SERUM GLUTAMATE DEHYDROGENASE DETERMINATION

IN

CHRONIC ALCOHOLICS

by

Richard Jan Pivon

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

Master of Science

Department of Experimental Surgery

McGill University

Montreal, Canada

August 1985.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

Résumé

DETERMINATION DE LA DÉSHYDROGENASE DU GLUTAMATE (GDH) SERIQUE CHEZ LES ALCOOLIQUES CHRONIQUES

Les facteurs sériques GDH, GGTP, Alk. Phos., SGOT et SGPT furent étudiés chez 72 patients souffrant d'alcoolisme chronique. Au cours de projets pilotes impliquant 12 patients, une modification fut apportée à la méthode de détermination de la GDH et les autres enzymes furent analysés. Cette méthode modifiée de détermination du facteur GDH (Koch et Pivon) s'est avérée plus précise et plus simple que la méthode utilisée jusqu'alors. Chez 60 alcooliques chroniques admis dans un centre de réhabilitation, les niveaux d'enzymes furent déterminés aux 1er et 21ième jours de leur séjour et mis en corrélation avec les différents niveaux d'éthanol sérique. Ceci fut établi d'après le procédé de jaugeage (Kapur et Israel - 1985). Lors de crises intenses d'alcoolisme, la hausse du taux de la GDH sérique était directement reliée au niveau d'éthanol dans le sang (Sensibilité: 81.25%; Spécificité: 90.91%; Valeur de prédiction positive: 76.47%; Valeur de prédiction négative: 93.02%). Au 21ième jour, tous les taux de la GDH se situaient dans les limites normales. Même au-delà de 48 heures après le retour à la normale des taux de la GDH, le facteur GGTP se révéla un bon indicateur de la condition hépatique. Cette méthode modifiée de détermination de la GDH sérique chez les alcooliques chroniques semble apporter un nouveau paramètre pour évaluer l'état de ceux qui, lors d'épisodes aigus d'alcoolisme, présentent des lésions aux mitochondries des cellules du foie et possiblement pour dépister d'autres maladies hépatiques.

Abstract

SERUM GLUTAMATE DEHYDROGENASE DETERMINATION IN CHRONIC ALCOHOLICS

Serum GDH, GGTP, Alk.Phos., SGOT and SGPT were studied in 72 chronic alcoholic patients. In pilot studies on 12 patients, the modification of GDH determination was developed, and the other enzymes tested. The modification of GDH determination (Koch and Pivon) was found to be more accurate and simple than previous methodology. In 60 chronic alcoholics admitted to a half-way center, the enzyme levels were determined at days 1 and 21 and correlated to serum ethanol levels, determined by dipstick methodology (Kapur and Israel 1985). During acute alcoholic episodes, serum GDH elevation was directly related to ethanol blood level (Sensitivity: 81.25%; Specificity: 90.91%; Positive Predictive Value: 76.47%; Negative Predictive Value: 93.02%). At day 21, all GDH values were within normal range. GGTP was found to be a good indicator of liver status beyond 48 hours, when GDH values return to normal. Serum GDH determination in chronic alcoholics appears to add a new parameter to the assessment of chronic alcoholics, reflecting mitochondrial injury in the acute episode and possibly in other liver diseases.

PREFACE

Since my graduation in medicine, the subject of liver function has fascinated me. I remember several professors stressing how little is known about the "metabolic factory" of the body. I have also wondered how we can assess liver status prior to surgery, without performing laparatomy or biopsy. It is important for the surgeon to know as much as possible about the patient before placing him on the operating table, so that decisions taken during the operation have a more valid biochemical background.

While working at the Biology Department of McGill University, my "research instincts" were encouraged by the Chief of the Department, Dr. G. Maclachlan, who used to say: "If you don't know, you have to find out for yourself and the best way to do it is to carry out a controlled experiment" This saying stuck in my mind, so that after graduation, I decided to pursue a research career. After working one year as a volunteer in the Division of Clinical Investigation of the Department of Surgery, Dr. Skoryna, Director of the Unit and the Gastro-intestinal Research Laboratory, suggested to me that I

take formal training at McGill. When I asked him whether there were any projects dealing with the liver, he said that this is one of the current concerns of many surgeons, particularly with reference to the assessment of liver function. The question then came up as to what subjects should be studied. I suggested that there was a need for a group of patients with demonstrable liver injury who could be studied for a prolonged period of time. At this point, I had also met Dr. Paul Koch of the Montreal General Hospital, where I previously worked as a student. With his help, I was able to design the project, after contacting Dr. Jose Negrete, Chief of the Alcohol Research Unit of the Hospital, whose patients were studied initially. Further studies were carried out on patients from Maison Jean Lapointe, whose program director, Mr. Jacques Perras, was most cooperative, not only in obtaining the samples but also in analyzing psychometric data on these patients.

In addition to these individuals who guided my studies, I must thank a number of persons who helped me to complete the project. I have enjoyed attending the research seminars and surgical rounds at St. Mary's

Hospital and the Montreal General Hospital. I became acquainted with the wide scope and diversity of research projects in the Department of Surgery, which added significantly to my pool of general knowledge. I wish to thank particularly Dr. D.B. Tonks, Chief of the Dept. of Laboratories, and Dr. David S. Mulder, Chief of the Dept. of Surgery of the Montreal General Hospital, for their encouragement and cooperation in establishing the techniques of serum enzyme determination at the Special Clinical Testing Laboratory of the Hospital. Miss Pauline Reed, Chief Technician, instructed the author in methodology and was extremely helpful in the initial stages of the investigation. Mrs. Marta Fuska, Chief Technician of the Clinical Investigation Service of St. Mary's Hospital, continuously helped in further enzyme analysis. Miss Huguette Bacon and her associates at La Maison Jean Lapointe processed serum samples from chronic alcoholics at required intervals in order to fulfil the research protocol requirements.

Dr. Jose Rodriguez, Surgeon-in-Chief, and Dr. John Keyserlingk, in charge of Surgical Teaching, at St. Mary's Hospital, were most cooperative in the course

of these studies. Dr. Julius Gordon, Director of Surgical Research of the Dept. of Surgery, and Miss Brenda Bewick, Administrative Assistant, were very cooperative in completing all the necessary documentation for registration and submission of the thesis. I should also mention my colleagues, Dr. Gilles Hedderich at St. Mary's, and Dr. George Chuang at the Montreal General Hospital, who were very helpful in the course of my studies. Mr. Jan T.Z. Nolan, Computer System Integrator, helped the author tremendously in the statistical and mathematical analysis of the computerized data. Mrs. Jean Cornellier, Secretary of the Gastrointestinal Research Laboratory, is thanked sincerely for typing the manuscript. Mr. Carmen Cristofaro, Chief of the Audiovisual Department, and his associates provided all the photographic material. Finally, I must thank my wife, Joanna, for her patience when I was virtually absent from home in the course of my studies, and my mother, Alexandra, for her support. Without all this help, the work could not be done.

Albert Einstein once said that curiosity has its own reason for existence: "One cannot help but be in

awe when he contemplates the mystery of the marvelous structure of life and reality". Perhaps "life" should read "liver". I sincerely hope that, by establishing a new procedure for serum GDH determinations in chronic alcoholics, this work will contribute in a positive way to the development of research of liver diseases.

Richard J. Pivon, M.D.
Research Fellow
Division of Surgical Research
Department of Surgery
McGill University.

August 17, 1985.

INTRODUCTION

In the search of knowledge for tests reflecting liver function, the serum Glutamate Dehydrogenase activity was neglected for a relatively long period of time. The first test used clinically was probably urine bilirubin determination, based on the observation of Paul Ehrlich in 1883 (Henry et al 1974), who had noticed that urine of patients suffering from liver disease contained bilirubin. Alkaline Phosphatase activity in blood was demonstrated by Kay in 1930. The heterogenous nature of alkaline phosphatase that can be derived not only from the cells lining the biliary tract of the liver but also from bone osteoblasts, the mucosal cells of the small intestine and the placenta, renders the value of this assay imprecise, unless other enzymes are determined simultaneously. The determination of serum Glutamic Oxaloacetic transaminase (SGOT), Glutamic Pyruvic transaminase (SGPT) and Glutamyl transpeptidase was developed around the early fifties. The transaminases are used as indicators of diffuse hepatocellular injury; however, not only are they not organ specific, but they are found in large quantities in the myocardium, skeletal muscles and kidneys. The

gammaglutamyl transpeptidase, although not elevated in bone disease, is a sensitive indicator of hepatic disease, but is not specific since it is also elevated in pancreatic disease and carcinoma as well as inflammatory disease. It was only in the early fifties that Hogeboom and Schneider (1953), in the eleventh chapter of their monumental work on intracellular distribution of enzymes, described the glutamate dehydrogenase activity in the liver.

Since chronic alcohol abuse ranks high in the health effects problem due to high morbidity and associated socialogical and economic impact (Meyer et al 1981, Vierling 1983), it is not surprising that the literature on serum enzyme determinations associated with ethanol toxicity became voluminous. Lieber and his associates (1977) proposed to use the Glutamate Dehydrogenase determination as an indicator of liver damage during the acute phase of alcohol abuse. Obviously, since GDH is of purely mitochondrial origin, it appears to the author that a new parameter for the assessment of liver function has been found, namely the reflection of the extent of mitochondrial damage.

The significance of changes in serum Glutamate Dehydrogenase activity may play an important role in other forms of liver injury due to obstructive jaundice, various forms of hepatitis and the effects of hepatotoxic substances such as certain medications and anaesthetic agents. The mitochondria have a remarkable capacity to recover after injury, including that caused by the most potent physiological agent - the high concentration of intracellular calcium. It was rather fortunate that, under the supervision of Dr. Paul Koch, we were able to develop a new and accurate modification for serum Glutamate Dehydrogenase determination. This method has the potential to eventually become useful in the assessment of various surgical and non-surgical conditions affecting the liver; however, a considerable amount of studies remain to be carried out on Glutamate Dehydrogenase activity, taking into account the variability of factors affecting the clinical course of these processes. In this respect, the study of Chronic Alcoholics offered a rather unique opportunity for obtaining data from a relatively homogenous group of patients, in which the degree of damage

to the "powerhouse" of the cell, as reflected by serum GDH levels, could be related to the level of the toxic substance in the serum.

CHAPTER II - HEPATOTOXICITY

Trace substances that may adversely affect liver function in man include such diverse groups as trace elements, certain drugs, chemicals and ethanol. In a vast majority of cases, hepatotoxicity is due to trace substances, since all the compounds listed below are present in the blood in trace amounts. Alcohol affects the liver only when taken in excessive amounts; however, the resulting damage to the liver is due to acetylaldehyde, a trace product of ethanol metabolism. Small amounts of ethanol found in normal human subjects is believed to originate from bacterial fermentation in the gastrointestinal tract (Geokas, 1981). The purpose of this presentation is to present some of the problems related to the investigation of hepatotoxicity as well as to discuss the perspectives in further studies of diagnostic procedures. Repeated liver biopsies, although necessary, are impractical and difficult from the clinical viewpoint, particularly in chronic alcoholics. A battery of liver function tests is used today: BSP, which reflects the degree of cholestasis; Indocyanine Green (ICG), to determine improved conjugation of Bromsulfaphtalein;

PTT and PT to assess coagulation status. These are just a few examples.

The main area of developments occurred in the determination of serum liver enzymes, as an non-invasive method for assessment of liver function. This refers to both the use of serum enzyme ratios as well as enzyme levels per se and to some relatively new tests such as glutamate dehydrogenese determinations (GDH), introduced by Ellis and Goldberg (1972) and Lieber and his associates (1977) which reflects changes in mitochondrial metabolism. Needless to say, advancements in this field are of significance not only in the assessment of hepatotoxicity or trace substances but also encompass a much wider field. This refers to clinical evaluation of liver status (French and Burbige, 1979 and Sherlock, 1982) in medical and surgical conditions particularly since liver transplants and hepatic resections are being carried out with increased frequency.

Before we discuss the assessment of hepatotoxicity, it is useful to review the classification of hepatotoxicity. The basis of currently used classification of hepatotoxicity is that proposed by Zimmerman (1978,1983)

who has divided the hepatotoxic substances into those that have idiosyncratic effects and those that are intrinsic hepatotoxic substances. The compounds exerting idiosyncratic action concern a relatively small group of the patient population; their effects are bases on hypersensitivity reactions and, therefore, the incidence is relatively low. Idiosyncratic substances can produce both diffuse and zonal changes in the liver lobule. According to Zimmerman (1978) diffuse necrosis can be produced by such substances as C-methyldopa, Doxorubicin, Salicylates; massive necrosis may result in some cases from prolonged use of Isoniazid, Phenytoin, Ketoconazole and Selenium compounds (Zimmerman, 1978). In connection with the idiosyncratic hepatotoxicity, it should be emphasized that the level of dosage is of significance; certain medications, such as salicylates (Zimmerman, 1978), produce the idiosyncratic effect in a small group of patients, if the dosage is relatively high. Another point that should be mentioned with reference to idiosyncratic agents is that when small doses are used, the effect is usually diffuse and relatively mild; with higher doses, massive necrosis may result

in individuals who are immunologically less competent (Zimmerman, 1983). However, in a small group of patients, even therapeutic doses of Isoniazid, Phenytoin, Selenium and Ketoconazole can produce massive necrosis (Zimmerman, 1978).

