalyzes the transfer of the glutamyl residue from glutathione to an acceptor amino acid, with the formation of cysteinylglycine. The operation of the cycle involves hydrolysis and resynthesis of glutathione, with consumption of ATP.

study shows both the importance of a dietary source of sulfur-containing amino acids for glutathione maintenance, and the reciprocal relationship between glutathione and GGT, which is assumed to be increased as a way of producing cysteine for the liver from circulating glutathione.

Perfusion of guinea pig livers (which have higher GGT activity than rat liver)[45] showed that there was a great capacity to remove glutathione from the perfusate, with the production of equivalent amounts of cysteinylglycine and cysteine in the effluent. The inhibition of GGT activity led to a massive increase in glutathione in the effluent fluid, showing that GGT accessible to the perfusion fluid (and presumably on the surface of the hepatocytes) was breaking down nearly all the glutathione presented.

The events connecting glutathione concentration and GGT synthesis were investigated by Moriya *et al*.[46] Glutathione depletion in rats was produced by the administration of diethyl maleate, and hepatic glutathione, hepatic GGT activity, and hepatic GGT mRNA were followed over the subsequent 24 h. Glutathione fell initially but was restored in 24 h, while GGT activity was significantly increased

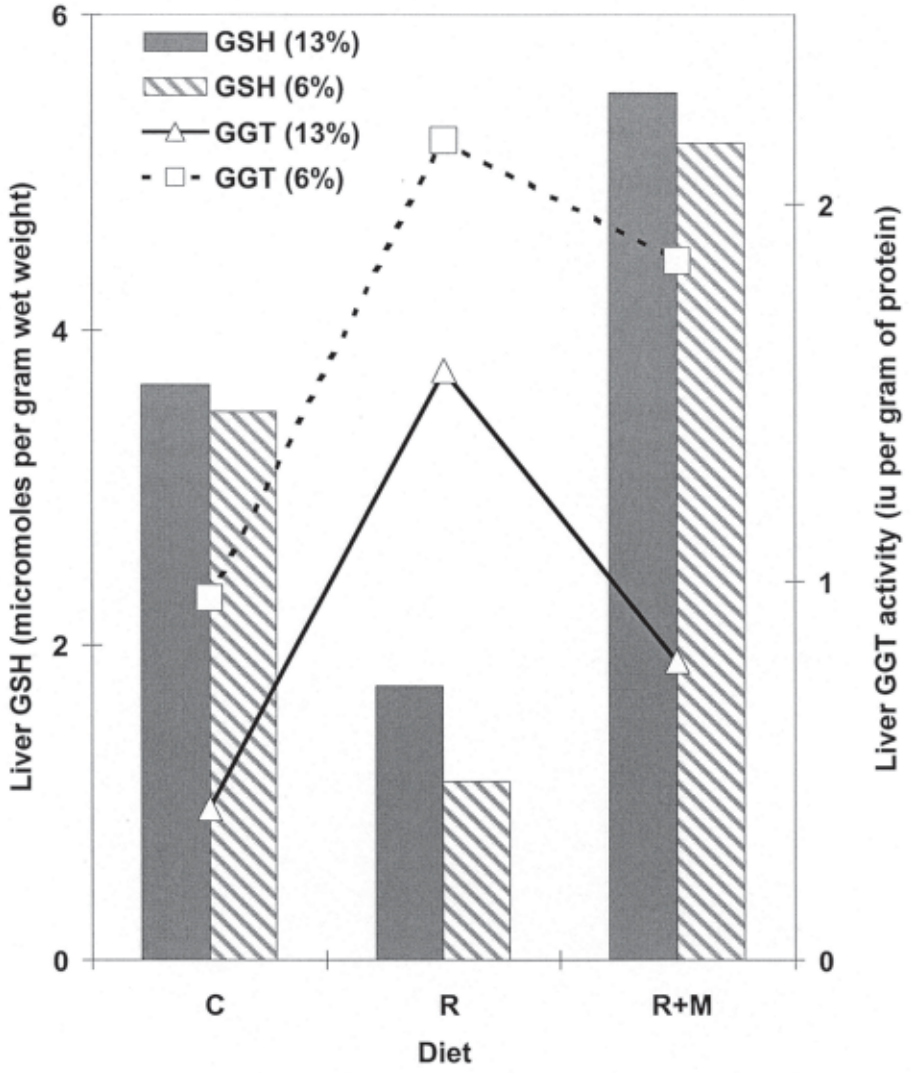

FIGURE 2. Effects of protein and sulfur-containing amino acids on hepatic glutathione (GSH) and GGT. Rats were fed diets containing differing amounts of protein (13 or 6%) and containing varying amounts of sulfur-containing amino acids (C = casein, R = rice-bean diet, R+M rice-bean diet with added methionine). Note the effects of dietary protein on GGT, and the inverse relationship between hepatic GSH and GGT. (Data from de Oliviera *et al.*[44])

at both 12 and 24 h. Semiquantitative measurement of GGT mRNA in liver tissue extracts produced equivocal results, but *in situ* hybridisation showed that 12 h after glutathione depletion the GGT mRNA was strongly visualized in most hepatocytes, and this reverted to the normal pattern of mainly biliary epithelial expression at 24 h. Therefore, both enzyme activity and mRNA changes are consistent with GGT activity being in part controlled by glutathione content.

D. Effects of GGT Deficiency

A small number of patients with GGT deficiency have been described, and mice with modifications to the GGT gene have been produced. As with many other gene defects, the occurrence of GGT deficiency provides information on the normal functions of the gene product.

Human GGT deficiency is rare and only five patients have been reported.[47–50] The family histories suggest recessive inheritance. The deficiency generally has been associated with intellectual impairment, but this may be because such patients tend to be screened for inborn errors of metabolism; in one case[50] no intellectual problems were present. In humans, GGT deficiency is compatible with life. In the GGT-deficient mice, on the other hand, growth retardation and early death are among the symptoms in the homozygous-deficient animals.[51,52] It is not clear why the effects of GGT deficiency are milder in the human patients than in the genetically engineered mice.

The biochemical features in both humans and mice are consistent with the major role for GGT being in glutathione metabolism. Urine of the patients contains abnormally large amounts of glutathione-related peptides,[53] showing that GGT is involved in the reabsorption of glutathione and/or its component amino acids from the glomerular filtrate. Tubular reabsorption of amino acids was found to be normal in two subjects,[49] which is against the hypothesis that GGT plays a general role in amino acid transport at cell membranes through the operation of the gamma-glutamyl cycle. In GGT-deficient mice[51] there was evidence of high extracellular (and urinary) glutathione but low levels within some tissues, including the liver and pancreas. Plasma cyst(e)ine concentrations were also low, consistent with glutathione and GGT normally being a major source of this amino acid in extracellular fluids. The examination of mRNA levels in the livers of affected mice[54] showed increased expression of gamma-glutamylcysteine synthetase, glutathione synthetase, and cystathionase (which normally lead to the production of glutathione, and of cysteine from methionine) and decreased expression of multidrug resistance protein 2 (which transports glutathione into the bile). These changes are consistent with regulation of these enzymes and proteins by hepatic thiol concentrations, which were found to be about one-third of wild-type levels in the animals with GGT deficiency.

Other findings in GGT-deficient mice include an increase in oxidative stress in the kidney,[55] and differences in the distribution of inorganic mercury and the excretion of methylmercury.[56] Again, a lack of glutathione within the tissues and cells is implicated. There is also evidence that the GGT deficiency leads, presumably via intracellular glutathione deficiency and lack of normal antioxidant defenses, to accumulation of DNA damage in the organs of these knockout mice.[57] DNA damage accumulates as the knockout, but not the wild type, animals age. In this study, glutathione concentrations in liver and lung were around half those found in the wild-type mice, and a smaller but still significant difference was found in the kidney.

Biochemical investigations on the livers of GGT-deficient mice showed a depletion of mitochondrial glutathione, which became more severe in parallel with deterioration in the animals' condition, and associated changes in mitochondrial function leading to impaired production of ATP.[58] The administration of *N*-acetylcysteine to the homozygous-deficient animals was able to reverse the growth retardation and abnormal coat color,[51] infertility,[59] cataracts,[60] and most of the biochemical abnormalities,[58] either through supply of cysteine alone or through restoration of intracellular glutathione as a result of the increased cysteine availability.

The range of abnormalities present in the GGT-deficient animals, and their reversal or reduction by supply of *N*-acetylcysteine, highlights the nature and significance of the normal physiological role of GGT.

III. CELLULAR BIOLOGY AND PATHOLOGY

A. Role in Carcinogenesis

The distribution and concentration of GGT in tumors show a number of differences from that found in normal tissues. Hanigan *et al*.[61] reported on a study of GGT in 451 human tumors using the immunohistochemical method they had applied previously to normal tissue (discussed above). A major object of this study was to compare GGT expression in the tumors with that in the normal tissues from which the tumors were derived, with a view to clarifying whether GGT expression was related to drug resistance. They found that most of the cancers derived from GGT positive organs were themselves GGT positive; in addition, carcinomas of the lung and ovary were generally GGT positive even though normal bronchial and ovarian surface epithelium were not. Most carcinomas were GGT positive. Melanomas and basal cell carcinomas, lymphomas, and sarcomas in general were nearly always GGT negative.

A more detailed investigation of breast tumors was published by the same group.[62] Whereas normal breast tissue, and benign lesions, all showed GGT immunoreactivity, 18% of *in situ* carcinomas and 29% of infiltrating carcinomas were negative. A paper on ovarian cancer[63] had found the opposite progression: from normal tissue through benign and low-malignant-potential changes to malignant neoplasms there was a significant increase in the proportion of GGT positivity. These results are presented to demonstrate that over the range of human tumor types, there is no constant relationship between malignant transformation and the expression of GGT.

However, GGT expression is very characteristic of chemically induced hepatocellular cancer in rats, and the production of GGT-positive foci has been taken as evidence of carcinogenicity of chemicals in this system. Similarities and differences between natural human hepatocarcinogenesis and experimentally induced

rat or other animal models have been discussed by Schaff *et al*.[64] Among other points, they showed that a virally induced hepatoma in chickens is GGT negative, and that GGT expression in human hepatomas is very variable. However, Hanigan *et al*.[61] found that human hepatocellular carcinomas (HCC) do show GGT activity. Mouse hepatomas are GGT negative, possibly because GGT promotor sequences that may respond to oxidative stress in the rat are not present in the mouse.[65] Therefore, GGT expression is not a universal feature of hepatomas.

The study of variation in human GGT mRNAs in HCC[66] revealed that in normal liver and livers with conditions other than HCC, the main GGT mRNA was one designated as type A (present in all samples) with some B (present in 11%) and C (present in 28%); however, all HCCs showed the presence of type B, 50% showed A, and none C. Interestingly, apparently normal tissue from livers containing HCCs showed a pattern of mRNAs different from normal liver. This was a significant difference and suggests that the shift in the pattern of GGT mRNA expression occurs at an early stage of development of HCC, or is associated with an environment in which development of HCC is able to occur.

Another study of HCC patients[67] provides complementary information on serum GGT activity and on the methylation status of sites within the GGT gene. So-called 'hepatoma-specific' GGT isoforms (HS-GGT) in serum were increased above the reference range in 86% of HCC patients, and there was a significant difference in the methylation status of a site in the 5´ noncoding region of the GGT gene between the HCC and the noncancerous samples.

Taken together, these papers suggest a sequence of events in human HCC from malignant transformation, through a change in methylation status and changes in the type of GGT mRNA to a different GGT isoform that appears in the serum. However, there are some unexpected features in this possible sequence of events. The GGT mRNAs are all believed to code the same amino acid sequence, and the HS-GGT differs from the usual form of GGT only in its glycosylation status. It is not apparent how variation in GGT mRNA type could affect the posttranslational modifications of the GGT protein, carried out by other enzymes, which lead to the presence of different isoforms of GGT in the serum.

Many features of hepatocarcinogenesis have been explored in rats, where standard protocols for the production of hepatic tumors have been developed, and GGT acts as a marker for the transformed or premalignant state. One significant question is whether GGT expression simply gives some advantage to cells so that they can survive with their transformed phenotype, or whether GGT also forms part of the chain of events that cause malignancy. It is clear that expression of GGT allows cells in culture access to a source of cysteine, from extracellular glutathione, which is advantageous when cysteine is present in low and growth-limiting concentrations as would occur *in vivo*. Transfection of mouse hepatoma cells with GGT gave the cells with GGT an enhanced ability to take up cysteine at physiological concentrations, and the ability to grow in glutathione-containing, cysteine-free conditions where GGT-negative cells could not do so.[68] It has also been shown[69]

that tumors can take up glutathione and cysteine from the circulation and that their glutathione uptake can be reduced by the GGT inhibitor acivicin. On the basis of these results, GGT seems to have a permissive role in tumor growth but not necessarily a fundamental one in malignant transformation. However, some of the evidence of GGT's involvement in oxidative damage (discussed below) suggests a more fundamental contribution.

Finally, there has been interest in whether expression of GGT allows tumors to resist the effects of chemotherapy. A number of drugs are conjugated with glutathione by glutathione-*S*-transferases, and the availability of glutathione could be dependent on GGT activity. Schadendorf *et al.*[70] found substantially lower mean GGT activity in human melanoma cell lines and melanoma metastases than in normal cells or nonmelanoma cell lines, but there was substantial variation within each group. No association could be shown between GGT activity, glutathione concentrations, or other glutathione-related enzyme activities and any clinical features. Studies on GGT and tumor growth, and sensitivity to cisplatin, were carried out by Hanigan *et al*.[71,72] The expression of GGT resulted in faster tumor growth when transfected human prostate cancer cells were injected into nude mice, and GGT-positive tumors were less sensitive to the effects of cisplatin.[71] However, in human patients with germ cell tumors there was no evidence of increased response to cisplatin in GGT-positive tumors.[72]

B. GGT as a Protection Against Oxidative Stress

Glutathione plays an important role in protecting cells against oxidants that are produced during normal metabolism. If oxidative stress increases, then so will the requirement for reduced glutathione, and conversely if glutathione is not available then the effects of oxidative stress will be greater. The importance of GGT in maintaining adequate levels of intracellular glutathione under normal conditions *in vivo* has been demonstrated through the studies on GGT-knockout mice described above.

Many studies have been carried out to investigate the response of GGT to experimentally induced oxidative stress in a number of tissues. The results suggest that GGT plays an important adaptive role in several tissues or organs in which it is not abundant and where it has not previously been thought of as clinically relevant. For example, the lung is constantly exposed to ambient oxygen concentrations, and oxidative damage may result. A series of studies on glutathione and GGT have been done with rat lung epithelial cells.

Oxidative stress was induced in rat lung alveolar cells in culture by Kugelman *et al*.[73] using menadione, a quinone compound that undergoes a cycle of reactions producing superoxide radicals and hydrogen peroxide. This treatment gives a dose-dependent cytotoxic effect. Exposure of cells to menadione was followed by a temporary fall in glutathione concentration, an increase in GGT activity that could

be blocked by the transcription inhibitor actinomycin D, and an increase in glutathione levels to above baseline. The increase in glutathione could be blocked by the GGT inhibitor acivicin, and GGT inhibition by acivicin also decreased the glutathione content of cells not subjected to menadione-induced oxidative stress. In addition to an increase in GGT enzyme activity, increases in GGT protein and GGT mRNA could also be shown.

Similar results were found when a different quinone, *tert*-butylhydroquinone, was used on these lung cells.[74] Again, the quinone increased GGT activity, and also the activity of gamma-glutamylcysteine synthetase, and increased cellular glutathione. Inhibitors of either gamma-glutamylcysteine synthetase or GGT prevented the increase in glutathione, but provision of a source of cysteine could overcome the effects of GGT inhibition. The cells also showed an adaptation to quinone exposure in that pretreatment with a subtoxic dose of *tert*-butylhydroquinone conferred relative resistance to its subsequent effects. This adaptation was diminished by inhibition of either GGT or gamma-glutamylcysteine synthetase, suggesting that it required the participation of the enzymes producing cysteine extracellularly and converting it to glutamylcysteine intracellularly. A subsequent paper[75] explored the regulation of GGT expression during exposure to quinones and concluded that more than one mechanism exists.

Similarly, the exposure of rats to nitrogen dioxide produced an increase in GGT in the lungs and particularly in the surfactant fluid,[76] as shown in Figure 3. GGT mRNA, protein, and enzyme activity all increased, with a change in the pattern of expression of the different mRNAs.

The induction of inflammation and lung injury by intratracheal administration of interleukin 1[77] caused an increase in production of hydrogen peroxide, changed lung morphology, and impaired arterial oxygenation; it also produced a small but significant increase in lung GGT activity. In humans, children with cystic fibrosis and pulmonary inflammation had higher GGT activity and lipid peroxide concentrations and slightly but nonsignificantly lower glutathione concentrations in bronchiolar lavage fluid than cystic fibrosis patients without inflammation or noncystic fibrosis controls.[78] Therefore, the cell culture results are supported by those from whole animals and from human patients.

The results from studies on the lung have been presented in some detail because they show the sequence of events from oxidative stimuli through the various stages of GGT induction. There is evidence of similar regulation of GGT by oxidative stress in other cells or tissues, including fibroblasts.[79] the epididymis,[80] and lymphocytes.[81] Therefore, it is reasonable to conclude that the protective action of GGT against oxidative stress is generalizable across a number of cell types, tissues, or organs.

It should be pointed out that the action of GGT on extracellular glutathione is not the only mechanism by which cells could maintain glutathione levels in the presence of oxidative stress. Direct glutathione transport has been demonstrated in kidney[82] and intestinal[83] cells. Oxidants are present at high concentrations during

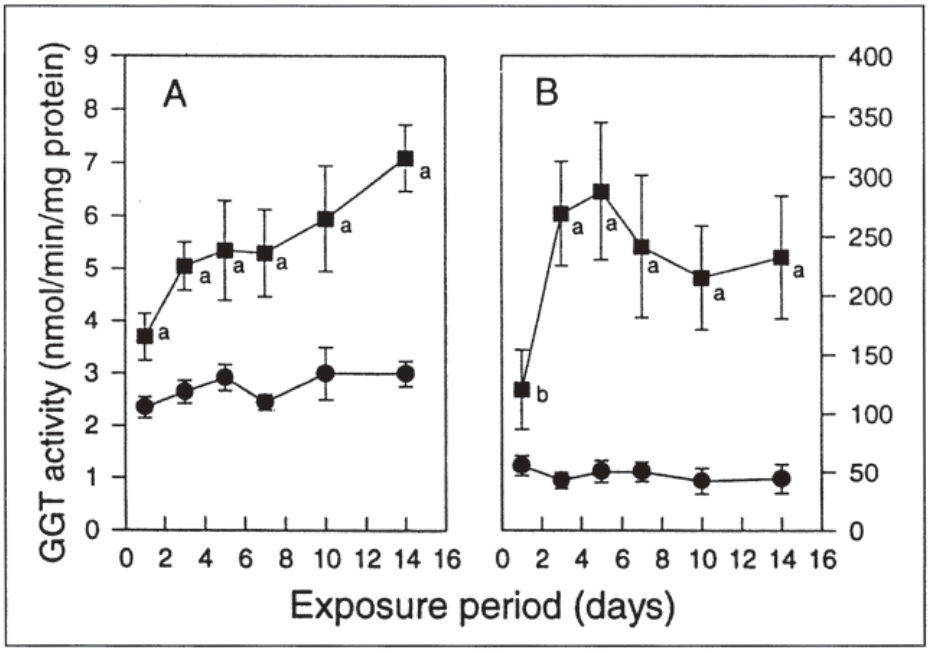

FIGURE 3. Protective effect of GGT against intracellular glutathione deficiency. Rats were exposed to 10 ppm NO_2 (squares) or clean air (circles). Animals were killed after the time periods shown, and GGT was measured in lung homogenate (A) and in the surfactant fraction from lung lavage (B). Error bars show standard deviations, n = 6 in each group. (Reproduced from Takahashi *et al.*[76] with permission from Academic Press.)

phagocytosis, and phagocytosing cells can substantially increase glutathione uptake from the medium directly and in the presence of the GGT inhibitor acivicin.[84] A glutathione transporter system with an affinity comparable to the plasma glutathione concentration *in vivo* was characterized in human monocytes. The existence of glutathione transport systems raises the interesting but unanswered question of why the more complex GGT-dependent system of extracellular glutathione breakdown and intracellular resynthesis prevails in most cell types.

C. Prooxidant Aspects of GGT Activity

It is clear from the studies described above that GGT induction can occur as a protective adaptation that allows cells access to more cysteine and thereby increases intracellular glutathione, which is protective against oxidative stress. On the other hand, there is evidence that GGT and glutathione, particularly in the presence of iron and copper, can lead to formation of free radicals, lipid peroxidation, and mutagenesis, and therefore GGT activity is potentially harmful. Both could be true, and the balance between them could depend on iron concentration or other unknown factors. It should also be noted that the protective effect is intracellular,

while the prooxidant effect is at least initially extracellular because it occurs at the outer surface of the cell membrane.

The possible prooxidant and carcinogenic effects of the glutathione-GGT combination were highlighted by Stark.[85] The high GGT activity of the probably preneoplastic hepatic foci produced in rats by chemical carcinogens prompted the investigation of mechanisms by which GGT might be a cause of malignant transformation, and evidence for such an effect has emerged in a number of subsequent papers.

Rat liver tissue from carcinogen-induced GGT-rich foci was tested for presence of lipid peroxides as a marker of oxidative damage.[86] It was found that lipid peroxides were produced in the GGT-rich regions when the tissue was incubated with glutathione and iron, and that this could be blocked by chelation of iron or omitting glutathione, by the incorporation of a free radical scavenger in the incubation mixture, or by inhibition of GGT activity.

The role of iron, or other transition metals, in GGT-glutathione-induced lipid peroxidation was examined in three further reports.[87-89] The balance between ferric and ferrous states of iron, the presence of iron chelators, and the significance of copper and ceruloplasmin were studied. GGT had substantial effects on both lipid peroxidation in rat liver microsomes and on the rate of reduction of ferric to ferrous iron in the system. Low concentrations of copper increased lipid peroxidation and iron reduction. Oxidative mutagenesis in the Ames test by the glutathione-GGT combination was also increased by copper and by ceruloplasmin (65% saturated with copper). High glutathione concentrations could overcome the requirement for copper, but GGT always enhanced mutagenesis. The reaction sequence leading to free radical generation was discussed, and it was thought likely that thiols (particularly the GGT reaction product cysteinylglycine) reduce copper, which in turn reduces iron, with production of superoxide, hydrogen peroxide, and hydroxyl radicals. Similar findings on the involvement of iron and copper emerged in a series of experiments using lipid peroxidation rather than mutagenesis as an indicator of oxidative damage.