The largest group of compounds, which exert hepatotoxic action, are those which have an intrinsic effect on the liver (Table I). These compounds can be classified clinicopathologically into those that produce either diffuse changes or exert an effect on the peripheral, middle or the central zone of the hepatic lobule. Substances which produce diffuse changes can be subdivided into those inducing only mild changes (Klatskin, 1974, Kristensen, 1981 and Vazquez et al, 1983) usually of inflammatory nature (such as those resulting from use of Ketoconazole (Klatskin, 1974)); moderate changes (Koch et al, 1976, Powell-Jackson et al, 1984 and Sippel and Agger, 1981) are related to subsequent steatosis and proliferation of fibrocytes; extensive changes (Black et al, 1975, Diaz-Rivera et al, 1950, Duarte et al, 1983, Patterson et al, 1983 and Witzleben, 1972) result in necrosis with consecutive development of cirrhosis. Zonal

Table I. Clinicopathological Classification of Liver Hepatotoxicity

			EXAMPLES
INTRINSIC HEPATOTOXIC TRACE SUBSTANCES	DIFFUSE HEPATOTOXIC EFFECT	MILD DIFFUSE EFFECT	KETACONAZOLE (Horn et al 1984) DISULFIRAM (Kristensen 1981) CYANAMIDE (Vazquez et al 1983)
		MODERATE DIFFUSE EFFECT	QUNIDINE (Koch et al 1976) NITROFURANTUIN (Duarte 1983) SODIUM VALPROATE (Powell-Jackson et al 1984)
		EXTENSIVE DIFFUSE EFFECT	NIACIN (Patterson et al 1983) KETOCONAZOLE (Duarte 1983) ISONIAZID (Black et al 1975)
	ZONAL HEPATOTOXIC EFFECT	PERIPHERAL ZONE EFFECT	ERYTHROMYCIN ESTOLATE (Lunzer et al 1975) CYANAMIDE (Vazquez et al 1983) DISULFIRAM (Kristensen 1981)
		MIDDLE ZONE EFFECT	NGANIONE (Zimmerman 1978) CARBON TETRACHLORIDE (Zimmerman 1978) BERILLIUM (Pepelko et al 1984)
		CENTRAL ZONE EFFECT	ACETYLALDEHYDE (Lieber 1981) ENFLURANE (Lewis et al 1983) KETOCONAZOLE (Delpre and Kallish 1984)

hepatotoxic effects of the peripheral zone of the lobule were reported following exposure to elemental phosphorus (Diaz-Rivera et al, 1950), manganese-bilirubin compounds (Witzleben, 1972) and erythromycin estolate (Lunzer et al, 1975). Ferrous sulfate when taken in high doses has also been reported to induce changes in the peripheral zone of the lobule (Horn et al, 1984). Beryllium toxicity due to industrial exposure is known to produce changes in the middle zone (Aldridge et al, 1949 and Pepelko et al, 1984); another example of hepatotoxic effects in this zone is the action of carbon tetrachloride and Nganione (Zimmerman, 1978). The central (perineural) zone of the hepatic lobule (Rappaport zone III) has a decreased oxygen tension (Rappaport et al, 1954). Hypoxia renders this zone more susceptible to hepatotoxic effects. Enfluorane has been reported to induce occasionally toxic changes in this zone (Lewis et al, 1983). Effects of ethanol received a great deal of attention, since Lieber (1981, and Lieber and Leo, 1982) pointed out that this zone is initially affected by chronic alcohol abuse, even if the intake of ethanol is not extremely high but

chronic. It should be stressed that some of these substances may affect concurrently different areas of the hepatic lobule or produce both a zonal change and a diffuse change. For instance, Ketoconazole produces initially central zone changes (Delpre and Kallish, 1984) but may also cause diffuse changes, ranging from mild to extensive (Duarte et al, 1983 and Klatskin, 1974). Erythromycin estolate and ethyl-succinate may affect occasionally both the central zone as well as the peripheral zone (Lunzer et al, 1975).

CHAPTER III - SERUM GLUTAMATE DEHYDROGENASE AS REFLECTING LIVER FUNCTION

A. Localisation and Development of Glutamate Dehydrogenase Determination

Interest in Glutamate Dehydrogenase localisation in hepatocytes was first developed following the studies of Hogeboom and Schneider and Pallade (1948). These investigators developed the methodology of differential centrifugation, which was subsequently improved by Schneider and Hogeboom (1950) and Hogeboom et al (1952-3). Differential centrifugation enabled them to establish the location of GDH in the mitochondria of the hepatocyte using spectrophotometry and a reaction mixture close to that later used by Ellis and Goldberg (1972) for serum enzyme determinations. During this study, it was established that GDH is confined to mitochondria and that initially minimal or no transfer into other intracellular compartments occurs without damage to the mitochondrial membrane. Studies by DeDuve's group (1955) further advanced liver cell fractionation. DeDuve et al (1955) had separated the mitochondrial layer into two fractions: a heavy one consisting of a dense bottom layer, and a light mitochondrial fraction was first isolated together

with the supernatant fluid and recovered with the microsomal fraction. DeDuve's work has concluded that the DPNH and TPNH cytochrome c reductase activities reflect the presence of two different systems associated with mitochondria and the microsomes respectively. Studies by Christie and Judah (1955) using the differential centrifugation method of Hogeboom and Schneider (1950), demonstrated the intracellular distribution of GDH by spectrophotometric methods. Mitochondria were found to contain 90% of the homogenate activity. Christie and Judah (1955) also confirmed the dependence of mitochondrial localisation of GDH on the intactness of the mitochondrial membrane. Water treatment "rendered" the co-factor or a substrate more accessible to the GDH system, suggesting that the "barrier" (mitochondrial membrane) must be disrupted before GDH can be released from the mitochondria.

A Montreal group, consisting of Allard, DeLamirande and Cantero (1957), separated the mitochondrial fraction of the hepatocyte and found that during fasting, GDH activity per mitochondria is increased. They reported GDH activity as a possible indicator of the rate of

gluconeogenesis from protein. In 1959, deDuve's group (Beaufay, Bendall, Baudhuim and deDuve, 1959) finally established the location of enzymes in the mitochondrial and lysosymal fraction, an issue that was contested prior to their publication. The GDH was found to be localised in the heavy (mitochondrial) fraction.

It appears that Schmidt and Schmidt (1962) were the first to correlate serum GDH levels with liver biopsies. The data from Schmidt and Schmidt (1962) in Table II show the amount of GDH present in various tissues (measured in mU/g of fresh tissue). In the liver tissue obtained from 30 subjects, the GDH content averaged 38610 mU/g, while the adrenal cortex occupied the second position with 6675 mU/g; brain cortex, with 4115 mU/g, was in the third position. It is of interest that the intestinal mucosa contains amounts ranging from 1481 mU/g in the stomach to 3658 mU/g in the sigmoid colon. According to Schmidt and Schmidt (1962), erythrocytes do not contain any GDH (Ø mU/g). The content of GDH in different types of tumours, listed in Table III, ranged from 1170 mU/g in testicular tumours to 6602 mU/g in colonic tumours.

Table II. GDH content in human tissues (after Schmidt and Schmidt 1962)

Organ	No. of samples	mU/g of fresh tissue	mU/g extracted protein
Liver	30	38610	398
Adrenal cortex	5	6675	120
Brain cortex	3	4115	250
Sigmoid colon mucosa	1	3658	164
Lymph nodes	5	2743	51
Thyroid	2	2469	203
Lungs	4	2469	43
Brain medulla	3	2304	190
Adrenal medulla	6	2286	45
Rectal mucosa	1	1737	42
Cerebellum	2	1554	93
Gastric mucosa	7	1481	27
Fat tissue	6	1280	95
Uterine muscle	3	1115	9
Heart muscle	6	1097	23
Gastric muscle	3	676	14
Skeletal muscle	5	548	5
Pancreas	4	530	14
Breast	2	438	13
Bronchial mucosa	1	365	33
Testicle	1	311	20
Erythrocyte	4	Ø	Ø
Skin	2	Ø	Ø

Table III. GDH content of tumour tissues in human subjects (after Schmidt and Schmidt 1962)

Organ	No. of samples	mU/g of fresh tissue	mU/g extracted protein
Colonic cancer	3	6602	86
Gastric cancer	4	3840	73
Lymphosarcoma	1	2578	42
Thyroid cancer	1	2396	38
Prostatic adenoma	2	2140	60
Metastasis (Hypernephroma)	3	1920	27
Seminoma	2	1170	42

Since the liver is the main source of GDH, the estimation of serum GDH has been used as an indicator of liver function for many years (Schmidt and Schmidt, 1962); however, the methodology of GDH determinations has been modified several times and it is only recently that the factors affecting the GDH level in serum are more clearly understood. Because of certain amounts of GDH found in cardiac and skeletal muscles, we have found in our studies that simultaneous determination of Creatinine Phosphokinase (CPK) and Creatinine are valuable adjuncts in the differentiation of hepatotoxic effects of various drugs and ethanol, from those due to cardiomyopathies and skeletal muscle sources (Table II).

B. Properties of Glutamate Dehydrogenase

According to the official nomenclature adopted by Enzyme Commission, the following classification was approved: EC 1.4.1.2. (Armstrong, 1983); (1) or Class 1 oxireductase; (4) or the group oxidized, in this case NH_4^+; (1) refers to the oxidizing agent or coenzyme NAD^+; (2) refers to the specific reaction or glutamate dehydrogenase, respectively. It was presumed that GDH was a Class II enzyme, but the use of inhibitor techniques has shown

that GDH is a Class I enzyme, whose mechanism for binding is totally random and not based on the amount of ammonia saturation (Purich 1983). The structural changes during transamination are shown in Fig.1. The Km values of GDH, according to Fersht (1983) are: 2×10^{-3} for OC-ketoglutarate, 5.7×10^{-2} for NH_4^+ and 1.8×10^{-5} for NADH. It appears from these values that GDH has the largest affinity for NH_4^+ production. GDH has a molecular weight of 2×10^6 (Ellis and Goldberg 1972). GDH is an allosteric enzyme and one of the most active amongst the dehydrogenases, whose activity is controlled by activators (ADP,AMP,GDP) and inhibitors (ATP,GTP,NAD(P)H) respectively (Smith and Thiers 1981, Powers and Meister 1982). It appears that a drop in NAD(P)H would enhance the oxidative process of GDH, while a rise in NAD(P)H levels could inhibit GDH activity. GDH catalyzes the oxidative deamination of L-Glutamate to OC-Ketoglutarate, a reversible reaction illustrated in the diagram (Fig.2).

Fig.1 Structural changes in serum GDH during transamination (after Rawn 1983)

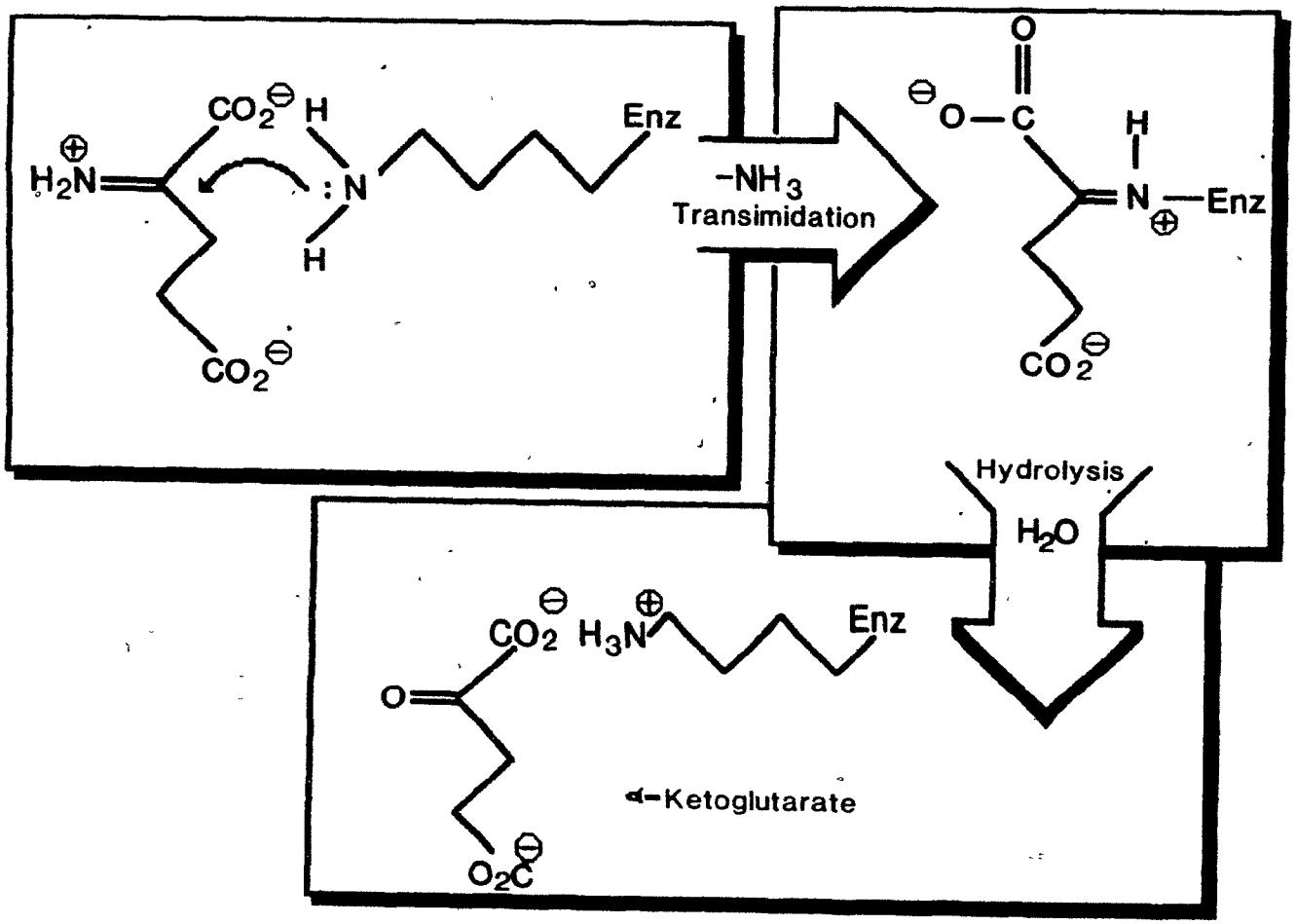
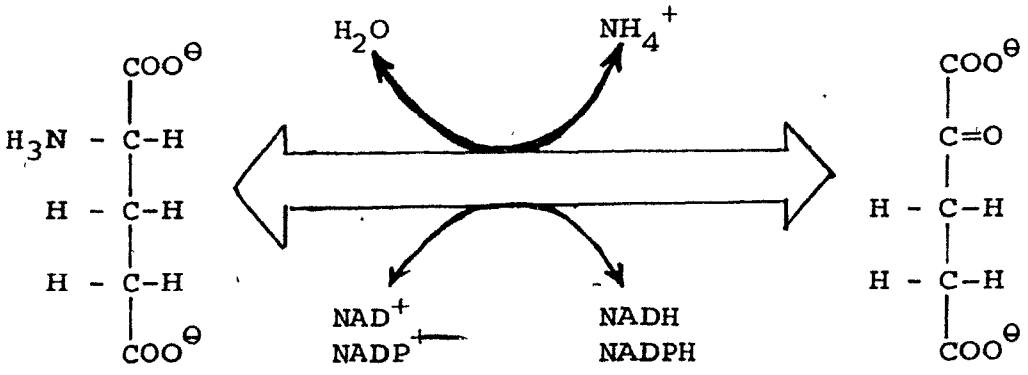


Fig.2 The reversible reaction catalyzed by GDH. The reaction involves a single step transamination of the α-carbon of α-Ketoglurarate.