Paolicchi *et al*.[90] studied lipid peroxidation associated with GGT activity in rat hepatocytes and in hepatoma cells of human origin. The addition of GGT to rat liver microsomes in the presence of glutathione and chelated iron stimulated lipid peroxidation in the microsomal membrane, particularly if GGT activity was enhanced by addition of the acceptor substrate glycylglycine. This could also be shown in isolated hepatocytes and in hepatoma cells and could be prevented by GGT inhibitors. The GGT-induced lipid peroxidation was dependent on the presence of iron. The authors proposed that the sulfydryl group of the product of the GGT reaction, cysteinylglycine, is better able to react with and reduce ferric iron than the sulfydryl group of the substrate glutathione. The ferrous iron produced can then catalyze the formation of reactive oxygen species that bring about lipid peroxidation.

Similar results were obtained by Drozdz *et al*.,[91] with the interesting demonstration that the iron transport protein transferrin could act as a source of iron for

these reactions. The production of reactive oxygen species was monitored by chemiluminescence of luminol, as seen in Figure 4. Either purified GGT or transfected cells expressing GGT, generated chemiluminescence in the presence of glutathione, glycylglycine, and transferrin. Cysteinylglycine, and smaller amounts of cysteine, were shown by HPLC to be generated in the reaction mixture containing the GGT-transfected but not the control cells. Cysteinylglycine and cysteine also produced chemiluminescence in the absence of GGT.

Approaching the issue from a rather different direction, Brown *et al*.[92] investigated the effects of iron overload. Rats were administered iron for 10 weeks, with consequent increases in hepatic iron, 4-hydroxynonenal (a fatty acid peroxidation

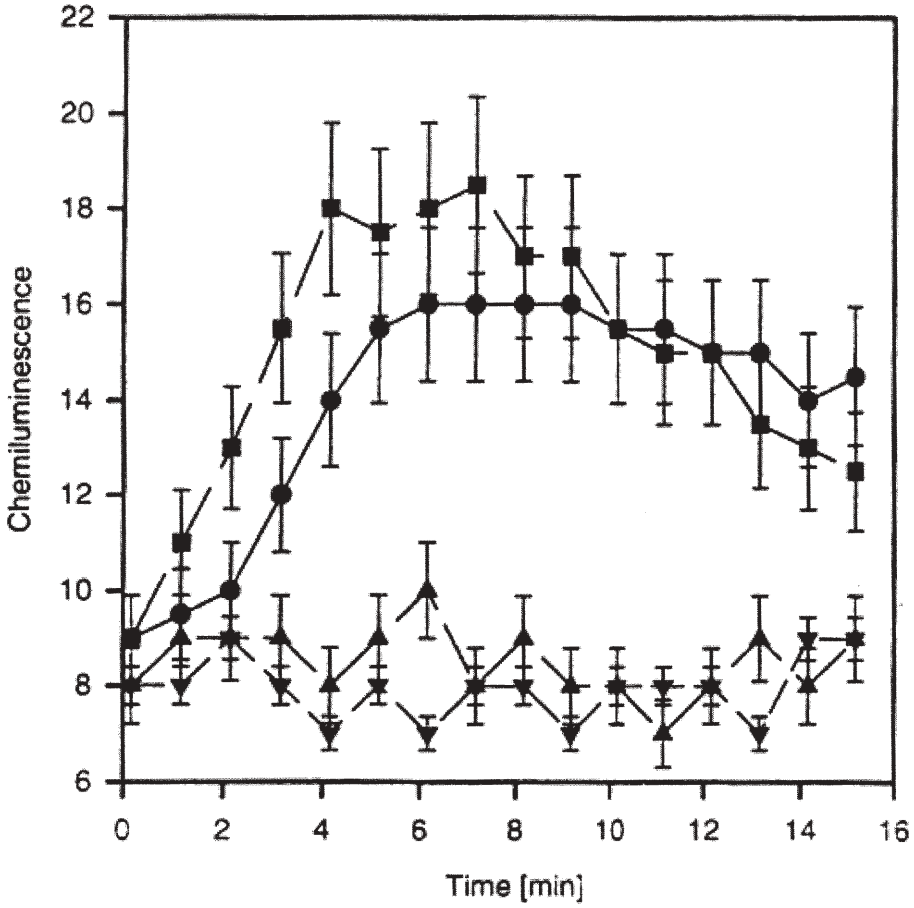

FIGURE 4. Prooxidant effect of GGT ectoactivity at the cell membrane. Chemiluminescence is used as a measure of the generation of reactive oxygen species through the action of GGT on glutathione in a cell-free system containing GGT, glutathione, glycylglycine, transferrin, flouresceine, and luminol in phosphate buffer pH 7.4. Triangles: controls with GGT omitted, circles: with GGT, squares: with GGT and 200 μM H_2O_2. (Reprinted from Drzdz *et al.*,[91] Copyright 1998, with permission from Elsevier Science.)

product), and hydroxyproline (a marker of collagen content). Hepatic GGT activity increased sixfold and was co-located with iron; GGT mRNA was also increased. There was a substantial decrease in activity of Cu-Zn superoxide dismutase, possibly due to its inactivation by hydroperoxides generated as a consequence of GGT activity in the presence of iron. The orientation of this paper is toward iron overload as a cause of oxidative stress and increased GGT as a protective response, but it is possible that the protective GGT response to iron generates further oxidative damage in the ways shown by other groups and presented above.

GGT-dependent lipid oxidation was further studied by Paolicchi *et al*.[93] in the context of atherosclerosis. First, an *in vitro* system containing low-density lipoprotein (LDL), chelated iron, glutathione, glycylglycine, and GGT was used to study LDL oxidation. This was increased by increasing either glutathione concentration or GGT activity in the presence of the other component. Glutathione-dependent iron reduction was also increased by GGT, indicating that GGT reaction products (in this defined system, cysteinylglycine) promoted iron reduction. Both LDL oxidation and iron reduction were inhibited by acivicin and serine-borate. Second, human atheromatous plaques obtained at carotid endarterectomy or *postmortem* were tested histochemically for GGT activity, which was detected in all samples. Examination of serial sections of the larger samples showed that GGT was found in the intima and corresponded to the location of accumulations of lipid, and high-magnification views showed that GGT was associated with foam cells. The lipid-rich GGT-positive areas also reacted with antibodies directed against oxidized LDL. The simulation of conditions within plaques showed that either purified GGT or cells of the macrophage line (which are precursors of foam cells) could lead to oxidation of LDL in the presence of glutathione, and of iron at concentrations expected *in vivo*. In view of the epidemiological associations between serum GGT and atherosclerotic disease, discussed later, these results are particularly interesting. It might also be speculated that the association between plasma lipoproteins and some isoforms of GGT could lead to oxidation of circulating lipoproteins *in vivo*.

Recent work has suggested a range of important implications of glutathione-GGT-associated oxidative processes. Del Bello *et al*.[94] investigated the stimulation of proliferation and inhibition of apoptosis by reactive oxygen species, and cited GGT as one source of reactive oxygen species because inhibition of GGT can lead to apoptosis in some circumstances. In monoblastoid cells in culture, inhibition of GGT caused a dose-dependent decrease in hydrogen peroxide production, arrest of cell growth, and DNA fragmentation characteristic of apoptosis. Chelation of endogenous or contaminant iron by desferrioxamine prevented the GGT-associated production of hydrogen peroxide. The authors suggested that ‘GGT-dependent generation of oxidant species might represent a basal antiapoptotic and proliferative signal for the cell’. If this proposal is correct, this could have implications for inappropriate survival and proliferation of cells in premalignant liver foci, or of endothelial cells in atheromatous plaques.

The ways in which oxidants generated as a result of GGT activity in the presence of iron might affect cell proliferation were investigated by Dominici *et al.*[95] They showed that GGT activity led to decreased levels of protein thiols on the cell surface because of oxidation or *S*-thiolation reactions. GGT inhibition not only negated this decrease in cell surface protein thiols but increased them above baseline, showing that basal GGT activity in control cells was affecting the state of the cell surface. Such variation in cell surface protein thiols could lead to variation in enzyme activity where cysteine is part of an enzyme's active site, or to variation in growth factor receptor-ligand interactions at the cell membrane. This concept was tested in further experiments,[96] which confirmed GGT-dependent hydrogen peroxide generation in melanoma cells and showed that stimulation or inhibition of GGT activity activated or inactivated the transcription factor NF-κB. This transcription factor is believed to be important in malignant behavior of cells and could indicate a direct rather than a permissive (selective advantage) role for GGT in experimental carcinogenesis.

The implications of GGT's prooxidant activity for cell growth *in vivo* for hepatic regeneration and cirhosis after injury, or for development of hepatocellular carcinoma, are still to be worked through, but it may be necessary to move from viewing GGT purely as a marker of a pathological process to an understanding of its role as an active participant.

IV. GGT AS A MARKER OF LIVER DISEASE

A. Reference Ranges for GGT

The reference range for serum or plasma GGT is similar across ages, although there are significant male-female differences, as can be seen in Figure 5. A summary of factors reported to influence the reference range is given in Table 1.

A number of physiological or demographic variables, shading into risk factors for disease, affect GGT, so the definition of a reference range is complex. When the object is to distinguish between subjects with and without liver disease, it is probably best to accept into the reference group all subjects able to engage in their usual activities and not known to have acute or chronic disease, which is the approach taken by the studies of blood donors and healthy subjects shown in Table 1. Several studies in which the same subjects have been tested on duplicate or multiple occasions have shown persistent differences between individuals,[113-115] which may be due to variation in family environment in younger subjects[116] and to genetic variation in older ones.[117]

Occasionally, subjects with high serum GGT but with no obvious cause are encountered. A study of three generations of an Italian family[118] showed apparent autosomal dominant transmission of serum GGT values of around 100 times normal; there were no ill effects reported, and one affected family member appears to have survived to at least the age of 92.

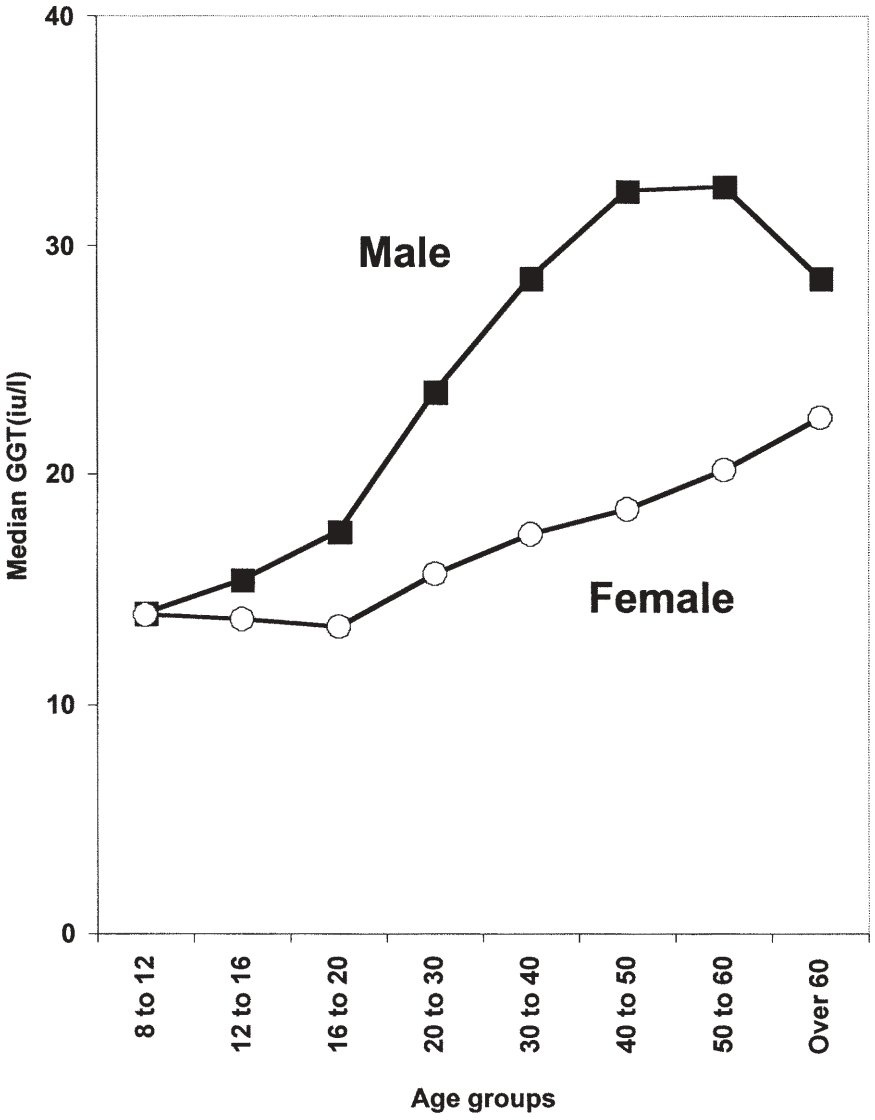

FIGURE 5. Age and sex effects on median serum GGT after exclusion of subjects with extreme alcohol intake and women on oral contraceptives. Note the similar values in male and female children and the divergence by age 20. (Data from Schiele *et al.*[105])

TABLE 1
Factors Influencing the Reference Range for Serum GGT

Factor Investigated	Findings	Ref.
Sex	Higher values in adult men than in adult women, for example,	
	• Central 90% range 13–51 for men, 11–35 for women, in London blood donors	97
	• Log-transformed mean ± 2SD 7-65 for men, 6–30 for women, in healthy adult Finns	98
	• Central 95% range 3-31 for men, 1–21 for women, in healthy adult white Americans	99
	• Central 95% range 11-58 for men, 8–42 for women, in healthy adult Finns	100
Age	Prenatal	
	• Cord blood activity showed no significant change with gestational age; mean 80 iu/l, SD 20 iu/l.	31
	• Cord blood GGT activity in foetuses at 20–26 weeks gestation (found at birth to be normal) averaged 24 iu/l	101
	• Cord blood GGT activity at 27 ± 6 weeks gestation was 157 iu/l in 72 foetuses investigated for possible abnormality. In 8 of these found not to have abnormalities the mean was lower at 106 iu/l.	102
	Neonates	
	• Median 90 iu/l, 95% range 24 to 313, in cord blood serum; same for males and females	103
	• Median 56 iu/l, 90% range 24 to 150, in cord blood; decreasing over the following 3 days	104
	Children	
	• Central 95% range 8–28 iu/l in boys and 8–25 in girls aged 8 to 12 years	105
	Adolescents	
	• Male, but not female, median GGT increases towards adult values across adolescence	105
	Adults	
	• 30% increase in mean between age groups 18–25 and 56–65 in both men and women	106
	• 38% and 29% increases in median between age groups 20–30 and 50–60 in males and females, respectively	105

TABLE 1 (continued)

Factor Investigated	Findings	Ref.
Pregnancy	Consistent with a decrease from nonpregnant values	
	• No change between 15 and 40 weeks gestation	31
	• No change between 15 and 40 weeks gestation	107
	• No significant change between 15 and 40 weeks	108
	• 86% of control values in first, 77% of control in second, and 63% of control in third trimester(lower in pregnancy)	110
Childbirth	Mean increase of 62% (8 iu/l) at 5 or 10 days after delivery, in part reflecting a return to pre-pregnancy levels. Greater increase (mean 92%) after cesarian section	111
Race	Higher values in black subjects: 95th centiles were 43 and 25 for black men and women, compared with 23 and 15 for white men and women, in healthy subjects aged 18 to 30 years.	99
Smoking	Mean value 24% higher in smokers (adjusted for age, race, sex, and BMI)	99
Oral contraceptive use	• Median value 20% higher in women using oral contraceptives	105
	• Mean value 10% higher in women using oral contraceptives (adjusted for age, race, sex and BMI)	99
Exercise	No short-term effect of strenuous physical exercise. No preexercise difference between athletes and reference group	112

B. GGT and Liver Disease

GGT was investigated and adopted as a liver function test or liver enzyme in the 1960s and 1970s. An early paper by Szczeklik *et al*.[119] gave average values in different types of liver disease and showed examples of changes in serum GGT with time, comparing it with other enzymes. The development of more convenient methods led to a substantial number of further papers.[120–126] It is a sensitive test, being abnormal in most patients with liver disease regardless of cause, although higher values are found in patients with cholestasis. The problem lies in a lack of specificity, given the wide range of diseases or other conditions (pancreatitis, diabetes, obesity, excessive alcohol intake, use of enzyme-inducing drugs) that can also cause high serum GGT.

Apart from its general use in the assessment of liver disease, GGT has been evaluated in a number of common or significant clinical settings. The roles of GGT in hepatitis C and alcohol-related disease are considered in subsequent sections. In Porphyria Cutanea Tarda (PCT) there is a very high prevalence of abnormal GGT;[127,128] this is rather unexpected for a metabolic liver disease with little biliary involvement. GGT decreases with treatment of PCT, whether the treatment is

through abstinence from alcohol or iron removal,[127] chloroquine,[128] cimetidine,[129] or following treatment of hepatitis C,[130] which is a known risk factor for PCT. The elevation in GGT may in part be due to the high frequency of alcohol abuse in PCT, and perhaps to the iron overload and associated oxidative stress. Some porphyrinogenic drugs have the effect of increasing hepatic or serum GGT.[131-135] The striking increase in hepatic GGT produced by hexachlorobenzene, and the close relationship with the increase in porphyrins and porphyrin precursors, can be seen in Figure 6.[132]

There are likely to be common features between the increase in hepatic GGT in this experimental porphyria and the induction of GGT by microsomal enzyme-inducing xenobiotics, discussed below. However, the connections between risk factors for PCT, the development of biochemical changes in the porphyrin biosynthetic pathway during active disease, and the increase in GGT are still unclear. Diagnostically, the porphyrin results are much more important than the GGT.

Nonalcoholic steatohepatitis (NASH) is a hepatic disease associated with diabetes and obesity and shares many features with alcoholic liver disease. GGT is increased in most patients with this condition,[136-138] but it is not of diagnostic use. The main interest in relation to GGT is the fact that the condition is similar to alcoholic liver disease but without the alcohol, and that it may represent the extreme end of the epidemiological associations between GGT and obesity, insulin resistance, diabetes, and fatty liver described later in this review. Once again, the details of the processes leading to GGT elevation are unknown.

Trends in GGT have been reported to be useful in detection of rejection of transplanted livers,[139] but single measurements were found to be of no value.[140]

C. Serum GGT in Cancer

Early clinical studies showed a high prevalence of abnormal GGT in patients with primary or secondary liver cancer. Taken in conjunction with the experimental studies that use tissue GGT as a marker of neoplastic transformation, this raises the question of whether GGT has a role in detecting primary hepatic cancers or differentiating them from secondary hepatic cancer.

A number of publications have sought to test whether GGT isoforms are associated with liver cancer, particularly hepatocellular carcinoma (HCC, hepatoma). Several promising reports have appeared, but unfortunately they are not easy to compare. The variable number of isoforms separated by different techniques, and the lack of a standard nomenclature, add to the confusion. In most cases, a form of GGT not associated with lipoproteins, and having greater electrophoretic mobility, was associated with HCC.

Sensitivity (and specificity) of the possibly HCC-specific isoform for HCC was reported to be 59% (~100%);[141] 90% (97%);[142] 75% for both primary and secondary cancers as a single group (90%);[143] and 58% (83%).[144] HCC-associated

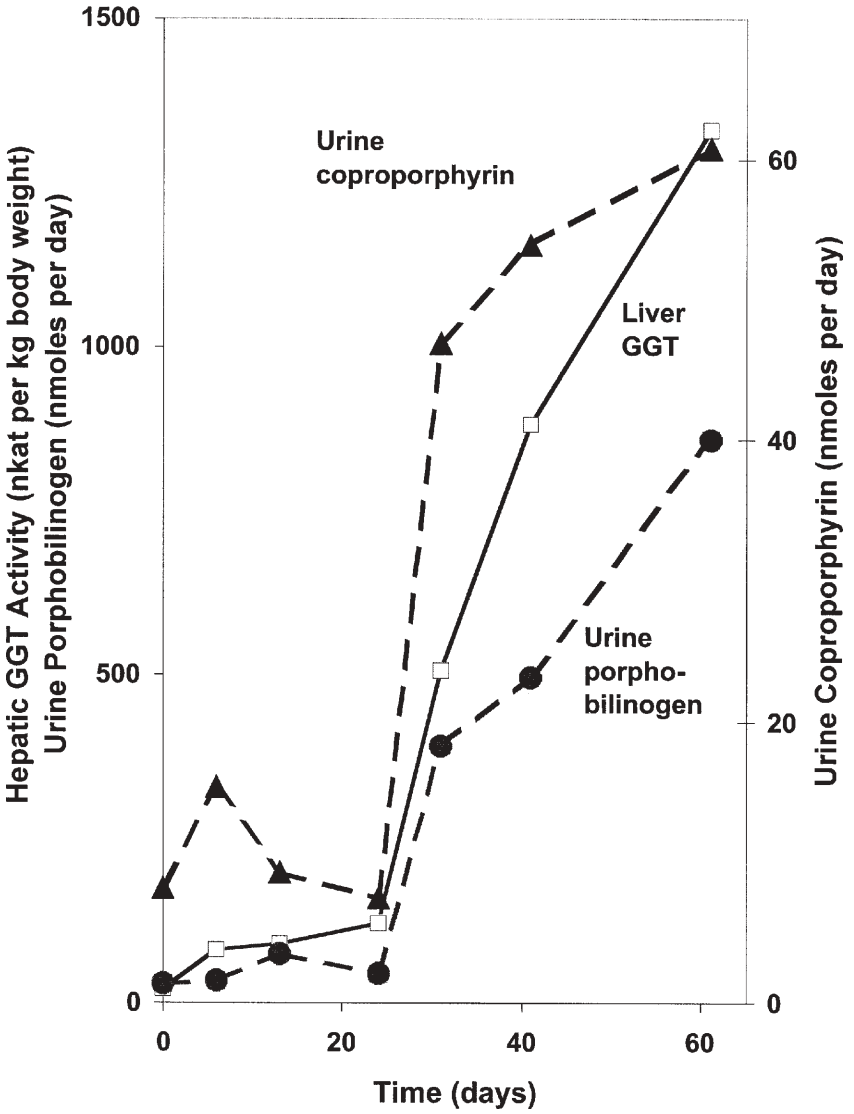

FIGURE 6. Effects of hexachlorobenzene on porphyrins and porphyrin precursor excretion in rats, showing parallel changes in hepatic GGT activity. (Data from Adjarov *et al.*[132])

GGT could be positive in patients with hepatoma who had normal α-fetoprotein. Follow-up of some patients who were at first thought to represent false negatives for this test[145] showed that many developed HCC over the 10 years following initial testing, especially if they showed persistent elevation of this isoform on repeat testing.

An investigation of the characteristics of GGT isoforms from human HCC tissue and a comparison with GGT from normal or cirrhotic livers showed reduced electrophoretic mobility.[146] However, the mobility varied between samples from

different HCC patients. There was also a difference in isoelectric point between the HCC and control GGTs. Both the electrophoretic and isoelectric point differences were reduced by neuraminidase treatment, suggesting differences in sialic acid content. Lectin affinity studies suggested the presence of additional differences in carbohydrate structure.