L-Glutamate α-Ketoglutarate

According to Smith and Thier (1981), the reaction involves a single step transamination during which an amino group is transferred to the α-carbon on α-keto-glutarate to replace the α-keto group to form L-Glutamate; in reverse, NH_4^+ is given off and α-ketoglutarate is formed. Catabolism of proteins leads to a higher concentration of glutamate than that when ammonia and glutamate undergo oxidative deamination by GDH, even though this reaction is not directed towards an equilibrium (McGilvery, 1983; Powers and Meister, 1983). After extraction of NH_4^+ by GDH from L-Glutamate, the α-ketoglutarate formed can enter the Krebs Cycle to form urea. Armstrong (1983) pointed out that the synthesis of glutamate by L-glutamate dehydrogenase

is one of the pathways used for conversion of inorganic nitrogen into carbon-bound amino groups; in this way, the nitrogen enters the metabolic pathway. Since the reaction is reversible, the carbon atoms in excess of L-glutamate become available as α-ketoglutarate for catabolism in the Krebs Cycle. Armstrong (1983) also pointed out that after transamination of glutamate, α-ketoglutarate is regenerated and once more an inorganic NH_3 is incorporated by GDH.

Mitochondria disposes of excess glutamate by two processes: nitrogen transfer to another keto group or by GDH oxidation to release NH_4^+; in both cases the α-ketoglutarate is free to enter the Krebs Cycle (McGilvery, 1983). This reaction, according to Newsholme and Leech (1983), is analogous to a secondary alcohol being oxidized to a ketone, but with the exception of oxygen replacement by nitrogen, which is released in the form of ammonia when the amino acid is hydrolysed. The reaction catalysed by GDH represents a port of entry for certain amino acids, such as arginine, histidine, glutamine and proline, which can take part in the formation of glutamate and enter Krebs Cycle (Smith and Thier, 1981). Since glutamate dehydro-

genase can take part in the process of transamination (Fig.3) of many other amino acids, during which their amino groups can be transferred to α-ketoglutarate; according to Newsholme and Leech (1983), α-ketoglutarate plays a more significant role in the process of transamination than previously suggested. In order for deamination to continue, α-ketoglutarate must be maintained in a free form for transamination, a condition which Glutamate Dehydrogenase fulfills by catabolising glutamate to α-ketoglutarate. In the process of amino acid degradation, the amino acid is usually lost by one of two processes: either by oxidative reaction (referred to as deamination) or by transfer to an oxacid (referred to as transamination). Newsholme and Leech (1983) concluded that pyridoxal phosphate forms a transient covalent complex (Schiff's base) with the amino acid during transamination. A realignment then occurs and "the oxacid is split off, leaving the amino group attached to the pyridoxae phosphate or pyridoxine phosphate". Therefore, the oxacid involved in transmination is available for future reaction. Since glutamate dehydrogenase catalyses not only transamination but also deamination, according to

Fig.3 Glutamate Dehydrogenase Reactions with Different Transaminases after Rawn (1983)

Transaminase	Source	Reaction catalyzed
Glutamate-alanine	Animals, plants	L-Glutamate + pyruvate ⇄ α-ketoglutarate + L-alanine
Glutamate-aspartate	Animals, plants, bacteria	L-Glutamate + oxalacetate ⇄ α-ketoglutarate + L-aspartate
Glutamate-cysteine	Animals (especially liver)	L-Glutamate + mercaptopyruvate ⇄ α-ketoglutarate + cysteine
Glutamate-glycine	Animals, plants, bacteria	L-Glutamate + glyoxylate ⇄ α-ketoglutarate + glycine
Glutamate-leucine	Animals, plants, bacteria	L-Glutamate + α-ketoisocaproate ⇄ α-ketoglutarate + L-leucine
Glutamate-phosphohistidinol	Molds	L-Glutamate + imidazole acetol phosphate ⇄ α-ketoglutarate + histidinol phosphate
Glutamate-tyrosine	Animals, bacteria plants	L-Glutamate + p-hydroxyphenylpyruvate ⇄ α-ketoglutarate + L-tyrosine

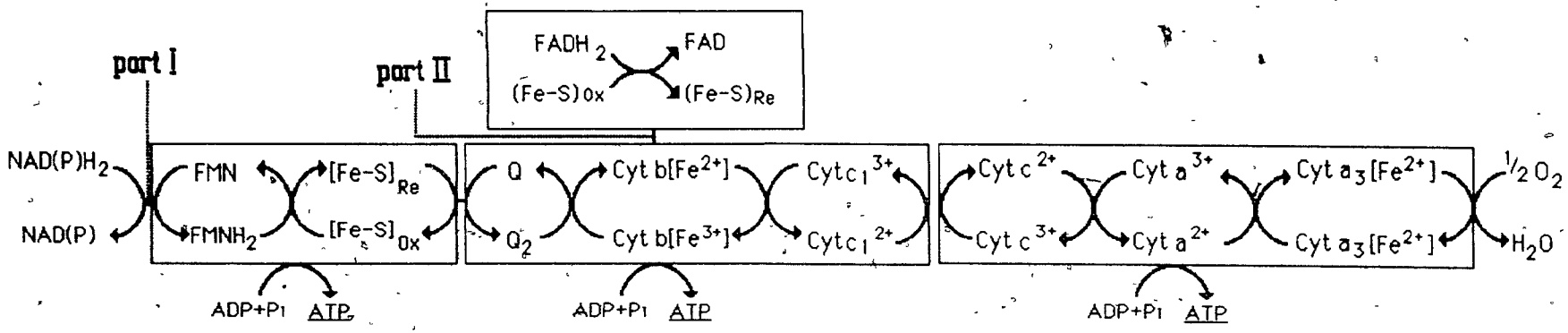
Newsholme and Leech (1983), the reaction is called transdeanimation; according to Krebs (Krebs et al, 1973), the reaction's direction is determined by the concentration of substrates involved. These reactions are apparently the reason for some of the accumulation of high energy bonds formed during mitochondrial oxidation of glutamate. According to McGilvery (1983), as many as 12 (ADP + P) are changed to 12 ATP. The Krebs Cycle is considered by Armstrong (1983) to be amphibolic, since the α-ketoglutarate is used not only as a port of entry into the Krebs Cycle, but also α-ketoglutarate, by the reverse reaction of GDH, can be used in L-glutamate synthesis. The significance of the Krebs Cycle in disposing of ammonia is evident in patients with alcoholic cirrhosis, where ammonia accumulation occurs and tremors may develop (Smith and Thier, 1981). It has been suggested by Smith and Thier (1981) that the ammonia is accumulated as a result of glutamate dehydrogenase catalysing the reverse reaction by which depletion of α-ketoglutarate occurs and interferes with the Krebs Cycle in carrying out its function, disposal of ammonia or urea synthesis. It is obvious that the reactions of transaminases (SGOT, SGPT)

and glutamate dehydrogenase must be in close equilibrium. However, as Newsholme and Leech (1983) point out, certain conditions must be met: oxacids, such as ketoglutarate, oxaloacetate, pyruvate, must be available; the process must be reversible; and the amino acids, such as glutamate, asparate and alanine, must not only be degraded but also synthesized.

Well balanced meals provide conditions for transdeamination, but as stated by Newsholme and Leech (1983), certain criteria have to be present: the ammonia and oxacids must undergo further metabolism; and the NAD(P)H must be oxidized to NAD(P) by passing through the electron transport chain, where it is first catalyzed by NADH dehydrogenase, according to Smith and Thier (1981), where 1 ATP is produced and two more ATP are generated along the chain giving a total of 3 ATP generated for 1 NAD(P)H oxidized (Fig.4).

One possibility that should be considered in GDH structure is that two biochemically different forms of Glutamate dehydrogenase may exist in human subjects: one form consisting of NADP-GDH, used for synthesis of glutamate and a second form consisting of NAD-GDH which

Fig.4 Diagram illustrating the two ports of entry into the mitochondrial respiratory chain. Port I shows entry of NADH; Port II shows site of entry of $FADH_2$. The pathway from Port I produces 3 ATP along the respiratory chain, while the pathway from Port II produces only 2 ATP through the respiratory chain. (Diagram modified from publications of White et al (1973), Harper (1973), Smith and Thier (1981).



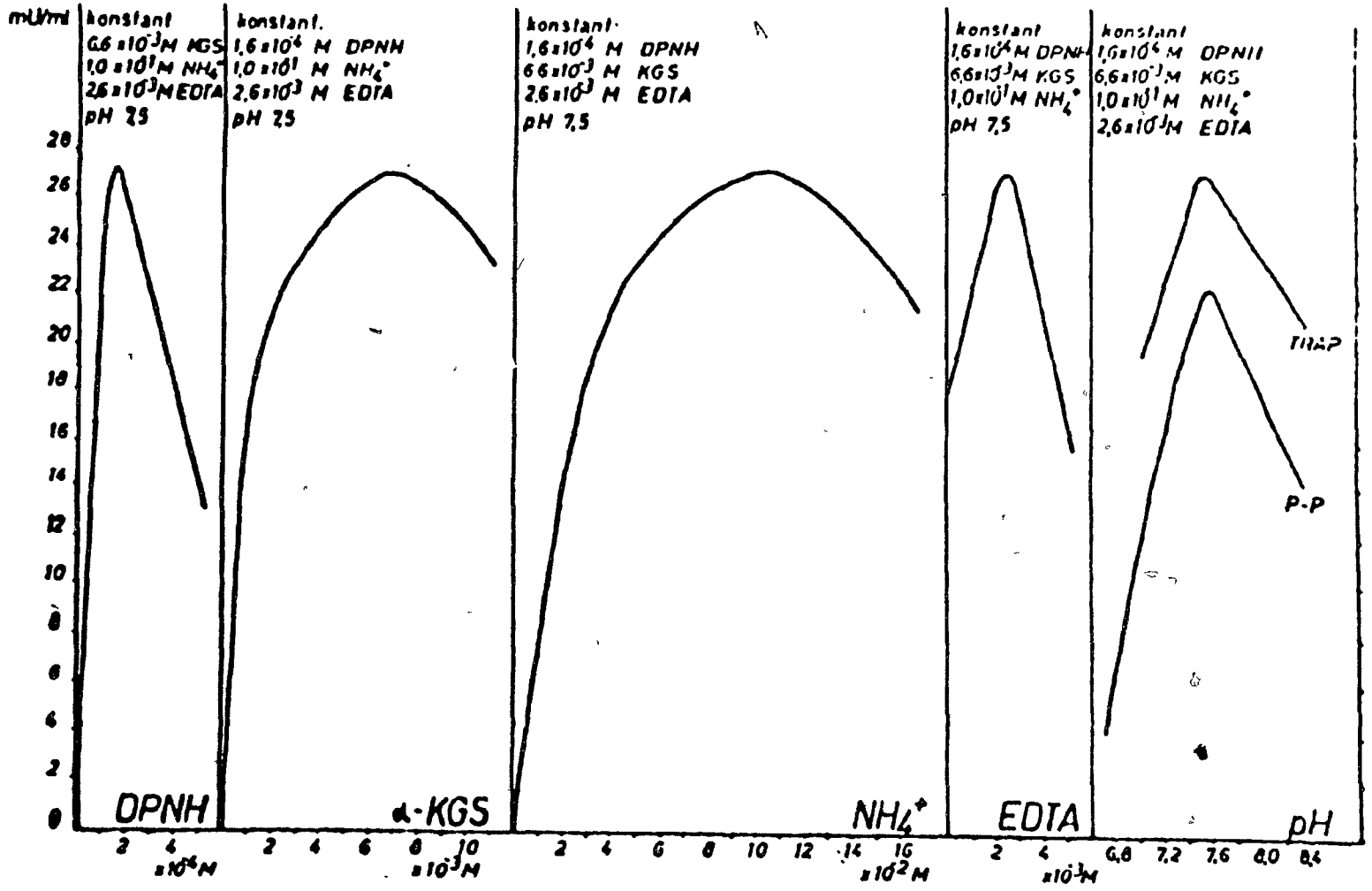
is used in the oxidative deamination of glutamate resulting in α-ketoglutarate formation. This presumption is strengthened by the finding of Hemmings (1984), who found that "yeast has the capacity to synthesize two genetically distinct glutamate dehydrogenases".

CHAPTER IV - METHODOLOGY

A. Methodology of Glutamate Dehydrogenase Determinations.

The methodology of serum GDH determinations was developed during the last 20 years. The original methodology has been modified several times and the results correlated with morphological findings, obtained from liver biopsy material. Schmidt and Schmidt (1962) used a reaction mixture of $DPNH_2$, TRAD (triethanolamine), EDTA and Ammonium Acetate with serum and found the serum GDH concentrations to be 100% ($\pm$5) on the first day and gradually declining to 70% ($\pm$21) within 12 days. When GDH was injected intravenously in dogs, the activity remained high in the first 2 hours (over 80%) and then declined to 20% over a period of 54 hours; a marked drop from 60% occurred at 11 hours. Schmidt and Schmidt (1962) studied 127 normal individuals and found the GDH activity to be below 1 mU/ml. They have shown the dependence of GDH activity on coenzymes, substrate, pH and EDTA concentration (Fig.5). Ellis and Goldberg (1972) assayed GDH activity and established the ultimate conditions of reaction mixtures, activators and

Fig.5 The Dependence of the speed of GDH reaction on co-enzyme substrate and EDTA concentration as well as pH level. (After Schmidt and Schmidt 1962)



reagents. They found that ADP was the best activator and that the pH should be optimally at 7.4 and the temperature at 37°C. Oxoglutarate was used in these studies as the initiator of the reaction. In previous studies, serum liver transaminase levels did not correlate with histological findings obtained in the course of liver biopsies (Kallai et al, 1964). It was VanWaes and Lieber (1977) who first carried out GDH measurements and correlated them with liver biopsy material obtained from chronic alcoholics. Worner and Lieber (1980a) carried out a study to determine the clinical application of GDH determinations and use as a marker in assessing liver damage produced by alcohol abuse (Worner and Lieber, 1980b). This study was carried out on alcoholics who were admitted for detoxification or treatment of complications arising from severe alcohol abuse. GDH was chosen as an indicator of alcohol toxicity to liver mitochondria. A very high concentration of GDH was observed in liver tissue extracts of these patients. It was found that not only does ethanol exert its main effect on the central zone of the hepatic lobule, but also that GDH appears to

play a major role in the derangement of mitochondrial metabolism in chronic alcoholics. The half-life of GDH is short, therefore measurements of GDH were carried out on blood samples obtained within 24 hours of the intoxication episode. Since cellular lesions persist longer than serum GDH elevations, the biopsies were still positive at day 10 after admission. Other tests carried out by Worner and Lieber (1980a,b) included PT, SGOT, SGPT, GGTP and bilirubin. No significant or consistent relationship was found between these parameters and liver biopsies, because of the large overlaps and perhaps due to differences in time, when serum samples were taken.

The liver biopsies were carried out using double blind methodology (VanWaes and Lieber, 1977; Worner and Lieber, 1980a,b). The morphological changes observed included Mallory bodies, fibrosis and necrosis, as well as steatosis and inflammatory changes. VanWaes and Lieber (1977) graded the degree of necrosis as follows: 0-absence of necrosis and parenchymal inflammation; 1^{+} occasional cell drop-out often shown by the inflammatory reaction, mononuclear in type;

2^+ scattered foci of necrotic cells in the parenchyma with polymorphonuclear infiltration predominantly in the centrolobular area (classified as "mild alcoholic hepatitis"); 3^+ diffuse parenchymal necrosis with polymorphonuclear infiltrates (classified as "frank alcoholic hepatitis")(VanWaes and Lieber, 1977).

The serum GDH determinations were classified into two groups: patients without necrosis (GDH < 12 IU or < 2½ times the upper limit of normal) and patients with 2^+ or 3^+ necrosis (GDH > 12 IU). This division showed a good correlation between serum GDH values and liver biopsies (VanWaes and Lieber, 1977). In group 1^+ necrosis, the GDH determinations varied from normal to clearly elevated, but not over the preset limit of 12 IU. The highest elevations of GDH were at the time of admission; at days 2-3, a marked decrease was noted and at days 5-9, the majority of patients had GDH values which returned to < 12 IU (VanWaes and Lieber, 1977). The results obtained from serum samples, taken after 5 days of alcohol abstinence, showed no correlation between GDH levels and morphological

changes observed during biopsies; while the results of serum GDH levels determined on blood samples drawn within 48 hours of alcohol abuse correlated well with the liver tissue biopsy results. The findings were confirmed in two separate studies by WanWaes (1977) as well as Worner and Lieber (1980 a,b). A group of 32 patients were followed for up to 15 months after discharge. The following parameters were evaluated: hepatomegaly, weight, jaundice, ascites, edema, anemia and coagulopathies. Blood tests included: Ht, SGOT, SGPT, PT, bilirubin and GDH determinations.

When our studies of GDH determinations in chronic alcoholics commenced, it became obvious that GDH determinations are not carried out in Quebec hospitals, nor are they included in the Governmental laboratory tests list. It became necessary to develop a suitable methodology which would be both simple and practical. The author has carried out these studies at the Laboratories of the Montreal General Hospital under the supervision of Dr. Paul Koch. Previously used methods of Schmidt and Schmidt (1962), WanWaes and Lieber (1977) and Worner and Lieber (1983) were initially tested in 10 patients; in each patient, the samples were tested five

times. The reaction mixture with serum was used as a "blank" (Ellis and Goldberg 1972). The purging was carried out using an automatic analyzer in which the absorbence is automatically set to zero. Using this method, it was found that the variation range between readings varied from 1.5 to 2.2.

Therefore, the same patients were tested using the method proposed by Dr. Koch (Koch and Pivon 1985), introducing changes which, according to our results, have improved the accuracy of GDH determinations. It was realized that the variability of results using standard methodology may have been due to the fact that automatic resetting of absorbence reading to zero does not take into consideration whether there is any residual enzyme left from the previous measurement which may influence the current reading. Manual methodology permitted us to bring the absorbence reading to zero prior to each reading; therefore, the number of purgings required varied but the result was uniformly constant. Deionized water was used for purging instead of reaction mixture with serum. Instead of pipetting the four ingredients (triethanolamine buffer, NADH

solution, ADP solution, ammonium solution) separately into a reaction tube, the ingredients were premixed and their concentrations adjusted so that the final reaction mixture volume was 2 ml; 0.5 ml of patient serum instead of 0.6 ml was then added. After 15 minutes, the reaction was then triggered by adding 0.1 ml of OC-Ketoglutarate (instead of 0.15 ml of Oxoglutarate) solution adjusted to account for the decrease in volume. Six-timed readings at 30-second intervals were taken on a 300N Gilford spectrophotometer. The multiplication factor was calculated according to Ellis and Goldberg (1972) taking into consideration the changed volumes. The modified methodology was repeated on the same samples from 10 patients and, as stated above, the results were more accurate; in the repeated reading of each sample, the variability ranged between 0.00 to 0.1.

B - Studies in Chronic Alcoholics

1. Studies in Normal Subjects

The serum of normal subjects was analyzed for baseline data utilizing the procedure outlined above. After blood samples were drawn (test tubes without heparin or EDTA), approximately 8 ml, they were left to stand for 20 minutes, after which they were centrifuged at 2,500 r.p.m. for 15 minutes. The serum was immediately pipetted and placed into two new test tubes. The serum of one tube was analyzed for standard serum values (SMA16). The serum in the second tube was used for determining the GDH level with a Gilford Stasar III Spectrophotometer and the modification developed by Koch (Koch and Pivon, 1985). The GDH level was calculated with a modified multiplication factor as follows: $\Delta E_{340} \times 2 \times 836$, where ΔE_{340} = the average difference between the six readings obtained at 30-second intervals. The use of this multiplication factor yields the mean GDH value, using the mean difference in ΔE_{340}, in IU per liter. An automated sampler was not employed; instead the whole procedure of GDH determinations was manually controlled.

The medical history of the normal control subjects was reviewed in order to rule out alcohol abuse as well as any medical condition or medication which might have an influence on our results. After a normal SMA 16 was obtained, the GDH determinations were carried out on the serum. Since the results of GDH tests were found to be close to the previously established normal range (Worner and Lieber, 1980 a,b), the results were tabulated. The total number of control subjects obtained was 80. The values of their GDH determinations can be seen in Table III in the chapter on Results.

2. Patient Population of Chronic Alcoholics

a) Studies at the Montreal General Hospital

The initial studies of serum GDH determinations and other serum liver enzymes, such as SGOT, SGPT, GGTP and Alkaline Phosphatase, were carried out on 12 subjects, who were treated as chronic alcoholics at the Alcohol Treatment Unit of the Montreal General Hospital. The age of the population ranged from 44-59 in men and from 34-55 in women. All subjects had been followed from 2 to 9 months (average 6 months) and detailed histories were taken at the hospital with reference to

chronic alcohol abuse, which was found to last from 10-42 years (average 25 years). The methodology of GDH determinations, established previously using normal control subjects in whom alcohol abuse was completely excluded, was utilized in chronic alcoholics to ascertain the relationship between GDH determinations and other enzyme levels.

Israel (1983) pointed out that alcoholism represents a full range of diseases including physical and pharmacological criteria, such as tolerance and dependence. A questionnaire was used to obtain information pertaining to the patient's alcohol abuse and included the following details: how long has alcohol been abused, the pattern of alcohol abuse, preference of alcoholic beverages; are withdrawal symptoms experienced and are hepatotoxic medications being taken? The questionnaire forms are not incorporated here.

The blood samples were obtained at admission to the Treatment Unit. Nine patients were seen at the peak of their alcohol abuse, while 3 cases were seen within 24 hours of the acute episode. The blood was drawn into a vacutainer, left to stand for 20 minutes, centrifuged

for 15 minutes at 2,500 r.p.m. and the serum pipetted into two unused test tubes for determinations. The SMA 16 was usually carried out within an hour of obtaining the sample. The GDH determinations were carried out either the same day or within a few days, since frozen serum, according to Ellis and Goldberg (1972), can be stored for several weeks with no loss of activity. During the follow-up visits, GDH determinations were carried out the same day or within two days, as well as certain other tests such as: CPK, Creatinine, LDH, bilirubin, Urea nitrogen, Uric acid, Triglicerides, glucose, protein and albumin, and also certain oligoelements such as strontium and zinc. A follow-up questionnaire was administered to determine their alcohol abuse since the last visit, as well as changes in weight or apetite.

b) Studies on patients admitted to Half-Way Center

The study carried out at the Montreal General Hospital permitted us to develop the new methodology for serum GDH determination as well as to streamline the procedure for testing other serum enzyme levels. However,

it was realized from the beginning that the levels of enzyme activity have to be correlated with ethanol blood levels. Fortunately, at the time of this study, Drs. Kapur and Israel offered us samples to conduct clinical testing with the new methodology of alcohol serum level determination, prior to release for general usage. The method has already been validated in the original laboratory by comparing the results with those obtained by standard gas chromatography and ultra-violet spectroscopy. Studies were undertaken on consecutive cases admitted to La Maison Jean Lapointe, where patients abstained from ethanol intake during their 21-day stay. Blood samples were obtained by the nursing staff of the Center under the supervision of Mr. Jacques Perras. The blood was centrifuged, the serum was extracted and immediately frozen. In order to maintain the double-blind method, the Center labelled the samples under a medicare number without any further information. The serum alcohol level was measured by a different technician, using Alcohol Dipstick Methodology and the scale described in section (3) (Tables IV and V). GDH was determined on the Gilford Stasar III according to the method described above (Koch and Pivon 1985 In Press).

Serum GGTP, Alkaline Phosphatase, SGOT and SGPT were determined simultaneously on the same apparatus. The data was entered in the appropriate file on the IBM computer and stored in the following manner: each patient was assigned a file number and a letter according to the time of sample procurement: A = acute phase, D = discharge at 21 days and J = interval period. Data were then subjected to statistical analysis, taking into consideration serum enzyme levels and serum ethanol level.

The following groups of patients were studied:

I. Serum GDH, GGTP, Alk.Phos., SGOT, SGPT levels on admission compared to the ethanol blood level (acute and subacute phases).

II. Serum GDH, GGTP, Alk.Phos., SGOT, SGPT at day 21.

III. Serum GDH, GGTP, Alk.Phos., SGOT, SGPT during the interval phase.

All patients admitted to La Maison Jean Lapointe had a SMAC carried out and psychometric testing to determine their alcohol abuse pattern, dependence and family history. These studies are not the subject of this thesis, but they will be reported in a separate paper in a correlative fashion (Perras et al, In Preparation).

3. Dipstick Methodology of Serum Alcohol Determination

Current methods require not only specific instruments but also trained personnel to determine the alcohol level in body fluids. The dipstick technique was developed in order to make alcohol determination simple and useful even for technically untrained personnel. Although alcohol level was usually determined in the blood, it has been shown that the urine and saliva can also be used with the dipstick methodology. The dipstick method is a sensitive and semiquantitative method for assessing alcohol in body fluids. It is a simple, efficient and rapid thchenique which could be used in almost any situation and requires only one minute to carry out.

The dipstick methodology uses a competitive inhibitor of alcohol dehydrogenase (ADH), pyrazole (Reynier, 1969), at the site of reaction with ethanol. The inhibitor is used because ADH has a low Km (Sund and Theorell, 1963) and would become saturated at low concentrations of alcohol. The enzymatic reaction can, therefore, proceed in a quasi-linear mode with respect to substrate concentration in virtually any desired concentration

range of alcohol. The addition of diaphorase and iodo-nitrotetrazolium chloride, an electron-accepting chromogen (Lim and Buttery, 1977), allows for a visual detection of alcohol presence. The visible reaction is possible because of the photo-sensitive reaction of the tetrazolium, but it is pH dependent and, therefore, a TRIS buffer is used (Gella et al, 1981). Semicarbazide traps the acetaldehyde generated and the reaction can proceed in the proper direction. The reaction is summarized in Fig. VI.

Fig. VI The Dipstick Reaction (Kapur and Israel, 1985)

1. $\text{Ethanol} + NAD^+ \xrightarrow{\text{ADH}} \text{Acetaldehyde} + NADH + H^+$

2. $NADH + H^+ + \text{Tetrazolim Salt} \xrightarrow{\text{Diaphorase}} NAD^+ + \text{Formazan}$
(colourless) (coloured)

The enzyme stabilizer is albumin from bovine serum, with either dithiothreitol or dithioerythritol. A predetermined amount of buffered enzyme-cofactor-inhibitor is used to impregnate strips of filter paper, which are lyophilised and attached to a plastic strip at one end. This allowed for determining alcohol in the range of 5-160 mg/dl (1-35 mmol/L and 20-120 mmol/L) by comparing to a scaled chart the color change observed (from light pink to dark red) on the strip tested. The dipsticks were first checked on a PMQ 2-Zeiss Chromatogram-Spectrophotometer at 530nm, with light absorption relating well to the square root of alcohol level (Table IV). It turned out that the naked eye could also discriminate colour changes for the specific ranges considered, using a six-point colour chart (Tables V and VI). Patients from various centers were tested. Dipstick analysis was carried out on 931 urine samples and 631 serum samples. For urine, alcohol level verification (Table VII) was carried out using a U.V. spectrophotometer and the correlation coefficient was found to be 0.90 for ranges 0-160 mg/dl and 2-160 mg/dl. The serum alcohol levels were verified by gas chromatography (Table VIII) with a correlation coefficient

of 0.90 for the ranges of 0-140 mg/dl and 3-140 mg/dl. Saliva specimens were also studied on 51 subjects and the correlation coefficient was greater than 0.90.

The sensitivity was found to be 98.8%, specificity was 97.5% and efficiency 98%. The alcohol dipstick can also be converted into a quantitative method by using reflectance spectrophotometry to measure the colour intensity. The false positives were found to be below 2.2 mM (10 mg/dl). The dipstick at $-15^{o}C$ is stable for 12 months; at $4^{o}C$ for 7 months and at room temperature for up to 31 days.