GGT isoenzymes may be useful in detecting, monitoring, or even predicting HCC, but so far there are not sufficient results available to be confident about the claims of high sensitivity. There is also a need for improved and standardized methods for measuring GGT isoforms.

D. Viral Hepatitis and the Response to Treatment with Interferon

Serum GGT is elevated in most, but not all, patients with the chronic liver disease associated with hepatitis C infection. Itoh and Nakajima[147] found that hepatic GGT was also increased, although the proportionate change was less than for serum; this suggests that both increased synthesis and increased release of GGT occur. The response to interferon treatment of chronic hepatitis C infection has been studied by many groups, and several papers have appeared showing that pretreatment serum GGT is predictive of the biochemical and/or virological response. In this context, a biochemical response consists of a reduction in serum AST and/or ALT to normal levels, while a virological response is a reduction to undetectable amounts of the hepatitis C virus RNA in serum.

Battezzati *et al*.[148] reported on the 3-month outcome after interferon treatment in 263 patients with non-A, non-B hepatitis. Mean initial values of several liver function tests (GGT, AST, and alkaline phosphatase) were lower in responders than nonresponders, when response was judged by a fall in ALT to 1.5 times the upper limit of the reference range or below. Multivariate analysis showed that among the biochemical tests only GGT was an independent predictor of response; the presence of cirrhosis and of obesity were also adverse predictors. Although the results showed an impressive statistical association with outcome, the success of GGT in predicting response was imperfect because sensitivity was calculated to be 87%, but specificity was only 27%.

Similar results were obtained by Camps *et al*.[149] GGT was the only significant biochemical predictor of response, together with age, sex, and obesity and the presence of cirrhosis. When patients were grouped by the presence of cirrhosis, GGT was only associated with a response to interferon in the noncirrhotic patients. Mazzella *et al*.[150] found that low GGT and female sex were positive predictors of response. Conversely, Chemello *et al*.[151] found that elevated GGT was associated with lack of response, and a significant negative association was also reported by Olaso *et al*.[152] Van Thiel *et al*.[153] separated patients into full, partial, and nonresponders and found that GGT at entry to the study was significantly lower in full than in partial or in nonresponders. In addition, they found that hepatic iron

content was lowest in full and highest in nonresponders, although this trend was not quite significant.

A slightly different approach was taken by Mihm *et al*.,[154] who assessed response not only by a fall in AST and ALT but also by undetectable viral RNA. They used the ratio of serum GGT to ALT and were able to show that in untreated patients with hepatitis C infection this ratio was near-constant across time for any individual. The GGT/ALT ratio differed by viral genotype, being lower in type 3a than in 1a or 1b, even though ALT, AST, and GGT levels themselves did not differ significantly. The virological but not the biochemical response to interferon therapy varied according to viral genotype, with a response being commonest with type 3a, and response was also more likely in patients with a low pretreatment GGT/ALT ratio. Although viral genotype 3a and low GGT/ALT ratio were associated with each other, the ratio was an independent predictor because it was lower in the responders than the nonresponders within each genotype group. In this study, serum GGT alone did not have predictive value and the ratio of the two enzymes was required for successful results. These authors calculated the sensitivity of the ratio, in combination with knowledge of the viral genotype, as 0.71 and the specificity as 1.0.

Pawlotsky *et al*.[155] assessed both the biochemical response to interferon (after 3 and 6 months treatment) and the virological response 6 months after the end of treatment. Although pretreatment GGT was predictive of biochemical response during treatment, only the viral load and presence/absence of anti-HCV core IgM antibodies were associated with sustained virological response.

An algorithm to predict sustained response was developed and assessed by Noventa *et al*.[156] This was constructed using data from 307 patients and tested against a further 200 with consistent results. Variables included were pretreatment GGT and ALT and viral genotype. The area under the curve from ROC analysis was 0.79 (0.78 in the replication group). This paper indirectly confirms the relevance of the relationship between GGT and ALT and the potential usefulness of their ratio, because both were independently predictive of response but in opposite directions.

A report on the GGT/ALT ratio[157] in a new sample of 48 patients supported its predictive value. The ratio was significantly lower in virological responders than in nonresponders, both in patients with viral genotype 1b and those with 3a. GGT on its own did not differ significantly between responders and nonresponders. All 17 patients with a favorable prognostic ratio showed clearance of viral RNA for at least 3 months, while 25 of the 31 patients with higher ratios failed to respond. It should be noted that the cut-offs used for GGT/ALT ratio varied with viral genotype, so this rather good prognostic performance depended on having pretreatment information about both the infective agent and the host characteristics.

The reason why high GGT (alone or as a ratio) is associated with nonresponse to interferon treatment is not known. Patients with higher GGT presumably have, on average, more longstanding or severe liver disease. Neither GGT/ALT ratio nor

treatment response were significantly associated with pretreatment viral load.[154] It is possible that the ratio reflects the balance between necrotic and cholestatic effects of the chronic infection on the liver. As far as the development of cirrhosis is concerned, most of the reports led to the conclusion that both high GGT and cirrhosis predict a lack of response, but GGT is not simply acting as a marker of cirrhosis.

The interactions between hepatic iron overload, serum GGT, and the response to interferon have been studied by several groups. Piperno *et al*.[158] found that GGT was significantly and quite strongly correlated ($r = 0.51$, $p < 0.001$) with serum ferritin in patients with chronic hepatitis C infection, but that both GGT and liver iron concentration differed between responders (who had lower pretreatment values for each) and nonresponders and were independently predictive in multivariate analysis. Unfortunately, removal of iron did not improve the long-term response to interferon, and although it did reduce ALT levels in the short term it was not reported whether GGT also fell. The same group found[159] that in patients without iron overload (assessed by normal transferrin saturation), GGT and liver iron again differed between responders and nonresponders. Subsequently, differences in both serum GGT and serum ferritin between responders and nonresponders to interferon have been confirmed.[160]

A study of long-term outcomes in patients with hepatitis C following interferon therapy[161] showed that high serum GGT at entry did not predict the progression of cirrhosis, but that it did marginally predict development of hepatocellular carcinoma and was significantly predictive of death or liver transplantation.

In summary, there is substantial evidence that patients with high serum GGT or high GGT/AST ratio are unlikely to have a sustained virological response to interferon treatment. However, the prediction of nonresponse is less than perfect and in the absence of alternative treatment the biochemical results are not likely to be used to exclude use of interferon.

E. Mechanism of GGT Increase in Liver Disease

The ways in which liver diseases lead to an increase in serum GGT are not well understood. Experimental work has mostly used rats, who have substantially lower serum and hepatic GGT activity than humans; Teschke *et al*.[162] found 20-fold higher values in normal humans than in control rats. As so much work is based on these animals, the assumption that the difference is purely quantitative is rather a crucial one.

The relationship between hepatic and serum GGT in human patients has been studied by several authors. A sensitive method for measurement of GGT activity in needle biopsy samples was developed by Satoh and colleagues,[163] who found that in 57 patients with a variety of liver diseases either serum or hepatic GGT, or both, or neither, could be elevated. Among those patients with both serum and liver

GGT increased, higher values were found in alcoholic or drug-induced hepatitis than in viral hepatitis, and in the former group serum GGT showed a greater increase above normal (approximately sixfold) than liver GGT (around threefold).

In another human study that included patients with several types of liver disease,[164] only the group with alcoholic liver disease had mean hepatic GGT increased significantly above normal even though all types showed increased serum levels. In patients with alcoholic liver disease the hepatic GGT approximately doubled, but the serum GGT increased about sevenfold. In individual patients, the correlation between hepatic and serum GGT activity was poor and nonsignificant. Changes in hepatic GGT in response to alcohol are considered in more detail later.

Two conclusions follow from these results. First, there seems no doubt that hepatic GGT is increased in at least some types of liver disease; the increase in serum GGT is not simply due to release of enzyme from damaged cells. The sequence of events, from the original stimulus to the increased expression of the gene or to the decreased breakdown of the protein, has not been fully explored. Because serum GGT increases more than hepatic GGT, it is probable that the enzyme expressed on the cell surface is released into the circulation more readily than usual, and some authors have suggested that this is the result of bile acids acting on the cell membrane. Second, the 'induction' of GGT appears to some extent specific for alcohol-related disease, although we cannot rule out the possibility that the near-normal levels in hepatic tissue in other types of disease are a consequence of more rapid loss of newly synthesized GGT from the cell surface into the circulation.

F. Glutathione and Oxidative Stress in Obstructive Liver Disease

Many papers have described the changes that occur in the liver following experimental bile duct ligation in rats, particularly the effects of this form of obstruction on lipid peroxidation and free radical activity and in hepatic glutathione content.[165-172] All have found evidence of increased lipid peroxidation or free radical activity, and most have found a decrease in hepatic glutathione.

The interpretation of the glutathione results is complicated by the fact that liver weight increases after bile duct ligation and results vary according to whether glutathione content is related to mass of tissue or total content per liver. Some authors have measured reduced glutathione only, while others have measured the reduced and disulfide components or total glutathione. There can be two processes taking place at the same time: a change in redox state and a change in the total concentration of glutathione. One paper[171] showed that initially reduced glutathione rose and oxidized glutathione fell, but after a few days oxidized glutathione increased, and subsequently reduced glutathione fell.

A measure of protection against the prooxidant effects of bile duct ligation could be obtained by giving either *N*-acetyl cysteine[168] or *S*-adenosyl methionine.[169] Either of these were able to eliminate or reduce the lipid peroxidation or free radical activity and restore hepatic glutathione concentrations. In the study where *S*-adenosyl methionine was administered, it was also found to attenuate the postligation increase in serum GGT. This was probably a nonspecific effect on liver function rather than a specific one on GGT, because serum bilirubin and alkaline phosphatase were also improved.

V. ENZYME INDUCTION

A. Anticonvulsants and Microsomal Enzyme-Inducing Drugs

Increases in serum GGT in patients receiving anticonvulsant drugs were first reported in the early 1970s.[173] Phenytoin and phenobarbitone are the main drugs associated with abnormal GGT results. The proportion of patients with elevated GGT is high: 75%,[173] 84%,[174] 74%,[175] 72%,[176] or 91%.[177] Comparisons among these drugs[176,178] suggest that the proportion is slightly less in those taking phenobarbitone alone. Before and after comparisons have shown increases in 90% of patients on phenytoin, and both the pre- and posttreatment GGTs were higher in patients drinking even small amounts of alcohol than in abstainers.[175] There is only a weak correlation between drug dose or plasma drug concentration and GGT in subjects on these anticonvulsants.[174,176,178] Younger patients (aged less than 20 years) tend to have lower GGT results.

Several other drugs are associated with increased GGT in at least some patients: these include carbamazepine and possibly valproate,[178] although another report[179] found no effect of valproate alone. Among other classes of drug, aminopyrine produced a significant increase,[180,181] and rifampicin produced a 50% but nonsignificant increase.[181] The insecticide DDT and the fungicide hexachlorobenzene can also produce elevated GGT.[132,182,183] It is evident that a wide range of chemicals can produce increased GGT, and most of them also produce induction of hepatic microsomal drug-metabolizing enzymes.

Several groups have compared the effects of drugs or xenobiotics on GGT on drug half-lives and on other indices of hepatic microsomal induction. Although it is evident that the same stimuli can lead to increased GGT and also to changes in drug metabolism, and some striking parallels can be drawn, the evidence suggests that there are differences between the events leading to increased GGT and those producing hepatic microsomal enzyme induction.

A study with human volunteers given phenobarbital[184] showed increased GGT over 2 weeks accompanied by expected changes in glucaric acid and 6-β-hydroxycortisol excretion and in aminopyrine half-life. There was a moderate correlation between the GGT and the urine glucaric acid, and smaller ones with

6-β-hydroxycortisol excretion and with aminopyrine half-life, but the number of subjects was small and none of the correlations was significant. No significant correlation was found between GGT and glucaric acid excretion in a larger group of 59 epileptic children treated with phenytoin or valproate.[179] Similarly, there were no significant correlations between GGT and antipyrine clearance or 6-β-hydroxycortisol excretion in children treated with carbamazepine,[185] or between GGT and glucaric acid excretion in adult patients receiving multiple drugs, including barbiturates, when those with liver disease were either excluded or considered separately.[186]

Although enzyme-inducing drugs have their greatest effect on GGT, there is some evidence of the effects on other liver function tests. Aldenhovel[177] found abnormalities of alkaline phosphatase, ALT, and AST in patients on phenytoin or, to a lesser extent, carbamazepine. Increases in alkaline phosphatase were also reported in patients taking phenytoin or carbamazepine.[187] However, increases in alkaline phosphatase are not necessarily evidence of liver dysfunction in patients taking anticonvulsants, because bone alkaline phosphatase may be increased through the effects of anticonvulsants on vitamin D metabolism.

The effects of phenobarbital on hepatic glutathione and GGT were investigated by Braide.[188,189] A single dose of phenobarbital to rats led to a decrease in hepatic glutathione, followed by an increase in hepatic GGT, as shown in Figure 7.

This suggested that GGT was induced by the drop in glutathione (although the temporal relationship does not prove causation), and this would be consistent with the function of GGT in maintaining hepatic glutathione. The manipulation of hepatic glutathione levels showed that a decrease in glutathione produced by administration of diethyl maleate daily for 4 days was accompanied by a significant increase in GGT, whereas increasing hepatic glutathione by administering cysteine produced no significant alteration in GGT.[189] Phenobarbital alone paradoxically increased both GGT and glutathione, but the combination of cysteine and phenobarbital led to a nonsignificant fall in GGT below control levels. These results support the concept that GGT is induced when enzyme-inducing drugs cause a fall in hepatic glutathione; GGT induction subsequently may lead to higher than normal glutathione (as with the phenobarbital-only treatment), but when glutathione does not fall (as with the cysteine + phenobarbital treatment) GGT is not induced.

VI. ALCOHOL

A. Elevation of GGT with Hazardous or Harmful Alcohol Intake

In the early 1970s a number of authors recognized that as a liver function test, GGT was particularly sensitive to alcoholic liver disease.[190] An extension of this

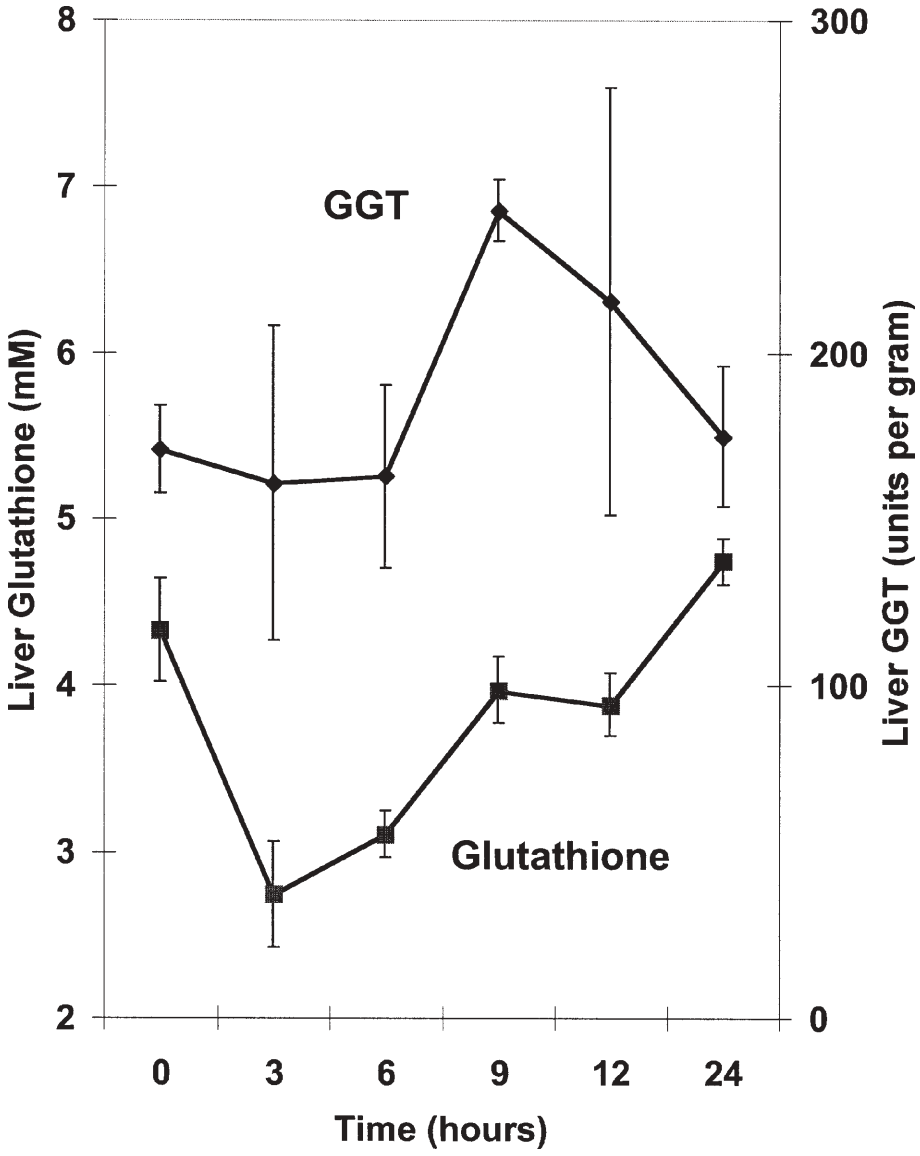

FIGURE 7. Effects of administration of phenobarbital on hepatic GGT and glutathione in rats. Note that the fall in glutathione precedes the increase in GGT activity. (Data from Braide.[188])

idea led to the discovery that GGT was elevated in a high proportion of alcoholics not currently showing the symptoms of liver disease.[191-193]

At about the same time, health screening programs incorporating biochemical tests were being evaluated, and it became clear that many seemingly healthy people had GGT results above the reference range. High GGT was statistically associated with higher alcohol intake.[194 -201] If the intention was to detect asymptomatic liver diseases, the association of high GGT with alcohol use might require separate, higher, reference ranges for people drinking large amounts of alcohol.

Data illustrating the association between alcohol intake and changes in the frequency distribution of GGT results in a screening-clinic sample of men and women are shown in Figure 8, and the greater proportionate increase in GGT than in AST or ALT brought about by alcohol consumption is illustrated in Figure 9.

A synthesis of these two ideas about GGT being sensitive to alcoholic liver disease and about the causes of variation in GGT values in the general population led to the suggestion that GGT might be used for the detection of potentially harmful drinking and that a combination of an effective screening test and early intervention for drinking problems could reduce the harm caused by alcohol. Another use suggested around this time was in monitoring known alcoholics for relapse from abstinence. Both of these potential applications of GGT led to evaluations of its sensitivity and specificity, and to comparisons with other putative markers of alcohol intake or with combinations of markers (see below). Such comparisons have continued to the present, with progressively larger studies being mounted or with combination of data across multiple studies by meta-analysis techniques.[203,204]

B. Human Studies of the Short-Term Response to Alcohol

Several studies of the short- to medium-term effects of alcohol consumption on serum GGT in humans have been carried out. Results appear to depend on the dose and duration of the experimental alcohol consumption, but probably more on the characteristics and previous drinking habits of the subjects tested.

A single dose of alcohol has no effect on serum GGT.[205 -207] Three doses of 0.75 g/kg (equivalent to about 40 to 50 g) on successive days were reported to produce a small increase in GGT: around 25% from a low baseline (5 units/l at 30°C) 60 h after the third dose.[208] The administration of the same dose of alcohol with periods of abstinence between test sessions also resulted in similar minimal elevations of GGT.[209] Consumption of 63 g per day for 5 weeks produced a significant increase from a pretreatment mean of 27 u/l to a 5-week mean of 52 u/l in eight young male volunteers.[210] However, in other studies on subjects described as ‘moderate drinkers’ or as ‘healthy’ consumption of 40 g of ethanol per day for 4 or 6 weeks,[211,212] or 60 g per day for 3 weeks[213] produced no detectable effect or a small (~15%) increase.