In our studies, the dipstick methodology was used to correlate ethanol levels in body fluids with serum liver enzyme concentrations, both of which were determined on the same individual serum sample of each patient. This permitted correlation of serum liver enzyme levels with actual concentration of alcohol in serum in the same sample.

TABLE IV. Verification of Dipstick Methodology as checked for reflectance densitometry on a PMQ 2-Zeiss Chromatogram Spectrophotometer at 530 nm. The square root of the ethanol level was compared to peak heights of deflection for urine, serum and saliva (after Kapur and Israel 1985)

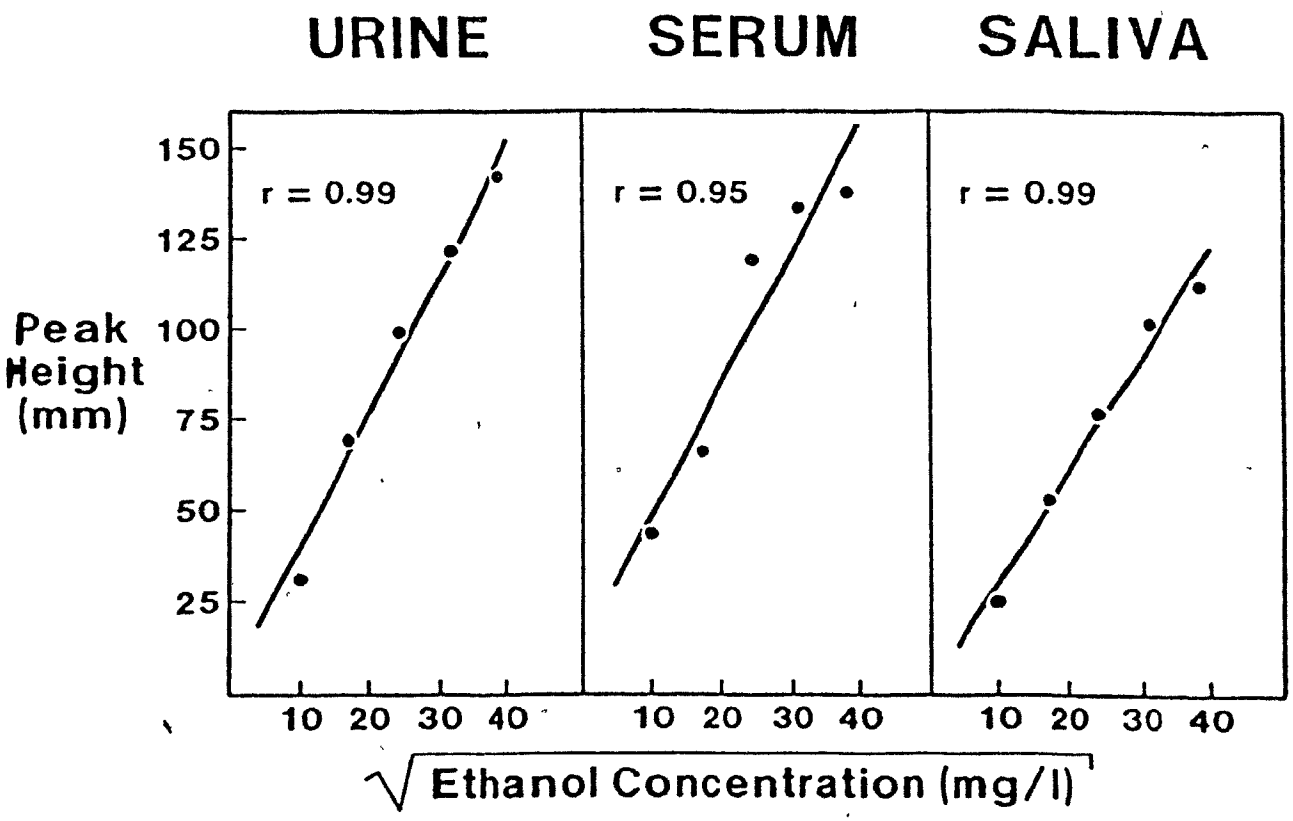
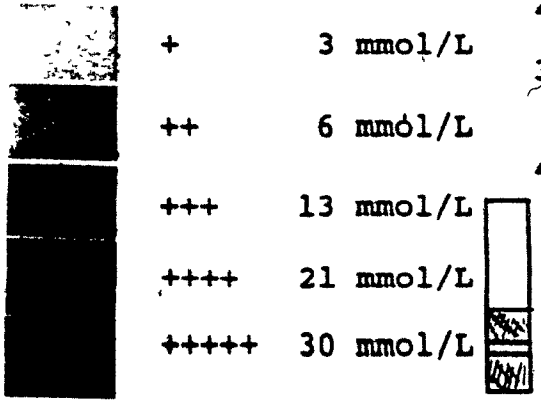


TABLE V. Classification of Alcohol Dipstick Results
Scale for Low Serum Ethanol Content (After Kapur and Israel 1985)



NEG	
+	3 mmol/L
++	6 mmol/L
+++	13 mmol/L
++++	21 mmol/L
+++++	30 mmol/L

ALCOHOL DIPSTICK
DIP-AND-READ TEST FOR
URINE/SALIVA ETHANOL

DIRECTIONS:

1. Dip test area of strip in fresh sample.
2. Tap edge of strip against container to remove excess.
3. Compare test pad with reference pad. If positive compare it with color chart at exactly 60 seconds.
4. If test pad is negative at 60 seconds continue observing for another 60 seconds. If at the end of this time it is positive then a trace amount of ethanol is present.

← Reference Pad
← Test Pad

Retighten cap immediately
Store at 0-4°C
Lot #

TABLE VI. Classification of Alcohol Dipstick Results
Scale for High Serum Ethanol Content (After Kapur and Israel 1985)

NEG	
+	10 mmol/L
++	20 mmol/L
+++	40 mmol/L
++++	80 mmol/L
+++++	120 mmol/L

ALCOHOL DIPSTICK
DIP-AND-READ TEST FOR
URINE/SALIVA ETHANOL

DIRECTIONS:

1. Dip test area of strip in fresh sample.
2. Tap edge of strip against container to remove excess.
3. Compare test pad with reference pad. If positive compare it with color chart at exactly 60 seconds.
4. If test pad is negative at 60 seconds continue observing for another 60 seconds. If at the end of this time it is positive then a trace amount of ethanol is present.

Retighten cap immediately
Store at 0-4°C
Lot #

TABLE VII. Comparison of urine ethanol determination by Alcohol Dipstick Methodology with that obtained by U.V. Methodology using standards with pre-determined optimal ranges (after Kapur and Israel 1985)

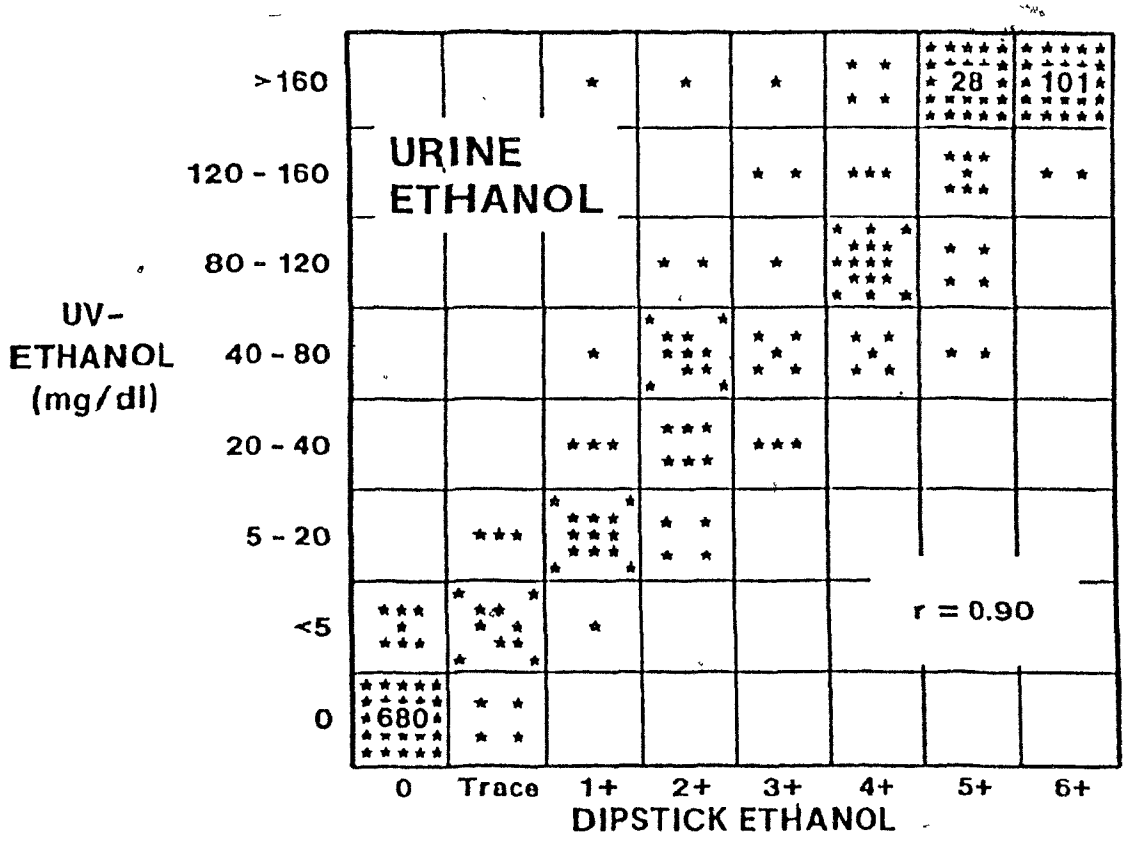
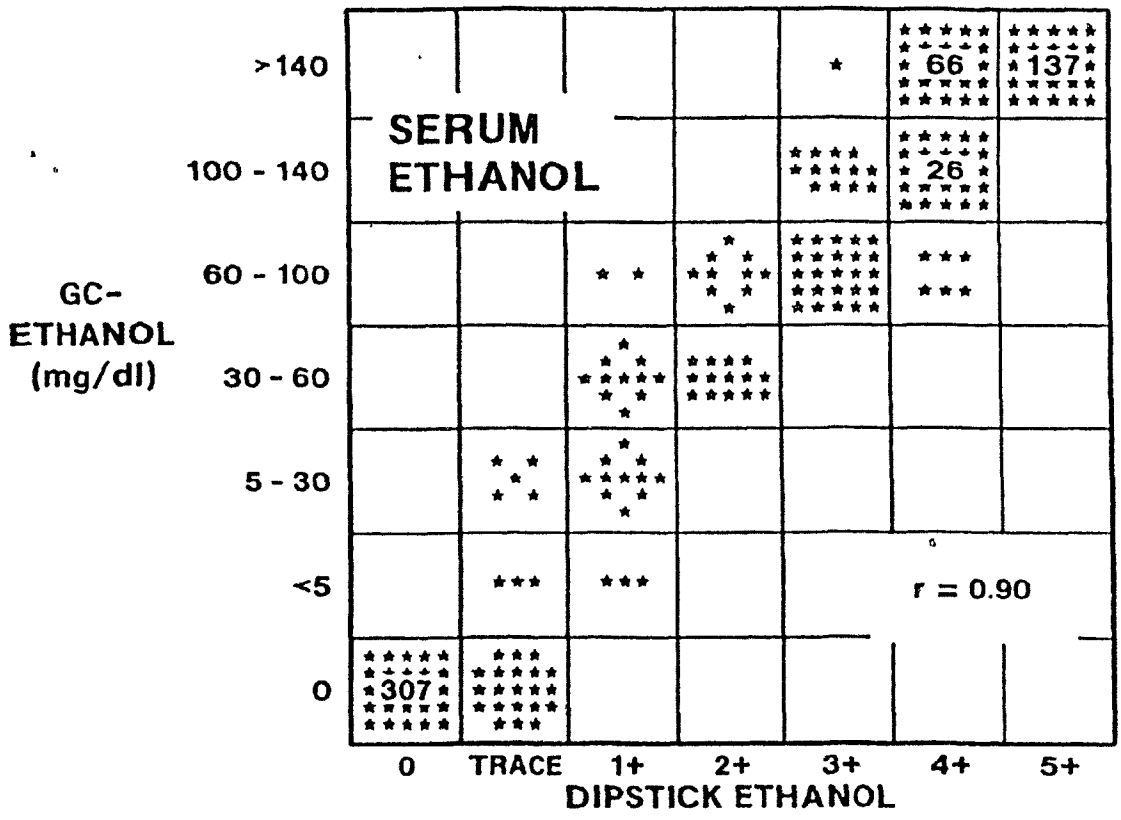


TABLE VIII. Comparative study of alcohol levels using Dipstick Methodology and Gas Chromatography carried out on serum standards with pre-determined optimal ranges (after Kapur and Israel 1985)



CHAPTER V - RESULTS

A. Serum GDH and Other Liver Enzyme Determination in Control Subjects.

Prior to studies in chronic alcoholics, the serum enzymes were determined in 80 control subjects. The subjects studied were all hospitalized without evidence or history of liver disease, which was verified by hospital records.

Table IX. Normal Values of serum enzyme levels as reflecting hepatic function.

ENZYME	VARIATION RANGE	MEAN VALUES
GDH	0.33 - 5.68 U/L	MEAN: 2.24
GGTP Al.Ph.	7 - 55 U/L 30 -110 U/L	GGTP/Al.Ph. MEAN : 0.29
SGOT SGPT	8 - 40 U/L 3 - 45 U/L	SGOT/SGPT MEAN : 0.8

B. Serum GDH and Other Liver Enzyme Determination in Chronic Alcoholics.

1. Pilot Studies

The results of the initial longitudinal studies on twelve chronic alcoholic patients at the Montreal General Hospital and St. Mary's Hospital are listed in Table X. These results demonstrate clearly that GDH values are markedly elevated during the acute phase, the mean being approximately 10 times higher than in control subjects. The samples were obtained on a monthly basis for a period of 6 months or more. When alcohol abuse was continued, the values remained elevated, variability depending on the amount of alcohol intake. It is significant that none of these patients had GDH value within the normal range.

As far as serum levels of GGTP and Alkaline Phosphatase are concerned (Table XI), both were significantly elevated. SGOT and SGTP levels varied, overlapping with normal subjects and no conclusions could be reached. Unfortunately, during this period, the alcohol dipstick methodology was still in the process of development and the alcohol serum levels were not determined.