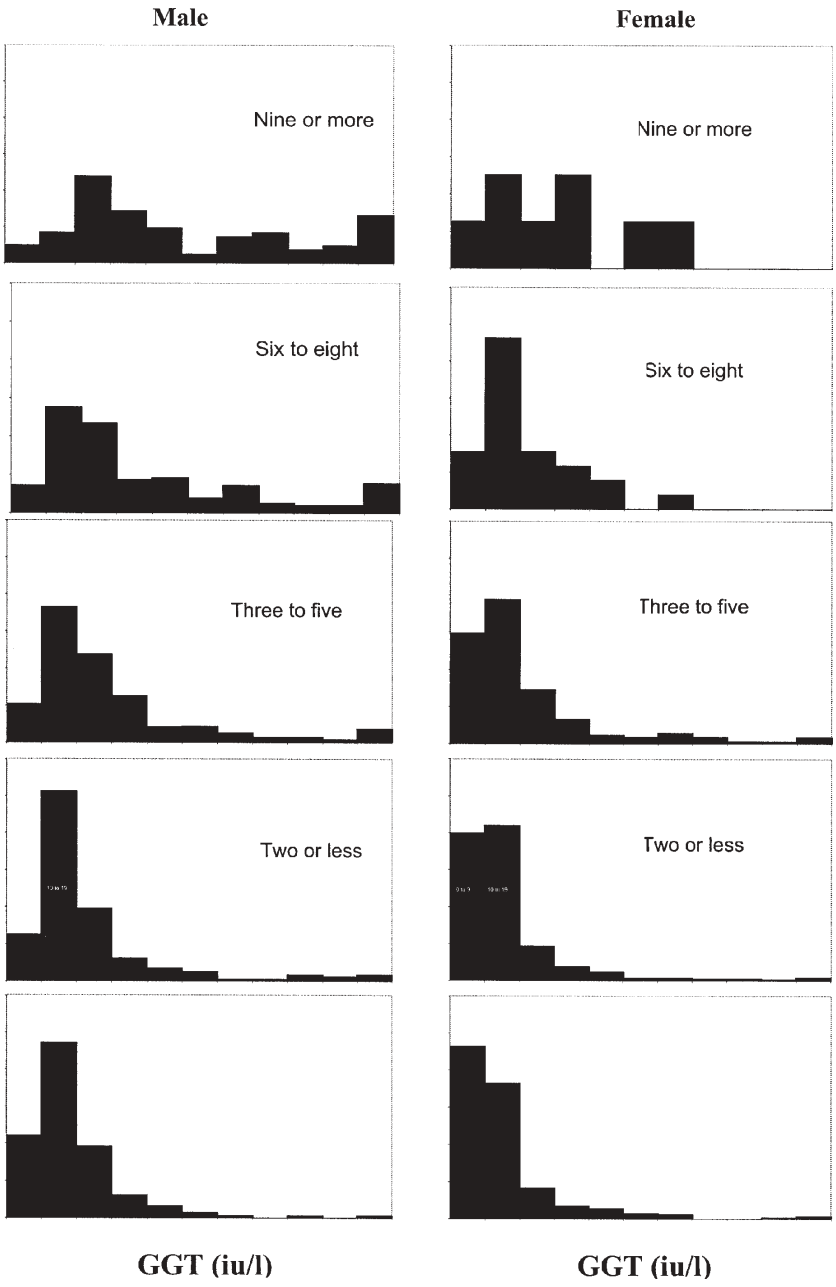

FIGURE 8. Variation in the frequency distribution of serum GGT according to the number of drinks taken on a typical drinking day. Within each panel, the abscissa represents GGT results in groups of 10 iu/l and the ordinate shows the percentage of subjects in that GGT group. The left-hand column shows results for men and the right-hand column for women, and the subjects' alcohol intake increases from the bottom row to the top. Note that about half the subjects in the heaviest drinking category have results within the range found in abstainers. (Adapted from Whitfield *et al.*[197])

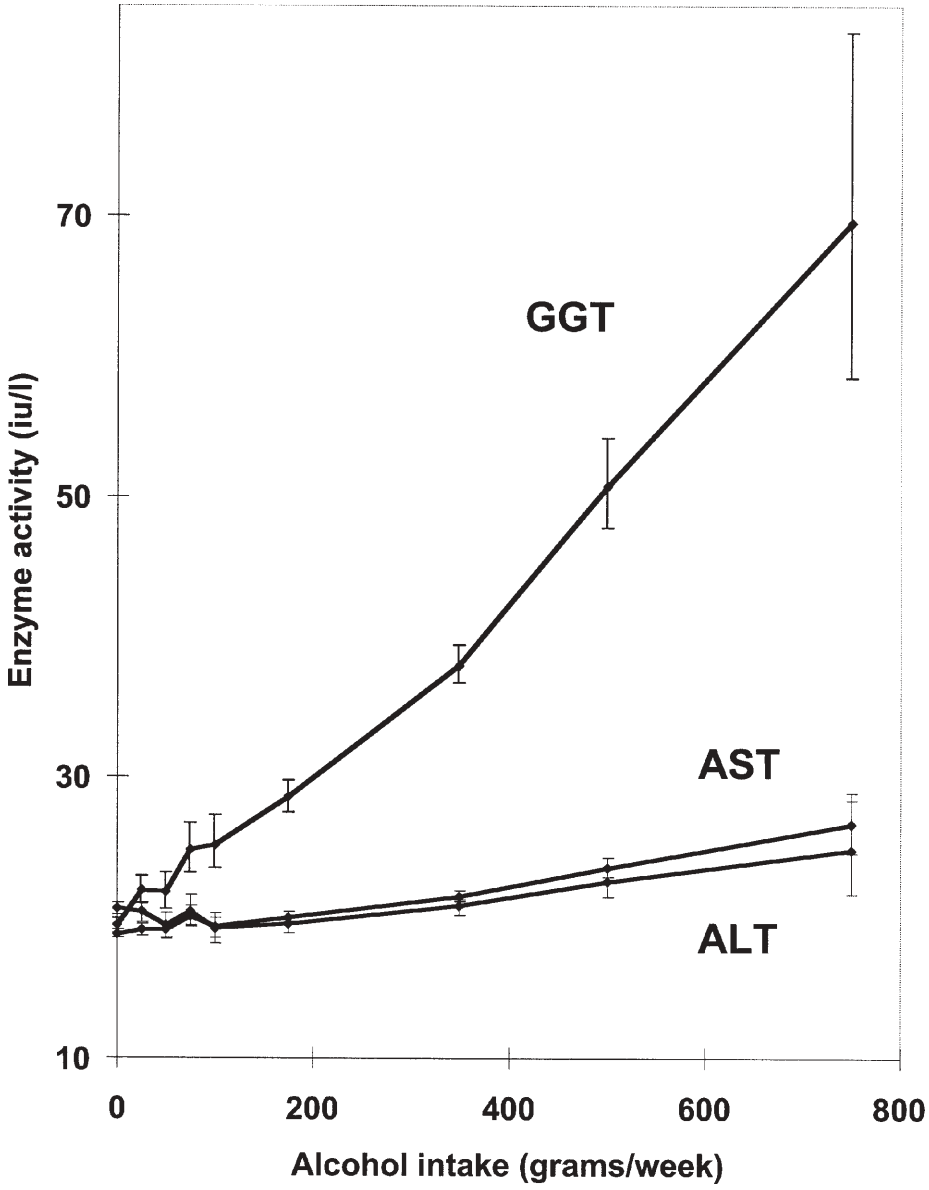

FIGURE 9. Relationship between alcohol intake and mean values for GGT, AST, and ALT in middle-aged Japanese men. Error bars show 95% confidence intervals about the mean. The increases in enzyme activity with increasing alcohol intake are close to linear, but much greater for GGT than for AST or ALT. (From data of Nagaya *et al.*[202])

When ‘moderate’ drinkers (usually drinking 60 to 80 g/day) abstained for 4 weeks and were then tested with a single dose of 1 g/kg an increase from 33 to 69 U/l at 24 h was found; however, after the same dose was given to nondrinkers the increase was much less (from 18 to 24 U/l).[214] It is notable that these allegedly ‘moderate’ drinkers had a preabstinence mean serum GGT of 86 U/l, and 9 of the 14 subjects had some abnormality on liver biopsy. In addition (although the authors did not comment on this), the peak post-alcohol GGT value correlated significantly with the subjects’ values before the period of abstinence from alcohol. This can be seen in Figure 10.

Therefore, it seems that GGT can be increased by experimental alcohol consumption, but mainly in subjects who are accustomed to drinking potentially hazardous amounts of alcohol and/or have an abnormal GGT value at entry to the study. This is consistent with the increase in GGT that occurs when alcoholics in treatment relapse (see below), although there are no experimental studies of the GGT response to a known dose of alcohol in alcoholics. There may be an effect of prior hazardous alcohol intake, which has been suggested as the reason for the lower sensitivity of GGT as a test for high alcohol intake in younger subjects.

C. Change on Abstinence from Alcohol After Dependent Drinking

As noted above, GGT decreased when moderate drinkers abstained from alcohol for a month. Decreases are also the rule when alcohol-dependent subjects enter treatment, and a number of papers have reported the time course and extent of these changes. The apparent half-life of serum GGT after cessation of drinking varied between 5 and 17 days.[215] In alcoholic patients with decompensated cirrhosis, half-lives varied between 11 and 54 days.[216]

In 32 alcohol-dependent patients who abstained for 8 weeks the mean GGT half-life was 26 days,[217] but all these patients had “alcoholic liver disease of different degrees of severity”, and this may have caused overestimation of the half-life. A comparison of alcoholic patients with and without liver disease[218] showed a 50% decrease in GGT with 8 weeks abstinence in patients with no history or clinical evidence of liver disease but essentially no decrease in patients with “obvious liver disease” (ranging from fatty liver to cirrhosis). Similarly, patients with alcohol dependence and hepatitis C infection showed a lesser decrease in GGT with 4 weeks’ abstinence than hepatitis C-negative alcoholics.[219] A variation in the rate of decrease in GGT is to be expected, and the frequently quoted half-life of 25 days should not be interpreted too rigidly.

The absolute value of the decrease on abstinence is related to the initial GGT value,[219-221] and the magnitude of the increase on relapse is correlated with the value during abstinence.[217] Some decrease in GGT during abstinence may occur even when the initial value is in the normal range.[219,222]

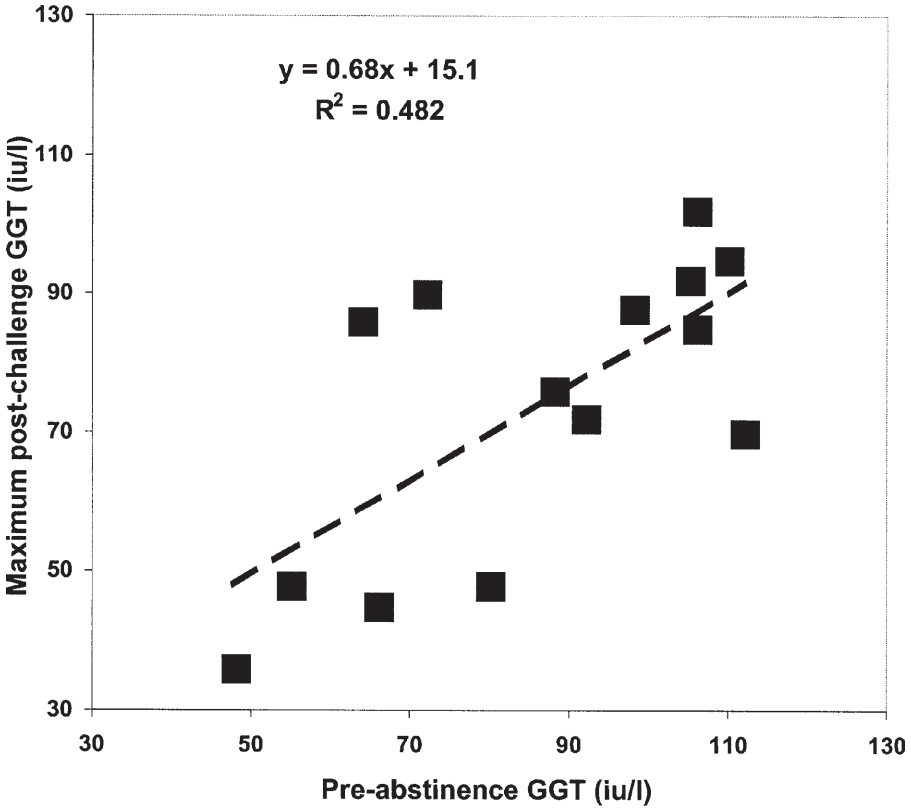

FIGURE 10. GGT before and after experimental abstinence and alcohol challenge. Subjects abstained from alcohol for 4 weeks and were then tested with a single dose of 1 gram/kg of ethanol. Note the correlation between the preabstinence (x-axis) and post-challenge (y-axis) values. (Data from Nemesanszky *et al.*[214])

Lamy *et al*.[215] pointed out that patients who remained abstinent at 1- and 2-year follow-up after detoxification attained normal GGT values, while those who relapsed had similar values at follow-up to those seen on presentation. Short periods of relapse (2 to 5 days of drinking in 6 months) seemed not to produce an increase in GGT, but frequent relapse (22 to 59 days drinking in 6 months) was associated with higher GGT.[223]

The question of whether the response of serum GGT to alcohol is greater in those who have previously been very heavy drinkers, or who have previously had high GGT, than in normal volunteers in the experimental studies discussed above is of interest but is difficult to address in practice. If a high GGT takes some years of drinking to achieve but is then reinstated by even a comparatively short period of relapse, then one could conclude that some change had occurred in the liver that "primed" it to increase GGT in response to alcohol. Such a change might involve development of fatty liver, or of iron overload, or even an immune reaction involving antibodies to acetaldehyde-modified proteins.

D. GGT Compared with Questionnaires and to Other Biological Markers

Many evaluations of the sensitivity and specificity of GGT as a marker of alcohol intake have appeared. They implicitly or explicitly raise several questions about the condition which is to be detected, the way in that subjects or patients are assessed and grouped, and the "gold standard" test against which GGT is to be compared. Furthermore, alcohol consumption in a population is a continuum, low-level alcohol consumption is not associated with increased risk of harm, and the threshold of alcohol consumption that it might be useful to detect with a biological marker cannot be defined with certainty.

Early evaluations tested the performance of GGT, or compared the performance of GGT and other approaches, by contrasting control subjects with patients undergoing treatment for well-established alcohol dependence. It is now recognized that such comparisons may be necessary in the early stages of developing a test but are not a useful measure of its value in practice. There is empirical evidence that case-control studies overestimate test performance.[224] In population studies a representative (or ideally random) sample is required, and in clinical studies the full spectrum of patients should be included.

Detection of undesirably high alcohol intake may be appropriate in two situations. First, when a patient has a disease and is seeking treatment, and alcohol may be a causative agent but high usage is not disclosed. In such situations dependence is likely to be present and levels of intake are high; the level of alcohol to be detected is often set at over 80 grams per day. Secondly, a number of controlled trials have shown benefits from early intervention with drinkers taking potentially hazardous amounts of alcohol (often defined as 40 grams/day for men and 20 grams/day for women), or binge drinking. This is a more difficult requirement for any test to meet and consequently the sensitivity of most tests is lower.

Another important issue is the distinction between alcohol intake and alcohol dependence. Biological markers are influenced by alcohol intake and only secondarily by the presence of alcohol dependence, whereas many of the questionnaires with which biological markers are compared are focussed on symptoms of dependence such as failure to cut down drinking despite attempts to do so, or morning drinking. Also, the questionnaires may cover a time-span of several months or even the whole lifetime, whereas the biological markers are affected by recent drinking. The questionnaires that have been compared against biological markers include MAST (Michigan Alcohol Screening Test), CAGE (a mnemonic based on the four questions related to Cut down, Annoyed, Guilty, and Eye-opener), and AUDIT (Alcohol Use Disorders Identification Test), and the results of these comparisons are discussed below.

The sensitivity and specificity of GGT, together with other markers of alcohol intake, have been reviewed or subjected to meta-analysis by several authors.[203,204,225,226] The original work covered by these reviews is not presented in

detail, but the main conclusions are discussed and more recent developments are included. The main issues are performance of GGT compared against questionnaires and against other biological markers, and performance of GGT at differing levels of alcohol intake. The important topic of GGT's performance in predicting outcomes is covered in a later section.

A comparison of approaches to the identification of excessive alcohol intake was published by Levine.[227] In his summary of papers on the MAST questionnaire, sensitivity in detecting "alcoholics" averaged 90% with specificity averaging 70%. GGT was quoted as showing sensitivity of 50 to 90% with specificity over 80%. However, the figures are not derived from the same patients so they are not directly comparable. A more direct comparison is available in the paper by Bernadt *et al.*,[228] which applied both tests (and a number of others) to consecutive patients admitted to a psychiatric hospital. In this context, the MAST and CAGE questionnaires had sensitivities in the range 85 to 93% for detection of either "excessive drinking" or "alcoholism" and specificity of 76 to 89%, while GGT sensitivity was 33 to 36% at a specificity of 87%. Another study on patients admitted to a psychiatric hospital[229] confirmed the superiority of the MAST. Seven percent of subjects had increased GGT only, 40% had increased MAST score only, and 10% had both. Of the subjects with normal GGT and abnormal MAST, 89% were classified as alcohol abusers, while only one of the (approximately 15) subjects with abnormal GGT and normal MAST was so classified.

Among patients admitted to a general hospital,[230] the sensitivity of GGT for alcohol dependence was rather better than that of the MAST (58% against 47%), but the specificity was notably poorer (70% against 99%). Moving further along the spectrum of recruitment contexts, Vanclay *et al*.[231] compared the performance of GGT and the short form of the MAST in distinguishing men from the general community drinking less than or more than 40 g of alcohol per day. At comparable specificity (90%) the sensitivities of GGT and MAST were 54 and 58%.

However, we may note that despite MAST's superiority in detecting dependence (but not in detecting hazardous consumption), GGT has advantages in detecting people who are likely to suffer consequences of their drinking. Rivara *et al*.[232] found that abnormal GGT was better able to predict repeat admission to a trauma unit (adjusted relative risk 2.4, 95% CI 1.5 to 4.0) than the MAST (adjusted relative risk 1.3, 95% CI 0.8 to 2.2). In the Malmo screening and intervention studies (discussed below), GGT was a better predictor than MAST of subsequent morbidity and mortality.

Laboratory tests that could replace or complement GGT in the detection of hazardous drinking or alcohol dependence include erythrocyte mean cell volume (MCV) and serum carbohydrate-deficient transferrin (CDT). The recognition of MCV as a potential marker of alcohol intake occurred at about the same time as for GGT, and a number of direct comparisons are available. Studies of CDT in this role started some 10 years later, and initially it appeared that it would be much better than GGT, but subsequently this view has been revised. Direct comparisons

on appropriate groups of subjects have revived the reputation of GGT and have stimulated investigations of the use of both tests.

Data on the sensitivity and specificity of GGT and MCV were summarized by Hoeksema and de Bock.[225] Results from seven case-control studies and four studies in nonselected family practice populations were presented, and the data are summarized in Figure 11.

Because of the interaction between sensitivity and specificity, the data from each study are shown as points on ROC plots. It is clear that neither test performs well, with sensitivity values around 40% at a cutoff giving specificity around 90%, and that their performance is very similar. The results from the unselected populations (Figure 11(b)) are similar to those from case-control studies (Figure 11(a)), except that specificity has been set to a high value so that sensitivity is lower.

Similar conclusions were reached by Conigrave *et al.*[226] For the detection of hazardous consumption, reports of GGT sensitivity were in the range 20 to 50% compared with 20 to 30% for MCV. For the detection of alcohol dependence, the figures were GGT 60 to 90% and MCV 40 to 50%. When results were stratified by the nature of the subjects studied, again GGT was slightly better than MCV, but not dramatically so.

A more recent study of excessive drinking in a substantial number of general practice patients[233] using ROC analysis found that MCV and GGT had very similar areas under the curve and no statistically significant difference was found. However, MCV had rather better sensitivity when a cutoff giving high specificity was used; an examination of the ROC plot shows a divergence between the GGT and MCV curves in the higher-specificity, lower-sensitivity region.

Comparisons of GGT and CDT are complicated by the wide range of methods used for estimation of CDT. Scouller *et al.*[204] performed a meta-analysis of 110 studies on CDT published up to June 1998, and one of the findings was that the modified Pharmacia anion exchange method (CDTect) gave results indistinguishable from those for GGT (see Figure 12).

However, data from papers using earlier methods (CDTect original, or isoelectric focusing) showed that CDT was significantly better than GGT. It is possible that the earlier papers, using the earlier and perhaps better CDT methods, suffered from design faults such as a comparison of known alcoholics with abstainers or very light drinkers.

Papers published since the cutoff date for that review have not changed the picture substantially. Sillanaukee *et al.*[234] found sensitivities for GGT and CDT of 33% and 39% in men and 34% and 29% in women. Meerkerk *et al.*[233] found that ROC curves for GGT and CDT were not significantly different ($p = 0.07$), although there was a tendency for GGT to show lower sensitivity than CDT at all cutoffs giving specificity over 70%. Anton *et al.*[235] suggested that GGT and CDT respond to slightly different stimuli, with GGT being more affected by drinking intensity and CDT by drinking frequency, at least among men.

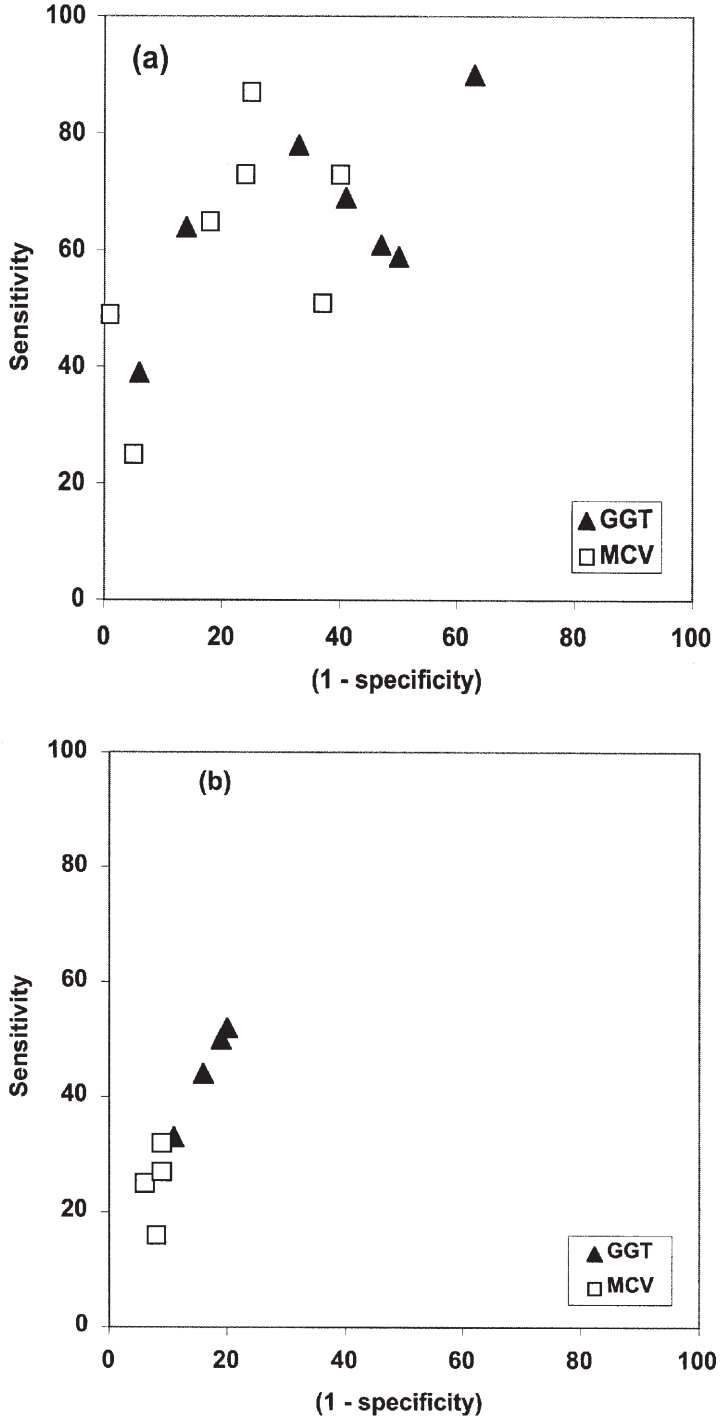

FIGURE 11. ROC plots summarizing data on sensitivity and specificity for GGT and MCV. Upper panel (a): reported results from seven case-control studies; lower panel (b) results from four studies from nonselected populations. (From data in Hoeksema and de Bock.[225])

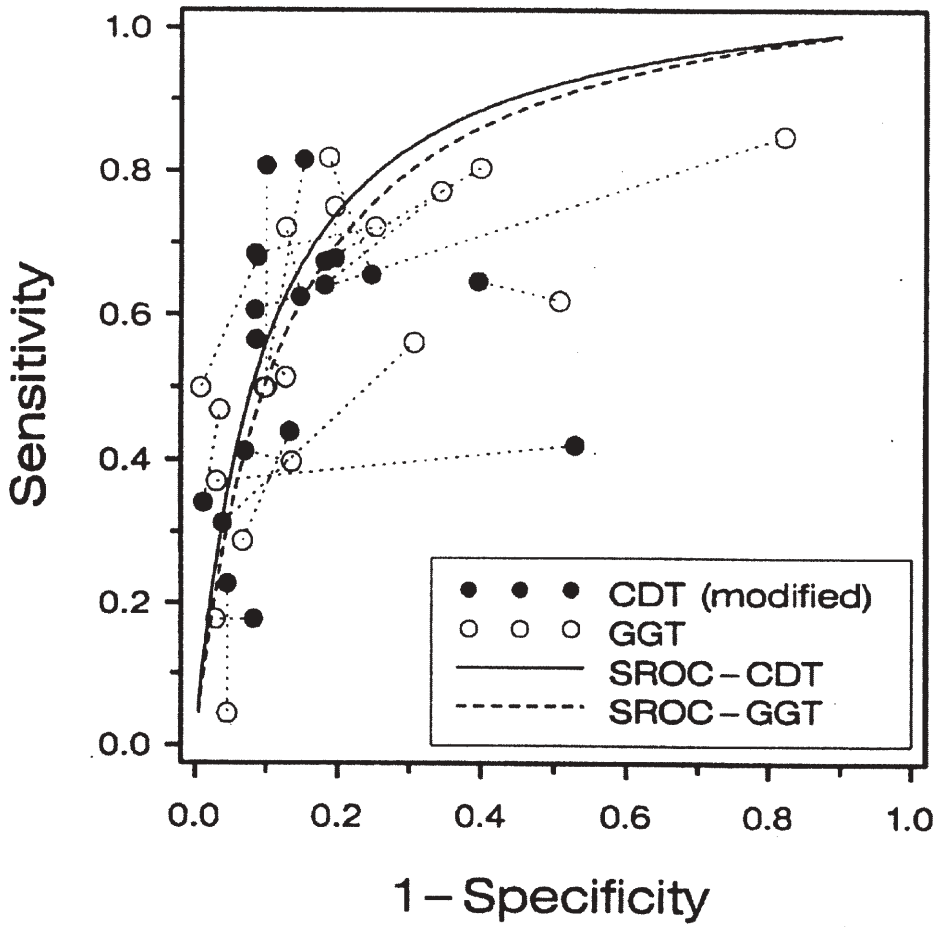

FIGURE 12. ROC meta-analysis comparing GGT with CDT measured by Pharmacia CDTect (modified). Each data point represents results from a single study and the continuous and interrupted lines are summary ROC (SROC) curves. Dotted lines join GGT and CDT results from the same study. (Reproduced with permission from Scouller *et al.*,[204] Copyright 2000 American Association for Clinical Chemistry.)

Salaspuro[203] reviewed papers comparing CDT with other markers, the commonest being GGT. He divided studies into those assessing test performance in alcoholics and heavy drinkers: in primary health care settings, in hospital settings, and in patients with liver diseases. In the first three categories, the performances of GGT and CDT were very similar. However, the poorer specificity of GGT, resulting from its known sensitivity to practically all forms of liver disease, made it a less reliable marker in the last of these groups.

A number of recent papers have explored the usefulness of combining GGT and CDT results. It has long been recognized that GGT and CDT are poorly correlated, and what correlation exists is caused by their common dependence on alcohol intake. Initial suggestions for their combined use were based on a simple rule that a patient

with abnormal values for either test should be regarded as positive. This would, of course, increase the sensitivity, but at the expense of specificity (if the cutoff values were unchanged) and there could be more false-positive results.

The effect of such a strategy can be assessed from data presented by Litten *et al*.[236] They collated data on the sensitivity and specificity of GGT, CDT, and (GGT or CDT) from seven studies. Average results for GGT and CDT are remarkably similar, with sensitivities of 41 and 44% and specificities of 92 and 91%, respectively. Counting an abnormal result in either test as positive increased the average sensitivity to 64% but at the cost of decreasing specificity to 83%. The gain in sensitivity is greater than the loss in specificity, but whether the application of the combined test produces an increase or decrease in the number of individuals classified correctly would depend on the prevalence of dependence or heavy drinking in the tested population. More encouragingly, data from Huseby *et al*.[237] showed an increased sensitivity (from 45% for GGT or 47% for CDT to 60% for the combination) at a constant specificity of 85%.

It may be possible to devise a more effective rule by modifying the cutoff values for positive results for the two tests, or by weighting one test more strongly than the other. Optimizing such a formula requires a substantial number of subjects, and the formula produced should be validated in at least one independent sample. Sillanaukee and colleagues[238] have developed and evaluated the formula γ-CDT = (0.8 * (ln GGT)) + (1.3 * (ln CDT)). Their evaluation covered results for both tests, and the combination, from almost 7000 subjects from an age- and sex-stratified but otherwise randomly selected sample in Finland. The test performance was assessed in a number of ways, and the overall conclusion was that the γ-CDT combination gave higher sensitivity for comparable specificity than either test alone, and that the areas under the curves in ROC analysis were significantly greater. For example, when men drinking more than 420 g/week (more than six drinks per day) were contrasted with abstainers the sensitivity results were CDT 48%, GGT 45%, γ-CDT 57%. However, because this was a population-based study and this category of very heavy drinkers was less than 2% of the total, the positive predictive value of the combined test was only 9% (CDT 7%, GGT 9%). These results confirm that CDT and GGT have independent value and combining them can be useful, but screening populations for hazardous drinking with these tests would not be cost effective.

A practical summary of the role of GGT in screening, investigation, and management of alcohol-related problems might be as follows. To screen for dependence, the first line should be a questionnaire such as the CAGE, MAST, or AUDIT. To screen for hazardous intake, or harmful intake, or for dependence with current drinking, the GGT + CDT combination will detect some subjects missed by questionnaires focused on dependence symptoms. At present the cost of CDT is an issue. To assess risk of morbidity and mortality, GGT is the only marker that has been shown to have predictive value. The lack of specificity makes GGT more suitable for the clinic or population setting (where liver disease will be rarer) than for hospital patients.

E. The Use of GGT in the Treatment of Alcohol Dependence or Hazardous Drinking

The suggestion that GGT might be used in monitoring of abstinence in alcoholics under treatment is longstanding. GGT, or other markers, could have two levels of usefulness: either in monitoring the response of individuals to therapy, or in assessing the effectiveness of the treatment itself. Because the latter would be based on information from multiple patients, the poor correlation between intake and marker results, on an individual patient basis, would not be a major issue. Examples of the use of GGT in each of these roles can be given.

The role of biological markers in monitoring abstinence was discussed by Borg.[239] He emphasized the importance of feedback, with personal information being more effective than general information. He also emphasized the usefulness of individual reference ranges: by this approach he and his colleagues were able to detect three times as many relapses among treated alcohol-dependent patients than would have been found with the much wider, general reference ranges. The related role of GGT results in motivating patients to initially acknowledge the harmful nature of their drinking, and to decide to change it, has been alluded to in a number of publications. The fall in GGT when drinking is reduced may also be helpful in reinforcing the reduction.[201] However, these are anecdotal reports, and at present there is no objective evidence that the discussion of the laboratory results offers advantages over the discussion of drinking and its effects in general.

A number of controlled trials have used differences in GGT values, either over time or between treatment and control groups, as a measure of the effectiveness of early intervention[240-242] or pharmacologically based treatment.[243,244] In this context, GGT or other markers may be less susceptible than reports of alcohol use to reporting bias. With the increasing demand for evidence of effectiveness of treatment, this may be an important role for biological markers of many conditions.

F. Application to Drink-Driving Issues

Many authors have investigated the frequency of abnormal serum GGT in drivers who have been either suspected or convicted of driving with blood alcohol concentrations above the allowable limit. Three overlapping issues have been highlighted: first, whether elevated GGT is associated with higher blood alcohol values; second, whether elevated GGT can be used to define a group of drink-drivers who are dependent on alcohol; and third, whether high-GGT drink-drivers are more likely to reoffend. If high GGT predicts repeat drink-driving offenses, it might be used in decisions about revoking or restoring driving licences.

A number of these papers show statistical associations between GGT results and characteristics of the offenders, although not to a degree that would permit practical stratification into high- and low-risk groups. GGT and blood alcohol

results were significantly correlated in a study of 300 drink-drivers in Germany.[245] The Tayside Safe Driving Project,[114] showed a significant but weak (R = 0.22) association between blood alcohol at the time of arrest and serum GGT in 521 drink-drivers. A subsequent report by the same group[246] showed that among men aged over 30 years arrested for drink-driving, 30% had abnormal GGT results and the proportion was significantly higher in drivers arrested after accidents than in drivers stopped for other reasons. However, blood alcohol levels were similar in accident-involved and accident-free drivers, and the authors suggested that GGT was acting as a marker of chronic deterioration in driving skills resulting from alcohol-induced neurological deficits. This idea gains support from the papers of Irwin *et al*.,[247] and Richardson *et al*.,[248] who showed significant associations between serum GGT and neuropsychological test impairment in alcoholics in treatment.

Results from a study in Norway[249] were similar to those from Scotland (the Tayside study discussed above). Drink-drivers aged 30 or more showed a significant association between high GGT and high blood alcohol results. The authors estimated that approximately 40% of these older drink-drivers had elevated GGT, and calculated from this figure and from data on sensitivity and specificity of GGT that a third of the drink-drivers habitually drank more than 80 g of alcohol per day (compared with less than 2% of the Norwegian population). Obviously, some drink-drivers will be regular heavy drinkers, while others will only consume small amounts and rarely exceed the legal limit for driving, but the GGT results allow an estimate of the relative proportions in these categories.

A subsequent study in Norway[250] used a prospective design to assess whether blood alcohol, GGT, or previous record of drink-driving arrests were able to predict further offenses over a 2-year follow-up period. There was a weak association between abnormal GGT and subsequent rearrest, but more detailed examination of the results shows that they did not reach statistical significance (Odds ratio 1.27, 95% CI 0.71 to 2.26).

Therefore, GGT was not able to predict which drivers were likely to be repeat offenders in the Scottish or Norwegian studies. However, the Malmo studies referred to below, in which middle-aged men underwent multiphasic health screening, including GGT estimation, showed that a conviction for drunken driving was five times more likely in men with GGT results above the 90th centile than in those with results below the median.[251] The analysis was retrospective, in that a search of court records was made covering the period before blood collection and GGT measurement occurred. In a slightly different context, GGT was the best indicator of readmission for trauma (not necessarily from vehicle accidents) in a prospective study from the USA.[232]

Because of its lack of both sensitivity and specificity, GGT is not a suitable basis for decisions on individual offenders. However, the measurement of GGT (or other markers) can produce useful information on the epidemiology of drinking and driving and on human factors contributing to traffic accidents.

G. Fetal Alcohol Syndrome

Because of the adverse effects of maternal alcohol use on fetal development, a reliable test for drinking during pregnancy should be useful. The low sensitivity of GGT in detecting unsafe drinking in women in general, and in young people in general, suggests that GGT is unlikely to fill this need but several groups have published results of their studies.

The first study[252] took the generally desirable approach of enrolling all pregnant women attending an antenatal clinic. Consequently, they had small numbers of excessive drinkers, and only two women had babies affected by alcohol. Using a lower than usual upper limit of the reference range, derived from women drinking less than 30 g of alcohol per day, GGT was abnormal in only 22% of women taking 30 to 125 g/day and 38% of those taking more than 125 g/day. The mothers of the two babies showing fetal alcohol effects had GGT values 4.5 and 5.7 times the upper limit of the reference range.

Three studies from Finland increased the number of adverse outcomes by concentrating on women known to be at high risk. Ylikorkala *et al*.[253] recruited 87 pregnant alcohol abusers and measured GGT on multiple samples throughout pregnancy. They defined three groups of drinkers, moderate (periodic consumption of up to 10 drinks), heavy (one to 10 drinks daily but not diagnosed as alcohol dependent), and alcoholic (two to 20 drinks daily with long histories of alcohol abuse and alcohol-related social problems). The rates of abnormality of GGT were 15%, 32%, and 59%, respectively, in these groups. Forty-two babies showed fetal alcohol effects, and it appears that within each of the three groups of women the GGT tended to be higher in their mothers. Unfortunately, no comparison of GGT by outcomes, controlling for alcohol intake, was reported.

Similarly, Halmesmaki *et al*.[254] recruited high-risk women. Twenty-five women taking at least 150 g of alcohol per week were compared with 20 abstinent pregnant controls; 13 of the 25 gave birth to affected infants. Mean maternal GGT was higher than control in high-risk mothers of unaffected babies, and higher again in the mothers of affected babies; means were 10, 19, and 32 units/l. High GGT (above the 95th centile for controls) was found in 33% of all samples from alcohol-abusing mothers and in 52% of those from mothers with affected infants. In a subsequent report by the same group,[255] 8 out of 44 high-risk mothers gave birth to an affected baby, and the GGT was significantly higher in those women, particularly in samples taken before 26 weeks of gestation. Sensitivity and specificity for detection of affected infants were no more than moderate, at 50 and 82%, respectively, but this was slightly better than the performance in detecting maternal drinking (31 and 79%). Performance of MCV was similar or slightly better than GGT, and CDT or acetaldehyde adducts were worse.

A further study[256] recruited unselected women attending prenatal clinics that seem to have served a disadvantaged and high-risk clientele. Results are difficult to interpret because they used four markers (whole-blood associated acetaldehyde,

CDT, MCV, and GGT), some of which seem to have been abnormal in very high proportions of nondrinking mothers and based most of their presentation on the number of abnormal markers present. Nevertheless, it appears that five of the 13 women with abnormal GGT results gave birth to affected infants, compared with 67 affected out of 407 infants overall. This yields an odds ratio of 3.17 (95% confidence interval 1.01 to 10.00), which is consistent with the other reports.

The use of GGT in screening for alcohol abuse in pregnant women would have poor sensitivity, and, because of the low prevalence of the condition, many false positives. Nevertheless, it is interesting that GGT is higher in women giving birth to babies with fetal alcohol effects or fetal alcohol syndrome. It is not clear whether the higher GGT in high-risk mothers of affected babies simply reflects higher alcohol intake, or a different and more harmful response to alcohol.

H. Factors Influencing the Relationship between Alcohol and GGT

The reference range for GGT differs between men and women, regardless of whether values are adjusted for covariates such as alcohol intake or obesity. In addition, the relationship between alcohol intake and serum GGT values (the dose-response curve) differs between men and women, and also probably varies with age, obesity, smoking, and other individual characteristics. There is unexplained individual variation in the response of GGT to alcohol; some people reporting heavy and regular alcohol use have normal GGT, while in other similar people the GGT is markedly increased, as can be seen, for example, in Figure 8 above.

Comparisons between men and women of the sensitivity of GGT in detecting heavy drinking are not easy; in many papers data from men and women from the same population are not directly compared. Similarly, comparative data on mean GGT values at similar levels of alcohol intake, or alcohol-GGT correlations, are sparse.

Differences between the GGT response to alcohol intake between men and women were reported by Whitfield *et al*.[257] Median serum GGT was higher in male than in female abstainers, and the absolute value increased with increasing alcohol intake to a greater degree in men than in women. Subsequent analysis of the same data[258] found correlations between reported alcohol intake and GGT of 0.36 in men and 0.20 in women, and these are significantly different ($p < 0.001$).

In a study restricted to female alcoholics, Hollstedt and Dahlgren[259] found elevated GGT in 42%. These patients reported a median daily alcohol intake "when drinking hard" of 120 g, and the proportion of male alcoholics with elevated GGT would be expected to be rather higher, but there was no information on the time between the last episode of heavy drinking and the time of blood collection for GGT, so the sensitivity value is difficult to interpret. Another study that only included women,[260] comparing outpatient alcoholics and college student controls, found a sensitivity of 38% at 90% specificity and an area under the GGT ROC curve of 0.68.

Anton and Moak [261] compared the performance of GGT and CDT in male and female alcoholics and controls. The area under the GGT ROC curves was similar for men and women (0.85 and 0.76, respectively) but at a cut-off that gave 100% specificity the male sensitivity was 65%, while the female sensitivity was only 44%. As with most such comparisons, the interpretation of this difference is complicated by substantially greater average daily alcohol intake in the male alcoholics.

A study in a remote Norwegian community[262] reported sensitivity and specificity figures for GGT (and other tests) in both men and women. Several different criteria for undesirable levels of alcohol intake were used, and these differed between the sexes. Taking the closest pair of criteria (male over 30 g/day, female over 22 g/day), and using similar specificities (92% and 89%), sensitivities of 12 and 9% were found for men and women.

Combining the available information, it seems that GGT is a less sensitive test for high alcohol intake in women than in men. In practice, test performance is made even worse by two factors: first, the level of alcohol intake that it is desired to detect is lower in women because of their greater vulnerability to alcoholic liver disease, and second, the lower prevalence of alcohol abuse in women decreases the predictive value of a positive result.

Age is another factor that has been found to influence the sensitivity of GGT in detecting high alcohol intake, and the correlation between intake and GGT. The Sydney study comparing male and female results referred to above[257] also provided a comparison of GGT values between lighter and heavier drinkers by age and found no difference in subjects aged below 30. Other reports led to the same conclusion. Nystrom *et al.*[263] found that in university students (mean age 22 years) the correlation between alcohol intake and GGT was 0.16 for men and 0.14 for women, and the sensitivity of GGT for detection of drinking over 40 g/day was 12% in men and 6% in women. Among young men in treatment for alcoholism, Bisson and Milford-Ward[264] found values above 48 iu/l in only 12%.

Details of alcohol intake/GGT correlation coefficients by age were provided by Nakajima *et al.*[265] As set out in Table 2, correlations were low in the youngest and oldest groups of men and highest in the fourth decade of life.

A similar age effect, but with lower correlations, was found for AST and ALT.

A subsequent paper[234] also confirmed an effect of age on the relationship between heavy alcohol use (> 280 g/week for men and > 190 g/week for women) and GGT, AST, and ALT. Sensitivities for GGT in the third, fourth, fifth, and sixth decades of life were 13%, 37%, 37%, and 40% for men, and 3%, 29%, 52%, and 43% for women. Similarly low sensitivities in subjects aged 20 to 30 years were shown for both AST and ALT.

The reason for lower sensitivity of GGT in detecting heavy drinking, or lower correlations, in younger people is not known. It is very difficult to resolve effects due to age per se, and effects related to the number of years a person has been drinking heavily.

Table 2
Effect of Age on the Correlation between Alcohol Intake and Serum GGT.

Age	Less than 30	30 to 39	40 to 49	50 to 59	60 and over
Correlation	0.18	0.44	0.35	0.32	0.13

Data from Nakajima et al.[265]

Comparisons between the response of GGT to chronic excessive alcohol use in groups of patients of differing ethnic origin, but all living in London, were performed by Clarke *et al*.[266] and by Wickramasinghe *et al*.[267] In the first report, patients from south Asia (India, Pakistan, Sri Lanka, Bangladesh) had been drinking for fewer years before admission to the Dependency Unit than patients of European descent and had GGT values that were four times higher (471 vs. 117 iu/l, $p < 0.01$). The second, and similar, study extended the range of alcohol intake markers used and confirmed both the shorter duration of drinking before coming to treatment and the significantly higher GGT in the South Asians. In the second study AST and ALT were measured, and also showed higher values in the Asians. These studies were oriented toward vulnerability to liver disease, and the higher enzyme results in Asians were interpreted as markers of more severe liver damage, which is subject to caveats about the time-course of GGT results in chronic alcoholic liver disease. However, the time-to-admission data do demonstrate a differential vulnerability between racial or ethnic groups. These differences between people of Indian and European background might be due to genetic differences, or cultural and dietary factors, or some combination of these.

The possibility that genetic variation contributes to the variation in biochemical responses to alcohol use has been substantiated in recent work from Japan. In this work, genetic variation at a known locus was shown to be associated with differing alcohol-GGT relationships. Takeshita *et al*.[268] studied 385 male workers from a metal plant in Japan and obtained information on the quantity and frequency of alcohol consumption. Serum GGT was measured and aldehyde dehydrogenase 2 (ALDH2) genotype was ascertained using DNA from blood samples. Subjects were divided into three groups of alcohol intake and three groups by ALDH2 genotype, and mean GGT values were compared. The results are illustrated in Figure 13; there were significant differences in GGT by genotype in the second and third alcohol intake groups (6.8 to 31.5 ml per day, and > 31.5 ml per day) but not in the lowest-drinking group (0 to 6.8 ml per day).

These results are striking but somewhat counter to expectations; there is some evidence to suggest that when ALDH2-deficient subjects (i.e. those with the ALDH2*12 or *22 genotypes) do drink excessively they are more (not less) likely to get alcohol-related liver disease. Here, however, there was evidence of less liver abnormality in the ALDH2*22 or *12 subjects than in the *11 homozygotes with full enzyme activity.

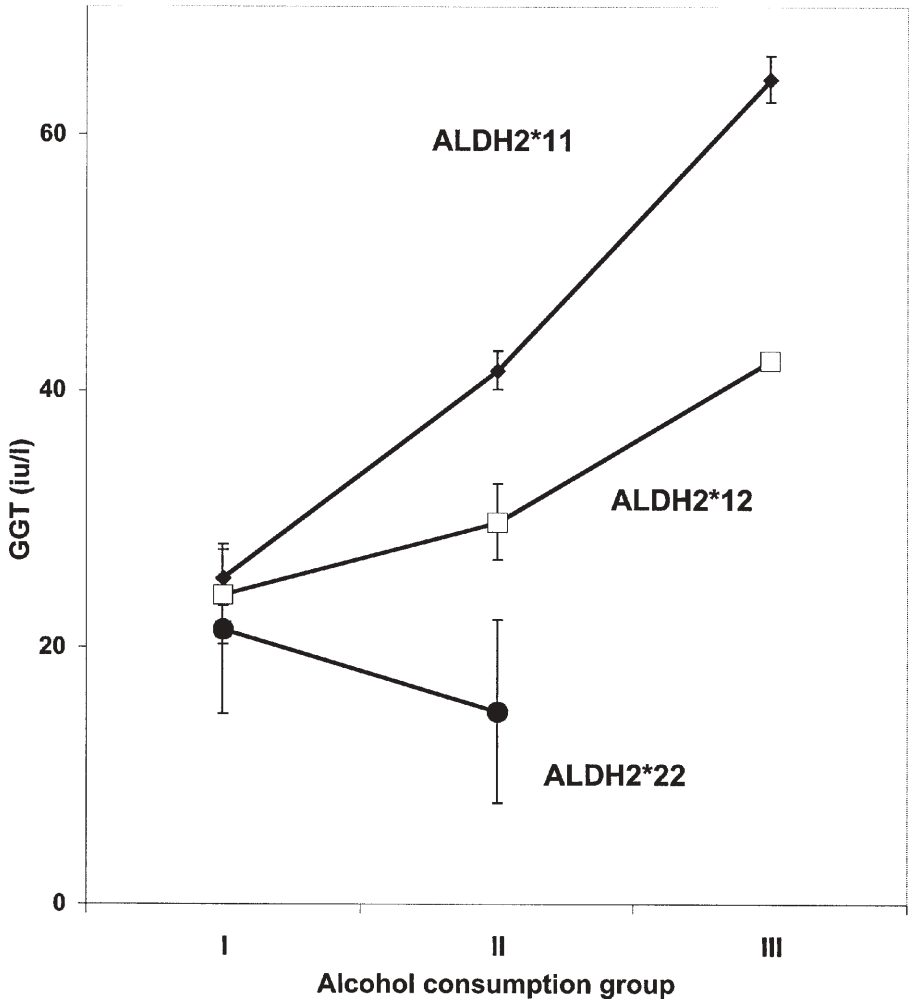

FIGURE 13. GGT results from a study of 385 male Japanese workers by alcohol intake and by *ALDH2* genotype. Alcohol consumption was divided into three groups: I. 0 to 6.8 ml/day, II. 6.8 to 31.5 ml/day, III. more than 31.5 ml/day. Bars show standard errors. (Data from Takeshita *et al.*[268])

As a complement to this evidence of a specific gene effect on GGT's response to alcohol, there is evidence that dietary differences can also be significant. Nakajima *et al.*[265] studied the carbohydrate content of the diets of over 2000 healthy men in Japan, as well as their drinking habits and serum GGT activities. As expected, GGT activity was positively related to alcohol intake: it was negatively related to carbohydrate and fruit consumption. When subjects were divided into three groups according to their sugar intake (low, < 16 g/day, medium, 16 to 32 g/day, and high, > 32 g/day), the GGT activity increased more with increasing alcohol use in the low-sugar group. The regression equations and confidence intervals for the estimated slopes and intercepts are shown in Table 3.