TABLE X. Serum GDH determinations in 12 chronic alcoholics

PHASE	VARIATION RANGE	MEAN
NORMAL CONTROL	0.33 - 5.68	2.24
ACUTE PHASE	6.69 - 66.88	22.0
INTERVAL PHASE	2.34 6.02	3.86
CONTINUOUS ABUSE	12.70 - 37.79	22.73

TABLE XI. Serum GGTP and Alk.Phos. in 12 chronic alcoholics

NORMAL CONTROL SUBJECTS

ENZYME	VARIATION RANGE	MEAN
GGTP	7 - 55	21
Al.PH.	30 - 110	79
RATIO	0.073 - 0.692	0.29

ACUTE ALCOHOL PHASE

ENZYME	VARIATION RANGE	MEAN
GGTP	135 - 555	367
Al.PH.	56 - 133	101
RATIO	1.5 - 5.09	3.7

CONTINUOUS ALCOHOL ABUSE

ENZYME	VARIATION RANGE	MEAN
GGTP	117 - 425	283
Al.PH.	77 - 126	104
RATIO	1.5 - 3.7	2.64

INTERVAL or ABSTINANCE

ENZYME	VARIATION RANGE	MEAN
GGTP	56 - 137	77
Al.PH.	33 - 97	59
RATIO	1.14 - 2.08	1.25

2. Studies of serum enzyme levels in chronic alcoholics admitted to the Half-Way Center

Sixty chronic alcoholics consecutively admitted to La Maison Jean Lapointe were studied. In addition to the determination of serum liver enzyme levels (GDH, GGTP, Alk.Phos., SGOT, SGPT), psychometric studies were carried out on these patients, although the results are not part of this thesis. It is hoped that eventually the assessment of psychologic status of the chronic alcoholic personality could be correlated to the biochemical effects of ethanol abuse on the liver.

There were 44 males and 16 females in this group. The age of male subjects varied between 20-72, and that of female subjects between 25-66. In all cases, serum ethanol level was determined using the method of Kapur and Israel (1985). The serum ethanol level was divided into four ranges: 1) normal range: 0-5 mmol/L; 2) slight elevation: 5-20 mmol/L; 3) moderate elevation: 20-30 mmol/L; 4) marked elevation: 30+ mmol/L.

Two parameters were studied relating the ethanol serum level to the serum liver enzyme levels: a) GDH levels; b) GGTP, Alk.Phos., SGOT and SGPT levels.

a) Relationship between the different ranges of serum alcohol level and the serum GDH concentration ranges.

The average value in each range is reported (Table XII). Although the GDH range values overlap to a certain extent in the four groups of ethanol levels, there is a significant trend toward parallelism between serum ethanol level and serum GDH levels when the averages are considered. There were twenty-six patients who had serum ethanol levels within normal range and whose GDH values were also normal. In five patients, both the GDH ($<$ 10 U/L) and the serum ethanol levels ($<$ 20 mmol/L) were slightly elevated. When the serum ethanol level was higher than 20, none of these patients had normal GDH level and only three had slightly elevated GDH; six patients had either moderate or marked elevation of GDH level ($<$ 10 U/L). When the serum ethanol level was higher than 20, all seven patients had serum GDH values greater than 10 U/L and five of these were $>$ 30 U/L with the highest value being 76.24. At day 21 when ethanol blood levels were normal, all serum GDH values were within normal range. Fig.7 shows the mean serum GDH levels in the four groups of serum ethanol levels.

Fig.7 Blood Alcohol Level vs Serum GDH (60 cases)

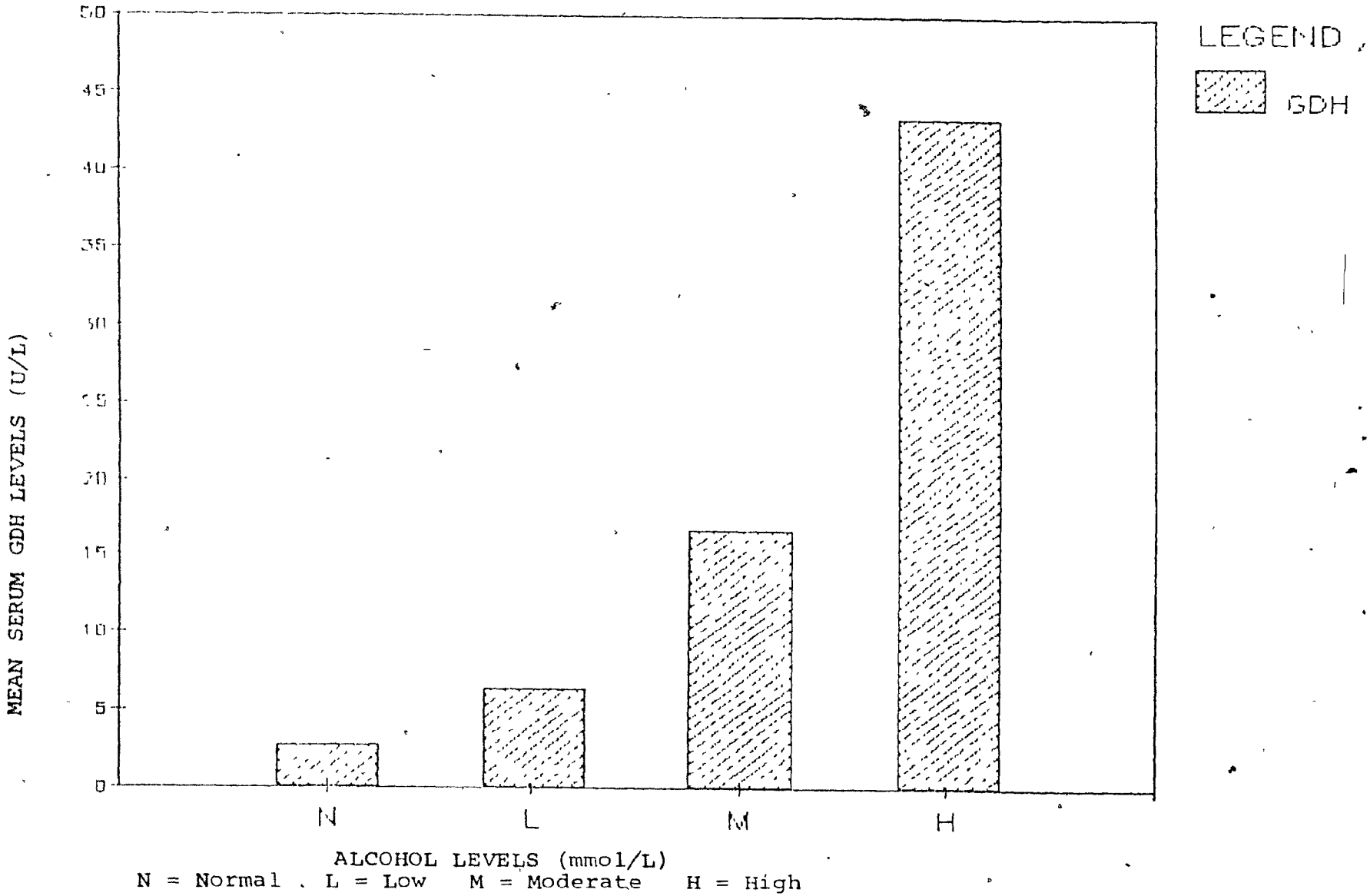
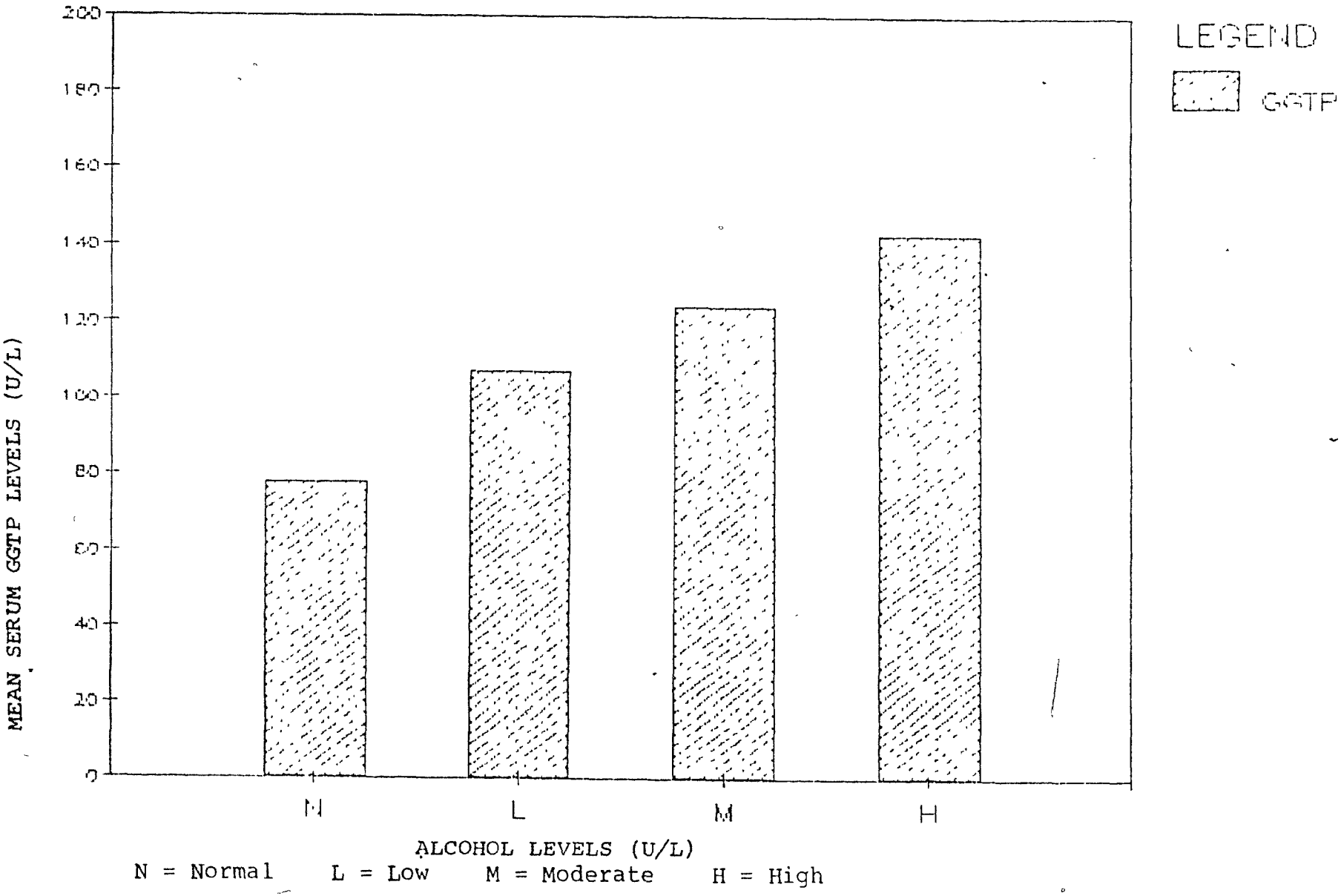


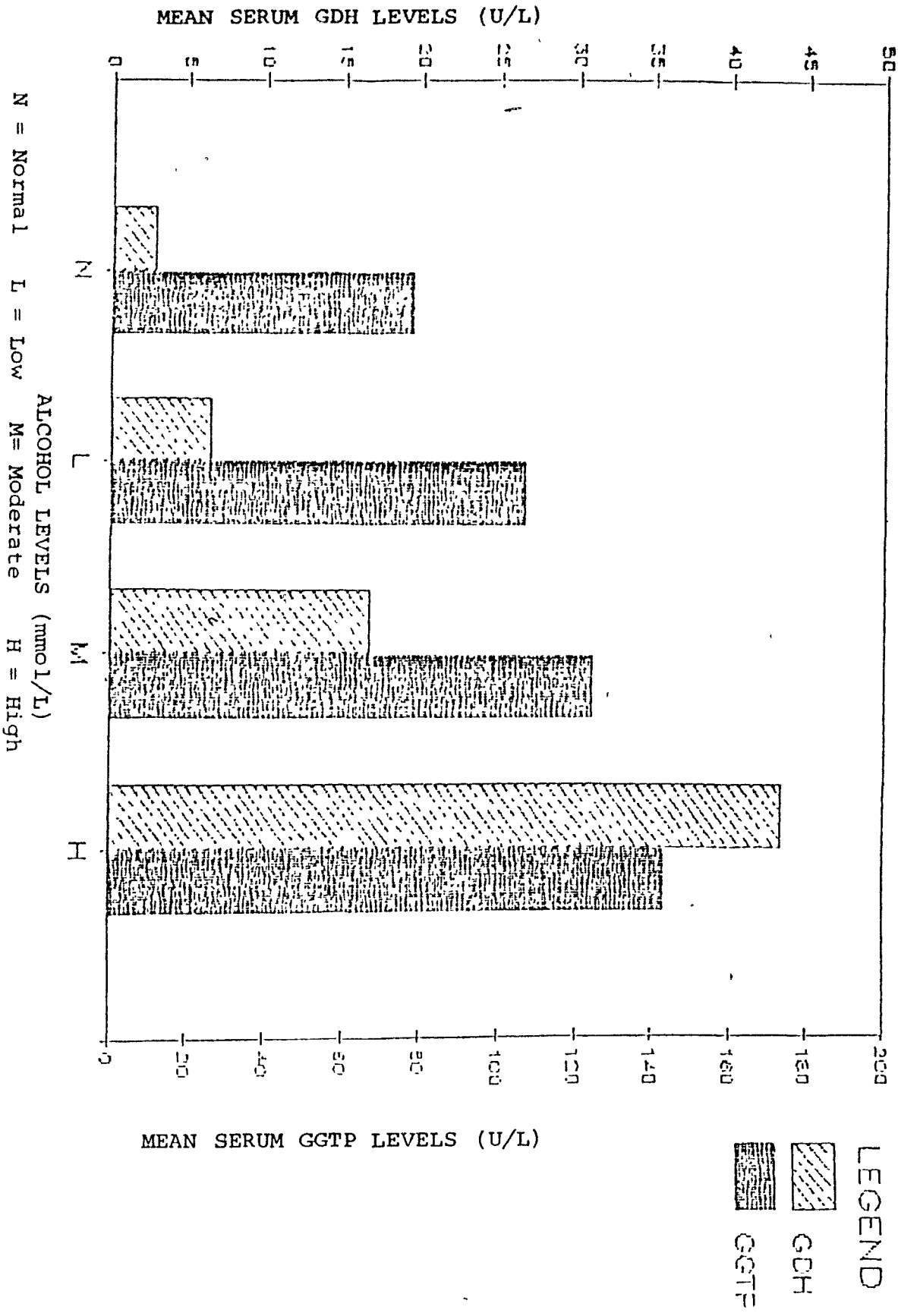
Table XII. Relationship between Serum GDH level and serum Alcohol level on admission (60 cases)