TABLE 3
Regression Coefficients for the Relationship between Alcohol Intake (independent Variable) and Serum GGT (Dependent Variable) Showing the Variation in Slope with Variation in Dietary Sugar Intake

	Slope	95% CI of slope	Intercept	95% CI of intercept
Low sugar	0.97	0.76 to 1.18	28.5	22.4 to 34.7
Medium sugar	0.86	0.70 to 1.01	27.5	23.2 to 31.9
High sugar	0.59	0.45 to 0.73	26.5	22.8 to 30.1

Data from Nakajima et al [265]

Therefore, we can conclude that high sugar intake protects against, or low sugar intake promotes, increased GGT in heavier drinkers but not in abstainers or very light drinkers. There are some similar data from animal experiments, as discussed below in the section on hepatic GGT activity. The explanation is unclear, but in discussing the results of their human studies, Nakajima *et al*.[265] considered that low sugar intake promoted the depletion of hepatic glutathione and consequently led to a greater increase in GGT.

I. Characteristics of Drinkers with High GGT

The fact that some heavy drinkers have elevated serum GGT, while others do not, leads to the question of whether this difference is caused by differences in susceptibility to liver damage from drinking. This can be judged in two ways: by comparison of GGT results and liver biopsy appearances in heavy drinkers or by prospective studies in hazardous drinkers with alcoholic liver disease as the endpoint. A small number of papers have compared GGT between groups of alcoholics classified by liver histology,[269-271] and those with abnormal serum GGT were found to be more likely to show fatty liver, fibrosis, or cirrhosis. Information from follow-up studies is presented in a later section.

Several reports on features of patients, or subjects' characteristics, at the time GGT was first measured are also relevant. The debate, at least initially, was whether a high GGT indicated a potentially harmful degree of liver damage or a presumably innocuous induction of GGT activity by alcohol analagous to the induction found in patients on anticonvulsant drugs. The strong correlation between serum GGT and AST results in heavy drinkers[272] suggests that hepatocyte necrosis is occurring in those with a high GGT. On the other hand, an equally strong correlation between serum GGT and urinary D-glucaric acid excretion in alcoholics has been reported,[273] suggesting an association between the processes of hepatic microsomal enzyme induction and either induction of GGT or its release from the liver.

An intriguing association between serum GGT and fatty liver was discovered by Allaway *et al*.[274] Hepatic density, measured by computer tomography and taken

as a measure of liver fat content, was highly significantly associated with serum GGT in occasional and heavy drinkers attending a health screening facility. In view of the epidemiological associations of GGT (discussed later), it is important that GGT may be a marker of fatty liver in comparatively healthy subjects.

J. Tissue Studies on GGT Response to Alcohol

Most studies on hepatic or other tissue GGT responses to alcohol have been done with rats, but studies not requiring experimental manipulation have been done on biopsy or autopsy material from human alcoholics. On the whole, similar results have been found for the effects of alcohol on GGT activity in the two species and this gives encouragement for extrapolation of other rat results to humans. As discussed above, hepatic GGT activity is on average greater in human alcohol-related disease than in nonalcoholic liver disease or in control tissue, but it is uncertain whether this is due to greater expression or to differences in the dynamics of GGT synthesis, membrane binding and release, or degradation.

Ivanov *et al*.[275] found that hepatic GGT activity in alcoholics was about double that found in control subjects. Unlike serum GGT, hepatic GGT was not related to the severity of liver damage, and there was no correlation between serum and hepatic activities. Teschke *et al*.[162] concentrated on alcoholic fatty liver. Again, there was a significant increase in hepatic GGT, with a doubling of the mean value, but the mean serum GGT increased some 15-fold in these patients.

The paper by Yamauchi *et al*.[276] reported rather different findings. They found that hepatic GGT activity was equally elevated in both alcoholic and nonalcoholic liver disease, although the serum GGT was substantially higher in the alcoholics. This may reflect the types or severity of liver disease in the nonalcoholic patients. Within the alcoholic group, they were able to show a significant correlation ($r = 0.66$, $p < 0.01$) between hepatic and serum GGT activity. Ishii *et al*.[277] also found that liver and serum GGTs were significantly correlated, but this appears to be because they combined data from both alcoholic and nonalcoholic liver disease patients. Those patients with alcoholic liver disease had higher values for both serum and liver GGT.

These human results show that alcohol tends to increase hepatic GGT, and that this is not just an effect common to all kinds of liver disease. Alcohol also tends to increase GGT in the intestine,[278-280] so the effect of alcohol is not confined to one organ.

Despite the species differences in GGT, a number of studies that require dietary manipulation, time-series data, or extensive analysis of liver tissue have to be done in experimental animals. Several groups have produced increases in hepatic GGT in rats by administering alcohol, and the results are similar to those from humans.[281-283] Rambabu *et al*.[284] obtained results consistent with both an increase in hepatic GGT synthesis and a decrease in hepatic GGT degradation in

ethanol-fed rats compared with controls. GGT could also be induced by alcohol in rat hepatoma cells,[285] and ethanol may modify the carbohydrate component of GGT in such cells.[286]

In relation to dietary effects, several groups have studied the influence of carbohydrate content of the diet on hepatic GGT and the response of hepatic GGT to alcohol. Teschke and Petrides[287] found that feeding alcohol to rats as part of a liquid diet resulted in a doubling of hepatic GGT activity compared with controls on an equicaloric alcohol-free diet. Increasing or decreasing the carbohydrate content and the calorific value of the diet in the absence of alcohol did not produce significant changes in hepatic GGT, but a combination of alcohol and high carbohydrate diet produced no difference from controls. The authors concluded that extra carbohydrate was able to prevent the alcohol-induced rise in GGT activity, but no mechanism was suggested. Conversely, Misslbeck *et al*.[288] found that alcohol did not increase hepatic GGT in rats fed a low-fat diet, but did in rats on a high-fat diet. High fat but no alcohol did produce an increase in hepatic GGT above the low-fat, no-alcohol group. Both alcohol and fat produced decreases in hepatic glutathione. Separating out the effects of carbohydrate and fat is difficult because in isocaloric diets a decrease in one is accompanied by an increase in the other.

A similar study by Yamada *et al*.[289] showed that hepatic GGT increased in rats fed alcohol as part of a liquid diet but variation in the other components of the diet, was also important. Hepatic GGT increased (in the absence of ethanol) as the carbohydrate content of the diet decreased or the fat content increased, although variation in carbohydrate/fat content did not increase hepatic GGT to ethanol-treated levels. This finding of a carbohydrate effect in the absence of alcohol is different from the earlier findings of Teschke and Petrides[287] but compatible with those of Misslbeck and her colleagues. Moreover, one type of standard solid rat food produced hepatic GGT activity considerably greater than the ethanol-liquid diet combination, but it did not increase serum GGT to the degree seen after ethanol. Two conclusions can be drawn from these results: first, that diet affects GGT activity within the liver, and second, that the increase in serum GGT produced by ethanol is the result of two events, an increase in hepatic enzyme and a release of this enzyme into the circulation. The solid diet produced the first of these but not the second.

Another set of animal experiments involved perfusion of the liver with a chromogenic substrate for GGT (gamma glutamyl *p*-nitroanilide) and measurement of the reaction product in the perfusate leaving the liver.[290,291] This experimental design allows the measurement of the GGT activity on the cell surface exposed to the circulation, and only this fraction of the GGT is active in hydrolyzing circulating glutathione. Chronic ethanol feeding led to increases in the GGT ectoactivity (measured as reaction product in the effluent perfusate) and also in GGT in homogenised liver tissue. In the ethanol-fed rats, the ectoactivity was approximately half the total hepatic GGT activity in the homogenate and the two

measurements correlated very well. In addition, the hydrolysis of glutathione added to the perfusate was increased by chronic alcohol administration and this too correlated well with the GGT ectoactivity.

The authors commented "Although the present studies do not unveil the metabolic consequences for the hepatocyte of an increased capacity to remove circulating glutathione, this could prove to be of importance as a contributing mechanism in the maintenance of hepatic glutathione", and they compared this to the role of renal GGT in recovering glutathione from the glomerular perfusate. This study is also important as a complement to histochemical studies of GGT, which tend to emphasize increases in GGT in the biliary system.

K. Alcohol, Glutathione, and GGT

This concept of an adaptive role for GGT in recycling the constituent amino acids of glutathione so they can be reused by the liver is consistent with the literature on the effects of alcohol on hepatic glutathione. Chronic administration of alcohol to rats, for 8 weeks, was found to double the rate of glutathione turnover in the liver but increased the glutathione concentration.[292] The activity of GGT in the liver was increased fourfold by the alcohol treatment, and the capacity to synthesize glutathione from its constituent amino acids, cysteine, glutamate, and glycine, in the presence of ATP was also increased. There was a positive relationship between glutathione turnover rate and GGT activity in the alcohol-treated rats, while the control rats had substantially lower rate and activity. A similar study[293] in which perfused livers from alcohol-fed and control rats were used showed an increased loss of glutathione in the alcohol-treated group that was due to loss from the sinusoidal surface of the hepatocytes rather than in the bile. Biliary loss of glutathione was decreased slightly by the alcohol pretreatment. Despite the increased loss of glutathione from the alcohol-treated rats' livers during perfusion, the reduced glutathione content at the end of the experiment was the same in each group. In this study, hepatic GGT was increased by 60% in the alcohol-treated group, but the increase was not significant. The two studies together suggest that the increased turnover of glutathione is a consequence of increased loss into the circulation, accompanied by increased synthesis that compensates or in some circumstances overcompensates for the loss. A later paper[294] showed that the glutathione efflux was not due to changes in extrahepatic glutathione uptake.

However, a group of papers from another group reported a decrease in hepatic glutathione after alcohol, and no increase in hepatic GGT. Fernandez-Cheka *et al*.[295] showed a 24% decrease in cytosolic glutathione and a substantially greater decrease (65%) in mitochondrial glutathione in rats fed alcohol for 6 weeks compared with pair-fed controls. Sinusoidal efflux of glutathione from perfused livers of alcohol-fed rats was increased by a third above controls. It is not clear why hepatic glutathione was decreased in this study but increased in others, but it may

be because a different strain of rats was used and they did not increase hepatic GGT activity in response to alcohol. However, the increased sinusoidal efflux of glutathione in the perfused livers from the alcohol-fed animals was consistent across the various reports.

Subsequent papers[296-298] reported that the greater glutathione efflux in the alcohol-fed animals was due to a change in K_m of the transport system rather than in V_{max}. Investigation of the relationship between cytosolic and mitochondrial glutathione showed that transport of glutathione from cytoplasm to mitochondria was impaired in alcohol-fed rats, particularly in perivenous (rather than periportal) hepatocytes. This was associated with impairment of mitochondrial function in these cells.

More recently, Battiston *et al*.[299] studied the acute effects of oral and intraperitoneal alcohol in fed and fasted rats. Prealcohol hepatic glutathione concentrations were substantially higher in the fed animals, and the administration of alcohol by either route to the fed animals decreased glutathione to fasting levels. In the fasting animals, alcohol decreased glutathione only slightly. Over the following 24 h glutathione increased to or toward prealcohol concentrations: quickly and completely after oral alcohol and more slowly after intraperitoneal alcohol in the fed rats, and to the lower initial values in the fasted group. The differences produced by the two routes of administration in the fed animals were ascribed to the differences in blood alcohol concentration arising from gastric metabolism of the oral alcohol.

Taken together, these papers show that alcohol affects hepatic glutathione turnover and, in some circumstances, concentration. The differences in hepatic GGT response to alcohol may be due to the different strains of rat used by the three groups; if so, they imply that genetic differences (between the strains) affect the process by which GGT can increase in response to glutathione efflux and the ability to maintain or increase hepatic glutathione concentration after chronic alcohol exposure.

VII. EPIDEMIOLOGICAL ASSOCIATIONS

A. GGT in Prospective Studies of Mortality and Morbidity

Prospective studies with GGT as a predictor variable commenced with a focus on alcohol-related disease as the outcome, but it became apparent that cardiovascular disease should also be considered. In some cases cardiovascular risk factors such as lipids and blood pressure were measured, while in others the focus was on GGT in comparison with other biological markers of alcohol use. The prospective studies in which GGT was shown to predict outcome (death, health or social indicators, or the onset of a specific disease) will be considered first, followed by studies on the associations between GGT and other risk factors and the issue of whether GGT is an independent predictor.

The first and most influential study was a multifaceted project to implement and evaluate health screening that commenced in Malmo, Sweden, in 1974. Men aged 46 to 48 years at screening were invited to participate. These subjects were followed for up to 8 years, and the resulting information has appeared in multiple papers. Taken chronologically, the first finding was that subjects with higher GGT values at screening had greater numbers of days on sickness benefits over the preceding 21 years.[300] The median number of days per person increased across five categories of increasing GGT results, from 84 days in subjects with GGT up to 0.29 μkat/l (17.4 U/l) to 191 days in subjects with GGT over 1.40 μkat/l (84 U/l). Of the subjects in the highest category, 72% admitted high alcohol consumption.

In the same year, the first of several reports on mortality in these subjects was published.[301] Although the numbers of deaths were small, a clear trend of increasing death rates with increasing GGT was found, particularly in the top two quintiles. The authors attributed 61% of the deaths to alcohol-related causes.

More detailed analysis of the relationships between GGT values and indicators of social or medical problems was published by Kristenson *et al*.[251] Convictions for drunkenness or drunken driving were three times as prevalent in subjects with GGT in the top decile of results as in those with GGT below the median. The results for days of sickness benefit (see above) were confirmed, and it was noted that the number of sick days per year had increased in the high-GGT group over the 10 years before screening. Injuries, and mental disorders, were commoner reasons for sickness benefits among those with high GGT. Information about marital status also suggested a higher rate of social and personal problems among men with high GGT, with more of them being divorced, widowed, or never married. An intervention program for men with high GGT, aimed at reducing their alcohol consumption, showed considerable success in reducing sickness, time spent in hospital, and mortality in the treated group.[302]

Later papers from the Malmo group, with more subjects and longer periods of follow-up, confirmed that GGT was a strong predictor of death.[303] However, it predicted not only alcohol-related illness but also myocardial infarction. This was an unexpected and thought-provoking finding, because alcohol consumption (at least up to 80 g/day) is usually associated with decreased cardiovascular risk. Taking subjects with GGT below the median as the reference group and calculating the relative risks of death for other GGT groups, the results on 12,550 men were as shown in Table 4.

A breakdown of the rates of myocardial infarction by GGT and by serum cholesterol showed that infarction risk trended upward with increasing GGT independently of cholesterol value (Table 5).

The cardiovascular effect showed a 70% increase in risk when subjects in the top decile of GGT were compared with those below the median, whereas the risk of alcohol-related hospital admission increased around 800% between these two groups. Some of the associations between GGT and other risk factors that might account for this cardiovascular finding are discussed later.

Table 4
Relative Risk of Death from All Causes Except Cancer by Centiles of GGT at Screening

Centile of GGT	Less than 50	50 to 80	80 to 90	90 to 95	95 to 97.5	97.5 to 99	Above 99
Relative Risk	1	2	4	5	7	10	21

Data from Hood et al.[303]

Table 5
Prospective Risk of Myocardial Infarction By Serum GGT Result at Screening and By Quintiles of Serum Cholesterol

Rates of Clinical Myocardial Infarction by

		Cholesterol quintiles					
		First	Second	Third	Fourth	Fifth	All
GGT:	Below median	0.8	1.5	1.8	1.8	3.5	1.88
	50-80 centile	0.6	1.7	3.6	2.8	4.8	2.70
	81-89 centile	1.7	2.2	3.9	4.6	3.7	3.22
	90-100 centile	1.8	2.5	1.7	5.0	5.3	3.26

Data from Hood et al.[303]

These Swedish studies were initially based on the premise that GGT was acting as an indicator of excessive alcohol consumption, and that the damage was a consequence of the alcohol use. The results of the intervention study, and the patterns of morbidity, supported this assumption. However, there was no data available on the quantity and frequency of alcohol consumption at screening among these men, and so it was not possible to determine whether a raised GGT was simply a marker for alcohol consumption, or whether it indicated damage from alcohol (or perhaps from other causes) and might thereby be a better predictor of problems than consumption measures alone. Some studies in which both serum GGT activity and quantitative data on alcohol use were available are considered below.

One study that was able to compare the predictive value of GGT and self-report of alcohol consumption was by Conigrave *et al*.[304] This had a smaller number of subjects and a shorter period of follow-up, but still showed significant results. 330 subjects (212 men and 118 women) were entered into the study and 250 (76%) were evaluated after a mean period of 32 months. GGT was predictive of death, liver disease, or trauma during the follow-up period among men but not women. In line with the approach taken in the Swedish studies, subjects with GGT values above the 80th centile were compared with those with GGT results below the

median: among men the relative risk of death was 7.3 of liver disease or gastrointestinal bleeding 4.9, and of trauma (broken bones, head injuries, or road traffic accidents) 1.8. Alcohol-related illness was significantly associated with initial GGT results: for each unit increase in $\log_{10}$GGT there was a 13.4-fold increase in risk and controlling for age, sex, smoking, BMI, and alcohol intake left a relative risk of 9.3 (95% CI 2.4 to 35.1). Health-care utilization (hospital admission, outpatient attendance, or general practitioner consultation) was significantly predicted by GGT results at entry, even after controlling for age, sex, smoking, BMI, and alcohol intake.

The main point to emerge from this study was that GGT is an independent predictor and not merely a marker of alcohol intake. Subjects with abnormal GGT either have early tissue damage or an inherent biological susceptibility to the harmful effects of alcohol (or both). In any case, it was clear that subjects with high GGT did worse on a number of measures than subjects drinking a equivalent amount of alcohol but with a normal GGT. This was one of the few papers to include women; either because women had fewer alcohol-related problems than men, or because the relationships between GGT and outcome differ between the sexes, GGT had little or no predictive value for women. This is a point that needs further investigation.

Two further papers relevant to this topic were from Brenner *et al*.[305] and Arndt *et al*.[306] These examined slightly different aspects of a study of 8043 male construction workers in Germany, who were followed for approximately 7 years. The first paper concentrated on causes of variation in GGT and on mortality as an outcome in the prospective study, while the second brought in data on AST and ALT and also on early retirement as a measure of ill health. The data from these two papers on causes of variation in GGT are considered in a later section. Consistent with previous results, death rates increased with increasing GGT values at entry. The magnitude of the estimated risk depended on whether results were adjusted for other risk factors present in the subjects. This was done in two stages: first by including age, nationality, occupational group, smoking, body mass index (BMI), diabetes, hypertension, and preexisting ischaemic heart disease, and second, by retaining these variables and also including alcohol consumption. The results are illustrated in Figure 14; both crude and adjusted death rates rose across categories of GGT result, with an increase in risk of approximately 240% (crude) or 124% (adjusted) from the lowest to highest category.

Several conclusions can be drawn from these results; part of the association is due to mortality from diabetes, hypertension, and heart disease and part to alcohol use because adjustment for these factors reduced the strength of the GGT/mortality relationship. Nevertheless, GGT provides information about risk that is not fully accounted for by its known associations with these risk factors, and again it is reasonable to conclude that GGT is acting as an index of harm caused by these risk factors or by some unmeasured variable.

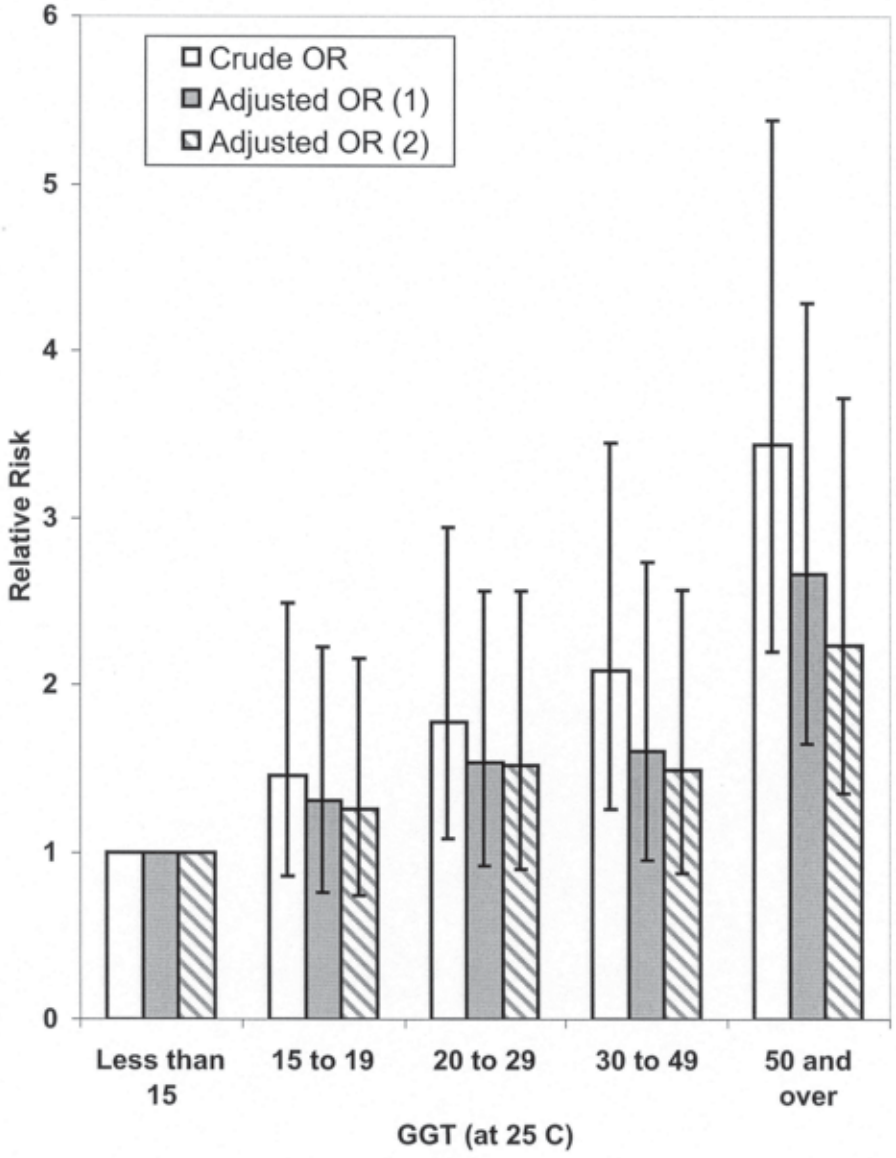

FIGURE 14. Relative risk (compared with the group with GGT less than 15 u/l) of death from all causes by serum GGT at entry. Crude OR: odds ratio without adjustment. Adjusted OR (1): adjusted for age, nationality, occupational group, smoking, body mass index, and pre-existing diabetes, hypertension, and ischaemic heart disease. Adjusted OR (2): also adjusted for alcohol consumption. Bars show 95% confidence intervals for the odds ratios. (Data from Brenner *et al.*[305])

The second of these papers[306] showed that rates of early retirement and disability also increased with increasing GGT, even after adjustment for the risks mentioned above. Data on AST and ALT values were also included, showing that they too were predictive of early retirement and all-cause mortality and suggesting that the effects associated with high GGT are mediated through pathways shared by these other markers of liver dysfunction.

Finally, a prospective study on a selected group of patients was reported by Karlson *et al.*[307] 714 subjects admitted to hospital in Goteborg, Sweden, with suspected myocardial infarction were evaluated 4 weeks after admission with a panel of tests, including GGT, and followed over the next 10 years. Their median age at entry to the study was 63 years and 67% were male and 33% female. In multivariate analysis with death as the endpoint, history of previous myocardial infarction, smoking, GGT, age, and glucose (in decreasing order of risk ratios) were identified as independent predictors. GGT was significantly predictive of death from all causes, cardiac death and noncardiac death. The 10-year mortality by quartile of initial GGT was 21% (lowest quartile, GGT < 19.2 IU/L), 27%, 35%, and 34% (highest quartile, GGT > 48.6 IU/L). Glucose and triglyceride (but not cholesterol) were highly significant predictors in univariate analyses, but the significance of glucose was much diminished in the multivariate analysis and triglyceride was no longer a significant predictor. Although this was a selected group of patients with proven or clinically suspected ischemic heart disease, the results support the conclusions from the population-based studies discussed above.

B. GGT in Prospective Studies of Individual Diseases

The papers discussed in the preceding section were focused on death or disability from all causes, with some expectation that excessive alcohol use and alcohol-related disease would be the cause of most adverse events associated with high serum GGT. Other papers have widened this perspective and examined the relationships between GGT and the development of heart disease, hypertension, stroke, and non-insulin-dependent diabetes mellitus (NIDDM).

Studies by Shaper and colleagues in the United Kingdom have followed a cohort of 7600 men recruited through their general practitioners for 11 years or more. The first of their papers relevant to this review[308] examined the relationship between initial GGT values and death from all causes — as other authors had done — but added data on specific causes of death. As with other studies, increased GGT (particularly in the top quintile of results) was significantly associated with increased all-cause mortality. This is shown in Table 6.

This could be broken down into categories of ischaemic heart disease and noncancer, noncardiovascular mortality, which were associated with GGT levels, and cancer mortality, which was not. This pattern is consistent with the Malmo results. An adjustment for expected risk factors reduced but did not eliminate the effect.

Table 6
Overall and Cause-Specific Mortality by Quintiles of Serum GGT at Entry

Quintile of initial GGT	First	Second	Third	Fourth	Fifth
Age-adjusted mortality per 1000 person-years					
All causes	8.0	9.2	9.5	9.8	13.4
Ischaemic heart disease	3.3	3.2	3.8	4.0	6.2
Other cardiovascular	0.9	0.9	1.1	1.0	1.2
Cancer	2.4	3.7	3.2	3.7	3.5
Other	1.4	1.4	1.4	1.0	2.3

Data from Wannamethee et al.[308]

The consideration of preexisting heart disease led to the important conclusion that in men without heart disease the increased mortality was due to noncardiovascular causes. On the other hand, in men with preexisting heart disease mortality from this cause was associated with higher GGT levels, particularly in men with more severe disease. In order to exclude the possibility that GGT was acting as a marker of subclinical disease or congestive cardiac failure, the analysis was repeated, excluding men who died in the first 5 years of the follow-up. This did not reduce the association of GGT with cardiovascular mortality in men with preexisting heart disease, but it did attenuate the association with noncardiovascular deaths in the heart disease-free group.

In considering the reasons for the associations between GGT and mortality from various causes, the authors considered and rejected the associations between GGT and other risk factors or preexisting disease. They drew attention to the role of GGT in glutathione metabolism and suggested that it is either a marker of abnormal glutathione metabolism (which might increase risks from oxidative stress), or a response to oxidative stress in which GGT is induced as a protective mechanism to maintain glutathione levels that would otherwise be depleted.

The same cohort of subjects, followed for up to 13 years, was a source of information about GGT as a predictor of the development of NIDDM.[309] The authors suggested that GGT is a marker for abdominal obesity and fatty liver that leads to insulin resistance. This hypothesis was tested by relating initial GGT values to the risk of development of clinically diagnosed NIDDM during the period of follow-up. The initial GGT values were highly significantly greater in men who subsequently developed NIDDM, and conversely the risk increased progressively with increasing GGT (Table 7).

TABLE 7
Rates of Development of Noninsulin-Dependent Diabetes (NIDDM) by Quintile of Serum GGT at Entry

Quintile of initial GGT	First	Second	Third	Fourth	Fifth
New NIDDM, cases per 1000 person-years	0.6	1.3	1.7	3.2	4.3

Data from Perry et al.[309]

The risk gradient was decreased by adjustment for other risk factors, including age, BMI, physical activity, smoking, alcohol consumption, and blood glucose, but the risk for subjects in the highest GGT quintile was still 3.5 times that for the lowest quintile and the trend was still highly significant. Other liver function tests (AST, alkaline phosphatase, and albumin) showed weak associations with NIDDM risk.

The authors' initial hypothesis was supported by these results, and they concluded that GGT "should be added to the cluster of vascular risk factors which form the insulin resistance syndrome". The associations between GGT and BMI, waist-to-hip ratio, hepatic steatosis, and serum insulin, reported in other studies, were cited in support of this concept.

Another cardiovascular risk factor, possibly also related to insulin resistance, was the subject of a prospective study in Japanese men. Miura *et al*.[310] showed that the development of hypertension in subjects with initially normal blood pressure was related to GGT levels. The number of subjects was small (77 men), but significant results were obtained. Although initial blood pressures, and alcohol intake, were strong predictors of the development of hypertension the GGT effect persisted after adjustment for initial blood pressure and for alcohol intake. Subjects were divided into three categories on GGT result, 0 to 9, 10 to 19, and 20 or more, and the adjusted risk was 5.82 (95% CI 1.83 to 18.6) in the highest group compared with the lowest. Although alcohol is a known risk factor for hypertension, this study provided further evidence that even after taking alcohol consumption into account, GGT is a marker for aspects of cardiovascular risk.

It was pointed out above that there is an element of paradox in GGT being predictive of cardiovascular risk, even though alcohol is on average cardioprotective. If, however, GGT is a marker of the propensity to become hypertensive, or a marker of insulin resistance, then subjects who have these characteristics will tend to have a high GGT and also a high cardiovascular risk. GGT may be able to offer some discrimination between subjects who decrease their cardiovascular risk, and those who increase it, through alcohol use.

Recently, a study from Finland on GGT and prospective risk of stroke was published.[311] This study included both men and women, and a significant effect of GGT on risk of all strokes, and of ischaemic strokes, was found in both men and women. Results on hemorrhagic strokes (which were less numerous) were equivo-

cal. The GGT effect remained significant after adjustment for other risk factors, including age, smoking, cholesterol, blood pressure, and BMI. Alcohol intake, which was presumably a significant contributor to variation in GGT, did not show any clear effect on stroke risk. It follows from the absence of an alcohol effect, and the existence of increased risk in subjects with a high GGT, that risk should be reduced in drinkers with normal GGT; however, the authors do not comment on this, and the published data do not allow this hypothesis to be examined in any detail.

C. Associations with Identified Risk Factors

Chronologically, the recognition of associations between GGT and other risk factors for cardiovascular disease overlapped with the prospective studies that established GGT as an apparently independent risk factor. However, the significance of these prospective studies justifies their priority in the review of evidence. This and the following sections retrace the recognition of associations between GGT and known risk factors for cardiovascular disease or NIDDM (obesity, blood pressure, and biochemical characteristics) and discuss how far these associations can be explained as aspects of a common underlying cause.

Most large studies that have examined the relationship between serum GGT and obesity have used body mass index (BMI) as the measure of obesity. As there is reason to believe that insulin resistance is the intermediate characteristic connecting BMI and GGT, it would be preferable to measure insulin resistance or associated measures such as the waist-to-hip circumference ratio directly, but such studies are few and smaller. Another complication is that many of the risk factors potentially associated with high GGT are themselves correlated, or clustered, so that subjects are likely to have several risk factors. The strength of the association of each with GGT can only be assessed by multiple regression techniques, and even then the direction of cause and effect may be uncertain. The relative importance of each risk factor, and of alcohol in particular, will depend on the characteristics of the sample studied and the prevalence of very high alcohol intake or of extremes of obesity. For this reason, the few studies on nondrinkers are particularly useful.

D. Associations with Cardiovascular Risk Factors: Obesity

Two papers have described the determinants of GGT in two waves of subjects from Tromsø, Norway.[312,313] The second included almost 22,000 subjects, men and women aged from 12 to 62 years. Multiple regression analysis showed that in both men and women, BMI had the strongest association with GGT, greater in this population than the effects of alcohol use, blood pressure, physical activity, or serum lipids. The authors commented that alcohol consumption was lower in

Norway than in other countries. They also reported that the association between BMI and GGT was the same, or stronger, in teetotalers as in drinkers. It appears from the regression coefficients presented in the first paper, and a figure in the second, that BMI had a greater effect on GGT in men than in women; this might reflect different distributions of body fat in men and in women.

A study of comparable size, but confined to men attending a screening service in London, was reported by Robinson and Whitehead.[314] Again, BMI was the most important influence on GGT, with a greater partial correlation than for alcohol intake, exercise, or smoking. Obesity had a significant effect on GGT in physically active subjects only, teetotalers or social drinkers only, and nonsmokers only, as well as in the whole group of subjects. As with the Norwegian men, GGT increased progressively across all classes of BMI from <20 kg/m^2 (mean GGT 20.7 IU/L) to >35 kg/m^2 (mean GGT 54.3 IU/L). BMI also affected ALT and (to a lesser but still significant extent) AST, and the authors considered that the effects of obesity on all three enzymes were due to fatty liver.

Similar results, confirming significant associations between BMI and GGT, have been reported from Italy,[315] Japan,[316,317] UK,[308] Sardinia,[318] USA,[319] and Finland.[311] The number of studies, and the high level of statistical significance achieved in most of them, makes it clear that body composition has a substantial effect on GGT or that they are both affected by common factors.

Some results from one of the Japanese studies,[316] which showed independent effects of obesity and alcohol, are illustrated in Figure 15. In this study obese subjects, even among the nondrinkers, also had higher AST and ALT values and a high prevalence of CT-demonstrated fatty liver.

A smaller but important study from the Netherlands[320] suggests that fat distribution is relevant in determining GGT values in men. GGT was more strongly correlated with the waist/hip circumference ratio than with BMI, although the difference in correlations (0.48 vs. 0.36, n = 69) is not significant.

Another study with interesting features was by Poikolainen and Vartinainen.[321] They found an overall association between GGT and BMI in 6010 subjects from Finland, but also reported a significant interaction between BMI and alcohol intake. The probability of an abnormal GGT (50 U/l or greater) increased more steeply with increasing alcohol intake in subjects with BMI of 27 or more than in subjects with BMI less than 27. This point is illustrated in Figure 16, based on their published data. It is also notable that in nonobese subjects, the probability of an elevated GGT result (and by inference the mean GGT) was lower in subjects taking moderate amounts of alcohol than in lifelong abstainers.

E. Associations with Cardiovascular Risk Factors: Blood Pressure

Many of the studies on obesity also examined the relationship between blood pressure and GGT, either across the entire range encountered in the population or

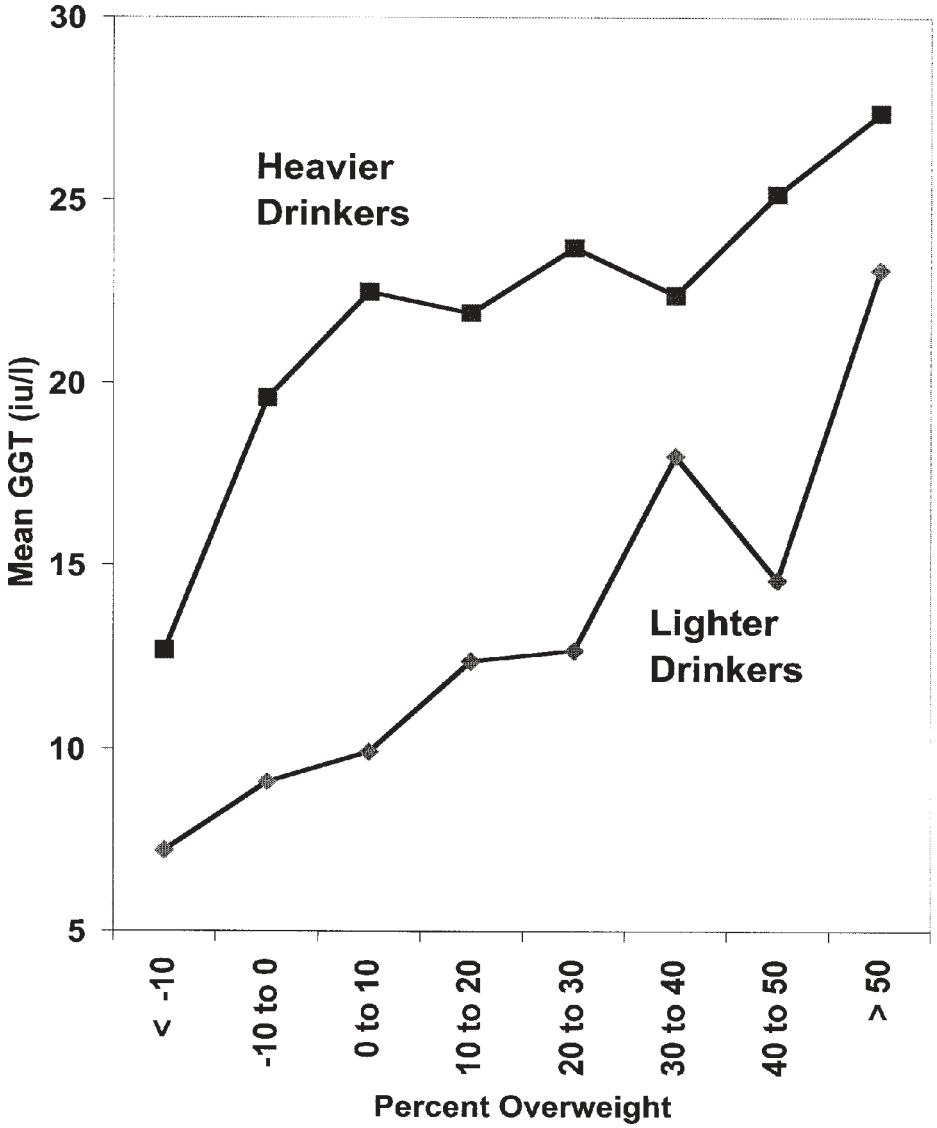

FIGURE 15. Effects of obesity on mean serum GGT in occasional and daily drinkers. (Data from Nomura et al.[316])

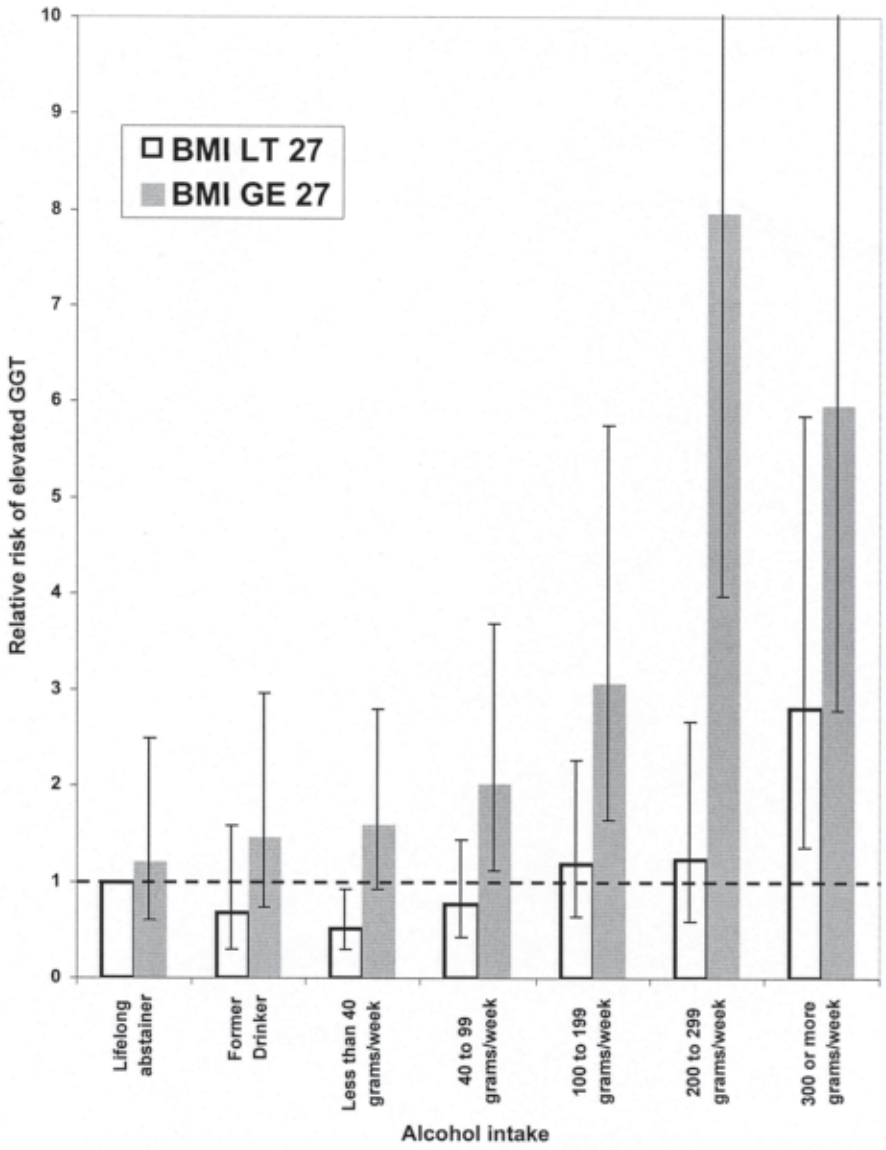

FIGURE 16. Probability of high GGT by alcohol intake and BMI. Note the interaction between alcohol intake and BMI, particularly around the 100 to 300 g/week region (from around two to four drinks a day), and also the dip below 1 in nonobese subjects drinking less than 40 g/week. Elevated GGT was defined by the authors as 50 IU/L or more. ORs are adjusted for sex, age, smoking, blood pressure, exercise habits, and coffee intake; bars show 95% confidence intervals. (From data of Poikolainen and Vertilainen.[321])

by contrasting hypertensive and normotensive subjects. An association undoubtedly exists, but it is weakened or made nonsignificant by the adjustment for other risk factors.

In the Malmo studies, a highly significant association between GGT and systolic or diastolic blood pressure was reported by Peterson *et al*.[301] Because there was no suitable data on alcohol consumption, it was not possible to determine how far the association was due to their common dependence on alcohol use. Similarly, Kornhuber *et al*.[322] showed a progressive increase in mean systolic blood pressure with increasing GGT, even within the 'normal' range of 9 to 25 U/l at 25°C (equivalent to about 16 to 45 U/l at 37°C).

GGT was strongly correlated with systolic and diastolic blood pressures among Japanese subjects;[323,324] obesity and age also affected blood pressure, but the effect of alcohol intake was much less. Multiple regression showed that the relationship between GGT and blood pressure was independent of age, obesity, and alcohol intake. GGT also emerged as a significant predictor of blood pressure in nondrinkers. A subsequent study contrasting nondrinkers and daily drinkers[325] showed that the relationship between hypertension and GGT was parallel but not identical in the two groups; for any value of BMI and GGT the prevalence of hypertension was predicted to be slightly higher in the daily drinkers, but GGT was a stronger predictor than alcohol intake. In the Third Tromso Study,[313] systolic blood pressure was found to be a significant independent predictor of GGT, for both men and women, in multiple regression analysis which also included BMI and alcohol use. Diastolic blood pressure was not an independent predictor, presumably because of its strong correlation with the systolic pressure. In addition, blood pressure and GGT had a strong association in teetotalers, supporting the conclusion that the correlation is not due to a common association with alcohol intake.

A study of 3315 Japanese nondrinkers[317] confirmed significant correlations between systolic or diastolic blood pressures and GGT in both men and women, independent of age and body mass index. Moreover, changes in blood pressure over 5 years in a subset of the male subjects correlated significantly with changes in GGT, independent of age, BMI, or initial blood pressure. The authors suggested that both GGT and blood pressure are affected by variation in fat distribution, fatty liver, and insulin resistance. A further study by the same group,[326] this time including drinkers supported this concept by showing that blood pressures were more strongly related to plasma insulin levels during a glucose tolerance test than to GGT, and that GGT was no longer significantly correlated with blood pressure after adjustment for insulin levels.

The UK study,[308] which showed an association between GGT and mortality, also showed significant positive correlations between GGT and both systolic and diastolic blood pressures. An adjustment for alcohol intake, BMI, smoking, physical activity, and other covariates reduced these correlations, but they remained highly significant. The association between GGT and mortality was reduced by adjusting for blood pressure but remained significant for both total and cardiovas-

cular mortality; the relative risk for noncardiovascular mortality was greater but nonsignificant, probably due to fewer deaths from these causes.

The stroke risk study of Jousilahti *et al.*[311] also found a highly significant association between GGT and both systolic and diastolic blood pressures at the time of initial assessment.

On the other hand, although significant univariate blood pressure-GGT correlations were found in a group of 38-year-old Dutch men,[320] these became nonsignificant in multiple regression analysis. In this case, waist/hip circumference ratio as well as BMI was included, and this measure of fat distribution may be represent a factor (such as insulin resistance) that affects both blood pressure and GGT. Similarly, the Sardinian group[318] found highly significant univariate correlations between GGT and both systolic and diastolic blood pressures in men and women, but blood pressure did not emerge as an independent factor in multiple regression analysis. In this case blood lipid and lipoprotein results were included as independent variables, as well as alcohol use, BMI, smoking, age, and physical activity level. Finally, the Finnish study[321] showed positive results in multiple regression analysis, this time for diastolic rather than systolic pressure.