SERUM ETHANOL LEVEL (mmol/L)	0 - <5	5 - <20	20 - <30	30 +
GDH RANGE (U/L)	0.66 - 11.73	2.01 - 13.04	5.68 - 14.46	12.04 - 76.24
GDH AVERAGE (U/L)	2.82	6.56	16.84	43.43

b) Relationship between the different ranges of serum alcohol levels and the GGTP, Alk.Phos., SGOT and SGPT levels on admission (60 cases)

Taking into consideration the normal range of values of these enzymes, the GGTP was considered to be significantly elevated when the serum level was 100 U/L or higher. Fig.8 shows the GGTP levels in the four groups of serum ethanol level. It is evident that even when ethanol level was low (5-10 mmol/L), the GGTP was elevated in 7 cases; in these cases, the GDH was elevated in only 3 cases; since significant GDH elevation occurs only during the acute phase and is also of much shorter duration than that of GGTP, it appears that these 3 cases were admitted at least 48 hours and possibly longer after the acute episode (Fig.9). GGTP was significantly elevated


LEGEND
GGTP
MEAN SERUM GGTP LEVELS (U/L)
200
180
160
140
120
100
80
60
40
20
0
N
L
M
H
ALCOHOL LEVELS (U/L)
N = Normal L = Low M = Moderate H = High


MEAN SERUM GDH LEVELS (U/L)
0
5
10
15
20
25
30
35
40
45
50
N
L
M
H
ALCOHOL LEVELS (mmol/L)
N = Normal L = Low M= Moderate H = High
0
20
40
60
80
100
120
140
160
180
200
MEAN SERUM GGTP LEVELS (U/L)
LEGEND
GDH
GGTP

(over 100 U/L) in 5 cases, when serum ethanol level was over 20 mmol/L; there were 13 cases of elevated GDH in the same category of ethanol level. This finding suggests, if confirmed in a larger number of cases, that the rise in GGTP following an acute alcoholic episode is much slower than that of GDH.

Alkaline phosphatase does not seem to be a good indicator of hepatic injury by alcohol. When the Alk. Phos. level of 150 U/L or more is considered abnormal, it was found to be elevated in only 2 cases of serum ethanol level of 20-30 mmol/L, and in 1 case of ethanol level over 30 mmol/L. Perhaps in this higher range of simultaneous GGTP and Alk.Phos. elevation, the GGTP/Alk. Phos. ratio may be useful as far as judging the severity of the injury.

The SGOT was considered elevated when the serum value was higher than 75 U/L; in this category, there were 8 cases; in all of these, other enzymes were also significantly higher to various degrees. SGPT was elevated on admission (over 80 U/L) in only patient out of 60 cases studied; in this instance, all other enzymes were also elevated.

The number of cases in low and high level groups of GDH, GGTP, Alk.Phos., SGOT and SGPT, in the four groups of serum ethanol concentrations as determined by the method of Kapur and Israel (1985), are listed in Table XIII.

Table XIII. Number of cases with Normal (N) and significantly elevated (H) enzymes in the four groups of serum ethanol concentrations on admission (60 cases).

GROUP I : SERUM ALCOHOL RANGE = 0 - < 5 CASES = 32

GDH	GGTP	AP	SGOT	SGPT
N = 31	N = 23	N = 31	N = 32	N = 32
H = 1	H = 9	H = 1	H = 0	H = 0

GROUP II : SERUM ALCOHOL RANGE = 5 - < 20 CASES = 12

GDH	GGTP	AP	SGOT	SGPT
N = 9	N = 7	N = 11	N = 10	N = 12
H = 3	H = 5	H = 1	H = 2	H = 0

GROUP III : SERUM ALCOHOL RANGE = 20 - < 30 CASES = 9

GDH	GGTP	AP	SGOT	SGPT
N = 3	N = 6	N = 7	N = 7	N = 9
H = 6	H = 3	H = 2	H = 2	H = 0

GROUP IV : SERUM ALCOHOL RANGE = 30 + CASES = 7

GDH	GGTP	AP	SGOT	SGPT
N = 0	N = 5	N = 6	N = 3	N = 6
H = 7	H = 2	H = 1	H = 4	H = 1

CHAPTER VI - DISCUSSION

1. Methodology of GDH Determination.

The history of GDH determination, reviewed previously, shows a progression in the development of understanding the origin and role of Glutamate Dehydrogenase as reflecting mitochondrial function. Hogeboom (1953) determined the mitochondrial origin of GDH and was able to isolate the enzyme from liver homogenates. Using spectrophotometric methods, he determined the GDH level in the serum of normal subjects. VanWaes and Lieber (1977) were the first to determine serum enzume levels in chronic alcoholics and have demonstrated a consistent elevation of GDH corresponding to morphological changes in liver biopsy material. In the current study, the methodology of GDH determination was modified at the Montreal General Hospital Laboratories under the supervision of Dr. Paul Koch. The α- Ketoglutarate was used as an initiator of the reaction mixture instead of oxoglutarate employed in previous methodology. In our studies, fresh α- Ketoglutarate was used. Studies using initiator kept frozen for up to 7 days showed a decrease of 0-10% in GDH values.

Instead of pipetting the ingredients separately, they were premixed and their concentrations were changed to produce the final reaction mixture volume of 2.6 ml instead of 3.0 ml ; 0.5 ml of serum was used. The reaction was triggered by the addition of 0.1 ml of α - Ketoglutarate. The multiplication factor was calculated in the following manner:

$$U = \frac{2.6 \text{ ml (volume measured)}}{0.5 \text{ ml (serum volume)}} \times \frac{10^3}{6.33} \times \Delta E_{340} / \text{min.}$$

Since the readings were taken every 30 seconds, the following multiplication factor was used:

$$\frac{\text{(sum of readings)}}{\text{(number of readings)}} \times 2 \times 836 = 1672$$

All GDH assays were carried out manually, since it was found that purging the system did not constantly result in zero absorbence reading prior to the next measurement and, therefore, sometimes required extra purging; this is a definite advantage of manual methodology over an automated system. As observed in 80 normal subjects (controls) the variation range was from 0.33 to 5.68 U/L; when this range is compared to previously used methodology (Worner and Lieber 1977),

the scale is wider towards the lower values, indicating increased accuracy in the low value range.

In repeated testing of the same samples, the differences in absorbence were the same, indicating the accuracy of the method.

The minimal water bath time was found to be 15 minutes and the maximum 180 minutes without influencing the results.

As far as the period between procurement of centrifuged serum samples and the time of GDH determination is concerned, we found that if the serum sample is immediately frozen and stored at -20^{O}C for a period of 14 days, the results are not adversely affected. When longer freezing periods are used, the results are less accurate. When the serum sample was obtained in a hemolized state, the GDH Assay readings were higher on the average 15-20%; to omit the possibility of error in our studies, only the non-hemolized samples were employed.

These studies on methodology of GDH determination were carried out on 12 chronic alcoholic patients at the Montreal General and St. Mary's Hospital and the results are listed in the preceding chapter. The

methodology of determination of GGTP, Alk.Phos., SGOT and SGPT was also streamlined in our investigation on these patients in order to obtain a total picture of the serum enzyme levels in ethanol abuse cases. The group is obviously too small to draw any statistically significant data; as seen in the preceding chapter, only two cases in this group could be classified as severe alcoholics. However, the value of this pilot study consists of the fact that in some of these cases, the study was extended over a period of nine months. We were able to determine the differences in serum enzyme levels between the acute phase and the interval periods, as well as when the ethanol abuse was continued. It was observed that the GDH values remain elevated up to 48 hours after the acute episode; however, the serum level of GDH gradually decreases after this period. The highest GDH values were observed in intoxicated patients. When patients were too intoxicated to report to the hospital, the sample was obtained at home, naturally with full agreement of the subject.

2. Assays of GDH and other serum enzymes in Chronic Alcoholics

As previously mentioned in the course of this year, we were able to obtain the cooperation of the program director of La Maison Jean Lapointe, M. Jacques Perras, who provided us with access to carry out serum enzyme determinations of serum enzyme levels in 60 consecutive cases of chronic alcoholism admitted to the Institute. Drs. Kapur and Israel, of the Addiction Research Foundation of Ontario, provided us with the sufficient number of dipsticks to measure alcohol serum levels at the same time when the serum enzymes were determined. This enabled us to fulfill the objective of this work, namely to correlate the serum GDH levels with that of alcohol blood level.

In severe alcoholics, where the GDH values were higher than 20 U/L, the serum alcohol level ranged from 30 to 120 (or more) mmol/l; since all these cases were intoxicated, this confirms our initial observations made at the Montreal General Hospital that GDH value reflects accurately the extent of mitochondrial damage during the acute phase. When the GDH values are

compared with those obtained at day 21, it becomes obvious that GDH levels should return to the normal range after abstinence. Since all these patients had no alcohol intake under the strict supervision of the personnel at La Maison Jean Lapointe, this confirms the conclusion of many investigators of chronic alcoholism, that abstinence is the ultimate goal of treatment. In 8 cases, a moderate increase in the serum GDH level was observed, ranging from 10.03 to 16.7 U/L; all these values, according to our proposed classification, were considerably higher than the normal range (0.33 - 5.68 U/L).

The serum alcohol level in these subjects ranged between 12 mmol/l and 60 mmol/l. In the remaining 44 cases, the GDH serum level was within normal range (0.66 - 5.68 U/L) or insignificantly increased (6.00 - 9.40 U/L). These cases, though classified as chronic alcoholics, were obviously admitted in the interval phase without evidence of acute intoxication. The mitochondria have a remarkable power for rapid recovery; if pathologic mitochondrial swelling were observed in these cases, it would probably start to

recede within 48 hours after the last intake of ethanol containing beverages.

When marked or moderate increase in GDH is considered as an indication of severe mitochondrial damage, the incidence of heavy alcohol abuse would be around 25%. This figure is in agreement with observations on the incidence of liver damage in chronic alcoholics, which is stated to be approximately 20 - 30% (Vierling 1983).

Although the primary objective of this study was to develop the methodology of serum GDH determination and to correlate these with serum alcohol levels, it was observed that the determination of other serum liver enzymes is a valuable adjunct in the assessment of liver damage in chronic alcoholics.

According to our results, the GGTP is the best indicator of liver damage in the interval phase; values ranging from 102 - 209 U/L were observed in patients. These values, according to our classification, were at least twice higher than the normal range (11.0 - 50.0 U/L in men and 7.0 - 32.0 U/L in women). When the serum was taken in the acute phase of alcohol abuse, as indicated by serum ethanol levels,

the GDH was extremely elevated. However, when GDH was normal or slightly elevated, in some cases the GGTP level was still markedly increased.

Alkaline phosphatase levels were elevated in 5 cases, when values higher than 150% of the normal range are considered to be significant. Alkaline phosphatase does not appear to be a good indicator of liver damage in chronic alcoholics. In 2 of these cases, the serum liver enzyme levels were normal, while in 3 cases, they were concommitantly increased. The GGTP/Alkaline Phosphatase ratio should perhaps be included as a parameter when assessing the chronic alcoholic, since in the majority of cases of liver damage, the alkaline phosphatase is also increased, although to a lesser extent, but for a longer period. This statement, though not fully qualified because of the small number of cases, is based on our preliminary observations made on the chronic alcoholics treated at the Montreal General Hospital and St. Mary's Hospital.

The SGOT values were increased in 8 patients, the values ranging from 95 - 256 U/L; this corresponds to

100% above the normal value range (0-37 U/L in males and 0-31 U/L in females). The SGPT serum levels in this study show no correlation to either ethanol blood level or levels of other enzymes including GDH. This may indicate that in chronic alcohol abuse, the derangement in transdeamination occurs towards the oxaloacetate pathway rather than the pyruvate pathway.

One final comment should be made about the sex ratio in chronic alcoholics. In this study, the male to female ratio was approximately 5:1 in 60 cases (44 vs 16). When only the severe cases are included, the ratio is approximately 3:1 (15 vs 4). If the trend shown continues in a large population group, it appears that the extent of liver damage in the acute phase of alcohol abuse would be more severe in male subjects.

3. Perspectives in GDH assay in liver diseases.

After the new modification for GDH assay was developed, it was realized that the determination of GDH adds a new parameter in the assessment of liver

function, namely that reflecting mitochondrial injury. GDH may become useful in other surgically and medically treated liver diseases. GDH was determined in several patients admitted in the Department of Surgery; since the number is small, the results have no statistical significance. However, it may be worth-while to give at least one example of the results obtained.

A 56-old male was admitted with a history of long standing back pain and recent obstructive jaundice. A diagnosis of pancreatic carcinoma was made on the basis of ultrasound and endoscopic examinations. The serum enzyme determinations were carried out prior to surgery and at intervals after the operation and are shown in Table XIV . The serum GDH value prior to surgery was 100.65 U/L and immediately after surgery, consisting of choledochojejunostomy with complete relief of biliary obstruction, the GDH level fell to 35.11 U/L, signifying a significant drop of 65% (as for bilirubin and other enzymes, the percentage drop ranged from 4 - 21%) and gradually returning to well within normal (12.04 U/L on the fifth postoperative day, 11.7 U/L on the eighth postoperative day and 9.36 U/L on the fourteenth post-

Table XIV Serum GDH and other liver enzyme values in a patient with obstructive jaundice.

ENZYMES	FEB-8	* FEB-13 *	FEB-18	FEB-21	FEB-27	MAR-14
GDH	100.65	35.11	12.04	11.70	9.36	4.68
GGPT	950	800	268	198	145	86
ALK.PHOS.	700	650	320	263	190	117
RATIO	1.36	1.23	0.84	0.75	0.76	0.73
SGOT	137	107	44	32	20	18
SGPT	245	218	73	60	28	15
RATIO	0.56	0.49	0.60	0.53	0.87	1.20
BILIRUBIN	9.1	8.7	3.0	2.4	1.6	0.8

Footnotes: FEB - February GDH - Glutamate Dehydrogenase

February-13: is the day of choledochojejunostomy.

operative day) by day 27 when the GDH was 4.68 U/L (normal < 5.68 U/L). GGTP and Alkaline Phosphatase were also markedly elevated, as were SGOT and SGPT; however, it should be stressed that the decrease of the other liver enzymes was much slower, especially Alkaline Phosphatase. This patient recovered rapidly after surgery. The implication is that if the fall in serum GDH is rapid after surgery, this would indicate recovery of mitochondrial function and a good prognosis for patient recovery; on the other hand, persistence of high serum GDH values would indicate irreversible damage to mitochondrial structure.

CONCLUSIONS

1. Serum GDH determination is a valid method for assessment of liver injury in chronic alcoholics during the acute episode reflecting the extent of mitochondrial damage.

2. Serum GDH values on admission to the half-way center were found severely elevated in 8 patients (22.00 - 76.24 U/L) and moderately elevated in 9 patients (11.70 - 16.70 U/L). In 45 patients, the GDH values were either normal or slightly elevated.

3. The GGTP was markedly elevated in 19 patients (103 - 441 U/L) at admission; on departure, six of these patients still had high levels of GGTP (102 - 209 U/L). In two patients, the GGTP was increased at day 21 when compared to the level of GGTP on admission. One patient had normal GGTP level on arrival but high value at day 21 (32 - 128 U/L). The differences between GDH and GGTP values on admission can be explained by the variation in time lapse between the last acute alcoholic episode and the time of admission.

4. Serum GDH values correlate well with ethanol blood level, determined in the same samples (Sensitivity: 81.25%; Specificity 90.91%; Positive Predictive Value: 76.47%; Negative Predictive Value: 93.02%).

5. Studies on serum liver enzyme patterns in chronic alcoholics may contribute to the assessment of the drinking pattern as well as the extent of liver injury.

6. In any proposed treatment regime, serum GDH levels and other liver enzymes may be useful in assessing the results obtained, without resorting to invasive techniques, such as repeated liver biopsies which are difficult in chronic alcoholics.

7. Although the goal of treatment in chronic alcoholics appears to be abstinence, assessment of subjects, who continue to abuse alcohol, remains a valid objective.

8. The possibility of using serum GDH determination in other surgical and medical conditions affecting the liver are briefly outlined.

REFERENCES

Aldridge W.N., Barnes J.M. and Denz F.A. 1949. Experimental beryllium poisoning. Brit. J. Exp. Pathol. 30, 376-389.

Allard C., de Lamirande G. and Cantero A. 1957. Behaviour of enzymes in liver of starved rats. Exp. Cell Res. 13: 69-77.

Armstrong F.B. 1983. Biochemistry, 2 ed., Oxford University Press, 134-313.

Beaufay H., Bendall D.S., Baudhuin P. and de Duve C. 1959. Intracellular distribution of some dehydrogenases, Alkaline Deoxyribonuclease and rat-liver tissue. I - Tissue Fractionation Studies. Biochem. J. 73: 623-628.

Black M., Mitchell J.R., Zimmerman H.J., Ishak K.G. and Epler G.R. 1975. Isoniazid-associated hepatitis in 114 patients. Gastroenterology 69: 289-302.

Christie G.S., Judah J.D. 1953. Intracellular distribution of enzymes. Proc. Roy. Soc. B141: 420-433.

De Duve C., Pressman B.C., Gianetto R., Wattiaux R. and Appelmans F. 1955. Tissue Fractionation Studies. Intracellular distribution patterns of enzymes in rat liver tissue. Biochem. J. 60: 604-617.

Delpre, G. and Kallish U. 1984. Ketoconazole and the liver: further considerations. Gastroenterology 87: 985-986.

Diaz-Rivera R.S., Callazo J.P., Pons E.R. and Torregrossa M.V. 1950. Acute phosphorus poisoning in man: a study of 56 cases. Medicine 29: 269-298.

Duarte P.A., Chow C.C., Fimmons F. and Ruskin J. 1983. Fatal hepatitis associated with ketaconazole therapy. Arch. Inter. Med. 144: 1069-1070.

Ellis G., Goldberg D.M. 1972. Optimal Conditions for the Kinetic Assay of Serum Glutamate Dehydrogenase Activity at 37°C. Clin. Chem. 18: 523-527.

Fersht A. 1985. Enzyme Structure and Mechanism. 2 ed. W.H. Freeman and Company, New York, 136-616.

French S.W. and Burbige E.J. 1979. Alcoholic hepatitis: Clinical, morphological, pathogenic and therapeutic agents. pp. 557-559. In: Progress in liver disease, Popper H. and Schaffner F., Eds., Vol.6, Grune and Stratton, New York.

Gella F.J., Olivella M.T., Pegueroles F. and Gener J. 1981. Colorimetry of Diaphorase in Commercial Preparations and Clinical Chemical Reagents by Use of Tetrazolium Salts. Clin. Chem. 27: 1686-1689.

Geokas M.C. 1981. Ethanol, the liver and the gastrointestinal tract. Ann. Intern. Med. 95: 193-203.

Harper H.A. 1973. Review of Physiological Chemistry. Lange Medical Publication, Los Altos, California, 171-179.

Hemmings B.A. 1984. The role of protein phosphorylation in the regulation of the NAD-dependent glutamate dehydrogenase from yeast, pp. 155-165. In: Enzyme regulation by reversible phosphorylation - further advances. Cohen P., Ed., Elsevier Science Publishers, B.V.3.

Henry R.J., Cannon D.C. and Winkelman J.W. 1974. Clinical Chemistry, 2nd Ed., New York, Harper and Row, 1043.

Hogeboom G.H. and Schneider W.C. 1953. Intracellular Distribution of Enzymes, XI Glutamic dehydrogenase, J. Biol. Chem. 204: 233-238.

Hogeboom G.H., Schneider W.C. and Pallade G.E. 1948. Cytochemical studies of mammolian tissues, I: Isolation of intact mitochondria from rat liver. Some Biochemical properties of mitochondrial and submicrosomal particulate material. J. Biol. Chem. 172: 619-635.

Hogeboom G.H., Schneider W.C. and Striebich M.J. 1952. Cytochemical studies, V: On the isolation and biochemical properties of liver cell nuclei. J.Biol. Chem. 196: 111-120.

Horn J.L., Skinner H.A., Wanberg K. and Foster F.M. 1984. Alcohol use questionnaire (Alcohol Dependence Scale). Add. Res. Found., Toronto, Ont., 1-4.

Israel Y. 1981. Early Identification of Alcohol Abuse: Definitional Issues and Diagnostic Dilemmas. Introductory Remarks, In: International Alcohol Research Seminar, Washington, D.C.

Kallai K., Hahn A., Roder V. and Zupanic V. 1964. Correlation between histologic findings and serum transaminase values in chronic diseases of the liver. Acta Med. Scand. 175: 49-56.

Kapur B.M. and Israel Y. 1985 (In Press). Alcohol Dipstick - A Rapid Method for analysis of Ethanol in Body Fluids. Res. Monograph No 17, U.S. Dept. of Health and Human Services.

Kay H.D. 1930. I - Methodology of determination: Some properties of the enzyme. J. Biol. Chem. 89: 235-247.

Klatskin, G. 1974. Drug-induced hepatic injury. pp.163-175. In: The liver and its diseases, Schaffer F. and Sherlock S., Eds., Int. Med. Books, New York.

Koch P. and Pivon R.J. 1985 (In Press). Modified method for Glutamate Dehydrogenase determination.

Koch M.J., Seeff L.B., Crumley C.E., Rubin L. and Burns W.A. 1976. Quinidine hepatotoxicity. Gastroenterology 70: 1136-1140.

Krebs H.A., Hems R. and Lund P. 1973. Accumulation of amino acids by the perfused rat liver in the presence of ethanol. Biochem. J. 134: 697-705.

Lewis J.H., Zimmerman H.J., Ishak K.C. and Mullick F.G. 1983. Enflurene hepatotoxicity. Ann. Intern. Med. 98, 984-992.

Lieber C.S. 1981. Pathogenesis of alcoholic liver injury. pp.199-203. In: Ethanol, the liver and the gastrointestinal tract, Geokas M.C., Ed., Ann. Intern. Med. 95.

Lieber, C.S. and Leo M.A. 1982. Liver injury by ethanol. pp.259-312. In: Major problems in internal medicine, Lieber C.S. and Leo M.A., Eds.

Lim H.H. and Buttery J.E. 1977. Determination of ethanol in serum by enzymatic PMS-INT Colorimetric Method, Clin. Chem. Acta, 75: 9-12.

Lunzer M.R., Huang S.N., Ward K.M. and Sherlock S. 1975. Jaundice due to erythromycin estolate. Gastro-enterology 68: 1284-1291.

McGilvery R.A. 1983. Biochemistry: A Functional Approach. Saunders W.B., Philadelphia, 576-643.

Meyer R.E., Glueck B.C., O'Brien J.E., Babor T.F., Jaffe J.H. and Stabenan P., Eds., 1981. Evaluation of the alcoholic: implications for research, theory and treatment. Proc. Farmington Conf. U.S.H.H.S., Res. Mono 5: 409.

Newsholme E.A. and Leech A.R. 1983. Biochemistry for the Medical Sciences. John Wiley & Sons, Toronto, 398-629.

Patterson D.J., Dew E.W., Gyorkey F. and Graham D.Y. 1983. Niacin hepatitis. South. Med. J. 76, 239-241.

Pepelko W., Dourson M.L. and Stara J.F. 1984. Ambient water quality criteria for berillium and health assessment. EPA 440-5-80-024 and 600-8-84-026A, Env. Prot. Agency Publ., Washington, D.C.

Pivon R.J. and Skoryna S.C. 1985. Clinicopathologic classification of hepatotoxic effects of trace substances. Trace Elem. Med. In Press.

Powell-Jackson P.R., Tredger J.M. and Williams R. 1984. Hepatotoxicity to sodium valproate: a review. GUT 6, 673-681.

Powers S.G. and Meister A. 1982. Ureal Synthesis and Ammonia Metabolism. pp.251-263. In: The Liver: Biology and Pathobiology. Arias I., Popper H., Schacter D. and Shafritz D.A., Eds., Raven Press, New York.

Purich D.L. 1983. Contemporary Enzyme Kinetics and Mechanism. Acad. Press, New York, 71.

Rappaport A.M., Borowy Z.J. and Lotto W.N. 1954. Subdivision of hexagonal liver lobules into structural and functional unit: role of hepatic physiology and pathology. Anat. Rec. 119, 11-33.

Rawn J.D. 1983. Biochemistry. New York, Harper and Row. 822-826.

Reynier M. 1969. Pyrazole Inhibition and Kinetic Studies of Ethanol and Retinol Oxidation Catalyzed by Rat Liver Alcohol Dehydrogenase. Acta Chem. Scand. 23: 1119-1129.

Schellenberg F., Weill J.,and Lamy J.N. 1983. Place de la glutamate déshydrogénase dans le dépistage biologique des beuveurs excessifs. Ann. Biol. Clin. 41: 255-262.

Schmidt E. and Schmidt F.W. 1962. Methode und Wert de Bestimmung der Glutaminsaure - Dehydrogenase - Aktivitat in Serum ein Beitrag zur Bedentung Untersuchung von Enzym-Relationen in Serum. Klin. Wochenschr.,40: 962-969.

Schneider W.C. and Hogeboom G.H. 1950. Intracellular Distribution of Enzymes, V: Further study on the Distribution of Cytochrome c in rat liver homogenates. J.Biol.Chem. 183: 123-128.

Sherlock S. 1982. Alcohol related liver disease: Clinical aspects and management. Brit.Med. Bull.38: 67-70.

Sippel P.J. and Agger W.A. 1981. Nitrofurantoin-induced granulomatous hepatitis. Urology 18: 177-178.

Smith L.H. Jr. and Thier S.O. 1981. Pathophysiology: The Biological Principles of Diseases, Saunders W.B., Philidalphia, 496-1721.

Sund H. and Theorell H. 1963. Alcohol Dehydrogenases, pp.25-86. In: The Enzymes, Boyer P.D., Lardy H.A. and Myrback K., Eds., New York, Acad. Press 7.

VanWaes L. and Lieber C.S. 1977. Glutamate Dehydrogenase: A reliable marker of liver cell necrosis in the alcoholic. Br.Med.J. 2: 1508-1510.

Vazquez J.J., Diaz de Otazu R., Guillen F.J., Zozaya J. and Pardo F.J. 1983. Hepatitis induced by drugs used as alcohol aversion therapy. Diagn. Histopathol. 6: 29-37.

Vierling J.M. 1983. Alcoholic liver injuries - update 1983. Am. Counc. Life Ins. (Med) 8: 87-93.

White A., Handler P. and Smith E.L. 1973. Principles of Biochemistry (5th ed), McGraw Hill/A. Blakiston Publication, Montreal, 357.

Witzleben C.L. 1972. Physiologic and morphologic natural history of a model of intrahepatic cholestasis. Am. J. Path. 66: 577-588.

Worner T.M. and Lieber C.S. 1980a. Plasma Glutamate Dehydrogenase: Clinical Application in Patients with Alcoholic Liver Disease. Alcoholism: Clinical and Experimental Research 4: 431-434.

Worner T.M. and Lieber C.A. 1980b. Plasma Glutamate Dehydrogenase: A Marker of Alcoholic Liver Injury. Pharmacology Biochemistry and Behaviour 13(1): 107-110.

Worner T.M. 1983. Blood Tests in Alcoholic Liver Diseases. Hematological Correlates of Alcoholism. In: International Alcohol Research Seminar, Washington, D.C.

Zimmerman H.J. 1978. Hepatotoxicity. Appleton - Century - Crofts, New York, 144.

Zimmerman H.J. 1983. Hepatotoxic evaluation methodology, pp.159-194. In: Diagnostic procedures in the evaluation of hepatotoxic diseases. Laboratory and Research Methods in Biology and Medicine. Zimmerman H.J., Ed., Alan R. Liss, New York.