In view of these conflicting results, the conclusion seems to be that high blood pressure and high GGT are found together more often than can be explained by chance, but whether the correlation can be abolished by taking other variables into account depends on which variables are included and, perhaps, on the population studied.

F. Associations with Cardiovascular Risk Factors: Lipids and Lipoproteins

High GGT is also associated with an unfavorable plasma lipid profile. Correlations between GGT and triglyceride values were found in patients attending a lipid clinic[327] and in diabetics.[328] In untreated (newly diagnosed) diabetics GGT was also correlated with plasma glucose, however, not with cholesterol, but in treated patients there were significant correlations between GGT and both cholesterol and urate, but not with glucose.

In male subjects attending a health-screening center, a significant correlation between GGT and triglyceride was also found.[272] The correlation was independent of alcohol intake, in that it was found in nondrinkers and persisted in a partial correlation on all subjects allowing for the variation in alcohol intake. There was a similar pattern of correlation between GGT and urate.

A wider range of lipid and lipoprotein data was collected in the Malmo study and reported by Fex *et al.*[329] and Janzon *et al.*[330] In the first of these papers, a significant positive correlation between GGT and triglyceride was found, but there was no significant correlation between GGT and total cholesterol, HDL cholesterol, or apolipoprotein A1. In the second paper there were more subjects and there

were progressive and statistically significant increases in total cholesterol, triglyceride, and apolipoprotein A1 across quintiles of GGT values.

In the Tromso study of almost 20,000 subjects,[313] GGT showed highly significant positive associations with total and HDL cholesterol, and triglycerides, in multiple regression analysis. This was true for both men and women, and after an adjustment for BMI, alcohol, blood pressure, physical activity, and coffee consumption. Even in the far smaller study of Dutch men,[320] GGT was significantly correlated with total and LDL cholesterol and with triglycerides (but not with HDL cholesterol). Total cholesterol also correlated with GGT in the studies by Wannamethee *et al*.,[308] Pintus and Mascia,[318] Poikolainen and Vartiainen,[321] and Jousilahti *et al*.[311] Comparison of GGT and CDT by Nikkari *et al*.[331] showed that increased CDT was associated with a favorable lipid profile, but increased GGT was associated with an unfavorable one, even though higher values of both were associated with higher alcohol intake. When subjects were grouped into quartiles of GGT, total and LDL cholesterol and triglyceride increased, and HDL cholesterol decreased, with increasing GGT.

G. Associations with Cardiovascular Risk Factors: Diabetes or Insulin Resistance

High rates of abnormal GGT in diabetics were discovered soon after its introduction as a liver function test. Early reports were summarized by Colloredo-Mels;[332] the prevalence of abnormal GGT was high among diabetics, but most studies had omitted to investigate the prevalence in controls. These authors compared the prevalence in diabetics and controls, finding no difference, but they excluded diabetics with abnormal AST or ALT results. A subsequent study[333] confirmed an increased prevalence of raised GGT in diabetics (compared with reference ranges from laboratory staff), and some of the diabetic patients also had increased AST or ALT. They found no difference in the rate of GGT abnormality by degree of glycaemic control, type of treatment (diet, oral hypoglycemic agents, or insulin), or duration of diabetes. However, there appears to be an effect of type of diabetes:[334] insulin-dependent diabetics had a median plasma GGT equal to control subjects, but in NIDDM the median GGTs in both men and women were twice those in same-sex controls. Significant correlations between body mass index and GGT were found in both controls and diabetics.

Associations between the presence of diabetes at the time of entry to the study, and GGT level, were also described in two of the prospective studies of GGT and mortality discussed above.[305,308]

Relationships between glucose tolerance and GGT in asymptomatic subjects were investigated by Umeki *et al*.[335] The prevalence of abnormal GGT results, and the mean GGTs, were significantly greater in subjects with either impaired glucose tolerance or diabetes mellitus than in subjects with normal glucose tolerance test

results. The results were unclear on the question of differences in GGT between the impaired glucose tolerance and the diabetes groups, as abnormal GGT was commoner in the former group, but the mean GGTs were similar.

Several groups have investigated the effects of experimental diabetes on hepatic and plasma GGT, and on hepatic glutathione, in rats. Shoukry[336] found that plasma GGT increased in streptozotocin–diabetic rats, and that this could be reversed by insulin treatment when the insulin dose was sufficient to normalise the blood glucose. McLennan *et al*.[337] showed that streptozotocin-induced diabetes increased hepatic GGT and treatment with insulin also restored this to normal levels. Total glutathione in the liver did not change unless the diabetic rats were restricted to the food intake of untreated animals, but the glutathione content of bile decreased with diabetes and increased to control levels with insulin. These results suggest that adaptive changes (increase in GGT, reduction in glutathione excretion) were able to maintain near-normal hepatic glutathione levels. Hemmings and Pekush[338] confirmed that hepatic GGT was substantially increased by steptozotocin treatment while insulin at least partially reversed this effect. In plasma, the streptozotocin treatment was followed by an increase in GGT, but paradoxically insulin treatment increased plasma GGT further. Hepatic glutathione was not measured in this study. Watkins *et al*.[339] found that diabetes increased hepatic but not renal GGT activity, and insulin-treated animals had values equivalent to controls. However, no significant increase in hepatic GGT mRNA or protein were found and the authors concluded that posttranslational changes to GGT affected the enzyme activity.

These experiments provide insight into the increase in plasma GGT found in some diabetics, suggesting that uncontrolled diabetes tends to decrease hepatic glutathione and that GGT is increased to maintain near-normal levels. However, they do not entirely explain the variation in GGT found among diabetics, and the lack of association between GGT and glycemic control in humans is unexplained.

Insulin resistance has been included in several of the epidemiological studies of causes of variation in GGT in humans. Because of the difficulty in testing large numbers of subjects with the glucose clamp protocol, insulin resistance generally has been assessed from the plasma insulin concentrations measured during an oral glucose tolerance test, or from fasting plasma insulin measurements.

The Malmo studies, which were designed to be multifactorial in their approach to health screening, produced data on the relationship between GGT and glucose and insulin results after a glucose load. Among 4763 men, those with low GGT (below the median) tended to have below-average glucose and insulin concentrations at 2 h post-glucose, while those with higher GGT had higher glucoses and insulins at both time zero (fasting) and at 2 h.[340] Similar results were reported by Kornhuber *et al*.:[322,341] serum insulin showed a highly significant increase with higher GGTs. They emphasized that this association was found within the reference range for GGT, or at least the range observed in the general population. The mean insulin (fasting, 1- and 2-h values in a glucose tolerance test) was approxi-

mately twice as high in subjects with GGT 16 to 25 U/l at 25°C compared with those with GGT of 8 U/l at 25°C or less.

Relationships between GGT and aspects of the insulin resistance syndrome were also examined by Rantala *et al*.[342] Just over a 1000 middle-aged men and women, half of them on treatment for hypertension, were studied. Highly significant associations were found between GGT and fasting or post-glucose insulin levels, and also with other insulin resistance-associated variables such as waist-to-hip ratio, triglycerides, and LDL cholesterol. As expected, GGT values were correlated with alcohol consumption and BMI, but correlations with components of the metabolic syndrome persisted after correction for BMI and also when only results from nondrinkers were analyzed. These authors considered two mechanisms for the association: first that insulin resistance is associated with fatty liver and this increases GGT, and second, that disturbed glucose metabolism might affect GGT more directly.

So far, insulin resistance has only been directly associated with increased GGT in a few studies, but it provides a potential unifying factor to connect GGT with such areas as obesity and fat distribution, hypertension, dyslipidaemias, smoking, exercise (as found in the cross-sectional studies), and total mortality or cardiovascular and cerebrovascular risk (as found in the prospective studies). This aspect has been emphasised by Shaper and colleagues and is discussed further below.

H. Cross-Sectional Associations with Cardiovascular Risk Factors: Exercise

Inverse associations between GGT and physical activity have been reported by many groups. Robinson and Whitehead[314] found about 20% difference in mean GGT between active and inactive categories, but their study was only on men. Nilssen *et al*.[313] found significant negative correlations with physical activity at work and in leisure activities in men but not in women in multiple regressions, including BMI, lipids, blood pressure, alcohol use, and age. Pintus and Mascia[318] did not find significant correlations between GGT and exercise, but results were marginally significant in multiple regression when other variables affecting GGT were included. Several papers arising from the British Regional Heart Study[308,309,343] have reported an inverse association between GGT and exercise. Again, only male subjects were studied. There was a difference of about 15% between inactive subjects and those undertaking vigorous exercise after adjustment for age, BMI, and alcohol. This was thought to be due to exercise having beneficial effects on insulin sensitivity, and higher GGT being associated with insulin resistance.

I. Cross-Sectional Associations with Other Known Risk Factors: Smoking

Positive associations with smoking have been reported by most investigators, although it is not always clear whether this is secondary to the association between

smoking and drinking. Robinson and Whitehead[314] studied only men and found a graded increase in GGT across categories of number of cigarettes per day. The effect was still significant but very small when results were adjusted for BMI, alcohol, and exercise. A subsequent paper with increased numbers of subjects[344] showed that smoking produced a significant increase in GGT activity in all drinking categories except teetotallers, and the smoking effect was strongest for those smoking more than 20 cigarettes per day.

Wannamethee *et al*.[308] found a small but highly significant increase in mean GGT across smoking categories, with or without adjustment for other factors. Pintus and Mascia[318] reported that smoking had a significant effect on GGT in multiple regression analysis in women but not men. Steffensen *et al*.[345] found that daily smoking increased the risk of raised liver enzymes in women. Jousilahti *et al*.[311] found that the prevalence of smoking increased significantly across quartiles of GGT in both men and women; this was in a univariate analysis, not adjusted for other factors affecting GGT.

J. Associations with Iron Overload

Serum ferritin concentration was reported to be higher in male subjects with high GGT than in those with GGT values close to the median.[346] However, there was no linear correlation between ferritin and GGT, and there was insufficient information on alcohol use to determine whether the result was due to their common association with alcohol intake. Among women,[347] GGT was significantly correlated with ferritin, but once again it was not possible to determine whether this was independent of alcohol use or other potential confounders. More recently a population-based study in Australia (Whitfield *et al*., in press) found a highly significant correlation between GGT and ferritin values in both men and women, which persisted after adjustment for alcohol intake.

Ferritin is widely used as a marker of iron status, but it is an indirect measure and is also affected by inflammatory processes. Therefore, it is likely, but not certain, that high GGT is associated with high hepatic iron stores. Because iron is a potential source of free radicals, and iron overload has been shown to be associated with liver damage, the association between GGT and ferritin can be counted as another one of GGT's multiple associations with increased risk. There is a substantial if rather conflicting literature on iron overload and cardiovascular risk.[348–351] The relationships between experimental iron overload, increased hepatic GGT, and the generation of free radicals and lipid peroxides were discussed in a previous section.

K. Cross-Sectional Associations with Coffee Consumption

It has been found consistently that higher coffee consumption is associated with lower GGT values.[312,313,318,321,352 -356] Other sources of caffeine do not produce

this effect. It has been described in both men and women, although individual reports differ on this point. Coffee consumption also decreases aminotransferases[356] or even alkaline phosphatase and bilirubin as well as ALT,[352] suggesting a general effect on the liver rather than a specific one on GGT. The effect of coffee may be greater among heavy drinkers of alcohol,[318,354,356] or possibly in those who drink or smoke more and have higher BMI.[357] It is not clear how coffee exerts this effect, or which component is the active one.

L. Can the Associations with Known Risk Factors Account for the Mortality Results?

This important point has been addressed explicitly in many of the prospective risk studies, or relevant data can be found in the papers cited. For example, good evidence on this point comes from Wannamethee *et al*.[308] The relative risk of death in men with elevated GGT compared with normal GGT was 1.52 when age was the only other factor considered, and 1.22 when adjustment was also made for social class, smoking, physical activity, alcohol intake, BMI, preexisting CHD or diabetes, blood pressure, and total and HDL cholesterol. Although the relative risk was substantially reduced, the 95% confidence interval on the adjusted estimate still excluded one so GGT had an independent effect on mortality. Similarly, Brenner *et al*.[305] found that the relative risk for death in the subjects with GGT results in the highest quintile, compared with the lowest, was reduced from 3.44 to 2.24 after adjustment for age, nationality, occupation, alcohol consumption, smoking, BMI, and several types of preexisting disease. Jousilahti *et al*.[311] found that adjustment for age, smoking, cholesterol, blood pressure, and BMI reduced but did not eliminate the association between GGT and risk of all types of stroke.

These results show that the associations with measured risk factors only partly account for the mortality results. However, the mortality studies do not include and adjust for all the risk factors associated with high GGT. An assessment of insulin resistance, iron overload, or oxidative stress, and inclusion of these in the model might change the conclusion about whether GGT is an independent risk factor.

M. GGT, Mortality, and Cardiovascular Risk

Many causes of raised GGT, in the liver or in the serum, have been shown to be associated with hepatic glutathione depletion or with free radical generation that in the absence of countervailing factors would tend to deplete GGT. This is an acceptable explanation for the associations between GGT and alcohol, but it is less established as an explanation for the effects of obesity and of insulin resistance syndrome, or of the other cardiovascular risk factors discussed above that are connected with insulin resistance.

One possible link between insulin resistance and GGT is fatty liver; it was noted earlier that high GGT was associated with hepatic fat content in human subjects.[274] The association between insulin resistance and fatty liver has been demonstrated in patients with nonalcoholic hepatosteatosis,[358–360] and fatty liver has been found to be associated with multiple features of the insulin resistance syndrome[326,361] or with waist-to-hip ratio.[362]

A possible link between fatty liver (and therefore the insulin resistance syndrome) and free radical generation is documented by Blazovics *et al*.[363] They produced hyperlipidaemia and fatty liver in rats by feeding a fat-rich diet and showed that homogenates of liver from the high-fat group showed evidence of greater free radical generation than control animals. This was demonstrated through increases in conjugated dienes (evidence of lipid peroxidation) and increased chemiluminescence (evidence of superoxide or hydroxyl radicals or hydrogen peroxide). Scavengers of free radicals were able to counter this effect. Experimental results from the chemiluminescence measurement are shown in Figure 17.

Therefore, a plausible chain of events can be constructed linking cardiovascular risk and GGT. Insulin resistance, and the other risk factors associated with it, promote cardiovascular disease. Insulin resistance is associated with fatty liver, which in turn is associated with free radical generation. Free radicals deplete intracellular glutathione, and GGT is induced in order to protect glutathione levels. The increase in GGT at the sinusiodal membrane of hepatocytes leads to an increased release of GGT into the circulation.

These ideas have prompted a number of clinical and epidemiological studies examining the steps in the causative chain, as discussed above, and results have on the whole been consistent with the hypothesis. The significance of the epidemiological evidence (of a link between GGT and cardiovascular mortality) is strengthened by being able to match it with biological plausibility in this way.

CONCLUSIONS

This review has been based on the proposition that the physiological functions of GGT, which are centered on glutathione metabolism, can help to explain the changes in tissue and serum activity that are found in disease and in predisease states. In particular, the changes in GGT activity associated with alcohol use, obesity, liver disease, enzyme induction, and cardiovascular risk factors are believed to be based on glutathione depletion and on the protective function of GGT induction in maintaining appropriate hepatic glutathione levels. These ideas are not new, but have been strengthened by recent studies.

In many of these situations there is experimental evidence that glutathione depletion does occur or would occur if corrective processes were not available: in obstructive liver disease, during the use of microsomal enzyme-inducing drugs, and after alcohol use. In relation to cardiovascular risk factors such as obesity,

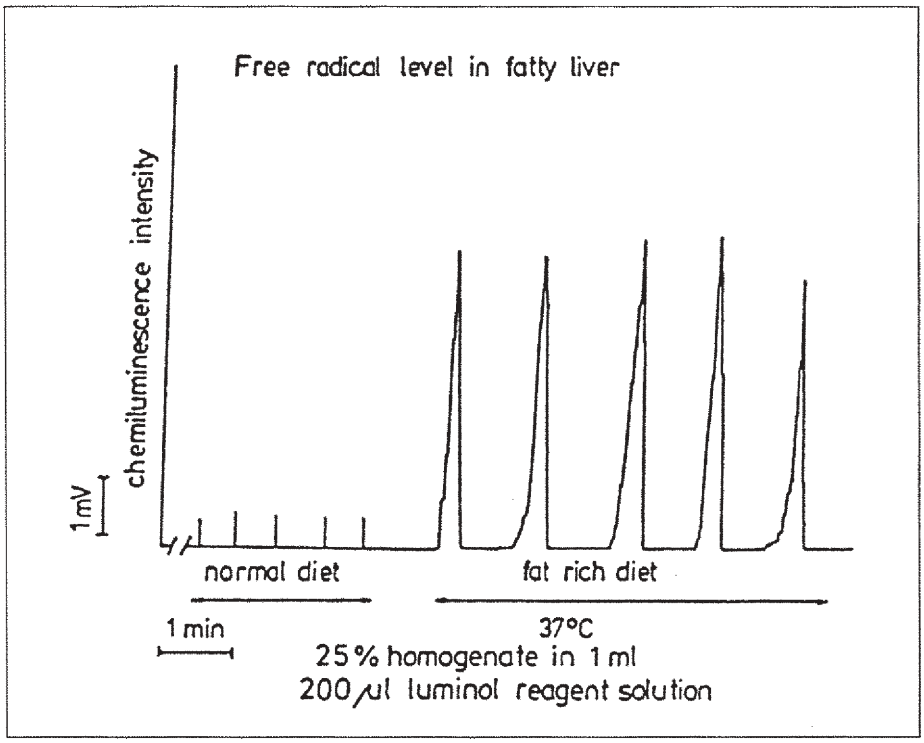

FIGURE 17. Fatty liver and oxidative stress. Homogenates of livers from control rats and rats fed a fat-rich diet were tested for free radical production in a system containing luminol and the chemiluminescence was recorded. (Reproduced with permission from Blazovics et al.,[363] Copyright 1992, Springer-Verlag.)

hypertension, and dyslipidaemias, the path from these factors or from the metabolic syndrome through glutathione depletion to GGT induction is less clear, but there is evidence that these are associated with fatty liver and that fatty liver produces oxidative stress. The overall connections between these processes are summarized in Figure 18.

If this is accepted as a working hypothesis, several further lines of enquiry can be identified. Iron overload occurs in both alcoholism and obesity and is known from cellular studies to be important for the prooxidant effects of GGT activity. Further *in vivo* studies of patients or community-based samples, measuring liver fat content by noninvasive techniques and relating them to GGT, insulin resistance, iron stores, and other cardiovascular risk factors are warranted to test whether the risk factors and the paths between them are correctly understood. At a cellular level, some aspects of GGT induction by GSH depletion need to be studied further. The mechanisms by which decreased glutathione affects GGT gene expression through promotors and the control of transcription are still unknown. Work is needed to elucidate the changes in growth factor receptor interactions, and in the balance between proliferation and apoptosis, which have been shown in a few

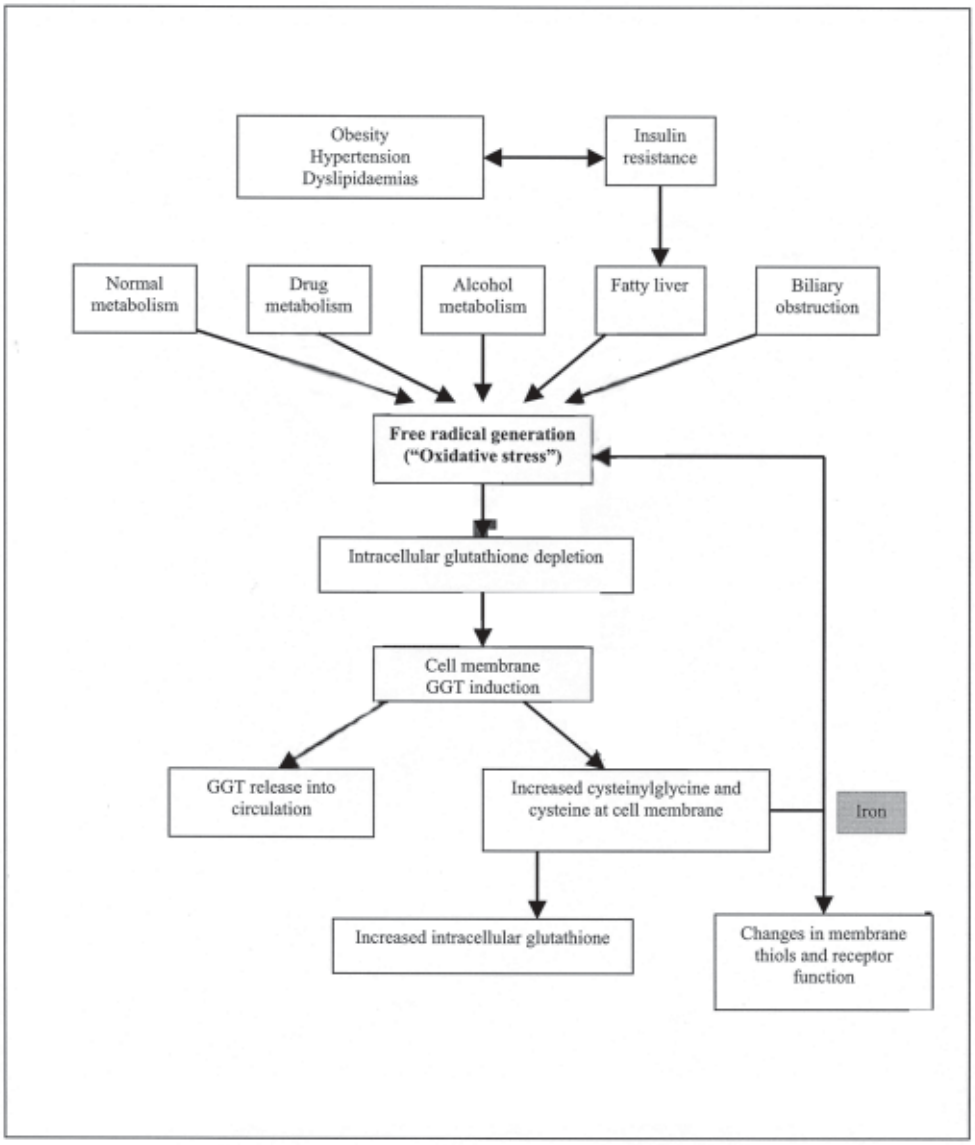

FIGURE 18. Summary of the postulated and established interactions between causes of free radical generation and the induction of GGT activity.

recent studies to result from GGT activity in the presence of transition metals, especially iron. This in turn may produce insights for epidemiology or for studies on cell proliferation within human atheromatous plaques. Meanwhile, GGT will continue to be a useful clinical laboratory test and a useful marker of alcohol intake and its consequences.

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